

Ehlers-Danlos syndrome type IV: phenotypic consequences of a splicing mutation in one COL3A1 allele

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Abstract

The features of a child with Ehlers-Danlos syndrome type IV (EDS IV) resulting from a mutation in one COL3A1 allele were studied. The child was heterozygous for a G- to A-transition at the splice donor site of intron 41. It resulted in the splicing out of the exon 41 encoded sequence from $\alpha 1(\text{III})$ mRNA and the deletion of 36 amino acids from glycine⁷⁷⁵ to lysine⁸¹⁰ of the triple helical domain of $\alpha 1(\text{III})$ chains of type III collagen. The amount of type III collagen in the dermis was only about 11% of normal. The child had the acrogeric form of EDS IV. He had the characteristic facies with a pinched nose, thin lips, and prominent eyes. These facial features, his aesthetic build, thin skin, prominent subcutaneous veins, and aged hands produced a 'cachectic' appearance. These features were evident in early childhood and worsened up to 12½ years when he was last reviewed. Spontaneous bruising, bleeding from the large bowel, constipation, and delayed gastric emptying were other features. In cross section, the dermal collagen fibrils were round and measured 93.3 ± 11.5 nm in diameter which was not significantly different from control values of 102.5 ± 13.4 nm. The serum type III procollagen amino-terminal propeptide level of 25.5 ng/ml was within the normal age matched values of 15.5 ± 7.7 ng/ml despite the low production of type III collagen

by cultured fibroblasts. The child probably had a spontaneous new mutation in one COL3A1 allele as only normal sequences were obtained from the corresponding amplified region of the parent's leucocyte DNA.

The Ehlers-Danlos type IV syndrome (EDS IV) is a heterogeneous group of disorders resulting from abnormalities in type III collagen.¹ Unlike the other types of EDS, joint hypermobility is restricted to the small joints of the hands and hyperelasticity of the skin is largely confined to the dorsal aspect of the elbows and the sides of the neck. Severe bruising and arterial fragility also set EDS IV apart from the other Ehlers-Danlos syndromes.¹⁻⁵ The recognition that the severity of EDS IV varies widely, from almost insignificant disease through to an extremely severe disease with episodes of life threatening ecchymosis and vascular rupture, has focused attention on a group of patients whose underlying disorder may not be readily apparent when joint hypermobility and cutaneous hyperelasticity are used as the sole diagnostic criteria of the Ehlers-Danlos syndrome.⁴⁻⁶

In this paper we describe the phenotypic consequences of a previously reported COL3A1 mutation in a child with EDS IV.⁷ The child was shown to be heterozygous for a GT- to AT- transition at the splice donor site of intron 41 of the COL3A1 gene. This mutation generated an abnormally spliced mRNA in which sequences of exon 40 and 42 were joined together with maintenance of the reading frame. Deletion of the 108 nucleotides encoded by exon 41 resulted in the deletion of 36 amino acids, from glycine⁷⁷⁵ to lysine⁸¹⁰ of the triple helical domain of the $\alpha 1(\text{III})$ chain of type III collagen. The peptide deletion included the mammalian collagenase cleavage site at glycine⁷⁸¹-isoleucine⁷⁸², which accounted for the resistance of the mutant type III collagen to mammalian collagenase digestion. Cultured dermal fibroblasts produced a reduced amount of type III collagen which was secreted poorly. The molecules included normal homotrimers, mutant homotrimers, and mixed heterotrimers. However, the dermis only contained about 11% of the normal amount of type III collagen.

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Case report

The proband was the third child of his 26 year old mother. His father was aged 24. His unrelated parents did not show any clinical features of EDS IV and there was no family history of the disease. He was born at term after a normal vaginal delivery. The birth weight was 2800 g (50th centile) and length 54.5 cm (50th centile).

Poor appetite and weight gain were observed from birth and he had a 'cachectic' appearance from early childhood (fig 1). He had a flat midface with a pinched nose and thin lips. His longitudinal growth was maintained on the 10th to 25th centile and his weight gain followed the 3rd to 10th centile. His 'cachectic' appearance and the presence of multiple bruises resulted in the diagnosis of 'child abuse' on many occasions before the diagnosis of EDS IV was made at 5 years of age.

