EDS IV (acrogeria): new autosomal dominant and recessive types[1]

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Summary: Evidence is presented that type IV of the Ehlers-Danlos syndrome (EDS IV) is genetically variable. A benign autosomal dominant form and two autosomal recessive variants are described with clinical and biochemical features that are distinct from classical acrogeria.

Introduction

Ehlers-Danlos syndrome (EDS) is a heterogeneous, inherited disorder of connective tissue with characteristically fragile hyperextensible skin, loose jointedness, tissue fragility and a liability to paper tissue scars. At least seven types can be separated clinically. Since Pinnell *et al.* (1972) described hydroxylysine-deficient collagen in an EDS patient who had retinal detachment and kyphoscoliosis, there have been great advances in the molecular understanding of the disease. Specific biochemical abnormalities have subsequently been found in types IV, V and VII (Table 1). EDS IV is an especially dangerous disease; Barabas (1967) and Beighton *et al.* (1969) first realized that the extensive bruising and extreme arterial fragility set this variant apart from the others. They called it the ecchymotic or arterial type which McKusick (1978) later classified as EDS IV. EDS IV is itself heterogeneous. It includes patients with thin faces, prominent eyes and prematurely aged limbs who have been described previously in the European and British dermatological literature as having acrogeria, which was regarded as a

Table 1. Biochemical abnormalities and EDS

Type	Synonym	Inheritance	Biochemistry	Reference
EDS IV	Acrogeria	Autosomal recessive and dominant	Type III collagen deficiency (total or variable)	Pope *et al.* (1975, 1977)
	Ecchymotic or arterial type	Autosomal dominant	Unknown	
EDS V		Sex-linked recessive	Lysyl oxidase deficiency Normal lysyl oxidase levels	Di Ferrante *et al.* (1975) Siegel *et al.* (1979)
EDS VI		Autosomal recessive	Hydroxylysine-deficient collagen (lysyl hydroxylase deficiency)	Pinnell *et al.* (1972)
EDS VII	Arthrogryphosis multiplex	Autosomal recessive	Procollagen peptidase deficiency	Lichtenstein *et al.* (1974)
	Arthrogryphosis congenita	Autosomal recessive	Extension peptide mutation	B Steinemann (personal communication)

[1] Based on case presentations to Section of Dermatology, 19 April 1979. Accepted 8 October 1979

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quite separate condition from EDS (Rook *et al.* 1972). Acrogeria refers to the particularly obvious thin skin of the acral parts (hands, feet, face) which gives a prematurely aged appearance. The characteristic faces with a pinched delicate nose, prominent eyes and unusually thin skin with easily visible veins have been clearly described (Basex & Dupré 1955). We have previously identified (Pope *et al.* 1975) a specific deficiency of type III collagen in tissues (skin, artery, lung, intestine) and cultured skin fibroblasts from such patients. It was later shown (Pope *et al.* 1977) that heterozygotes have significantly reduced amounts of type III collagen in tissues and in fibroblast cultures. Here we present evidence that the disease is clinically and genetically variable. We describe a benign autosomal dominant form and an autosomal recessive variant less dangerous than classical EDS IV, with distinct clinical and biochemical differences which allow them to be separated from the original form of the disease.

Case reports

Patient 1: This 29-year-old woman was diagnosed as acrogeric when aged 7 years and described at the Section of Dermatology of the Royal Society of Medicine (Morris 1957). She originally presented with small stature, easy bruising, cutaneous fragility, slow growth and a prematurely aged appearance, but remained essentially well for the next 17 years. In 1975 she developed sudden, acute abdominal pain with severe clinical shock. Laparotomy showed a large retroperitoneal haemorrhage from a ruptured splenic artery and a splenectomy was performed with good effect. She later had recurrent pneumothoraces which required two separate pleural strips on opposite sides. In 1978 she again developed sudden acute abdominal pain and clinical shock and an emergency laparotomy showed retroperitoneal and intraperitoneal bleeding from a ruptured left renal aneurysm. Shortly afterwards she spontaneously aborted in the 14th week of her first pregnancy. The fetal tissues clearly showed adequate type III collagen indicating that it would have been heterozygous for the gene. Since then she has remained well but recently had an osteoma of her left talus excised.

