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ORIGINAL ARTICLE

Defect in proline synthesis: pyrroline-5-carboxylate reductase 1 deficiency leads to a complex clinical phenotype with collagen and elastin abnormalities

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Abstract Pyrroline-5-carboxylate reductase 1 (PYCR1) catalyzes the last step in proline synthesis. Deficiency of PYCR1, caused by a defect in *PYCR1*, was recently described in patients with cutis laxa, intrauterine growth retardation, developmental dysplasia of the hips and mental retardation. In this paper, we describe additional six patients (ages ranging from 4 months to 55 years) from four Iranian families with clinical manifestations of a wrinkly skin disorder. All patients have distinct facial features comprising triangular face, loss of adipose tissue and thin pointed nose. Additional features are short stature, wrinkling over dorsum of hand and feet, visible veins over the chest and hyperextensible joints. Three of the patients from a large consanguineous family do not have mental retardation, while the remaining three patients from three unrelated families have mental and developmental delay. Mutation analysis revealed the presence of disease-causing variants in *PYCR1*, including a novel deletion of the entire *PYCR1* gene in one family, and in each of the other patients the homozygous missense mutations c.616G>A (p.Gly206Arg), c.89T>A (p.Ile30Lys) and c.572G>A (p.Gly191Glu) respectively, the latter two of which are novel. Light- and electron microscopy investigations of skin biopsies showed smaller and fragmented elastic fibres, abnormal morphology of the mitochondria and their cristae, and slightly abnormal collagen fibril diameters with irregular outline and variable size. In conclusion, this study adds information on the natural course of PYCR1 deficiency and sheds light on the pathophysiology of this disorder. However, the exact pathogenesis of this new disorder and the role of proline in the development of the clinical phenotype remain to be fully explained.

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Introduction

The term cutis laxa refers to a heterogeneous group of skin disorders characterised by loose and inelastic skin that can be either inherited or acquired. In inherited forms, the defect arises mostly from the synthesis and/or assembly of abnormal elastic fibres, whereas acquired forms result from destruction of normal elastic fibres. Inherited forms of cutis laxa are characterised by generalised cutaneous laxity

which is present at birth or develops soon after. Other organs such as the lungs, aorta, gastrointestinal tract and genitourinary system are generally involved. The classification of the inherited forms of cutis laxa comprises different categories based on the mode of inheritance (Table 1).

In the autosomal dominant group (MIM #123700), skin laxity can already be present at birth or can be late in onset, and the disorder can be caused by mutations in the elastin gene, *ELN* (MIM *130160) (Morava et al. 2009; Tassabehji et al. 1998) or in *FBLN5* (MIM *604580) (Markova et al. 2003). The X-linked recessive form, also known as occipital horn syndrome (MIM #304150), which is allelic to Menkes disease (MIM #309400), is due to mutations in a copper-transporting ATPase encoded by *ATP7A* (MIM *300011) (Kaler et al. 1994). The third and most heterogeneous category is defined by an autosomal recessive mode of inheritance and comprises two types. Type I (MIM #219100) is associated with early pulmonary emphysema, poor prognosis and can be caused by missense mutations in *FBLN4* (also known as *EFEMP2*; MIM *604633) and *FBLN5* (MIM *604580) (Hucthagowder et al. 2006; Loeys et al. 2002). Autosomal recessive cutis laxa type II (ARCL2; MIM #219200) is sometimes referred to as cutis laxa with growth and developmental delay or as cutis laxa with joint laxity and retarded development. Related disorders, the group of so-called wrinkly skin disorders, include gerodermia osteodysplastica (GO) or Walt Disney dwarfism (MIM #231070) and the wrinkly skin syndrome (WSS; MIM #278250).

Definitive criteria for distinguishing these conditions unambiguously are lacking, leaving a subjective element in clinical diagnosis. Thus, molecular diagnosis is proving increasingly useful in resolving these phenotypically similar disorders.

In individuals with cutis laxa type II, mutations have been identified in two genes, specifically H(+)-ATPase V0 subunit A2 encoded by *ATP6V0A2* (MIM *611716; classified as cutis laxa type IIA) (Kornak et al. 2008) and SCYL1 binding protein encoded by *SCYL1BP1* (MIM *607983) (Hennies et al. 2008).