The first abnormality noted by the parents was bright bleeding from the rectum at 9 weeks of age. Fresh and old blood were separate from the stool and sometimes mixed with it such that the bleeding was presumed to be coming from the lower gastrointestinal tract. This proposal was confirmed at 2 years of age by colonoscopy which showed fresh red blood in the rectum and very friable rectal mucosa. Bleeding from the rectum continued intermittently for three years and was not associated with constipation. In later childhood he was constipated with bowel actions once every three to four days. It was unrelieved by liquid paraffin, psyllium hydrophilic mucilloid, or domperidone.

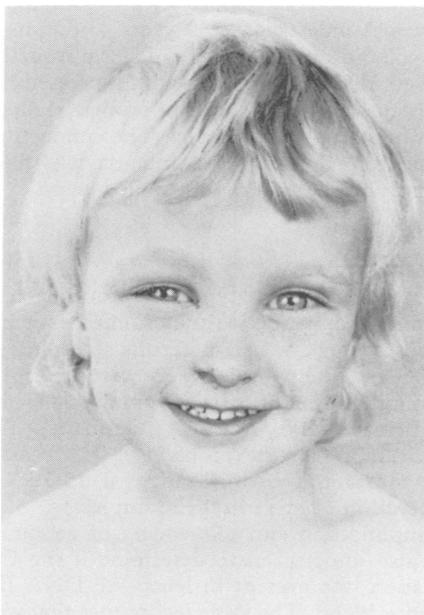


Figure 1 Facial appearance at 2 years 3 months of age showing a pinched nose and thin lips.

Bruising on the trunk and limbs occurred spontaneously from 10 months of age. While blunt trauma led to severe bruising, there was no history of life threatening ecchymoses or haemarthrosis. The cause of his easy bruising was extensively investigated at five different medical centres before the diagnosis of EDS IV was made and no abnormality of coagulation or platelet function was found.

The skin on the ventral surface of his body and limbs was soft, thin, and translucent, and the underlying veins were prominent. The skin over his lower abdomen was finely wrinkled. These features were first noted in infancy. Cutaneous hyperelasticity was only present at the base of the neck and behind both elbows. The skin on the dorsum of the hands was finely wrinkled and prematurely aged at 2 years 3 months (fig 2). The skin became more atrophic and violaceous with age.

Skin fragility and poor wound healing were not apparent. Minor lacerations on his shins had healed normally. Two lacerations required suturing and they also healed without dehiscence or abnormal scarring.

He was re-examined at the age of 12½ years. He still had spontaneous bruising and constipation. The physical findings were similar to those observed in early childhood but were more marked. There were dark rings around his eyes which were deeply set and he had a pinched nose and thin lips. These facial features and his aesthenic build gave him a 'cachectic' appearance which was more marked than in early childhood (fig 3). His height was 140 cm (10th centile), arm span 143 cm, and upper segment/lower segment ratio 0.97.

The hyperelasticity of the skin was still confined to the neck and elbow skin. There was no scarring of the forehead or knees. There were no inguinal herniae and the testes were fully descended. His elbows and wrists were hypermobile but the range of movement in his fingers, knees, and ankles was normal. He had postural flat feet. There was no clinical spinal deformity.

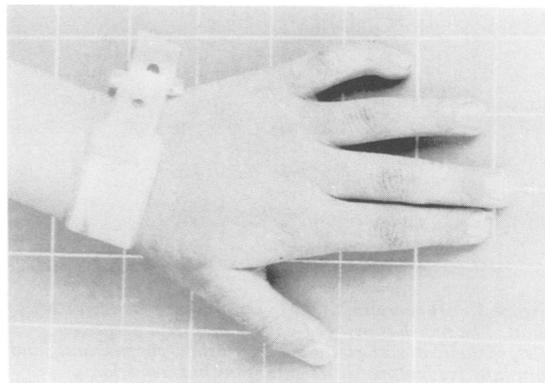


Figure 2 Hand at the age of 2 years 3 months showing a finely wrinkled, aged appearance.