Examination showed a small (1.53 m) female with a characteristically thin skin, especially of the forearms, hands, arms and legs. She had very prominent eyes and a thin, pinched, curved nose (Figures 1 & 2). There was a minimal nail dystrophy and the first terminal digits showed acro-osteolysis (Figure 3). Full clinical examination was otherwise normal.

Patient 2: This patient was diagnosed when the 7-year-old proposita was referred to the Haemophilia Centre at Newcastle upon Tyne with persistent and unexplained bruising. Haemophilia was excluded but EDS diagnosed because of slight loose-jointedness and rather soft skin with somewhat prominent veins. The face is normal (Figure 4) and she is a large-eyed pretty child. Apart from the bruising she is entirely well and has had no major medical problems.

Patient 3 (Figure 5): The 37-year-old father of Patient 2 was closely examined because of his daughter's abnormalities. He was obviously prematurely aged but his face was quite different from Patient 1. In particular he lacked the bulbous, prominent eyes, thin nose and lips. His skin was markedly thin with a prominent venous network and his hands and feet showed the premature ageing typical of acrogeria. There was a history of spontaneous pneumothoraces which had necessitated pleural stripping. The family tree is shown in Figure 6.

Examination of collagens

Four-millimetre punch biopsies were taken from the right upper arm under local anaesthesia. The biopsies were divided to take samples for light and electron microscopy, examination of tissue collagens and establishment of fibroblast cultures. Tissue collagens were examined after digestion of samples with cyanogen bromide in 70% formic acid under nitrogen at 30°C for 4 hours. The resultant peptides were recovered by removal of the volatile side products first by evaporation *in vacuo* and then lyophilization. They were then examined by polyacrylamide

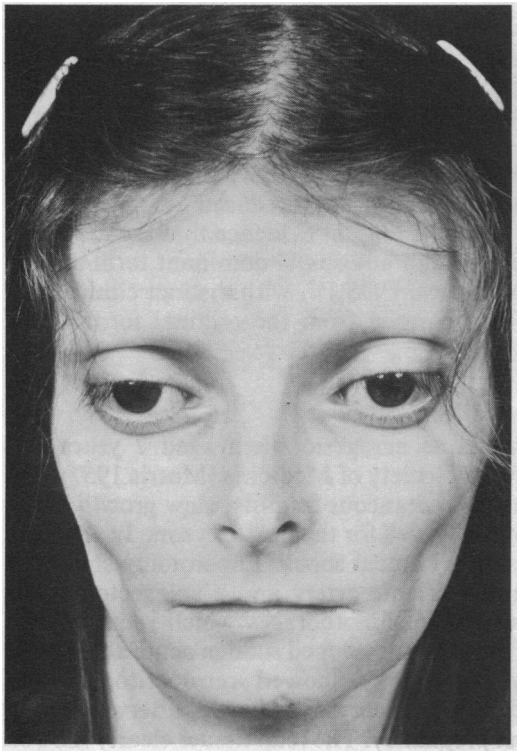
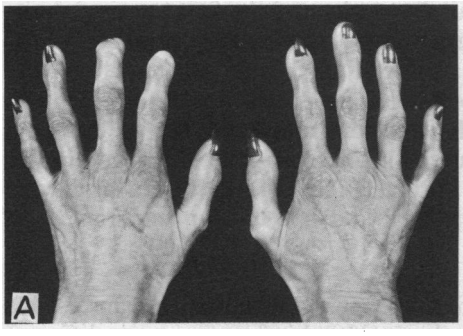


