Chapter 9

The Ehlers-Danlos Syndrome

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SUMMARY

- **The Ehlers-Danlos syndrome (EDS) is a clinically and genetically heterogeneous group of connective tissue disorders affecting mainly skin, joints, and ligaments but in some cases also arteries, intestine, and the gravid uterus. Its hallmarks are hyperelasticity of the skin, hypermobility of the joints, and tissue fragility.**
- **The classical type of EDS (EDS I and II) is the most common form and is characterized by marked skin hyperextensibility and fragility and by joint hypermobility. The disorder is most often inherited as an autosomal dominant trait, but there is considerable locus heterogeneity.**
- **The hypermobile type of EDS (EDS III) is characterized by marked joint hypermobility, moderate skin involvement, and an absence of tissue fragility. It is inherited as an autosomal dominant trait; the underlying defect is unknown.**
- **The vascular type of EDS (EDS IV) is the most severe form of the disorder, with a markedly reduced life span due to spontaneous rupture of internal organs such as arteries and intestine. It is inherited as an autosomal dominant trait and caused by mutations in the *COL3A1* gene coding for collagen III. Unlike in the classical type, the skin is not hyperelastic but thin, translucent, and fragile, joint hypermobility is restricted to the minor joints, and affected subjects may have a characteristic facial appearance.**
- **The kyphoscoliotic type of EDS (EDS VI) is characterized, in addition to the features of the classical type, by severe muscular hypotonia after birth, progressive kyphoscoliosis, a Marfanoid habitus, osteopenia, and occasionally rupture of the eye globe and great arteries. It is inherited as an autosomal recessive trait and caused by deficiency of collagen lysyl hydroxylase activity (EDS VIA). A subgroup of patients with similar clinical features does seem to exist, however, in whom lysyl hydroxylase activity appears to be normal (EDS VIB).**
- **The arthrochalasic type of EDS (EDS VIIA and VIIB) is characterized by congenital bilateral hip dislocation, severe generalized joint hypermobility, moderate skin involvement, and osteopenia. It is inherited as an autosomal dominant trait and caused by mutations leading to the loss of the amino-terminal telopeptide of one or other of the two distinct α-chains of the heterotrimeric collagen I molecule.**
- **The dermatosparactic type of EDS (EDS VIIC) is characterized by redundant and fragile skin, prominent herniae, joint laxity, and dysmorphic features. It is inherited as an autosomal recessive trait and caused by a deficiency of procollagen I N-terminal proteinase.**
- **Other classified forms of the EDS, the existence of which as separate entities is questionable, include X-linked EDS (EDS V), the periodontotic type of EDS (EDS VIII), and fibronectin-deficient EDS (EDS X). Further types of EDS include the progeroid form, unspecified types, and chance associations as confounding factors.**
- **Animal models — naturally occurring, transgenic, and experimentally induced — are useful models for the elucidation of physiological and pathophysiological processes.**

INTRODUCTION

The Ehlers-Danlos syndrome (EDS) is a heterogeneous group of heritable disorders of connective tissue characterized by articular hypermobility, skin hyperextensibility, and tissue fragility affecting skin, ligaments, joints, blood vessels, and internal organs. Its clinical and historical aspects have been reviewed in the classical monographs of Beighton [1] and McKusick [2]. In the preface to his book, Beighton [1] writes as follows:

> "Until recent years, no fairground was complete without an 'Elastic Man'. Audiences would be amazed by his ability to stretch his skin for a prodigious distance, before allowing it to snap back into place. He often had hypermobile joints, and would happily place his left great toe in his right ear, or his feet in his trouser pockets.
>
> As medicine became increasingly scientific, the appellation 'Ehlers-Danlos syndrome' was used and he is now more likely to be encountered in a clinical meeting than in a circus side show."

Connective Tissue and Its Heritable Disorders, pages 431–523

Figure 1. First description and picture of an individual with EDS published by Job Janszoon van Meek'ren. **Foreground**: Spaniard with hyperextensible skin. **Background**: On the table, a woman with a distended abdomen because of ascites and an ovarian tumor; she was the wife of Govert Flinck, a famous Dutch painter, and her case was also described in the same book [9]. Van Meek'ren was a notable surgeon of Amsterdam, who was a pupil of Tulp, the original of Rembrandt's ''Anatomist'' [12]. His observation made in 1657 was first reported posthumously in ''Belgian'' in 1668 [9] and translated into German in 1675 [10]. Below follows a translation from the German by Michael Roth and Ursula Zeller von Murg of Zürich. The original Belgian report was also translated into Latin in 1682 [11], and an English translation of this last is given in McKusick [2].

''Of a Soft-Skinned Spaniard''

''Out of pressing needs and unforgettable sufferings our ancestors raised us, as it were, from the cradle with the belief that there is no wilder, more merciless and cruel people to be found in the world than the Spanish. As the history books, especially the chroniclers of the Dutch wars and the American barbarities to excess prove.[1] In spite of this we must confess that we have not seen a softer or more lithe Spaniard than Gregorius Albes, begotten by Spanish parents and on a Canary Island born. However, in his skin alone was he such. We saw him together with the famous professors, Johann von Horne, Francisco Sylvio, Guil. Pisone, and Francisco von der Schaagen in the year 1657 in the large hospice. He was a young fellow, twenty-three years of age, healthy in body and build. In our presence he took with his left hand the skin from his right shoulder and pulled it to his mouth, like an archer pulls the string on a cross-bow. The skin, however, from the chin he pulled with both his hands into a point like a beard, to his breast, from whence he then pulled the self same skin over his head, covering his eyes in a manner such that we could no longer see them. Even more of a wonder was how his skin, when he let go of it, fell back immediately into its proper place in such a manner as if it had never been touched. In just such a way he pulled the skin from his right knee up and down about half an arm's length. And once he let go of it, a man could not notice that it had once been pulled up.

At the same time we were astonished to discover that the skin on his left shoulder and knee in no way let itself be pulled, as it was in these places so fixed and firm, it would have been impossible.

What however the causes of the soft parts as well as the firm parts were remains to us till this very hour unknown.''

Chapter 29 in ''Rare and Fantastical Observations of the Surgical and Healing Arts, as they were first made public five years ago, shortly after the passing away of the author, after much urging and desire to please the students of the Healing Arts; now for the advantage of High German speakers truly translated and printed. Throughout adorned with copper engravings and supplied with a complete register. Set and printed by Paul Fürstens, Art and Book dealer, Late Wittib and Successors, Nürnberg, An 1675'', pp 186–188. (Reproduced from [11] with permission of the British Library.)

Note that, most interestingly, there is no mention of joint involvement or scarring and that the left side is not involved.

[1] The author points out that Spaniards are the ''most cruel'' people in the world, a remark to be interpreted in the light of the fact that the Dutch had just undergone an 80-year war with Spain.

Figure 2. First known photographic documentation of a person with EDS. Felix Wehrle, the ''Elastic Skin Man,'' who, besides having the power to stretch his skin could readily bend his fingers backward and forward, was photographed by Charles Eisenmann[2] in about 1880 (Cabinet card photograph, private collection). The same person was photographed in 1888 in Budapest and his picture was published in 1896 [3]. Wehrle was apparently consigned to the ''museum circuit,'' where he did contortions along with skin stretching. His career seems to have been overshadowed by the more spectacular feats of James Morris, ''The India Rubber Man.'' An Eisenmann portrait in the files of the Museum of the City of New York shows that Morris was able to pull the skin of his throat up over his eyes, an achievement that won him long-standing contracts with various Barnum shows.

The changes in social structure and the advances in biomedical sciences have had as consequences that affected persons earlier enjoyed collectively as curiosities [3,4] are now regarded as individuals suffering from a disease and that the anecdotes and picturesque descriptions with their undoubtedly high didactic value (for examples, see [1]) have been replaced today by more scientific clinical, morphological, biochemical, and functional characterizations published in papers or monographs (for reviews, see [5–8]).

The first partial description of the syndrome was provided by Job Janszoon van Meek'ren (1611–1666), a surgeon from Amsterdam, who in 1657 described a 23-year-old Spaniard named Georg Albes in a report published posthumously in 1668 in ''Belgian'' [9], which was translated into German in 1675 [10] and Latin in 1682 [11], containing one and two etchings (Fig. 1), respectively. It is of note, however, that no mention was made of abnormal scarring or joint laxity and that the left side is not involved. Photography as a new method of clinical documentation started after 1850, and it was in about 1880 that the first known photograph of an individual with EDS was taken, the subject being a traveling showground performer (Fig. 2).

Merit for the classical description of the syndrome should be assigned to the Russian dermatologist A.N. Tschernogubow, who presented at the first meeting of the Moscow Dermatological and Venereological Society, November 13, 1891, a 17-year-old sporadic case with all the hallmarks of the syndrome [13] (translated by Denko [14]; abstracted by Lanz [15] and von Trautvetter [16][3]). He described the skin as pale, lusterless, velvety, thin, hyperextensible (''it is easily pulled away, far beyond normal limits, and rapidly like elastic it regains its normal position''), and friable, with scar formation in a ''strange fashion'' and with secondary wound dehiscence because all sutures cut through the skin; the joints had an extreme degree of mobility, and there was subluxation of a hip and an elbow; there were ''fleshy tumors'' mainly on protruding areas such as the elbows, and cysts on the buttocks and knees; upon palpating the skin, especially that of the extremities, there were round subcutaneous nodules, fine, small, quite hard, and painless, which on removal were found to be deposits of mineral salts; the patient was born feeble, had recurrent convulsions starting in infancy, began to walk only during his third year, and continued to fall frequently; he was rather intelligent, with a ''good head on his shoulders''; gentle natured, as a rule, with a ''good frame of mind and spirit.'' Tschernogubow then concluded that, in the future, ''there might be an opportunity to clarify the observed looseness of the connective tissue that impaired the generalized development of all connective tissue components... It is possible that the development of this looseness is due in part to a deficiency in the supporting structures with a resulting diminution in the ability of the skin to resist deformation.'' In the Russian literature, this condition is called today, quite appropriately indeed, ''Tschernogubow syndrome,'' but because the report was written in Russian, it was not known to most European and American dermatologists. Tschernogubow did not describe further patients (N.P. Bochkov, Moscow, personal communication, 1990).

In 1901, Edvard Ehlers (1868–1937) (Fig. 3), a dermatologist from Copenhagen, reported a patient with ''cutis laxa,'' a marked tendency to hemorrhages, and loose-jointedness [17]. Henri-Alexandre Danlos (1844–1912)

[2]Charles Eisenmann, ''the popular photographer'' as he called himself, was born in the then independent German State of Würtemberg in 1850 and moved to New York, where he opened his studio, in 1879. Eisenmann's interest in human grotesques was reflected in the world of second-string vaudevillians and first-rank freaks; he was the official photographer of the dime museum *demi-monde*. The term ''portraits taken instantaneously'' refers to the collodion process, which transformed the minutes of the daguerreotype exposure into approximately as many seconds and arose when early users of the wet plate system discovered that the process allowed them, for the first time, to record street scenes with arrested movement [4].

[3]Both abstracts are entitled ''cutis laxa'' instead of the original ''cutis laxae,'' and von Trautvetter [16] reported erroneously a 50-year-old woman who was, in fact, the healthy mother of the 17-year-old proband described by Tschernogubow, thereby giving the misleading impression to readers, including Beighton [1] and McKusick [2], that there were actually two different case reports.

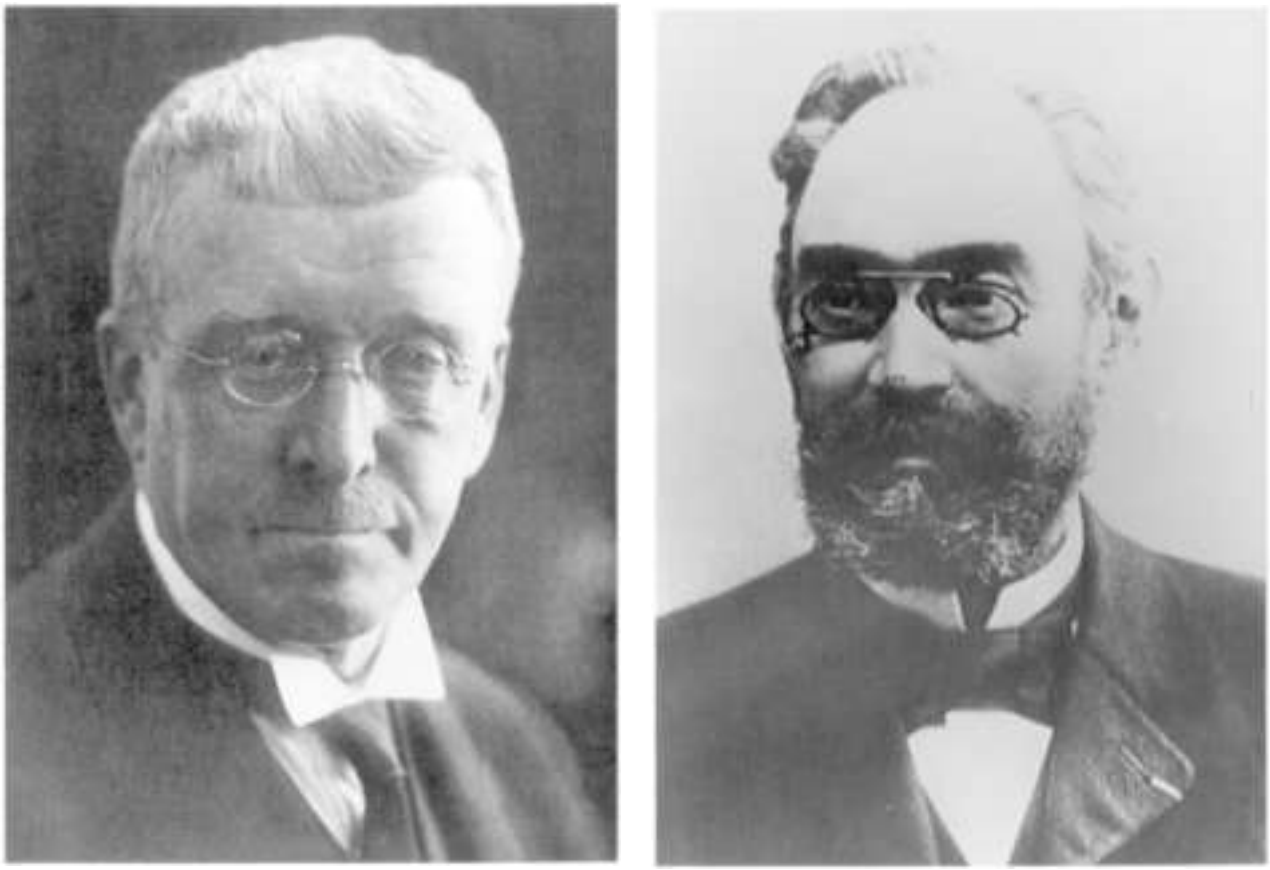

Figure 3. Portraits of Edvard Ehlers and Henri-Alexandre Danlos. Edvard Ehlers (1863–1937) (**left**) presented in 1899, at a meeting of the French Dermatological Society, a law student with lax skin, a tendency to hematoma of the skin, and generalized loose jointedness; he published this case in 1901 [17] but subsequently refrained from doing more work on the condition later bearing his name. Henri-Alexandre Danlos (1844–1912) (**right**) discussed in 1908, at a meeting of the French Dermatological Society, the nature of heaped-up lesions on the elbows and knees of a boy in whom a diagnosis of juvenile pseudodiabetic xanthoma had previously been made by his colleagues, Hallopeau and Macé de Lépinay. Danlos recognized that the skin of this patient was fragile and extensible, and he pointed out the similarity of these features to those of the patient presented by Danlos to the same society in 1899; he published the case the same year [18]. In 1900, Morris presented a similar case, a lad with ''elastic'' skin and numerous cutaneous nodules [19]. (Courtesy of Dr. Pierre Maroteaux, Paris.)

(Fig. 3), a dermatologist from Paris, described in 1908 another patient with hyperelastic, thin, and fragile skin and explained the molluscoid pseudotumors as chronic contusions of the vulnerable tissues [18]. The terminology became complex and, apart from general terms such as ''dystrophia mesodermalis'' and ''fibrodysplasia elastica generalisata,'' many terms that were descriptive of various features of the syndrome were employed, such as ''dermatorrhexis,'' ''dermatolysis,'' ''Gummihaut,'' ''cutis pendula,'' ''cutis hyperelastica,'' ''chalasodermia,'' ''human pretzel,'' and ''India rubber man.'' Between 1932 and 1936, the condition received its eponymous title and (by this) achieved scientific respectability [20–23]. In 1936, Sack described a patient with excessive friability of the arteries and called the condition ''status dysvascularis'' [24]. Dominant inheritance was mentioned as early as 1888, and from then on occasionally [25–28]. In 1949, Johnson and Falls [29] demonstrated autosomal dominant inheritance on the basis of their study of an extensive kindred containing 32 affected persons over five generations. Six years later, Jansen [30] suggested that the defect affected the collagen ''wickerwork,'' which he described as being excessively loose in this disorder.

In 1960, McKusick reported genetic heterogeneity of the EDS in the second edition of ''Heritable Disorders of Connective Tissue'' [2b]. In 1967, Barabas [31] delineated three clinical types, including the arterial-ecchymotic type, later also called the Sack-Barabas syndrome and now known as the vascular type of EDS, or EDS IV. One year later, Beighton [32] distinguished five clinical forms, and in 1972, McKusick listed seven types [2], including EDS VI, the ocular-scoliotic type, due to lysyl hydroxylase deficiency [33], EDS IV, the arterial-ecchymotic type, due to collagen III deficiency [34], and EDS VII, arthrochalasis multiplex congenita, which has turned out to be caused by a failure of removal of the amino-terminal globular propeptides of procollagen I [35,36,305].

The importance of identifying correctly the type of EDS with which a patient is affected cannot be stressed enough because the natural history and mode of inheritance differ among the types. Unfortunately, much of the older literature does not differentiate clearly between types, and the severe complications of EDS IV are often cited as characteristic of the syndrome as a whole, thereby creating unnecessary anxieties. In this chapter, we try to cover the EDS in a

comprehensive and authoritative way, to weigh the different aspects as objectively as possible, and to clarify earlier concepts and conclusions that today are no longer tenable. To give the reader an idea of how knowledge was gained, references are often cited in a historical sequence.

GENERAL ASPECTS OF THE EHLERS-DANLOS SYNDROME

Common Signs and Symptoms

The signs and symptoms listed in this section may be encountered in various of the different types of EDS, although to variable degrees, and are therefore described together (see Table 1).

Dermatological Features, Facies

The skin is of a white color and lusterless. It is thin, smooth, soft, and velvety, like "wet chamois leather," a "fine sponge," "the skin you love to touch," or "marshmallow," with a doughy feel. The thinness of the skin is best appreciated clinically on the dorsal aspect of the proximal phalange of the fourth finger. The face may be characteristic, with its pale, pastel-colored appearance resembling a portrait by Manet, with irregular scars over the forehead and chin, which are remnants from early childhood (Figs. 4b, 5b, 6a), epicanthic folds, or, later in life, telecanthus, secondary cutis laxa of the eyelid, a crooked nose with a soft cartilage, crowded teeth, and lopsided and floppy ears. In elderly patients, the forehead shows both horizontal and vertical creases (Fig. 6a), giving a peculiar reticular pattern.

Cutaneous hyperextensibility. This is a cardinal feature of EDS, except EDS IV. The skin, and also the mucosa, is hyperelastic[4]; that is, it extends easily and snaps back after release (unlike lax, redundant skin as in cutis laxa). It seems loosely attached to the subcutaneous tissues, and when traction is applied there is a sensation felt by the applier of the skin "coming away." This is particularly striking over areas such as the thenar, the upper part of the chest, and other parts of the body where the skin is usually tightly fixed (Figs. 2,4a, 25d). A "man who could hide behind his skin" is shown in Figure 1 ([11], from which the figure is reproduced).

Skin hyperelasticity should be tested at a neutral site, meaning one that is not subjected to mechanical forces or scarring, such as the volar surface of the forearm. Although in infants hyperelasticity of the skin is difficult to assess because of abundant subcutaneous fat, in the adult, with time, loose folds of skin may appear over the elbow joints like a dewlap (Fig. 4c); the palms may be peculiarly wrinkled (Fig. 25d), the skin of the hands resembles loose gloves, and the soles of the feet appear loose-fitting and resemble moccasins or oversized ankle socks (Figs. 5c–e). Cutaneous hyperextensibility varies with the site of the body, and simple clinical scores have been established to assess it [44,45]. Elderly individuals may also develop abnormally lax skin (acquired cutis laxa), such as of the eyelids (blepharodermatochalasis, see below). *In vivo* methods of measuring skin elasticity have been developed by Grahame and Holt [46], as discussed by Daly [47], and applied by Henry et al. [48]. In both normal individuals and affected persons, the module of elasticity increases with age, always being higher in females than in males, and clinical evidence of Langer's lines shows that skin is not mechanically isotropic [49]. Tensile testing shows this anisotropy to be related only to the magnitude of the initial large extension region and not to the low and final high stiffness [47].

Cutaneous fragility (dermatorrhexis). This is manifested by splitting of the dermis or mucosa following relatively minor trauma, which occurs mainly over pressure points (knees, elbows) and areas prone to trauma (shins, forehead, chin). The wounds often present a gaping, "fish-mouth" appearance with protruding subcutaneous fat lobules and usually bleed little because the edges retract due to the elasticity of the adjacent skin, thereby compressing the ends of the blood vessels. Stitches may hold poorly because the thread cuts through the skin, and dehiscence may occur; in addition, wound healing *per se* is delayed. Stretching of the scars after apparently successful primary wound healing is a characteristic of all EDS types; they become wide, thin, and shiny, with a "cigarette paper," papyraceous, or "burn scar"-like appearance (Figs. 4b, d; 5b, f; 25a, b, f). Due to repeated trauma, the scars often become darkly pigmented or violaceous and usually corrugated by fine wrinkles, and telangiectasia may form within their borders. Many lacerations occur during childhood when the child takes its first steps and then more frequently in boys than girls. Proneness to skin lacerations is later aggravated by the unstable joints and muscular hypotonia, which cause stumbling and falling. The tendency toward skin-splitting sometimes decreases as the patient grows older, but it is difficult to know whether this represents a change in the connective tissue itself or whether it is due to less frequent exposure to trauma when the patient reaches adult life. It has been shown that patients with the thinnest skin have the greatest tendency toward skin-splitting [50]. As a rule, the greater the degree of scarring, the more readily soft tissues inside the body will tear on operation.

Hematoma and bruising. Easy bruisability is common and is frequently the presenting complaint to the pediatrician. It is probably due to the friability of the perivascular connective tissue and the walls of the small blood vessels. Child abuse is often suspected (see legend to Fig. 7). Bleeding from the gums following brushing of the teeth and profuse bleeding after tooth extraction are frequent; gastrointestinal bleeding and hemoptysis, however, are rare (with the exception of EDS IV). The bleeding tendency may lead to an extensive search for a coagulopathy. The Rumpel-Leede test may be positive, indicating capillary fragility. Although a variety of plasma clotting factor or platelet abnormalities have been recognized as a result of a greater awareness of EDS, these are probably chance associations, and most patients will be normal in this regard (see EDS X).

Molluscoid pseudotumors and spheroids. The peculiar mechanical properties of the skin in EDS may lead to a

[4] "Elastic" is used synonymously with "stretchy" to indicate a rubberband-like property — that is, the ease with which a material is reversibly deformed — as opposed to the formal physical definition described by Hooke in 1670:

$$\Delta l = \frac{F \cdot l}{E \cdot q}; E = \frac{l \cdot F}{\Delta l \cdot q}$$

where Δ l is the change in length (l) of a material with cross section (q) when a certain force (F) is applied; E, the elastic modulus, is the constant, characteristic for the material. E is larger the less the material is deformed (e.g., steel) and is a measure of the resistance of a particular material against deforming forces. For a historical discussion about the difference between the terms "popular" and "physical" elasticity, see Cohn [43].

TABLE 1. The Ehlers-Danlos Syndrome

Nomenclature		Signs and Symptoms: Skin			Signs and Symptoms: Joint Laxity	Signs and Symptoms: Other Distinctive Features, Complications	Inheritance	Primary Defect	Relative Frequency	MIM No.
New*	Type	Hyperelastic	Fragile	Bruisable						
Classical type	I	+++	+++	++	+++	Vascular and intestinal complications occasionally	AD	Collagen V-defects, other defects	Common	130000
	II	++	++	+	++				Common	130010
	II/III					Normal wound healing	AR	Tenascin-X deficiency	Rare	
Hypermobile type	III	+	−	+	+++	Arthritis	AD	?	Common	130020
Vascular type	IV	−	++++	+++	+	Rupture of arteries, intestine, uterus; pneumothorax; acrogeria; periodontitis; thin skin with easily visible venous pattern; characteristic facial expression	AD	Abnormal and/or reduced type III collagen	Not so rare	130050 (225350) (225360)
Kyphoscoliotic type	VI A	+++	++	++	+++	Muscular hypotonia, kyphoscoliosis, osteoporosis, microcornea, ruptures of arteries and the eye globe	AR	Deficiency in lysyl hydroxylase (normal enzyme)	Rare	225400
	(VI B)									(229200)
Arthrochalasic type	VII A	++	+(+)	+	+++	Congenital hip luxations, osteoporosis, Wormian bones, fractures	AD	Missing N-telopeptide of α1(I) or α2(I) chains of collagen I	Rare	130060
	VII B	++	+(+)	+	++					(225410)
Dermatosparactic type	VII C	−	++++	+++	+	Skin doughy and lax	AR	Procollagen I N-proteinase-deficiency	Rare	225410
Other Types	(V)	++	+(+)	+	+		XL ?	?	Rarissim	(305200)
	(VIII)	+	++	++	++	Periodontal disease, early loss of teeth	AD	?	Rare	(130080)
	IX**	+/−	+	−	+	Skin lax rather than hyperelastic; bladder diverticula; osteoporosis, exostoses, ''occipital horns''; mental retardation	XL	Copper-transporting P-type ATPase↓	Rare	304150
	(X)	+	+	+	++	Platelet dysfunction, petechiae	?	Defect in fibronectin ?	Rarissim	(225310)
	XI**	−	−	−	++	Arthritis	AD	?	Not so rare	147900
Progeroid type							AR	Galactosyltransferase II↓	Rare	130070
Unspecified type							AR/AD			130090
Unspecified type										225320

MIM numbers [37] refer to the predominant form of each type for which the inheritance is given; MIM numbers in parentheses refer to less common variants, the existence or inheritance of which may be questionable. Inheritance: AD, autosomal dominant; AR, autosomal recessive; XL, X-linked.

*The new nomenclature is defined in [39]; see also Footnote 5, below.

**EDS IX, originally called X-linked cutis laxa, and EDS XI are included for historical completeness but, as mentioned in the text (see also [38,39]), have been reclassified as the occipital horn syndrome, a disorder of copper transport (see Chapter 14, this volume), and the familial joint hypermobility syndrome, a disorder of unknown cause, respectively. The existence of EDS V, VIII, and X as separate entities is questionable (see the text), as indicated by parentheses. For a description of EDS VIB, which clinically resembles EDS VIA but is characterized by normal lysyl hydroxylase activity, see the text.

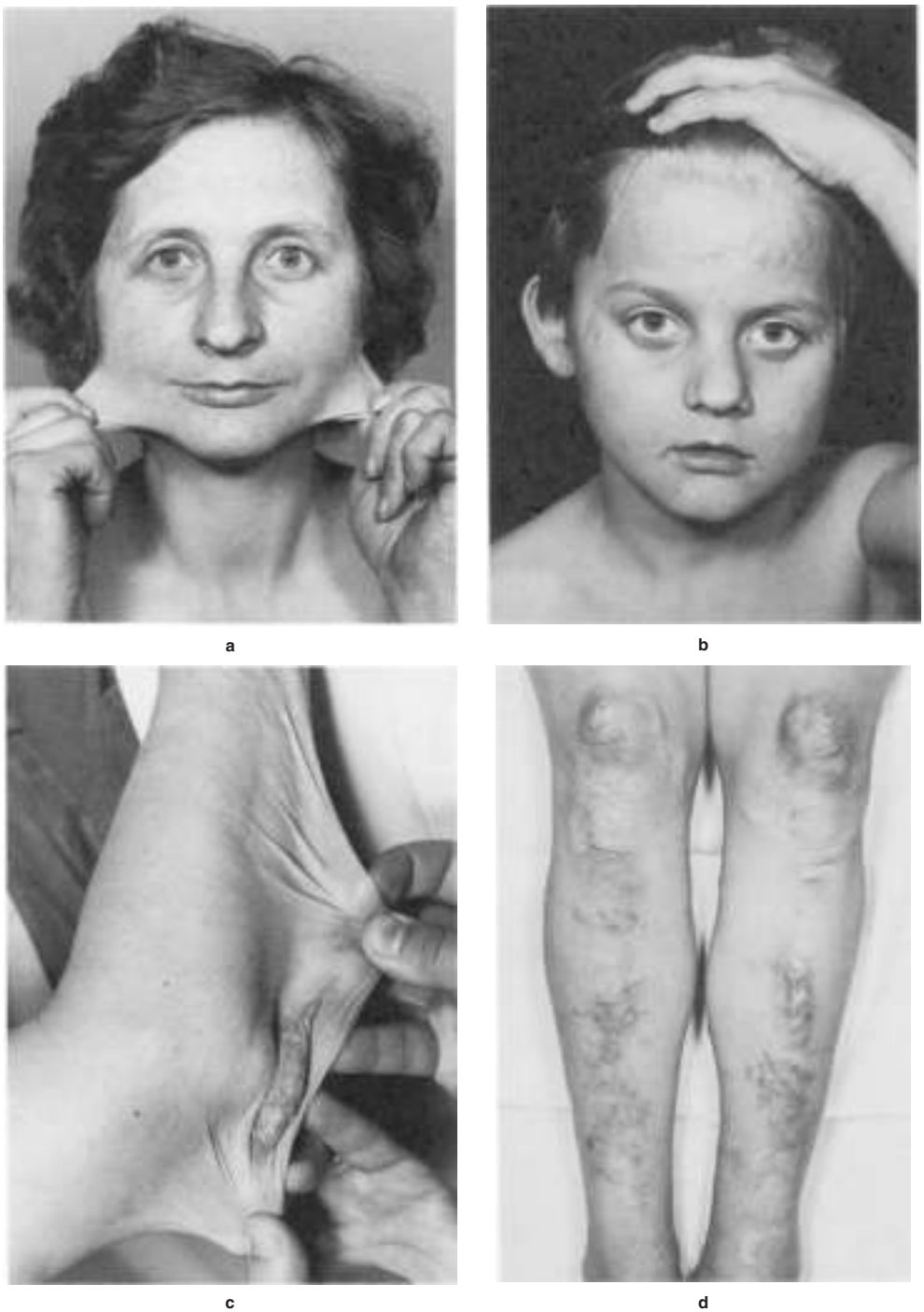

Figure 4. Classical type of EDS (EDS I). A.V. (4 Jul 1933) and her son U.V. (29 Dec 1964) are two of four affected members of a three-generation Swiss pedigree who were nicknamed ''those with the worn-out skin.'' Ultrastructural analysis of the skin of U.V. and A.V. has been reported (patients 1 and 2 in Vogel et al. [40]). (**a**) Hyperextensible skin, moderate scar formation on the forehead, and discrete telecanthus in A.V. (43 years). (**b**) Extensive atrophic broad scars on the forehead and over the nose and cheekbones, which are remnants from early childhood (U.V., 12 years). (**c**) Hyperelasticity and loose folds of skin resembling a dewlap, and a soft, fleshy molluscoid pseudotumor over the elbow (A.V., 43 years). (**d**) Atrophic and hypertrophic hemosiderotic scars in the doughy-feeling skin over the knees and shins (A.V., 43 years).

As a schoolgirl, A.V. had recurrent posterior luxations of the left tibia, the last time being at the age of 16 years. Deliveries of her first two, unaffected children were 2 and 7 weeks before term, unexpectedly and rapidly on the couch at home. Her third child, U.V., and her fourth, unaffected child were born at term. She has had no striae gravidarum. At age 42 years, she had a hysterectomy because of prolapse of the uterus with urinary incontinence; postoperative wound healing was normal.

Follow-up at age 58 showed that this intelligent and witty person had had few further complications; she commented that even her ''loose mouth'' was firmer than her connective tissue.

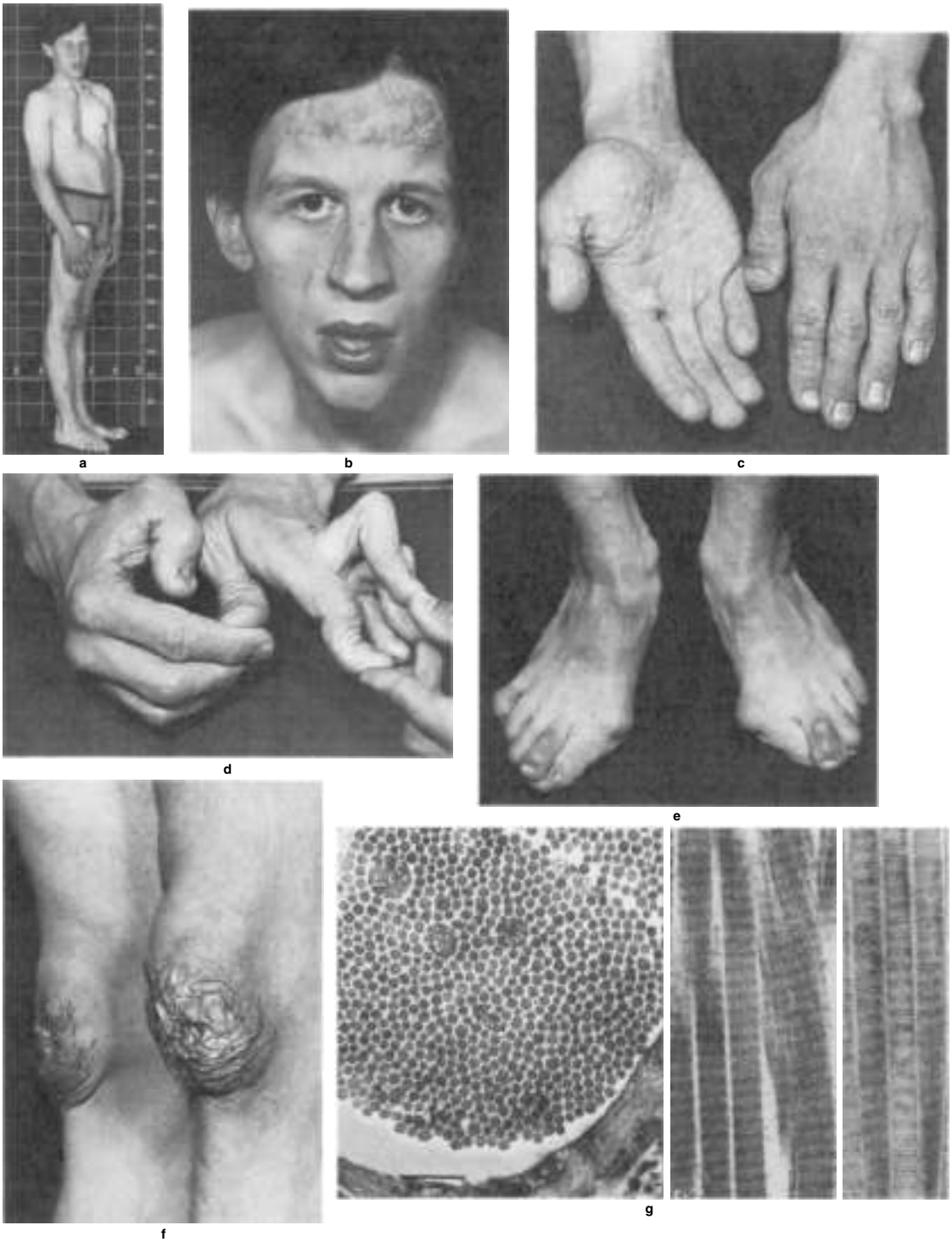

Figure 5. Classical type of EDS (EDS I). B.S. (5 May 1955), at the time of the photographs a 21-year-old man, mentally retarded and institutionalized; nothing is known about his perinatal history; his father is also affected. A heterozygous mutation in *COL5A1* (IVS 28 + 1G > A) predicts a premature stop codon in the mRNA after the insertion of 178 bp of intronic sequence (see ''EDS43'' in [41]). (**a**) Marked pectus excavatum and moderate kyphoscoliosis. (**b**) Atrophic and hypertrophic scars on the forehead. (**c**) The skin over the hands is abnormally wrinkled and redundant, resembling a pair of oversized gloves. (**d**) Hyperlaxity of the small joints of the fingers. (**e**) Dynamic flat foot, hallux deformity, and formation of pseudotumors over pressure points. (**f**) Large, papyraceous, and hemosiderotic scars over both knees. (**g**) Electron microscopic pictures of the dermis of B.S., showing large composite fibrils with an irregular contour and an abnormally wide range of diameters of the round collagen fibrils in cross section (left), and a spiral-like appearance of the composite fibrils in longitudinal section (middle); for comparison, a site-matched skin from an age and sex-matched control is included (right). (Case 4 in Vogel et al. [40] copyright by U.S.-Canadian Academy of Pathology Inc., reprinted with permission; a–f reprinted with permission from Steinmann [42].)

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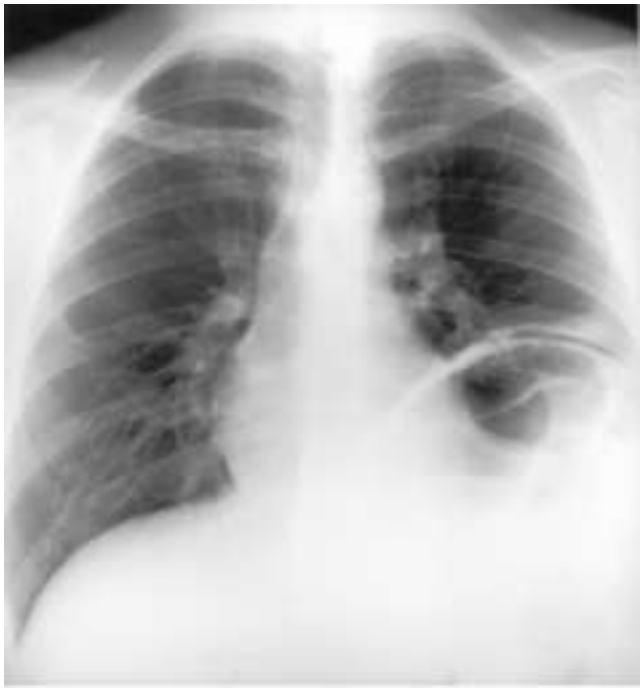

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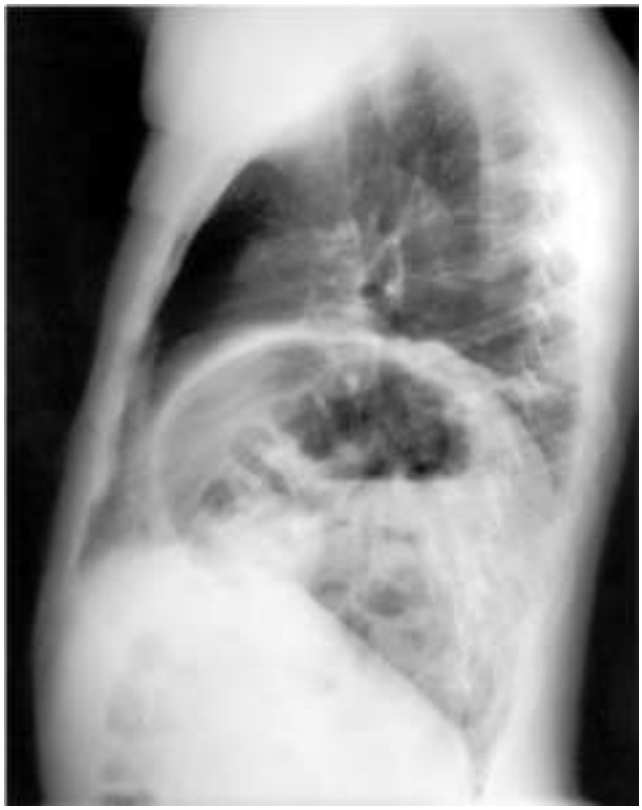

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Figure 6. Classical type of EDS (EDS II). (**a**) A three-generation family with EDS II. The grandmother is 55 years old, the father (E.C., born 29 Jan 1959) 32, the girl 9 (to the left), and the boy 6. Note the multiple scars on the forehead and the horizontal and vertical creases on the grandmother's. (**b**) Anteroposterior and lateral (**c**) radiographic views of the thorax of E.C. demonstrate an elevated diaphragm (relaxatio diaphragmae) on the left side. After a trivial accident with his motorcycle at age 35, E.C. complained of chest pain, and upon the radiological finding of an elevated diaphragm, rupture of the diaphragm with eventration of the stomach or paralysis of the phrenic nerve were considered. A diagnostic thoracotomy revealed relaxatio diaphragmae due to weak tendinous structures. One year later, he rapidly became anemic and had profuse melena due to a Dieulafoy ulcer, which necessitated ligation of the enlarged artery below the muscularis mucosae.

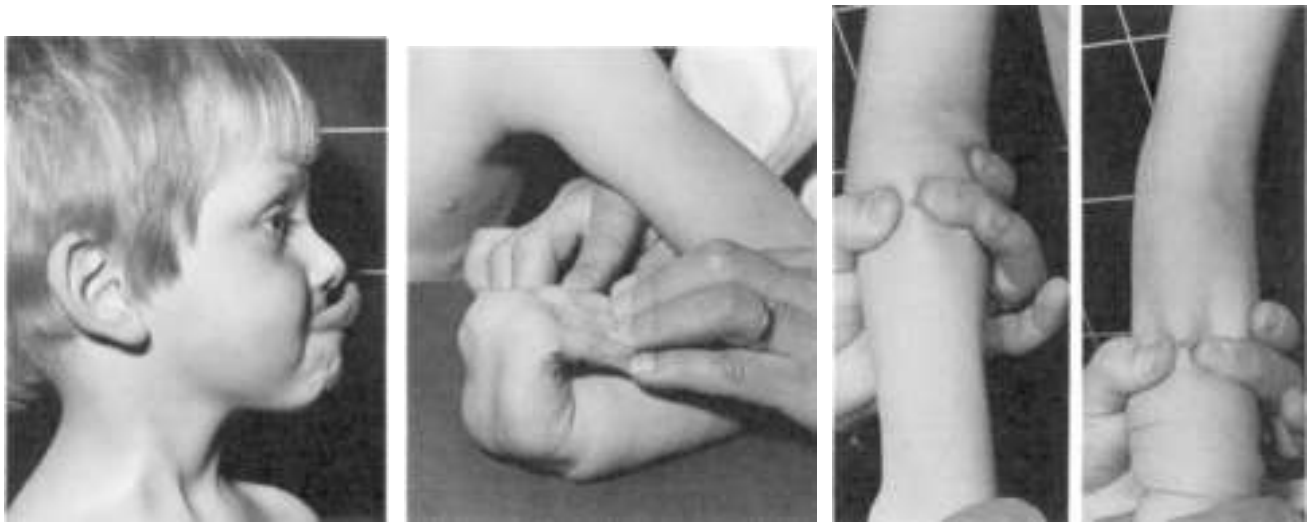


Figure 7. Classical type of EDS (EDS II). (**a**) Positive Gorlin sign (i.e., the ability of a patient to extend the tongue to the tip of the nose). (**b**) Hyperextensibility of the fingers and hands. (**c**) The skin can be displaced up (**left**) and down (**right**) over the subcutaneous fat of the forearm to a great degree. F.K. (20 Jan 1975) was hospitalized at age 2 years and 4 months because of suspected child abuse presenting with multiple hematoma and hemarthros. The diagnosis of EDS was only made at the age of 5 years (Figs. a–c). He is a sporadic case.

Because of recurrent luxations of the right shoulder while exercising, and especially during sleep, and the right patella, he was operated on successfully at the ages of 10 and 12 years, respectively. He luxated his left knee numerous times between the ages of 10 and 12 years but has not done so since. His skin feels doughy but is not excessively fragile.

Suspected child abuse, late diagnosis in a sporadic case, and the difficulty of classifying a patient into a defined group are characteristic of the situation with many individuals with EDS. ((**a**) reprinted with permission from Steinmann [41]).

variety of secondary lesions that develop with time in areas exposed to frequent microtrauma and pressure. Molluscoid pseudotumors are fleshy, heaped-up lesions associated with scars over pressure points such as the elbows (Figs. 4c, 25c) and knees, which usually appear after entering kindergarten (they were already described as features by Tschernogubow in 1891 [13] and Danlos in 1908 [18]). Redundant knuckle pads may develop over deformed and hyperflexed toes. These probably develop through frequent microtrauma, bleeding, and fibrotic scarring and may spontaneously rupture and discharge necrotic material. Spheroids (or spherulae) are small, cyst-like, hard shot-like nodules, freely movable in the subcutis over the bony prominences of the legs and arms. They occur in about one-third of patients [51], are numerous, and feel like hard grains of rice or small pieces of shot. On x-rays, they present an outer calcified layer with a translucent core. In contrast, phleboliths, although presenting a similar appearance, usually are not superficial and are rarely as numerous as spheroids, while calcified cisticerci taper in shape and are found in muscles rather than subcutaneous tissues [51,52]. The spheroids represent subcutaneous fat lobules that have lost their blood supply and then become fibrosed and calcified [53]. Alternatively, they may present as "subcutaneous mobile encapsulated lipoma" with or without tenderness [54].

Other dermatological features. These comprise hyperkeratosis follicularis; piezogenic papules, which are small, painful, reversible herniations of underlying adipose tissue lobules through the fascia into the dermis, such as on the medial and lateral aspects of the feet upon standing (Fig. 8) [55]; elastosis perforans serpiginosa (Miescher elastoma, [56]; MIM 130100) (see EDS IV), not to be confused with mycosis; acrocyanosis and chilblains; and a conspicuous lack of striae gravidarum, which are focal tears in the dermis perpendicular to the direction of stress without disruption of the overlying epidermis.