Mutations have also been found in a member of the aldehyde dehydrogenase family, namely *ALDH18A1* (alias delta1-pyrroline-5-carboxylate synthetase, P5CS; MIM *138250); this gene was found to be defective in patients described with a so-called neurocutaneous syndrome closely resembling cutis laxa type II (Baumgartner et al. 2000, 2005; Bicknell et al. 2008). Remarkably, this condition (MIM *612652) is associated with metabolic changes suggestive of an inborn error of metabolism, in particular resembling findings of a urea cycle defect (hypoprolinemia, hypoornithinemia, hypocitrullinemia, hypoargininemia and hyperammonemia). P5CS is a bifunctional ATP- and NADPH-dependent mitochondrial enzyme which catalyses the reduction of glutamate to delta1-pyrroline-5-carboxylate (P5C), a critical step in the biosynthesis of proline, ornithine and arginine (Phang et al. 2001). P5C is the immediate precursor for both proline and ornithine, reactions catalysed by pyrroline-5-carboxylate reductase (P5CR, encoded by *PYCR1*; MIM *179035; classified as cutis laxa type IIB) and ornithine aminotransferase (encoded by *OAT*; MIM *613349), respectively (Phang et al. 2001). The biochemical pathway of P5CS and P5CR is depicted in Fig. 1.

Recently, mutations in *PYCR1* were described in patients with a phenotypic spectrum comprising wrinkly skin, osteopenia and progeroid appearance with or without mental retardation (Guernsey et al. 2009; Reversade et al. 2009). The pathophysiological basis appears to be an impaired mitochondrial function leading to developmental defects through increased apoptosis.

Table 1 Differential diagnosis of cutis laxa disorders

Cutis laxa disorders and syndromes
Autosomal dominant: defect in *ELN* or *FBLN5*; present at birth or late-onset skin laxity, no mental retardation
X-linked: occipital horn syndrome; defect in *ATP7A*; copper deficiency; mild mental retardation
Autosomal recessive:
Type I: defect in *FBLN4* and *FBLN5*; early pulmonary emphysema, some with mental retardation
Type II: defect in *ATP6VOA2*; caused by abnormal N- and O-glycosylation; with mental retardation
Δ^1-Pyrroline-5-carboxylate synthetase: defect in *ALDH18A1*; low proline, citrulline, arginine, ornithine; mental retardation (but only two families described)
Pyrroline-5-carboxylate reductase: defect in *PYCR1*; normal amino acids; some with normal mental development
Gerodermia osteodysplastica: defect in *GORAB* (*previous symbol: SCYL1BP1)*; precocious skin aging and osseous changes, no mental retardation
Costello syndrome: defect in *HRAS*; facio-cutaneo-skeletal syndrome; mental retardation
De Barsy syndrome: defect unknown; with corneal clouding and mental retardation; ATP6V0A2-CDG in some patients
Cantu syndrome: defect unknown; with osteochondrodysplasia and cardiac anomalies; some with mild mental retardation

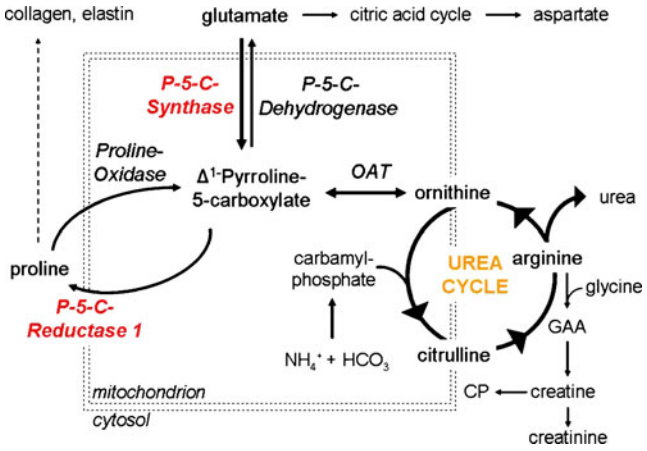

Fig. 1 Pathway of proline biosynthesis and its connection to the human urea cycle. *P5C* Δ^1-Pyrroline-5-carboxylate, *OAT* ornithine aminotransferase, *GAA* guanidinoacetate, *CP* creatine phosphate

In this paper, we describe an additional six patients with clinical manifestations of a wrinkly skin disorder in whom disease-causing variants in *PYCR1* were found. The paper focuses in particular on light- and electron microscopy investigations which showed alterations in collagen and elastic fibres.