Figure 1. Face of classical EDS IV

Figure 2. Face of autosomal recessive variant



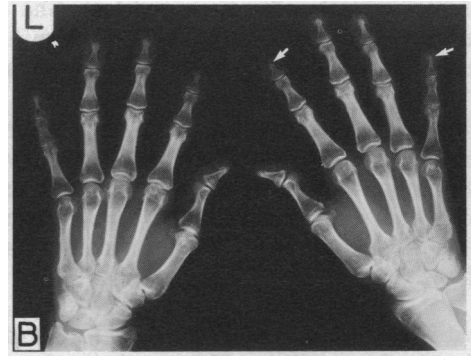


Figure 3. A, hands showing typically aged appearance; B, X-ray of hands showing erosion of terminal phalanges (arrowed)

slab gel electrophoresis by the method of Laemmli (1970). After staining with Coomassie Blue 10%, the gels were visually examined for peptide differences (but not scanned).

Cultured fibroblasts were grown from explants in McCoy's 5A medium containing 10% fetal calf serum. Cultures were labelled by 24-hours incubation with one microcurie/ml of [^{14}C]glycine and [^{14}C]proline in depleted medium containing 50 μg/ml of ascorbic acid and 50 μg/ml of beta-aminopropionitryl (BAPN). Following dialysis against 0.15 mol/l saline pH 7.4 and then 0.5 mol/l acetic acid, carrier calf skin collagen (30 mg) and pepsin were added and incubated at 15°C for 16 hours. The digestion was stopped by raising the pH to 8.0 for 30 minutes and collagens precipitated by dialysis against 10% NaCl in 0.5 mol/l acetic acid. The

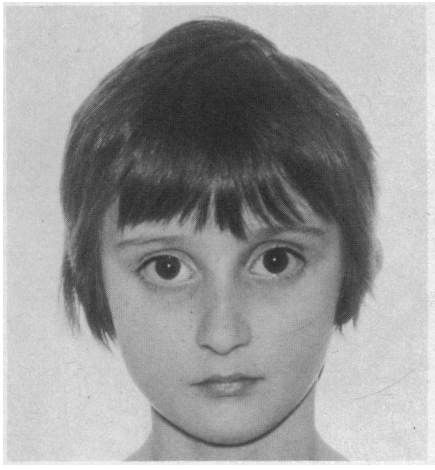
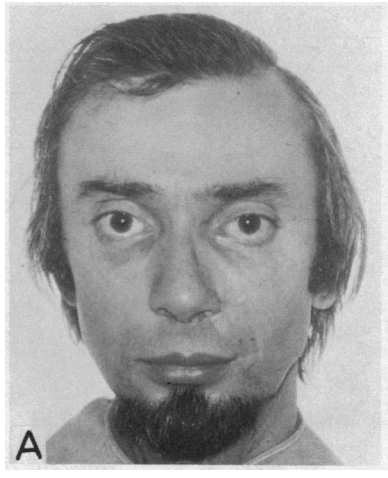


Figure 4. Proposita (Patient 2) with normal face



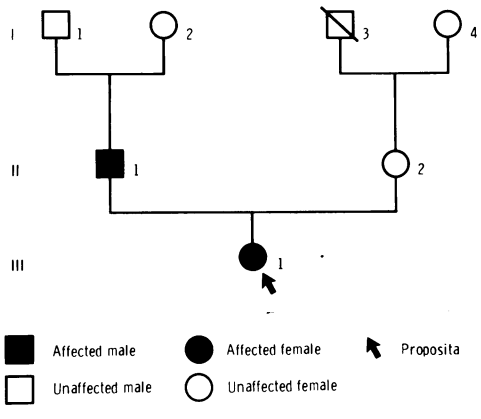


Figure 6. Family tree of Patients 2 and 3 showing autosomal dominant inheritance

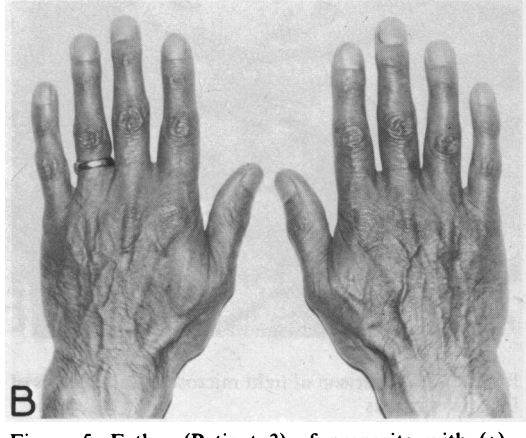


Figure 5. Father (Patient 3) of proposita with (A) normal face and (B) acrogeria of hands