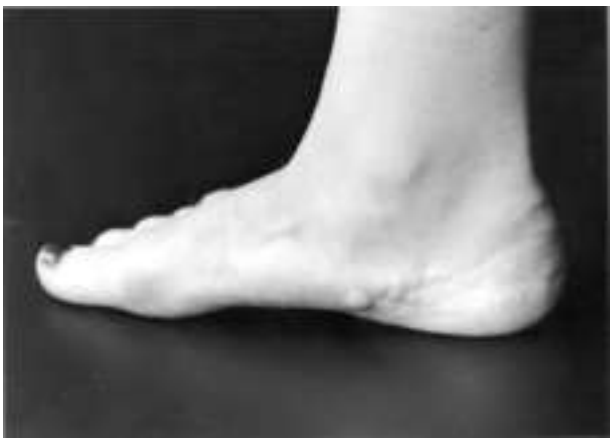


Figure 8. Classical type of EDS (EDS II). Piezogenic papules in a 20-year-old woman, M.M. (21 May 1973). These are small herniations of adipose tissue lobules through the fascia on the medial or lateral aspect of the foot upon standing, which may be painful and require support or special shoes.

Orthopedic Aspects

Significant orthopedic abnormalities are, in decreasing order of frequency: pes planus, joint dislocations, spinal deformity, joint effusions, thoracic cage deformity, osteoarthritis, talipes, and congenital hip dislocation [57].

Hyperextensibility or hyperlaxity of the joints. This is also frequently called "hypermobility" of the joints and is a result of laxity of the joint capsules, ligaments, tendons, and possibly muscular hypotonia. It may be a source of enjoyment to patients to show their double-jointedness, and in the past certain individuals amused people as contortionists in sideshows (for anecdotes, see [1,2]). On the other hand, it may cause the patient considerable discomfort in walking, writing, or performing other skilled functions. Questions such as "Are you double-jointed?" and "Can you do tricks with your fingers?" may elicit a remarkable display of maneuvers from a patient with EDS, such as finger contortions, placing the ankles behind the neck, or placing the head between the knees while bending backward. As a rule, joint laxity is generalized, affecting both large and small joints (with the exception of EDS IV), and is usually noted when the child starts to walk. It also depends on age, sex, and race, and on the dominant side of the body, especially with regard to the hands, and increases during pregnancy and decreases with the passage of time [58]. Several grading systems for the objective semiquantification of joint hypermobility using a simple score have been proposed [39,58–60], among which the "Beighton score" [58] has become the most widely accepted screen for detecting generalized hypermobility and correlates well with more quantitative, instrument-dependent methods (Fig. 9).

Ligamentous laxity may be observed by pulling on the distal phalanx of a finger, which results in a considerable lengthening of the finger ("telescoping"), with radiological widening of the joint spaces [61]. Dynamic swan neck deformity is occasionally observed.

Dislocations. Occasional or habitual dislocation of the shoulder, patella, digits and thumbs, hip, radii, and clavicles is common [62], and its frequency is proportional to the degree of joint laxity, although in some persons a surprising range of joint movement can occur without any problem. Dislocations are usually resolved spontaneously and are easily reduced by the patient. Dislocation of the shoulder may follow a simple everyday movement, such as putting on a coat or raising an arm during a school class. Dislocation of the interphalangeal joints of the thumb leads to difficulty in using a pinch grip (Fig. 20).

Joint effusions. These are common and may be persistent or recurrent. They are associated with activity, affect mainly joints of weight-bearing parts (in decreasing order: knees,

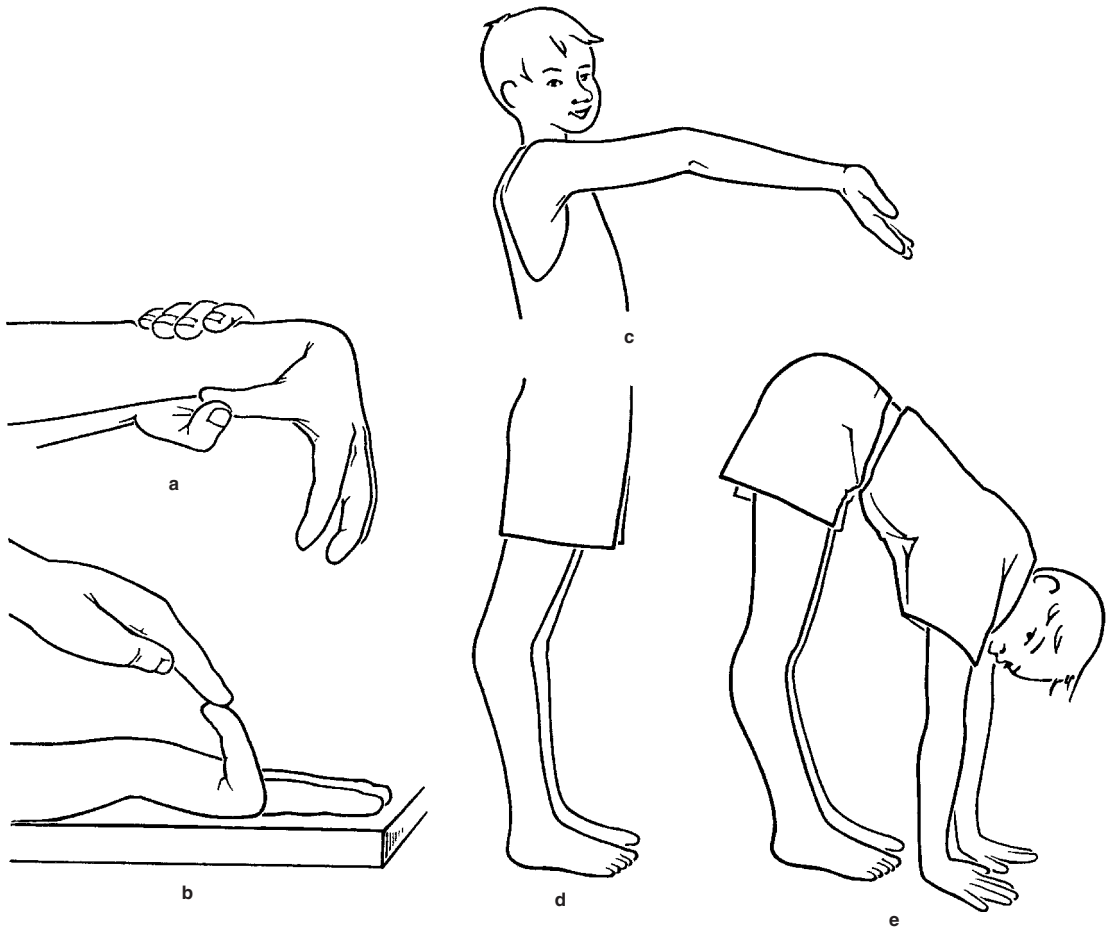


Figure 9. The "Beighton-score" to assess joint hypermobility. (**a**) Passive apposition of the thumb to the flexor aspect of the forearm; one point for each hand. (**b**) Passive dorsiflexion of the little finger beyond 90°; one point for each hand. (**c**) Hyperextension of the elbow beyond 10°; one point for each elbow. (**d**) Hyperextension of the knees beyond 10°; one point for each knee. (**e**) Forward flexion of the trunk with the knees fully extended so that the palms of the hand rest flat on the floor; one point.

A score of 5/9 or greater defines joint hypermobility [58]. (Figure drawn by Susanne Staubli, University Children's Hospital, Zürich.)

ankles, elbows, and also digits), and commonly appear at the end of the day. The tendency for joint effusions to develop is again proportional to the degree of joint laxity. Hemarthrosis may occur, mainly in EDS IV.

Joint instability. Joint instability results from lax ligaments and poor muscle tone. Children tend to walk late and fall easily because of the difficulty in controlling their unstable limbs. Unstable ankles and knees frequently need supportive bandages. The gait is typical: the feet are placed firmly and flatly on the ground, and the hips are hyperextended during weight-bearing to counteract the genu recurvatum. Running or wearing high heels may be impossible. The handshake may be characteristic and sometimes allows a diagnosis at the first encounter; the musculoskeletal structure of the hands seems to collapse on pressure, and the hand feels "like a bag of little bones." Trapeziometacarpal abnormalities lead to instability of thumbs and fingers and hence difficulty in picking up large or heavy objects with one hand or unscrewing bottle tops [63]. One of our patients never wanted to wash dishes for friends because she often crashed the plates — a not unprecedented excuse also for normal individuals — and, like this, other aspects of clumsiness in EDS may be explained, at least in part, by disturbed proprioception. Spinal deformity is usually due to strains and confined to thoracolumbar kyphoscoliosis, which progresses with age (Fig. 29e). Chest deformity such as pectus excavatum or carinatum does occur (Fig. 5a). Spondylolisthesis is not uncommon (see legend to Fig. 11). Foot deformities are the most prevalent signs: congenital clubfoot due to intrauterine malposition, progressive static and dynamic pes planus, talipes equinovarus, and hallux valgus (Figs. 5e, 23d, 25 g, 29c, 30d).

Osteoarthritis. Osteoarthritis as a consequence of joint instability is a major problem. In one study, 20 out of 100 patients were affected, or as many as 16 out of 22 patients over 40 years of age [57], the main complaints being the knees, hands, ankles, general joints, and shoulder. Here we might cite Beighton's patient, who said "When I was a baby they called me a floppy infant, and now the doctors tell me that I am a loose woman" [62], and might add that she may soon be a stiff elderly lady due to osteoarthritis.

Bursae. Over the olecranon and prepatellar region, enlarged bursae are often encountered, and these have to be distinguished from hematoma or molluscoid pseudotumors, which occur at the same sites. The excision of these bursae usually yields reasonably satisfactory results [1].

Bones. Bone involvement was not considered a feature of EDS until it was recognized that osteopenia is a constant feature of EDS VI, VIIA, VIIB, and VIIC, and a propensity to fractures has been reported in the latter group (see in EDS VII). In a case-control design study of 23 EDS individuals (10 with classical EDS and 13 with EDS III), Dolan et al. [64] showed that EDS subjects have a previously unrecognized tendency to fracture characterized by a low bone mass and abnormal bone structure, and concluded that this finding is likely to be multifactorial, with an inherited structural element accentuated by reduced mobility and exercise, and the possibility of a proprioceptive defect of the hypermobile joints. Carbone et al. [65] come to the conclusion that their 23 cases with EDS III had a significantly decreased bone mineral density at the femoral neck compared with controls but that this difference disappeared after adjustment for body height, weight, and physical activity levels.

Chronic joint and limb pain. This is a common complaint, but skeletal radiographs are normal. Frequently, it is difficult to establish the precise anatomical localization of the pain [39]. Osteoarthritis is more pronounced and pain is worse in the nondominant hand. Pain is worse during pregnancy because there is normally an increase in joint mobility in this state. Pain, swelling, dislocation of joints, a history of surgery of the joints, and impaired ambulation seem to be more frequent and more pronounced in patients with EDS III than in those with EDS I, II, or IV (see below, the hypermobile type of EDS [66]).

Dural ectasia. Enlargement of the spinal canal, with or without clinical complaints such as weakness in the lower extremities and abnormal gait, has occasionally been reported [67,68]. Dural ectasia and spinal canal widening seem to be more prevalent and pronounced in Marfan syndrome than in EDS, and their extents were found in two small series of ill-defined patients to be independent of age, sex, or symptoms, such as low back pain or sciatica [69]. Dural ectasia seems to be a major diagnostic criterion in Marfan syndrome [70].

Pregnancy, Obstetric, and Gynecological Features

The pregnancy of a woman with EDS bears risks for the newborn as well as the woman. During pregnancy, joint laxity may increase, but women enjoy the absence of striae gravidarum. Varicose veins in the legs and varicosity of the vulva may become prominent. Cervical insufficiency may lead to miscarriage or premature birth. Gross abdominal hernia and spinal deformity with backache may develop.

Fetal complications include prenatal rupture of the membranes (if the fetus is affected) and prematurity. Out of 18 children with nonspecified EDS, 14 were premature, and 13 of these had premature rupture of the membranes; in contrast, normal siblings were never premature, demonstrating that the fetal membranes are affected to the same extent as the fetus [71]. Breech presentation is more frequent if the baby is affected, and therefore hypotonic, and may lead to dislocation of the hips or to arms with injury of the plexus brachialis (Erb palsy).

Delivery may be unusually smooth or even precipitous (see legend to Fig. 4); however, severe pre- or post-partum hemorrhage may occur through perineal tears, the extension of episiotomic incisions, tearing by forceps, or prolapse of the uterus and bladder, all of which occur mainly in EDS I and IV. Rupture of the uterus and large arteries is a typical complication of EDS IV. The pubic symphysis may distract and cause pain for several months. Dehiscence of sutured incisions of skin, mucosa, or the uterine wall is frequent in EDS.

Dyspareunia and sexual dysfunction are occasional complaints in the classical and other types of EDS [72,73] and result from tissue fragility leading to small tears and recurrent infection in the vaginal mucosa and the skin of the external genitalia. Coital vulval laceration has been described occasionally, is possibly underreported for various reasons, and may be erroneously considered to be due to rape [74].

Gastrointestinal Complications

Gastrointestinal manifestations in the EDS can be subdivided into those that are a consequence of tissue extensibility and those that result from tissue fragility (see EDS IV). They include gastric, duodenal, and colonic diverticula, visceroptosis, gastric atony, megaesophagus, and megacolon. Constipation is a common complaint and probably results from flaccidity of the large bowel. Gastroesophageal reflux and irritable bowel syndrome are common complications of classical and hypermobile

EDS [75]. Inguinal and umbilical herniae are frequent and may recur after surgical correction. Femoral, incisional, hiatal, and diaphragmatic herniae, relaxation of the diaphragm (Fig. 6b), and even its eventration have been reported. Gastric, duodenal, jejunal, and colonic diverticula may lead to bleeding or perforation [76–78]. Recurrent rectal prolapse usually resolves before the age of 4–5 years ([79]; own observations).

Neuromuscular and Psychological Features

Primary muscular hypotonia in the newborn, and especially the premature, is frequent and may be so severe, especially in EDS VI, that affected infants cannot be breast-fed and need teats with especially large holes. Neuromuscular disease is often suspected, especially when clubfoot or hip dislocation coexist. A complete neuromuscular workup is usually performed in such "floppy infants," with unrevealing results. Hypotonia is also considered responsible for the increased frequency of breech presentation, which, together with the laxity of ligaments and joints, favors Erb palsy and congenital hip dislocation. In children, joint hypermobility and hypotonia may cause delayed motor development, problems with ambulation (delayed walking, frequent stumbling), and mild motor disturbance ("clumsiness"). Consequently, hypotonia may be maintained through the poor development of muscles resulting from an avoidance of exercise and activity because of the laxity and instability of the joints.

Fatigue is a frequent complaint. Muscle cramp in the calves, especially at night, is common in children but gradually disappears. Histology reveals that the connective tissue in the muscles is very sparse, so that the muscle bundles are hardly held together [1]; it may be speculated that the muscle fibers might lose their parallel orientation when they contract, thus decreasing mechanical efficiency. That dysfunction of the Golgi corpuscles embedded in the loose tendons may play a role in determining hypotonia is another possibility. Impaired proprioception has been documented in the hypermobility syndrome (MIM 147900), but its underlying mechanism remains open [80].

Genuine intelligence is normal; however, prematurity predisposes to birth trauma and complications, such as intracranial hemorrhage, respiratory distress syndrome, or sepsis, which may lead to epilepsy, as observed also in the patient described by Tschernogubow in 1891 [13]; epilepsy, however, has occasionally been reported in EDS [81]. The social consequences of disfiguring scarring, particularly of the face, and musculo-skeletal deformity may lead to personality problems and psychosocial distress, which is also common in chronic illnesses in general [73]. As a rule, there are many more affected women than men in EDS support groups (as is the case for other support groups as well). This may reflect a difference between the sexes in coping with the handicap rather than any true difference in clinical severity; however, this fact is important for the critical evaluation of studies based on circular letters, which may be skewed by selection bias [66,82]. Living with fear, living with pain, feeling stigmatized, and experiences of nonaffirmation in health care are identified as conditions leading to limited self-actualization in all areas of daily life [83].

Nerve compression and traction of the brachial and lumbar plexuses through hematoma and luxations at birth or later have occasionally been reported ([1,84–87]; H.A. with EDS VI, case 19 in legend to Figs. 22 and 23c). Occasionally, patients complain of cutaneous hyperesthesia/hyperalgesia when friction is applied to the skin, such as while drying with a towel or during the measurement of blood pressure [88]. Some patients report insufficient analgesia during minor surgery (e.g., dental and oral surgery) and may be characterized as hysterics [89,90]; it has been shown that the lack of effectiveness of local anaesthetic solutions is not due to their rapid dispersal through the loose connective tissues and therefore is unlikely to be compensated for by simply increasing the amount used [91]. An ill-defined polyneuropathy in two siblings of consanguineous parents together with typical signs of EDS VI has been described [92].

By far the most severe neurological complications are those due to intracerebral hemorrhages, such as transient aphasia or amaurosis, and hemiplegia or fatal stroke-like events caused by multiple arterial aneurysms or arteriovenous fistulae [93,94], which are typical findings of EDS IV.

Cardiovascular Features

Structural cardiac malformations are rare and are probably rather chance associations (for a review, see [95]). Mitral valve prolapse and, less frequently, tricuspid valve prolapse are due to redundant chordae tendineae and valve cusps [96]. However, stringent criteria should be used for the diagnosis of mitral valve prolapse [97]. Mitral valve prolapse and proximal aortic dilatation should be diagnosed by echocardiography, computed tomography, or magnetic resonance imaging. Mitral valve prolapse is a common manifestation, and aortic root dilatation may not be as uncommon as previously thought [98], although Dolan et al. [99] reported that none of their 12 patients with EDS I and II or 18 patients with EDS III had mean aortic diameters outside the normal range. In a small proportion of patients with EDS, aortic dilatation may be progressive [96]. Dilatation of the aortic root should be diagnosed when the maximum diameter at the sinus of Valsalva exceeds the upper normal limits for age and body size [100,101]; in such a case, annuloaortic ectasia needs to be considered in the differential diagnosis. Tortuosity of the aorta and its major thoracic divisions, including the coronary arteries, and peripheral pulmonary stenoses [102], aneurysm of the sinus Valsalvae [103,104], aortic root dilatation, and dissection of the aorta, sometimes familial, have been observed ([2c,105,106], own observation). Spontaneous rupture of large arteries [31,107,108] and intracranial aneurysms and arteriovenous fistulae [93,94] are all typical of EDS IV, and to a lesser degree of EDS VI, but may also occur in EDS I, sometimes even in persons with minimal external findings [109].

Acrocyanosis is a common complaint. The association of EDS II and III with orthostatic intolerance and chronic fatigue syndrome has been reported and tentatively explained by the connective tissue abnormality leading to excessive venous pooling and an exaggerated hemodynamic response [110].

Ophthalmological Aspects

It is not surprising that such a complex and delicate organ as the eye should be affected in many different ways, as tabulated by Pemberton et al. [111]. Extraocular signs are not uncommon. In a series of 100 affected patients, 27 had epicanthic folds (which lessen or disappear with age, or change to telecanthus, giving the impression of widely spaced eyes), seven had blueness of the sclerae, seven had strabismus (probably due to laxity of the tendons of the extrinsic muscles of the eyes), and eight had myopia, while redundant skin on the upper eyelids (blepharodermatochalasis, not to be confused with acquired or inherited blepharochalasis, in

which the whole eyelid is lax [112]; MIM 109900, MIM 110000), which makes putting on mascara difficult, and ease of eversion of the upper lid (Méténier's sign) were frequently encountered; none of the patients in this series, however, had any serious ophthalmological lesion [113]. Ocular signs are not frequent and include keratoconus (forward bulging and thinning of the central part of the cornea, which is a rare finding [114]; however, see also [115]); megalocornea; microcornea, which occurs mainly in EDS VI but occasionally also in EDS I (see [116]); myopia, due to distortion of the eye-globe or, very rarely, to subluxation of the lens; brittleness of the sclera and cornea; angioid streaks; retinal detachment; and retinitis proliferans due to hemorrhage. Eye fatigue may be a result of inappropriate eye movements because of increased concentration, tension, general fatigue, immobilization, or pain, such as a stiff neck. It is of interest that in the majority of individuals with sight-threatening lesions, autosomal recessive inheritance is likely (see EDS VI). Pulsating exophthalmos in EDS IV is due to retrobulbar arteriovenous fistula. It is of note that vision is not impaired by any corneal deficiency in patients with the classical type of EDS resulting from mutations in the collagen V genes. Since abnormal cauliflower-like collagen fibrils are observed in dermis from these patients (see classical type of EDS below) and in dermis and cornea of pN-knockout mice (see "Animal Models and Lathyrism" below), it is remarkable that the cornea, an organ rich in collagen V, seems to be normally translucent in these patients (see also Chapter 1, Part V, this volume).

Additional Features

Hemoptysis, hematothorax, and spontaneous and recurrent (hemato-)pneumothoraces, with or without mediastinal and subcutaneous emphysema, are typical of EDS IV [117]. Tracheobronchiomegaly (MIM 275300) has been reported [118,119].

Anatomical abnormalities of the urinary tract, such as urinary bladder diverticula, are common and usually asymptomatic. Cuckow et al. [120] reviewed 24 cases of bladder diverticula, all observed in males aged between $1\frac{1}{2}$ and 49 years, with 80% presenting before 16 years (for a further review, see [121]). Vesico-ureteral reflux may lead to recurrent urinary tract infection and renal insufficiency [116]. Multiple and large bladder diverticula are especially typical of the cutis laxa syndromes and the Menkes/occipital horn syndrome (EDS IX) (Chapters 10 and 14, respectively, this volume).

Oral aspects of the EDS have been described by Barabas and Barabas [122] (see also "Recent Developments"). The oral mucosa is fragile, easily bruisable, and often presents hemorrhagic blisters; bleeding from the gums is a common complaint. The gingival tissues are more liable to injury, leading to periodontal disease with early loss of teeth. The teeth are often crowded but are otherwise normal [1]; hypoplastic areas of enamel, the formation of pathologic dentin, and teeth with high cusps and deep occlusal fissures, stunted and deformed roots, and large pulp stones have been reported [8,123]. These manifestations, however, are not clearly associated with a particular type of EDS. Histological abnormalities include changes in the amelo-dentinal and cemento-dentinal junctional areas, irregular and ill-formed secondary dentinal tubules, vascular dentinal inclusions, and fibrinoid gingival deposits. Chronic habitual luxation and arthrosis of the temporo-mandibular joint may occur but rarely needs condylectomy [124]. The Gorlin sign (i.e., the ability of the patient to extend the tongue to the tip of the nose; Fig. 7a) is rather more astonishing or amusing than specific, 50% of EDS patients having this ability compared with 10% of controls [8]. A high prevalence of speech and swallowing difficulties has been reported [125]. In some individuals, inappropriate use of the voice, such as by shouting, may cause recurrent aphonia for which systematic voice training may be needed.

Radiological aspects of EDS have been reviewed (see above and [51,61,126,127]) and include telescoping fingers, calcified spheroids, and spondylolisthesis. Osteoporosis is a prominent feature of EDS VI and to a lesser degree also of EDS VII; exostoses may be observed in occipital horn syndrome (EDS IX) and EDS VII, acroosteolysis in EDS IV, and Wormian bones in EDS VII (see "EDS VII").

The fragility of the skin and most organs (except the skeletal system) may come as a bad surprise for the unaware surgeon at operation and may be remarked upon by the pathologist during subsequent autopsy. The friability of organs has frequently been described as being like "wet blotting paper" and making surgical incisions as "like cutting with a knife through butter" or through "cold porridge."

Diagnostic Approach to the Patient

As with most other genetic disorders, the first step in making the diagnosis of EDS is clinical awareness. The possibility of EDS will be brought to mind by complaints and physical findings such as hyperextensible joints, hyperelastic skin, and abnormal wound healing, or by personal history such as easy bruisability, habitual luxations, joint effusions, and recurrent herniae. The next step then consists of looking for elements that can reinforce the diagnostic suspicion, positive family history, and a more detailed personal history with regard to possible connective tissue disease such as prematurity, breech presentation, hypotonia, and delayed motor development. The importance of comprehensive anamnestic and clinical assessment of individuals cannot be overemphasized.

If the suspicion of a connective tissue disorder can be substantiated, an attempt should be made to understand whether it is a "genuine" connective tissue disorder such as the EDS or whether it is part of a "broader" genetic disorder or syndrome. Although exceptions exist, the presence of significant mental retardation and/or overt dysmorphic features argues against EDS. If clinical and anamnestic evidence points to an isolated connective tissue disorder such as EDS, consultation of textbooks, experts, and synoptic tables (see Table 1) may help in establishing which EDS type is more probable. This is crucial for further diagnostic investigations, such as skin ultrastructure and/or fibroblast culture and/or urinary cross-link products.

The clinical assessment of individuals with hyperextensible and thin skin and/or with lax joints needs some clinical experience, not so much in the case of patients with marked findings but rather in those with moderate findings; clinical experience is required especially in the latter group because the findings are graded according to age, anatomical location, sex, and race, as they are in normal individuals. Skin hyperelasticity [44,45] and thickness, and joint hyperextensibility [59,60,62], can be assessed semiquantitatively with scoring systems or with physical devices (see also Fig. 9). Unless diagnostic findings such as congenital bilateral hip dislocation and generalized joint laxity are present (EDS VII), the diagnosis of EDS is difficult in neonates and infants because the adipose tissue is normally abundant in healthy subjects and masks

hyperelasticity of the skin, because muscular hypotonia is frequent and unspecific, and because bruising and splitting of the skin do not occur until the child begins to walk and fall. In the adult individual, the diagnosis of EDS is usually made easily and can sometimes be made instantly — the characteristic handshake upon the first encounter in the office or at a cocktail party may be diagnostic; the velvety touch of the dorsal skin on a darkened dance floor may immediately enlighten the clinical geneticist.

Since all the signs are graded and age-, sex-, and race-related, semiquantitative assessments of the extensibility of the skin [44,45] and joints [59,60,62] must be compared by the same observer with the appropriate controls, and because these parameters also vary with anatomical location, identical sites should be chosen for better comparison, such as the skin over the fourth metacarpal, the thenar, the dorsum of the wrist, the neck, and so forth.

The diagnosis of EDS is difficult in newborns or infants because the laxity of the joints may be indistinguishable from that of infants with unrelated muscular hypotonia, hyperelasticity of the skin may be masked by the abundant subcutaneous tissue, and other skin changes are not easily assessed because the bruising and skin-splitting tendency do not usually become apparent until the child begins to walk and fall. In older persons, the skin is more redundant and somewhat lax, but atrophic, hemosiderotic scars easily distinguish the condition from cutis laxa.

Classification, Nomenclature, and Relative Frequency

The classification of EDS is first made on clinical grounds and substantiated by consideration of the most likely inheritance pattern and by biochemical and molecular analysis when possible. This is reflected in the historical development of EDS classification, which comprised three types in 1967 [31], five types in 1968 [32], and seven types in 1972 [2]. The formerly most generally used classification, which comprises ten or more types, is based on a combination of clinical, genetic, and biochemical criteria (Table 1) [37,38].

Over time, it became apparent that the diagnostic criteria established and published in 1988 [38] do not adequately discriminate between the different types of EDS or between EDS and other phenotypically related conditions. In addition, elucidation of the molecular basis of several types of EDS has added a new dimension to the characterization of this group of disorders. A revision of the classification of EDS, the "Villefranche Nosology, 1997," has thus been proposed, based primarily on the cause of each type [39][5]. Major and minor diagnostic criteria are defined for each type and complemented wherever possible with laboratory findings. This simplified classification will aid in accurate diagnosis of the types of EDS, thereby facilitating development and improvement in the following aspects: (1) diagnostic uniformity for clinical and research purposes, (2) natural history, (3) management, (4) genetic counseling, and (5) identification of potential areas of research [39].

In this revised Villefranche nosology [39], a simplified classification of EDS into six major types is proposed (Table 1). The guiding principle in formulating the classification was its usefulness to the "generalist." For each type, major and minor diagnostic criteria are defined. A major criterion has high diagnostic specificity because it is infrequent in other conditions and in the general population. The presence of one or more major criteria is either necessary for clinical diagnosis or highly indicative, warranting laboratory confirmation whenever possible. A minor criterion is a sign of lesser diagnostic specificity; the presence of one or more minor criteria contributes to the diagnosis of a specific type of EDS. However, in the absence of major criteria, minor criteria are not sufficient in themselves to establish the diagnosis. Their presence alone might be more suggestive of other EDS-like conditions, the nature of which will be elucidated when their molecular basis becomes known [39].

In this chapter, both nosologies are used interchangeably — the traditional one, using Roman numerals I to X, as well as the newly designated types — for reasons of convenience and completeness.

In many cases (20–40% as estimated by Hollister [128]), however, an unambiguous classification is not possible because there is a phenotypic continuum and, especially in children, the appearance of recognizable symptoms is age-dependent.

The relative frequencies of the different EDS types are not known precisely. In 1966, during a survey in southern England, Beighton examined a unique series of 100 patients with EDS. Seventy-one of them were members of 50 separate families, while 29 (17 males and 12 females) were sporadic cases [1]. A follow-up of eight of the 17 sporadic males revealed that three had produced children with EDS I, two had normal offspring, and three had no children [129]. Among the 50 families, EDS I had occurred in 22, EDS II in 17, EDS III in six, EDS IV in three, and EDS V in two. Although these figures are quite realistic in genetic terms, they are prone to inherent ascertainment bias, the more severely affected patients being most likely to have been diagnosed and hence reported in the literature, whereas patients with milder symptoms (EDS II) are more likely to have been able to escape medical attention and malpractice; on the other hand, severe cases leading to early death (EDS IV) are frequently missed, especially when sporadic. Individuals at both ends of the spectrum of severity may therefore be underrepresented.

Differences between individuals with the same type of EDS may reflect inter- or intra-familial variability or genetic heterogeneity (see EDS I, II, IV, VI, VII, IX). The definition of a genotype may then help to describe better the phenotypic spectrum. The Villefranche nosology will doubtless have to be revised and extended in the future as distinct, specific clinical signs and molecular defects are recognized and as genotype-phenotype correlations become clearer.

Prevalence and Epidemiology

There are no well-founded figures for the prevalence of EDS. While Beighton [1] gives a figure of 1:156,000 for southern England, McKusick states that EDS is one of the more frequent heritable disorders of connective tissue [2]. It seems likely that only a fraction of patients are ever diagnosed, owing to the lack of symptoms alarming to both patient and doctor and to the fact that single symptoms, such as habitual luxation of the patella, easy bruisability, or recurrent inguinal hernia, may not be considered as part of one generalized disorder. With increased medical awareness, however, the presumed rarity seems likely to disappear. At the other end of the spectrum, Holzberg et al. [45] claim a frequency of 9% in a predominantly black population. The aggregate frequency of EDS may be about 1:5,000 births,

[5]In this chapter, we designate the different types of EDS with adjectives throughout rather than a mixture of nouns and adjectives as given in [39].

with no racial or ethnic predisposition. The syndrome has been observed all over the world [1,2].

THE CLASSICAL TYPE OF EDS — EDS TYPE I (MIM 130000) AND EDS TYPE II (MIM 130010)

Diagnostic Criteria

These two types of EDS are described together because they differ only in their degree of involvement and occasionally are allelic. They comprise about 90% of all cases of EDS and correspond to the first historical descriptions, hence the term "classical." Locus heterogeneity has been demonstrated. The classical type of EDS is inherited as an autosomal dominant trait and characterized as follows [39]:

- Major diagnostic criteria
  - Skin hyperextensibility
  - Widened atrophic scarring (manifestation of tissue fragility)
  - Joint hypermobility
- Minor diagnostic criteria
  - Smooth, velvety skin
  - Molluscoid pseudotumors
  - Subcutaneous spheroids
  - Complications of joint hypermobility (e.g., sprains, dislocations, subluxations, pes planus)
  - Muscular hypotonia, delayed gross motor development
  - Easy bruisability
  - Manifestations of tissue extensibility and fragility (e.g., hiatal hernia, anal prolapse in childhood, cervical insufficiency)
  - Surgical complications (postoperative herniae)
  - Positive family history

Severity, Special Signs, Features, and Complications

EDS I (MIM 130000). In EDS I, skin involvement is marked and joint laxity is generalized and gross, with musculoskeletal deformity and diverse orthopedic complications. This form is also called the "*gravis type*" (severe type) according to an earlier nomenclature. Its frequency has been estimated to be 1:20,000 [5]. Prematurity occurs in ~50% of cases. It is in this severe (gravis) group, in addition to EDS IV, that internal complications such as aortic and bowel rupture may occasionally occur.

EDS II (MIM 130010). EDS II is probably the most prevalent type of EDS. It has all the stigmata of EDS I to a minor degree, and some patients may easily remain undiagnosed. This form is also called the "*mitis type*" (mild type) according to an earlier nomenclature. Joint laxity is limited and may be confined to the hands and feet. Skin involvement is less evident. Prematurity does not occur more frequently than normal. Mitral valve prolapse is rare.

The "late onset of EDS" [130,131] may represent mild conditions that become manifest only in adulthood; other cases may be acquired, especially when the symptoms appear late in life and are restricted to skin in a particular area [132].

Genetics

The first pedigrees compatible with autosomal dominant inheritance were published as early as 1888 [25–28,133]. In 1949, Johnson and Falls [29] demonstrated autosomal dominant inheritance on the basis of an extensive kindred containing 32 affected persons (21 males and 11 females) over five generations. Among them, a consanguineous couple of affected cousins had eight children, three of whom were unaffected, three of whom had the same stigmata as their parents, and two of whom had the condition to a very severe degree. The authors concluded that the latter two may have been homozygous in which case all of their children should have been affected; unfortunately, no follow-up is available. The heterozygous mother also had four miscarriages, which, according to the authors, might also have represented homozygous, severely affected fetuses or might have been due to cervical insufficiency, which, as we now know, is quite typical of EDS. A severe, presumably homozygous form of EDS has also been observed in a highly inbred kindred with EDS type I [134]. Jansen [135] reviewed the literature and came to the same conclusion of autosomal dominant inheritance. Parental gonadal mosaicism may explain why neither parent of the two affected sisters, cases 6 and 7 in [40], has the syndrome.

In Beighton's series of 100 patients with EDS, 63 were from 20 families and inherited the condition as an autosomal dominant trait [1,136]. The 29 sporadic cases were clinically indistinguishable from the familial cases, and no parental age effect was evident, paternal age being 30.7 versus 29.3 years and maternal age 29.3 versus 28.4 years; birth rank did not deviate from normal.

Segmental cases, confined to one or more body segments, were already described by van Meek'ren [9] (Fig. 1) and Du Bois [137] and have subsequently been reported by Beighton [1] and Cullen [132]. These may represent somatic mutations similar to the reported case of asymmetric Marfan syndrome [138]. However, no mention is made of articular involvement in these cases.

Interfamilial variability may be due to genetic or locus heterogeneity, and intrafamilial variability may be due to differences in genetic background (i.e., differences in the composition of other connective tissue components inherited in a multifactorial way), age dependence, and, apparently, differences in the assessment of clinical severity. Allelism in EDS I and II has been demonstrated [139,140].

Basic Defect(s) in EDS I/II

In EDS types I and II, the basic defects were unknown until recently and are heterogeneous. In 1955, Jansen [30] described a loose, disorderly arrangement of apparently normal collagen fibers and postulated that the collagen fibrils might be abnormally cross-linked in such a way that the interlacing network of connective tissue was deficient. This theory would account for the changes in the physical properties of the skin in the classical form of EDS and for the lack of specific histological abnormalities. In the past, urinary excretion of hydroxyproline, uronic acid, mucopolysaccharides (glycosaminoglycans), and amino acids, serum levels of elastase inhibitor, and amino acid profiles of skin and elastin in skin were measured (for references, see Beighton [1]), but no consistent abnormality has been described. An abnormal cross-link profile was observed in the dermis of one patient, but no clinical data were given [141].

Occasionally, chromosomal anomalies have been found in EDS, most of which have been considered insignificant [142,143]. Scarbrough et al. [144] speculated that genes located in the area of breakpoint 6q27 or 13q11 may be responsible for EDS II. Linkage analysis has excluded changes in the genes for collagens I, II, and III as being responsible for the classical type of EDS [145–147].

Mutations in the Collagen V Genes

Evidence for *COL5A1* on chromosome 9q34 and *COL5A2* on chromosome 2q24.3-q31 as candidate genes for EDS I and II came from the following studies, which indicated the role of collagen V in regulating the formation of heterotypic fibrils in the extracellular matrix. First, the high concentration of collagen V in the chick corneal stroma is one important factor responsible for the small, uniform collagen fibrillar diameter observed in this tissue in contrast to other collagen I-containing tissues with larger diameter fibrils, as shown by *in vitro* collagen fibrillogenesis [148]. $[\alpha1(V)]_2\alpha2(V)$ heterotrimers are localized within the mature heterotypic fibrils, with their N-telopeptides exposed at the fibril surface, and modulate fibril diameter by a steric or electrostatic mechanism [149]. Second, genetically altered homozygous mice lacking the exon 6-encoded N-terminal telopeptide of the α2(V) chain demonstrated collagen fibrils displaying abnormal diameter, contour, and packing in skin and, especially, cornea, and presented with EDS-like features (see "Animal Models and Lathyrism" below; [150]).

Linkage studies in man strengthened this concept [139,140,151], which has now been proven by the direct demonstration of structural mutations in *COL5A1* [116,152–155] and *COL5A2* [156,157], which exert a dominant negative effect. In one patient, however, EDS I was found due to haploinsufficiency of collagen V caused by a translocation that disrupted one *COL5A1* allele [158]. It must be stressed, however, that mutations in *COL5A1* and *COL5A2* account for only a minor fraction of EDS-causing mutations because evidence for a collagen V defect was found in only six patients in a series of 35 patients/families [159].

Protein chemical demonstration of abnormal α1(V) or α2(V) chains produced in fibroblast cultures is, unfortunately, rather ineffective. For documented mutations in *COL5A1* and *COL5A2*, gel electrophoretic changes have been reported only rarely [116,152,154,157,159a]; these include faint doublets, reduced mobility of the chains, or decreased amounts of collagen V. This last is not unexpected, in any case, given the low amount of collagen V deposited in the cell layer and the variability in the relative amounts of collagens I, III, and V deposited from experiment to experiment. In the previously mentioned series of 35 patients/families, only two showed abnormal collagen V profiles, fewer than the six detected as having *COL5A1* and *COL5A2* mutations by molecular means [159]. Despite linkage, cDNA abnormalities of *COL5A2* and *COL5A1* were found in only four and eight, respectively, among 28 families, and the different authors concluded that the classical type of EDS is mainly due to haploinsufficiency caused by nonsense mutations [41,157,159a,160] in one-third of the cases of *COL5A1* [41,160].

Intrafamilial clinical variability in a three-generation family with the classical type of EDS has been explained by the finding that the more severely affected propositus was a compound heterozygote carrying the disease-causing mutation Gly1489Glu and an additional, putative disease-modifying substitution Gly530Ser in *COL5A1* (Fig. 10; [116]). The hypothesis that the heterozygous Gly530Ser substitution is indeed disease-modifying seems to be strengthened by the observation that the same substitution in the homozygous state appears to be disease-causing. Giunta et al. found that a Turkish boy with classical EDS born to consanguineous parents was homozygous for the Gly530Ser substitution and had no further mutations in the *COL5A1* and *COL5A2* genes, and linkage to the genes coding for

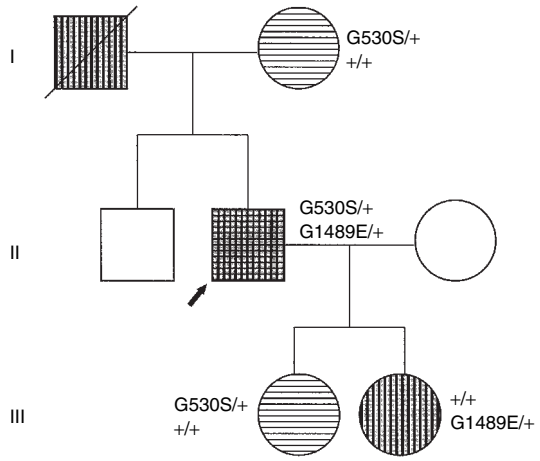


Figure 10. Intrafamilial variability in the classical type of EDS (EDS I) due to compound heterozygosity for the *COL5A1* genes. In each affected individual in family K, the genotype for the disease-causing mutation in exon 58 of *COL5A1* (Gly1489Glu) is represented by bold vertical lines and the disease-modifying substitution in exon 13 (Gly530Ser) by horizontal lines. The compound heterozygous propositus G.K., indicated by the arrow, is the most severely affected individual among the three with classical EDS. (Taken and corrected from Giunta and Steinmann [116] with permission.)

the α3(V) chain, decorin, thrombospondin 2, and tenascin-X was excluded by homozygosity mapping [161].

Because the α3(V) chain is primarily expressed in the epimysial sheaths of developing muscles, in nascent ligaments to forming bones, and in joints [162], *COL5A3* is a candidate locus for at least some cases of classical EDS in which *COL5A1* and *COL5A2* have been excluded and for at least some cases of the hypermobile type of EDS (see below).

Tenascin-X Deficiency

The tenascins (see Chapter 5, this volume) constitute a family of large extracellular matrix proteins. Although they have been implicated in several cellular processes, such as cell adhesion to collagen, no function has been clearly established for any of them. Tenascin-X (TN-X; MIM 600261) is a collagen fibril-associated protein that is highly expressed in dermis, tendon, and ligaments and in the connective tissue of striated muscle. The human *TNXA* gene is on chromosome 6p21.3 at the MHC class III locus, and its 3′ end overlaps the steroid 21-hydroxylase gene on the opposite strand of DNA. In 1997, Burch et al. [163] described a new contiguous gene syndrome, involving the *CYP21B* and *TNXA* genes, that resulted in 21-hydroxylase deficiency combined with a connective tissue disorder resembling EDS II, which is inherited in a presumed recessive fashion.

The proband was a 26-year-old man in whom a diagnosis of severe salt-wasting congenital adrenal hypoplasia due to 21-hydroxylase deficiency had been made at $1\frac{1}{2}$ weeks of age. He complained of a 2–3 year history of worsening joint pain and easy bruisability of many years duration. His history was significant for the release of a trigger finger at age 10, the repair of testicular torsion at age 15, and bronchiectasis confirmed radiographically at age 23. He had always had slow healing of wounds. Physical findings included micrognathia; a high, narrow palate; patellar chondromalacia; talipes planus; hypermobile joints of the fingers, wrists, and knees; a soft, doughy, hyperextensible

skin; and vascular fragility as evidenced by multiple small bruises and ecchymoses. The only family history of connective tissue disease was in his father, who also had hyperextensible finger joints but was otherwise normal [163].

Unusual for EDS II were the ultrastructural findings in his skin [163] but not in skin of further TN-X-deficient patients (see below). The collagen fibrils were not irregular in contour, had a diameter somewhat smaller than normal, and were reduced in number in the reticular dermis, where tenascin-X is mainly expressed. Also, there were abnormal elastin bodies beneath the dermo-epidermal junction. Capillaries in the papillary dermis were prominent and demonstrated increased perivascular matrix, and cutaneous nerves showed abnormal packing of the lamellae of myelin sheaths.

Biochemical and molecular studies of the patient [163] showed that he had a *TNXA*-null phenotype: no mRNA for tenascin-X was detectable, and tenascin-X was absent from skin and fibroblasts. He, as well as his father and one of his two sisters, carried a novel 30 kb deletion arising from the recombination of *TNXA* and its partial duplicate gene, *XA*, precluding TN-X transcription. The nature of his second maternal *TNXA*-null allele remains unresolved.

The patient's distinct histopathologic status suggests a novel mechanism of disease. Hence, TN-X may regulate the macromolecular organization and distribution of matrix elements such as fibronectin, collagen, and elastin; it could interact with cell receptors, modulating the cellular deposition of matrix; it may cross-link matrix fibers or proteoglycans, thereby strengthening the matrix; or, alternatively, it may play a primary structural role in skin, joints, and vascular connective tissues [164]. Definition of the precise role of TN-X in the EDS phenotype will require the identification of other TN-X-deficient patients and *Tnx* knockout mice, as well as the further study of this complex protein.

A quite similar but unrelated patient has been identified [165], who, however, does not present the same morphological findings (L. Smith, personal communication, 1999). A further patient, heterozygous for the *CYP21/TNXA* deletion with an unidentified inactivating mutation on the second *TNXA* allele, and three patients with isolated such mutations have been reported [166], but it remains unclear whether EDS resulted from TN-X deficiency alone and what proportion of patients with EDS might be accounted for by *TNXA* mutations [167].

The same group identified a 140 kDa carboxyl-terminal fragment of TN-X in serum of normals by western blotting, which facilitated the large-scale screening of 151 patients with EDS (35 classical, 87 hypermobile, 1 vascular, and 28 unclassified types of EDS). Among these, TN-X was absent in five individuals with unclassified EDS in each of whom the mutation was identified [167,168]; examination of TN-X-deficient fibroblasts confirmed the failure of TN-X synthesis and showed abnormal deposition of collagen I into matrix *in vitro*. The phenotype of the five patients is characterized by joint laxity, hyperelasticity of the skin, easy bruising, and absence of atrophic scarring. Ultrastructural examinations of the dermis did not show the typical cauliflower collagen fibrils observed in dermis of individuals with EDS caused by mutations of the genes coding for collagen V, nor was the density of the collagen fibrils reduced as observed in the tenascin-X knockout mouse (see "Animal Models and Lathyrism" below). Obligate heterozygotes had approximately 50% of normal TN-X levels in serum and conditioned culture medium.

