

A Novel Q378X Mutation Exists in the Transmembrane Transporter Protein, *ABCC6* and its Pseudogene - Implications for Mutation Analysis in Pseudoxanthoma Elasticum (PXE)

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Introduction

◆ Pseudoxanthoma elasticum (PXE) (OMIM 264800)

- is the classical Mendelian disorder that affects the elastic tissue by progressive calcification of elastic fibers in skin, retina, and the cardiovascular system. While the cutaneous lesions may have mainly cosmetic implications and diagnostic relevance, it is the ocular and cardiovascular manifestations that can cause serious morbidity.
- Mutations in the ATP-binding transmembrane transporter gene *ABCC6* were recently shown to cause PXE.
- We had previously identified the starting point of a large genomic segmental duplication within the *PXE* locus in the cytogenetic interval defined by the *Cy19* and *Cy185* somatic cell hybrid breakpoints on chromosome 16p13.1.
- This starting point mapped to BAC clone *CTA-962b4* (*U91318*) within the genomic sequence of *ABCC6*.

◆ In an ongoing mutation screen

- in *PXE* families we identified the *Q378X* (*1132C→T*) nonsense mutation in exon 9 of the *ABCC6* gene in diseased and haplotype negative members of our *PXE* families in apparent contradiction to the families' haplotype. Screening 192 chromosomes of a control population revealed that all these individuals were heterozygous for the *Q378X* mutation questioning its disease relevance for *PXE*.



Figure 1: *Pst*I restriction fragments of the *E09-RFLP* (see Fig. 2) after amplification of *ABCC6* and ψ *ABCC6* sequences in family A and controls. The 200bp fragment represents the normal (*1132C*) allele, the 295bp product represents the disease- or pseudo- (*1132T*) allele. Family A: Lane numbering corresponds to individuals' ids in the pedigree (see results)

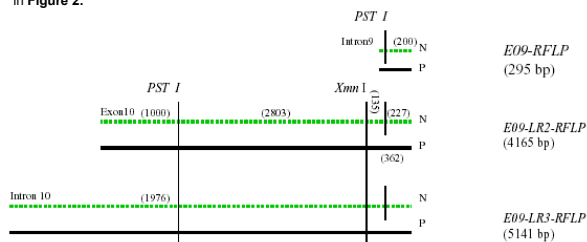
Methods

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

- 192 affected and 380 non-affected individuals from a total of 81 families,
- one affected individual per family and 4 unaffected members (that carried the reciprocal familial haplotype of their affected siblings) of different families were selected.

◆ Sequence and genotype analysis

- Fluorescent dideoxyterminator sequencing was carried out on ABI 377, ABI 3100 and ABI 3700 automated sequencing devices, on appropriate PCR-amplified fragments of *ABCC6*.
- Quality-score-based sequence comparisons were done using the "Sequencher" software tool version 4.05 (Gene Codes Corporation, Ann Arbor, MI).
- For the *Q378X* (*1132C→T*) mutation we developed 3 distinct PCR-RFLP screening assays shown in **Figure 2:**



◆ Somatic Cell Hybrid Panel Analysis

- Five cell lines containing sequential breakpoints informative for the *PXE* region, *Cy19*, *Cy185*, *Cy183*, *Cy163*, *Cy11* (ordered from 16p12 and towards 16q12) plus one additional clone, *Cy180(D)*—*deletion - Cy180(P)* for either verification or exclusion of additional ψ *ABCC6* copies telomeric to this region, was used for physical mapping of STSs of exon 1 to 10 of *ABCC6*.

Results

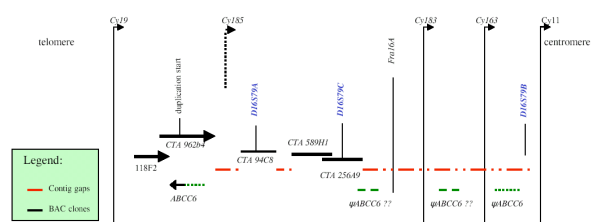
◆ Sequence alignment of BAC clone *CTA-962b4* with the cDNA of *ABCC6*

- suggested the existence of a partial homologue or a pseudogene.

◆ Somatic cell hybrid mapping

- identified at least one, possibly several copies of an exon 1 to 9 containing *ABCC6* pseudogene, ψ *ABCC6*, within the previously identified large segmental duplication on chromosome 16p [8] within the immediate vicinity of the *PXE* locus.
- Evidence for several *ABCC6* pseudogene copies is provided by several distinct copies (A-C) of *D16S79* in different somatic cell hybrid breakpoint intervals in physical proximity to either *ABCC6* or ψ *ABCC6*

Figure 3: Regional Partial Contig Map of Chromosome 16p13.1

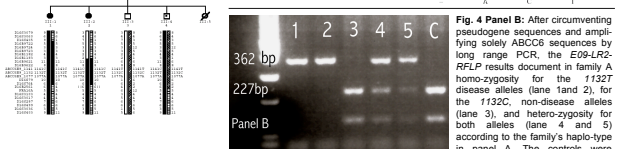


◆ DNA sequencing, long range PCR and RFLP analysis

- demonstrated the presence of the *Q378X* mutation in the *ABCC6* gene in five *PXE* associated chromosomes that showed altogether two distinct family haplotypes for 3 SNPs of exon 9 (*1077A→G*, *S359S*; *1132C→T*, *Q378X* and *1141T→C*, *L387L*) and also
- identified this *Q378X* variant at the analogous position in the ψ *ABCC6* gene, but **not** in *ABCC6* in the general population (100 control chromosomes).

Table 1 Sequence analysis of allelic variants of SNPs in exon 9 of *ABCC6* and ψ *ABCC6*

Chromosome	PCR primer	Sequencing primer	Phenotype	Mutation	E99 at 1077	E99 at 1132	E99 at 1141
<i>ABCC6</i> and ψ <i>ABCC6</i>							
Family A	E09	E09	PXE	Q378X	A	T	C
Family B	E09	E09	PXE	Q378X	A	C	T
Family C	E09	E09	PXE	Q378X	A	T	T
Family D	E09	E09	PXE	Q378X	A	C	T
Family E	E09	E09	PXE	Q378X	A	C	T
Controls (50)	E09	E09	Unaffected	-	A	C	T
<i>ABCC6</i>							
Family A	E094LR2	E09	-	Q378X	A	T	C
Family B	E094LR2	E09	-	Q378X	A	C	T
Family C	E094LR2	E09	-	Q378X	A	T	T
Family D	E094LR2	E09	-	Q378X	A	C	T
Family E	E094LR2	E09	-	Q378X	A	C	T
Controls (50)	E094LR2	E09	-	-	A	C	T



Conclusions

- The *Q378X* mutation in *ABCC6* is relevant for the *PXE* phenotype.
- It contributes to the phenotype in either a homozygous or compound heterozygous allelic state.
- It is derived by gene conversion from the silent *Q378X* variant in ψ *ABCC6*.
- Therefore gene conversion plays a role in at least two mutation events (two distinct conversion breakpoints), which lead to pseudoxanthoma elasticum.

J Mol Med (2001) 79:536-46

<http://link.springer-ny.com/link/service/journals/00109/bibs/1079009/1079053>