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Early and severe amyloidosis in a patient with concurrent familial Mediterranean fever and pseudoxanthoma elasticum

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Summary

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None declared.

A young woman patient had early and extensive familial Mediterranean fever (FMF)-related amyloidosis and pseudoxanthoma elasticum (PXE). She had the novel G1042S mutation in the ATP-binding cassette subfamily C member 6 (*ABCC6*) gene, responsible for PXE, and the mutation M694I in *MEFV*, the FMF gene. Both mutations were homozygous, in agreement with consanguinity in the parents. ABCC6 deficiency may have increased the severity of amyloidosis by increasing the deposition in target tissues of heparan sulphate, which colocalizes spatially and temporally with amyloid proteins, and/or by decreasing the therapeutic activity of colchicine.

Familial Mediterranean fever (FMF, MIM 249100) is an autoinflammatory disease with autosomal recessive transmission due to mutations in the *MEFV* gene.[1–4] *MEFV* maps on 16p13.3 and encodes pyrin (marenostrin), the biological function of which is probably to downregulate cytokine-mediated inflammatory pathways and to stimulate apoptosis.[5] FMF predominantly affects Jewish, Armenian, Turkish and Arabic populations from the Mediterranean basin, and is characterized by periodic and acute bouts of fever with painful arthritis and polyserositis. Development of renal amyloidosis indicates a poor prognosis. Prophylactic treatment with colchicine has been demonstrated both to avoid inflammatory episodes and to prevent amyloidosis-related renal involvement.[6,7] The mechanisms of the therapeutic effectiveness of colchicine in FMF remain to be determined, but it is of note that pyrin colocalizes with leucocyte microtubules, the subcellular target.[8]

Pseudoxanthoma elasticum (PXE, MIM 264800 and 177850) is a heritable systemic disorder of connective tissue characterized by cutaneous, vascular and ocular changes that result from the accumulation of mineralized and fragmented elastic fibres (elastorrhexic fibres).[9] Skin lesions consist of yellowish confluent papules and plaques on the neck, axilla, antecubital fossa, groin and periumbilical areas, leading to loss of cutaneous laxity. Similar elastic fibre changes within the internal elastic lamina of medium-sized arteries are frequently associated with early onset of peripheral occlusive vascular disease. Angioid streaks on fundoscopic examination of the eye result from elastic changes in Bruch's membrane. They may be complicated by subretinal neovascularization and haemorrhage, and lead to loss of central vision. Changes in other extracellular matrix components, such as collagen fibrils or proteoglycans, have also been demonstrated in patients with PXE.[10,11] Serum amyloid antigen (SAA) has been immunocolocalized with mineralized elastin, and also with collagen fibrils and microfilamentous aggregates in the vicinity of damaged elastic fibres.[11] The ATP-binding cassette subfamily C member 6 (*ABCC6*) gene, mapped on 16p13.1, has recently been demonstrated to be responsible for PXE.[12–15] More than 100 mutations have been identified to date, and PXE is now considered to be recessively inherited in most cases.[9] The substrate(s) of the transmembrane transporter ABCC6 remain(s) unidentified. *ABCC6* is predominantly expressed in the liver and kidneys, suggesting that PXE may be a primary metabolic disorder.[16]

We report a young woman who had both extensive FMF-related amyloidosis and PXE. The responsibility of ABCC6 deficiency in the severity of FMF in this case is discussed.

Case report

An Algerian woman, born in 1972 of healthy consanguineous parents, was diagnosed with FMF at 16 years of age. Febrile inflammatory attacks involving the abdomen, joints and chest had been occurring for 10 years, at a frequency of 20–30 per year, with durations of 5–9 days. Examination revealed spleen enlargement. In addition, nephrotic syndrome and renal failure were present. Renal amyloidosis was demonstrated by a kidney biopsy (Fig. 1a). Treatment with colchicine (1·5 mg daily) was initiated in 1988. It was only partially effective for the inflammatory attacks but led to a decrease in the splenomegaly. Treatment compliance was good, but the dosage was limited because of profuse and intractable diarrhoea. Renal failure necessitated haemodialysis (1998) and then kidney transplantation (2004). A liver biopsy was obtained during laparotomy for cholecystectomy because the liver looked macroscopically abnormal. In addition, intestinal mucous membrane biopsies were performed because of recurrent diarrhoea. Despite colchicine therapy, the liver and intestinal biopsies (Fig. 1b) also showed extensive amyloidosis. Thyroid, heart and carpal tunnel amyloidosis was also suspected because of thyroid enlargement, heart failure and painful fingers, respectively. Plasma SAA was 30 mg L^{-1} (normal, 0–15) (Dr A.L. Debard, Lyon, France) in 2002, a couple of days after performing haemodialysis. After informed consent, mutation analysis of the *MEFV* gene demonstrated the M694I point mutation.