Mutations in Collagen I

α2(I)-deficient collagen I. Patients with a total α2(I) deficiency are extremely rare — only four have been described so far — and they show markedly different clinical phenotypes. Sasaki et al. [169] described a 30-year-old man, born to parents who were second cousins, with hypermobility of the joints and hyperextensibility of the skin, who was operated on because of severe aortic regurgitation. Hata et al. [170] reported a 35-year-old woman with EDS II (hypermobility of the joints, hyperextensibility of the skin, with easy bruising and a tendency toward scar formation) with slightly blue sclerae and mitral valve regurgitation. Her unrelated parents and her son are healthy. At age 42, she was operated on for mitral valve replacement; by electron microscopy, dermal collagen fibrils were more variable than normal in diameter and contour [171]. Nicholls et al. [172] described a 9-year-old girl with normal height, generalized joint laxity, pes planus, and valgus heels leading to a secondary shortening of the Achilles tendon. Her skin was normal, her sclerae were pale blue, and her teeth were without signs of dentinogenesis imperfecta. She was born prematurely. There was a history of recurrent patellar dislocations and fractures of the skull, clavicle, fingers, and a toe. Her parents were consanguineous. Skin fibroblasts from the three patients synthesized no proα2(I) chains, produced only half the normal amount of collagen, and degraded newly formed collagen intracellularly to a greater extent than normal. In the first and third cases, α1(I) chains were reported to be overmodified (i.e., to show excessive post-translational modification of lysyl and hydroxylysyl residues), apparently as a result of slowed helix formation of the α1(I) homotrimer [169]. In the first two cases, mRNA for the proα2(I) chain was present at less than 10% of the normal amount [170,173] and was interpreted as being unstable because the rate of transcription was normal [173]. Neither stimulation of transcription of the *COL1A2* gene nor increase in the level of mRNA was observed after activation by ascorbic acid 2-phosphate, in contrast to the situation with *COL1A1* in the patient and both genes in controls. From Southern blot analyses, it was further concluded that the patient was homozygous for a functionally defective *COL1A2* gene [174]. The third case had a homozygous substitution in IVS46+2T>C of *COL1A2*, which leads to the use of a cryptic donor splice site 17 bp upstream of the normal splice site; as a result of this, the last 17 bp of exon 46 are deleted and the resultant frame shift introduces a new termination codon just three codons further downstream.

The three patients have normal bones, in striking contrast to the patient with severe osteogenesis imperfecta type III in whom a homozygous 4 bp deletion in the C-propeptide of the proα2(I) chain prevents these chains from assembling and being incorporated into normal heterotrimers [175]. Although studies on collagen extracted from skin and bone have not been performed in either of the patients, the discrepancy may well be explained by tissue-specific differences in expression of the proα2(I) chain. (Similar tissue-specific differences in collagen gene expression have been documented in fibroblasts, odontoblasts, and osteoblasts derived from the Mov-13 mouse [176,177]). Given the relatively benign EDS phenotype without bone involvement on the one hand [169] and the severe OI phenotype on the other [175], we speculated that the EDS patient may have lacked a putative tissue-specific transcription factor in skin, ligaments, and aorta, transmitted as an autosomal

recessive trait, but that a different transcription factor was present in bone and thus allowed the production of normal heterotrimeric collagen I and the formation of normal bones. However, polyethyleneglycol-induced hybridization of the EDS and the OI fibroblasts failed to result in complementation of the α2(I)-deficient cell strains in heterokaryons (C. Giunta and B. Steinmann, unpublished experiments).

Mutations in the *COL1A1* and *COL1A2* genes. Different mutations in three unrelated families led to skipping of exon 9 in *COL1A2* (A. Nichols, personal communication, 1999), and in two sporadic cases substitution of the conserved arginine in exon 14 led to an Arg134Cys substitution in the α1(I) chain, which also resulted in the classical type of EDS [178].

Other Candidate Genes

Decorin and lumican, as well as thrombospondin-2, play an important role in collagen fibrillogenesis. Targeted disruption of the decorin gene led to viable homozygous mice whose skin was fragile, with a markedly reduced tensile strength. Ultrastructural analysis revealed abnormal collagen morphology in skin and tendon, with coarser and irregular fibril outlines [179]. Mice homozygous for null mutations in lumican [180] and thrombospondin-2 [181] displayed skin laxity and fragility similar to that in EDS (see "Animal Models and Lathyrism" below). Thus, decorin, lumican, and thrombospondin-2 are candidate genes for human EDS.

Physical and Morphologic Properties of Connective Tissue and Pathogenesis of the Disorder

Skin elasticity. In normal individuals, elasticity has been assessed using a "pinchmeter" by determining the ease with which a fold of skin from the dorsum of the wrist could be raised by a spring-loaded caliper, or by a suction cup with increasing negative pressure, and recording the resulting distortion [46]. Using the latter method, it was determined that the modulus of elasticity is significantly higher in female than in male control individuals [50] and that it rises progressively with age in both sexes [46]. Mechanical properties of skin have also been evaluated in 17 children aged 3–10 years with EDS I, II, or III and compared with normal values from 63 healthy age-matched children; prominent increases in skin extensibility and elasticity were the most distinctive and diagnostic features, being the greatest in EDS I and the least in EDS III [48].

Stress-strain analysis carried out on skin from EDS patients has shown that the initial lag phase is prolonged and followed by a phase in which the stress-strain relationship is linear and parallel to that of the control tissue [50,182]; that is, the fibrils must be cross-linked and virtually inextensible at this stage. The long lag phase is therefore not due to an absence of cross-links within the fibrils but to an abnormal packing of these fibrils into tight bundles. This interpretation is supported by observations made by scanning electron microscopy that the fiber bundles are less tightly packed than normal [183]. Under tension, considerable extension will be required before the majority of the bundles are aligned in the direction of the tension, at which point they will be tightly packed and resist further extension. If a defect in cross-linking had been present, such as that which occurs in lathyrism or in dermatosparaxis in cattle, continuous extension of the fiber would have occurred as the fibrils slipped past one another, up to the point of rupture. In such conditions, no restoring force would be present, and the effect would not be reversible, whereas in EDS subjects the skin returns to its normal position after hyperextension [183]. These studies have provided support for the previously unsubstantiated proposal by Jansen [30] that skin hyperextensibility in EDS is due to a defective "wickerwork" of apparently structurally normal collagen fiber bundles.

In vitro studies of skin have shown that its tensile strength depends on its water content, the texture of the dermis, and the strength of each single collagenous fiber, and that it increases with age. In a 35-year-old patient with EDS, the tensile strength of the skin was 0.34 kg/mm^2 versus 1.61 ± 0.08 in controls [184], whereas the difference in tensile strengths of the tendons was less marked, namely 4.3 kg/mm^2 versus 9.0 in controls [185].

Skin thickness. Skin thickness can be assessed *in vivo* by the Harpenden caliper [50,186–188], radiographically [189], or by ultrasound [188]; all three methods give quite similar results. There is a direct relationship between dermal thickness measured *in vivo* or histologically and the content of collagen; skin collagen in normal individuals decreases linearly with age by about 1% per year throughout adult life and is less in females at all ages [190]. The skin of 14 patients with EDS studied was thinner than that of controls (0.8 mm versus 1.15 mm), and there was a significant inverse relationship between thickness and the tendency toward skin-splitting as evidenced by the degree of scarring [50].

Morphology of skin. For a historical review of the morphology of the skin, molluscoid pseudotumors, spheroids, and tendons, and of the cardiovascular, gastrointestinal, and pulmonary systems in EDS, see references in Beighton [1] and Wechsler and Fisher [191].

Light and electron microscopic studies of skin from patients with inherited connective tissue disorders are excellently reviewed by Holbrook and Byers [192]. Their generalizations indicate that mutations rarely affect only a single aspect of macromolecular function and that, because of the interactions of matrix components in this complex organ, they often disturb the organization of the entire dermis. Because similar structural abnormalities may result from different molecular defects, most morphological findings are not specific and are often subtle due to a limited repertoire of the dermis for changes. Structural alterations of matrix components include collagen fibrils that are excessively large and irregular in cross-sectional appearance, which have been called composite fibrils or flower or cauliflower figures, and mixed populations of large- and small-diameter fibrils; these changes were first recognized in individuals with EDS I by Vogel et al. [40] (Fig. 5g). Although structural alterations in connective tissue fibers are rarely specific for a given disease, there are characteristic patterns of structural changes in the matrix that may be used to confirm a diagnosis. Hausser and Anton-Lamprecht [193] found a certain correlation between the ultrastructural changes and the severity of the phenotype. In EDS I, ultrastructurally abnormal fibrils were present immediately below the dermo-epidermal junction and, in contrast to EDS II and III, there were almost no normal fibrils. In EDS II, the upper dermis contained only a few abnormal fibrils, in contrast to the middle and deep dermis, which had many abnormal fibrils. In EDS III, on the other hand, isolated smaller collagen "flowers" were only present in the reticular dermis. Such a morphologic gradient, however, needs to be confirmed by genetic analysis.

What is intriguing to us is the fact that the morphology of the collagen fibrils is so patchy and heterogeneous. Why are the cauliflower fibrils sparse and scattered widely apart, and why are there normal-appearing fibrils at all instead of a homogeneous distribution of abnormality? Is haploinsufficiency for collagen V not operative in all sites due to local differences? In this context, it is of note that vision seems not to be impaired by any alteration in translucency of the cornea, an organ rich in collagen V, because abnormal cauliflower-like collagen fibrils are observed in dermis and cornea of pN-knockout mice (see "Animal Models and Lathyrism" below and Chapter 1, Part V, this volume); ultrastructural studies on the cornea of patients with the classical type of EDS have not been reported.

Elastic arteries. The mechanical properties of elastic arteries have been measured by noninvasive monitoring of the pulsatile changes in diameter of the distal abdominal aorta and the common carotid artery by the use of an electronic echo-tracking instrument; however, the diameter and stiffness in several EDS I and II patients were not different from those in the reference population, and the study was unable to demonstrate any alteration in wall mechanics as a sign of disturbed vessel wall integrity [194].

In conclusion, the whole spectrum of tactile, mechanical (log phase), histological and ultrastructural (cauliflower collagen fibrils), and biochemical changes (decreased collagen and increased elastin content) are not yet well-understood.

Diagnosis and Differential Diagnosis

For most practical purposes, diagnosis remains syndromic thus far (see Table I). For specific practical or research purposes, laboratory investigations include a skin biopsy for the following analyses: (1) Ultrastructure quite often may suggest disturbed collagen fibrillogenesis [40,192,193], as in most cases with mutations in the collagen V genes, or other causes such as decorin deficiency, which has not yet been observed in man (see "Animal Models and Lathyrism" below). (2) Analysis of collagens produced by cultured fibroblasts may indicate abnormal collagen V, although this is not a sensitive assay, or, exceptionally, may reveal α2(I)-deficient collagen. Frozen cells may be useful for later studies. (3) A piece of skin should be stored frozen for immediate or later studies. (4) In large pedigrees, linkage analyses should be done to include or exclude known genes responsible for EDS or to map candidate genes.

Joint laxity may be found in the Marfan syndrome (MIM 154700), the Marfanoid hypermobility syndrome (MIM 154750), the familial articular hypermobility syndrome(s) (MIM 147900; the distinction between this syndrome and EDS III may be artificial), the Larsen syndrome (MIM 150250, MIM 245600), certain forms of osteogenesis imperfecta, and muscular hypotonia of various causes. Contortionists may achieve extraordinary hypermobility of the large joints by training. Stickler's hereditary arthroophthalmopathy (MIM 108300), the osteoporosis-pseudoglioma syndrome (MIM 259770), pseudoachondroplasia (MIM 177150, MIM 264150), Morquio syndrome (MIM 253000, MIM 253010), spondyloepimetaphyseal dysplasia with joint laxity (MIM 271640), Desbuquois syndrome (MIM 221880), and hyperlysinuria (MIM 238700) share with EDS hyperextensibility of the joints but are readily differentiated by other distinct features. Ligamentous laxity is also a feature of many other genetic disorders, such as the fragile X-syndrome (MIM 309550), trisomy 8-mosaic syndrome, and multiple endocrine neoplasia type 2b (MIM 162300). Joint laxity and soft skin are frequent in the renal-coloboma syndrome (MIM 120330) and the Langer-Giedion syndrome (MIM 150230). Hyperelasticity of the skin and joint laxity together with mental retardation and cataracts have been described in siblings with an autosomal recessive disorder due to impaired proline synthesis (MIM 138250) [195,196]. Skin and joint laxity has been reported together with lengthening and tortuosity of systemic, pulmonary, and coronary vessels and an elongated facies [197]. In the fetal anticonvulsant syndrome, joint laxity is a frequent finding, involves all sizes of joints, and is part of a generalized connective tissue disorder [198].

Hyperelastic skin can be found in the Noonan syndrome (MIM 163950) and should be distinguished from that observed in cutis laxa syndromes (MIM 123700, MIM 219100, MIM 219150), the De Barsy syndrome (MIM 219200, MIM 304150), gerodermia osteodysplastica hereditaria (MIM 231070), and Menkes disease (MIM 309400), in all of which the lax redundant skin, which is not fragile, hangs in loose folds and, although it may be stretched, will return only very slowly to its former position. Such patients have a "bloodhound facies" and look considerably older than they are. In the autosomal recessively inherited wrinkly skin syndrome (MIM 278250), the skin is wrinkled over the dorsum of the hands and feet, and the palms and soles, and the abdomen [199] (see also Chapter 10, this volume).

Increased skin fragility is found in osteogenesis imperfecta and in senile people, and may be mimicked by self-affliction by retarded or hysterical persons (Münchhausen syndrome). Excessive bruising is found in thrombo- and coagulopathies (see EDS IV) and in nonaccidental injury ("battered child") (see legend to Fig. 7).

Cardiovascular anomalies similar to those in EDS I may be found in familial mitral valve prolapse (MIM 157700) and annuloaortic ectasia Erdheim (MIM 132900), and are frequently associated with adult polycystic kidney disease (MIM 173900) [200], sometimes together with a Marfanoid habitus [201]. Dilatation of the aorta is typical of the Marfan syndrome (MIM 154700), aortic arch arteritis Takayasu, and other forms of arteritis and aortic malformations. For multiple arterial aneurysms, see EDS IV.

Musculoskeletal deformities, especially in EDS VI (Figs. 24,25), may resemble those in the Marfan syndrome.

Keratoconus was found in 22 of 44 patients with hypermobility, mainly of the fingers and thumbs, but without hyperelasticity or fragility of the skin; this condition is compatible with autosomal dominant inheritance and, according to the author in question, with EDS II [115].

Management and Genetic Counseling of EDS I and II

Because causal therapies are not available, medical intervention is limited to symptomatic therapy, prophylactic measures, and counseling.

Medical Therapy

Ascorbic acid, a cofactor of prolyl and lysyl hydroxylases, has been given to some patients with EDS but in spite of anecdotal reports of a beneficial effect does not change the basic clinical picture. The same holds true for zinc therapy [202,203]. Mild antirheumatic drugs may be indicated in patients with articular pain. Patients with mitral valve regurgitation require antibiotic prophylaxis for bacterial endocarditis and should carry a medical identification card with the appropriate instructions. The bleeding tendency is thought to be caused primarily by tissue

and capillary fragility rather than an intrinsic platelet or plasma defect. DDAVP (vasopressin) has been successfully used to reduce blood loss in patients without connective tissue disorders undergoing heart surgery and may be useful in EDS patients with chronic bruising and epistaxis, or perioperatively (e.g., for tooth extraction), in whom bleeding time is normalized by DDAVP (P. Tuchschmid and B. Steinmann, unpublished, 1996; [204]).

Physical Therapy, Orthopedics, and Prevention

A physiotherapeutic program is important in children with hypotonia and delayed motor development. In milder cases, light, non-weight-bearing muscular exercise (e.g., swimming) is useful to correct hypotonia and promote development of the musculature and muscular coordination, thus stabilizing the loose joints; gymnastics and sports with heavy joint strain are to be discouraged. Sports instructors should be informed about those with EDS. High-impact activities and hyperextension of all joints are to be avoided. If there is significant joint laxity and hyperextension at the interphalangeal joints, the occupational therapist should evaluate patients for ring splints. Bracing of the lower limb is indicated when joint instability and hypotonia prevent walking and physiotherapy has not been effective; ultimately, surgery is indicated when other means have failed. Special shoes may be required by patients with flat feet, other foot deformities, or painful piezogenic papules. Osteopathy and relaxation for pain control and management may be helpful.

Surgery

Because of tissue fragility, any type of surgery may be more difficult in patients with EDS, and it is not unusual for the original problem (e.g., herniae) to recur after intervention. Surgery may be indicated for fixation of a particularly unstable or habitually luxated joint, for the correction of dislocated hips (see EDS VII), for the repair of herniae or diverticula, and for scoliosis or cardiovascular problems [205]. Wounds should be closed without tension, preferably in two layers. Deep stitches should be applied generously and cutaneous sutures left in place for twice as long as usual. Long-lasting cutaneous analgesia may be difficult to obtain [89]. Tooth extraction may be difficult because of dilated irregular and poorly formed terminal roots, and prolonged post-extraction hemorrhage should be anticipated because of the poor vascular retraction. From a retrospective study, based on the patients' reports of their own experiences after surgical procedures to the shoulder, the elbow, the knee, or the ankle for pain, instability, poor range of motion, or a combination thereof, Weinberg et al. [206] conclude that surgical complications are common in EDS.

Prophylaxis of Skin Changes

Young children with pronounced skin fragility should wear protection in the form of athletes' pads or bandages over the forehead, knees, and shins in order to avoid skin tears. Later, the child may learn to avoid violent sports. When skin tears do occur, irregularly frayed wound margins should be excised and precisely adapted to allow (rapid) healing without dystrophic scarring, which is especially important in the case of facial wounds. Numerous fine, atraumatic stitches should be used and left in place for twice as long as usual. Additional fixation of the adjacent skin with adhesive tape is very helpful in preventing stretching of the scar; however, removal of adhesive material has to be done carefully to avoid secondary dehiscence. Hyperpigmentation and premature aging of both the scars and normal skin are of concern to many patients.

Other

The majority of patients with EDS learn to cope with their disease and eventually adapt well to daily life. However, some patients are troubled by hypotonia and joint instability, and many by chronic joint pain. These handicaps should be recognized and discussed, and lifestyle and professional choices should be adapted accordingly; strenuous physical activity should be avoided. Behavioral and psychological therapy may be indicated. Patients may gain valuable up-to-date information, confidence, emotional support to accept and cope with their handicap, and much practical advice from meeting other patients who are members of self-help organizations (see "Patient Support Groups" below).

Genetic Counseling

EDS types I and II are inherited as autosomal dominant traits. Affected persons have a 50% risk of transmitting the disorder. Affected first-degree family members should be examined to obtain an estimate of intrafamilial variability and thus the phenotypic range to be expected should the child be affected. Prenatal diagnosis has not been attempted.

THE HYPERMOBILE TYPE OF EDS — EDS TYPE III (MIM 130020)

Diagnostic Criteria

The hypermobile type of EDS is inherited as an autosomal dominant trait and characterized as follows [39]:

Major diagnostic criteria
- Skin involvement (hyperextensibility and/or smooth, velvety skin, absence of tissue fragility)
- Generalized joint hypermobility

Minor diagnostic criteria
- Recurring joint dislocations
- Chronic joint/limb pain
- Positive family history

EDS III (hypermobile type) (MIM 130020) is characterized by quite severe, generalized joint laxity (Fig. 11) and the sequelae thereof, such as dislocations, effusions, and precocious arthritis. Certain joints, such as the shoulder, patella, and temporo-mandibular joints, dislocate frequently. Pain, swelling, dislocation of joints, a history of surgery of the joints, and impaired ambulation were reported by questionnaire during a National Ehlers-Danlos Syndrome Foundation Conference to be more frequent and pronounced by patients with EDS III than by patients with EDS I, II, or IV; the authors came to the conclusion that EDS III is the most debilitating form with respect to musculoskeletal function, although the data reflected an inherent bias because the patients were voluntary attendees at the educational symposium [66]. Skin hyperextensibility is variable; because tissue fragility is absent, the presence of atrophic scars, spheroids, or molluscoid pseudotumors with joint hypermobility suggests diagnosis of the classical type. The frequency of prematurity is not increased. Mitral valve prolapse is more prevalent than normal.

Musculoskeletal pain in EDS III is early in onset, chronic, and sometimes debilitating [207]. Its anatomical distribution is wide, and tender points can sometimes be elicited (a tender point is defined as an area that, when palpated with the thumb or two or three fingers, will be painful at a pressure of 4 kg or less [208]). In rheumatology practice, large numbers of patients present with generalized joint hypermobility.

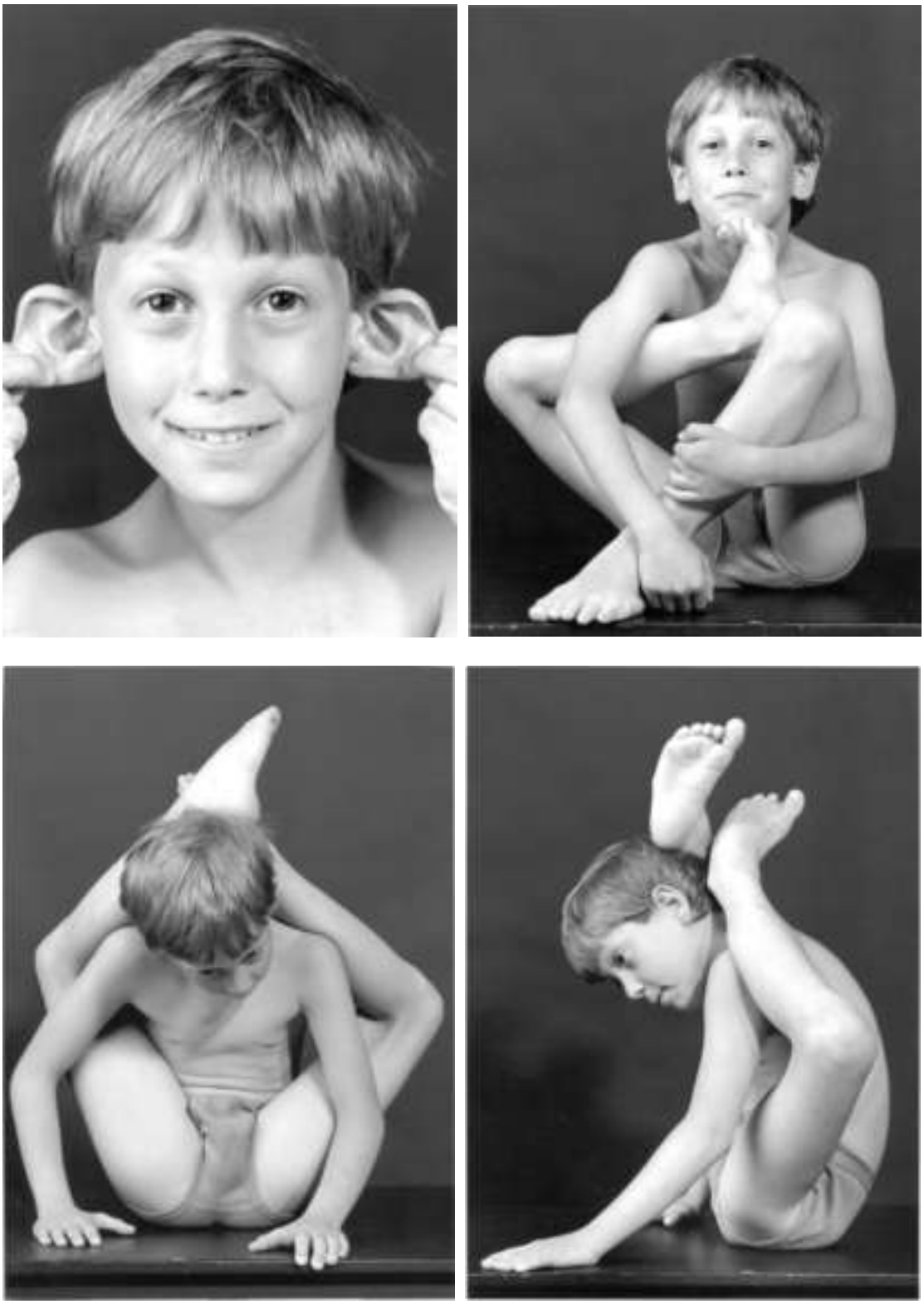


Figure 11. Hypermobile type of EDS (EDS III). General joint hypermobility in a 10-year-old boy (A.T., 18 Apr 1981) with hyperelastic, smooth, velvety skin, which is not fragile and has no abnormal scars, and hyperelastic, floppy ears.

He is a sporadic case and had several episodes of rectal prolapse starting at age 2.5 years, which was operated on; rectal prolapse has not recurred but it remains open if this is due to the operative fixation or simply represents the natural history. Because of chronic back pain, spondylolisthesis L5/S1 was operated on by dorsal spondylodesis, and an indwelling urinary catheter was inserted, which led to urethral strictures needing several bougienages. He suffered from several hemorrhages into the knee joints and complained of chronic fatigue.

For clinical research purposes, it is important to distinguish these individuals from those affected with the hypermobile type of EDS. There is considerable debate as to the causal interrelationship, if any, between the two phenotypes.

EDS III is similar to, although by no means as severe as, EDS VIIA and VIIB, but congenital hip dislocation does not usually occur. Furthermore, a separate autosomal dominant condition, familial articular hypermobility syndrome, the former EDS XI (MIM 147900; Table 1), which is characterized by severe joint laxity, occasional congenital hip dislocation, but no skin changes, has to be distinguished. Whether the familial articular hypermobility syndrome represents the upper end of the normal spectrum of variation of joint mobility or reflects a mild connective tissue disorder remains open, and in the absence of biochemical and genetic markers, the nosologic relationship between it and EDS III remains unclear.

Surprisingly, Narcisi et al. [209] described a family in which a point mutation in the *COL3A1* gene (Gly637Ser) was associated with a phenotype they described as EDS type III, a condition that is unlikely to be confused with EDS IV; it may well be that individuals of this family will have a later onset of significant symptoms of EDS IV because at description most were well below the age at which major complications would have been expected. Only the first affected individual was over 60 years of age, and mosaicism for the mutation was not excluded.

COL5A3 is a candidate gene for at least some cases of the hypermobile type of EDS (and some cases of classical EDS in which *COL5A1* and *COL5A2* have been excluded, see above) because the α3(V) chain is primarily expressed in the epimysial sheaths of developing muscles, in nascent ligaments to forming bones, and in joints [162].

To control the pain, which is sometimes considerable in this type of EDS, and the consequent impairment of well-being, behavioral, physical, medical, and psychological therapy may be indicated (see classical type of EDS above).

VASCULAR TYPE OF EDS — EDS TYPE IV (ARTERIAL-ECCHYMOTIC TYPE OF SACK-BARABAS) (MIM 130050)

Diagnostic Criteria

The vascular type of EDS is inherited as an autosomal dominant trait and is caused by structural defects in the proα1(III) chain of collagen III encoded by *COL3A1*. It has the worst prognosis and is characterized as follows [39]:

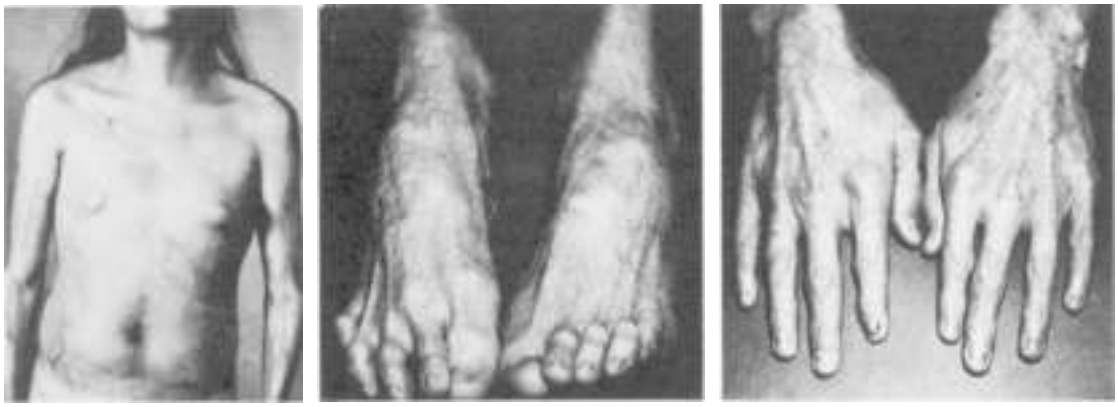
- Major diagnostic criteria
  - Thin, translucent skin
  - Arterial/intestinal/uterine fragility or rupture
  - Extensive bruising
  - Characteristic facial appearance (Fig. 13)
- Minor diagnostic criteria
  - Acrogeria
  - Hypermobility of small joints
  - Tendon and muscle rupture
  - Talipes equinovarus (clubfoot)
  - Early-onset varicose veins
  - Arteriovenous, carotid-cavernous sinus fistula
  - Pneumothorax/pneumohematothorax
  - Gingival recession
  - Positive family history, sudden death in (a) close relative(s)

Note: The presence of any two or more of the major criteria is highly indicative of the diagnosis, and laboratory testing is strongly recommended.

Historical Introduction

EDS IV is a life-threatening and disabling disease, "dramatic, deceptive and deadly" [210]. In 1967, Barabas [31] pointed out that the extensive tendency toward bruising and bleeding and the extreme arterial fragility distinguished this variant from other forms of EDS. Sack [24], and probably others, had described the same entity many years before. Affected individuals have only slight joint hypermobility, usually limited to the digits, and skin hyperextensibility is minimal or absent [142], hence the designation ecchymotic or arterial type (or Sack's type) [1,2,211], now the vascular type of EDS [39]. It is unfortunate that the complications typical of EDS IV are sometimes cited in texts as characteristic of other types of EDS, thus creating unnecessary anxieties.

EDS IV was the second of the disorders of collagen to gain a biochemical identity. It was recognized that "patients with Ehlers-Danlos syndrome type IV lack type III collagen" [34], a description still confusing to clinicians less familiar with the terminology. The possibility that the manifestations of EDS IV could be caused by an absence of collagen III was first raised by Pope et al. [34]. They found that in the aorta and skin of their patient P.P. (Figs. 12b and 13a; also Fig. 6–10 in [2]), the total amount of collagen was markedly decreased and neither collagen III nor its CNBr-derived peptides were detectable. Fibroblasts grown from this and four additional patients did not secrete medium proteins that eluted on DEAE-cellulose column chromatography in the region expected for procollagen III. Furthermore, cells in culture from two of the five patients failed to stain by immunofluorescence with antibodies specific for procollagen III [212]. These authors concluded, wrongly as it happens (see below), that the defect was absent synthesis of procollagen III rather than a post-translational abnormality of procollagen secretion. Because clonal cells from dermatosparactic animals produce both collagens I and III [231], and because normal fibroblasts react with antibodies to both procollagens I and III, it was considered unlikely that there was a deficiency of a specific cell type synthesizing procollagen III [212]. Studying another patient by more sensitive methods, Byers et al. [232] found that the amount of procollagen III in fibroblast culture medium was indeed reduced to 10–15% of that in controls but that it was retained within the cells both *in vitro* and *in vivo* [233]. The finding that the stored collagen III was of higher molecular weight than expected suggested to them that the procollagen might have extensive post-translational modifications, possibly secondary to some structural alteration of the proα1(III) chains. They further proposed that the relatively low amount (10%) of normal collagen III secreted would be approximately that predicted if only one allele at a single locus were normal: because procollagen is a homopolymer containing three identical chains, only one-eighth of the assembled molecules would be normal if the molecules were assembled randomly from a pool containing equal numbers of normal and mutant chains. The seven-eighths of abnormal collagen III molecules might then be retained and/or degraded intracellularly. Finally, it could be predicted that such an abnormality would be inherited as an autosomal dominant trait [232]. Their concept of heterozygous mutations leading to structural defects of collagen α-chains and instability and/or nonsecretion and degradation of the abnormal



a b

Figure 12. Vascular type of EDS (EDS IV). (**a**) Thin, translucent skin with an easily visible venous pattern in E.A., born 1949 (Johns Hopkins Hospital #1287186), at 12 years of age. She also had acrogeria and acrocyanosis with atrophy of the fingertips and toe tips.

She was the second child born to healthy parents at term; her four brothers were unaffected. Labor was induced because of ruptured membranes before the onset of uterine contractions. Birth weight was low (1.8 kg), in contrast to that of her brothers, which ranged from 3.2 to 3.6 kg. She had pyloric stenosis operated on at 7 weeks, easy bruisability and skin fragility from infancy, splenic rupture at age 12 years, intramural hematoma of the colon at 17 years, and bleeding into the peritoneal cavity at 19 years. At age 25 years, she had recurrence of colonic hematoma, which required surgical intervention. She had extensive postoperative bleeding into the rectus muscle sheet and the abdominal cavity, was reoperated on twice, and died in shock ([2], and follow-up by B. Steinmann, 1978). (**b**) Acrogeria in P.P. (born 11 Nov 1957, died 17 Nov 1973) at age 14 years; see also Fig. 13a. (For references, figures, and cell strains see [34,212–220]; Fig. 6-10 in [2]; CRL 1145, and CRL 1243).[6] ((**a**) is (Fig. 6-21 in McKusick [2], reprinted with permission.)

procollagens — a process that later became known by the somewhat misleading term "protein suicide" coined and propagated by Prockop [234] — proved not only to be correct but crucial also for the understanding of mutations of procollagens I, II, IV, V, VI, VII, IX, X, XI, XVII and XVIII, leading to osteogenesis imperfecta and various other connective tissue disorders described in this volume.

Clinical Findings, Natural History, and Prevalence

Unlike in other types of EDS, affected individuals have nonhyperelastic, thin, translucent skin, through which the venous pattern over the chest, abdomen, and extremities is readily visible (Fig. 12a). Bruising is marked and wound healing delayed. Joint hypermobility is minimal and limited to the small joints of the hands and feet, although shoulder dislocation may occur. The skin covering the hands and feet may be extremely thin, finely wrinkled, and look "older" (so-called acrogeria — see Fig. 12b; some individuals with acrogeria in the older literature [235–239] probably had EDS IV). In contrast, tightness of the skin over the face may result in a "younger" appearance, not unlike after a face-lifting procedure (Fig. 13). The nose is thin, delicate, and pinched, the lips are thin, the cheeks are hollow, and the eyes appear prominent and staring because of a paucity of adipose tissue; the pinnae of the ears are firm and tight and frequently without a free ear lobule (Fig. 13h); there is a tendency to alopecia. The facies is therefore often quite characteristic (Fig. 13a–g) [220–222].

Congenital club foot and inguinal hernia are common, as are keratoconus [240], periodontal disease, and venous varicosity and thrombophlebitis. Elastosis perforans serpiginosa ([241–243]; MIM 130100) is not uncommon in EDS IV and presents as a circular rash with raised rough edges and a clear center, sited over the neck and flexures. This is the result of fragmented elastic fibers that are extruded through the epidermis.

Keloid formation (Fig. 14a) [246], Raynaud phenomenon, acroosteolysis [222,247,248], and skull defects [249] have been reported. Adult patients have been subdivided into ecchymotic and acrogeric varieties [250]. This distinction is more confusing than helpful because all patients with EDS IV tend to have ecchymoses, whereas only a proportion will show the additional feature of overt acrogeric changes of hands and feet. In children, even if at risk, it is often difficult to make the diagnosis on clinical grounds alone unless bruising is severe or the venous pattern particularly noticeable [222]. Although mitral valve prolapse was observed in most affected individuals from a large pedigree with EDS IV [251], it does not appear to be a consistent finding.

The hallmarks of EDS IV are the severe, life-threatening internal complications, that usually occur after puberty and include spontaneous rupture of the arteries, the colon, and the gravid uterus, and (hemato-)pneumothorax [252]. There does not appear to be any familial predisposition for a certain type of complication because different catastrophic events can occur in different individuals within one large family (Fig. 18) or sequentially in one person [210,253].

Arterial complications have been collated in a large collective review of literature published over 20 years, from 1975 to 1995, in which only patients for whom biochemical confirmation was available are included; not considered were intracranial vascular complications [254]. The 45 vascular

[6]Cell strains detailing the cells studied in the original papers are given in the 1st edition of this volume; these cells, designated CRL, are commercially available from: American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852.

[7]The known tendency toward keloid formation in patients with EDS IV is somewhat at odds with the observation that keloid tissue in other subjects contains 32% collagen III relative to collagen I, as compared with 21% collagen III present in dermis of normal skin [245].

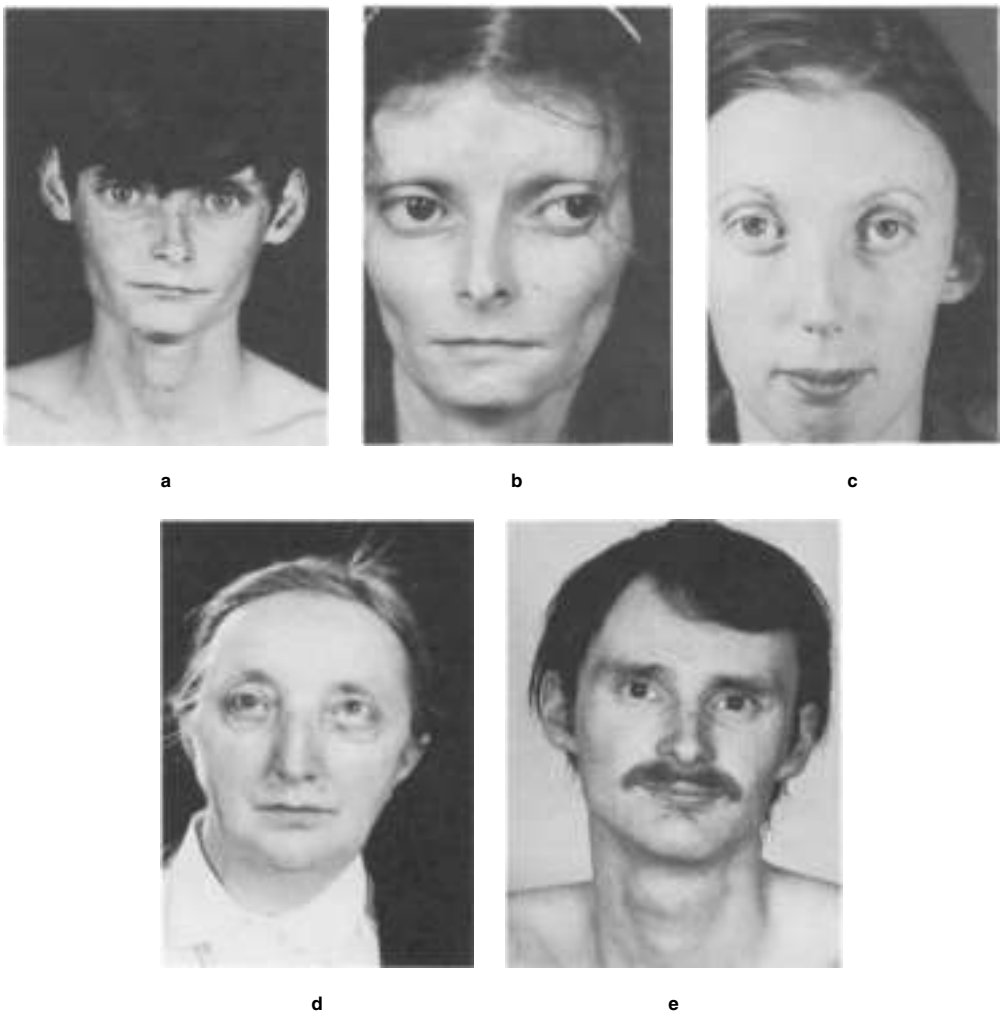


Figure 13. Facial appearance in the vascular type of EDS (EDS IV). The facies in EDS IV patients is often quite typical (**a**, **b**, **f**), with a thin, delicate, and pinched nose; thin lips; tight skin; hollow cheeks and prominent and staring eyes because of a paucity of adipose tissue; and tight, firm, lobeless ears. In some patients (**e**), however, the facial characteristics are less apparent. For further illustrations, see [220]. (**a**) P.P. at age 14 years (see also Fig. 12b). His molecular defect is a Gly1021Arg substitution in the α1(III) chain [220]. (**b**) and (**c**). The defects are a Gly1021Glu substitution and skipping of exon 43, respectively [120]. (**d**) M.D. (2 Jan 1933) at age 55 years. Her molecular defect is a Gly910Val substitution (for further references, see [223–225]). (**e**) D.S. (born 27 May 1964, died 27 Sep 1990) with a multiexon deletion [226,227] at age 22 years (for further references, see [213,219,228,229]). (**f**) Baby, childhood, and adult facies of a woman with EDS IV who died at age 33 years from a rupture of the splenic artery. The authors claim that ''the diagnosis is unmistakable in all three pictures''; although we do agree that the adult face is typical, the two younger facies would be quite inconspicuous to a pediatrician. This demonstrates that in children it is often difficult to suspect the diagnosis on clinical grounds unless bruising is severe and/or the venous pattern particularly noticeable, or unless there is a positive family history. Her molecular defect is a Gly1003Asp substitution [220]. (**g**) R.I. (11 Feb 1976) at age 18 years. He was born to healthy unrelated young parents after premature rupture of the membranes three weeks before term. At three months, bilateral inguinal herniae were operated on and his skin was noted to be unusually fragile with an easily visible venous pattern. Subcutaneous fat was sparse and, according to his mother, he never ''looked like a baby.'' At 5 months, hydrocephalus due to chronic subdural hematoma was diagnosed, and it was drained at 9 months. Easy bruising was a continuous complaint. At age 17 years, after an excess of alcohol, vomiting provoked pneumothorax with subcutaneous emphysema and rupture of the lower esophagus (Boerhaave syndrome) with consequent mediastinitis and chronic pericarditis responsible for heart failure. Dilatation of the esophageal stricture — before the diagnosis was appreciated — led to pneumomediastinum and massive symptomatic pneumoperitoneum that required needle aspiration. Over a year later, he had recurrent left inguinal hernia repaired, an operation that was complicated by severe intraoperative hemorrhage from extremely dilated, tortuous, and fragile veins at the level of an almost nonexistent fascia transversalis. He developed pterygium-like contractures of the knees, which made ambulation difficult. His molecular defect was a single nucleotide exchange (IVS42+5G>A), which led to an insertion of ten amino acids. At age 23 years, he died the victim of a tragic car accident [230]. (**h**) Ear of L.R. at age 62 years, which is poorly modeled, lobeless, and has a very tight feel. Her molecular defect is a G to A transition at nucleotide 730, which leads to a Gly43Asp substitution [230] (see also legend to Fig. 20). ((**a**) reprinted from Pope et al. [221] with permission; (**b**) and (**c**) reprinted from Pope et al. [222] with permission; (**d**) and (**e**) reprinted from Steinmann [42] with permission; (**f**) reprinted from Pope et al. [221] with permission.)

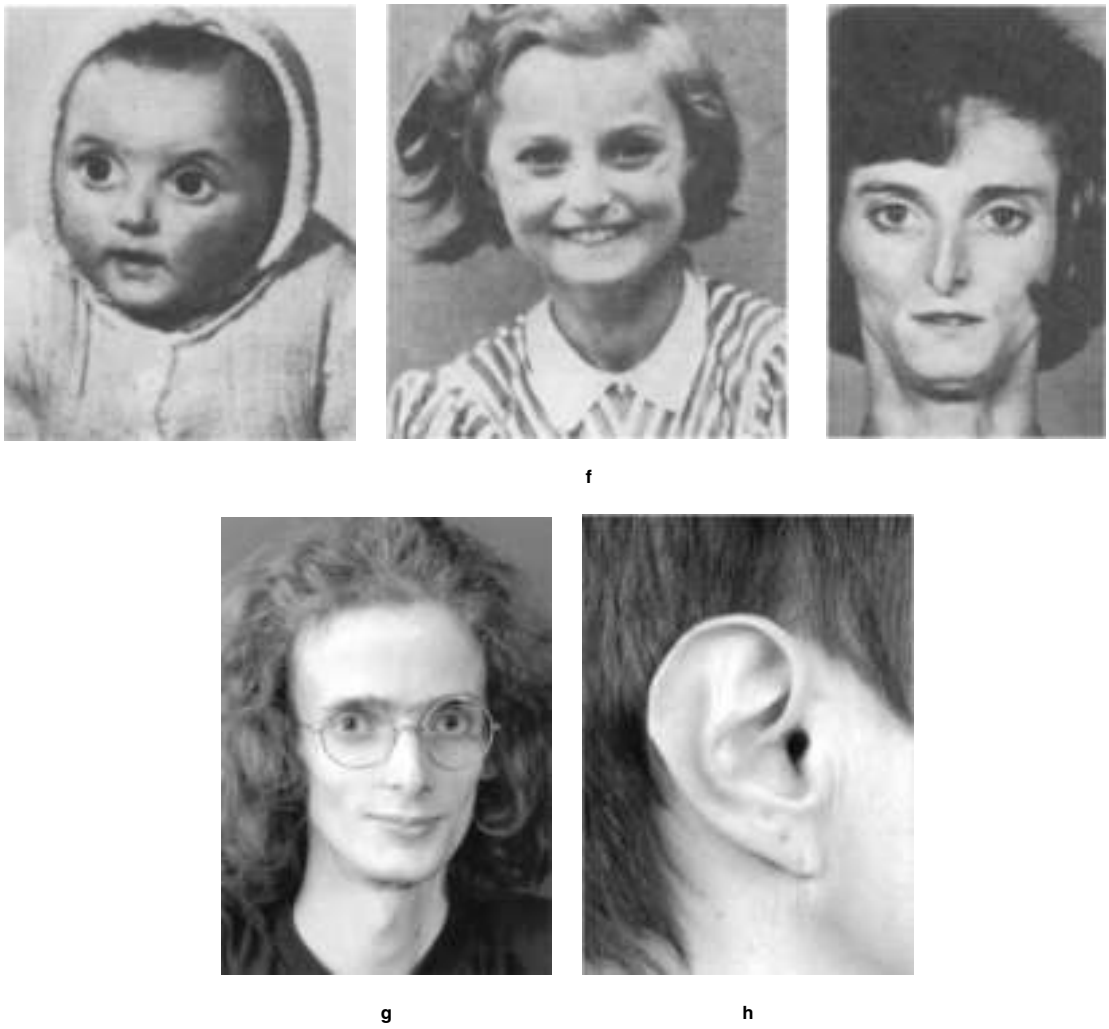


Figure 13. (*Continued*)

complications reported comprise 22 spontaneous hemorrhages, 17 aneurysms, five arterial dissections, and one arteriovenous fistula. The patients, 20 males and 20 females, had a mean age of 27.2 ± 10.9 years (range 11–63 years).