Dental examination showed a normal class I occlusion with rotation of the upper lateral incisors.

INVESTIGATIONS

Coagulation studies were performed on numerous occasions. At 5 years of age, the skin bleeding time was mildly increased to 13 minutes (normal range 2.0 to 9.5 minutes). The platelet count was normal and platelets aggregated normally with ADP, adrenalin, collagen, and ristocetin. Factor VIII Rag and Von Willebrand factor levels were normal.

A skeletal survey at 12½ years showed relatively slender long bones, minimal platyspondyly of the lower lumbar vertebrae, and minor scoliosis in the lumbar spine. The hands did not show acro-osteolysis.¹ His bone age was within normal limits. A CT scan of his head, undertaken at 5 years of age because his head circumference was 46 cm (less than the 3rd centile), was normal.

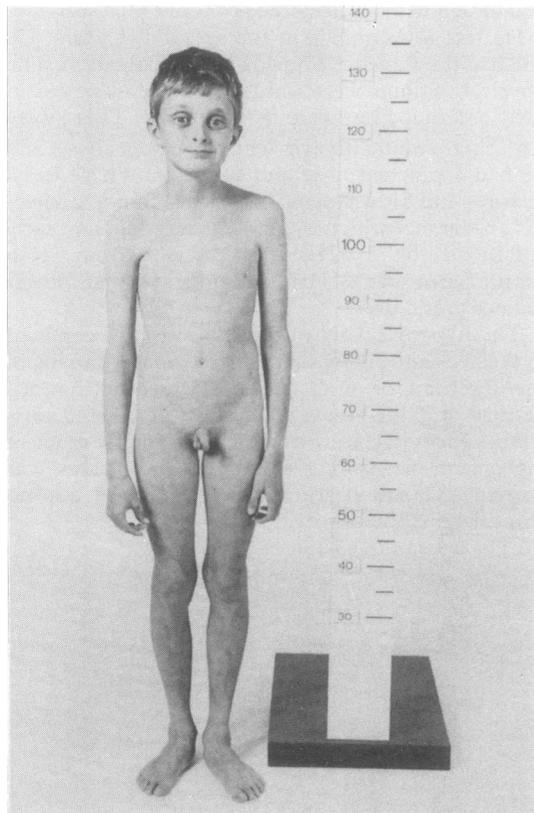


Figure 3 Appearance at 12½ years showing aesthetic body build. The face has an aged appearance with deeply set eyes, violaceous periorbital discolouration, pinched nose, and thin lips. He had moderate cutaneous hyperelasticity, particularly of the neck and elbows. The hands have an aged appearance with atrophic and transparent skin. There was minimal scarring. There was mild distal joint laxity.

Echocardiography with 2D Doppler scanning in the 'M' mode showed that the heart, heart valves, and major blood vessels were normal. A Doppler abdominal ultrasound also showed that the liver, pancreas, gall bladder, and kidneys were normal.

While a barium meal and follow through examination did not show any abnormality, a nuclear medicine gastric emptying study showed slow movement of the tracer from the stomach to the small bowel with a clearance (t_1) of 128 minutes (normal = 37 ± 5 minutes).

SKIN HISTOLOGY

A punch biopsy was obtained from his thigh at 5 years 3 months for electron microscopy. The skin was soft and thin and the biopsy hole immediately retracted to produce an abnormally large defect which bled profusely.

The biopsy was processed for electron microscopy as previously described.⁸ The dermal fibroblasts appeared to be normal (fig 4). The mid-dermal collagen fibril cross sectional profiles and elastin structure and staining were within normal limits (fig 5). The mean cross sectional collagen fibril diameter was 93.3 ± 11.5 nm ($n = 390$). The mean fibril diameter was marginally smaller than the mean value of 102.5 ± 13.4 nm ($n = 1000$) obtained from a closely age matched control sample of inguinal skin.

