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Pseudoxanthoma Elasticum Is a Recessive Disease Characterized by Compound Heterozygosity

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Pseudoxanthoma elasticum (PXE) is caused by mutations in the *ABCC6* gene. Historically, PXE has been suggested to be inherited either in an autosomal dominant or autosomal recessive manner. To determine the exact mode of inheritance of PXE and to address the question of phenotypic expression in mutation carriers, we identified seven pedigrees with affected individuals in two different generations and sequenced the entire coding region of *ABCC6* in affected individuals, presumed carriers with a limited phenotype and unaffected family members. Two allelic mutations were identified in each individual with unambiguous diagnosis of PXE, as well as in those with only minimal clinical signs suggestive of PXE but with positive skin biopsy. Missense mutations were frequently detected in the latter cases. In conclusion, PXE is inherited in an autosomal recessive manner and presence of disease in two generations is due to pseudodominance.

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INTRODUCTION

Pseudoxanthoma elasticum (PXE; OMIM 264800 and 177850) is a heritable multisystem disorder with cutaneous, ophthalmologic, and cardiovascular manifestations (McKusick, 1972; Neldner, 1988; Uitto and Pulkkinen, 2002). There is marked clinical heterogeneity, and patients even within the same family may differ in disease severity as well as in the number of organ systems involved. PXE is caused by mutations in the *ABCC6* gene located on chromosome 16p13.1 (Bergen *et al.*, 2000; Le Saux *et al.*, 2000; Ringpfeil et al., 2000). This gene encodes MRP6, an ATP-binding cassette transmembrane transporter protein expressed primarily in the liver and the kidneys (Kruh and Belinsky, 2003).

Since the discovery of *ABCC6* as the gene harboring mutations in PXE, molecular studies have suggested an autosomal recessive mode of inheritance in the majority of PXE families studied (Le Saux *et al.*, 2001; Ringpfeil *et al.*, 2001b; Chassaing *et al.*, 2004, 2005; Plomp *et al.*, 2004). However, the exact mode of inheritance of PXE has remained controversial for several reasons. First, early clinical and epidemiologic studies, particularly in the United Kingdom, suggested that a large portion of families display autosomal dominant inheritance, based on clinical observations of affected individuals in two subsequent generations (Pope, 1974). Second, more recent studies have suggested that heterozygous carriers of *ABCC6* mutations in families with a history of PXE may have subclinical disease, that is, positive skin biopsy in the absence of overt clinical lesions and with asymptomatic or minimally symptomatic angioid streaks (Bacchelli *et al.*, 1999; Sherer *et al.*, 2001). In addition, the pattern of inheritance is often difficult to decipher from the family pedigree owing to delayed onset of symptoms, frequently not evident until the second or third decade of life (Neldner, 1988).

We have identified seven families with clinical features suggestive of PXE in individuals in two generations. We performed mutation analysis by direct sequencing of the *ABCC6* gene in these families to clarify the exact mode of inheritance at the molecular level.

RESULTS

Clinical features

The nuclear pedigrees of the seven families with affected individuals in two generations are depicted in Figure 1. All patients were examined at least by one of the authors, and investigations included skin biopsy and ophthalmologic examination with fluorescent angiography. The clinical findings in the affected individuals, including skin findings, the presence of angioid streaks, and evidence of vascular disease, together with skin biopsy results are summarized in Table S1. Families 1–3 included presumed obligate heterozygous carriers with asymptomatic or mildly symptomatic angioid streaks and positive skin biopsy in the parent generations (for clinical details of Family 1, see Ringpfeil *et al.*, 2000). In Family 4, a father and a son were clearly affected with PXE. Family 5 depicted a clinically affected paternal grandmother of an affected child (Larralde *et al.*,

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Abbreviation: PXE, pseudoxanthoma elasticum