Bleeding seems to be more frequent in young males [255,256] and in patients during the postoperative period, and may occur at virtually every possible site and lead to symptoms such as sudden death, cerebral stroke, hemoptysis, hematemesis [244,253,257], renal colic and hematuria (see legend to Fig. 20) [219], acute abdomen, respiratory distress, retroperitoneal bleeding, and muscle swelling and shock. The most common locations of arterial bleeding are in the abdominal cavity and involve the small arteries rather than the aorta itself (Fig.15). In some individuals, there is evidence of aneurysmal dilatation and dissection or of arterio-venous fistulae, whereas in others, slit-like defects detectable at autopsy may occur in arteries that appear normal by angiography. In a review of the literature, Bergqvist found 112 cases in which vascular complications had been reported, and aortic dissection had occurred in 12 of these [261]. Acute myocardial infarction is a rare complication, reported in only eight cases, and is due to coronary dissection or rupture (see [262]).

Tortuosity of arteries has also been documented. Arterial rupture accounts for most deaths in EDS IV because it is frequent, the hemorrhage is rapid, and repair, even when timely, is difficult due to the marked friability of the tissues [255]. Carotid-cavernous sinus fistula formation and resultant exophthalmos has been described in several individuals [72,93,94,263–267]. Of 212 patients with carotid-cavernous fistulae treated by Halbach et al. [268], four had both EDS IV and spontaneous onset of their fistulae. Out of 202 individuals from 121 families with EDS IV proven by biochemical or molecular studies, 19 had cerebrovascular complications at a mean age of 28.3 years (range 17–48), of whom four died [269].

EDS IV has been proposed as a model of more common forms of aortic and intracranial aneurysms which often cluster in families, but, to date, most studies have excluded the *COL3A1* gene as the locus for such mutations in the absence of some findings of EDS IV [270–272]. However, there is evidence that a small number of cases of familial aortic aneurysm may be caused by collagen III deficiency [273–276] in the absence of significant skin changes. However, it must be noted that familial aortic dissection has been reported in EDS I as well [2]. The first

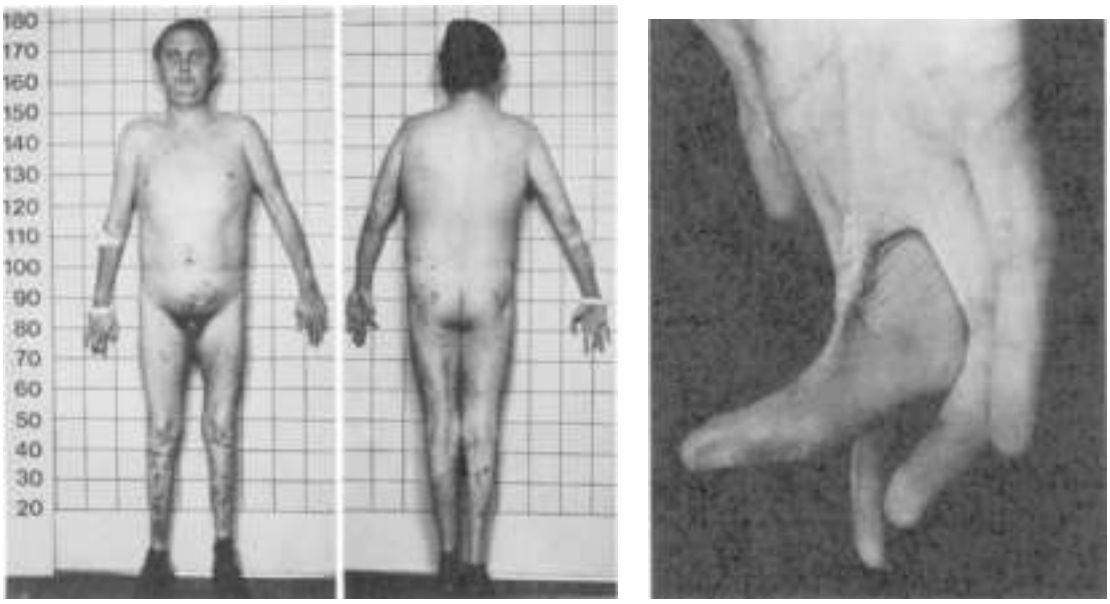


Figure 14. Vascular type of EDS (EDS IV). C.E. (3 Jul 1946, died ~35 years), at age 27 years showed atrophic scars on the shins, keloid formation[7] on the abdomen over the symphysis, numerous scars on the forehead and over the chin (**a**), and pterygium-like cutaneous flexion contractures of the thumb and the third finger of the right hand (**b**). For further references, see [34,212–214,216–219,244] and CRL 1299 and CRL 1409[6]. His molecular defect was a single nucleotide substitution (IVS25+5G>T) leading to skipping of exon 26 [227]. Sporadic case, bilateral clubfoot at birth; easy bruising since boyhood, several episodes of hemorrhage. (Reprinted from Steinmann [42] with permission.)

demonstration of microangiopathy in EDS IV is presented in Figure 16.

Rupture of the colon, usually in the sigmoid region, is the most common of the bowel problems and occurs at sites where the bowel surface appears normal. Constipation seems to play an important role in the pathogenesis of colonic perforation [279]. In a literature review spanning 20 years (see above), 41 colonic perforations among 44 gastrointestinal complications were cited: 33 of the sigmoid, four of the descending, two of the ascending, and one of the transverse colon, and one of the rectum. The mean age of patients with gastrointestinal complications was 31.5 ± 16.9 years (range 7–66 years), and the sample included 17 males and 13 females [254]. The small intestine rarely ruptures (for exceptions, see [279,280]), but intramural hemorrhage may lead to recurrent abdominal pain. Spontaneous esophageal rupture after vomiting — Boerhaave syndrome — has been observed only exceptionally [230,281,282].

Complications in late pregnancy, or during or after delivery, are not so rare and include vascular, intestinal, or uterine rupture (Fig. 17), vaginal lacerations, prolapse of uterus and bladder, and premature delivery because of cervical insufficiency or fragility of membranes [1,241,283–288]. In the largest survey of "classical" EDS IV individuals, pregnancy-related complications led to death in 9–15% of women who became pregnant [256].

Pulmonary complications may arise either from a primary defect in the lung parenchyma or from primary intrathoracic vascular rupture. Lung disease has been reviewed by Dowton et al. [289], who concluded that no death has been recorded as solely due to pulmonary manifestations of EDS IV.

Liver rupture is rare. A report describes the spontaneous rupture of a transplanted donor liver, which was most probably derived from an unrecognized individual with EDS IV [290]. After completing the caval and portal anastomoses, the liver was revascularized; within seconds, the donor liver developed multiple large subcapsular hematoma that spontaneously ruptured with extrusion of liver parenchyma. Despite efforts to obtain hemostasis, the liver continued to fragment, requiring hepatectomy (later retransplantation with another liver was successful). The donor was a 38-year-old woman with brain death secondary to subarachnoid hemorrhage. During collection of several internal organs, the right renal hilum was torn during removal of the kidney, and it was noticed that the heart valves were fragile and had to be discarded. Little was known about her personal and familial history except that her cousin had died from rupture of a visceral aneurysm. Attempts to culture fibroblasts failed, but electron micrographs showed that collagen fibrils from small hepatic arteries were smaller than normal in diameter and irregularly packed [290].

Descriptions of tissues as "fragile like wet blotting paper" are not uncommon in surgical and autopsy reports [2,291]. In the father of the propositus D.S. (Figs. 13e and 16), open chest cardiac massage resulted in avulsion of the heart from the superior vena cava and death [226]. For a similar case, see Krog et al. [292]. In two patients with shoulder dislocation, closed reduction and surgery resulted in the rupture of the brachial plexus (see also legend to Fig. 15) and rupture of both brachial plexus and brachial artery, respectively [85]. The degree of tissue friability differs among individuals and even in the same individual with aging [210]. An unusual combination of multiple aneurysms, a hepatic artery to portal vein fistula, and diverticula of the biliary passages, the sigmoid colon, and bladder has been reported in a patient possibly affected by EDS IV [293].

Natural history. In the largest survey [256] available of patients with "classical" EDS IV (comprising 220 index

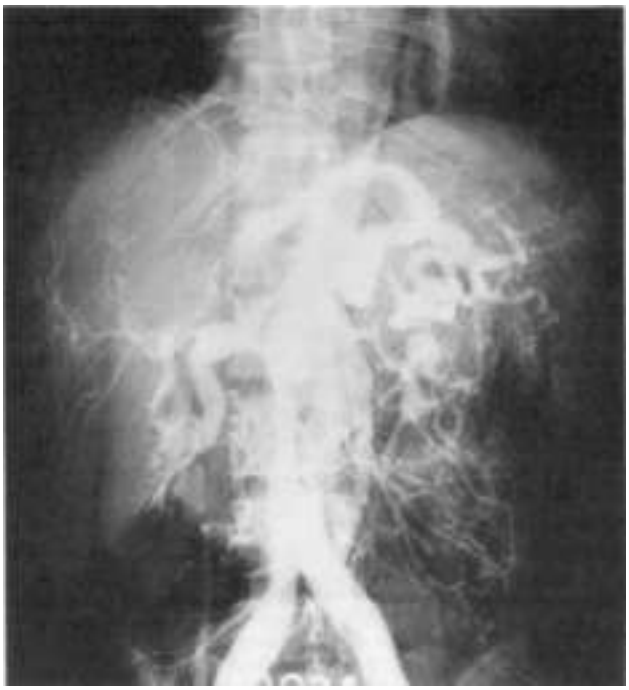


Figure 15. Diffuse dilatations of abdominal arteries and dissecting aortic aneurysms in vascular type of EDS (EDS IV). Y.P. (2 Dec 1941), mother of F.P. (see below), spent a normal childhood and adolescence and practiced many sports, including judo and horseback riding. Two deliveries at ages 24 and 29 years were uneventful. She always had skin of ''bad quality,'' a tendency to keloids and scar dehiscence; alopecia started at age 30 years. She had bilateral club feet, as has her daughter, needed early dental prostheses, and had hyperextensible elbow, knee, and foot joints (requiring arthrodesis), and recurrent luxations of both shoulders. Surgery on the left shoulder resulted in rupture of the brachial plexus at age 37 years.

At age $37\frac{1}{2}$ years, because of suspected inguinal adenopathy and deep venous thrombosis on the left side, she was given antibiotics, anticoagulants (Marcumar®), and anti-inflammatories (Tanderil®). Shortly thereafter, she was in deep shock, with bilateral hematothorax and bleeding in the abdominal cavity, the retroperitoneum, and the subpericardial and hepatic subcapsular spaces. After resuscitation, angiography was done (the diagnosis was still unknown at that time) disclosing diffuse dilatations of abdominal arteries and dissecting aortic aneurysms.

At age 42, she had a spontaneous rupture of the right posterior tibial artery, which was diagnosed by arteriography of the right femoral artery performed in a peripheral hospital. Because of the fragility of the artery, a repair with sutures was impossible and a simple ligation was performed; nevertheless, the leg remained viable [258]. She also had vertigo due to a vertebro-basilar syndrome; radiographically, both vertebral arteries were markedly calcified. Four years later, she suddenly experienced a heavy pain in the right groin and died in shock two hours later. Autopsy was not performed (own observation).

Her daughter, F.P. (21 Aug 1965), was shown to have a Gly595Cys substitution, which resulted in the formation of unusual trimers that contained, in addition to the normal disulfide bonds at the C-terminus, two proα1(III) chains which were disulfide bonded in the middle of the chains and, probably for ill-understood conformational reasons, migrated faster than the regular normal homotrimers [259]. By direct collagen analysis of chorionic villus biopsies, two prenatal diagnoses predicted normal fetuses, and she delivered vaginally and without further complications two babies who were confirmed to be normal [260].

Comments: (1) The first hemorrhagic event in Y.P. occurred shortly after beginning therapy with anticoagulants, nonsteroidal anti-inflammatory drugs, and penicillin. These drugs are likely to have promoted the hemorrhage and are contraindicated in EDS IV. (2) Although the second arteriography was uneventful, the lethal hemorrhagic event 4 years later could have been promoted by this intervention. (3) The general risk of arteriography was recognized in the university hospital but was not conveyed to the peripheral hospital; therefore, this type of information should be marked on a medical identification paper. (4) The risk of injuring the plexus brachialis while operating on the shoulder should also be noted.

patients with biochemically confirmed disease and 199 of their affected relatives), complications were rare in childhood; 25% of the index patients had a first complication by the age of 20 years, and more than 80% had had at least one complication by the age of 40 years. The calculated median survival was 48 years (range 6–73) and did not differ between men and women. The most frequent complications were, in decreasing order, arterial rupture (79%), organ rupture (uterus, heart, liver, spleen) (10%), and gastrointestinal rupture (8%). Bowel rupture was often amenable to surgical treatment and thus rarely fatal, while arterial or organ rupture was associated with higher mortality with or without surgical intervention. The different types of complications were not associated with specific mutations in *COL3A1* (Figs. 18 and 19).The relative frequencies of arterial and gastrointestinal complications were similar for the first and second complications; thus, the nature of the first complication does not predetermine the nature of the following complications. We have documented the condition in a woman, L.R., with a Gly43Asp substitution (Figs. 13 h and 20) [230], who reached the age of at least 62 years in spite of numerous complications, whereas another report describes sudden infant death [296]. Gilchrist et al. [297] described a large kindred with a low risk of pregnancy complications and

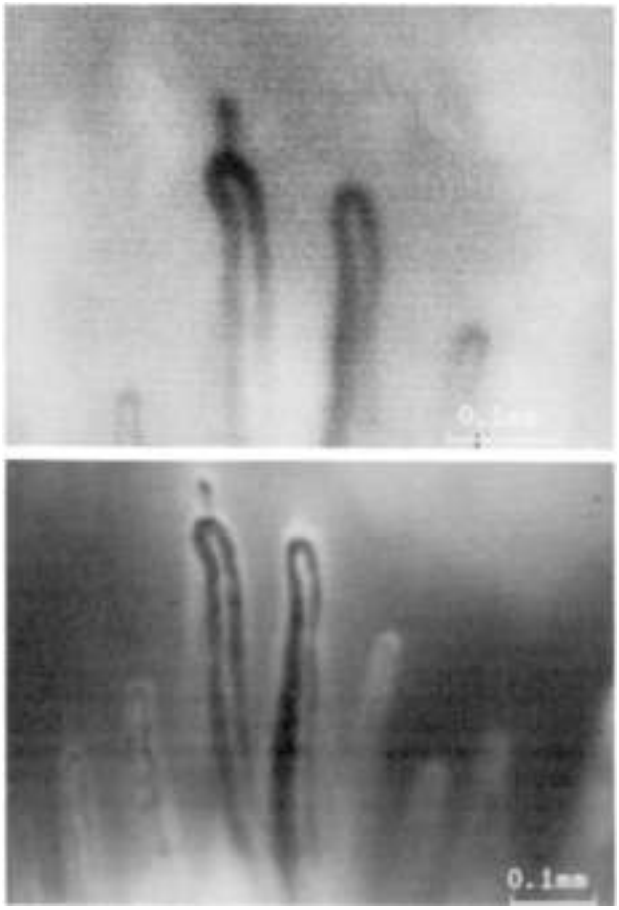


Figure 16. Microangiopathy in vascular type of EDS (EDS IV). In patient D.S. (see also Fig. 13e), intravital fluorescence microscopy of the nailfold was carried out with a fluorescence videomicroscopy system [277]. Before the application of dye, the morphology was examined under incident white light (**top**). Transcapillary diffusion was studied after i.v. bolus injection of sodium fluorescein (**bottom**), and the diameter of the capillaries of the arteriolar and venular limbs was measured after i.v. injection of indocyanin green (Cardiogreen®).

The microangiopathy consisted of multiple microaneurysms located at the apices of the capillary loops, which had a smaller neck and a larger head (indocyanin green); enlargement of the apical, arteriolar, and venular limbs of several loops; presence of microbleedings; and increased transcapillary diffusion of sodium fluorescein. Similar results were obtained in F.P. (legend to Fig. 15) [278].

The demonstration of microangiopathy in EDS IV indicates that the disease is not restricted to large vessels and thus points to a role of collagen III in the function of the capillary vessels.

unexpected longevity in some affected relatives; no deaths and few significant complications occurred among the eight affected women over a total of 30 full-term deliveries. For a family with a *COL3A1* mutation and apparent EDS III [209], see above.

The prevalence of EDS IV is not so low and is currently estimated to be 1:50,000. This estimate suggests that the frequency of mutations in the *COL3A1* gene is about the same as that of mutations in the collagen I genes that result in osteogenesis imperfecta, that the pleiotropic effects of the mutations obscure the diagnosis, and that, most unfortunately, knowledge about the disorder is still not widespread.

Defect and Pathogenesis

EDS IV is due to mutations in the *COL3A1* gene, located at 2q31-q32, which contains 51 exons distributed over 44 kb. The gene encodes a protein of 1,467 amino acids, of which 1,029 are located within the central triple-helical domain. The triple-helical domain is encoded by portions of 44 exons, of which 42 are cassettes that begin with a glycine codon and end with a Y-position codon, so that deletion of a single exon would result in an in-frame but shortened protein. The majority of published mutations in *COL3A1* result in substitutions of single glycine residues within the triple-helical domain, approximately one-third result in exon skipping, and a small number are larger genomic deletions [220,256,298] (for a list of over 200 mutations and numerous polymorphisms in *COL3A1*, see the Web site http://www.le.ac.uk/genetics/collagen/col3a1.html — see "Appendix", below).

Because procollagen III is a homotrimer, the synthesis of an equal number of normal and mutant α1(III) chains results in 7/8 of the collagen III molecules produced being abnormal and containing one, two, or three mutant chains. The abnormal collagen III leads to a quantitative deficiency of collagen III and sometimes, more deleteriously, to disturbed fibril formation by the remaining normal collagen III molecules. Therefore, tissues normally rich in collagen III, such as skin, blood vessels, and internal organs, are affected, in contrast to bone and cartilage, for example, which lack collagen III. In all cases studied at the molecular level, and in many less well-characterized patients, structural defects in collagen III lead to impaired secretion, intracellular storage and degradation, or lower stability of the secreted molecules, or both.

Different types of mutation most certainly have different consequences.

(**1**) In the case of glycine substitutions, triple-helix formation of molecules containing one, two, or three mutant α1(III) chains seems to be delayed for sterical reasons, as shown directly for abnormal collagen I in fibroblasts from patients with osteogenesis imperfecta [299] and suspected for collagen III in cells from EDS IV patients [300]. As a result, abnormal collagen III is overmodified and secreted only slowly or not at all and is degraded intracellularly to a significant extent [301,302].

(**2**) In the case of mutations leading to exon skipping, the abnormal molecules contain one, two, or three shortened chains, there is efficient secretion only of normal homotrimers and abnormal, shortened homotrimers, which are deposited efficiently into the extracellular matrix. Among individuals with exon skipping due to a single base substitution, Schwarze et al. [303] identified only two among 28 in whom the splice-acceptor site was affected, Pope et al. [220] two among five, and Giunta and Steinmann [230] one among four. The underrepresentation of splice-acceptor site mutations suggests to the current authors that the usual consequence of such mutations is the use of an alternative acceptor site that creates a null allele with a premature termination codon and thus leads to a 50% reduction in normal collagen III and a phenotype that may escape medical attention (see "Recent Developments").

(**3**) Splicing efficiency has been shown to be temperature-dependent in one patient, C.E., in whom a G to T transversion at position +5 of intron 25 resulted in skipping

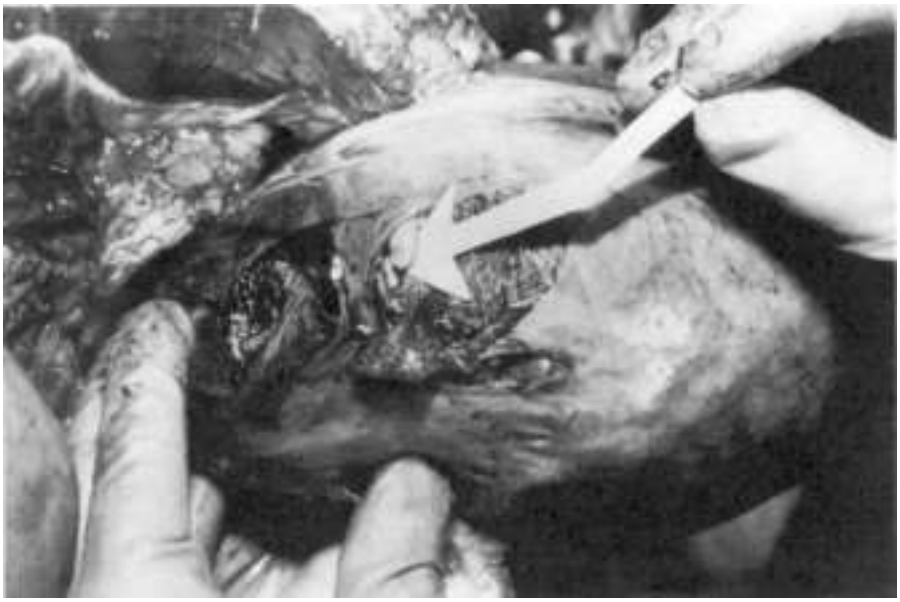


Figure 17. Rupture of the gravid uterus in vascular type of EDS (EDS IV). N.K., with a negative family history of EDS IV, had suffered spontaneous rupture of her sigmoid colon after an 8 week gestation at age 31 years; a therapeutic abortion was performed at the time of operation. Two years later, she was readmitted at 28 weeks gestation in premature labor and died shortly thereafter. At necropsy, the tissues were unusually easy to dissect. The thoracic aorta was torn transversally above and below a large dissection. The uterus had a complete, irregular 4 cm tear of the myometrium. The arrow points to fetal fingers stretching out of the torn uterus. It is of note that death may also occur several days or weeks postpartum [230]. (Reprinted from Rudd et al. [283] with permission.)

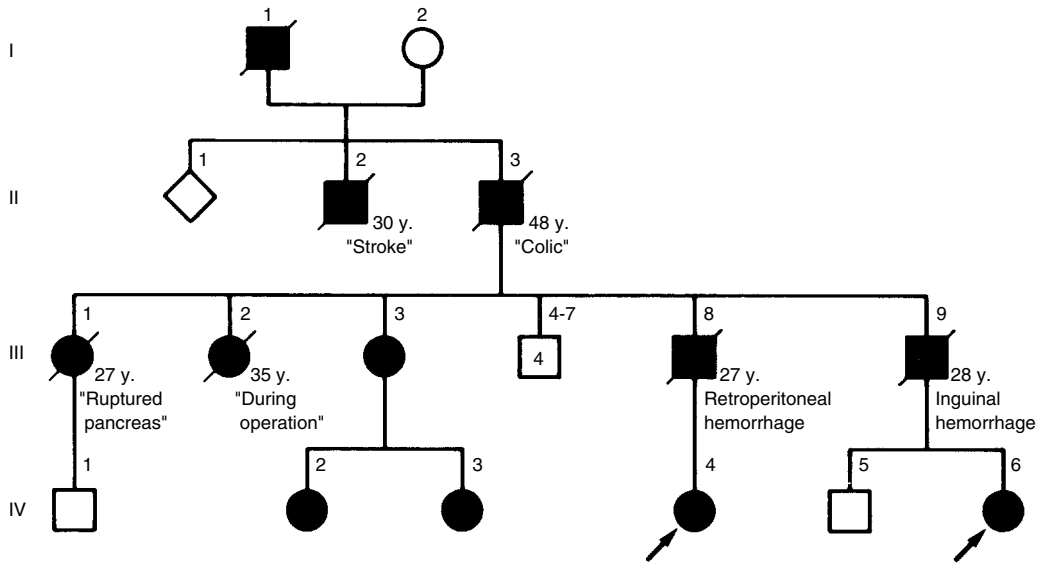


Figure 18. Pedigree of a family with vascular type of EDS (EDS IV). The index cases (**arrows**) came to medical attention in childhood because of easy bruising; the clinical diagnosis of EDS IV had earlier been made in subject III-9, who presented with recurrent bacterial meningitis due to bony defects in the skull [249]. The pedigree illustrates the manifold causes of death in EDS IV at various ages. It is also noteworthy that III-1 and III-3 brought pregnancies to term without major complications. The unaffected wife of individual II-3 could readily tell who among her nine offspring had the disease.

The molecular defect in this family consists of a Gly1003Asp substitution, which leads to a markedly reduced secretion of collagen III with intracellular retention and overmodification [259]; serum collagen III aminopropeptide is low in the two index patients [228]; for morphological studies, see [294,295].

This family originates from a small village in southern Italy where it was known as ''the family with the thin skin.'' The social implications of the disease are illustrated by this nickname and by the fact that the father of two young women tried (in vain) to dissuade them from marrying two brothers from the family (III-8 and III-9) because it was known that ''something was wrong'' with this family.

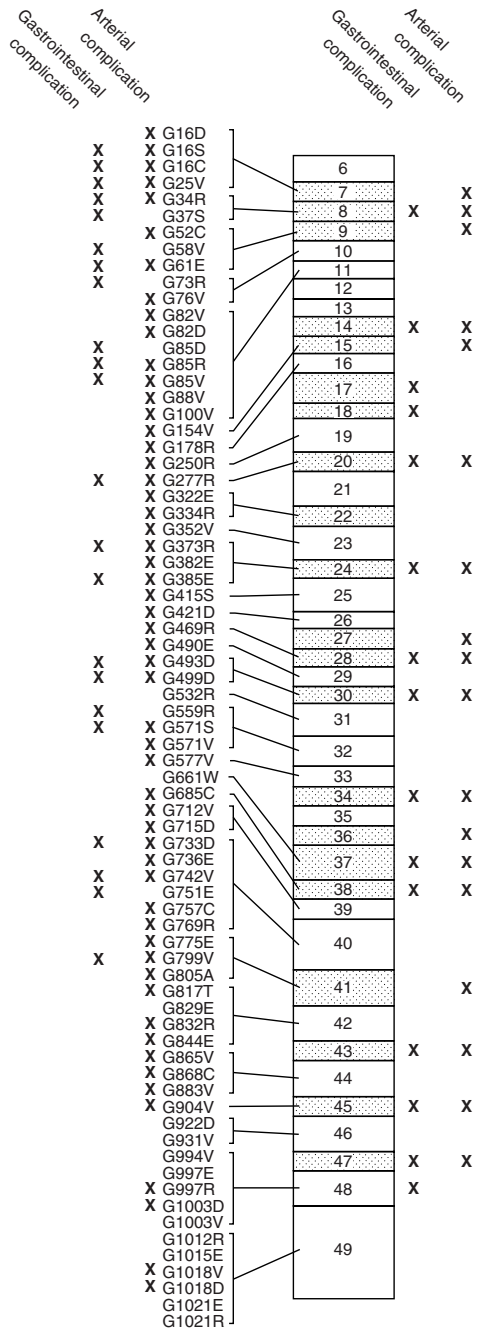


Figure 19. Lack of relationship between the nature and location of mutations in *COL3A1* and the types of complications in the vascular type of EDS (EDS IV). Causative mutations in the *COL3A1* gene were identified in 135 index patients, among whom there were 73 different mutations in 85 index patients that resulted in the substitution of some other amino acid for glycine (G) within the triple helix. Mutations in 41 patients led to skipping of a single exon (indicated by the stippling). The remaining mutations were more complex. The presence of an X at the site of a mutation indicates that one or more patients with that mutation had a complication of the indicated type. The amino acids are numbered from the first glycine of the major triple helix, which is residue 168 of the preproα1(III) chain. A denotes alanine; C, cysteine; D, aspartic acid; E, glutamic acid; R, arginine; S, serine; V, valine; W, tryptophan; and T, threonine. (Reprinted from [256] with permission.)

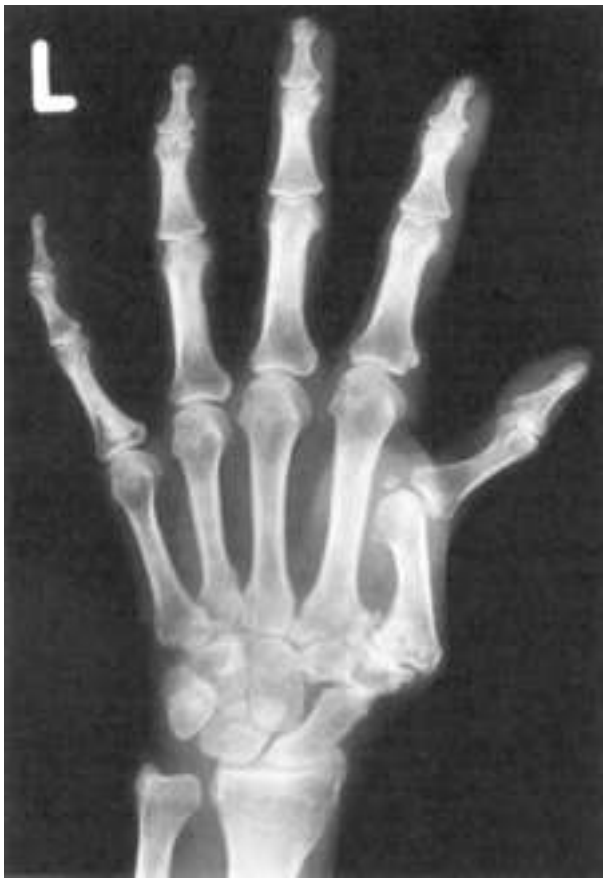


Figure 20. Vascular type of EDS (EDS IV). The x-ray of the left hand of L.R. (8 Jul 1928), at age 62 years, shows a chronically dislocated carpo-metacarpal joint of the thumb, marked arthritic degeneration of all joints, and a lack of adipose tissue.

Since the age of 50 years, recurrent luxations of the first metacarpal joint and arthritis of the trapezio-metacarpal joints had precluded a pinch grip, the picking up of large or heavy objects, and unscrewing bottle tops.

The patient was a sporadic case, born at term with a birth weight of 1.8 kg. Since early childhood she had suffered from easy bruisability, skin fragility, and mild joint laxity. She had had strabismus, hemopneumothorax, and hip dislocation after a car accident at age 29 years, the uneventful delivery of a healthy girl, an episode of ''nephrolithiasis'' due to a dissecting renal artery at age 45 years, several stroke-like episodes, and diverticulosis of the large intestine. Now she presented with a younger-looking face, often being asked if she had had a face-lift, with tight and lobeless ears (Fig. 13h); her skin was thin, and she had acrogeric hands and feet and suffered from the Raynaud phenomenon (for references and cell strains, see [213,214,217–219,228,230] and CRL 1384 and CRL 1397)[6]. Her condition was due to a Gly43Asp substitution [230].

of exon 25, which was almost corrected when his cells were incubated at a lower temperature [244]. The fact that his cells produced more collagen III at a temperature lower than 37°C is therefore due to a combination of reduced thermal stress on helix formation (see below), and hence rescue of mutant collagen [213], and a decrease in exon skipping [244]. An inverse temperature-splicing relationship has also been reported in a further patient [304]. (Another case of temperature-dependent splicing has been described in a patient with EDS VIIB [36,305].)

(4) Larger in-frame deletions may also lead to the secretion of two main populations of collagen III molecules, normal-sized and shortened ones. Patient B.H. had a 9.0 kb deletion spanning from intron 33 to exon 48 of *COL3A1*, and half of her α1(III) chains lacked the sequence corresponding to residues 586–999 [219,306,307]. Procollagen III molecules composed of either three normal length or three shortened chains were thermally stable and efficiently secreted, whereas those containing one or two shortened chains were unstable and completely excluded from secretion. Failure to secrete unstable molecules, and a possibly residual functional role of the stable "minicollagen III," which lacks the collagenase cleavage site, may explain the milder phenotype of this patient [306,307] compared with another EDS IV patient, D.S., who bore a similarly sized deletion toward the amino-terminal end of the α1(III) chain [226,227]. However, this concept of a possible role of the shortened homotrimeric collagen III *in vivo* may be challenged by the report of a 14-year-old sporadic patient [308] whose mutant collagen III lacked exon 41 and hence also the collagenase cleavage site; his mutant homotrimeric collagen III was efficiently secreted, not cleaved by collagenase, and had normal thermal stability. In the small skin biopsy specimen available, there was only 11% of the normal amount of collagen III; however, there was no evidence that the mutant homotrimers, which would be resistant to mammalian collagenase digestion, had accumulated in the dermis [308]. In another cell strain showing skipping of exon 17, Chiodo et al. [309] showed that the mutant shortened homotrimers were secreted normally and had a normal thermal stability but were not incorporated into the extracellular matrix of an *in vitro* model of dermis.

The stability of the triple helix of procollagen III in EDS IV is reduced as judged by its lower melting temperature when probed with proteinases. Approximately one-eighth of procollagen III molecules, composed of normal α1(III) chains, are secreted normally, which indicates that there is no defect in any putative secretory mechanism for procollagen III. Incubation of cultured fibroblasts at 30 °C decreases the thermal stress on helix formation and can increase the secretion of molecules that contain the abnormal chain for some, but not all, mutations [213]. The possibility that the presence of acrogeria correlates with the secretion of a thermally unstable collagen III, the extremities having a lower temperature than the core of the body, is intriguing but is at variance with findings in fibroblasts from the "acrogeric" patient, P.P. (Figs. 12b and 13a), from which no secretion of such unstable procollagen III could be demonstrated. One mutant cell strain did secrete abnormal collagen III almost as efficiently as normal [214]; however, it was extremely sensitive to proteases due to its Gly790Ser substitution (for further references concerning this case, see [228,310,311]).

Morphologic and Functional Aspects

Morphologic aspects. There have been only a few autopsies in which EDS IV-specific changes have been studied. Collagen III deficiency is most evident in the connective tissue scaffold of the blood vessel walls, dermis, intestine, lungs, and liver, and even in intervertebral disc tissue, where normally only little collagen III is present. The total amount of collagen extractable from lung and liver expressed per gram dry weight is similar in patients and controls, whereas the fraction of collagen III in pepsin-solubilized material is lowered to 3–4% of total collagen as compared with ~20% in control tissues [252,312,313]; in uterine leiomyoma tissue from an EDS IV patient, collagen III as determined by CNBr digestion was ~25% of that of controls [314]. Immunofluorescent staining of retained procollagen III with anti-procollagen III antibodies has been demonstrable in most fibroblasts [232,315], except in a few earlier reports [212,316]. In one case, C.E., the failure to stain [212] is clearly at variance with the biochemical evidence of retention of procollagen III by his fibroblasts [213].

Although collagen III constitutes only 10–22% of the total collagen in normal skin [317,318], at all levels of the dermis [319], its reduction in patients with EDS IV has a dramatic effect on both the thickness and architecture of their skin. In some patients, the skin measures only a quarter of the normal thickness and the reticular dermis is nearly identical in architecture to normal papillary dermis [233]. Collagen fiber bundles are small, and fibril diameters are either uniformly small or show marked variation. Elastic fibers are relatively abundant because of the decreased amount of collagen in the dermis. Because in the skin of normal fetuses below 20 weeks of gestation collagen III is the major collagenous protein present [317], it seems likely that it is important for the formation of a normal scaffold that can be elaborated upon by the subsequent synthetic activity of dermal fibroblasts and that its deficiency leads to the abnormal tissue structure observed in affected individuals. This notion is supported by the finding that all collagen fibrils in skin contain both collagens I and III, so-called heterotypic collagen fibrils [320]. The solubility of collagen in the lungs is markedly increased, which might also reflect an altered fibrillar organization resulting from a decreased content of collagen III [252].

Laurent and Agache [321] performed an early ultrastructural study demonstrating an engorged rough endoplasmic reticulum in fibroblasts from the dermis of an affected individual and suggested defective secretion of an unknown substance. This observation was crucial for the concept of Byers et al. [232,233,246] of impaired secretion of structurally abnormal procollagen III. Since then, dilatation of the endoplasmic reticulum in fibroblastic cells from skin [226,233,294,295,322] as well as in the lung [252] has been demonstrated in numerous other reports. Smith et al. [323] studied skin from 22 individuals with EDS IV in whom the *COL3A1* mutation had been identified. Dermal thickness ranged from 0.66 to 1.54 mm in affected skin compared to a mean of 1.25 ± 0.25 mm in 14 controls, elastic fibers were proportionally increased in relation to collagen fibers, and the collagen fibers themselves were finer and more loosely organized, as observed by scanning electron microscopy. The study suggested that different mutations in the *COL3A1* gene may have different effects on such parameters as secretion, fibrillogenesis, and skin architecture, depending on the nature and location of the sequence changes they induce. Substitutions for glycine and exon-skipping mutations at the C-terminal end of the triple helix led to very marked intracellular accumulation of mutant collagen III in the rough endoplasmic reticulum and to a considerably smaller than normal collagen fibril diameter (65–80 nm versus 92 ± 7.5, range 95–110 nm, respectively), as had been observed previously [193]. In contrast, mutations near the N-terminal end were associated with a more variable fibril diameter (85–120 nm), and there was less evidence of intracellular retention of abnormal collagen III [323]. The cross-sectional shape of fibrils often deviated from the regular rounded profiles of controls, but the changes were relatively subtle and different from the composite fibrils found in EDS I and

II [40,192,193]. In postcapillary venules in EDS IV skin, there was increased perivascular matrix and pericytes had dilated rough endoplasmic reticulum [323]. Why smooth muscle and endothelial cells, which also normally synthesize collagen III, do not have a similarly altered rough endoplasmic reticulum in the same patients is not clear [233,323]. Several features of EDS IV, namely the characteristic facial appearance, the frequently observed low birth weight [221], and clubfoot, are suggestive of a primary, morphogenetic defect. If one considers the preponderance of collagen III in the embryo, it is surprising that even more severe defects are not observed.

Because arterial ruptures are the most severe complications in EDS IV, arterial morphology is of interest. Arterial vessels may have a small bore and a thin wall, and their total collagen content is markedly reduced [34,324,325]. The adventitia is thin, and in the media collagen fibrils are diminished in number, while elastic fibrils are irregular, fragmented, and accumulated. The elastin appears accordion-pleated with a high waviness index, this being the ratio of the length of elastin in the internal elastic lamina to its circumference [326]. The average collagen fibril cross-sectional area was decreased in the media of all arteries and in the adventitia and intima of some arteries, whereas it was increased throughout the vena cava [325]. Immunofluorescence studies on tissues from patients whose fibroblasts secreted only 10% of the normal amount of collagen III did not reveal an abnormal distribution of collagen III, nor was the intensity of staining indicative of the amount of material deposited in the tissues of L.R. and C.E. [327] (K. von der Mark and B. Steinmann, unpublished observations, 1985). There have been only a few studies of the architecture of the gastrointestinal tract or of most other organs. Collins et al. [328] reported that even in areas away from rupture sites, the bowel wall was thin because of diminished submucosa and muscularis propria; similarly, the walls of the blood vessels in bowel submucosa and elsewhere in the abdomen varied in thickness and contained quite striking frayed and fragmented elastic tissue fibers.

Functional aspects. Only a few studies have been devoted to functional aspects of tissues from EDS IV patients. Nemetschek et al. [329] have shown by electron microscopy that the elastic lamellae of the media of an affected aorta seem to be rather isolated from the surrounding collagen fibrils, in contrast to control specimens, in which they are in close contact with the collagen fibrils. Because stress-strain curves show an increased stiffness of the affected arterial wall, the authors speculated that collagen III is involved in the optimal integration or anchoring of elastin in the arterial wall. In contrast, Østergaard and Oxlund [330] found an increase in the extensibility of the middle cerebral artery of patients not specifically classified as having EDS IV but dying from the rupture of intracranial saccular aneurysms at stress values between 100 and 200 mm Hg, but no such extensibility was found in brachial arteries of the same patients despite a deficiency of collagen III. Holzschuh et al. [331] determined by Doppler sonography the pulsatility index (i.e., the difference in flow velocity between systole and diastole compared with an overall mean flow velocity throughout the cardiac cycle) in the cerebral arteries of a 34-year-old woman and showed that it was lower than that in controls; they concluded that the elasticity was increased, which may favor the formation of aneurysms.

Abnormal myoelectric activity was measured *in vitro* in sigmoid and descending colon from an affected individual with colonic perforation, which suggested to the investigators concerned a possible link between abnormal myogenic activity and colonic perforation [332].

Genotype-Phenotype Correlation

At present, there is no clear correlation between the nature and location of mutations within the *COL3A1* gene and the clinical features of EDS IV. The most likely explanation of failure to find such a correlation is that the criteria for inclusion in studies require that most of the clinical findings be met. Thus, the range of phenotypic variation is likely to be small and may be limited to minor features such as acrogeria.

The phenotype to be expected for *COL3A1* null alleles is uncertain. In a small number of families, the synthesis of procollagen III is reduced, perhaps to only about half the normal level, and there is no evidence of intracellular storage. Such a biochemical phenotype would be expected to result in a milder clinical phenotype than classical EDS IV [333]. If these preliminary studies are confirmed, this type of defect would establish a distinct subclass analogous to mild osteogenesis imperfecta (OI type I, see Chapter 8, this volume). The negative results of two large studies of individuals with abdominal aortic aneurysms [271] and cerebral aneurysms [272] make it unlikely that *COL3A1* null alleles are frequent causes of these conditions. Also, heterozygous mice with a *COL3A1* null allele generated by targeted gene inactivation appear phenotypically normal [334] (see "Animal Models and Lathyrism" and below). Given that null alleles of the *COL1A1* gene result in the mildest form of osteogenesis imperfecta [335], type I, milder or late-onset visceral involvement may yet be a candidate phenotype for the *COL3A1* null allele phenotype. It will be interesting to see whether the so far underrepresented mutations that affect the splice-acceptor site, supposedly leading to null alleles [220,230,303], will be detected (see above); such an approach will have to be made by direct molecular genetic means because the ability to discern heterozygous null cells by protein assay is limited (see "Diagnosis"). A putative "mild" *COL3A1* mutation (Gly637Ser) has been associated with an EDS type III-like phenotype without arterial disease in a single family, but the phenotypic characterization of this family was incomplete [209] (see "Recent Developments").

Genetics

EDS IV is a dominantly inherited disorder, with a significant proportion (~50%) of cases representing new mutations [215]. As a rule, each patient or family has its own specific mutation. No paternal age effect has been demonstrated. Early linkage analysis studies showed cosegregation of the phenotype with *COL3A1* alleles [336,337].

Parental mosaicism has been documented in several instances. A clinically healthy father with an affected daughter was shown to be mosaic for a 2 kb deletion in *COL3A1*, which was present in only 10% of his leukocytes but 40% of his fibroblasts [280]. This difference in proportions of normal and mutant alleles between white cells and skin fibroblasts could have resulted from a difference in the allocation of cells early in embryogenesis or, alternatively, may reflect sampling from a clonally derived region in skin enriched for cells containing the mutant allele. If progenitor cells that contained the mutant allele were not selected against, and if they divided at the same rate as normal cells, then the proportion of cells with mutant alleles in blood indicates that the mutation occurred prior to lineage determination. Further cases of mosaicism

have also been documented [310,338,339] or have to be presumed or postulated because of the oligosymptomatic founder [209,230].

Autosomal recessive inheritance has never been demonstrated. On the basis of a study of two cases in which the putatively heterozygous parents were found to have apparently decreased levels of collagen III in skin compared with controls, and whose fibroblasts in culture seemed to secrete lower than normal quantities of procollagen III, it was suggested that the mode of inheritance of EDS IV was autosomal recessive [216]. This proposal is, however, invalid because it relied on the use of DEAE-cellulose column chromatography, which is markedly insensitive as a quantitative technique because of incomplete recoveries. More recent reinvestigation by SDS-polyacrylamide gel electrophoresis has disclosed that the cells of the first case, P.P., secrete approximately 10–15% of the normal amount of procollagen III and that small amounts of heavily overmodified procollagen III are retained within the cells. Normal amounts of mRNA with normal translation activity are detectable [217,226]. The demonstration of a heterozygous Gly1021Arg substitution in this case ends this debate definitively [220]. The second case, C.E., has since been defined as heterozygous for a point mutation leading to exon skipping [244]. The existence of a recessively inherited variant of EDS IV remains to be proven and, if such were confirmed, would appear to be unrelated to defects in collagen III [340].

The division originally proposed by Byers et al. [246] into subtypes A, B, C, and D is no longer meaningful because distinct biochemical and ultrastructural phenotypes can arise from different mutations at the *COL3A1* locus.

Diagnosis

This type of EDS has the worst prognosis, and biochemical confirmation should be sought, although analysis is labor-intensive and expensive. For this reason, careful examination of the patient is mandatory. The diagnosis is made by demonstration of structurally abnormal collagen III and/or direct mutation analysis (see "Recent Developments").

(1) Structurally abnormal collagen III produced by ascorbate-stimulated cultured skin fibroblasts or cells derived from noncutaneous tissues, such as artery, vein, or peritoneum [225], leads first to decreased overall production, defective secretion into the culture medium, and retention within the cells; second, to post-translational overmodification as judged by a slower electrophoretic mobility on SDS-polyacrylamide gels, especially of the portion retained within the cells; and, third, to lowered thermal stability and abnormal sensitivity toward proteases (Fig. 21). Procollagens or pepsin-treated collagens from culture medium and cell layers are best analyzed separately after radiolabeling the cells at 30 °C in addition to the normal temperature of 37 °C [213]. Culture conditions should also be standardized because cell density influences the ratio of collagen III to collagen I synthesized [218,342]. Because an apparent reduction of collagen III may occasionally be found in control fibroblasts, the demonstration of collagen III with abnormal stability or electrophoretic mobility is a better diagnostic criterion than quantitative measurements. Collagen III produced by fibroblasts from one large family with EDS IV was originally reported to be normal [336]; however, a more extensive analysis of the CNBr-derived peptides of collagen III revealed the abnormal migration of peptide α1(III)CB5 [343] due to a 27 bp deletion in exon 37 [344]. It should again be stressed that heterozygotes for a null allele would most probably be missed by this approach and that this may be one reason why such individuals have never been diagnosed; of course, an alternative explanation is that such individuals do not present an abnormal phenotype and thus escape medical attention (see "Recent Developments").

(2) Searching for a reduced amount of collagen in pepsin or cyanogen bromide extracts of skin is not recommended because of the excessive amounts of material required and the unreliability of results with regard to quantitative changes.