precipitate was collected by centrifugation (35 000 g) and redissolved in 0.5 mol/l acetic acid, dialysed to remove salts and lyophilized. The sample was chromatographed on carboxymethyl cellulose (Whatman) at 42°C using a linear sodium chloride gradient to elute bound collagens (Miller 1971). Fractions of the eluate were monitored for radioactivity and UV absorbance of the carrier collagen.

Results

Light microscopy showed a relative increase of elastin but the classical variety had much less collagen than the two autosomal dominant patients (Figure 7). Chromatography on carboxymethyl cellulose of collagens synthesized by fibroblasts in culture showed no type III collagen in fibroblasts from Patients 1, 2 and 3. The mother of Patient 2 showed normal amounts of this protein. Collagen peptide patterns from whole-skin samples showed differences between the patients. Patient 1 showed α1(III)CB 5/9 but lacked α(III)CB 6/8 at normal gel loadings (tracks 2–4 Figure 8A). The smaller peptides (CB 6/8) appeared at higher loadings. In contrast Patients 2 and 3 lacked, or at least contained only very slight traces of, both peptides (tracks 6 & 7 Figure 8B). The mother of Patient 2 (track 8 Figure 8B) showed both peptide bands which excluded the possibility that she is heterozygous for this gene. Her peptide pattern was identical to the surviving grandparents (tracks 4 & 5 Figure 8B).

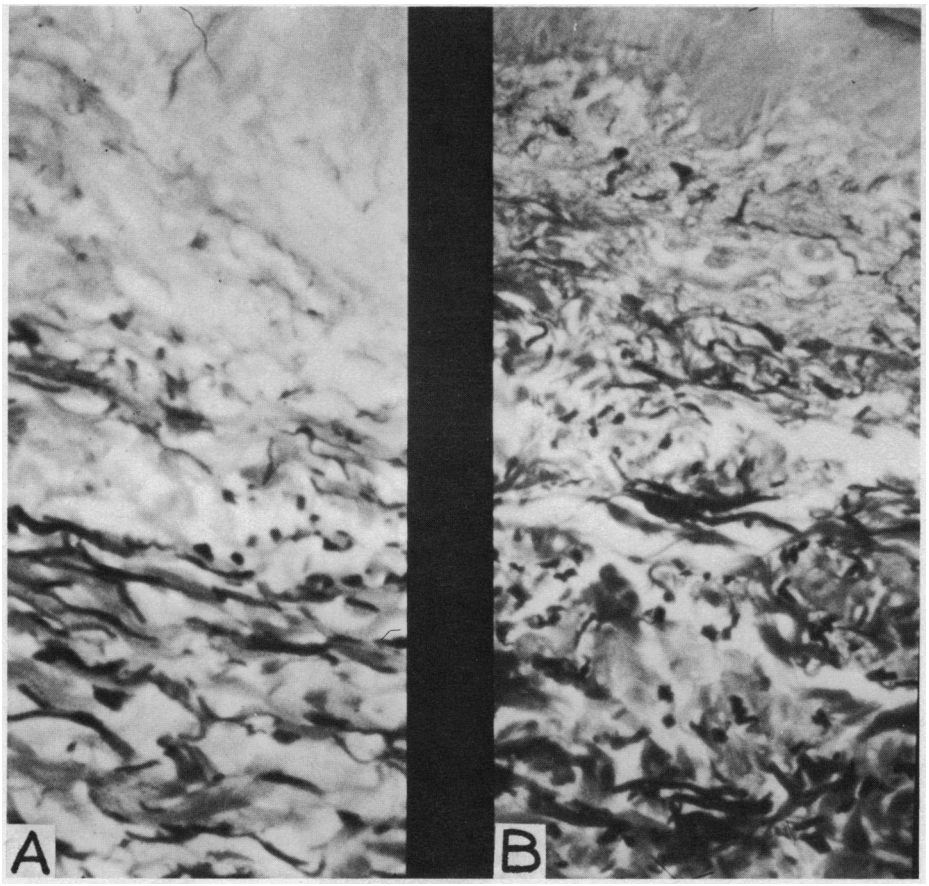


Figure 7. Comparison of light microscopical changes of (A) classical, mild recessive and (B) dominant EDS IV variants