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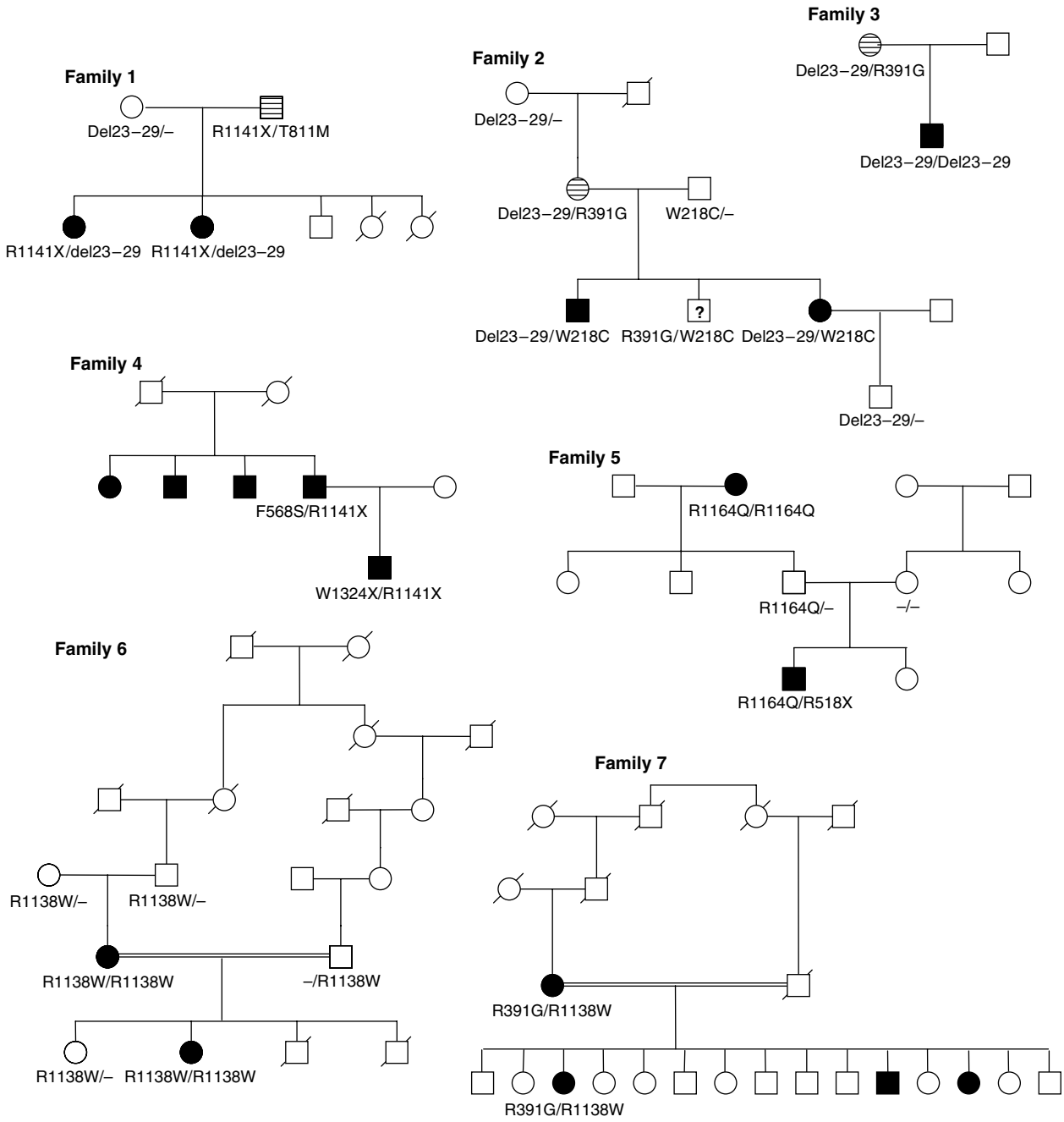

Figure 1. Pedigrees of seven families with PXE in two generations. Solid symbols denote a definite diagnosis of PXE; shaded symbols reflect minimal cutaneous and/or ophthalmological symptoms or a histopathological diagnosis of PXE based on skin biopsy. Double lines denote consanguinity. The corresponding mutations are indicated below each individual examined. The diagnosis of an individual marked by ? in Family 2 is uncertain.

2002). Families 6 and 7 were consanguinous and featured clinically affected individuals in two subsequent generations (for details of Family 6, see Ringpfeil *et al.*, 2000).

Genotypic data

Mutation analysis of the *ABCC6* gene resulted in detection of two allelic mutations in each affected individual as well as in those presumed to be heterozygous carriers with subclinical phenotype (Figure 1 and Table S1). A total of 11 distinct *ABCC6* mutations were discovered in the 34 alleles of these individuals. Five of the alleles harbored the recurrent R1141X mutation, which is prevalent in Caucasian populations, six of the alleles contained the R1138W mutation, and seven alleles harbored the deletion mutation del23–29, all of which have been previously reported in a number of families with PXE (Le Saux *et al.*, 2001; Ringpfeil *et al.*, 2001a; Uitto *et al.*, 2001; Pulkkinen *et al.*, 2002; Chassaing *et al.*, 2004). Among the other mutations identified, three were novel missense mutations: W218C, T811M, and R1164Q. In addition, a previously unpublished nonsense mutation W1324X was

noted in the proband of Family 4. The previously undescribed missense mutations were considered pathogenic, as they were not found in at least 200 alleles of unrelated healthy controls.