(3) Many cases of EDS IV can be detected by quantitative radioimmunoassay of the serum level of the procollagen III aminopropeptide (PIIINP), which is released during the conversion of procollagen to collagen. Indeed, the amount of procollagen III aminopropeptide is low in a major subset of patients with EDS IV whose fibroblasts secrete only little procollagen III [228]. However, this approach is experimental and not generally recommended because of biological variability, confounding concomitant conditions, and the analytical modification of the assay necessary for the detection of low serum procollagen III aminopropeptide levels [39,228]. Whether measurement of PIIINP in skin interstitial fluid (i.e., suction blister fluid), which is independent of its clearance by the liver, is superior remains to be proven [345]. Prolonged bleeding time has been used at the beginning of a study to ascertain family members [346], but this approach is to be discouraged because bleeding time is prolonged in most types of EDS and because it is an unreliable test.

(4) Direct molecular genetic analysis of the *COL3A1* gene will become faster and will provide a high analytical efficiency, especially when combined with the biochemical analyses obtained using cultured cells [230].

Differential Diagnosis

Excessive bruising is found in several coagulation disorders (hemophilias; von Willebrand disease [MIM 193400, MIM 277480]), platelet disorders (including leukemia), scurvy, and nonaccidental injury ("battered child") [347].

Poor wound healing is a feature of scurvy, dysfibrinogenemia (MIM 134820), and factor XIII deficiency (MIM 134570).

Ruptures of arteries occur in the Marfan syndrome (MIM 154700), arterial tortuosity (MIM 208050), isolated multiple cerebral aneurysms with or without polycystic kidney disease (MIM 173900) (for discussion, see Leblanc et al. [270]), arterial dissection with lentiginosis (MIM 600459), different forms of cerebral cavernous malformations (CCM1, CCM2, and CCM3; MIM 116860, MIM 603284, and MIM 603285, respectively), and hereditary cerebral hemorrhage with amyloidosis (MIM 105150) and have also been documented in mild osteogenesis imperfecta [348]. Spontaneous coronary artery dissection (MIM 122455), a rare event, occurs in relatively young persons, with a striking predilection for women. The association of lengthening and tortuosity of systemic, pulmonary, and coronary vessels together with skin and joint laxity and an elongated face seems to be an autosomal recessive disorder [197].

Recurrent pneumothoraces occur in the Marfan syndrome or as an isolated familial disorder (MIM 173600). Periodontal disease is also a major finding in the questionable entity EDS VIII [39]. Acrogeria of Gottron (MIM 201200) [349] and mandibuloacral dysplasia (MIM 248370) [350] are characterized by atrophic skin localized to hands and feet, but without translucent thin skin, mottled hyperpigmentation of

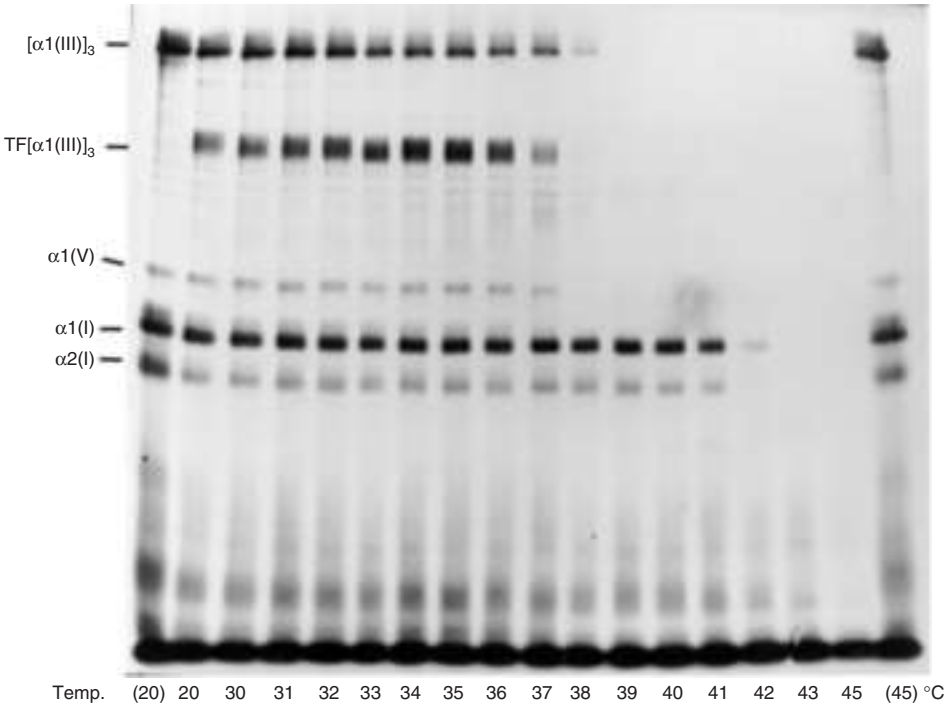


Figure 21. Abnormal thermal stability of collagen III in a patient with vascular type of EDS (EDS IV). Thermal stability of collagens produced at 37 °C in the cell layer of fibroblast cultures from Ch. W. Aliquots of pepsin-purified collagens were heated to the temperatures indicated, incubated with trypsin for 2 minutes, subjected to SDS-polyacrylamide gel electrophoresis, and visualized by fluorography (as a control, trypsin was omitted from the samples incubated at 20 °C and 45 °C, indicated by brackets). The results are summarized as follows.

First, the proportion of collagen III in relation to collagen I is increased, indicating cellular retention of abnormal collagen III molecules [341].

Second, the band representing collagen III is broadened as a result of overmodification caused by slower helix formation.

Third, incubation with the proteolytic enzyme produces a tryptic fragment (TF[α1(III)]$_3$) of abnormal molecules not seen in control preparations. This fragment loses helical conformation at approximately 37 °C. Moreover, [α1(III)]$_3$ trimers in this preparation are completely digested at 37–38 °C, whereas control [α1(III)]$_3$ trimers are resistant up to 39 °C (not shown).

Fourth, the thermal stability of collagen I (41.5 °C) and collagen V (37 °C) is identical to that in control preparations and serves as an internal control. (Reprinted from [259] with permission.)

the skin, thickened nails, micrognathia, and atrophic skin on the tip of the nose, there is no bruisability and no tendency to rupture of internal organs or vessels, and collagen III production is normal [351]. In the literature, the term acrogeria of Gottron has, unfortunately, often been used synonymously with EDS IV and *vice versa* [235,352,353]. Occasionally, patients with similar phenotypes, which may be dominantly or recessively (MIM 225350; MIM 225360) inherited, but with apparently normal collagen III metabolism, may be found ([215,340]; own observations).

Treatment and Management

Because causal therapies are not available, medical intervention is limited to symptomatic therapy, prophylactic measures, and counseling. Because of the danger of sudden arterial hemorrhage and bowel perforation, and the high risk associated with surgery, patients with EDS IV should carry a medical identification paper noting information about their diagnosis, possible complications, and blood group. General recommendations for anesthesia [265] and surgery [328] have been formulated; these include cross-matching of adequate amounts of blood, avoidance of intramuscular premedication, establishment of an adequate peripheral intravenous access and avoidance of arterial lines and central venous catheters if possible, control of hypertension, and gentle intubation maneuvers.

EDS IV represents a formidable challenge to the cardiovascular surgeon. The management of bleeding should be conservative as long as possible, especially when it is interstitial (muscular, retroperitoneal) [254,255,292]; bleeding in the abdominal cavity usually requires immediate transfusion and surgery. When operative therapy is required, minimal vessel dissection with balloon catheter or tourniquet occlusion should be performed, and vessel loops and vascular clamps, which frequently produce injuries that cannot be repaired, should be avoided. Standard anastomoses similarly often fail because of the poor tensile strength of the blood vessels. Primary arterial repair, if attempted, should be tensionless, using interrupted horizontal mattress sutures reinforced with pledgets. Vessel ligation with umbilical tapes, however, appears to be the safest operative therapy. Bypass grafting is then performed only if distal ischemia develops [254]. A review of the treatment of spontaneous carotid-cavernous fistula and outcome is given in [354].

Angiography should be avoided because of possible severe morbidity and frequent mortality [94,95,254,255,292] and replaced by ultrasonography [355] and/or subtraction angiography, although even this procedure is not free of risks [356].

The stripping of varicose veins may be difficult because they tear readily [312]. Surgical repair or embolization of carotid-cavernous fistula has been attempted with success in some patients [268,357].

Because of the high recurrence rate of distal colon perforation (15 cases out of 41 [254]), it is advisable to perform total colectomy and ileostomy when the first episode of perforation occurs [254,284].

Pregnancy in women with EDS IV should be closely monitored. Whether bed rest after 32 weeks of gestation and elective Cesarean section before incipient labor, to avoid the risks associated with vaginal delivery, should be recommended is still unclear [220,283,358]. It is worthy of note that sudden death may also occur several days postpartum. In the largest survey of "classical" EDS IV individuals, pregnancy-related complications led to death in 9–15% of women who became pregnant [256].

Because cerebrovascular complications occur in a minority of patients, screening for aneurysms is not warranted, and the risk of surgery would preclude intervention before the development of symptoms. Nevertheless, the occurrence of neurological symptoms, including headache, in individuals with EDS IV should alert the physician to exclude intracranial vascular pathology by noninvasive methods.

The management of hemopneumothoraces and thoracic cysts seems to be satisfactory using standard approaches [289].

Cough and constipation should be prevented by the timely use of antitussives and high-fiber diets and laxatives; enemas should not be given because colonic distention may result in perforation.

Many commonly used drugs interfere with platelet function, either alone (e.g., acetylsalicylic acid, nonsteroidal anti-inflammatory drugs) or in combination (penicillins and cephalosporins), and should be avoided or used with caution; paracetamol, pyrazolone derivatives, or synthetic opioids can be used instead. Anticoagulation therapy for venous thrombosis, or for the prevention of thrombosis during prolonged bed rest, should be avoided because it may lead to fatal bleeding (own observation; see legend to Fig. 15). In one patient, the administration of aspirin had to be discontinued because of a marked increase in spontaneous bruising [359].

Because atheromatous plaques, as observed in a patient described by Kontusaari et al. [275], may initiate arterial rupture, the prevention of atherosclerosis may be potentially beneficial.

Heavy physical exercise (isometric exercise, weightlifting), or procedures that lead to increased intrathoracic pressure such as blowing the alpenhorn or French horn [360], and contact sports are contraindicated. Psychological support may be important.

Genetic Counseling and Prenatal Diagnosis

In EDS IV, dominant inheritance should be assumed in sporadic cases also unless proven otherwise. When a low serum procollagen III aminopropeptide level or a defect in collagen III metabolism has been documented in the index case, this may be used for screening other family members [228], with the caveats outlined above. Linkage of EDS IV to *COL3A1*, direct demonstration of the gene defect, or protein studies on a chorionic villus biopsy are all methods of prenatal diagnosis. We have been able to rule out EDS IV in two pregnancies of one case (F.P.) by excluding the presence in a chorionic villus biopsy of a structurally abnormal collagen III previously observed in fibroblasts from the mother [260].

KYPHOSCOLIOTIC TYPE OF EDS — EDS TYPE VI (OCULAR-SCOLIOTIC TYPE) (MIM 225400)

Diagnostic Criteria

The kyphoscoliotic type of EDS is caused by a deficiency of lysyl hydroxylase 1 (PLOD1), a collagen-modifying enzyme, due to homozygosity or compound heterozygosity for a mutant *PLOD1* allele(s). It is characterized as follows [39]:

Major diagnostic criteria
- Severe muscular hypotonia at birth
- Generalized joint laxity
- Kyphoscoliosis at birth, which is progressive
- Scleral fragility and rupture of the ocular globe

Minor diagnostic criteria
- Tissue fragility, including atrophic scars
- Easy bruising
- Arterial rupture
- Marfanoid habitus
- Microcornea
- Radiologically considerable osteopenia
- Family history (i.e., affected sibs)

Note: The presence of three major criteria in an infant is suggestive of the diagnosis, and laboratory testing is warranted. In the majority of cases, the condition is caused by the enzyme deficiency and specified as EDS VIA, whereas a rarer, similar condition with normal lysyl hydroxylase activity is designated EDS VIB (see Table 1 and below), the biochemical/molecular basis of which is still unclear.

Historical Introduction

The existence of an autosomal recessively inherited, "ocular" form of EDS was suggested by Beighton in 1970 on the basis of case 3 in the legend to Fig. 22 (Fig. 6.16 in [2c]) because of the overall rarity of sight-threatening eye complications, which were, however, clustered in families with only sibs affected and/or with consanguineous parents [113]. Investigation of such a family led to the first definition of a chemical abnormality of collagen in man, a milestone achieved by Krane and his colleagues [33]. In 1970, they studied two sisters (cases 1 and 2 in the legend to Fig. 22) who, in addition to typical EDS, had marked muscular hypotonia, severe progressive kyphoscoliosis from birth, microcornea, and fragility of ocular tissues leading to rupture of the globe or retinal detachment. The mother ruptured her membranes while 3 months pregnant with the older child yet still managed to carry the pregnancy to term. The younger affected girl required enucleation following a relatively mild trauma to the eye. Biopsy specimens of skin from both children contained collagen that was normally soluble in nondenaturing solvents (such as dilute acid) but more soluble than normal in denaturing solvents (such as 4 M $CaCl_2$ or 9 M KSCN). In view of this, it was assumed that there was some defect in the intermolecular cross-linking of the dermal collagen. The most striking chemical abnormality, which came as a "surprise to the authors" (S. Krane, personal communication, 1991), was the decreased hydroxylysine content in skin (0.2 to 0.3 residues per 1,000 amino acid residues; 5–7% of normal). Because the hydroxylysine content of the skin of the clinically normal parents and older sister was normal, it was assumed that the disorder was inherited as an autosomal recessive trait

and was due to a reduced enzymatic hydroxylation of lysyl residues [33,362]. Indeed, when in a later study enzyme extract from the cells of case 3 was mixed with that from a control, the activities were essentially additive [374], an observation that favored the conclusion that a structural defect of the enzyme rather than a defect in the activation step or the presence of an inhibitor led to the deficient enzyme activity.

Because these patients had both features of the EDS and ocular abnormalities, but a distinct biochemical defect, it was suggested that they be classified as a new subtype, EDS VI, the ocular type [2], or the ocular-scoliotic type [37e], now the kyphoscoliotic type [39], of EDS because ocular signs, though dramatic, are less frequent features in larger series than was initially reported [39,363].

It remains to be explained how the chemical abnormality relates to the clinical manifestations because the presence of typical symptoms even in patients with an apparently normal or only somewhat decreased content of hydroxylysine in skin, and normal lysyl hydroxylase activity in fibroblasts ([88,92,409,410]; cases 4 and 5 in [383,411]), suggests a more complex relationship between the known molecular defects and clinical manifestations than has been thought up to now. As mentioned above, patients with a phenotype resembling that of the kyphoscoliotic type but with normal lysyl hydroxylase activity do exist, and these individuals are now classified as EDS VIB [37f].

Clinical Findings

The legend to Figure 22 lists the 57 reported and unreported patients with EDS VIA known to the authors, derived from 49 families, who have been described clinically and confirmed biochemically. Those newborns whose neonatal history is available were usually described as floppy, with a poor cry, difficulty in sucking, and delayed motor development (cases 1, 2, 5–8, 10, 12, 14–16, 18–20, 22–29, 51). Sometimes poor fetal movements were noted by the mothers. Because of the severe muscular hypotonia, many of the patients had undergone a neuromuscular workup. Leg cramps are a common complaint.

Kyphoscoliosis is often present at birth and is progressive and severe (cases 1–4, 7–14, 16, 18–20, 22–24, 33, 51) (Figs. 23a,b and 24). It is probably the result of muscular hypotonia together with ligamentous laxity because the vertebral bodies are structurally normal, and is often resistant to external bracing. Thoracic cage deformity and hypotonia

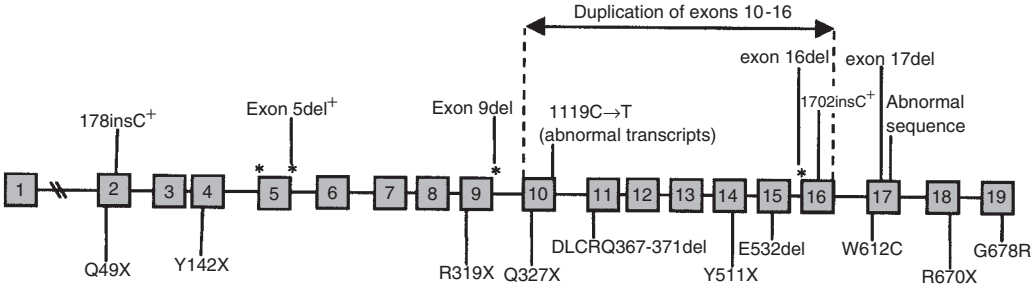


Figure 22. Structure of the *PLOD1* gene coding for lysyl hydroxylase 1 and mutations leading to the kyphoscoliotic type of EDS (EDS VI). The shaded boxes represent the 19 exons in the *PLOD1* gene joined by single lines indicating introns (not drawn to scale). The effects of point mutations and deletions on the amino acid sequence are shown below the line; above the line, the effects of other mutations are shown in the DNA: mutations that produce downstream preterm termination codons, PTCs, (+); splice-site mutations that result in exon skipping (*); as well as the common duplication of exons 10–16 (↔). (Adapted from [361] with permission.)

All biochemically proven patients known to the authors are listed below. The case numbers are followed by the mutation(s), sex, initials, and date of birth in parentheses, the ratio of urinary pyridinolines (LP/HP), and the references describing them, if the information is complete. Dup refers to the prevalent duplication of seven exons (exons 10–16) due to (a) homologous recombination event(s), Ter to a stop codon, PCT to preterm termination codon, and 0 to a functional null allele.

Cases 1 and 2, dup/dup, sisters (born 3 Dec 58 and 4 Jul 61) [33,362]; cases 7 and 8 in [363–373]; *cases 3 and 4*, dup/dup, sister and brother (J.L., 1924 and G.B., 1915), see Fig. 6-16 in [2], [37,111,113,373–378]; *case 5*, male [369,379,380]; *case 6*, male (J.D.H.), case 5 in [363,381,382]; *case 7*, del exon 16/del exon 17, male (9 Jan 76), case 1 in [383], [384–386]; *case 8*, male (18 years), case 2 in [383], [387–389]; *case 9*, male (33 years), case 3 in [383]; *case 10*, male (19 years), case 6 in [383]; *case 11*, del Glu532/Gly678Arg, male (A.T., 28 Jan 78), case 4 in [363], [390–393]; *case 12*, female (2 years) [392]; *case 13*, male (42 years), case 10 in [363], [426]; *cases 14–18*, cases 1,2, 3,6,9 in [363]; *cases 19 and 20*, Arg319Ter/Arg319Ter, sisters (H.A., 30 Jul 76 and N.A., 31 May 81), LP/HP 5.3 and 5.9 [394,395]; *case 21*, Tyr511Ter/del exon 5, male (SF 996, 3 Jul 89) [396,397]; *cases 22 and 23*, sisters (O.A., 29 Jun 84 and M.A., 16 Dec 94), LP/HP 6.3 and 5.9 [398]; *case 24*, dup/dup, male (A.R., 13 Mar 80), LP/HP 5.1 [373,399]; *case 25*, dup/dup, female (S.B., 12 Apr 77), LP/HP 5.3 [389,400,401]; *cases 26 and 27*, dup/?, brothers (A.B., 15 Sep 86 and H.B., 15 Oct 91), LH/HP 6.8 and 6.0 [373]; *cases 28 and 29*, dup/dup, brothers (T.D., 15 May 81 and A.D., 31 May 85), LP/HP 5.7 and 5.7 [373]; *case 30*, del exon 9/del exon 9, female (D.I., 18 Jan 90), LP/HP 4.3 [402]; *case 31*, dup/dup, male (A.R., 19 May 79) [401]; *case 32*, Trp612Cys/0, male (A.K., 14 Feb 80) [401]; *case 33*, male (T.O., 4 Oct 92), LP/HP 5.7 [own observation]; *case 34*, Tyr511Ter/Tyr511Ter, male (D.B. (1122), 34 years) [403]; *case 35*, D367LCRQdel/Gln49Ter, male (J.H., 3 Mar 84) [404]; *case 36*, D367LCRQdel/abnormal transcripts, female (C.C.) [404]; *case 37*, c.1702insC/c.1702insC, PTC in exon 16, female (K. (1072)) [405]; *case 38*, Tyr511Ter/Tyr511Ter, female (L.T., 17 May 70) [405]; *case 39*, Gln327Ter/Gln327Ter, female (A.C., 3 May 94) [405]; *case 40*, Tyr142Ter/del exon 14, female (A.F., 12 Aug 71) [405]; *case 41*, Arg670Ter/dup, male (M.C., 7 Feb 94) [405]; *case 42*, Tyr511Ter/Tyr511Ter?, male (J.H. (716)) [405]; *case 43*, IVS4-2A del/c.353insC, female (N.) [406]; *case 44*, Tyr511Ter/?, female [407]; *case 45*, female (D.A., 22 Apr 90), LP/HP 6.5 [own observation]; *case 46*, (A.A., 12 Mar 82), LP/HP 7.7 [own observation]; *case 47*, female (N.A-T., 31 Mar 95), LP/HP 6.9 [own observation]; *case 48*, female (Y.I., 10 Jun 93), LP/HP 7.8 [own observation]; *case 49*, male (J.S., 14 Jun 71), LP/HP 8.1 [own observation]; *Cases 50 and 51*, sisters (S.G., 14 Jun 70 and C.G., 23 May 65), LP/HP 4.6 and 7.8 [408]; *cases 52 and 53*, brother and sister (S.F., 12 Feb 80 and A.F., 28 Dec 81), LP/HP 3.9 and 4.1 [own observation]; *case 54*, female (F.Al-M., 9 Nov 00), LP/HP 8.1 [own observation]; *case 55*, female (K.K., 11 Oct 73), LP/HP 5.47 [own observation]; *case 56*, male (S.K., 20 Jul 93), LP/HP 3.5 [own observation]; *case 57*, male (D.D., 3 Oct 91), LP/HP 4.38 [own observation]; *case 58*, female (M.K., 9 Dec 95), LP/HP 3.4 [own observation].

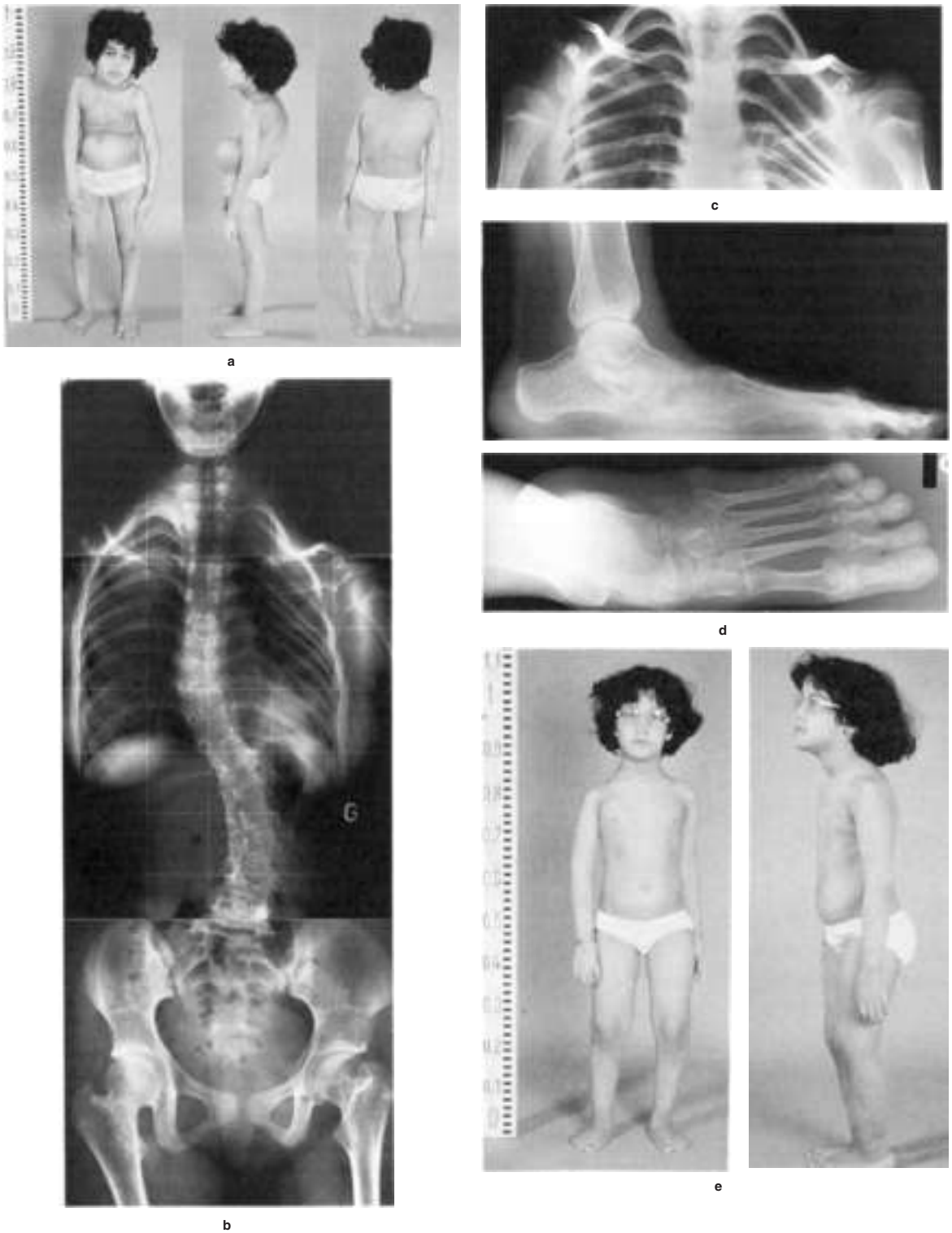


Figure 23. Kyphoscoliotic type of EDS (EDS VIA). H.A. (30 Jul 1976) and N.A. (31 May 1981) (cases 19 and 20 in the legend to Fig. 22) are the third and fifth children of healthy parents from Qatar who are first cousins. This family was the first in which the disorder was characterized at the molecular level [395]. (**a**) H.A. ($5\frac{1}{2}$ years old): severe kyphoscoliosis, dislocation of the right shoulder, flat feet in valgus position, microcornea. (**b**) X-rays of the spine 4 years after Harrington rodding and 3 years after removal of the rod because of its displacement due to premature loading (11 years). (**c**) X-ray of the shoulder showing dislocation of the right humerus (11 years). (**d**) X-ray of the foot showing marked osteoporosis and flat feet (11 years). Because of joint instability, arthrodesis of the lower ankle joint was required 2 years later. (**e**) N.A.: younger affected sibling who has a much milder phenotype, at age 5 years, shown for comparison, and thereby demonstrating marked intrafamilial variability. ((a), (e) reprinted from Steinmann [42] with permission.)

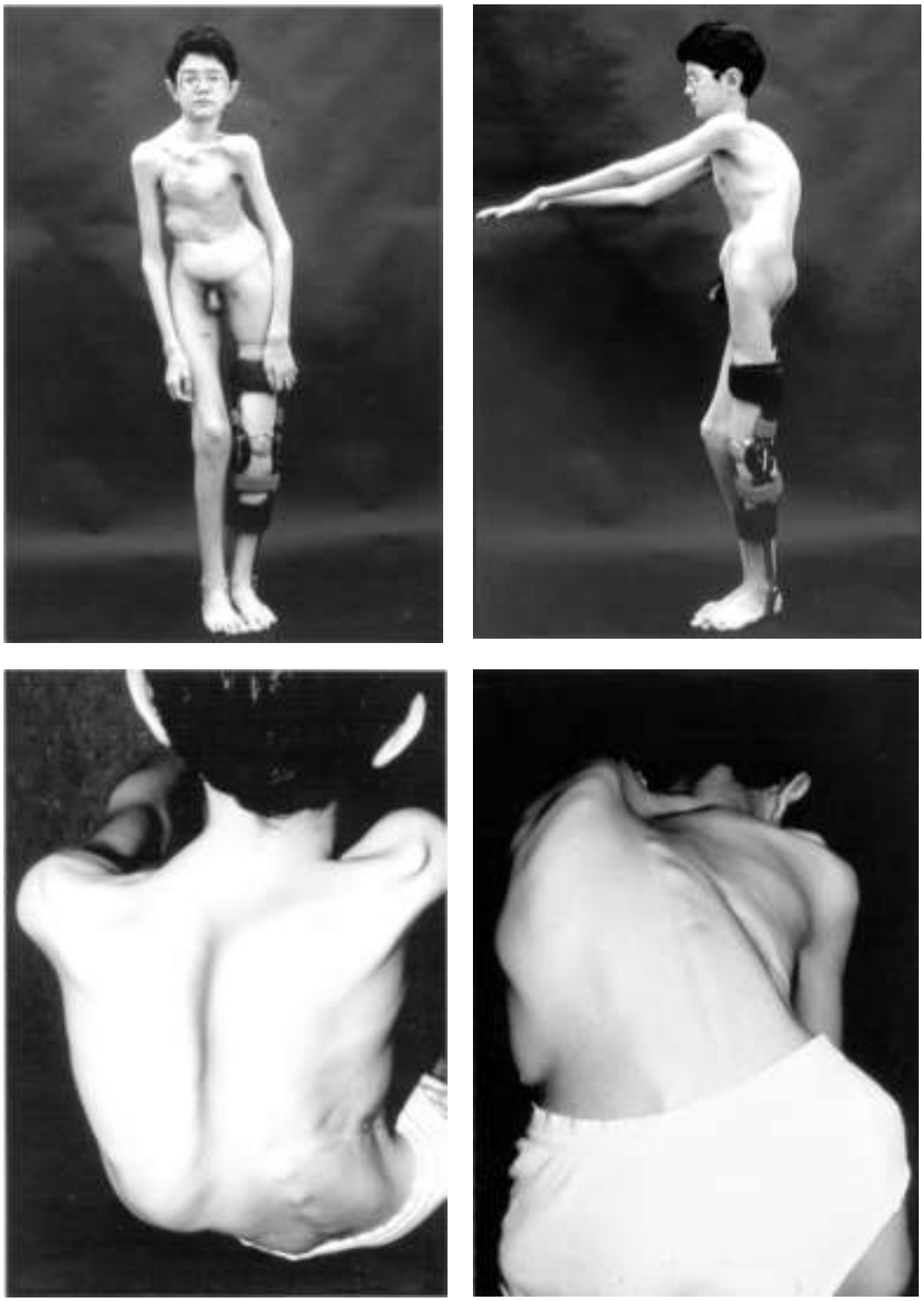


Figure 24. Kyphoscoliotic type of EDS (EDS VI). A.R., aged 13.3 years (case 24 in the legend to Fig. 22), with severe thoracic kyphoscoliosis and a Marfanoid habitus. He was only able to walk with the upper part of the body bent forward and preferred sitting in a wheelchair. He had microcorneae, brownish sclerae with decreased rigidity, and tortuous retinal arteries. Generalized joint hypermobility, habitual luxation of the shoulder, hypotrophic muscles, and marked skin hyperextensibility were present. There was mild aortic regurgitation, and lung function was severely restricted with a vital capacity of 30%. Only at age 13 years was EDS VI suspected and appropriate diagnostic procedures initiated.

He was born to consanguineous Iranian parents in the breech position. He was a weak baby and had difficulty in sucking. Treatment of the progressive kyphoscoliosis with a brace was not effective. Extensive and invasive neuromuscular workup remained noncontributory. During a ventral derotation spondylodesis from T11 to L5 at age 10.9 years, the inserted catheter led to an ectasia of an internal jugular vein, which was later resected. (Reprinted from Heim et al. [399] with permission.)

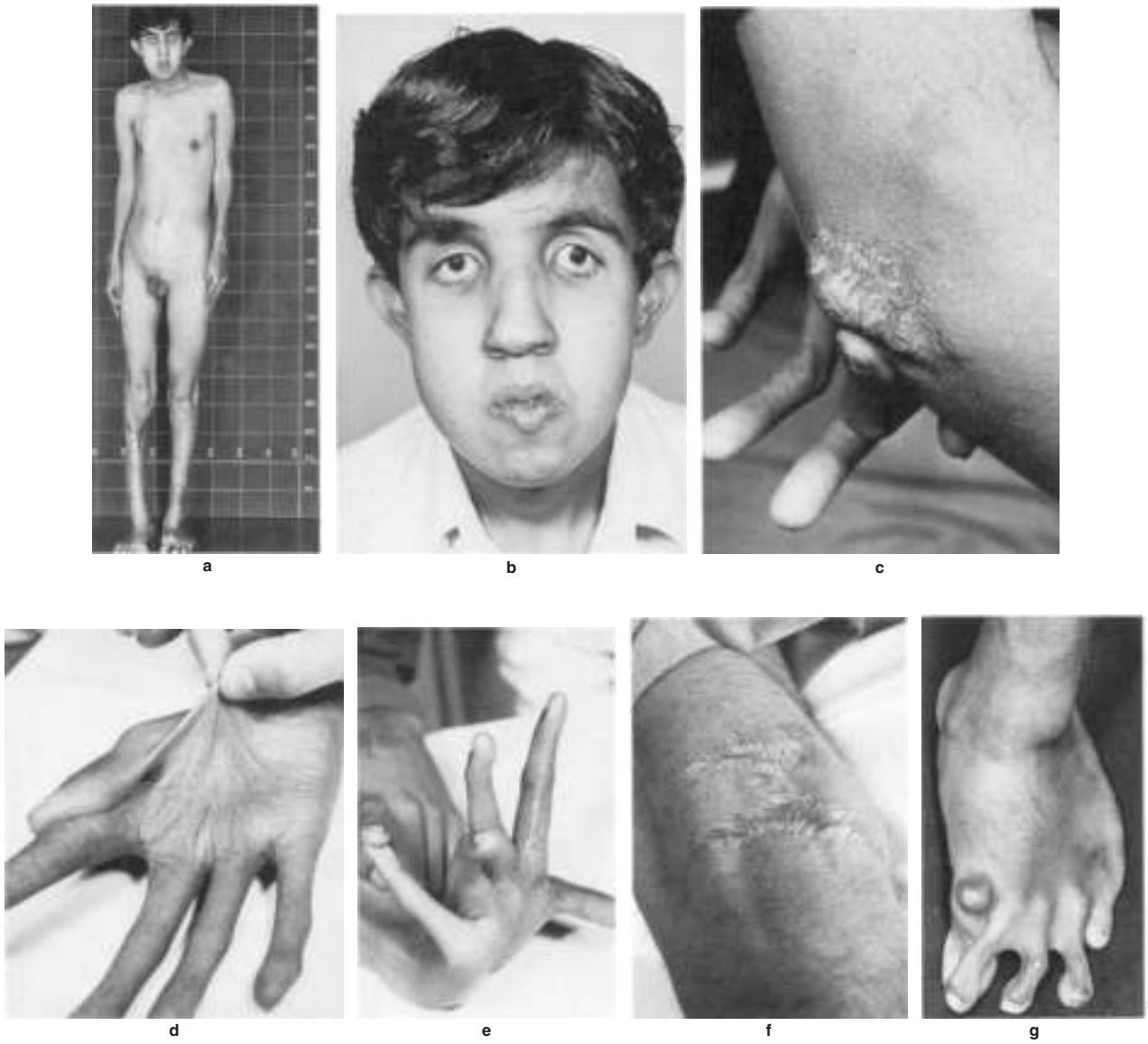


Figure 25. Kyphoscoliotic type of EDS (EDS VIB). A.S. (27 Apr 1956), 17 years old, the younger of two affected children born to healthy young parents from Pakistan who were first cousins. (**a**) Marfanoid habitus, moderate kyphoscoliosis and chest deformity, atrophic hemosiderotic scars, especially over the right shin, foot deformity, and mitral valve prolapse and regurgitation as judged by auscultation and echocardiography. His head circumference was normal (55 cm), and thus he differs from the cases with macrocephaly described by Cadle et al. [411] and Judisch et al. [409]. (**b**) Microcornea (9 mm in diameter), droopy eyelids, epicanthal folds, atrophic scars over the saddle of the nose, slightly lopsided and quite floppy ears. (**c**) Molluscoid pseudotumor over the elbow and spider-like fingers. (**d**) Hyperelasticity of the skin over the thenar and excessively wrinkled skin over the palm and fingers. (**e**) Hyperextensibility of the fingers. (**f**) Cigarette-paper-like scars over the knee. (**g**) Foot deformity and pseudotumor. Clinical follow-up: A.S. died at age 28 years of heart failure due to acute noncontrollable, bacterial endocarditis in his mother country (B. Steinmann, unpublished). (Reprinted from Steinmann et al. [88] with permission.)

lead to a decrease in pulmonary function and favor recurrent episodes of pneumonia, cardiac insufficiency, and early death (this occurred in case 4 at 54 years; in case 8 at 20 years; and in case 13 at 43 years). A Marfanoid habitus is quite common (Figs. 24, 25a). Osteoporosis seems to be a common finding but without any tendency toward fractures (Fig. 23d).

Ocular fragility leads to retinal detachment (case 3) and bleeding (case 3) and rupture of the globe, rather than of the cornea as in the brittle cornea syndrome, after minor trauma (cases 2–4, 9). Attempts to suture scleral tissue are often in vain (case 4). Microcornea is common (Fig. 25b) (cases 1–3, 6, 8, 10, 19, 20), the diameter in normal individuals being approximately 11.7 mm (range 11.0–12.5 mm) [412]; however, it also occurs in EDS I (as in the propositus and his affected daughter, Fig. 10). The sclerae are often bluish, in contrast to those in EDS I, II, and III, which are of normal hue.

Arterial rupture may be another prominent finding in EDS VI [363; personal observations]. Case 3 died at 51 years with symptoms typical of dissecting aneurysm of the

aorta; however, autopsy was not performed [37e]. Case 4 had a cerebrovascular accident in the distribution of the right middle cerebral artery at the age of 19 years [374]. Other impressive clinical descriptions of patients with classical features of EDS VI who were not studied biochemically have been reported in the older literature [2,119,413]. The natural history in relation to the vascular system is still not well-known. An ill-defined polyneuropathy in two siblings of consanguineous parents together with typical signs of EDS VI has been described [92].

To the authors' knowledge, affected females with proven EDS VI (see legend to Fig. 22) have not been reported to have given birth; in contrast, case 4 fathered four healthy children (see "Recent Developments").

Life span is markedly reduced because of arterial rupture and cardiopulmonary insufficiency due to severe kyphoscoliosis; there are, however, no precise data on mean age of survival (see above).

Obligate heterozygotes are clinically normal in our experience.

Macrocephaly has been reported in six patients with EDS VIB from three separate families and claimed to be a feature of a distinct syndrome [411] (R.G. Cadle, personal communication, 1990) (MIM 229200), but macrocephaly is clearly not present in other patients with EDS VIB (Fig. 25a) [88,440].

Interfamilial variability of EDS VI is quite considerable within EDS VIA, and even more so if EDS VIB, with its unknown genetic defect, is included. Intrafamilial variability as observed in the pairs of siblings (cases 1 and 2, 3 and 4, 19 and 20, 22 and 23, 26 and 27, 28 and 29, 50 and 51, 52 and 53; see legend to Fig. 22, Figs. 23a and 23e) further indicates that environmental factors and complications, such as neonatal brain hemorrhage in case 50 [408], and genetic factors other than mutations in the gene coding for lysyl hydroxylase are also important in determining clinical expression; the phenotype in case 51 is unusually mild [408].

Genetic Defect

The Gene, the Enzyme, and Physiology

Lysyl hydroxylase 1 (LH1) (EC 1.14.11.4; procollagen-lysine, 2-oxoglutarate 5-dioxygenase; PLOD1; MIM 153454) is a homodimer consisting of subunits with a molecular weight of about 85,000, each comprising 709 amino acid residues and a signal peptide of 18 amino acids. The C-terminal region is especially well-conserved, with a 139 amino acid region, residues 588–727, being 94% identical between the human and chick at the amino acid level, and with a 76 amino acid region coded for by exons 18 and 19, residues 639–715, being 99% identical [414,415]. By site-directed mutagenesis, it has been shown in expression systems that three residues, histidines 656 and 708 and aspartate 658, provide the three ligands required for the binding of Fe^{++} to a catalytic site, whereas the role of a third critical histidine (residue 706) remains to be established [416]. The enzyme appears to be a true resident of the endoplasmic reticulum despite the fact that it does not contain any known endoplasmic-reticulum–specific retention signals in its primary structure [417].

The ascorbate-dependent enzyme requires α-ketoglutarate, Fe^{2+}, and O_2 as cosubstrates and cofactors [418] and catalyzes the formation of hydroxylysine in collagen chains and other proteins with collagen-like amino acid sequences by the hydroxylation of lysyl residues in Gly–X–Lys sequences (for a review, see [419]). The hydroxylysyl residues formed have two important properties: first, their hydroxyl groups serve as sites of attachment for a monosaccharide or a disaccharide — galactose or glucosylgalactose, respectively — the function of which is unknown so far, although they may play a role in recognizing and activating collagen receptors in the cell membrane [420]; second, they are essential for the stability of the intermolecular collagen cross-links that provide tensile strength to most soft tissues and bone.

Cross-linking involving lysyl and hydroxylysyl residues occurs after the processing of procollagen and secretion of the molecules into the extracellular space. The first step in cross-linking is the oxidation of specific lysyl and hydroxylysyl residues in the telopeptide regions at both ends of the collagen molecule to form aldehydes [421]. This reaction is catalyzed by the extracellular copper-dependent enzyme lysyl oxidase (protein lysine 6-oxidase, EC 1.4.3.13, MIM 153455). When hydroxylysyl residues are present in the telopeptides, the hydroxylysine-derived aldehyde, hydroxyallysine, reacts with a specific peptidyl lysyl or hydroxylysyl residue in the triple-helical domain of a neighboring molecule in a fibril to form a bifunctional reducible intermediate that matures into a trifunctional nonreducible pyridinium cross-link through the addition of a third residue of hydroxylysine [421]. Two forms of pyridinium cross-link have been identified: hydroxylysyl pyridinoline (HP, or "pyridinoline"), formed from three hydroxylysyl residues, and lysyl pyridinoline (LP, or "deoxypyridinoline"), formed from one lysyl and two hydroxylysyl residues. These pyridinolines are especially abundant in bone, cartilage, and dentin [422]. Both compounds are biologically and chemically stable and are excreted in urine in free and peptide-bound forms as products of collagen degradation. Their urinary excretion correlates with bone turnover in acquired and inherited disorders affecting bone (see Appendix II, this volume), whereas a highly increased ratio of LP to HP is diagnostic for EDS VI (see below).

Lysyl hydroxylase 1 is coded for by the gene *PLOD1* (MIM 153454) on chromosome 1p36.2→p36.3 [415], which contains 19 exons and a 5′-flanking region with characteristics shared by housekeeping genes; the constitutive expression of the gene in different tissues suggests that lysyl hydroxylase has an essential function [423]. From sequencing the introns, where many mutations and rearrangements have been found to be concentrated, Heikkinen et al. [424] demonstrated extensive homology of intron 9 and intron 16 resulting from five and eight Alu sequences, respectively, contained therein (see Fig. 22).

The Mutations in the Gene Coding for Lysyl Hydroxylase

The mutations so far elucidated in lysyl hydroxylase in EDS VIA are indicated in Figure 22. Of special interest is the high frequency of the large duplication of nucleotides 1176 to 1955 corresponding to amino acids 326 to 585 in the normal sequence [415]. The seven-exon duplication in *PLOD1* is 8.9 kb in size, is caused by an Alu–Alu recombination in introns 9 and 16 of the gene, does not disturb the splicing of introns, and leads to a duplication of amino acids 326–585 [415,424]. This duplication was found in 19% of mutant alleles among 35 EDS VI families, but haplotype analysis showed variations in the sequence of the DNA region surrounding the duplication, thereby excluding a founder effect [373] and suggesting that it must be a frequent, independent event.

Pathogenesis

EDS VIA was shown by Krane et al. [33] to be due to a marked deficiency of lysyl hydroxylase. Residual activity in cultured skin fibroblasts ranges from 2% to 50% of normal using radiolabeled chicken protocollagen (unhydroxylated procollagen) as a natural substrate. These figures have to be treated with caution because the results may vary considerably from assay to assay, and even more so from laboratory to laboratory, making direct comparison difficult (e.g., in cases 1 and 2, residual activity was variously 14% and 10% [33] and 14.6% and 26.6% [363], respectively, in the same patients). Only a few studies of the kinetic and physicochemical properties of crude enzyme preparations from patients and controls have been performed. In one study using cultured fibroblasts from the two siblings above (cases 1 and 2), a mutant enzyme had optimal activity at 30 °C, rather than 34 °C, as for the wild-type enzyme, and did not form high-molecular-weight aggregates in low-ionic-strength buffers as did the normal enzyme. It also exhibited a higher apparent K_m for ascorbate (20 μM) than the wild-type enzyme (4 μM), and its activity was stimulated threefold or more by prior dialysis against buffer solutions containing 10 mM dithiothreitol, but no such effect was observed with the normal enzyme [364]. These differences in kinetic properties suggest that there is not simply a deficiency of enzyme protein in affected individuals but that an enzyme protein is present with abnormal properties. Miller et al. [382], in their study of cultured fibroblasts from case 6, found that the mutant enzyme had the same K_m for ascorbate (0.1 mM) as the wild type but that its apparent V_{max} was reduced to 25% of the control figure.

As a consequence of the lysyl hydroxylase deficiency, the hydroxylysine content of collagen is diminished, with a concomitant increase in lysine, although to variable degrees in different tissues. The amount of hydroxylysine is only 5% of normal in skin [362], 14–28% in fascia [362], 55% in tendon [387], 10–20% or 43–100% in bone, depending on the mode and site of sampling [363,365,387,425], and ~90% of normal in cartilage [362]. The level of hydroxylation in the C1q component of complement was initially reported as being 77% of normal by Pinnell [366], but a later study found it to be normal [384]; in both studies, the C1q was functionally normal. The analysis of different collagen types extracted from various tissues of case 8 and their respective chains gave similar results [387]. The extent of lysyl hydroxylation of collagen I extracted from skin was 0%, from bone 17%, tendon 36%, kidney 71%, and from lung 73% of normal. Collagen III extracted from skin and lung contained 0% and 50%, respectively, of the normal content of hydroxylysine, whereas collagens II, IV, and V from cartilage, kidney, and bone, respectively, were almost normally hydroxylated [387]. The authors of this latter study concluded that the varying degree of impairment of the hydroxylation of lysine residues correlated well with the severity of the clinical manifestations. Furthermore, cyanogen bromide cleavage of dermal collagen revealed that the hydroxylysine content was not uniformly low in the peptides examined but rather that it was zero in some peptides containing normally hydroxylatable lysyl residues and measurable, although still reduced, in others. Just one possibility to explain this is that the altered enzyme protein acts on different lysyl residues to different extents as a function of the variations in surrounding amino acid sequence [367]. Cultured skin fibroblasts from patients with EDS VI produce collagens I and III (and V), which are hydroxylated to a greater extent (~50% of normal) than their dermal counterparts formed *in vivo* [368,375,383,385,426]; overhydroxylation of collagen produced in culture is also observed in control cells [368] and may indicate that the rate of polypeptide elongation and/or helix formation in culture is delayed, thus allowing more extensive hydroxylation of lysyl residues. It is also clear that the content of hydroxylysine glycosides in collagen is likely to be markedly reduced. The ratio of glucosylgalactosyl hydroxylysine to galactosyl hydroxylysine in diseased bone appears abnormally high (1.04 and 1.24 in two cases versus 0.39 and 0.47 in two controls) [365]. However, the function of the sugar residues normally linked to hydroxylysine is not known.