Discussion

Classical acrogeria (EDS IV) is caused by a specific deficiency of type III collagen which is neither present in any body tissue nor produced by cultured skin fibroblasts. Such patients die in their second or third decade from lethal arterial rupture. Inheritance is autosomal recessive (Pope *et al.* 1977) and parents are obligate heterozygotes (unless one or both are newly mutated). The original patients described (Pope *et al.* 1975, 1977) have a dangerous, prematurely fatal disease. It has since become apparent that there are less severe forms and that the disorder is both clinically and genetically heterogeneous. It should always be suspected when arterial aneurysms affect young people. Some patients survive into middle age (and possibly beyond) and there are subtle clinical differences which separate these patients from the classical form of the disease (Table 2).

Byers *et al.* (1979) have recently suggested that four sub-groups can be separated on the basis of the electron microscopical findings of collagen fibre size and distribution. However, the published clinical details do not allow us to determine whether their patients are directly comparable with ours. The CB-peptide patterns of the patients described here show that at least three varieties are clearly separable on clinical and biochemical criteria. This does not exclude the possibility that other type III collagen abnormalities occur and, for example, alterations in type III collagen structure (due to lengthening, shortening or amino acid substitution within the chain) are possible. The essential clinical and biochemical differences between our groups are shown in Table 2.