INTESTINAL HISTOLOGY

Colonic and rectal biopsies were obtained at colonoscopy undertaken at 2 years 4 months of age. The colonic biopsies, at 15 and 25 cm from the anus, showed mild inflammation with increased numbers of plasma cells in the lamina propria, oedema, and areas of attenuated epithelia. The connective tissue of the colonic and rectal mucosa was finer than normal and there were thin walled veins.

BIOCHEMISTRY

The level of serum type III procollagen amino-terminal propeptide was determined as it has been proposed to be a useful serum screening test for EDS IV.⁹ The peptide levels were determined by radioimmunoassay using a commercially available kit (Behringwerke, Marburg, Germany). The level of 25.5 ng/ml in the proband at 12½ years of age was within the normal range of 15.5 ± 7.7 ng/ml observed in 15 age matched samples.

Amplification and sequencing of genomic DNA was also undertaken to determine if the COL3A1 mutation was present in leucocyte DNA from the parents' blood. The same techniques that were used to define the genomic DNA mutation in the proband were used.⁷ Only normal DNA sequences were



Figure 4 Dermal fibroblast with normal rough endoplasmic reticulum and collagen fibrils in longitudinal cross section.

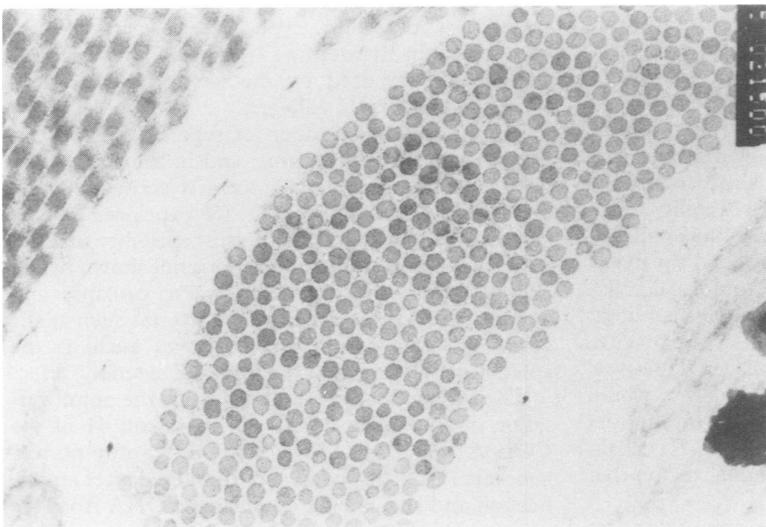


Figure 5 Dermal collagen fibre bundle with normal cross sectional fibril pattern.

obtained from the polymerase chain reaction products. The sequence extended from exon 41 to exon 42 of the COL3A1 gene and included the splice donor site of intron 41, the site of the mutation in the proband.⁷ Neither parent showed any evidence of the mutant sequence in the amplification products.

Discussion

The proband had the typical features of the acrogeric form of EDS IV.¹ He had the characteristic facies with a pinched nose, thin lips, prominent eyes,

and violaceous eye lids. These facial features, his aesthenic build, thin skin, prominent subcutaneous veins, and acrogeric hands produced a 'cachectic' appearance. These features were evident in early childhood and progressively worsened up to the time of review at 12½ years of age.

His failure to thrive, bleeding from the rectum, and frequent bruising were suspected initially to be the result of 'child abuse'. The proband did not have the marked joint hypermobility and cutaneous hyperelasticity that are characteristic of most types of EDS.¹⁰ The numerous hypoplastic scars on the

forehead, elbows, knees, and shins in EDS types I and II were not present in the child reported here. Similarly, generalised joint hypermobility, a prominent feature of EDS types I to III and V to VIII, was not observed in this child except for mild hypermobility in his elbows, wrists, and feet.