The considerable extent of hydroxylation of collagen lysine residues that occurs in certain tissues in EDS VI patients may also explain why the urinary excretion of hydroxylysyl glycosides was only moderately decreased (62% of normal) in the two patients studied in this regard [362].

The above-mentioned wide variability in the extent of tissue-specific post-translational modification could be explained by several possibilities or a combination thereof:

1. Differences in the residual activity of lysyl hydroxylase in various tissues, resulting from a relative increase of either synthesis or activation of proenzyme. Krane et al. [369] reported that the ratio of lysyl to prolyl hydroxylase in dermal cells was 8–10% but that it was as high as 46–49% in bone cells of case 2.
2. Tissue-specific differences in the rate of collagen synthesis and triple-helix formation in the presence of limiting lysyl hydroxylase activity, as discussed above for the *in vitro* model.
3. Different affinities of the mutant enzyme for different collagens. Risteli et al. [388] found that the residual activity of lysyl hydroxylase in EDS VI fibroblasts (case 8) was 10% using underhydroxylated chicken collagen I as substrate but that it was as high as 35% using collagen IV from mouse sarcoma. The previously mentioned observation that the underhydroxylation of lysyl residues of CNBr-derived peptides is not uniform [367] could also be explained in this way.
4. Tissue differences in critical cofactor concentrations.
5. The existence of multiple forms of lysyl hydroxylase. This possibility may be supported by the unpublished results that lysyl hydroxylase activity in platelets from the two original patients was normal (cited in [367]) and the observation that cultured osteoblasts express a relatively higher residual activity than fibroblasts [369]. The urinary excretion of pyridinolines (see below) strongly indicates the existence of a lysyl hydroxylase activity other than the "classic type" [427]. Whether the isoform lysyl hydroxylase 2 (LH2), with its two tissue-specific splice forms, LH2a and LH2b [428], encoded by *PLOD2* located at chromosome region 3q23-q24 (MIM 601865) [429], is responsible for a substantial amount of the hydroxylysine formed is not clear because its tissue distribution has not yet been sufficiently studied, although it has been suggested that PLOD2 is associated with lysine hydroxylation in the nontriple-helical domains of collagen I in bone [430]. The same uncertainty holds true for isoform 3, lysyl hydroxylase 3 (LH3), encoded by *PLOD3* (MIM 603066) at

chromosome region 7q36 [431,432] — which has interestingly, in addition to lysyl hydroxylase activity, collagen glucosyltransferase activity [433] — and the putative lysyl hydroxylase 4 (LH4); all four isozymes are highly homologous at the C-terminal end and other parts of the molecules [431,432] and seem to be derived from an ancestral gene by two duplication events [434]. No differences in catalytic properties are found between the recombinant lysyl hydroxylase 3 and 1 isoenzymes using an artificial substrate, but the data do not exclude the possibility that differences might exist with respect to the hydroxylation of different collagen types [432]. It is possible that a deficiency of lysyl hydroxylase 2 and 3 isoforms may lead to some other variant of EDS or to some other heritable connective tissue disorder such as Bruck syndrome (see Chapter 26, Part IV, this volume).

6. The questions of why mRNA levels for LH1 do not correlate with lysyl hydroxylase activity measured in cells (R. Myllylä, unpublished data, cited in [431]) and why removal of the most conserved portion of LH1 does not totally abolish the enzyme activity [395] remain open.

The pathophysiology of this disorder is becoming clearer. Whereas early studies found no pyridinolines in normal skin [422], Pasquali et al. [435] re-examined skin, the major organ affected in EDS VI, tendon, and bone from controls and patients; the total amount of pyridinolines in patients was only ~50% of normal in skin but normal in tendon and bone, although the ratio of LP to HP was increased in the three organs. Therefore, although a qualitative abnormality in pyridinoline cross-links occurs in all tissues, only the skin of patients with EDS VI has reduced amounts of total pyridinolines, with absent hydroxylysyl pyridinoline. In long-term cultures containing ascorbic acid of fibroblasts from patients with EDS VI, pyridinium cross-links, when present, had a greatly increased LP to HP ratio (6.39 ± 3.86 versus 0.22 ± 0.10 in controls), comparable to that determined in urine from patients and controls, respectively [436] (see below)[8].

It appears that the solubility of collagen in denaturing solvents is inversely proportional to its residual content of hydroxylysine. Dermal collagen with a low (5% of normal) content of hydroxylysine is excessively soluble in 4 M $CaCl_2$ or 9 M KCSN [362,374], whereas collagen from bone (10–20% of normal hydroxylysine content) and cartilage (90% of normal hydroxylysine content) are extractable in these solvents in only moderately increased or normal amounts, respectively [362]. Because of the Amadori rearrangement and the formation of ketoamines, cross-links derived from hydroxylysine aldehydes are chemically more stable *in vitro* than those derived from lysine aldehydes, but this does not necessarily imply greater stability *in vivo*. Eyre and Glimcher [365] found an abnormal profile of reducible cross-links in skin and bone collagens, but again it is not clear how this is related to the mechanical weakness of the connective tissues because the lysine aldehyde-derived cross-links should have made equally effective intermolecular bonds. Two nonreducible trifunctional collagen cross-links, lysyl pyridinoline and hydroxylysyl pyridinoline, based on a 3-hydroxypyridinium ring, are derived from hydroxylysine and its aldehyde. Indeed, Eyre [370] has found decreased levels of the pyridinoline cross-links in the annulus fibrosus of a patient with EDS VI and the presence of a newly observed, more basic hydroxypyridinium compound that could be a lysine analog of the normal compound. It was shown that the ratio of total lysyl to hydroxylysyl pyridinolines in bone of case 8 (legend to Fig. 22) was 11.9 and 12.5 instead of 0.13 and 0.25 as in controls [389,435].

In EDS VI patients, the skin is unusually thin. This most probably is due to a reduced amount of collagen relative to elastin because the ratio of hydroxyproline to desmosine is only 1/4 of normal [365]. Why the amount of dermal collagen is reduced in these patients remains speculative but could be explained by its higher solubility, which favors rapid turnover and degradation. Bones are more radiolucent; osteoporosis seems to be a direct consequence of the qualitative change in pyridinolines and their glycosides in an as yet undefined manner and may also be enhanced by epigenetic factors such as muscular hypotonia and poor physical activity rather than to the underlying molecular cause (cases 19, 20; Fig. 23d).

Finally, it remains to be emphasized that residual enzyme activity and dermal hydroxylysine content correlate poorly with the severity of the phenotype. This is best exemplified by certain patients with EDS VIB, such as those described by Steinmann et al. [88,379].

Genetics

EDS VIA is inherited as an autosomal recessive trait. This is supported by the occurrence of affected siblings from unaffected parents (legend to Fig. 22), by the occurrence of affected children from consanguineous parents (cases 7, 8, 13, 19 and 20, 22 and 23, 24, 28 and 29, 50 and 51) and by there being intermediate enzyme activity in obligate heterozygotes who are clinically normal. However, in some parents, heterozygosity cannot be established using the usual enzymatic assay (father of cases 1 and 2, parents of case 5, father of case 11). Therefore, the disorder in the corresponding patients was thought to be most probably due to compound heterozygosity because it is the rule rather than the exception in very rare autosomal recessive disorders when there is no parental consanguinity, until it was discovered that the Alu–Alu repeats lead to frequent duplication events (legend to Fig. 22). The autosomal recessive mode of inheritance of EDS VIB is based only on formal genetic considerations [88,92,409,410] cases 4 and 5 in [383,411,440].

Diagnosis

The clinical diagnosis is still generally suspected very late and only after a negative workup of neuromuscular disorders [395,398,399]. This is mainly due to the rarity of the disease and its unfamiliarity to most physicians.

[8] Until recently, it was not known how pyridinoline cross-links are formed in cases such as cases 19 and 20 with proven absent activity of the "classical" lysyl hydroxylase [427]. Indeed, not only is lysyl pyridinoline formed — in excessive amounts, apparently in proportion to the decrease in hydroxylysyl pyridinoline — which requires hydroxylysine in telopeptides, but also some hydroxylysyl pyridinoline, which requires hydroxylysine within the triple helix, is formed as well. The most plausible explanation for pyridinoline formation is the existence of the predicted N-telopeptide-specific lysyl hydroxylase [437,438], which, however, has been shown to be bone-specific [430,439]. Furthermore, such an observation implies an alternative source of hydroxylase activity for at least some triple-helical region lysyl residues in collagen as well as the telopeptide hydroxylase activity [427] (for details regarding lysyl hydroxylase isoforms, see the text).

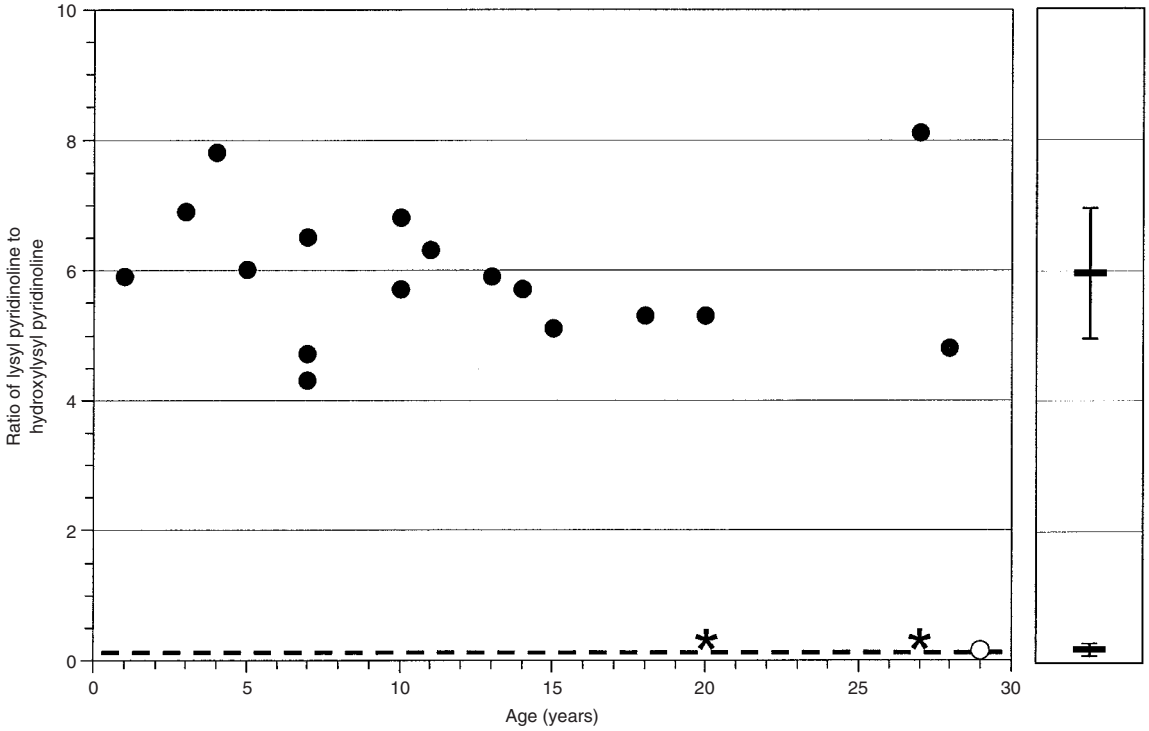


Figure 26. Urinary pyridinolines in patients with kyphoscoliotic type of EDS (EDS VI) and controls. The ratios of urinary total lysyl pyridinoline (LP) to total hydroxylysyl pyridinoline (HP) are given for each of 17 individuals with EDS VIA (•) as a function of age and compared with the mean of those of controls (▬ ▬) (**left panel**). Means of both groups of all ages are shown in the **right panel** and are 5.97 ± 0.99 (±1 SD, range 4.3–8.1) for EDS VIA and 0.2 ± 0.1 (±1 SD, range 0.1–0.4) for controls. The intraindividual variation of the ratio in 14 urinary samples collected over a time span of 7 weeks by one of the EDS VIA patients, T.O., was small (5.64 ± 0.31, range 5.21–6.22) [442]. The ratio in the female patient with EDS VIB (○, [88]), however, was 0.15 and indirectly confirmed the normal activity of lysyl hydroxylase [379]. The symbols (*) indicate two of the three patients studied with alkaptonuria.[9]

Note: the urinary pyridinoline ratio is independent of age and distinguishes both groups without overlap (for further comments, see Footnote 9).

Sometimes, confounding factors or unrelated associated diseases [398] detract from its recognition.

(1) The recommended, simple, reliable, and noninvasive laboratory test is the measurement by HPLC of total urinary lysyl pyridinoline ("deoxypyridinoline") and hydroxylysyl pyridinoline ("pyridinoline") cross-links after hydrolysis, a test that has a very high degree of sensitivity and specificity [389,427,435,441]. The ratio of the two compounds is in an abnormally high, narrow range, 5.97 ± 0.99 (mean ± 1SD; range 4.2–8.1) in 17 proven cases, as opposed to the low, narrow value of 0.20 ± 0.10 in controls, and is age-independent [442] as it is in controls [425] (Fig. 26). Obligate heterozygous parents have normal values [427]. Furthermore, this high ratio is not observed in other inherited or acquired collagen disorders, and the normal ratio is not changed by ascorbate or penicillamine and is only minimally influenced by renal dysfunction [441][9].

(2) The determination of the hydroxylysine content of dermis freed from the epidermis using an amino acid analyzer following hydrolysis [88,445] is also easy and quick, although more invasive. The results may be expressed as lysyl residues/1,000 amino acid residues (normal mean 3.85 ±

[9]The urinary total lysyl pyridinoline (LP) to hydroxylysyl pyridinoline (HP) ratio is unchanged in alkaptonuria (see Fig. 26) and under ascorbate and penicillamine treatment; however, it is abnormally low in Bruck syndrome (MIM 259450, [439]).

Purified lysyl hydroxylase has been reported to be inhibited by homogentisic acid in a linear, noncompetitive, and reversible way, with a K_i of 120–180 μM, and biosynthesis of hydroxylysine-derived intermolecular collagen cross-links in organ cultures of embryonic chick calvaria was inhibited by homogentisic acid in a dose-dependent manner [443]. The authors speculated that these results may explain the predilection of alkaptonuric complications for hydroxylysine-rich tissues such as articular cartilage, heart valve, vascular wall, and bone. To address the question of potential inhibition of hydroxylation of helical and nonhelical lysyl residues by homogentisic acid and related metabolites, we measured urinary total lysyl pyridinoline and hydroxylysyl pyridinoline in three alkaptonuric adult individuals, the oldest one symptomatic (M.Y.) and the two younger ones asymptomatic (A.A., G.A.-L.). Pyridinolines were normal in total amounts excreted and relative proportions (i.e., 0.16–0.18) [444]. These results imply that under *in vivo* conditions the claimed inhibition of lysyl hydroxylase by homogentisic acid or its derivative benzoquinoneacetate seems not to be operative, at least not in the organs that are the main source of urinary pyridinolines.

An 11-year-old girl (P.J.) with Wilson disease had an unchanged urinary LP to HP ratio of 0.22 before and after 16 months of treatment with penicillamine 3 × 300 mg daily; the ratio was 0.16 after a 12-month period in which penicillamine was replaced by oral zinc (100 mg/kg body weight) and 0.15 after a 12-month period of combined treatment with the same doses of zinc and penicillamine.

For details regarding the lack of influence of ascorbate on the urinary LP to HP ratio, see "Treatment" in EDS VI.

0.42, range 3.0–4.24, $n = 8$), as the ratio of hydroxylysine to hydroxyproline (normal mean 0.042 ± 0.006, range 0.033–0.047, $n = 8$), or as the ratio of hydroxylysine to lysine (normal mean 0.15 ± 0.02, range 0.11–0.18, $n = 8$) (values calculated from Steinmann et al. [88]).

(3) The determination of enzyme activity in cell extracts is done on a research basis only. The assay is not very sensitive and requires fresh, biosynthetically [^{14}C]- or [^{3}H]-lysine-labeled unhydroxylated collagen (protocollagen) prepared from embryonic chick calvariae or tendon cells as a natural substrate [418]. Because enzyme activity depends on cell density [367], the specific activity of the substrate, and cofactor composition, appropriate control cells have to be included in each assay. The results may be expressed as the amount of hydroxy[^{14}C]lysine formed, as the amount of tritium released, determined as [$^{3}H_2O$], or as the extent of decarboxylation of 2-oxo[1-^{14}C] glutarate, per number of cells or μg of protein in the cell extract or, preferably, as the ratio of lysyl hydroxylase activity to prolyl hydroxylase activity, the latter serving as a reference enzyme [418,432].

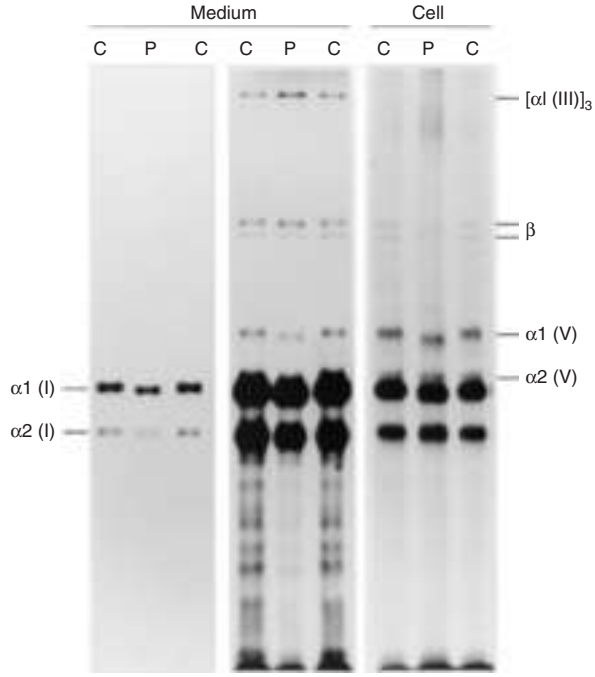


Figure 27. Lysyl residues in collagens from patients with kyphoscoliotic type of EDS (EDS VI) are underhydroxylated. Pepsin-treated radiolabeled procollagens from medium and cells synthesized by fibroblasts from case 23 with EDS VIA (P) and two controls (C) were electrophoresed on a 5% SDS-polyacrylamide gel, processed for fluorography, and exposed to x-ray films for 3 hours (**left panel**) or 7 days (**middle and right panels**). The α1(I), α2(I), and α1(V) chains and the β11 and β12 components from the patient's cells migrate slightly faster than those from the controls; the faster electrophoretic mobility is not apparent in the large collagen III molecules consisting of three disulfide-linked α1(III) chains but is obvious after reduction to free α1(III) chains (not shown). This is an indirect demonstration that collagens are underhydroxylated. Similar results were obtained after electrophoresis of collagen extracted from skin and visualized by silver staining; direct measurement of amino acids in dermis from the patient showed a marked deficiency in hydroxylysine (not shown) and proved the indirect conclusions derived from these observations. (Reprinted from Jarisch et al. [398] with permission.)

A synthetic tridecapeptide containing [^{3}H]-labeled lysine and corresponding closely to residues 98–110 of the collagen α1(I) chain has also been used as a substrate to determine residual activity in case 11 [390].

(4) It is difficult to demonstrate a decrease in the extent of hydroxylation of lysine residues in collagen produced by cultured fibroblasts from patients with EDS VI because the level of collagen hydroxylation in cultured cells is normally higher than it is *in vivo* (see above). However, a somewhat faster electrophoretic mobility of α chains on SDS-polyacrylamide gel electrophoresis is noticed due to relative underhydroxylation and underglycosylation (Fig. 27, [398]).

(5) Mutation analysis of the *PLOD1* gene will become easier and will be especially useful for prenatal diagnosis and research.

The diagnosis of EDS VIB is based so far on the typical clinical findings, together with a normal hydroxylysine content in dermis, normal lysyl hydroxylase activity, and a normal ratio of urinary pyridinolines as shown in the female of the sibs described in [88] (Steinmann et al., unpublished results) (Fig. 26).

Differential Diagnosis

EDS VI is a rare differential diagnosis in the neonate with severe muscular hypotonia; neuromuscular disorders will have to be considered first. Later, the brittle cornea syndrome [445], fragilitas oculi with joint hypermobility (MIM 229200, under which condition are included several patients also designated by McKusick [37] as having EDS VIB), and other causes of corneal rupture [410] have to be considered. Cameron et al. [446,447] reported EDS VI-like patients who presented with corneal thinning, keratoglobus, blue sclerae, corneal rupture, joint hypermobility, similarly affected sibs, and often consanguineous parents. Investigations by Steinmann et al. (unpublished results) revealed that several of the patients in these latter two reports were identical and that their normal urinary ratios of pyridinolines excluded EDS VIA. Whether this condition should be labeled as EDS VIB or instead brittle cornea syndrome, because of the fragility of the cornea as opposed to the sclera, remains open. Pemberton et al. [111] reported on six of the seven members of a three-generation family who were afflicted with retinal detachment; four of these six had features of EDS and one died of a dissecting aortic aneurysm. Biglan et al. [448] reported five patients from two families with keratoglobus, blue sclerae, hyperextensibility of the small joints, sensorineural hearing alterations, and mottling of the teeth; corneal perforations developed in seven of their ten eyes after minimal trauma.

Special Management and Genetic Counseling

Anticipatory management of individuals with EDS VI includes the following organ systems.

The musculoskeletal system. Referral to, and regular follow-up by, an orthopedic surgeon for management of kyphoscoliosis. Severe kyphoscoliosis may be resistant to bracing and require surgical intervention. Such patients may be at high risk for neurologic and vascular complications consequent upon surgery for scoliosis, necessitating careful perioperative evaluation and management [449,450]; halogravity traction may be indicated before surgery or between stages of surgery in patients with severe curves, but it must be instituted carefully to avoid brachial plexus injury and other

neurologic deficits [87]. Physical therapy should be instituted for older children, adolescents, and adults to strengthen large muscle groups, particularly at the shoulder girdle, and to prevent recurrent shoulder dislocation. Routine examination for inguinal hernia and surgical referral should be carried out as necessary. The management of other complications is similar to that in EDS I, II, and III (see above).

The eye. Routine ophthalmological examination for management of myopia and early detection of retinal detachment and glaucoma. Surgical repair of a ruptured eye globe may be difficult because of the friability of the scleral tissue (e.g., case 4). Primary vitrectomy permits the successful treatment of retinal detachment if a buckling procedure cannot be performed because of scleral atrophy. However, serious complications may occur and therefore other surgical procedures should be considered, such as pneumatic retinopexy, a temporary balloon, or a dura patch with episcleral pocket [451].

The cardiovascular system. Measurement of aortic root size by echocardiogram at the time of diagnosis or by the age of five years; it may be advisable to repeat an echocardiogram at five-year intervals even if the initial examination is normal. Patients with mitral valve prolapse or valvular insufficiency should follow guidelines for antimicrobial prophylaxis. Vigilant observation and aggressive control of blood pressure is necessary to reduce the risk of arterial rupture; vascular surgery is fraught with danger.

Others. The use of pharmacological doses of vitamin C, a cofactor of lysyl hydroxylase, has been investigated and found to result in an increased urinary excretion of hydroxylysine. Over a two-year period, one patient's wound healing and muscle strength improved and the corneal diameter increased; joint laxity, skin fragility, and the hydroxylysine content of the skin, however, did not change [381]. These results are at variance with those obtained with two pairs of sibs, cases 22 and 23, cases 26 and 27, and case 33 (legend to Fig. 22), who received vitamin C 2–4 g/day in two daily doses for two weeks. The ratio of urinary pyridinolines was indistinguishable in the six urinary specimens obtained from each during each of the periods of preloading, treatment, and washout (Steinmann et al., unpublished results)[9].

Genetic counseling and prenatal diagnosis. The parents of an affected child are obligate heterozygotes and the risk of recurrence of another affected child is 25%.

Krane et al. [33] observed that lysyl hydroxylase is readily detectable in normal cultured amniotic fluid cells, raising the possibility of prenatal diagnosis. Dembure et al. [391] found that lysyl hydroxylase activity in amniotic fluid cells, whether amniocytes (AF) or fibroblasts (F), corresponded to that in dermal fibroblasts, whereas prolyl hydroxylase activity varied markedly depending on whether F or AF cells were examined and in each case was only a fraction of that in dermal fibroblasts. This apparently differential expression of prolyl hydroxylase activity in amniotic fluid cells and dermal fibroblasts is not understood. Therefore, in a pregnancy at risk, lysyl hydroxylase activity in amniotic fluid cells was determined without reference to prolyl hydroxylase. Normal activity was found, and a healthy baby was born [391]. To our knowledge, chorionic villus biopsies, as opposed to cultured chorionic villus cells, have not been investigated as an enzyme source but have been used as a source of DNA [397].

ARTHROCHALASIS TYPE OF EDS — EDS TYPES VIIA AND VIIB (ARTHROCHALASIS MULTIPLEX CONGENITA) (MIM 130060)

Diagnostic Criteria

The arthrochalasic type of EDS is inherited as an autosomal dominant trait and is caused by mutations leading to deficient processing of the amino-terminal end of proα1(I) (EDS type VIIA) or proα2(I) (EDS type VIIB) chains of collagen I because of partial loss or complete skipping of exon 6. It is characterized as follows [39]:

- Major diagnostic criteria
  - Severe generalized joint hypermobility, with recurrent subluxations
  - Congenital bilateral hip dislocation
- Minor diagnostic criteria
  - Skin hyperextensibility
  - Tissue fragility, including atrophic scars
  - Easy bruising
  - Muscular hypotonia
  - Kyphoscoliosis
  - Radiologically mild osteopenia, occasionally fractures

Historical Introduction

Hass and Hass, in 1958 [452], suggested that there is a distinct entity of loose-jointedness, which they called arthrochalasis multiplex congenita (congenital flaccidity of the joints), which "represents, to some extent, the antithesis of arthrogryposis multiplex congenita", and which may occur with or without skin changes. This disorder was classified as EDS VII [2]. In 1973, three patients with EDS VII were reported who accumulated procollagen in their skin and tendon, and it was thought therefore that their disorder resembled dermatosparaxis in cattle (see below) [453,454]. Because it was felt that the accumulation of procollagen was most likely to be due to a defect in the conversion of procollagen to collagen, the activity of the converting proteinase was determined in the culture medium of fibroblasts from these patients and found apparently to be reduced to between 10% and 40% of normal [453,454]. However, the marked clinical and ultrastructural differences between the disorder in man and that in cattle, later reported also in sheep, led Steinmann et al. [35,36] to reinvestigate one of these patients. Analysis by SDS-polyacrylamide gel electrophoresis of collagen extracted from skin or produced by fibroblasts from the youngest of the three reported patients [453] (case 5 in Table 2) disclosed, in addition to α1(I) chains, the presence of pNα2(I)-like chains, in a 1:1 ratio with normal α2(I) chains, and an absence of pNα1(I), pCα1(I), and pCα2(I) chains. The pNα2(I) chain was resistant to pepsin, α-chymotrypsin, and N-procollagen proteinase, and, further, procollagen N-proteinase activity in cell extracts was normal. Rather than there being an autosomal recessively inherited N-proteinase deficiency as in dermatosparaxis, it was concluded that there was an extensive structural abnormality in that portion of the proα2(I) chain that is normally cleaved by N-proteinase (and other proteinases), and that the patient was a sporadic heterozygote arising from a new mutation because both parents, who were phenotypically normal, lacked the mutant pNα2(I) chain in extracts of both skin [36] and cultured fibroblasts (B. Steinmann, unpublished observations). The authors speculated further that, for sterical reasons,

TABLE 2. Arthrochalasic Type of Ehlers-Danlos Syndrome (EDS Types VIIA and VIIB)

Case	Patient initials	Sex	Congenital bilateral hip dislocation	Scoliosis	Additional findings	Family history*	Gene	Mutation	References
1.	(A)	f	+			+	*COL1A2*	Intron 5 −2A>G	457
2.	(B)	m	+	+		−	*COL1A2*	Intron 5 −1G>A	457
3.	R.B.	f	+	+	Fractures	+	*COL1A2*	Intron 5 −1G>C	458,459
4.	N.D.	f	+		Wormian bones	+	*COL1A2*	Intron 5 −1G>C	460
5.	S.N.	f	+		Fractures	−	*COL1A2*	Exon 6 −1G>A	36,305,453,454,461–464
6.	(E.)	f	+			?	*COL1A2*	Exon 6 −1G>A	457
7.	M.F.	m	+	+	Wormian bones; occipital horn	−	*COL1A2*	Intron 6 +1G>A	465
8.	T.T.	f	+	+		−	*COL1A2*	Intron 6 +1G>A	466,467
9.	P.S.	m	+	+		+	*COL1A2*	Intron 6 +1G>A	468,469
10.	K.H.	f	+			−	*COL1A2*	Intron 6 +1G>A	470,471
11.	(- -)	m	+			−	*COL1A2*	Intron 6 +1G>A	472
12.	S.M.	f	+		Fractures; Wormian bones	±	*COL1A2*	Intron 6 +1G>A	473,474
13.	(C)	f	+			−	*COL1A2*	Intron 6 +1G>T	457
14.	(D)	m	+		Fractures	−	*COL1A2*	Intron 6 +1G>T	457
15.	K.K.	m	+			±	*COL1A2*	Intron 6 +1G>T	475
16.	L.G.	m	+		Wormian bones; large head and wide patent fontanelles	−	*COL1A2*	Intron 6 +2T>C	465
17.	W.A.	f	+			−	*COL1A2*	Intron 6 +2T>C	461,476,477, Figure 2 in 456;478–480
18.	L.W.Y.	m	+			−	*COL1A2*	Intron 6 +2T>C	481
19.	(F)	f	+	+	Fractures	−	*COL1A2*	Exon 6 deletion	457

(*Continued Overleaf*)

TABLE 2. (*Continued*)

Case	Patient initials	Sex	Congenital bilateral hip dislocation	Scoliosis	Additional findings	Family history*	Gene	Mutation	References
20.	K.D.	f	+	−	Fractures	−	*COL1A1*	Genomic deletion of exons 5+6	475
21.	?	?	+			−	*COL1A1*	Intron 5 −2A>G	482
22.	(G)	f	+		EDS VII C-like features	−	*COL1A1*	Intron 5 −1G>A	457
23.	S.E.	m	+	+	Fractures, Wormian bones, dentinogenesis imperfecta	−	*COL1A1*	Intron 5 −1G>A	475
24.	?	?	+			−	*COL1A1*	Intron 5 −1G>C	482
25.	T.H.	m	+	?	Fractures, osteopenia, dentinogenesis imperfecta, Wormian bones?, rectal prolapse	−	*COL1A1*	Intron 5 −1G>T	483
26.	N.W.	f	+	+	Fractures	−	*COL1A1*	Exon 6 −1G>A	484
27.	R.M.	f	+	+	Fractures	−	*COL1A1*	Exon 6 −1G>A	455,485–487
28.	?	?	+			−	*COL1A1*	Exon 6 −1G>C	482
29.	E. D.	f	+			?	nc		Fig. 6-15 in 2;36,453,461
30.	S.E.	f	+			−	nc		Fig. 6-28 in 2;36,453,454,461,464
31.	A.P.	m	+				nc	normal *COL1A1* and *COL1A2* genes	460
32.	Ma San	f	?				nc		36,461,464
33.	Mel Neg	m	?				nc		36,461
34.	Es Ho	f	?				nc		36,461

*The symbols designate positive (+), negative (−), or questionable (?) family history; the symbol (±) refers to possible parental mosaicism. nc denotes normal structure of collagen produced by fibroblast cultures as judged by SDS-polyacrylamide gel electrophoresis.

molecules containing pNα2(I) chains would interfere with fibrillogenesis and cross-linking, thus leading to abnormal collagen fibrils and increased solubility of collagen. After the description of a similar patient, in whom the condition was due to a mutant pNα1(I) rather than pNα2(I) chain [455], the classification of EDS VII was subdivided into types VIIA and VIIB, respectively, depending on whether the α1(I) or α2(I) chain was affected [38]. The classification EDS VIIC (MIM 225410) is reserved for the procollagen N-proteinase deficiency analogous to dermatosparaxis in animals. Details of the patients discussed below are summarized, with case numbers and initials, in Table 2.

Clinical Findings

The hallmark of EDS VII is the involvement of ligaments and joint capsules. The disorder is characterized by severe multiple joint hypermobility, recurrent subluxations and luxations of both small and large joints, and ligamentous tears. All patients are ascertainable in the newborn period, although the correct diagnosis is still frequently only made years later. Congenital bilateral hip dislocation is the rule, and muscular hypotonia is prominent (Figs. 28–30); both factors predispose to the high incidence of breech presentation (cases 2, 7, 15, 22, 29; see Table 2) and delayed gross motor development. Short stature, if present, is due to severe thoracolumbar scoliosis and hip dislocation (cases 27, 29). In striking contrast to dermatosparaxis, the skin is only moderately affected; it is usually thin, velvety, and moderately extensible, and is occasionally affected by poor healing of wounds, with atrophic and hemosiderotic scars, especially in the adult. The facies appears to be normal, despite descriptions of a chubby appearance, epicanthal folds, hypertelorism, a depressed nasal bridge, and micrognathia (Figs. 28–30). Judging by six affected members from a three-generation family, intrafamilial variability is slight (case 1) [457].

The newly recognized occurrence of recurrent fractures, Wormian bones, and abnormally wide fontanelles (Table 2) indicates that the EDS VII phenotype includes bone changes and fragility similar to those reported in mild osteogenesis imperfecta [465]. Wormian bones were noted initially in one family (case 12), seducing the observers into calling into existence still another form of EDS [473], but have since also been observed in cases 4, 7, 16, and 23 (Table 2). X-rays of one of our patients disclosed bone changes such as discrete occipital horns, exostoses, a thickened calvarium, and Wormian bones [465] (Fig. 30; case 7).

Defect and Pathogenesis

The molecular defect in all 28 cases elucidated to date is remarkably homogeneous and results in the loss of exon 6, or part of it, of the mature mRNA coding for one of the α1(I) chains or the α2(I) chain (Table 2). The deleted peptide is the junctional segment (N-telopeptide) linking the N-propeptide to the major triple-helical domain. Loss of this short segment, 24 and 18 amino acid residues encoded by exon 6 in *COL1A1* and *COL1A2*, respectively, results in union of these latter domains and a lack of the N-proteinase cleavage site (Pro–Gln and Ala–Gln at positions 4–5), the critical cross-linking lysyl residue (at positions 13 and 9, respectively), and the cleavage sites for proteinases such as pepsin and α-chymotrypsin. The apparent paradox of a deletion yielding a larger than normal protein is thus resolved.

Studies by Wirtz et al. [476] showed that in EDS VIIB (case 17), the α1(I) N-propeptide, although cleaved from the α1(I) chain by the N-proteinase or (less likely) by nonspecific proteases, is retained by noncovalent association with the mutant pNα2(I) chain in native mutant collagen molecules, both *in vivo* and *in vitro* (Fig. 31); the α1(I) N-propeptide was readily demonstrable by western blotting of skin extracts, and rotary shadowing of pepsin-treated collagen produced in culture disclosed kinked molecules, which were longer than their normal counterparts (Fig. 31E). These data suggest that the retention of a partially cleaved but essentially intact N-propeptide in mutant collagen may play a critical role in the pathogenesis of this disease.

As a consequence of the persistence of the N-propeptide and/or the missing critical cross-linking lysyl residue, the solubility of collagen is increased three to four fold [36,453,470,477] (cases 5, 10, 17) and the ratio and pattern of β-components is abnormal [36,455,470] (cases 5, 10, 27). Only one-quarter of the normal content of histidinohydroxymerodesmosine, the major residue derived from skin collagen upon borohydride reduction, is found, but there is a similar to normal content of the mature cross-links, hydroxylysyl and lysyl pyridinoline, in fascia and bone [470]. Extracts of bone contain significantly more pNα2(I) than normal α2(I) chains. This solubility difference between bone and the soft tissues probably results from basic differences in the chemistry and molecular sites of their collagen cross-links [470].

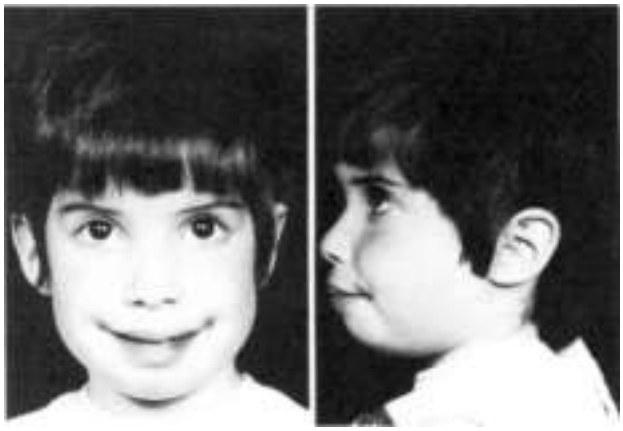


Figure 28. Arthrochalasic type of EDS (EDS VIIB). S.N. (28 Dec 1968), case 5 in Table 2, is the first patient in whom a structural mutation in proα2(I) chains was demonstrated [35,36]. At the age of 4 years, she had a ''dish-like'' face and mild retrognathia, which, however, cannot be considered typical of the syndrome because it is absent in cases 17 and 7 (Figs. 29, 30) and is not apparent in the patient herself at a later age.

She had a neuromuscular workup because of breech presentation and hypotonia at age 2 months, did not sit until age 1 year, stood with support at age 2 years, and when she began walking was noticed to have a waddling gait, which led to the diagnosis of bilateral hip luxation, necessitating several surgical interventions. The ligamentum teres of the right hip was found to be torn at surgery. She also had generalized, pronounced joint laxity.

Follow-up at age 22 years revealed an adult height of 157.5 cm (her target height was 158.5 cm); joint laxity and muscle strength had improved after puberty, whereas her skin had remained soft, velvety, and fragile and tended to form keloids. She was studying to become an elementary teacher (C. Nash, personal communication, 1991). Follow-up at age 30 years disclosed that she had had three luxations of the patella and two fractures of the femur after minor trauma in the preceding 8 years (S. Nash, personal communication, 1999).

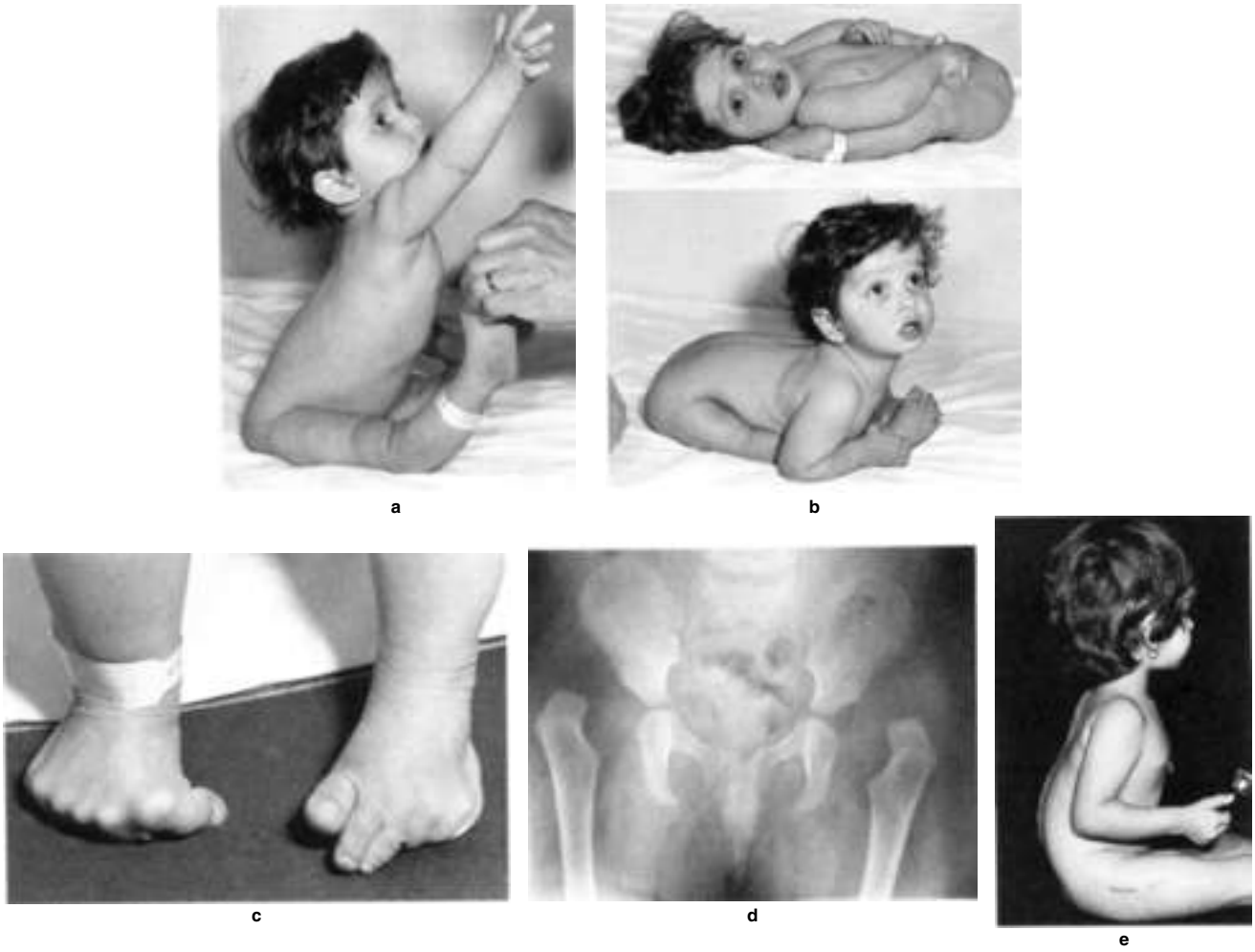


Figure 29. Arthrochalasic type of EDS (EDS VIIB). W.A. (17 Feb 1982), case 17 in Table 2, a 12-month-old girl, born to healthy, unrelated parents from Libya. Her younger brother is healthy, and cultured amniotic fluid cells obtained during his delivery produced normal collagen I. (**a**) Subluxations of both knees, moderate thoraco-lumbar kyphosis. (**b**) Congenital hip luxations. (**c**) Marked hyperlaxity of the small joints. (**d**) Radiograph showing bilateral hip dislocation with lateralization and elevation of both femurs. (**e**) Thoracolumbar kyphosis and keloid formation at age 2.7 years, one-and-a-half years after successful operative correction of the hip dislocation. (a–c are reprinted from Steinmann and Gitzelmann [456] with permission.)

Figure 30. Arthrochalasic type of EDS (EDS VIIB). This 34-year-old subject with EDS type VIIB (M.F., case 7 in Table 2) was originally referred to us by Dr. Katarina Raslova (Bratislava, Slovakia). The patient was born from a breech presentation with dislocations of both hips and the left knee; although the hips were surgically reduced at the age of 2 years, he could not walk and had to crawl around on all fours until surgical correction of the left knee at age nine. Thereafter, he was able to walk without aid for a short period of time but soon had to use crutches because of recurrent dislocation of the right ankle, upon which surgical arthrodesis was finally performed. His skin has always been somewhat fragile, but wound healing is not delayed. His only internal complication has been a symptomatic bladder diverticulum, which was resected at age 17. In spite of these severe handicaps, he has an above-average intelligence and has led an independent life, doing much traveling; he drives an adapted car. He has had one unaffected child by a first wife and another healthy child by his second wife after negative prenatal diagnosis [260].

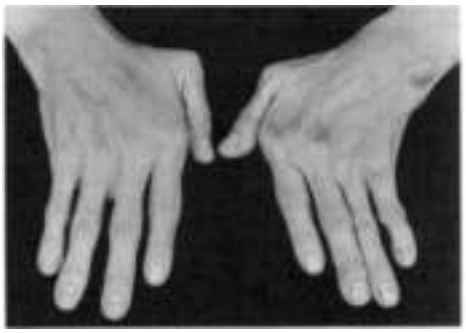
The photographs show the sagging skin around the eyes and mouth (**a**, **b**; the xanthoma in the left upper eyelid is probably due to coincident hyperlipoproteinemia); marked ulnar deviation of fingers II to V (which cannot be fully extended) and subluxation of the proximal interphalangeal joint of the right thumb (**c**); malposition and deformation of toes (**d**); arthrodesis has been performed on the right ankle joint. Note the normal stature (172 cm) and body proportions and the thin, hyperpigmented scars on both shins and knees (**e**).

X-rays show a peculiar combination of thickening of the calvarium with a pumice stone-like appearance, Wormian bones, calcification of the falx cerebri, and calcification of a tendon insertion in the occipital region (''occipital horns''; see arrow) (**f**, **g**); exaggerated lumbar lordosis with moderate osteoporosis of vertebral bodies (**h**); arthrotic degeneration of the knee joints with marked osteophyte formation at insertion sites on the patella (**arrows**) (**i**); reduction of articular cartilages and subluxation of several joints in both hands and feet (**j**, **k**). Note also the short metacarpals III–V, a small osteophyte (probably also in a tendon insertion) at the distal extremity of the ulna, and a previous fracture of the left fourth metatarsal.