PXE was diagnosed at 8 years of age. The skin areas involved were the neck (Fig. 2), armpits and abdomen. Bilateral angioid streaks were present on fundoscopy. There was no occlusive arterial disease. Examination of an axillary skin biopsy specimen demonstrated unequivocal PXE. Haematoxylin and eosin and von Kossa staining showed fragmented and calcified elastic fibres in the mid-dermis. Ultrastructural examination demonstrated both characteristic central foci of heavy mineralization within the core of the elastic fibres (Fig. 3) and filamentous material at their periphery. Evocative abnormal flower-shaped collagen fibrils were also visible. In addition, sporadic calcifications were present in the walls of small dermal arteries (not shown). Amyloid deposits were absent

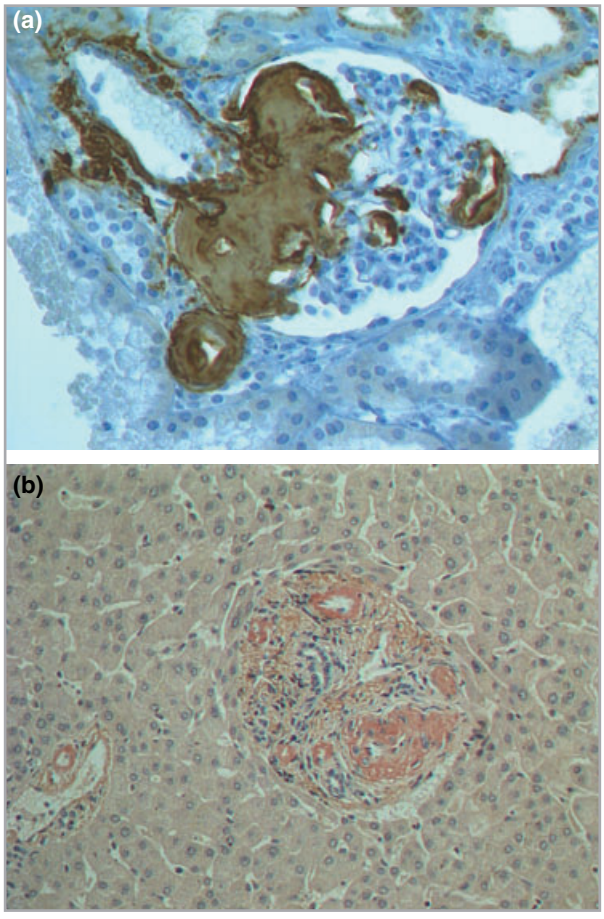

Fig 1. (a) Glomerular and vascular serum amyloid antigen (SAA) amyloid deposits in kidney (anti-SAA immunolabelling; original magnification × 100); (b) portal and vascular amyloid deposits in the liver (Congo red staining; original magnification × 100) (Dr F. Maître, Orléans, France).

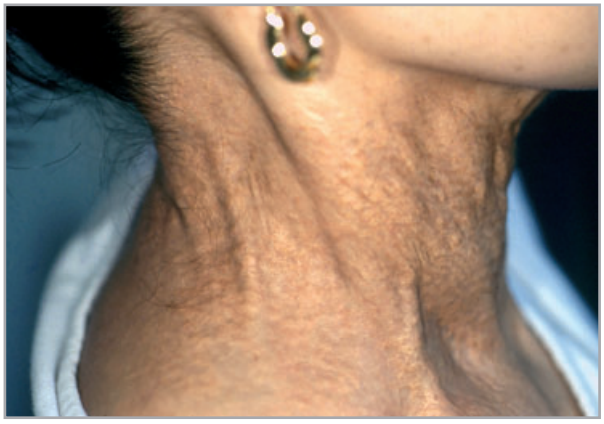

Fig 2. Pseudoxanthoma elasticum involvement of the skin of the neck.

