Mutations of the gene encoding the transmembrane transporter protein ABC-C6 cause pseudoxanthoma elasticum

Abstract We recently published the precise chromosomal localization on chromosome 16p13.1 of the genetic defect underlying pseudoxanthoma elasticum (PXE), an inherited disorder characterized by progressive calcification of elastic fibers in skin, eye, and the cardiovascular system. Here we report the identification of mutations in the gene encoding the transmembrane transporter protein, ABC-C6 (also known as MRP-6), one of the...
Pseudoxanthoma elasticum (PXE), the prototypical Mendelian disorder of elastic tissue, is characterized by progressive calcification of elastic fibers in skin, retina, and the cardiovascular system. Whereas the cutaneous lesions are mainly of cosmetic concern, the ocular manifestations result in various degrees of visual impairment in about half of all cases, brought about by retinal hemorrhage due to vascular friability, and the cardiovascular lesions, albeit rare, can lead to serious morbidity [1, 2].

Based on our previous genetic mapping studies in which we identified a 500-kb region on chromosome 16p13.1 as the localization of the gene associated with PXE [3, 4], we identified four putative candidate genes, which we identified a 500-kb region on chromosome 16p13.1 as the localization of the gene associated with PXE.

**Key words** Pseudoxanthoma elasticum · Membrane transporter proteins · ATP binding cassette proteins · ABC-C6

**Abbreviations** ABC: ATP binding cassette · ATP: Adenosine triphosphate · PCR: Polymerase chain reaction · PXE: Pseudoxanthoma elasticum

**Introduction**

Pseudoxanthoma elasticum (PXE), the prototypical Mendelian disorder of elastic tissue, is characterized by progressive calcification of elastic fibers in skin, retina, and the cardiovascular system. Whereas the cutaneous lesions are mainly of cosmetic concern, the ocular manifestations result in various degrees of visual impairment in about half of all cases, brought about by retinal hemorrhage due to vascular friability, and the cardiovascular lesions, albeit rare, can lead to serious morbidity [1, 2].

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Genomic and c-DNA sequencing of these four genes in a number of affected and unaffected members of PXE families revealed ABC-C6 as the disease-causing gene.

**Materials and methods**

**Collection of PXE-families and selection of samples for mutation screen**

The establishment of a large repository of 192 affected and 380 nonaffected individuals from a total of 81 families, and the techniques to extract DNA from blood samples obtained after appropriate informed consent have been previously described [3, 4]. Haplotypic homozygous affected and haplotypic homozygous unaffected members of four consanguinous families were selected for systematic sequence analysis of pM5, MRP1, NPIP, and ABC-C6.

**Sequence and genotype analysis**

Fluorescent dideoxyterminator sequencing was carried out on ABI 377 and ABI 3700 automated sequencing devices, on appropriate polymerase chain reaction (PCR) amplified fragments of all four genes. Quality score based sequence comparisons used the Sequencher 4.0 (Gene Codes, Ann Arbor, Mich., USA) software tool. For two of the mutations identified in ABC-C6 we developed PCR restriction fragment length polymorphism screening assays:

(a) The exon 24 C3421T single nucleotide polymorphism is associated with the loss of a BslI restriction endonuclease recognition site, resulting in major digestion products that differ by 13 bp in length. Briefly, 20 ng genomic DNA was PCR-amplified using primer pair E24 (Table 1) and incubated with 7 U BslI (New England Biolabs) in the appropriate buffer at 55°C for 6 h, with a subsequent inactivation at 80°C for 20 min, fractionation by submarine agarose (4% MetaPhor, FMC Bioproducts, Rockland, Me., USA) gel electrophoresis, and visualization by ethidium bromide staining.

(b) The exon 28 C4015T single nucleotide polymorphism is associated with the gain of a novel BslHKAI restriction endonuclease recognition site, resulting in major digestion products that differ by 72 bp in length. Briefly, 20 ng genomic DNA was PCR-amplified using primer pair E28 (Table 1) and incubated with 5 U BslHKAI (New England Biolabs) in the appropriate buffer with addition of bovine serum albumin at 65°C for 6 h with a subsequent inactivation at 80°C for 20 min, fractionation by submarine agarose.

**Table 1 ABC-C6 mutations**

<table>
<thead>
<tr>
<th>Exon</th>
<th>Mutation</th>
<th>Primers</th>
<th>Annealing temperature (°C)</th>
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<tbody>
<tr>
<td>15</td>
<td>nt 1896 C→A aa 632 His→Gln</td>
<td>E15F: TCC CTA AAA ACA TGA GGC TGG TTA CTA C E15R: CGG CCA GGT CAG GGG TCT C</td>
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<td>nt 4015 C→T aa 1339 Arg→Cys</td>
<td>E28F: CCC ACC ATG CCT CCC ATC TT E28R: GTA CAG CAG AAA GAT CCT CCC AAT AAA</td>
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