The level of the amino-terminal propeptide of type III procollagen in the serum has been reported to be low in patients with EDS IV.⁹ The propeptide is normally cleaved from the procollagen in the extracellular processing of procollagen to collagen. It has been proposed that some of the released propeptide enters the circulation and that its level can be used as an indicator of the level of type III collagen synthesis.⁹ The serum assay was normal in this child and would, therefore, not have been useful as a screening test for EDS IV. The normal serum level of the propeptide was not expected in view of the low amounts of type III collagen in the dermis and the low production and secretion of type III collagen by cultured fibroblasts.⁷

Increased susceptibility to bruising, vascular fragility and gastrointestinal abnormalities occur in many forms of EDS but they are major features of EDS IV.¹ In the proband, bleeding from the large intestine appeared to be because of fragility of the fine connective tissue stroma of the mucosa and mucosal vessels. There did not appear to be a major abnormality in platelet function or coagulation to account for the mucosal bleeding and bruising. However, the skin bleeding time was mildly increased which could have been the result of increased vascular fragility or an abnormality in the ability of the subintimal collagen to stimulate platelet aggregation. The child's platelets aggregated normally when exposed to normal collagen but there were insufficient amounts of mutant type III collagen available to enable these *in vitro* assays to be repeated with mutant collagen. However, it is likely that the mutant type III collagen would have reacted normally with platelets as the normal reactive domain is in the middle of the $\alpha 1(\text{III})$ chain and not in the inactive carboxy-terminal region that contained the deletion.¹¹

He was also constipated and gastric emptying was delayed. The mechanisms involved in these anomalies of intestinal motility were not investigated but they were likely to be the result of the abnormal connective tissue structure of the bowel wall.

The skin was clinically fine and thin in keeping with our previous finding that its type III collagen content had been reduced to 11% of normal.⁷ The collagen fibrils were round in cross section and there was an essentially unimodal distribution of fibril diameters with occasional small fibrils. It is likely that type I and III collagens normally occur together in dermal fibrils.¹² However, the mean fibril diameter in the proband's thigh dermis was not significantly reduced when compared to control samples

from the inguinal region. These results were difficult to interpret because variations in fibril diameter have been observed in normal samples from different sites.¹³ Others have reported reduced collagen fibril diameters and heterogeneity in fibril distribution with some large and irregular fibrils.^{13,14}

The reduced amount of type III collagen in the dermis from the proband was probably the result of decreased synthesis and secretion of type III collagen by the fibroblasts as observed *in vitro*. However, the rough endoplasmic reticulum of dermal fibroblasts was not distended as has been observed in other cases of EDS IV.^{3,15} In some of the latter cases, immunofluorescent studies have shown accumulation of type III collagen in the cisternae of the rough endoplasmic reticulum.¹⁵

Our previous results showed that mutant homotrimeric molecules were the major triple helical species of type III collagen produced by cultured dermal fibroblasts.⁷ They had a normal thermal denaturation temperature which suggested that the shortened molecules were able to form a stable triple helix. However, despite the apparent stability of these molecules and their resistance to mammalian collagenase digestion, type III collagen did not accumulate in the dermis. It appears that this outcome was prevented by the low production and secretion of type III collagen.

The normal distribution of type III collagen in the vascular system, dermis, and in the supporting connective tissues of the viscera is consistent with the clinical features of EDS IV expressed by the proband.¹ It also explains the susceptibility of EDS IV patients to other manifestations not shown by the proband, for example, mitral valve prolapse and aneurysms and rupture of major arteries such as the aorta and of medium sized arteries such as the splenic, renal, popliteal, and carotid arteries.^{11,16}

The proband was heterozygous for the point mutation at the splice donor site of intron 41 of the COL3A1 gene.⁷ In the present study, only normal genomic DNA sequences were obtained after amplification and sequencing of leucocyte DNA from the parents. It is likely, therefore, that the proband had a new dominant mutation, but gonadal mosaicism in one parent was not excluded as an alternative mechanism. The heterozygous mutation of type III collagen in the proband with EDS IV provides an explanation for his clinical phenotype. From our findings and other reports, it appears that the acrogeric type of EDS IV can arise from deletions or amino substitutions within the triple helical domain of $\alpha 1(\text{III})$ chains.^{14,17-19}

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