Materials and methods

Patients

Patient 1 is the first and only child of related Iranian parents. After an uneventful pregnancy, she was born at term by caesarean section due to breech presentation. Birth weight was 1,600 g (<3rd centile), but length and head circumference were not documented. She presented with developmental dysplasia of the hip. Examination at 5 years of age showed a normal cognitive development but mild facial dysmorphism with triangular face, deep set eyes, pinched nose, low-set ears, high arched palate and small teeth. She had wrinkled, thin and translucent skin with visible veins. Additional signs were pectus excavatum; hypermobility of finger, wrist, elbow, knee and toe joints; pes planus; hallux valgus; talipes equinovarus and generalised muscle weakness. Patient 1 was referred with a suspicion of a type of Ehlers-Danlos syndrome.

Patient 2 is a 49-year-old male and a relative of patient 1. There is not much information regarding pregnancy. Delivery was by normal vaginal delivery at term. We do not have exact measurements, but relatives claim that the infant was very small, both in weight and length. Head circumference was apparently normal. Milestones were delayed, but cognition was normal as was puberty. He was examined at 49 years. His measurements were as follows: weight 45 kg (<3rd centile for Iranian population), height 150 cm (<3rd centile) and head circumference 51 cm (<3rd centile). He had thin wrinkled face, loss of adipose tissue in face and limbs, pinched nose, prominent chin, widely spaced teeth and wrinkling of dorsum of hands and feet (Fig. 2). He had recurrent dislocation of his right shoulder, pes planus and hyperextensibility of joints, especially in hands and feet, which has improved over time. He has myopia. The patient is married and has two healthy children. Radiography of chest shows osteopenic rib bones.

Patient 3 is a 54-year-old female and the sister of patient 2. Information regarding pregnancy and infancy is not available. Delivery was at term by normal vaginal delivery, and although we do not have measurements at birth, the relatives claim that she weighed less and was much shorter than normal healthy infants. She had delayed milestones. She had normal puberty with menses starting at 15 years and menopause at 52 years. She was examined at 54 years. Her height, weight and head circumference were 145 cm (<3rd centile for Iranian population), 40 kg (<3rd centile) and 49 cm (<3rd centile), respectively. She had a thin long face, thin pinched nose, prominent chin and loss of adipose tissue in her face (Fig. 2). The hands and feet are very small with wrinkling on the dorsum. Pes planus is evident. The skin is thin and loose. Cognition is normal. She never married.

Patient 4 is the first and only child of first cousin Iranian parents. He was born spontaneously at 36 weeks of gestation, after an uneventful pregnancy. Birth weight was 2,000 g (3rd centile), length 42 cm (3rd centile) and head circumference 34 cm (<50th centile). At follow up at 5 years, the patient presented a thin, translucent, lax and wrinkly skin as well as hypermobile joints with developmental dysplasia of the hip and pes planus. Clinically, he showed distinct facial features with hollow cheeks, pinched nose, loss of adipose tissue, prominent low-set simple ears and thin lips. There were neither blue sclerae nor atrophic scars. Gross motor developmental milestones were delayed: he could sit at 12 months and walk unsupported at 2 years. His cognitive development was also delayed, and he could speak only a few words at 5 years of age. Figure 3 shows the phenotype of patient 4 at age 5 years.

Patient 5 was spontaneously born at term after an uneventful pregnancy as the second child of a consanguineous Iranian family. The parents and their first child were healthy. Birth weight was 2,300 g (<3rd centile), length 47 cm (3rd centile), and head circumference 33 cm (<50th centile). Thriving was regular, but he presented mild motor and mental retardation. Developmental dysplasia of the hip was present, and the skin was lax and wrinkled with prominent vessels over the chest and abdomen. In addition, he showed joint laxity, long fingers, pes planus, delayed bone age, osteoporosis and mild facial dysmorphism with a

