

Mutation Analysis in *ABCC6* Reveals no Evidence for Dominant Inheritance of Pseudoxanthoma Elasticum (PXE)



Berthold Struk^{1,8}, Sara Ibba^{1,8}, Amanda Lumsden², Ulf P Guenther¹, Dorothee Foernzler⁷, Stéphanie Christen-Zäch³, Carol Daugherty⁶, Raj Ramesar⁴, Mark Lebwohl⁵, Daniel Hohl³, Kenneth H Neldner⁶, Klaus Lindpaintner⁷ and Robert I. Richards²

¹Max-Delbrueck Centrum, Berlin, Germany; ²Department of Molecular Bioscience, The University of Adelaide, Adelaide, Australia; ³Department of Dermatology, University of Lausanne, Lausanne, Switzerland; ⁴Department of Human Genetics, Medical School, University of Cape Town, South Africa; ⁵Department of Dermatology, Mount Sinai School of Medicine, New York, NY; ⁶Department of Dermatology, Texas Tech University Health Sciences Center, Lubbock, TX; ⁷F. Hoffmann-La Roche Ltd, Roche Genetics, Pharmaceuticals Division, Basel, Switzerland; ⁸Charité, Franz-Volhard-Clinic, HELIOS-Klinikum, Humboldt University, Berlin, Germany.

Email: bstruk@mdc-berlin.de Web: www.napxe.org

Introduction

◆ Dominant Pseudoxanthoma elasticum (PXE) (OMIM 177850)

- was initially proposed by Pope in 1972 when he reported the observation of dominant inheritance for this disease in families with PXE from the UK. He never confirmed the disease in three consecutive generations of such families.
- PXE, however, is a solely autosomal recessive genetic disease which primarily affects the skin, retina and cardiovascular system (Neldner KH and Struk B 2002). This is in contrast to historic clinical (Pope 1974) and recent molecular claims (Bergen et al. 2000) that it is also dominantly inherited.
- PXE is characterized by progressive calcification of the elastic fibers in these organ systems with subsequent disintegration and destruction of the elastic tissue. While the cutaneous lesions have mainly cosmetic implications and major 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.
- The function of *ABCC6* and how perturbation of this function leads to PXE are still unknown.
- We performed a mutation screen in *ABCC6* through direct sequencing of 170 PXE chromosomes in 81 families with PXE and analyzed a subset of 9 families with the occurrence of PXE in more than 1 generation or in first degree cousins to gain insight into the molecular genetics of this clinically highly variable disease. These families would have been compatible with an apparent dominant mode of inheritance.

Methods

◆ Collection of PXE-families and selection of samples for mutation screening

- 81 families with PXE represented 170 distinct disease chromosomes that were analyzed.
- At least one affected individual per family and a total of 4 unaffected members (that carried the reciprocal familial haplotype of their affected siblings) of different families were selected for direct sequencing.

◆ Diagnosis of PXE was based on finding

- Category I diagnostic clinical criteria according to the consensus conference in 1993 that also required
- a positive skin biopsy of lesional skin (microscopic observation of fragmented calcified elastic fibers in the mid- and deep dermis after staining a histology slide of the skin specimen with von Kossa stain)

◆ Sequence and genotype analysis

- Fluorescent dideoxynucleotide sequencing was carried out on ABI 377, ABI 3100 and ABI 3700 automated sequencing devices, on appropriate PCR-amplified exonic fragments of *ABCC6* using primers complementary to neighboring intronic sequence.
- Quality-score-based sequence comparisons were done using the "Sequencher" software tool version 4.05 (Gene Codes Corporation, Ann Arbor, MI).
- Using the *ABCC6* flanking microsatellite markers *D16B9621* and *D16B9622*, downstream and *D16S79* and *D16S764* upstream from the gene sequence genotyping and haplotyping was performed as has previously been published.