Such radiological changes have not been observed in EDS VII so far, apart from Wormian bones (Tables 2 and 3), and may point to the natural history of the disorder as observed in this 34-year-old patient. ''Occipital horns'' and calcification of tendon insertions have been considered characteristic of EDS IX, now the occipital horn syndrome (see below and Chapter 14, this volume).

a

b



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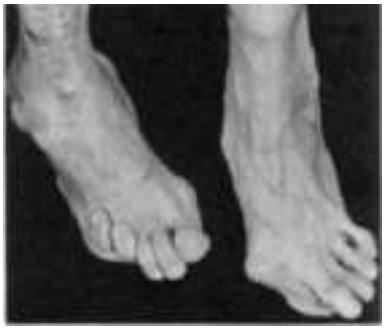

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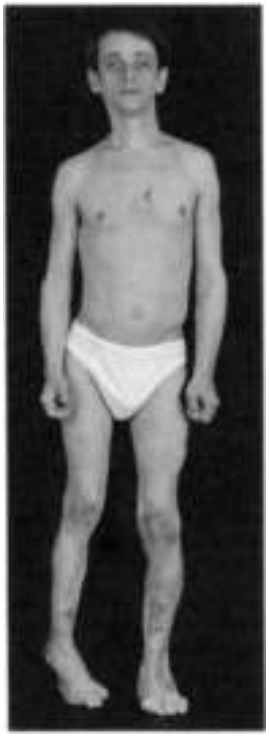

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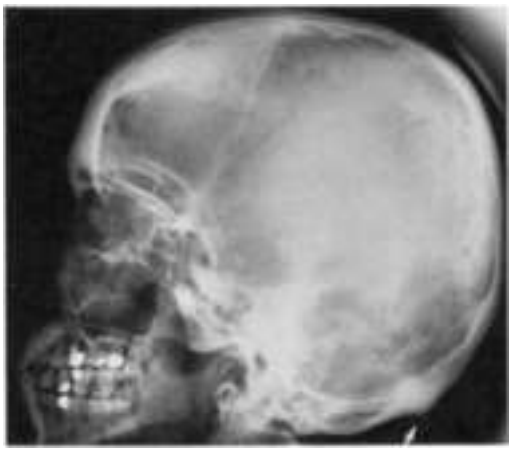

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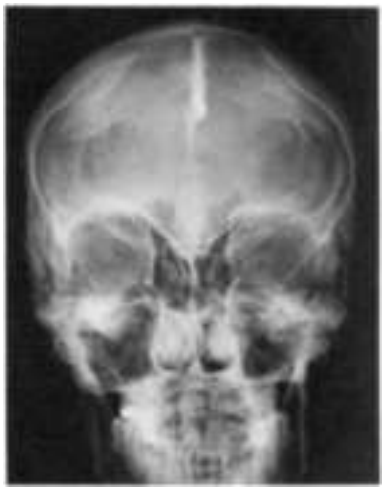

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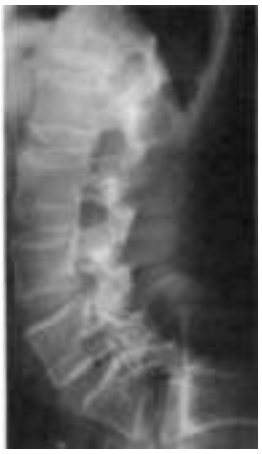

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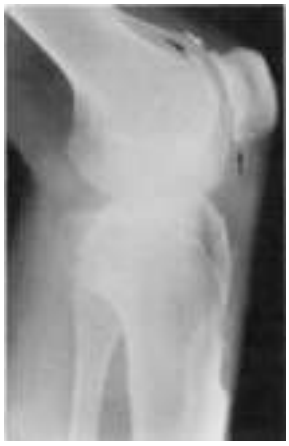

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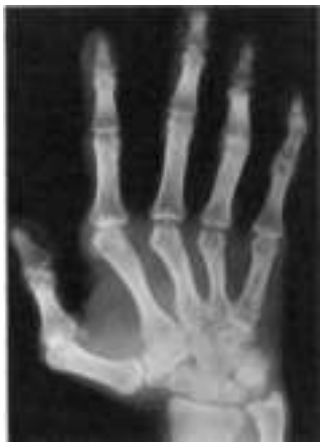

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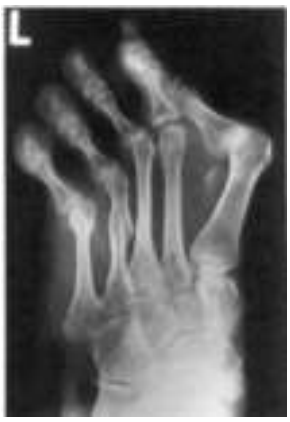

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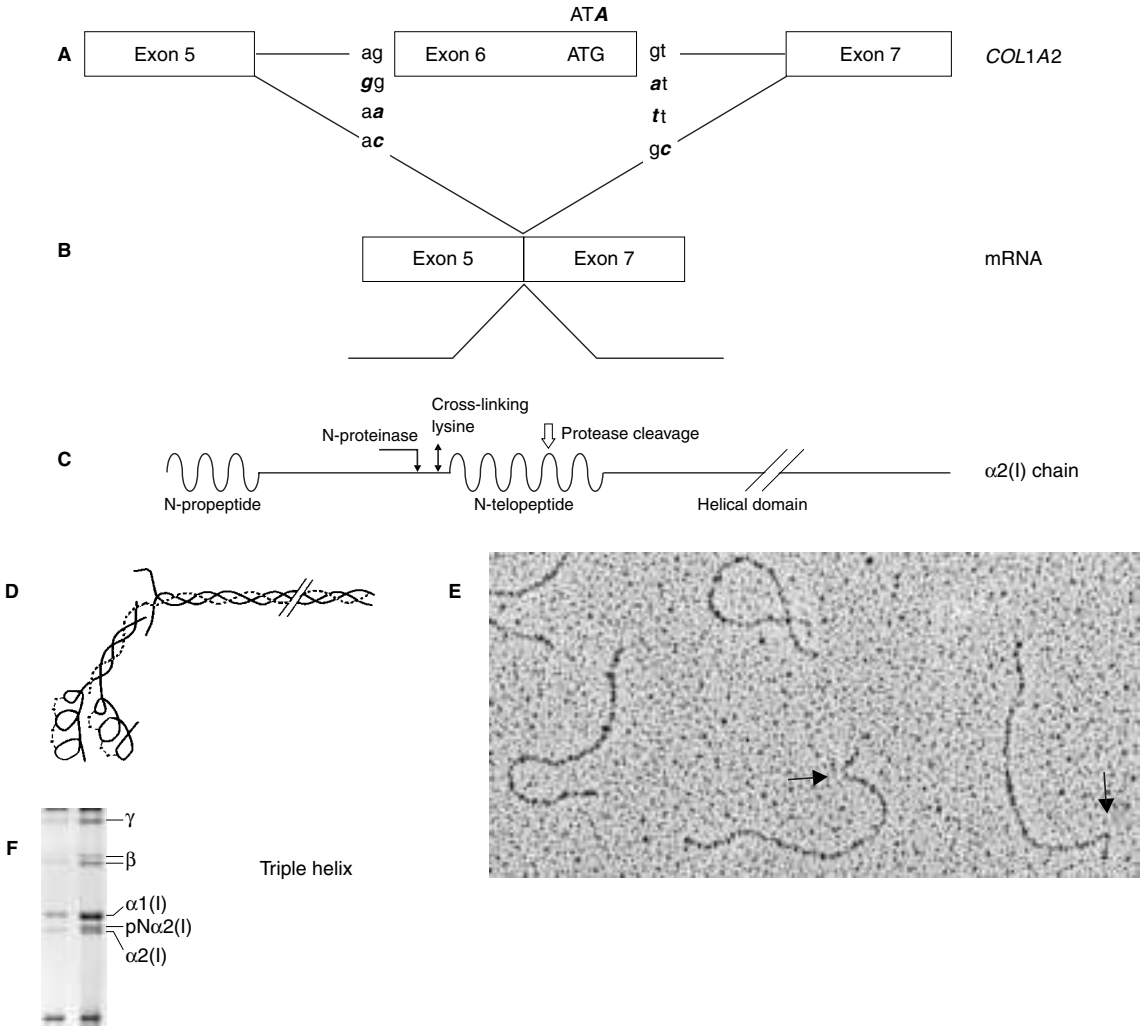


Figure 31. Schematic representation of the pathogenesis of arthrochalasic type of EDS (EDS VIIB). (**A**) Exon-intron structure of *COL1A2* with acceptor (ag) and donor (gt) splice sites adjacent to exon 6. In bold italics are mutations as listed in Table 2. (**B**) Skipping of exon 6 or deletion of exon 6 leading to fusion of exons 5 and 7 in the α2(I) mRNA. (**C**) The deletion of exon 6 removes the N-telopeptide, which contains the N-proteinase cleavage site, a lysine cross-link site, and protease cleavage sites. (**D**) Structural model of an abnormal collagen I molecule containing a mutant pNα2(I) chain, as indicated by a dotted line. Both cleaved N-terminal peptides of the α1(I) chains, as indicated by solid lines, are retained through triple-helical binding to the mutant N-terminal propeptide of the α2(I) chain and form a kink. (**E**) Rotary shadowing electron microscopy of pepsin-treated collagen produced by fibroblasts. Approximately one-half of the molecules present a kink (**arrows**) at their N-terminal ends and are somewhat longer than normal (312 vs. 297 nm). (**F**) SDS-polyacrylamide gel electrophoresis of collagen extracted by neutral salt from skin of patient S.N. [36] (**right lane**) and a control (**left lane**). The mutant pNα2(I) chain migrates more slowly than the normal α2(I) chain, and both chains are in a one-to-one ratio; pN collagen I, is more soluble than fully processed collagen I, as shown by the increased amount of its derived α, β, and γ chains. (Model in (D) adapted from experimental rotary shadowing data and reprinted from Wirtz et al. [476] with permission. (E) reprinted from Wirtz et al. [476] with permission.)

Two very interesting patients with EDS VIIB (cases 3 and 4) have been observed [458,460]. In both, a G to C change at the splice acceptor site of intron 5 activates a cryptic splice site in exon 6, removing 5 amino acid residues, including the N-proteinase cleavage site, but preserving the lysyl residue cross-link site. The fact that both mother and son, and father and daughter, respectively, were affected with typical EDS VII indicates that the persistence of the N-terminal propeptide is more deleterious than the absence of the lysyl residue or, alternatively, that in the presence of the propeptide, the lysyl residue is either not susceptible to lysyl oxidase activity or that following lysyl oxidase activity the lysyl aldehyde is unable to participate in cross-link formation for sterical reasons. However, studies of collagen solubility and cross-links have not yet been performed to answer this question.

A further instructive patient from a family with nine affected members in three generations was reported by Sippola et al. [488]. He presented features of both EDS VII and osteogenesis imperfecta (generalized osteoporosis,

platyspondyly, Wormian bones, blue sclerae, but no fractures) similarly to the patient shown in Figure 35, and carried a 19 bp deletion that caused skipping of exon 11 of *COL1A2* [489], thus deleting one of the lysyl residues involved in intermolecular cross-link formation. The presence of the shortened proα2(I) chain in procollagen synthesized by the proband's fibroblasts both lowered the thermal stability of the molecules and prevented or delayed their processing by procollagen N-proteinase [490]. The decrease in thermal stability was apparently not sufficient to produce exclusive degradation of the protein. Instead, the extreme laxity of joint ligaments in the proband was probably explained by the effect of the incompletely processed N-propeptide on fibril assembly [488]. Thus, the deletion disturbed both the helical domain and the cleavage site of the molecule, thereby resulting in the dual phenotype. The variable phenotypic expression in the proband's family was remarkable and may be explained by the possibility that the 19 bp deletion did not produce exon skipping *in vivo* with the same efficiency in everyone who inherited the defect [489], although *in vitro* the abnormal splicing was not changed by varying either the temperature of the fibroblast cultures or the salt concentration. In contrast, temperature-dependent alternative splicing has been demonstrated *in vitro* in case 5 [305] and subsequently in case 26 [484], but its significance *in vivo* remains unknown. A further case has been reported who had features of both osteogenesis imperfecta and Ehlers-Danlos syndrome [491].

We do not sufficiently understand why the abnormal collagen in humans produces major manifestations in joints and ligaments, whereas the skin, unlike that in dermatosparactic animals, is less affected; it may be that fibril formation is particularly disturbed by the pN-peptide where fibrils are tightly packed, as in tendons.

Genotype-Phenotype Correlation

Interfamilial and intrafamilial variability seems to be slight. In EDS VIIB, the heterotrimeric collagen I molecules consist of one population of normal collagen I and one population of abnormal collagen I containing the mutant α2(I) chain. In contrast, in EDS VIIA, three-quarters of collagen I molecules would be expected to be abnormal because they contain one or two mutant α1(I) chains, and thus the phenotype would be expected to be more severe than that of EDS VIIB. However, among the reported cases of EDS VIIA, three involve substitutions for the last nucleotide of exon 6 (cases 26–28, Table 2). This results in alternative splicing so that in part of the product the exon sequence from mRNA is deleted, whereas in the remainder normal splicing is permitted and the protein product contains an isoleucine rather than methionine at position 3 of the first Gly–X–Y triplet of the triple helix. The normally spliced product containing an amino acid substitution diminishes the dysfunctional effect of the three-quarters of abnormal molecules; this results in a less severe clinical phenotype that is indistinguishable from that of EDS VIIB. In contrast, in case 22, there is complete skipping of exon 6 from mutant proα1(I) chains; this leads to three-quarters of the collagen I molecules being abnormal and dysfunctional and explains the more severe phenotype, which almost resembles EDS VIIC, in which none of the collagen I molecules are processed [465].

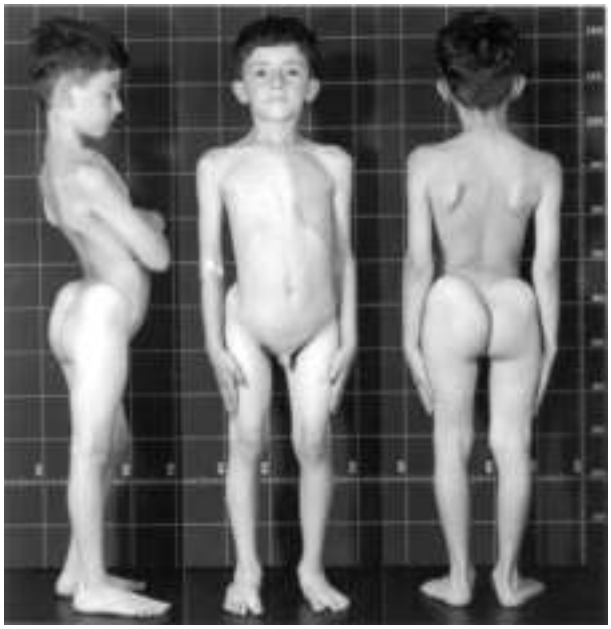


Figure 32. Arthrochalasic type of EDS (EDS VIID). A.P. (30 Sep 1993; case 31), at age 7 years, presents with bilateral hip dislocation, hyperlordosis, flat feet, pigeon-breast-like deformity, and scapulae alatae. His skin is moderately hyperelastic and soft, does not present abnormal scars, and has no tendency toward bruising. He has generalized joint laxity, muscular hypotonia, and a waddling gait due to congenital hip dislocation. The eyes and internal organs are normal, and height and weight are on the 50th centiles.

He is the fifth child of healthy, unrelated parents from Kosovo; the four older siblings are not affected. His birth and neonatal adaptation were normal, but it was noted that something was ''wrong'' with his hips; gross motor development was delayed.

Collagen fibrils in dermis were ultrastructurally normal in diameter and contour. The collagens produced by his fibroblasts were normal in amount and structure. These findings are similar to those in case 29 (see Fig. 33d) and unlike those observed in EDS VIIA, VIIB, and VIIC. He is therefore temporarily grouped as EDS VIID.

Hass and Hass [452] state that ''arthrochalasis multiplex congenita is a distinct clinical entity characterized by a multiple congenital flaccidity of the joints. It may be associated with laxity or hyperelasticity of the skin defined as Ehlers-Danlos syndrome, or it may exhibit hypermobility of the joints without involvement of the skin.''

Locus heterogeneity is exemplified by several patients (cases 29–34, Fig. 32) who are clinically similar to EDS VIIA/B but whose fibroblasts produce normal procollagen that is converted normally to collagen by pepsin or α-chymotrypsin [36]. Using the assay of Prockop and Tuderman [492], a somewhat lower N-proteinase activity has been determined, which reflects methodological problems rather than biological aberrations [36,461]. The normal structure of collagen I extracted from the skin of cases 29 and 31 and produced by fibroblasts, the absence of pNα1(I) and pNα2(I) chains in skin extracts, normal procollagen N-proteinase activity, and a normal electron-microscopic appearance of dermal collagen fibrils (Fig. 33d) clearly distinguish these patients from those with EDS VIIA and VIIB. It remains uncertain whether patients such as these are examples of EDS VII of unknown cause, temporarily called

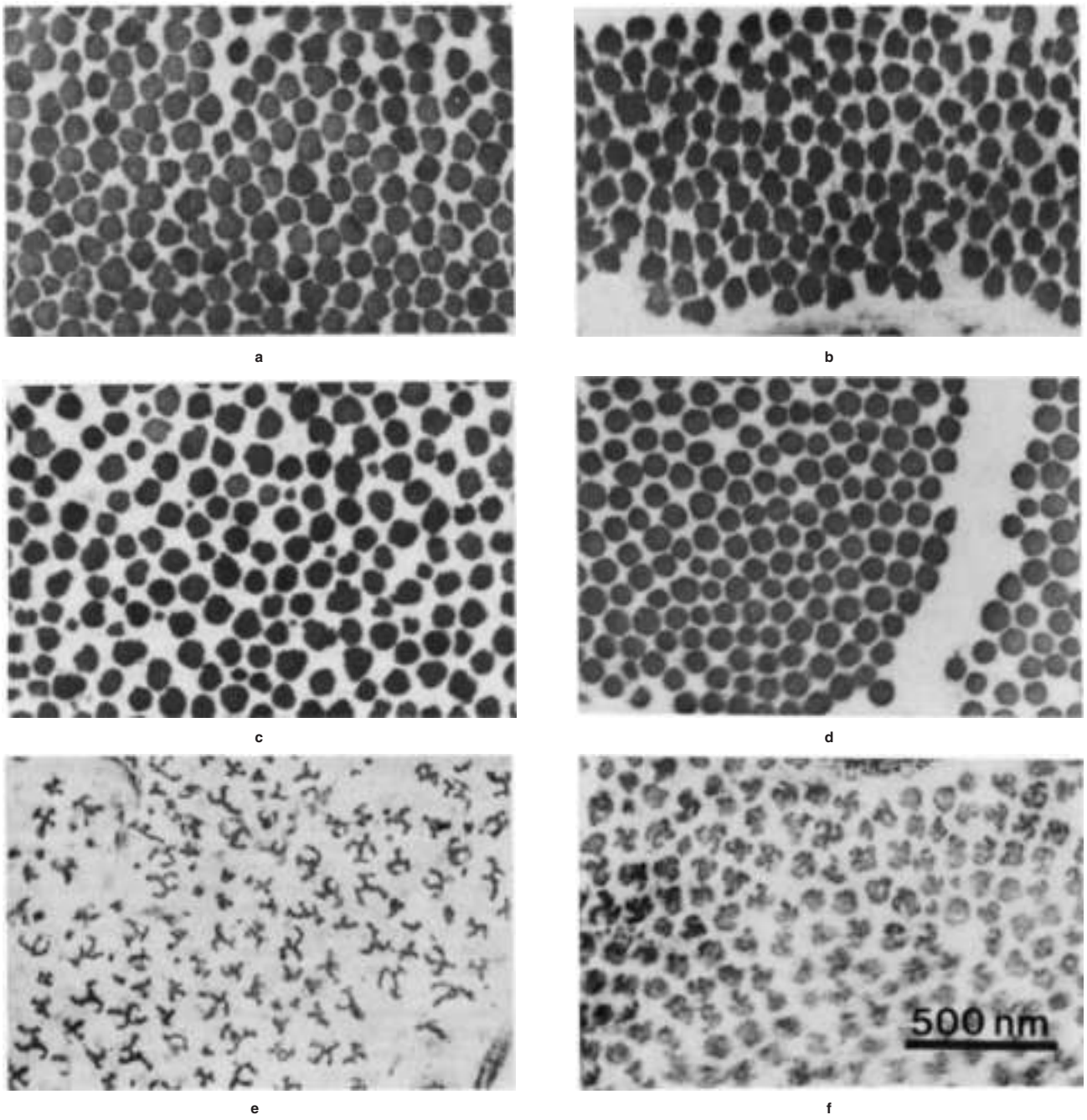


Figure 33. Ultrastructural appearance of collagen fibrils in the dermis of patients with arthrochalasic type of EDS (EDS VIIB and VIID) and in dermis and tendon of a dermatosparactic sheep. Electron-microscopic pictures of collagen fibrils in cross section from the dermis of patients with EDS VII (**a**–**d**), and the dermis (**e**) and tendon (**f**) of a dermatosparactic sheep. (**a**) Case 5, at age 10 years; (**b**) case 17, at age 1 year; (**c**) case 7; (**d**) case 29, at age 38 years (EDS VIID). Staining was with phosphotungstic acid and uranyl acetate, and the magnification is identical in all photographs. In the case of the patients, pictures were taken 0.5 mm below the epidermal-dermal junction, and the diameters of 1,000 fibrils were measured and are given below as mean ±SD.

In the three patients with defined EDS VIIB (**a**–**c**), the collagen fibrils are irregular in contour but have normal diameters with slightly increased variability: 89.0 ± 11.7 nm in (a), 86.5 ± 16.7 nm in (b); 79.5 ± 14.2 in (c). In contrast, both shape and diameter of the fibrils in the patient with EDS VIID (d) are normal and indistinguishable from controls (90.3 ± 12.4 nm).

The skin of the newborn dermatosparactic lamb was moist and thicker than normal, with a jelly-like consistency, while histologically the collagen consisted of loosely woven thin fibrils. On electron microscopy, the cross sections showed a multibranching appearance of the fibers, resembling ''hieroglyphics'' (**e**); longitudinal sections showed that the fibers had a twisted, ribbon-like appearance. In tendon, cross sections showed a more rounded appearance of the fibers with thicker and less-developed branches, particularly in areas where the fibers were packed more tightly together (**f**). (Figures a–d are unpublished pictures from Ursula Lüthi and A. Vogel, and Figs. e and f are from Fjølstad and Helle [493] with permission.)

EDS VIID, or whether they represent the extreme end of severity of EDS III. It may be of note that cases 29–31 have smooth, hyperelastic skin but without skin fragility.

Morphology

The skin collagen fibrils have a smaller and more variable diameter than normal. They are irregular in outline and also appear to be more loosely and randomly organized into fibrils resembling a loosely wound rope (Figs. 33a–c) [470,485]. A similar fibril outline is also evident in sections of fascia and decalcified bone [470]. All these changes, however, are much more subtle than those observed in dermatosparactic animals (see below) (Fig. 33e) and in cases 22 and 25, in whom three-quarters of collagen I molecules are abnormal.

Genetics

EDS VIIA and VIIB are inherited as autosomal dominant traits, and some of the sporadic cases have been shown to be heterozygous for a new mutation (Table 2). Mosaicism in the mother of case 12 is likely the cause of her milder phenotype as opposed to her affected children. The apparently higher incidence of EDS VIIB is puzzling but may have to do with differences in the structures of intron 6 in *COL1A1* and *COL1A2*.

The pedigree of case 30 (Fig. 6.15 in [2]) suggested autosomal recessive inheritance because of consanguinity of the parents and the presence of two apparently affected sisters. Re-evaluation, however, revealed that both sisters have normal joints, skin, and height and complain only about "troubles with the knees." It is therefore more likely that case 30 is a sporadic case also arising from a new mutation (B. Steinmann, own observation, May 1978).

Diagnosis and Differential Diagnosis

The diagnosis is first made on clinical grounds, supported by protein data pertaining to collagen extracted from skin or produced by fibroblasts (Fig. 31F), and proven by the demonstration of complete or partial loss of exon 6 in cDNA and of the mutation in genomic DNA. The existence of individuals with a clinical picture of arthrochalasis multiplex congenita but normal collagen I structure, normal procollagen N-proteinase activity (cases 29–34), and normal electron microscopic findings in skin (case 29, Fig. 33d) indicates further heterogeneity; these patients who do not fall into any one of the three type VII variants A, B, or C may temporarily be grouped as EDS VIID.

Congenital hip dislocation is also found in the Larsen syndrome (MIM 150250, MIM 245600), as an isolated disorder (MIM 142700), and in an as yet less well-defined disorder (i.e., dislocation of the hip, congenital, with hyperextensibility of fingers and facial dysmorphism) (MIM 601450).

The risk to healthy parents of having another affected child is small and due to the possibility of parental mosaicism. The mother of the four patients reported by Viljoen et al. [473] (case 12) was the least severely affected individual within the family and may represent a symptomatic mosaicism of the mutation in *COL1A2*, although the fibroblasts examined do not disclose any mosaicism; other of her cells, however, have not been investigated. Alternatively, her milder phenotype may simply be the expression of intrafamilial variability (G. Wallis, personal communication, 1991). The risk of an affected person transmitting the disorder is 50%, but prenatal diagnosis should be possible by either DNA or protein studies on a chorionic villus biopsy if the defect in the index case has been characterized. Biochemical analysis of a chorionic villus biopsy in a pregnancy at risk of the spouse of case 7 was normal and a healthy girl was born as predicted [260].

Management

The orthopedic outcome is often unsatisfactory because premature degenerative arthritis of the hips and other joints occurs.

The principal orthopedic problem in EDS VII patients is bilateral congenital dislocation of the hips. To gain an insight into the management of this problem, Giunta et al. [465] reviewed the treatments and their outcomes in 16 patients from 12 families. Several of the patients listed in Table 2 were not included because insufficient information was available from their published reports. Stable reductions were infrequently achieved following closed reduction with orthoses or hip spicas. Anterolateral open reductions with capsular plication, even in infancy, were also inadequate because most of the patients continued to have subluxated or dislocated hips. The poor outcome of the latter procedure is likely to be due to early stretching of the capsulorrhaphy sutures. In contrast, the addition of an iliac osteotomy of the Pemberton or Salter type with or without femoral osteotomy achieved some good results (cases 14, 17, 27).

From published results [494] and the findings of Giunta et al. [465], it appears that open reduction with capsulorrhaphy and iliac osteotomy, with the addition of femoral osteotomy in some cases, is required to achieve and maintain stable reduction. These requirements are similar to those shown to be necessary to achieve and maintain stable reduction in other laxity conditions such as Down and Larsen syndromes. As with the latter conditions, careful planning of osteotomy is required to ensure that adequate acetabular coverage of the femoral head is achieved in the functional positions of the limb [465].

Generalized joint hypermobility is worst in infancy, when marked muscular hypotonia accompanies the severe ligamentous laxity. Motor development is consequently slow, and aids such as knee-ankle-foot and ankle-foot orthoses are often required to stabilize the lower limb joints for standing and walking. As muscle tone improves, the knee-ankle-foot orthoses may be reduced to ankle-foot orthoses. Surgical procedures to stabilize the knees and the patellofemoral joints have been used occasionally. Orthotic stabilization is likely to be more reliable. Ankle or subtalar fusions have been undertaken in a few cases but, as shown in case 7, the arthrodesed joints need to be in an optimal position for this to be successful [465].

Recurrent and/or persistent dislocations, as well as hypermobility of upper limb joints, are also frequently disabling. Operative procedures appear rarely to have been undertaken in the upper limbs, and one would predict that capsulorrhaphy and osteotomy would not stabilize these joints [465]. Arthrodesis may be useful in stabilizing some small joints, such as the metacarpophalangeal joints of the thumbs, although fusion rates cannot be predicted.

Postural thoracolumbar kyphosis due to hypotonia and ligamentous laxity is a feature of infants (cases 16 and 17, Fig. 29). The spinal posture improves as the children gain in muscle power. However, structural scoliosis has been reported in eight cases (cases 2, 3, 7, 8, 9, 19, 26, 27). Spinal fusion was undertaken in cases 8 and 9, although few details are available concerning curve patterns and surgery. Spondylolisthesis of L5-S1 was noted in case 19.

The findings in the 20 molecularly proven cases of EDS VIIA and VIIB reviewed [465] show how difficult the

management is and how important early diagnosis may be. The homogeneous nature of the molecular defects allows laboratories with the appropriate expertise rapidly to establish the diagnosis, after which the clinical problems, in particular those relating to the dislocated hips, can be predicted. Adequate physical and occupational therapy and orthotic management can be given to assist with standing, walking, and activities of daily living. Appropriate surgical treatment of the dislocated hips should also diminish the frequency of hip redislocation, recurrent dislocation, avascular necrosis, and premature osteoarthritis. However, more experience correlating detailed orthopedic management and long-term outcome is needed before sound recommendations can be made [465].

Comment

In the initial study [453], it was found, and later confirmed [461], that affected fibroblasts produce more collagen in culture than normal cells. The authors speculated that the procollagen extension peptides might act as feedback inhibitors of collagen synthesis. Evidence in support of this hypothesis has been presented [495–497]. This is another example of findings in a disease leading to further general insight into biological control mechanisms.

DERMATOSPARACTIC TYPE OF EDS — EDS TYPE VIIC (MIM 225410)

Diagnostic Criteria

The dermatosparactic type of EDS is inherited as an autosomal recessive trait caused by deficiency of procollagen N-terminal proteinase as a result of homozygosity or compound heterozygosity of mutant alleles coding for the enzyme (in contrast to the arthrochalasic type, which is due to mutations involving the substrate sites of procollagen I chains) and characterized as follows [39]:

Major diagnostic criteria
- Severe skin fragility
- Sagging, redundant skin

Minor diagnostic criteria
- Soft, doughy skin texture
- Easy bruising
- Premature rupture of fetal membranes
- Large herniae (umbilical, inguinal)

Historical Introduction

Dermatosparaxis in man (see Table 3) has only recently been recognized and shown to be due to an enzyme deficiency, whereas in animals it was the first defect in collagen metabolism to be defined [507] (see "Animal Models and Lathyrism" below). An earlier suggestion of dermatosparaxis in man [453] was disproved by Steinmann et al. [36], who demonstrated a structural abnormality in the patient's proα2(I) chain, the substrate, rather than a deficiency of the enzyme, N-proteinase (see EDS VIIB above).

Clinical Findings

Clinical findings are summarized in Table 3. All six patients reported to date were born prematurely after rupture of the membranes, one of them (case 1) by Cesarean section because of breech presentation; he presented bilateral inguinal tears [498,499]. In all, the skin was soft, with a doughy feel, redundant, lax, and fragile. The skin was noted to bruise easily and avulsed from the underlying soft tissue after minor trauma; it was easily approximated, and healed well in a short time, with minimal scarring.

All patients had umbilical herniae and some dysmorphic features, consisting of micrognathia, puffy eyelids with excessive periorbital skin, sagging skin, and wide open fontanelles and osteopenia (Fig. 34). One had moderate joint laxity and had not yet walked at the age of 18 months.

Joint laxity seems to become a major clinical feature with increasing age, as illustrated by case 4, who had only a moderate degree of joint laxity as assessed over the first 3 years. Chondrodysplastic changes, however, seem not to be absent (see "Pathogenesis" below).

Genetic Defect

The defect is due to an autosomal recessively inherited deficiency of N-proteinase, as has been demonstrated by the direct measurement of enzyme activity and by indirect evidence such as pN-collagen extracted from skin or produced in fibroblast cultures, which could be cleaved by the addition of N-proteinase activity [502] or normal cell extracts [498], or, less specifically, other proteases [498,502].

Procollagen N-proteinase (ADAM-TS2, a disintegrin and metalloproteinase with thrombospondin repeats, EC 3.4.24.14, MIM 604539) is a large, neutral zinc metalloproteinase, which cleaves the amino-propeptides in the processing of pN-collagens I and II to collagens I and II, respectively. It has been isolated by affinity chromatography on immobilized collagen XIV [508]. The interaction between the enzyme and collagen XIV is highly specific, which suggests that collagen XIV might be a physiological ligand, the role of which might be to immobilize the enzyme in the close vicinity of collagen I fibers, thereby allowing the processing and subsequent polymerization of newly synthesized molecules under strict spatial control [508]. Partial amino acid sequences allowed the cDNA cloning of the enzyme. The N-proteinase consists of 1,205 amino acid residues with two sequences in the N-terminal domain that are substrates for furin-like enzymes; this suggests processing of the proenzyme to its mature form as is generally the case for metalloproteinases [509]. The enzyme is expressed in all tissues rich in collagen I [509]. The human gene is located in chromosomal region 5q35 [510].

Mutation analysis of the six cases is shown in Table 3 [500]. Five of them are homozygous for the identical mutation, although their ethnic backgrounds differ: three are Ashkenazi Jewish, one is Hispanic/Mexican, and one is from an American family of undetermined background.

Pathogenesis

Skin is more severely affected than other collagen-rich tissues (tendons, blood vessels, bones, and cartilage), and this is correlated with the extent of morphological alteration of collagen fibrils. This suggests that another enzyme, which has tissue-specific expression, can remove the amino-terminal propeptides of procollagens I and II (the latter especially in cartilage), although at a lower rate than the N-terminal proteinase.

The fragility of the skin is directly related to the concentration of precursors among the collagen polymers. The persistence of the N-terminal propeptide is known to prevent the use for intramolecular and intermolecular cross-linking of the lysyl residue located in the N-telopeptide. As a consequence, the skin collagen content in one case studied was lower than normal, and the extractability of collagen under nondenaturing conditions increased [502]; this indicates defective formation of stable intermolecular

TABLE 3. Dermatosparactic Type of Ehlers-Danlos Syndrome (EDS Type VII C)

Case no.	1	2	3	4	5	6
Reference	498–500	498,500,501	500,502–504a	500,505	500,506	500
Age at description (years)	2	2	2 and 9	15	0.5	0.7
Gestation (weeks)	28	35	29.5	32	30	31
Premature rupture of membranes	+	+	+	+	+	−
Fontanelles widely patent	+	+	+	+	+	?
Edema of eyelids	+	+	+	+	+	
Blue sclerae	+	+	+	+	+	+
Postnatal skin fragility (observed at month)	+(7)	+(0)	+(12)	+(9)	+(3)	
Easy bruisability	+	+	+	+	+	
Cutis laxa	+	+	+	+	+	+
Umbilical hernia	+	+	+	+	+	+
Joint laxity	+	+	?	+	−	
Osteopenia	−	+	+	+	−	
Wormian bones	?	+	+	?	(at birth)	
Micrognathia	+	+	+	+	+	+
Short limbs and fingers	+	+	+	+	+	
Short stature	+	+	+	+	+	+
Myopia	+	?	?	+	−	
EM ribbon-like fibrils	+	+	+	+	+	+
Defective conversion of procollagen I	+	+	+	na	+	+
pN-collagen extracted from skin	+	+	+	?	?	
Sex	f	m	f	f	m	m
Consanguinity	?	−	?	−	?	?
Genetic defect [500]	Q225X/Q225X	Q225X/Q225X	W795X/W795X	Q225X/Q225X	Q225X/Q225X	Q225X/Q225X

na, not analyzed; Q, glutamine; W, tryptophan; X, stop.

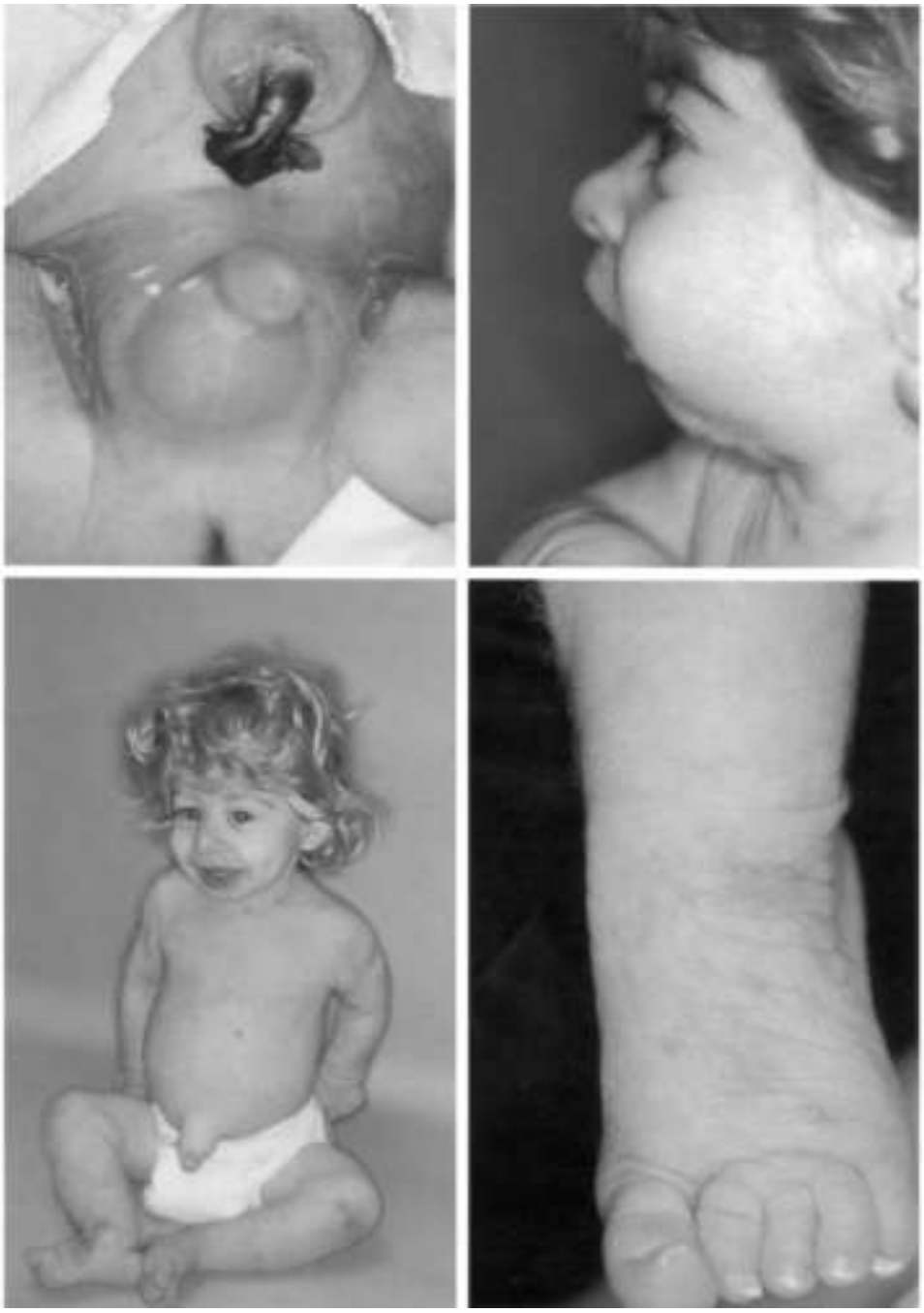
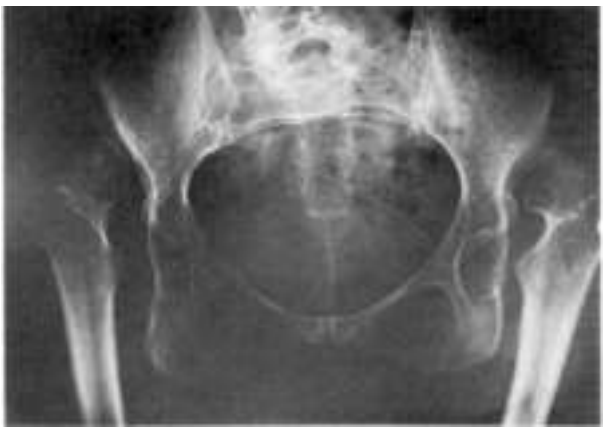


Figure 34. Dermatosparactic type of EDS (EDS VIIC). Boy with Ehlers-Danlos syndrome type VIIC. **Top left**, groin showing bilateral fissures at birth; **top right**, profile of face at 18 months of age (note the vellus hair and micrognathia); **bottom left**, frontal view at 18 months of age showing the symmetrically shortened extremities, prominently visible vasculature, multiple ecchymoses, excess skin folds, blue sclerae, and umbilical hernia; **bottom right**, closeup of left foot showing hyperconvex nails. (Reprinted from Petty et al. [501] with permission.)

cross-links, as observed in dermatosparactic animals [511]. The pattern of collagen polypeptides extracted from the skin of cases 1–3 (Table 3) comprised a large proportion of pNα1(I) and pNα2(I) chains in a 2:1 ratio, with a small proportion of completely processed α(I) chains [498,502]. The presence of apparently correctly processed collagen I is due to either residual enzyme activity or the presence of a poorly efficient alternative proteolytic pathway. Extracted pN-collagen I treated with purified N-proteinase or pepsin can be converted to collagen consisting of normal α(I) chains [502]. Extracts of normal cells cleaved procollagen I synthesized by cells from patients, demonstrating that the enzyme, not the substrate, was defective [498]; in contrast, cultured fibroblasts from three patients studied

a

b



c

Figure 35. Marked joint laxity and osteoporosis. This female patient, in her forties, has marked joint laxity (**a**, **b**) and severe scoliosis requiring Harrington rodding. X-rays showed untreated bilateral congenital hip dislocation (**c**) and diffuse osteoporosis. She has had some fractures, suggesting that she is affected by an overlap form between EDS and osteogenesis imperfecta. She is not married and has refused a skin biopsy (own observation). Two families with similar findings have been described [488,489,491].

cleaved the N-terminal propeptides from procollagen I poorly [498,502], and collagen synthesis in cells from case 3 was increased [502], probably because of a lack of feedback inhibition by the released N-terminal propeptides, as shown in studies on cells derived from animals [495–497,512].

The accumulation of pN-collagen in tissues, mainly the skin, leads to disturbed fibrillogenesis. Instead of forming bundles of polymers running in parallel, the procollagen fibrils are poorly oriented and the space between them is much enlarged with numerous cells, among them mast cells [503], and may contain an increased amount of hyaluronate and thus water, as in dermatosparactic animals [513]. Transmission electron microscopy of the dermis reveals ribbon-like collagen fibrils and hieroglyphic profiles (Figs. 33e and 33f) characteristic of those observed in the skin of dermatosparactic animals [498,502,505,506,514]; (for a scanning electron micrograph of dermal collagen, see [498]). Collagen fibrils of the sheets around vessels, nerves, and adnexa are similarly affected, whereas the dermo-epidermal junction basement membrane and anchoring fibrils appear normal, indicating that collagens IV and VII are not involved [498], thereby indicating substrate specificity of the N-proteinase.

Watson et al. [474] have proposed a model of fibril formation in EDS VIIC in which the intact N-propeptides are located at the surface of the hieroglyphic fibrils. Partial cleavage of the *in vitro*-synthesized abnormal collagen by N-proteinase allows the N-propeptide to be incorporated within the body of the fibrils with conversion of the hieroglyphic outlines to the ragged outlines characteristic of EDS VIIA and VIIB.

Skin fragility seems to decrease as a function of age, as exemplified by case 4, a tendency also observed in animals. This may be explained by the increasing density of collagen fibers in skin during development and the slower collagen turnover as observed at a more advanced age in animals [515].

Diagnosis and Differential Diagnosis

The diagnosis is suspected clinically. The ultrastructural findings of skin collagen fibrils are typical but may be almost indistinguishable from those in certain patients with EDS VIIA (see case 22 in Table 2). It must be emphasized that light microscopy does not allow detection of these changes. This is well-demonstrated by case 4 (Table 3), where routine paraffin sections of samples of skin and tendon taken on several occasions showed no remarkable features, whereas later, samples reprocessed for electron microscopy from paraffin blocks showed hieroglyphic collagen fibrils in dermal samples and, to a lesser extent, in tendon [506]. Biochemical confirmation is based on the electrophoretic demonstration of pNα1(I) and pNα2(I) chains of collagen I extracted from dermis in the presence of protease inhibitors or detected in fibroblast cultures in the presence and/or absence of added dextran sulfate [487]. Determination of N-proteinase activity is performed on a research basis only. However, direct mutation analysis has become feasible (Table 3) [500] and will allow prenatal diagnosis.

Comment

Dermatosparaxis represents a good example of the unity of biomedical research. The study of dermatosparaxis was initiated by clinical veterinarians, taken up by biochemists and cell biologists with the help of physical chemists, and continued by clinicians and molecular biologists to determine the cause of the disease in man. This collaboration has also largely contributed to the better understanding of the physiologic processes involved in collagen fibrillogenesis and the regulation of collagen biosynthesis.

OTHER TYPES OF EHLERS-DANLOS SYNDROME

The number of distinct types of EDS that have already been identified indicates great heterogeneity, but clearly that heterogeneity is not exhausted by the most widely used classification and presents a pasture for splitters — although the most recent classification reverses that trend. There is no place for the passionate defense of a purely clinical classification, which can only provide the framework for a further investigation of clinical problems. The question is whether a particular set of findings represents merely a chance association or whether they can be understood either as having a cause-and-effect relationship or as sharing a common pathogenesis.

Ehlers-Danlos Syndrome Type V — X-Linked EDS (MIM 305200)

The existence of EDS V (Table 1) as a separate entity is more than questionable [39]. Its phenotype is not well-defined because it is based on only eight members of two British families demonstrating apparent X-linked inheritance [136] and one additional case traced during a follow-up of these two families [129]. All nine patients had marked dermal extensibility but only mild to moderate tissue fragility and mild scarring and bruising. Spheroids and molluscoid pseudotumors were also found. The skin was of a soft and doughy consistency. Articular laxity was mild and most evident in the digits, yet orthopedic complications (effusions, recurrent sprains, arthritis) were frequent. It may have been noteworthy, at least to the authors, that all affected persons in both families had red hair [129]. In one 53-year-old affected man, a skeletal survey excluded occipital horns and other skeletal abnormalities, and serum copper and ceruloplasmin in affected males and obligate carrier females were normal [129]. The disorder is thus clearly different from the occipital horn syndrome (EDS IX) and is a nuisance rather than a handicap to the affected persons [129].

Di Ferrante et al. [516] reported a 9-year-old boy and two maternal cousins as having EDS V. All had congenital heart disease, floppy mitral valve syndrome, which in one of them progressed to severe insufficiency and death, herniae, short stature, stretchable skin, moderate joint hypermobility, musculo-skeletal weakness with dorsal kyphosis, genu valgum, and flat feet. An atrial septal defect with left-to-right shunt, and floppy mitral and tricuspid valves with significant regurgitation, were present. The nosology of this disorder remains uncertain, but in our view it more likely belongs to an X-linked cardiovascular disease type (MIM 314400; [517]).