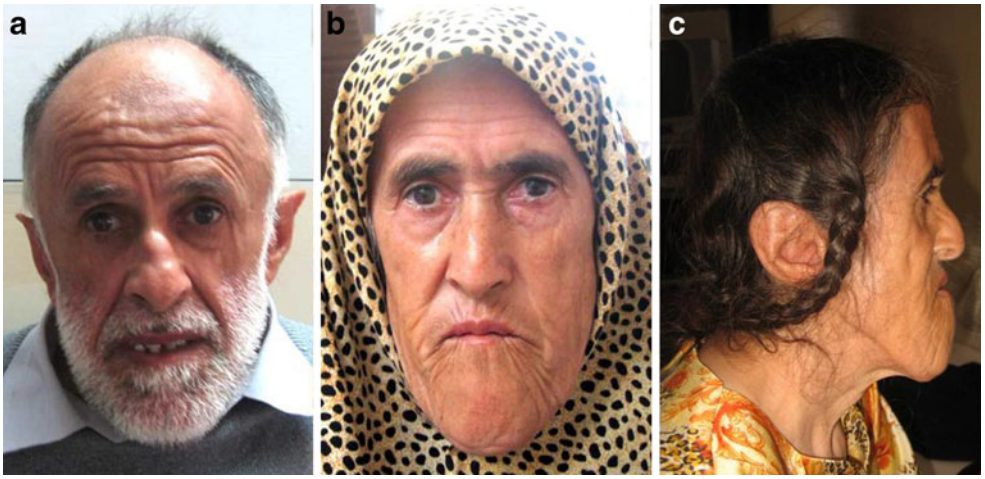

Fig. 2 Facial appearance of patients 2 (**a**) and 3 (**b**, **c**) at age 45 and 50 years, respectively. Note the facial similarities to the young patients (as shown in Figs. 3 and 4)

triangular face, arched eyebrows, and prominent simple ears. He had dental caries, strabismus and myopia and needed an operation for his right undescended testis when he was 7 years old. Plasma ammonia and concentrations of plasma amino acids were normal. This patient was referred with a suspicion of wrinkly skin syndrome. Figure 4 shows the clinical presentation of the patient at 1 and 8 years.

Patient 6 is the first and only child of first cousin parents. Sonography revealed severe oligohydramnios. Delivery was by normal vaginal delivery at 9 months. Birth weight and length were 1,400 g (<3rd centile) and 35 cm (3rd centile) respectively. At 9 months, weight was 4.5 kg (<3rd centile), height was 62 cm (<3rd centile) and head circumference was 39 cm (<3rd centile). He had a pinched nose, deep set eyes, blue sclera, cleft palate, pectus excavatum, hyperextensibility of fingers and wrists, prominent vessels (mostly on abdomen and chest), muscle weakness, developmental dysplasia of the hip, poor feeding, wrinkling over dorsum of hands and feet and muscle weakness. The child was developmentally delayed, showed head control at 9 months, could sit with support at 16 months but has not developed the ability to stand nor started saying single words. Ophthalmologic examination revealed blue sclera and mild myopic astigmatism. X-rays taken at 9 months showed developmental dysplasia of the hip, knee joint subluxation, wormian bones in the skull and decreased bone density.

The main clinical signs of all patients are summarised in Table 2.

Mutation analysis

Genomic DNA was isolated from peripheral blood cells by standard methods. The *ALDH18A1* gene was PCR amplified for each of the 17 coding exons including flanking intronic regions. Annealing temperatures were 60°C for exons 2–11 and 13–16, 62°C for exons 12 and 18, and 54°C for exon 17. Likewise, PCR was performed for the *PYCR1* gene amplifying all eight coding exons including flanking regions. Annealing temperatures were 58°C for exon 1, 62°C for exons 2–7, and 64°C for exon 8. Primer sequences can be obtained from the authors on request. PCR products were directly sequenced using the 3130*xI* Genetic Analyzer (Applied Biosystems, Life Technologies).

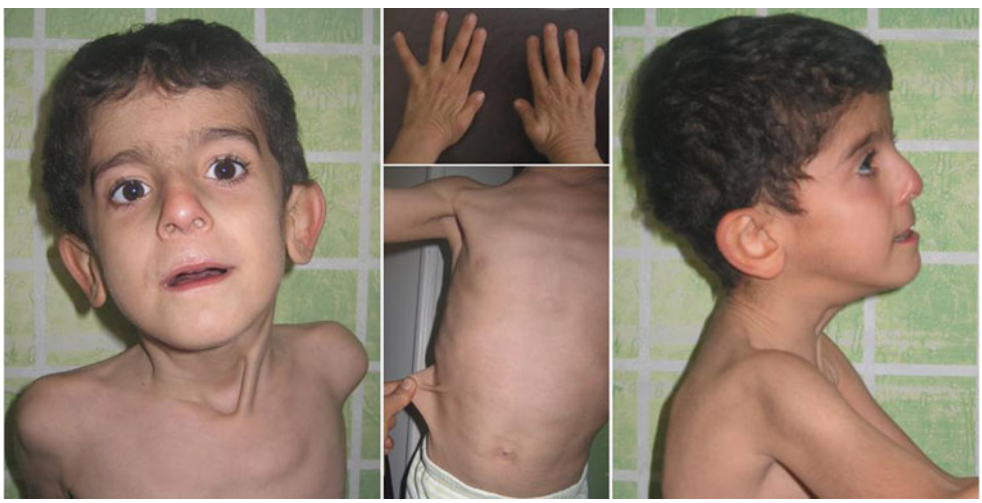

Fig. 3 Clinical phenotype of patient 4 at age 5 years. Note the facial dysmorphism with triangular face, pinched nose, thin lips and the lax, wrinkly translucent skin with visible veins