◆ Sequence variants were considered to be phenotype modifying when

- ABCC6* sequence variants result in nonsense or splice site alterations that
 - cosegregate with the disease haplotype in affected but not in unaffected members of all PXE families carrying the specific mutation, and
 - that are not detected in 200 control alleles of unrelated and unaffected individuals.
- Nucleotide variations result in amino acid substitutions that
 - firstly cosegregate with the disease phenotype and haplotype in all PXE pedigrees carrying it
 - secondly no other potentially disease-causing and familial haplotype specific allelic variant is identified by complete sequence analysis of *ABCC6*,
 - thirdly the variant was not found in the panel of 200 control chromosomes and
 - fourthly the amino acid substitution involved a conserved amino acid.

References:

- Pope FM (1974) Autosomal dominant pseudoxanthoma elasticum. *Journal of Medical Genetics* 11 (2):152-157.
 Neldner KH and Struk B (2002) Pseudoxanthoma Elasticum. In Royce PM, Steinmann B (eds) Connective Tissue and Its Heritable Disorders: Molecular, Genetic and Medical Aspects, 2nd ed. New York: Wiley-Liss.
 Bergen AA et al (2000) Mutations in *ABCC6* cause pseudoxanthoma elasticum. *Nat. Genet.* 25 (2):228-231.

Results

◆ Genetic mapping data

- from all families was consistent with a single genetic locus on chromosome 16p giving rise to the proposal that "recessive" and "dominant" PXE map to the same locus.

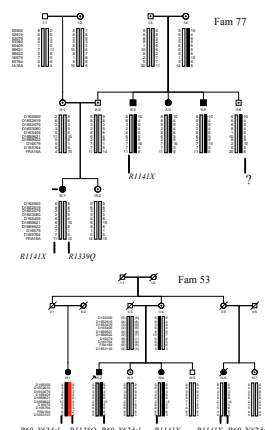
◆ Haplotype analysis

- did not identify affected sib pairs (according to category I diagnostic criteria) as heterozygotes at the gene locus.
- However, it showed that all of the PXE affected sib pairs in each family shared a maternal and paternal chromosomal haplotype (homozygous or compound heterozygous) at the locus (comparing familial haplotypes among affected siblings and also with their unaffected siblings where available) suggesting a recessive-only molecular mode of inheritance for PXE.
- Clinical carriers of the trait, with a haplotypic heterozygous allelic status for one of the familial disease alleles, may express a *form fruste* of the disease that can be characterized, in part, by category II diagnostic criteria.

◆ Mutation analysis confirmed a recessive-only mode of inheritance in PXE:

- In 93.8 % (76 out of 81) of the families, the affected individuals are either homozygous for the same, or compound heterozygous for 2 mutations.
- The remaining 5 families with one uncovered mutation show allelic compound heterozygosity for the cosegregating PXE haplotype.
- The transmission of the disease from an affected parent to the next generation always required two parental mutant allelic variants of *ABCC6*, independent of the clinical disease status of the parents consistent with a pseudo-dominant mode of inheritance.
- The types of mutations observed (in-frame termination codons, small and large deletions and their homozygous and compound heterozygous cosegregation with the disease phenotype) suggest a loss-of-function as the molecular mechanism for PXE.

Table 1. Clinical and molecular characteristics of the 81 families with PXE. The table includes the following columns: Index case, Age at onset, Phenotype, Haplotype, and *ABCC6* Mutations. The table is divided into two main sections: 'Families with compound heterozygosity for two mutations' and 'Families with compound heterozygosity for one mutation and one wild-type allele'. The table contains detailed data for 81 families, including the specific mutations found in each family and the clinical characteristics of the affected individuals.



Conclusions

- Despite other previous clinical and molecular claims our results show only evidence for recessively inherited PXE.
- The mechanism of disease transmission to the next generation is pseudo-dominant in all families that show the complete phenotype in more than one generation in our cohort.
- This has profound consequences for the genetic counseling of families with PXE:
 - no immediate disease risk to children of diseased individuals
 - genetic testing in families at risk can identify unrelated carriers prior to marriages