In Family 1, the two affected daughters of a clinically unaffected mother and an affected father were compound heterozygotes (R1141X/del23–29), and the parents were carriers of the corresponding mutations. In addition, the father with asymptomatic angioid streaks, evidence of coronary artery disease, history of gastrointestinal bleeding, and positive skin biopsy, but no clinically obvious skin involvement, was compound heterozygous for R1141X and a novel missense mutation T811M. In Family 2, the three children were compound heterozygotes with two different combinations (del23–29/W218C and R391G/W218C). The mother with minimal clinical signs but with a positive skin biopsy was compound heterozygous for two of these mutations (del23–29/R391G) and manifested with asymptomatic angioid streaks and hypertension. However, the father, a heterozygous carrier of the W218C mutation, as well as the maternal grandmother, carrier of the del23–29 mutation, were clinically unaffected. In Family 3, the clinically affected son was homozygous for the del23–29 mutation, whereas the mother manifesting with vision loss, intermittent claudication, stroke, and hypertension was a compound heterozygote for del23–29/R391G mutations. She had not been diagnosed with PXE prior to this study. The son had presumably inherited the other del23–29 allele from the clinically unaffected father. In Family 4, the two affected individuals, a father and a son, were compound heterozygotes, both for R1141X mutation in one allele, in combination with either F568S (father) or W1324X (son). In Family 5, the proband was compound heterozygote for R1164Q/R518X, the missense mutation being inherited from the clinically unaffected father while the nonsense mutation was either a *de novo* mutation or reflected germline mosaicism in the clinically unaffected mother whose peripheral blood DNA did not carry this mutation. The grandmother of the proband was homozygous for the R1164Q mutation. Segregation of the mutant alleles in Families 6 and 7 suggested that the homozygosity for the R1138W mutation (Family 6) and compound heterozygosity for the R391G/R1138W mutations (Family 7) in affected individuals in two subsequent generations were due to consanguinity, a conclusion supported by examination of the family pedigrees (see Figure 1) and by haplotype analysis (data not shown).

The presence of different combinations of mutations in these families could suggest that the carrier frequency of mutations in the *ABCC6* gene may be higher than previously suspected, resulting in occurrence of different compound heterozygous mutations in the same family, with a different degree of clinical involvement. However, probing the two most common mutations in *ABCC6*, namely R1141X and deletion of exons 23–29 in 254 control alleles of Caucasian origin, revealed no carriers (data not shown).

Collectively, our data indicate that individuals with unambiguous diagnosis of PXE, as well as those presenting with minimal manifestations suggestive of PXE and positive skin biopsy, examined in our study, harbor two mutations in the *ABCC6* gene.

DISCUSSION

Since the availability of DNA testing in PXE, an accumulating body of evidence has suggested that PXE is inherited primarily in an autosomal recessive inheritance pattern, and that autosomal dominant PXE in many cases reflects pseudodominance. Despite the overwhelming evidence to the contrary to date, some practitioners still believe in an autosomal dominant form of PXE and initiate work up and counseling accordingly. Those beliefs were fostered by early publications that suggested the presence of both autosomal dominant and autosomal recessive forms of PXE (Pope, 1974, 1975). In particular, studies canvassing the PXE patient population in the United Kingdom reported that the majority, $\sim$53% of the 121 families examined, showed autosomal dominant inheritance with disease presenting in two subsequent generations, as suggested by clinical observation but not verified in all cases by diagnostic skin biopsy (Pope, 1974). Subsequently, examination of a cohort of 100 patients in the United States concluded that the majority of cases, $>$90%, demonstrate autosomal recessive inheritance, and that autosomal dominant inheritance, as evidenced at the time by the presence of affected individuals in two generations, is rare (Neldner, 1988). Many of the cases were sporadic with an unknown mode of inheritance. In a recent report, Plomp *et al.* (2004) re-examined their PXE families from the Netherlands with previously reported autosomal dominant disease and reviewed the existing literature (1966–2003) on several other families. Through clinical, genealogical, and molecular genetic examination, they reclassified the mode of inheritance as autosomal recessive in all but one of their families, in which a second mutation could not be identified. They concluded that autosomal dominant inheritance in PXE may exist, but is much more rare than previously thought. As the current detection rate for mutations in the *ABCC6* gene by direct sequencing has been reported to be in the range of 70–100%, it is reasonable to assume that the second mutation in their family evaded detection, although this possibly was dismissed by the authors (Plomp *et al.*, 2004). On another note, reports on minimal symptoms in obligate carriers were based on establishing the presence of a single mutation in a first-degree relative of a PXE patient, yet the obligate carriers were not examined for additional, independently inherited coding changes in the *ABCC6* gene (Bacchelli *et al.*, 1999).