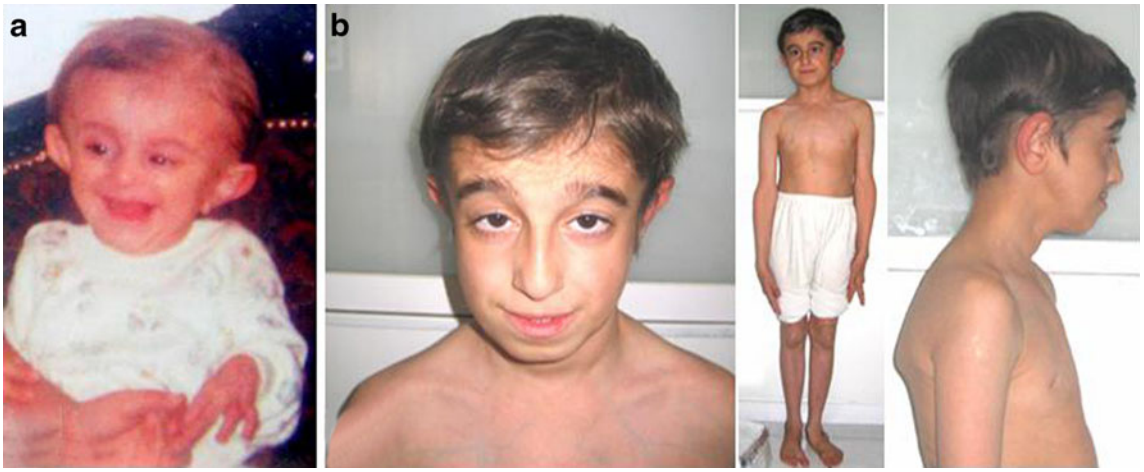

Fig. 4 Clinical phenotype of patient 5 at age 1 (**a**) and 8 (**b**) years. Note the facial dysmorphism with progeroid features at age 1 year (**a**) and the translucent skin with visible veins (**b**)

Genetic investigations were performed after written informed consent of the parents was obtained.

MLPA analysis

Three self-designed MLPA probes specific for exons 4, 5, and 8 of the *PYCR1* gene were used with a commercially available set of control probes (MLPA kit P300, MRC, Holland). The MLPA analysis was performed using DNA of patients 1, 2 and 3 as well as from other family members according to the standard method recommended by MRC Holland. Separation of the products was performed by capillary electrophoresis on a 310 Genetic Analyzer (Applied Biosystems).

Light and electron microscopy of skin

A portion of the skin biopsy from patients 1 and 5, taken to establish fibroblast cultures, was processed for light and transmission electron microscopy. For light microscopy, semi-thin sections (1 μm) were stained with methylene blue. Electron microscopy was performed as previously reported (Vogel et al. 1979). The biopsy site was the lateral upper thigh region.

Biochemical analysis of collagens

Cultured dermal fibroblasts were established from a skin biopsy obtained from patient 5. Cells were maintained under standard conditions and radiolabeled as previously described (Steinmann et al. 1984). At the end of the incubation, medium and cell layer were harvested separately, and collagen samples were prepared by digestion with pepsin, precipitated with ethanol, separated on a 5% SDS-PAGE and visualised by fluorography (Steinmann et al. 1984).

Results

Mutation analysis

In all patients from this study, two mutant alleles in *PYCR1* were identified.

In patient 1, we did not succeed in amplifying any of the *PYCR1* exons. The suspected occurrence of a novel homozygous deletion in the index-patient was confirmed by MLPA using three probes specific for *PYCR1*. The MLPA analysis also revealed the homozygous loss of the

Table 2 Main clinical signs in the patients of this study

Patient	IUGR	Thin, translucent, lax, wrinkly skin	Developmental dysplasia of the hip	Pes planus	Facial dysmorphism	Mental retardation	Delayed motor development
1	+	+	+	+	+	-	+
2	+	+	-	+	+	-	+
3	+	+	-	+	+	-	+
4	+	+	+	+	+	+	+
5	+	+	+	+	+	+	+
6	+	+	+	?	+	+	+

? Not known

PYCR1 gene in the fourth-degree cousins (patients 2 and 3), while all obligate heterozygous parents as well as additional family members were found to be carriers of this mutant allele (Fig. 5). The analysis of the neighbouring genes *MAFG* and *MYADML2* showed normal results in all family members, indicating that the deletion affects only the *PYCR1* gene (data not shown).