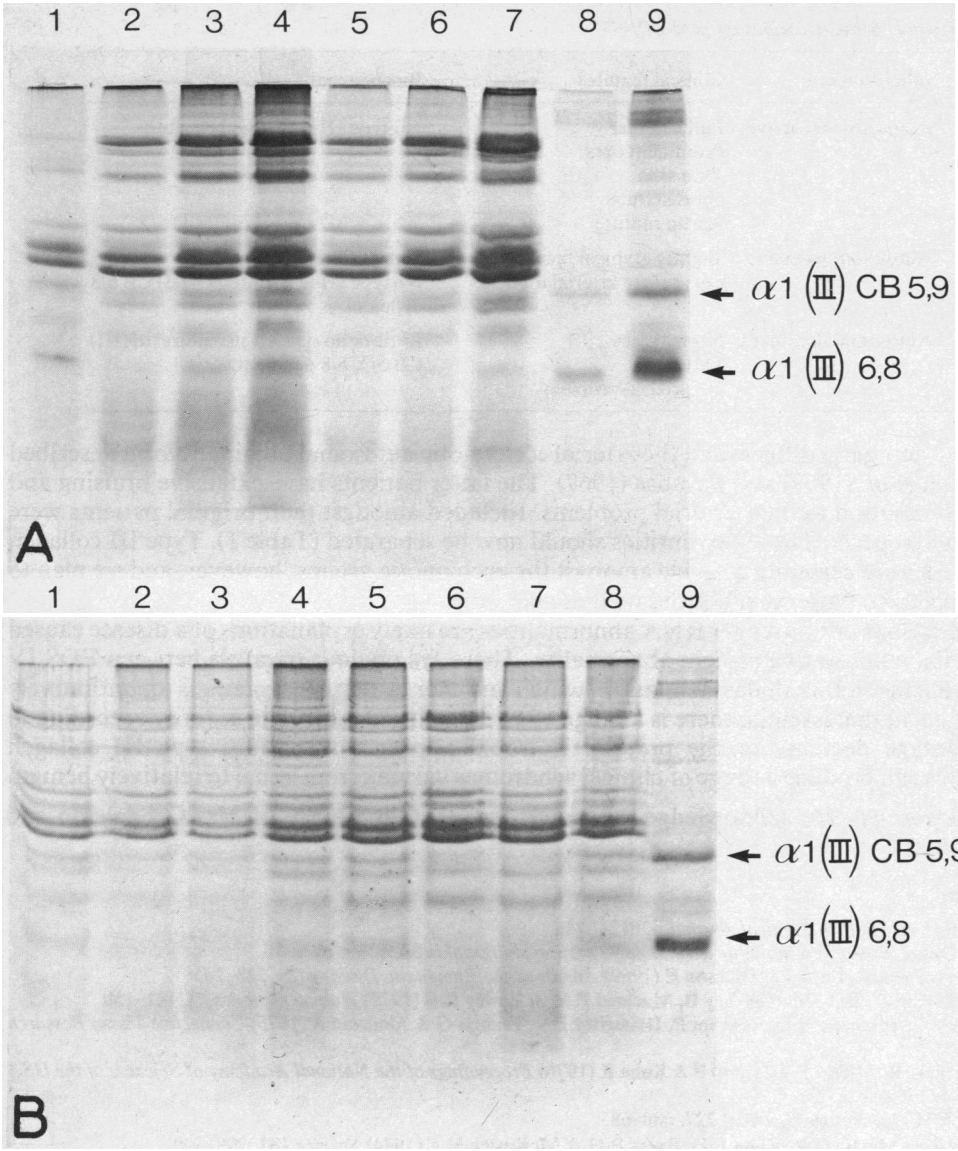


Figure 8. Slab gel electrophoretic patterns of cyanogen bromide derived peptides of skin. A, tracks 2, 3 & 4 are Patient 1 at increasing loadings. B, tracks 6 and 7 show Patients 2 and 3 respectively; tracks 4, 5 and 8 show respectively the paternal grandparents and mother of proposita. (In both A and B track 9 contains cleaved purified human type III collagen)

Patients with the most severe disease make no type III collagen (Pope *et al.* 1975, 1977, Gay *et al.* 1976), but its presence in variable amounts does not correlate directly with severity in the other patients. Thus Patients 2 and 3 who make little or no type III collagen in culture and who have little detectable type III collagen in tissues, have a relatively mild disease, whereas Patient 1, who has detectable quantities of this collagen in tissue has a tendency to small aneurysms of medium-sized arteries. On the other hand, the former have normal dermal collagen thickness whereas the latter does not. One possibility may be that different type III collagen mutations affect various tissues in different ways, so that some patients could have widely disparate amounts of type III in tissues such as skin as compared with say blood vessels.

Table 2. Differences between various types of EDS IV

Type	Inheritance	Clinical features	Biochemical
Classical (short-lived)	Autosomal recessive	Pinched face Prominent eyes Thin skin Short stature Aortic rupture	No type III in tissues or culture
Long-lived	Autosomal recessive	Slightly atypical face Less severe arterial disease	Some type III in tissues No type III in culture; α1(III) CB 6/8 deficient
Atypical	Autosomal dominant	Normal face Thin skin No arterial problems	Little or no type III in culture; α1(III) CB 5/9, 6/8 deficient

Classical acrogeria differs from the arterial ecchymotic autosomal dominant form described by Beighton *et al.* (1969) and Barabas (1967). The latter patients have extensive bruising and scarring in addition to their arterial problems. Included amongst their original patients were some with acrogeria. These two entities should now be separated (Table 1). Type III collagen abnormalities are certainly possible amongst the ecchymotic groups, however, and we plan to examine collagen patterns produced by these patients.

Gene deletions or messenger RNA abnormalities are likely explanations of a disease caused by a specific quantitative protein abnormality. There are obvious parallels between EDS IV and the haemoglobin thalassaemias in which another essential protein is quantitatively depleted. As in thalassemia, there is a range of abnormalities varying in severity according to the percentage decrease of the protein. It would appear that various type III collagen deficiencies can produce a range of clinical syndromes varying from lethal to relatively benign.

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