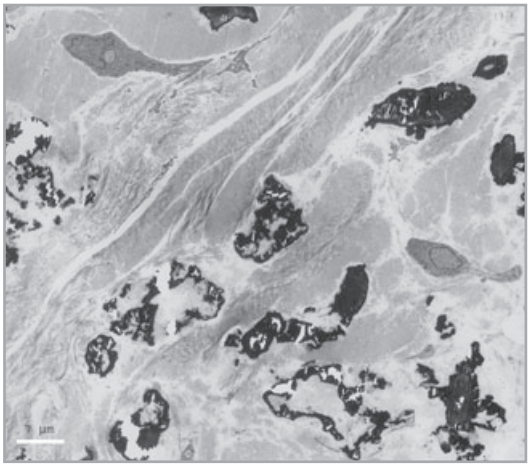

Fig 3. Skin electron microscopy (original maginification ×1200). Heavy mineralization (black deposits) within the core of the elastic fibres; absence of amyloid deposits (Dr B. Arbeille, Tours, France).

both on light microscopy (Congo red staining and immunohistochemistry using anti-SAA antibody) and at ultrastructural levels. The strategy for *ABCC6* mutation analysis was as previously described.[17] Sequencing of *ABCC6* exon 23 showed the G1042S variation. The latter was absent in a panel of 200 alleles from unaffected and unrelated control individuals.

As *ABCC6* and *MEFV* are both located in the 16p13 chromosomal region, we wanted to exclude hemizygosity for the mutations identified in association with a heterozygous deletion encompassing both genes. The latter was investigated using fluorescent *in situ* hybridization. BAC probes overlapping this region (RP-397B22 and RP-292B10) were used (data not shown). No deletion was found, and both mutations were considered homozygous.

Discussion

We report a young woman with early FMF-related amyloidosis and PXE. FMF-related amyloidosis has been described in childhood,[7] but such extensive disease prompted us to look for a link with the concurrent PXE.

The patient was a homozygote carrier of the *MEFV* M694I mutation. M694I seems to be specific to the Arab population.[18] M694V homozygosity has repeatedly been shown to be associated with the development of amyloidosis and severe phenotype.[19] M694I is much less frequent than M694V but has also been reported in association with renal amyloidosis.[18]

The patient was also homozygous for *ABCC6* G1042S. This novel missense point mutation is disease-causing according to usual criteria.[20] On the basis of alignment of the 12 human ABCC proteins G1042S alters a conserved amino acid within an intracellular domain (Fig. 4).[9] We assumed that the association of FMF and PXE in this case was due only to chance and was favoured by consanguinity.

Amyloidosis developed strikingly in this patient despite appropriate colchicine treatment, raising the possibility that ABCC6 deficiency might have contributed to the clinical presentation and severity of FMF. Two nonexclusive pathogenic explanations can be proposed: increased amyloid deposition in target tissues and/or decrease in colchicine activity.

Changes in glycosaminoglycan (GAG) metabolism are described in PXE. The presence of SAA within and close to dermal elastorrhexic fibres has been demonstrated using electron microscopy.[11] Large amounts of heparan sulphate have been immunolocalized in association with mineralized elastin, microfilament aggregates and collagen. In addition, sulphated GAGs were recently measured in the urine of 10 PXE patients.[21] The authors showed that urinary polysaccharides were significantly lower in PXE-affected patients, and that the relative proportions of chemical species were changed. Indeed, chondroitin sulphate was decreased, but heparan sulphate was increased, indicating a greater synthesis of the latter. Such results have also been demonstrated in our patient and published elsewhere.[22] The precise role of ABCC6 deficiency in these biochemical changes is currently unknown. However, as heparan sulphate colocalizes spatially and temporally with all amyloid proteins, and probably favours amyloidogenesis and amyloid fibril stabilization,[23] it may be hypothesized that in the context of *MEFV* mutation carriage, the high level of heparan sulphate associated with ABCC6 deficiency promotes visceral amyloidosis.