In patient 4, investigation of the *ALDH18A1* gene did not reveal any mutations. However, in *PYCR1* a previously described homozygous missense mutation c.616G>A (p.Gly206Arg) in exon 5 was found; both parents were carriers of this change (Reversade et al. 2009). The residue Gly206 lies within a highly conserved region of the *PYCR1* gene that is conserved in mammals.

DNA sequencing of all coding exons including the flanking regions of the *PYCR1* gene for patient 5 revealed a single novel homozygous missense mutation c.89T>A (p.Ile30Lys) in exon 2. The residue Ile30 is conserved in mouse, rat, dog, cattle and anopheles but not in chimpanzee.

In patient 6, the novel mutation c.572G>A (p.Gly191Glu) in exon 5 of the *PYCR1* gene was identified in a homozygous state with both parents being carriers for this mutation. The residue Gly191 lies within a highly conserved region of the *PYCR1* gene that is conserved in mammals, drosophila and anopheles.

Light and electron microscopy of skin

Light microscopy of a skin biopsy of patient 5 showed, distributed between collagen bundles, elastic fibres which were abnormally thin and which showed "jagged" contours. Overall, there was a reduction in elastic material and decrease in elastic fibre size when compared to a normal control sample (Fig. 6a, b).

Ultrastructural analysis of a skin biopsy of patient 5 revealed abnormally thin elastic fibres, and slightly abnormal collagen fibrils with irregular contours and variable sized diameters. Furthermore, the morphology of mitochondria and their cristae was altered (Fig. 6c, d; control shown in Fig. 6e, f).

Likewise, morphological analysis of a skin biopsy of patient 1 disclosed a reduced amount of elastic material (decreased size and fragmentation of elastic fibres). Ultrastructurally, the elastin component was surrounded by excessive elastic microfibrils. Collagen bundles, especially those adjacent to elastic fibres, were less compact and revealed variation in single fibril calibers.

Biochemical analysis of collagens

Radiolabelled collagens retained by cultured dermal fibroblasts and secreted into the culture medium were separated and treated with pepsin and subjected to SDS-PAGE and fluorography. In patient 5, we detected normal migration patterns for collagens I, III and V in both the medium and the cell layer (not shown).

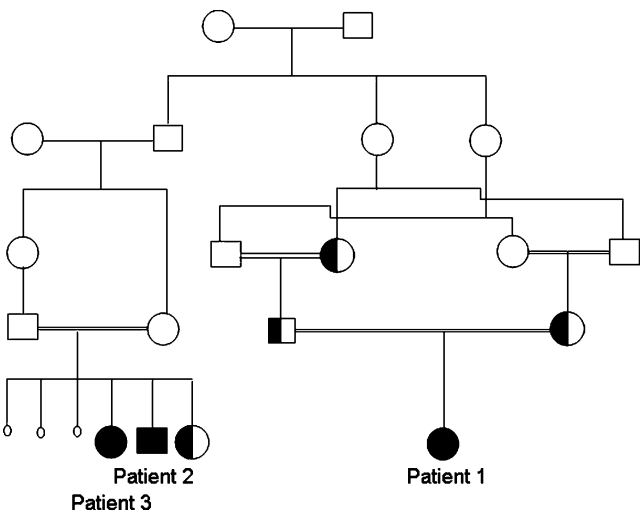

Fig. 5 Pedigree of family 1 indicating patients 1, 2 and 3 as well as obligate heterozygotes investigated in this study

Discussion

Although wrinkled and lax skin is an "inevitable consequence of normal human aging" (Kornak 2009), it is also a striking clinical presentation in patients with P5CR deficiency. The presentation of lax and wrinkled skin in a child often leads to the suspicion of Ehlers-Danlos syndrome, biochemically characterised by collagen abnormalities. However, lax and wrinkled skin rather point towards abnormalities of elastic fibres and their synthesis. This entity has been recently reviewed highlighting also a few metabolic disorders which can present with wrinkled skin or cutis laxa (Morava et al. 2009). As cutis laxa disorders overlap considerably, proper diagnosis is challenging.

