Mutations in the *GGCX* and *ABCC6* Genes in a Family with Pseudoxanthoma Elasticum-Like Phenotypes

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A characteristic feature of classic pseudoxanthoma elasticum (PXE), an autosomal recessive disorder caused by mutations in the *ABCC6* gene, is aberrant mineralization of connective tissues, particularly the elastic fibers. Here, we report a family with PXE-like cutaneous features in association with multiple coagulation factor deficiency, an autosomal recessive disorder associated with *GGCX* mutations. The proband and her sister, both with severe skin findings with extensive mineralization, were compound heterozygotes for missense mutations in the *GGCX* gene, which were shown to result in reduced γ-glutamyl carboxylase activity and in undercarboxylation of matrix gla protein. The proband's mother and aunt, also manifesting with PXE-like skin changes, were heterozygous carriers of a missense mutation (p.V255M) in *GGCX* and a null mutation (p.R1141X) in the *ABCC6* gene, suggesting digenic nature of their skin findings. Thus, reduced γ-glutamyl carboxylase activity in individuals either compound heterozygous for a missense mutation in *GGCX* or with haploinsufficiency in *GGCX* in combination with heterozygosity for *ABCC6* gene expression results in aberrant mineralization of skin leading to PXE-like phenotype. These findings expand the molecular basis of PXE-like phenotypes, and suggest a role for multiple genetic factors in pathologic tissue mineralization in general.

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INTRODUCTION

Pseudoxanthoma elasticum (PXE; OMIM 264800, 177850) is an autosomal recessive multisystem disorder characterized by dystrophic mineralization of soft connective tissues, particularly the elastic fibers, in a number of organs, including the skin, the eyes, and the arterial blood vessels (Ringpfeil *et al.*, 2001; Neldner and Struk, 2002; Uitto and Ringpfeil, 2007; Li *et al.*, 2008). The primary cutaneous lesions are small, yellowish papules on the predilection sites at flexural areas, and these lesions progressively coalesce into larger plaques of inelastic, leathery skin with a yellowish hue. Histopathology of the affected skin shows accumulation of pleiomorphic elastotic material in upper and mid dermis, which is mineralized as visualized by special histopathologic stains. The eye manifestations consist of angioid streaks, and bleeding from the retina can result in loss of visual acuity and, rarely, blindness. The cardiovascular manifestations result from mineralization of the arterial blood vessels, and include gastrointestinal bleeding, intermittent claudication, hypertension and, occasionally, early myocardial infarcts. Although the disease has considerable morbidity and mortality, the phenotypic spectrum is highly variable with both inter- and intra-familial heterogeneity. The precise incidence of this condition is undefined, but the estimates are in the range of 1 in 50,000–75,000.

Classic PXE is caused by mutations in the *ABCC6* gene, which encodes a putative transmembrane transporter protein, ABCC6 (also known as multidrug resistance-associated protein 6), a member of the family of ATP-binding cassette proteins (Bergen *et al.*, 2000; Le Saux *et al.*, 2000; Ringpfeil *et al.*, 2000; Miksch *et al.*, 2005; Pfendner *et al.*, 2007). The *ABCC6* gene is primarily expressed in the liver, to a lesser extent in the proximal tubules of kidneys, and at very low level, if at all, in tissues affected in PXE (Belinsky and Kruh, 1999; Scheffer *et al.*, 2002; Matsuzaki *et al.*, 2005). Although the mineral deposits in the affected tissues are known to consist of calcium and phosphate, the precise mechanisms leading to aberrant mineralization remain unclear, and specifically, the substrate specificity of ABCC6 *in vivo* is currently unknown.

Occasionally, PXE-like phenotypes have been reported in association with multiple coagulation factor deficiency (Rongioletti *et al.*, 1989; Le Corvaisier-Pieto *et al.*, 1996;

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Vanakker *et al.*, 2007), and molecular analysis of some of these patients revealed mutations in the *GGCX* gene, which encodes an enzyme required for γ-glutamyl carboxylation of gla proteins (Vanakker *et al.*, 2007). In this report we detail a family with PXE-like clinical features, and also with deficiency of vitamin K-dependent clotting factors, particularly Factor X. Strikingly, this family harbors mutations both in the *GGCX* and *ABCC6* genes, and combinations of these mutations in two patients with PXE-like skin phenotype suggest digenic inheritance.