ABCC6 belongs to the ABC superfamily of transmembrane transporters.[24] ABCs have the ability to transport various substrates. Some ABCs are involved in nonspecific resistance to antitumoral drugs, hence the name multidrug resistance proteins (MRPs). One of the most commonly studied MRPs in humans is ABCC1 (formerly MRP1), a transporter very close to ABCC2, 3 and 6, both structurally and genetically.[24] Dubin–Johnson syndrome is related to mutations in the *ABCC2* gene. It has been shown in this condition that in the situation of ABCC2 deficiency, other ABCC family members can be overexpressed, thus explaining cholestasis. Indeed, the absence of ABCC2 transporter on the apical (bile canalicular) membrane of the hepatocyte is accompanied by overexpression of ABCC3. However, ABCC3 is basolaterally distributed in hepatocytes, and this results in excretion of bile salts, not in bile ducts but in blood vessels.[25] A similar overexpression of ABCC1 may exist in PXE, particularly in the liver and kidneys where ABCC6 should be abundant and where amyloidosis occurs. The fact that ABCC1 transports colchicine and decreases the activity of this drug could be one explanation for the poor efficacy of colchicine in preventing the extension of amyloidosis in our patient.[26] A Dutch epidemiological study has demonstrated that R1141X, the most prevalent *ABCC6* mutation in Europe, was not rare in the general population (0·8%).[27] Accordingly, it can be speculated that *ABCC6*

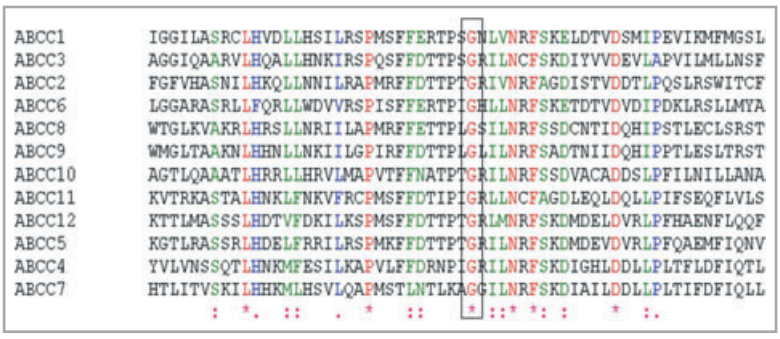

Fig 4. Alignment of 12 human ABCCs (ClustalW). Residues are described as identical (*), strongly similar (:), weakly similar (.). ABCC6 G1042 is in the middle of the figure.

mutations as a whole could be frequent in the general population, and might enhance the severity of some cases of FMF.

References

1 Samuels J, Aksentijevich I, Torosyan Y *et al.* Familial Mediterranean fever at the millennium. Clinical spectrum, ancient mutations, and a survey of 100 American referrals to the National Institutes of Health. *Medicine (Baltimore)* 1998; **77**:268–97.

2 Ozen S. Familial Mediterranean fever: revisiting an ancient disease. *Eur J Pediatr* 2003; **162**:449–54.

3 French FMF Consortium. A candidate gene for familial Mediterranean fever. *Nat Genet* 1997; **17**:25–31.

4 International FMF Consortium. Ancient missense mutations in a new member of the *RoRet* gene family are likely to cause familial Mediterranean fever. *Cell* 1997; **90**:797–807.

5 Schaner PE, Gumucio DL. Familial Mediterranean fever in the post-genomic era: how an ancient disease is providing new insights into inflammatory pathways. *Curr Drug Targets Inflamm Allergy* 2005; **4**:45–52.

6 Zemer D, Pras M, Sohar E *et al.* Colchicine in the prevention and treatment of the amyloidosis of familial Mediterranean fever. *N Engl J Med* 1986; **314**:1001–5.

7 Cakar N, Yalcinkaya F, Ozkaya N *et al.* Familial Mediterranean fever (FMF)-associated amyloidosis in childhood. Clinical features, course and outcome. *Clin Exp Rheumatol* 2001; **19** (5 Suppl. 24):S63–7.