All six patients presented in this study were referred to our centre for further work-up of a wrinkly skin syndrome. Based on clinical findings, in particular developmental delay, we considered the still short list of metabolic disorders presenting with cutis laxa.

In patient 1, MLPA analysis revealed the presence of a genomic deletion comprising the entire *PYCR1* gene with two further relatives also affected (patients 2 and 3 of this study) and obligate heterozygotes in the family found to be carriers of this deletion (Fig. 5). It is interesting that all patients from family 1 were the only ones in this study not affected by mental retardation although they all had a homozygous deletion of *PYCR1*.

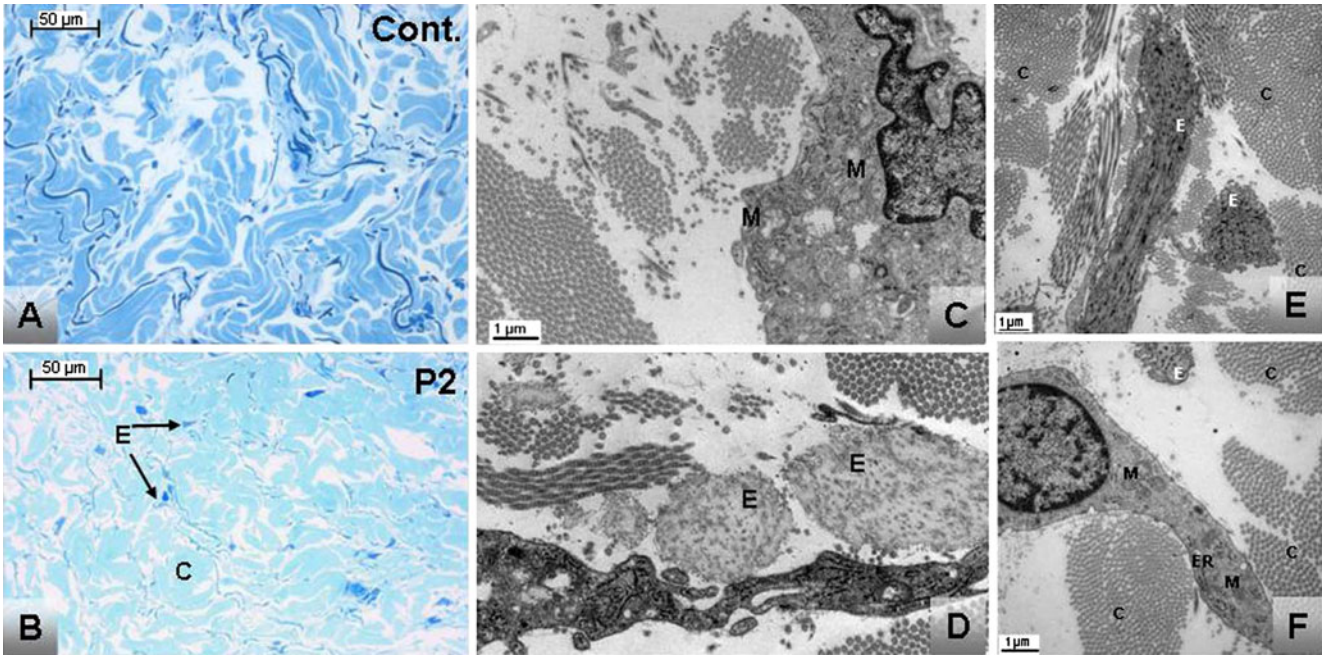

Fig. 6 Light and electron microscopy analysis in patient 5. **a**, **b** Semi-thin section, methylene blue staining. **a** Control: regular distribution of collagen bundles (*light blue*) and elastic fibres (*dark blue*). **b** Patient 5: fibres are small, thin and fragmented, so that the overall amount of elastic material is reduced; the number of elastic fibres is not substantially reduced. **c**–**f** Ultra-thin sections (magnification 8,800x). **c** Altered mitochondria (*M*). **d** Small fragmented elastic fibres within reticular dermis with marginal elastic microfibrils. **e** Normal female control aged 42 years. Elastic fibres (*E*) and collagen bundles (*C*) are shown; note that elastic fibres are larger and thicker in size than in the patients (**c**, **d**). **f** Normal female control aged 42 years: a dermal fibroblast [within normal collagen (*C*) bundles] is shown; in the cytoplasm ER cisterns (*ER*) and mitochondria (*M*) are depicted. An elastic fibre is shown only partially (*E*)

In patient 4, P5CS deficiency was investigated first but no mutation was found. Because of the considerable clinical overlap among the known disorders of proline biosynthesis, genetic investigation of the *PYCR1* gene was also performed and revealed the presence of a homozygous mutation within a highly conserved domain of *PYCR1*, with both parents being carriers. The same genotype was previously reported in a patient from the same ethnic background (Reversade et al. 2009).