RESULTS

Clinical findings

The proband (III-3 in Figure 1) is a 16-year-old woman who was initially evaluated in early childhood because of cardiac abnormalities, including supravalvular pulmonic stenosis and peripheral pulmonary artery stenosis. She has undergone two pulmonary valve replacement procedures. Following an episode of endocarditis, she had a stroke with vision loss in the right eye, but rigorous ophthalmologic examination with respect to angioid streaks has not been reported. She developed focal segmental glomerulosclerosis, thought to be immune complex mediated. At around age 10 years, she began developing progressively loose, sagging and redundant skin, primarily affecting the neck and trunk, and an initial diagnosis of cutis laxa was made (Figure 1a–c). She was also found to have a coagulation disorder with Factor X deficiency (Table 1). The proband has a 19-year-old sister (III-1) with similar skin changes beginning in her early teens, as well as a coagulation disorder; she has had a normal cardiac evaluation. They have a 17-year-old brother (III-2) who is clinically normal. Examination of other members of the nuclear pedigree (Figure 1g) showed that the father of the proband is clinically normal, whereas the mother (II-2), at the age of 40 years, has distinct folding of the skin, particularly in the axillary areas. She also has characteristic yellowish papules consistent with PXE on the sides of the neck and in the axillary areas (Figure 1d). The mother has a fraternal twin sister (II-3) with similar cutaneous findings (Figure 1e and f). Neither the mother nor her sister have evidence of a coagulation disorder (Table 1).

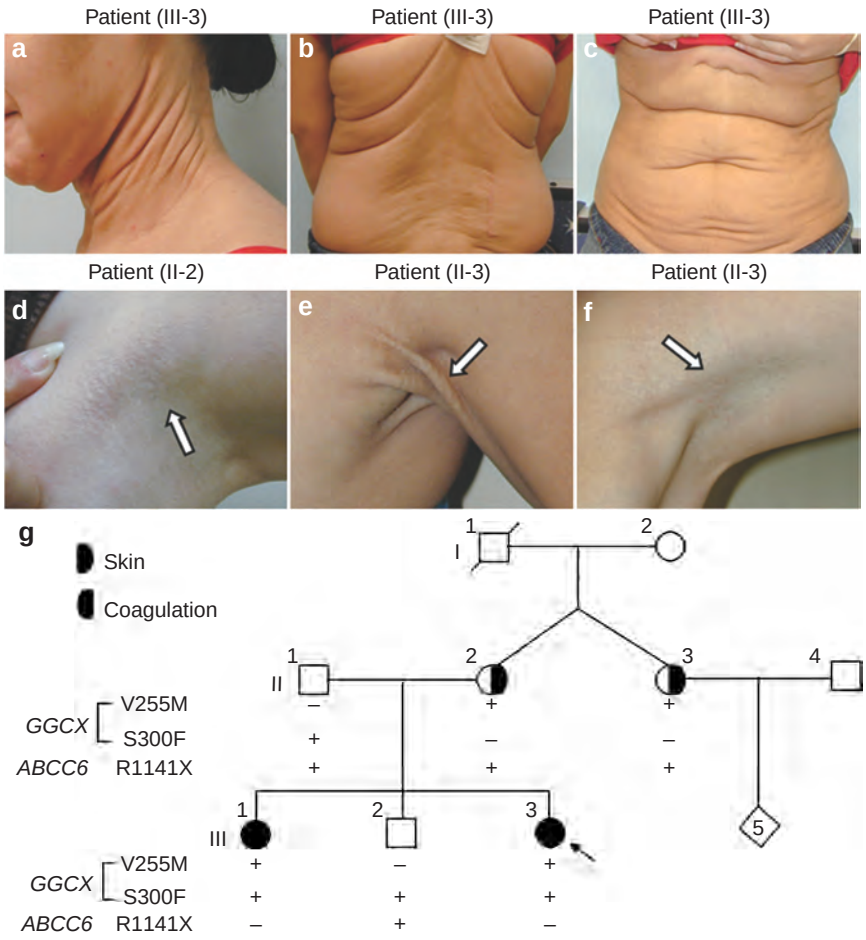

Figure 1. Cutaneous findings in a family with PXE-like phenotype. Note the loose and sagging skin in the 16-year-old proband (III-3, **a**–**c**). The proband's mother (II-2, **d**) and aunt (II-3; **e, f**), both 40 years of age, demonstrate redundant folding of the skin in the axillary area (**d**, **e**) and popliteal fossa (**f**), as well as small yellowish papules characteristic of PXE (arrows). The nuclear pedigree of the family with PXE-like skin findings and coagulation factor deficiency (**g**). The mutations in the *GGCX* and *ABCC6* genes identified in this family are indicated on the left of panel g. The proband is identified by an arrow.