8 Mansfield E, Chae JJ, Komarow HD *et al.* The familial Mediterranean fever protein, pyrin, associates with microtubules and colocalizes with actin filaments. *Blood* 2001; **98**:851–9.

9 Chassaing N, Martin L, Calvas P *et al.* Pseudoxanthoma elasticum: a clinical, pathophysiological and genetic update including 11 novel *ABCC6* mutations. *J Med Genet* 2005; **42**:881–92.

10 Pasquali-Ronchetti I, Volpi N, Baccarani-Contri M *et al.* Pseudoxanthoma elasticum: biochemical and ultrastructural studies. *Dermatologica* 1981; **162**:307–25.

11 Baccarani-Contri M, Vincenzi D, Cicchetti F *et al.* Immunochemical identification of abnormal constituents in the dermis of pseudoxanthoma elasticum patients. *Eur J Histochem* 1994; **38**:111–23.

12 Ringpfeil F, Lebwohl MG, Christiano AM, Uitto J. Pseudoxanthoma elasticum: mutations in the *MRP6* gene encoding a transmembrane ATP-binding cassette (ABC) transporter. *Proc Natl Acad Sci USA* 2000; **97**:6001–6.

13 LeSaux O, Urban Z, Tschuch C *et al.* Mutations in a gene encoding an ABC transporter cause pseudoxanthoma elasticum. *Nat Genet* 2000; **25**:223–7.

14 Bergen AA, Plomp AS, Schuurman EJ *et al.* Mutations in *ABCC6* cause pseudoxanthoma elasticum. *Nat Genet* 2000; **25**:228–31.

15 Struk B, Cai L, Zach S *et al.* Mutations of the gene encoding the transmembrane transporter protein ABC-C6 cause pseudoxanthoma elasticum. *J Mol Med* 2000; **78**:282–6.

16 Uitto J. Pseudoxanthoma elasticum – a connective tissue disease or a metabolic disorder at the genome/environment interface? *J Invest Dermatol* 2004; **122**:ix–x.

17 Chassaing N, Martin L, Mazereeuw J *et al.* Novel *ABCC6* mutations in pseudoxanthoma elasticum. *J Invest Dermatol* 2004; **122**:608–13.

18 Dodé C, Pecheux C, Cazeneuve C *et al.* Mutations in the *MEFV* gene in a large series of patients with a clinical diagnosis of familial Mediterranean fever. *Am J Med Genet* 2000; **92**:241–6.

19 Ben-Chetrit E. Familial Mediterranean fever (FMF) and renal AA amyloidosis – phenotype–genotype correlation, treatment and prognosis. *J Nephrol* 2003; **16**:431–4.

20 Cotton RG, Scriver CR. Proof of disease-causing mutation. *Hum Mutat* 1998; **12**:1–3.

21 Maccari F, Gheduzzi D, Volpi N. Anomalous structure of urinary glycosaminoglycans in patients with pseudoxanthoma elasticum. *Clin Chem* 2003; **49**:380–8.

22 Volpi N, Maccari F. Composition of urinary glycosaminoglycans in a patient with pseudoxanthoma elasticum and familial Mediterranean fever. *Clin Chim Acta* 2005; **359**:207–9.

23 Hart M, Li L, Tokunaga T *et al.* Overproduction of perlecan core protein in cultured cells and transgenic mice. *J Pathol* 2001; **94**:262–9.

24 Dean M, Rzhetsky A, Allikmets R. The human ATP-binding cassette (ABC) transporter superfamily. *Genome Res* 2001; **11**:1156–66.

25 Kullak-Ublick GA, Beuers U, Paumgartner G. Hepatobiliary transport. *J Hepatol* 2000; **32** (Suppl. 1): 3–18.

26 Rajagopal A, Pant AC, Simon SM, Chen Y. *In vivo* analysis of human multidrug resistance protein 1 (MRP1) activity using transient expression of fluorescently tagged MRP1. *Cancer Res* 2000; **62**: 391–6.

27 Trip MD, Smulders YM, Wegman JJ *et al.* Frequent mutation in the *ABCC6* gene (R1141X) is associated with a strong increase in the prevalence of coronary artery disease. *Circulation* 2002; **106**:773–5.