Because of the phenotypic similarity to patient 4, *PYCR1* mutation analysis was done in patient 5 and revealed the novel missense mutation c.89T>A (p.Ile30Lys) in a homozygous state. Since no other mutation was found within the *PYCR1* gene and because of the high grade of conservation across species, this mutation was considered likely to be responsible for the clinical phenotype of the patient.

In patient 6, the novel mutation c.572G>A (p.Gly191Glu) in exon 5 of the *PYCR1* gene was found affecting a residue which is highly conserved across species.

The assumption of a disease-causing role of the *PYCR1* mutations we detected was further supported by the results of light and electron microscopy. In patients 1 and 5, EM of a skin biopsy showed fragmentation, rarefaction and size reduction of the elastic fibres, as previously shown in P5CR deficiency (Reversade et al. 2009). Furthermore, it revealed mild alterations of the collagen bundles, mostly of those proximal to elastic fibres.

The pathophysiological mechanism underlying P5CR deficiency is still far from clear, but several observations suggest a role of P5CR in the cell's response to oxidative stress. Ultrastructural analysis of mitochondrial morphology in cultured fibroblasts revealed abnormal morphology of mitochondria and their cristae (Reversade et al. 2009), which was similarly found in our patients.

As depicted in Fig. 1 the enzyme proline oxidase (POX; also known as proline dehydrogenase, PRODH) catalyses the rate-limiting two-electron oxidation of proline to P5C. It has been suggested that the intracellular accumulation of proline is an adaptive stress response that affords oxidative stress protection in mammalian cells because overexpression of P5CS and P5CR observed after exposing cell lines to physiological H_2O_2 levels resulted in two-fold higher proline content, lower ROS levels and increased cell survival (Krishnan et al. 2008). Furthermore, lower expression of ornithine aminotransferase (OAT) was measured during H_2O_2 stress, indicating that the P5C-ornithine pathway was suppressed in order to funnel the P5C intermediate to proline synthesis (Krishnan et al. 2008).

The recent observation that a defect in GLUT10-mediated transport of L-dehydroascorbic acid (DHA) is

the pathophysiological mechanism underlying arterial tortuosity syndrome (Lee et al. 2010) might also shed light on the pathomechanism of P5CR deficiency. Parallels can be drawn between the protective mechanism against oxidative damage operating in vascular smooth muscle cells by mitochondrial GLUT10 transport of DHA and the protective mechanism operating in fibroblasts through the mitochondrial proline synthesis pathway. Thus, it can be hypothesized that enhanced transcription and activation of matrix metalloproteinases (MMPs) and, as a consequence of increased ROS, enhanced degradation of the extracellular matrix as shown in vascular smooth muscle cells (Satoh et al. 2009), may also occur in the tissues most severely affected by P5CR deficiency. This mechanism might account for the rarefaction and fractionation of elastic fibres as documented in the skin biopsies of P5CR-deficient patients.

The clinical picture of P5CR deficiency with progeroid appearance during infancy might be the result of apoptosis due to compromised mitochondrial function during prenatal development. In addition, the physiologically high activity of OAT during early infancy (Raiha and Kekomaki 1968) might result in low levels of P5C and proline because OAT serves to produce ornithine rather than proline and P5C in this period in life. When OAT works mostly in the other direction (i.e. to produce P5C from ornithine) later in life, the degree of P5C and proline drainage might be decreasing.

However, in contrast to P5CS deficiency where some patients have low proline, P5CR-deficient patients seem to have normal serum proline levels (Reversade et al. 2009). Unfortunately, we could only perform two single amino acid profiles in two of the patients of this study; the results were normal.

Clinically, the combination of progeroid appearance, wrinkled skin, developmental dysplasia of the hip, IUGR and neuromotor developmental delay should suggest the diagnosis of P5CR deficiency. EM of a skin biopsy might be a helpful diagnostic tool, in particular to distinguish P5CR from P5CS deficiency; however, since many of the cutis laxa syndromes are monogenetic disorders, mutation analysis may be warranted.

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