In this study, we have examined seven families with evidence of PXE in two different generations. In four of the families (nos. 4–7) there were clearly affected individuals in two generations, as determined by characteristic clinical findings, and positive skin biopsy demonstrating abnormal, calcified elastic fibers. In two of the families (nos. 6 and 7), pseudodominant inheritance was caused by consanguinity, a phenomenon that has recently been highlighted in other PXE families as well (Ringpfeil *et al.*, 2000; Chassaing *et al.*, 2004). In Families 4 and 5, compound heterozygosity due to two different combinations of mutations was observed. In

Families 1–3, individuals with clearcut diagnosis of PXE were noted, and in each family one of the parents had minimal clinical manifestations but positive skin biopsy and they were found to be compound heterozygous for *ABCC6* mutations. It is of interest that in Family 2, the eldest son and the daughter had a clearcut clinical diagnosis of PXE, yet the second son, upon examination by a dermatologist and an ophthalmologist, showed no clinical evidence of PXE at the age of 37 years even though he was compound heterozygous for R391G/W218C mutations. These mutations are apparently pathogenic in a recessive manner, as they, in combination with the del23–29 mutation, result in severe phenotype in the older and younger siblings of this individual (Figure 1). This individual, from whom no skin biopsy was available, may therefore represent a presymptomatic, clinically undetectable form of PXE, and his disease manifestations may be delayed beyond his current age. Thus, in each patient examined in this study, even in cases with the most limited form of the disease, the clinical manifestations are a result of the presence of two mutations in the affected individuals. These observations emphasize the phenotypic heterogeneity in PXE and suggest the possibility that intragenic *ABCC6* polymorphisms and/or other modulating genes influence the clinical presentations.

Collectively, our findings strongly imply that PXE follows strictly an autosomal recessive mode of inheritance. This conclusion has been reinforced by recent observations on mice in which the *Abcc6* gene has been inactivated by targeted ablation (Gorgels *et al.*, 2005; Klement *et al.*, 2005). Specifically, in a study reported by us (Klement *et al.*, 2005), heterozygous +/− mice, similar to their wild-type littermates, displayed no evidence of tissue calcification, while −/− mice readily showed extensive calcification of skin, mid-sized arteries, retina, and other tissues, recapitulating PXE.

The findings of our study have clear implications for genetic counseling. First, our studies establish that PXE is inherited in an autosomal recessive manner, thus providing basis for appropriate counseling for the risk of re-occurrence of PXE in families with at least one previously affected individual. In this context, it should be noted that identification of *ABCC6* mutations in an affected child with PXE and his/her heterozygous carrier parents allows presymptomatic diagnosis of this condition in siblings in whom the manifestations of PXE may be delayed even beyond the usual time span of manifestations in the second or third decade of life. Finally, our observations suggest that pseudodominance is relatively frequent in PXE, a notion that the health-care personnel providing genetic counseling should be aware of.

MATERIALS AND METHODS

Patient population

Seven families with either the diagnosis of PXE in two generations or PXE in one generation and an obligate heterozygote with limited phenotype in another were selected from a cohort of over 70 families with PXE in a research database. The diagnostic criteria for PXE consisted of cutaneous yellowish papules and plaques in predilection sites together with angioid streaks and/or evidence of cardiovascular disease. Individuals with positive skin biopsy and lack of clinical skin manifestations, but having asymptomatic angioid streaks and/or cardiovascular disease, were considered minimally affected. All families consisted of two or more affected individuals (Figure 1). Informed consent was obtained from all individuals conforming to the University's Institutional Review Board, and the experiments were performed according to the Helsinki Principles. Patients were subjected to dermatological, ophthalmological, and cardiologic examination (Table S1). All patients were seen by at least one of the authors and had a skin biopsy (hematoxylin and eosin and von Kossa stains). The presence of angioid streaks was verified by fundoscopy. Blood was obtained from all affected individuals and their unaffected family members when available. Control DNA was also obtained from 104 unrelated, population-matched controls using the above ethical guidelines.

Genotype analysis

Genomic DNA was isolated from peripheral blood leukocytes using standard protocols. The 31 exons and flanking intronic sequences of the *ABCC6* gene were amplified by PCR, followed by automated dideoxy nucleotide sequencing (Pulkkinen *et al.*, 2001; Ringpfeil *et al.* 2001b). New mutations were confirmed by restriction enzyme digests of 50 unrelated, unaffected controls. The segregation of pathogenetic mutations within families was determined by restriction enzyme digestion and/or direct nucleotide sequencing.

Haplotype analysis

Haplotyping was performed in individuals who were homozygous for *ABCC6* mutations to determine consanguinity. Twelve microsatellite markers spanning 3 cM around the *ABCC6* locus were PCR amplified. PCR products were end labeled with [γ-^{32}P]ATP and examined on 6% polyacrylamide sequencing gels. Up to 25 intragenic single nucleotide polymorphisms were analyzed by direct sequencing or restriction enzymatic digestion.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Table S1. Clinical features of affected individuals.

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