Other patients with EDS V have been mentioned [518], but no clinical or genetic data were given, and probably more sporadic cases have also been reported uncritically and erroneously as EDS type V [519–521].

The distinction of EDS V from autosomal dominant EDS is based solely on its apparently X-linked recessive mode of inheritance in two pedigrees. However, pedigree 2 [129,136] could be explained, as originally critically discussed [136], by autosomal dominant inheritance with incomplete penetrance in the supposed carrier female (II/5 in [129,136]). This view is fostered by the follow-up revealing that her affected son (III/1) has two children with minor stigmata of the syndrome: the 21-year-old daughter has generalized joint laxity and normal skin, the 19-year-old son has soft, extensible skin but no scars or joint laxity. In pedigree 1, the putative heterozygote mother (III/2 in [129,136]), when re-examined at the age of 60 years, had recently undergone surgery for degenerative arthropathy of the knee, which had been ascribed to articular instability. This problem, together with slow healing at the operation site, could represent minor syndromic manifestation in a female heterozygote or, again, incomplete penetrance. Close linkage to Xg blood groups and color blindness was excluded in the two families [136], and cytogenetic investigation, including high-resolution banding, yielded normal results [129]. X-linked inheritance therefore remains questionable, and thus the existence of this disorder as a distinct entity remains to be proven.

The biochemical defect was thought to be a deficiency of lysyl oxidase (protein-lysine 6-oxidase, EC 1.4.3.13; MIM 153455), the gene for which was later shown to be located on chromosome 5q23.3-q21.2 [522], a finding that is at variance with claimed X-linked inheritance of the disorder. The observation of low levels of lysyl oxidase in skin and other tissues from mice with an X-linked connective tissue disorder [523,524] was the stimulus for Di Ferrante et al., who reported a deficiency of lysyl oxidase in one patient [516]. However, methodological flaws make their measurements unreliable; lysyl oxidase, which is almost entirely secreted in culture, was estimated in fibroblast cell layer homogenate; furthermore, the activity in culture medium was measured after lyophilization and "redissolution" of the enzyme, which, however, is poorly soluble in buffers lacking urea; in the absence of any clinical or biochemical follow-up report, there remains a conspicuous silence about the nature of the disease in their patient, who also had two maternal cousins similarly affected. Siegel et al. [525], using appropriate techniques, could not confirm this result in four of the original patients reported [136]. They found greater than normal activity of lysyl oxidase and no diminution in the amount of immunologically cross-reacting material in extracts of skin from patient J.W. (subject III/2 in family 2 [136]), normal proportions of reducible cross-links in skin from all four patients studied (III/1 and III/2 of family 2 and IV/1 and IV/4 of family 1 [136]), and normal amounts of thermally stable cross-links as judged by the normal proportion of high-molecular-weight chains on SDS-polyacrylamide gel electrophoresis. Siegel et al. [525] also pointed out that an absence of cross-links would lead to continuous extension of the fibers as they slipped past each other under tension until rupture occurred and that in this situation there would be no restoring force, in contrast to what is found in EDS. Ultrastructural studies showed changes that were not qualitatively different from those in the skin of patients with EDS I or II [183]; other changes in undefined patients have been reported [518,519]. Note that Fig. 7 in [526] refers erroneously to a patient with EDS V instead of EDS IX, the occipital horn syndrome (K. Holbrook, personal confession, 1990).

Because there are no distinct clinical characteristics and no biochemical marker for EDS V, diagnosis depends solely on the mode of inheritance. In every sporadic male EDS patient, the family history should be carefully evaluated, with special emphasis on the males of the maternal tree (such as maternal uncles and maternal cousins). EDS V is readily differentiated from the X-linked EDS IX, the occipital horn syndrome, by the absence of cutis laxa and exostoses such as occipital horns and by the presence of normal amounts of serum ceruloplasmin and copper and from an X-linked congenital heart disease (MIM 314400) [517] by other criteria.

Beighton and Curtis [129] state that "the disorder is undoubtedly very uncommon; nevertheless, it has gained the asterisk of syndromic respectability in the catalogue of Mendelian inheritance in man." We feel that this is unjustified and would question the existence of this disorder as a separate entity despite the asterisk attributed to this entry in "Mendelian Inheritance in Man" [37].

Ehlers-Danlos Syndrome Type VIII — Periodontotic Type of EDS (MIM 130080)

The existence of EDS VIII (Table 1) as a separate entity is questionable [39]. On the last page of the chapter on the Ehlers-Danlos syndrome in "Heritable Disorders of Connective Tissue," McKusick [2] writes: "A condition unique in my experience, and apparently in the literature as well, is demonstrated by the wife of a colleague of mine and several of her relatives. Lesions on the shins suggest those of EDS, and slow-healing breaks in the skin at that site have occurred after blunt trauma. The skin is not generally fragile, and no unusual bruisability or stretchability of the skin has been noted. The joints are not hyperextensible. A second feature, apparently syndromally related to the lesions on the shins, is absorptive periodontosis, with early loss of the teeth. The dental and skin changes are present also in the proband's father, in several sibs of her father, and in a cousin. The lesions on the shins suggested necrobiosis diabeticorum on histologic study.... The small scars on the knees are somewhat like those of EDS, and the skin of the lateral aspect of the soles is wrinkled in the manner demonstrated by EDS-patients."

The description of this "unique condition," together with a second family, prompted Stewart et al. [527] to classify this new variant as EDS VIII. More cases have since been reported [528–533; D. Hollister, personal communication, 1990; K. Hinkel, personal communication, 1990] or have just been mentioned without clinical description [518]. In all, joint laxity, especially of the fingers, is mild to moderate, hyperextensibility is mild or absent, fragility of the skin is mild to severe, the scars are "cigarette-paper"-like, the palms and soles are excessively wrinkled [528], and there is no evidence of visceral involvement [529]. A Marfanoid habitus with arachnodactyly and slim limbs and fingers was noticed in two families [527,528]. Minor trauma produced ecchymoses that resolved normally except on the shins, where the pretibial skin healed with distinct hyperpigmented atrophic scars. Hoffman et al. [534] described a sporadic patient with EDS VIII in whom autoimmunity to collagen I appeared to be responsible for unusual features such as intractable vasculitis and resorptive arthritis and osteolysis.

Dental disease begins with numerous caries of the deciduous teeth, which are shed prematurely or normally; the permanent dentition erupts at the usual time. The onset of gingival inflammation in the second decade, and the progression of periodontal disease, leads to a generalized alveolar bone loss around all teeth, which results in the premature loss of all permanent teeth during the third decade. The teeth are morphologically normal [530]. A propensity for the rapid and extensive formation of calculus, particularly around the gingival portions of the posterior teeth, was noted [527].

In one sporadic case, histology of the gingiva exhibited the presence of peculiar, previously unidentified, cell-poor homogeneous masses underneath the epithelium with staining properties resembling those of fibrin. Whether these gingival fibrinoid deposits are a typical feature of EDS VIII will remain uncertain until similar studies in other cases have been performed [531]. Ultrastructural studies have shown a mixed population of large- and small-diameter fibrils with altered packing into fibers and fiber bundles in the reticular dermis [192,532].

EDS VIII seems to be a rare, dominantly inherited disorder of unknown cause. In three patients from a family with eight affected individuals over six generations (K. Hinkel, personal communication, 1990), collagen studies were normal (M. Raghunath, personal communication, 1991). In the large Dutch kindred reported by Hollister [530], linkage to *COL3A1* has been excluded [535]. Ultrastructural examination of skin from two patients did not show any abnormalities in the cells or matrix, and the collagen bundle architecture appeared normal [530].

Absorptive periodontosis occurs also in EDS IV. Therefore, collagen studies should be performed to exclude the latter condition with its dismal prognosis. The father of the initial propositus reported by Stewart et al. [527] died at the age of 39 years of an apparently spontaneous rupture of the duodenum with subsequent overwhelming peritonitis [530]. However, studies of fibroblasts from the propositus performed later showed normal collagens I and III (D. Cohn, personal communication, 1991), indicating that he may not have suffered from EDS IV. On the other hand, an initial diagnosis of EDS IV based on preliminary biochemical data in a separate case had to be revised following more careful studies [533].

The gene for an autosomal dominant form of juvenile periodontitis (MIM 170650) is located on chromosome 4 and linked to the trait for dentinogenesis imperfecta type III [536]; a recessive form may also exist (MIM 260950). Early loss of teeth occurs also in the Papillon-Lefèvre syndrome with keratosis palmoplantaris (MIM 245000), hypophosphatasia (MIM 146300 and MIM 241500) (see Chapter 18, this volume), and Werner syndrome (MIM 277700). Because periodontal ligaments are rich in collagen XII [537], the gene *COL12A1* could be a candidate for the disorder.

Occipital Horn Syndrome (Formerly Ehlers-Danlos Syndrome Type IX) (MIM 304150)

The phenotypic manifestations of the occipital horn syndrome (OHS) were first described by Lazoff et al. [538] in an 11-year-old boy and his two maternal uncles. The child presented with diarrhea, recurrent urinary tract infections, bladder diverticula, inguinal herniae, and peculiar "occipital horns." All three had educational problems but were not mentally retarded. One year later, a new form of X-linked cutis laxa with bladder diverticula and skeletal anomalies was reported [539]. The phenotype was associated with a deficiency of lysyl oxidase that was later inferred to result from disordered copper metabolism [540]. The condition was renamed Ehlers-Danlos syndrome type IX [126], but because of the skin laxity it was later withdrawn from the EDS nosology [38] and the accepted term is now the occipital horn syndrome. The proper term indeed should be mild Menkes disease with occipital horns (see Chapter 14, this volume, and Table 1).

Menkes disease (MD) is an X-linked inborn error of copper metabolism affecting several body systems, including the connective tissues. The clinical manifestations of MD can be quite variable, and both severe and mild forms exist. In the milder forms, the neurological symptoms are less severe, whereas connective tissue findings remain prominent. This group is likely underdiagnosed. The

classical, severe form of MD comprises 90–95% of all cases, and its clinical manifestations include progressive neurological degeneration, seizures, growth failure, arterial aneurysms, and skeletal defects, as well as characteristic hair changes (pili torti) and hypopigmentation, and death results in early childhood. The occipital horn syndrome is the mildest form of MD. Remarkable changes are inguinal herniae, bladder diverticula, skin laxity, and skeletal abnormalities, including a short trunk, deformed elbows, and genu valgum. A diagnostically important feature is the occurrence of bony protuberances on the occiput, the so-called occipital horns. Chronic diarrhea and orthostatic hypotension are other characteristic features.

MD and the occipital horn syndrome are allelic, X-linked, and due to mutations in the Menkes gene, *ATP7A*, which codes for a P-type ATPase, an enzyme located in the *trans*-Golgi network, where it transports copper into the lumen for incorporation into secreted and vesicular copper-requiring enzymes (see Chapter 14, this volume).

Ehlers-Danlos Syndrome Type X (MIM 225310) — Fibronectin-Deficient EDS

The existence of EDS X (Table 1) as a separate entity is questionable [39]. The problem of defining a new type of EDS is well-illustrated by the single family with EDS X to which McKusick has assigned a separate entry in the catalog, although without the "quality label" of the asterisk [37]. Arneson et al. [541] described four of six sibs born to apparently unaffected, unrelated parents with a mild form of EDS and dysfunction of plasma and cellular fibronectin, which was confirmed independently [542]. The proband, a 28-year-old female physician, had joint hyperextensibility, most prominently of the hands and feet and to a lesser extent of the elbows and shoulders, thin but not velvety skin with "fish-mouth" scars that was easily bruisable, petechiae, a positive Gorlin sign, mitral valve prolapse, an aortic root diameter at the upper limit of normal, and, surprisingly, striae distensae. She had once had a knee dislocation that reduced spontaneously (D. Hammerschmidt, personal communication, 1990). Electron-microscopic studies showed a lack of cohesion between collagen fibers within bundles in skin, where there was an unusually loose organization of the deep reticular dermis. Cauliflower collagen fibrils and large fibril diameters were also observed (Fig. 7a in [192]; Fig. 10 in [543]). The proband has had two uncomplicated pregnancies (D. Hammerschmidt, personal communication, 1990).

The platelet aggregation defect was correctable *in vitro* by the addition of normal human fibronectin; on the other hand, the patient's plasma failed to support the aggregation of gel-filtered platelets from controls in response to collagen. Because the measured level of immunoreactive fibronectin in plasma was normal and immunohistochemical studies have shown fibronectin to be present in the platelet α-granules, the authors assumed a minor structural or post-translational abnormality of the fibronectin molecule. In a preliminary experiment, the fibronectin was found to have an abnormal isoelectric behavior (D. Hammerschmidt, personal communication, 1990).

The inherited connective tissue disorder segregated with the platelet aggregation defect, and, since fibronectin is an important adhesive glycoprotein in connective tissues, the authors proposed that both the platelet malfunction and the mild EDS in this kindred might be explained by a defective fibronectin. This relationship, it seemed to them, was further supported by the observation that during the first pregnancy the ability of the proband's plasma to support collagen-induced aggregation of normal platelets markedly improved [544], which may have contributed to the surprising fact that hemostasis was normal, while the somewhat dilated aorta remained constant through both pregnancies and even episiotomy was needed, and healed normally (D. Hammerschmidt, personal communication, 1990). Plasma obtained six months postpartum behaved as did pregravid and first-trimester plasma samples [544].

The proband has two children, a girl and a boy. However, the girl had had approximately 75 luxations of the head of the radius by the time she was 8 years old but had no more in the subsequent two years. Her brother, as well as their two maternal cousins, has also had a luxation of the radius at least once. Studies of platelet aggregation or skin morphology have not yet been performed in them (D. Hammerschmidt, personal communication, 1991). Since no further cases have been observed and no biochemical or genetic studies have been performed, the primary defect and its mode of inheritance remain unclear. The linkage of two separate disturbances and a common underlying cause are both possible, and autosomal recessive or autosomal dominant inheritance, transmitted by one parental carrier of a gonadal mosaicism, are also both possible. If one considers the proband's daughter and son, as well as their maternal cousins, to be affected, dominant inheritance seems more likely.

Regarding the proposed association between the platelet aggregation disturbance and this mild form of EDS, it should be noted that altered production, assembly, and distribution of fibronectin has been observed in cultured fibroblasts from patients with several different types of EDS [545–547]. It is further appropriate to mention that in eight members of a three-generation family, half-normal levels of plasma fibronectin, which has a normal electrophoretic mobility, do not result in abnormalities of platelet aggregation or clinical signs of a connective tissue disorder [548].

Furthermore, there are numerous reports of EDS associated with more or less well-characterized platelet or coagulation dysfunctions [549–557]; most probably, these findings are simply coincidental, but they may have added to the bleeding tendency of the underlying EDS, thus prompting clinical investigation. In summary, the causal association between EDS, dysfibronectinemia, and platelet dysfunction remains to be established and thus we question the existence of EDS X as a separate entity in this single family.

Familial Joint Hypermobility Syndrome (Formerly EDS Type XI) (MIM 147900)

Familial joint laxity with recurrent luxations of the shoulder and patella, often with congenital hip dislocation, has been reported as an autosomal dominant condition [2,59,62,558–560]. Because of the lack of skin involvement, it has been recommended that this entity, formerly EDS XI (Table 1), be classified as a separate entity, familial articular hypermobility syndrome [37–39].

Progeroid Form of Ehlers-Danlos Syndrome (MIM 130070)

Hernandez et al. [561–563] described five sporadic male patients with mental retardation, short stature, a progeroid appearance with wrinkled facies, curly and fine hair, scanty eyebrows and eyelashes, and periodontitis in addition to typical signs of the EDS. Increased paternal age suggested to

them *de novo* mutations. A similar case has been reported by DeLozier-Blanchet et al. [564].

Kresse et al. [565] have described a five-year-old sporadic patient, born to nonconsanguineous parents, who represented a progeroid variant with signs of the EDS (hyperelastic skin and atrophic scars), developmental delay, dwarfism, craniofacial disproportion, osteopenia of all bones and dysplasia of some, and defective wound healing. Later, at age 8.4 years, it was noted that his growth rate had been normal during the last years and that he had made steady intellectual progress [566]. His fibroblasts were defective in the biosynthesis of the ubiquitous proteoglycan small dermatan sulfate proteoglycan (DS-PG II), or decorin, which consists of a core protein of M_r 36,319, with a single glycosaminoglycan chain on the serine residue at position 4, and either two or three asparagine-bound oligosaccharides (see Chapter 4, this volume). The fibroblasts synthesized normal amounts of core protein but converted it into mature proteoglycan only in reduced amounts; the remainder was secreted in a glycosaminoglycan-free form, the tyrosine residues of which were normally sulfated [567]. This abnormality is explained by the marked deficiency (~5% residual activity) of galactosyltransferase I (xylosylprotein 1,4-β-galactosyltransferase, XGALT1; EC 2.4.1.133) as measured in cell homogenates using artificial substrates [568]. This enzyme normally catalyzes the second glycosyl transfer reaction in the assembly of the xylose/serine-linked proteoglycans. The mutant enzyme was thermolabile and had K_m values different from normal for several substrates. Because the parents have intermediate enzyme levels, the disorder seems to be inherited as an autosomal recessive trait. The disorder has been shown by two groups to be due to compound heterozygosity for two mutant alleles, Ala186Asp and Leu206Pro, of β4Gal-T7 (*XGalT-1*), and enzymatic measurements in expression systems of both cDNAs showed 10–50% and 0% activity, respectively [569,570]. Why the activity of galactosyltransferase II (galactosylxylosylprotein 3-β-galactosyltransferase, EC 2.4.1.134) is reduced to ~20%, and whether the large dermatan and heparan sulfate proteoglycans are also affected, remains to be determined [568]. Quantitative immunogold electron microscopy of a skin biopsy revealed that the collagen fibrils of the patient were decorated with much less decorin than those from an age-matched control [566]. It was suggested that the glycosaminoglycan-free core protein present in the patient's tissues is more rapidly metabolized than intact proteoglycan. Unfortunately, no information is available about the thickness and morphology of the patient's skin. Further studies on this and future cases will certainly provide important insights into physiological and pathophysiological roles of decorin.

Reduced transcription and expression of decorin mRNA has been observed in conditions that have in common muscle hypotonia, little subcutaneous fat, joint and skin laxity, easy bruisability, and poor mineralization of bone, as observed in progeroid and progeria patients, and the neonatal Marfan syndrome (MIM 1154700) [604], as well as in the Wiedemann-Rautenstrauch syndrome (MIM 264090). However, in this last condition, such a defect was present only during infancy and hence should be considered as a secondary phenomenon, which leads to a fault in the regulation of decorin gene transcription [571].

Another example of a disturbance in proteoglycan synthesis is given by Fushimi et al. [572], who describe a sporadic case of isolated adrenocorticotrophic hormone deficiency with features characteristic of the EDS (fragility, hyperextensibility and easy bruisability of the thin skin with easily visible veins, molluscoid pseudotumors, but an absence of hypermobility of any joints). Immunohistochemical analysis using anti-dermatan sulfate-proteoglycan antibodies and studies of the biosynthesis of glycosaminoglycans in cultured fibroblasts suggested a defect either in the synthesis of a proteoglycan core protein or in the synthesis of dermatan sulfate side chains in the patient's skin. These results suggested that the dermal findings were due to a lack of dermatan sulfate proteoglycans. The reason for the thinness of the skin, and hence deficiency of collagen, in spite of the normal amounts and proportions of collagen produced by the fibroblasts, is unknown, but the findings suggest that dermatan sulfate proteoglycans play an important role in the formation, function, and maintenance of the structural integrity of the skin. The absence of joint laxity may be explained by the fact that the core proteins of dermatan sulfate proteoglycans in tendon and skin are genetically different [573], but dermatan sulfate in tendon could not be analyzed in this case. This report also illustrates that in the absence of joint laxity the nosology of the EDS itself becomes loose.

Unspecified Types of the EDS and Chance Associations

There are many enthusiastic claims reporting "new forms" of the EDS, such as that of Viljoen et al. [473] in members of a family later shown to have EDS VII [474] (see above), among others [104,109 (MIM 225320), 574,575 (MIM 130090)] waiting to be confirmed clinically and/or characterized biochemically or to be withdrawn because of a recognized chance association with EDS, such as occurred in cases of, for example, neurofibromatosis [576], α2-macroglobulin deficiency [577], α1-antitrypsin deficiency [578], epidermolysis bullosa [579], cystic fibrosis [398], and bilateral focal polymicrogyria [580]. Other authors have been caught in semantic traps of different syndromes [581].

ANIMAL MODELS AND LATHYRISM

The advantages of studying animals with heritable disorders of connective tissue are obvious: (1) they provide adequate quantities of material from different tissues for morphological, biochemical, and biomechanical studies and the establishment of cell cultures; (2) they allow the comparison of paired affected and unaffected littermates at all stages of embryonic, fetal, and postnatal development, with the assessment of early consequences of lethal mutations, as well as during aging within a reasonably short time period; (3) they allow experimental mating for prospective genetic studies to produce heterozygous, homozygous, and compound heterozygous animals; (4) they allow the comparison of similar diseases in different species. Although the eponyms of human diseases are not strictly applicable to the analogous diseases in animals, the causes and effects of these diseases appear to be directly comparable. For an overview of animals with dermatosparaxis or EDS-like conditions, see Table 4.

Dermatosparaxis

Dermatosparaxis in cattle was the first "true" collagen disease to be elucidated. Its characterization greatly stimulated research on the conversion of procollagen, led to the isolation, characterization, and sequence determination of the N-terminal propeptide extensions of proα1(I) and proα2(I) chains, and enabled the study of the regulation

TABLE 4. Natural Animal Models of EDS

Disorder and Species	Inheritance[1]	Defect	Phenotype	References (Selected)
Dermatosparaxis				
Cattle from Belgium	AR	Procollagen N-proteinase deficiency	Severe	464,500,507,511,513, 582–593
from Texas (Hereford)	AR (?)		Severe/moderate	594
Sheep from Norway (Dala)	AR	Defective conversion of pN-collagen		464,493,512,595–599,
from Australia	AR	Procollagen N-proteinase deficiency (25%)	Mild	487,600–605
from Finland	AR		Severe	606
from South Africa (white Dorper)	AR		Moderate	607
Cat	?		Mild	608–610
Dog	AR		Severe	611;G.D. Hegreberg, personal communication, 1991
Ehlers-Danlos syndrome-like conditions				
Dog	AD			612–618
	?			442,619–621
Mink	AD			613–615,622
Cat	?			623
	AD			616,618,624
Horse	ND			625
Cattle	AD?			626,627
Rabbit	?			628–631
Mottled mouse				
Blotchy mouse (Mo^{blo})	XR	Disturbed copper handling ⟶ deficient lysyl oxidase activity	Mild	523,524,632–636

[1] Inheritance: AR, autosomal recessive; AD, autosomal dominant; XL, X-linked recessive; ?, sporadic case; ND, autosomal recessive or dominant inheritance possible but more likely paternal mosaicism of an AD trait.

of fibrillogenesis and cross-link formation by the precursor peptide and the function of the cleaved peptide in the control of procollagen synthesis. Although it had been speculated for some time that high-molecular-weight precursors of collagen might exist [637,638], the actual demonstration of such molecules in tissue cultures of human fibroblasts [639], and in both rat calvariae [640] and chick embryo tendon cells [641], by three independent laboratories occurred at about the same time that dermatosparactic cattle were recognized to have an abnormal high-molecular-weight collagen precursor in skin. This immediately made it possible to prepare and purify the precursor chains in sufficient amounts and to gain the above-mentioned insights at a much higher speed.

The hallmark of dermatosparaxis (“torn skin”) is the excessive fragility of the skin with only mild or absent involvement of tendons and ligaments, which distinguishes this disorder from EDS VIIA and VIIB in man. In affected calves from Belgium, lambs from Norway, sheep from Australia, and cats, the conversion of pN-collagen to collagen seems to be disturbed, either because of a deficiency of procollagen N-proteinase activity [507,600,608] or possibly because of an absence of the collagen-binding protein annexin V (anchorin CII), which could function as a “presenter” of procollagen to the enzyme [595]. The amount of pN-collagen in skin roughly correlates with the extent of the enzyme deficiency, the clinical severity, and morphological abnormalities; for example, skin from the severely affected lambs contained almost exclusively pN-collagen [582], whereas skin from the less severely affected Australian sheep contained only approximately one-third of the collagen as pN-collagen [600]. The relatively low amount of pN-collagen in these latter sheep is due to a less severe reduction (~25% of normal) in procollagen N-proteinase activity as measured in skin extracts [600]. Within one animal, variable degrees of involvement of different organs are reflected in differences in their content of pN-collagen (70% and 15% in skin and tendon, respectively; [596]) and ultrastructure (Figs. 33e and 33f).

As a consequence of the persistence of the N-propeptide, collagen fibril formation is disturbed. This is reflected by the ultrastructural changes observed in all dermatosparactic animals. In cross-section, the fibrils have a spider-like or hieroglyphic-like appearance or a markedly serrated contour; longitudinally, the fibrils resemble ribbons twisting in both directions [493,583,594,601,609,611]. Abnormal cross-link formation [511], increased solubility of collagen, and, ultimately, decreased tensile strength are the consequences of disturbed fibril formation. *In vitro* experiments show that with increasing pN-collagen content, cross sections of the formed fibrils are progressively distorted from circular to

lobulated to thin and branched structures [642,643], which indicates that there is a dose-dependent relationship. Watson et al. [474] have proposed a model of fibril formation in EDS VIIC in which the intact N-propeptides are located at the surface of the hieroglyphic fibrils. Partial cleavage of *in vitro*-synthesized abnormal collagen by N-proteinase allows the N-propeptide to be incorporated within the body of the fibrils, with conversion of the hieroglyphic outlines to the ragged outlines characteristic of EDS VIIA and VIIB.

The variability of different organ involvement has not been adequately studied. It may be influenced by differences in local residual procollagen N-proteinase activity, collagen turnover, stringency in collagen packing, or a combination thereof. The fact that with aging there is an improvement in various properties of the skin may point to an influence of collagen turnover; Piérard and Lapière [515] observed several differences between two calves 3 and 6 months old. The sheet-like arrangement of the polymers was replaced by a bundle-like organization, the ratio of collagen to procollagen increased, and the biomechanical properties of the dermatosparactic skin also tended to become more normal.

In one strain of dermatosparactic calves in which the disease was first observed [584], the mutation consists of a 17 bp deletion that changes the reading frame of the message [500], and would, if the mRNA were stable, result in the synthesis of a truncated protein. Because the mutation would prevent the synthesis of active procollagen N-proteinase, the fact that different tissues contain variable amounts of processed procollagen I suggests that another enzyme, tissue-specifically expressed, can remove the N-terminal peptide of procollagen I, although at a lower rate than the N-proteinase. Two proteins that have a high sequence homology with the N-proteinase and contain similar domains, including properdin repeats, have been described (for references, see [500]). Their physiological roles have not yet been determined. However, the regulation of their expression and their tissue-specificity suggest various functions for members of this new subfamily, which are candidates for an alternative pathway of processing procollagens I and II [500].

Ehlers-Danlos Syndrome-like Conditions

All animals with "generalized cutaneous asthenia," "conditions resembling the EDS," or "fragility and hyperextensibility of the skin" seem to inherit these as autosomal dominant traits. Most reports focus on the description of the skin, others (e.g., [619]) mention also profound joint laxity, subluxations, and osteoarthritis; radiographic and microradiographic studies have revealed subclinical involvement of bone [617]. In all animals, the defect remains unknown. Ultrastructurally, abnormalities in the packing of collagen into fibrils and fibers have been noted [617,620,624,625]. Differences in the solubility and cross-linking of collagen have occasionally been reported [612,625], but these may also be accounted for by an increased rate of collagen turnover and thus be secondary to some other primary change.

In pregnancies resulting from matings between two heterozygous cats [624], the litter size was reduced and only 12 of 34 offspring were affected; furthermore, uterotomy at gestational days 20 and 44 (normal duration is 63 days) revealed that approximately one-quarter of the placental sacs were abnormally small and contained no embryo. It was concluded therefore that the homozygous state of the "defect in collagen fibrillogenesis" would be lethal during early embryonic development [618], perhaps shortly after implantation or during early gastrulation (R. Minor, personal communication, 1990). The apparently homozygous EDS cases in man [29,134] seem therefore to be basically different from those in this animal model.

Kobayashi et al. [644] reported a 4-month-old Holstein calf with soft and hyperextensible skin, skin fragility, and a history of delayed wound healing, but without joint hypermobility. Electron microscopy showed rarefaction of collagen fibers and an increased amount of dermal ground substance. Tajima et al. [645] showed that there was a deficiency in dermatan sulfate proteoglycans in skin caused by a presumably new heterozygous mutation, a G to A transition at nucleotide position 254, which resulted in a Ser-to-Asn substitution at amino acid position 85 of the bovine proteoglycan core protein. This substitution occurred in the highly conserved Ser–Gly dipeptide repeat sequences suspected to be the O-glycosylation site of dermatan sulfate side chains. The sire and grandsire did not carry the mutation, and the dam and granddam were not available for analysis but did not present these skin manifestations. It therefore appears that the calf was affected due to a newly occurring heterozygous mutation of the gene coding for the proteoglycan core protein.

Transgenic Animals

The use of gene targeting as a means of assessing the biological function of a protein is a powerful tool, although there are certainly many instances in which overlapping functions or gene compensation can obscure the function of the missing or defective protein under study, often making comparison between affected individuals and experimental animals difficult. Some examples are given here which illustrate that certain transgenic animals were seminal in the characterization of the human counterpart, while others should prompt research into the elucidation of hitherto unknown basic defects of diseases in man.

Targeted Mutation of the Col5a2 Gene in Mice — A Model of the Classical Type of EDS

This animal model was seminal in the elucidation of the classical type of EDS in man. Homozygous mice (pN/pN) with an in-frame exon 6 deletion in *Col5a2* survive poorly, possibly because of complications from spinal deformities, and exhibit skin and eye abnormalities caused by disorganized collagen I fibrils [150]. The deletion is outside the major helix of the collagen V molecule and does not alter the reading frame of the *Col5a2* transcript but prevents normal processing of the proα2(V) chains. It results in a heterotrimeric $[\alpha1(V)]_2\alpha2(V)$ that is assembled, secreted, and integrated normally into heterotypic collagen fibrils. The abnormal collagen V is structurally defective due to an alteration in the conformation of the N-globular domain, a region thought to play a key role in the interaction between collagen V and collagens I and III [149].

Heterozygous animals (pN/+) were phenotypically normal and fertile. Likewise, the homozygous mutant mice (pN/pN) appeared normal at birth. Soon after, however, many of them were cannibalized and the remaining ones began to show a progressive hunching of the back that affected mobility and respiration. By weaning age (~3 weeks), the survival rate was 5% and their weight was ~50% of normal. Radiology revealed varying degrees of spinal lordosis and kyphosis in all. The normal morphology of the vertebrae suggested that spinal deformity was probably secondary to loss of tensile strength of the ligaments. On the other hand, intravital

double-tetracycline labeling demonstrated that bone in the mutant mice grew at a slower than normal rate.

Skin was much more fragile and stretchable upon physical examination and biomechanical testing. The amount of collagen in the dermis of mutant mice was markedly reduced, whereas the hypodermis was 4–6-fold thicker and contained a large number of hair follicles not normally localized in this layer. Ultrastructurally, the collagen fibrils were more disorganized, less tightly packed, and more heterogeneous in size. Collagen fibrils in the cornea were disorganized and thicker: mean fibril diameters were estimated to be 25 nm in controls, 35 nm in heterozygous mutants, and 50 nm in homozygous mutants. Consistent with the ultrastructural findings, the stroma but not the epithelium of the cornea appeared collapsed and was thinner than that of controls.

The lack of an apparent phenotype in the heterozygous mice is consistent with the stoichiometry of collagen V, which predicts that only half of the trimers will have an abnormal conformation. This could also explain why size differences in fibrils were noted only in the cornea, a tissue that contains fibrils of equal size and with the largest proportion of collagen V. Alternatively, heterozygous animals were not examined with more stringent analyses and were under greater physiological stress, so clinical signs may have been missed.

In summary, the deletion of the α2(V) N-telopeptide leads to a change in the conformation of the collagen V heterotrimer and the elimination of the hinge-like region. This conformational change abrogates the collagen V regulation of the growth of collagen fibrils and their subsequent packing into fibers, affecting the spatial arrangement of the fibers into tissue-specific macroaggregates [150].

The tight skin mouse (TSK/+), a naturally occurring mutant, develops a generalized connective tissue abnormality that is transmitted as an autosomal dominant trait and is caused by a large duplication in the fibrillin1 gene (see Marfan syndrome, Chapter 12, this volume). The phenotype is characterized by marked hyperplasia of loose connective tissues, thickening of the skin, pulmonary emphysema, and cardiac hypertrophy. Although both pN/pN and TSK mice exhibit cutaneous thickening, there are striking differences between the two mutant strains. However, crossing TSK/+ mice with pN/pN mice resulted in an F1 progeny without cutaneous hyperplasia [646]. This interesting example of genetic complementation may serve as a model to explain lack of penetrance in double heterozygous individuals.

Tenascin-X Knockout Mouse — A Model of the Classical Type of EDS

Mao et al. [647] have produced a *Tnx* knockout mouse. The skin of TN-X-deficient mice becomes clearly hyperextensible at about 2 months of age, and hyperelasticity is progressive with advancing age. The best phenotypic indication, beginning at about 3 months of age, is that when such mice are held by the skin of the neck, they are still able to twist around to bite the investigator due to the hyperelasticity of their skin. The joints show no obvious hypermobility.

Biomechanical studies demonstrate reduced tensile strength (60% of that of age and sex-matched unaffected littermates) and hyperextensibility of skin. The dermis is less dense and thinner in TN-X-deficient animals than in normal littermates. Ultrastructurally, dermal collagen fibrils have a normal contour and a normal mean diameter, which is, however, more variable. The most striking finding is that the density of the collagen fibrils is reduced by 40% and that the alignment of fibrils with respect to one another appears to be less perfect. These data suggest that TN-X has a role in regulating collagen fibril architecture and that its lack can cause the skin manifestations of EDS with minimal alteration of fibrillogenesis.

Decorin Knockout Mice — A Model of EDS?

To study the function of decorin in collagen fibrillogenesis, Danielson et al. [179] created mice with a targeted disruption of the decorin gene. The decorin null mice presented no gross anatomical anomalies, were normal in size, fertile, without radiologically overt bone abnormalities, without obvious behavioral deficiencies, and with normal routine chemistry.

The skin was hyperelastic, thin, and fragile, with detachment of the tail skin, not unlike in dermatosparaxis (heterozygotes were indistinguishable from control animals). Histology showed a thin dermis, a loose connective tissue in the hypodermal layer, and a sharp detachment of the skin between the deeper dermis and the fascia, with clean, sharp edges along the dissection. Electron microscopy revealed less orderly packed collagen fibrils with a much larger diameter and irregular coarse outlines in cross section. Electron microscopy of longitudinally sectioned collagen fibrils from tendon showed that the typical 67 nm periodicity of collagen I was maintained; however, numerous d bands of the D-periodicity were not occupied by orthogonally arranged proteoglycan granules as in controls. The absence of overt abnormalities in the cornea of null mice, a tissue in which decorin is normally expressed, may be explained by the larger requirement for keratan sulfate in this transparent structure and by the presence of at least five members of the small leucine-rich proteoglycan family, which could control more tightly the fibril diameter necessary for transparency.

Dermatosparaxis and decorin knockout mice. A contributing factor to the skin fragility in dermatosparaxis was thought to be the steric exclusion of a factor from the gap zone of collagen fibrils by the N-terminal propeptide, which forms a hairpin and thus blocks this region [179]. In dermatosparactic skin, several proteoglycan filaments are not closely bound to collagen fibrils but rather float freely in the interfibrillar spaces [593]. Thus, dermatosparactic animals, through the presumed exclusion of access to decorin, and decorin null animals, through a lack of decorin, were assumed to share a common pathogenetic mechanism (i.e., the obstruction/deficiency of a key regulatory molecule in collagen fibrillogenesis). Further support for this concept was provided by the lowered dermatan sulfate to hydroxyproline ratio from 4.7 in normal skin to 3.6 in dermatosparactic skin [513]. However, later experiments showed that in dermatosparactic calf fibrils the equilibrium between fibrillar and soluble decorin was shifted in favor of enhanced fibril-associated decorin [648]. Thus, the results showed that N-propeptides can distort the morphology of fibrils, that they do not inhibit the binding of gap-associated macromolecules (such as decorin), and that normal mechanical properties of skin are strongly dependent on the close association of near-cylindrical fibrils, thereby enabling maximal fibril-fibril interaction [648].

Lumican-Deficient Mice

Mice homozygous for a null mutation in lumican display skin laxity and fragility with easy bruising resembling certain types of EDS [180]. In addition, mutant mice develop bilateral corneal opacification. Skin has a markedly increased compliance and reduced tensile strength independent of any differences in skin thickness. Its fibroblasts and collagen fibers are poorly aligned. There is a significant proportion of abnormally thick collagen fibrils in skin and cornea.

Thus, lumican, a prototypic leucine-rich proteoglycan (LRP) with keratan sulfate side chains that colocalizes with fibrillar collagens, has a crucial role in the regulation of collagen assembly into fibrils in various organs, especially the cornea [180]; other LRP members, such as biglycan, fibromodulin, epiphycan, osteoglycin, and keratocan, may have similar functions.

Thrombospondin-2-Deficient Mice — a Model of Ehlers-Danlos Syndrome

Thrombospondin-2 (TSP-2) is a member of a family of five secreted, modular glycoproteins whose functions in the extracellular matrix are diverse and poorly understood. TSP-2 is predominantly expressed in dermis, tendon, ligament, perichondrium, and pericardium; it is also present in smooth muscle cells and endothelial cells. In an attempt to determine the function of TSP-2, Kyriakides et al. [181] disrupted the *Thbs2* gene by homologous recombination in embryonic stem cells and generated TSP-2-null mice by blastocyst injection and appropriate breeding of mutant animals.

Thbs2 − /− mice are normal in size and overt appearance and reproduce normally. Juvenile mice often have subtle bends in their tails that become less distinct as they mature, but the tail becomes more flexible, enabling the investigator to tie its end into a knot, a manipulation that is not possible in normal mice. The phenotype of adult mice is characterized by increased laxity and fragility of skin, tendons, and ligaments, an increase in total bone density and cortical thickness, an increased density of medium and small blood vessels, and a bleeding diathesis.

These abnormalities in structure and function are supported by anatomical analysis and mechanical testing. The weave of dermal collagen fibers in the knockout mouse is disorganized, and collagen fibrils in skin and tendon are abnormally large and irregularly contoured when examined by electron microscopy. Tensile strength measurements of skin from mutant mice support indications of increased fragility, first suggested by the tendency of skin to tear. The skin ruptures at lower loads and has increased ductility; these findings explain its increased stretchability.

A possible clue to the pathogenesis of these findings lies in the decreased attachment of skin fibroblasts from these animals to various substrata and their increased sensitivity to trypsinization. Although the binding of TSP-2 to collagens has not been studied, defective cell adhesion to a suitable substratum through lack of TSP-2 may be the primary cause. This would explain abnormal collagen fibrillogenesis, osteopetrosis-like features through abnormal osteoclast adhesion to bone or failure to form a bone-resorbing ruffled border, and the bleeding diathesis through defective adhesion of platelet aggregates to the injured subendothelium. TSP-1 has been shown to bind to von Willebrand factor (vWF), and it is possible that TSP-2 is also required, either directly or indirectly, for the binding of vWF to one or more collagens in the subendothelium. The increase in blood vessel density in mutant mice is a strong argument for the function of TSP-2 as an inhibitor of angiogenesis *in vivo*. It is of interest that findings intermediate between those in wild-type and mutant animals are observed in skin from heterozygous *Thbs2*+/− mice; this suggests haploinsufficiency for the function of TPS-2, at least in skin [181].

Fibromodulin-Null Mice — A Model of Abnormal Fibrillogenesis as in the Ehlers-Danlos Syndrome

Homozygous mice lacking a functional fibromodulin gene do not show any gross anatomical defects, grow to normal size, are fertile, and have a normal life span. In fibromodulin-null animals, virtually all collagen fiber bundles are disorganized and have an abnormal morphology; also, 10–20% of the bundles in heterozygous mice are similar to those in homozygous null mice. Electron microscopy shows that the fibrils are thinner, with irregular and rough outlines. They have a fourfold increase in the content of lumican despite a decrease in lumican mRNA; this suggests that lumican and fibromodulin, both of which belong to a family of extracellular matrix glycoproteins/proteoglycans sharing a leucine-rich repeat (LRR), have the same binding site on collagen fibrils [649].

Procollagen N-Proteinase Knockout Mice — A Model for Dermatosparaxis

Transgenic mice that are homozygous for inactive alleles coding for procollagen N-proteinase (ADAM-TS2) have fragile skin, as expected; however, surprisingly, adult homozygous males are sterile and have a marked decrease in sperm count in semen and fluid expressed from the epididymis and in mature sperm in cross sections of the testes [650]. At the moment, it is unclear whether male sterility is confined to these transgenic mice or whether the enzyme is necessary for the modification of some other and still unknown substrate because three of the six reported cases of human dermatosparaxis (Table 3) have not reached the age of reproduction and because dermatosparactic sheep and calves did not reach puberty. Male individuals with EDS VIIB, however, were fertile and produced one affected and two unaffected children (father of case 4 and case 7, respectively).

Targeted Mutations of the Col3a1 Gene in Mice — A Model of the Vascular Type of EDS

To define the role of collagen III in fibrillogenesis, Liu et al. [334] generated *Col3a1*−/− mutant mice by gene targeting. Most homozygous mutants died in the perinatal period. The precise cause of neonatal lethality was not clear because the dead pups were cannibalized before they could be examined, and light microscopic histologic analysis of live newborn homozygous mutants did not detect any gross abnormality.

The phenotype of surviving homozygous mutant mice resembled the clinical manifestations of patients with EDS IV, whereas heterozygous mice appeared phenotypically normal. Mutant mice (−/−) displayed an average survival rate of 5% at weaning age (~3 weeks), with most deaths occurring within the first 48 hours after birth. Adult homozygous mutant mice appeared normal except that they were about 15% smaller than their wild-type littermates of the same sex. Their average life span, however, was about 6 months, or one-fifth of normal.

Autopsy showed that blood vessel rupture was the major cause of death of these mice. The sites of ruptured arteries with or without aneurysm were random. In addition, mutant mice showed frequent intestinal enlargement and occasional intestinal rupture. Two-thirds of −/− mice displayed spontaneous skin lesions. Light microscopy of skin, intestine, and internal organs, including liver and lung, did not detect any overt abnormalities.

Electron microscopy showed that collagen fibrils normally located between smooth muscle cells, or between smooth muscle cells and elastic fibers, were absent or severely reduced in the media of the aorta as well as in the intestinal submucosa and serosa. Most strikingly, in the adventitia of the aorta, skin, lung, and liver, where collagen I fibrils predominate,

the number of fibrils was reduced to approximately one-third and their mean diameter was approximately twofold and highly variable.

These studies show that collagen III is not only an essential component in tissues rich in collagen III but that it also plays a critical role in collagen I fibrillogenesis and thus in maintaining the functional integrity of the organs [334]. Also, heterozygous mice with a *Col3a1* null allele are phenotypically normal under the conditions examined and, as in putative EDS IV patients with a nonfunctional allele, a milder or late-onset visceral involvement may yet be found under stress conditions (see EDS IV above).

Targeted knock-in mutation in *Col3a1* in mice with defective cross-linking of collagen III. Toman et al. [651] generated transgenic mice by microinjection of a mutated mouse *Col3a1* harboring a Lys>Met substitution at the amino acid 939 cross-linking site within the triple-helical region of the α1(III) chain. Pregnant female mice expressing the transgene at levels of >30% in relation to the normal endogenous procollagen III showed greatly diminished ability to deliver pups at time of labor. Analysis of the uterine smooth muscle function of pseudopregnant transgenic mice showed an abnormal contraction pattern upon stimulation with oxytocin, and electron-microscopic analysis of the uterus indicated a marked disorganization of the collagen bundles disrupting the normal architecture of the myometrium. The authors concluded that the mutation that prevents cross-linking of the collagen III alters the normal functioning of smooth muscle in the uterus.

Mottled Mouse Mutants

Of the series of mouse mutants involving the X-linked locus mottled (Mo), so named because of its effect on coat color, the brindled (Mo^{br}) and blotchy (Mo^{blo}) variants have been studied particularly. The nature of the basic defect is a disturbance in copper handling as a result of mutations in *Atp7a*, the murine homolog of *ATP7A*, which codes for ATP7A, a member of the copper ATPase family, and is known to be affected in Menkes disease and the occipital horn syndrome. Consequently, intracellular copper is unavailable for enzymes requiring copper [632,633,652]. For a more detailed consideration, see Chapter 14 of this volume.

Hemizygous brindled mutant males normally die at 10–14 days of age with severe neurological deterioration without showing any signs of connective tissue disorder [523,653]. Although lysyl oxidase in extracts of skin is secondarily reduced to about 60% of normal [524,654], there is perhaps insufficient time for effects on connective tissue to manifest themselves before the neurological involvement takes its toll. The brindled mutants are considered to be a model of Menkes disease in the human [633], in severe cases of which connective tissue abnormalities may be prominent [655].

The blotchy male mutant, on the other hand, shows a variety of connective tissue features, including emphysema [656], and tends to die from aortic aneurysm at about 150 days of age [523,657]. The connective tissue defects appear to be secondary to deficient cross-linking of collagen [658] and elastin, consequent on inadequate activity of the copper-dependent lysyl oxidase which functions to initiate the process and is present in the tissues at only about one-quarter of normal levels [524,654,659]. The blotchy mutant has been suggested as a model of EDS IX [633], now reclassified as the occipital horn syndrome (see Chapter 14, this volume).

Experimental Animals for the Study of Tissue Fragility

Lathyrism

The condition in which deleterious effects in connective tissues are caused by chronic ingestion of the sweet pea *Lathyrus odoratus* is called lathyrism and is due to the inhibition of lysyl oxidase by β-aminopropionitrile (β-APN), with consequent inhibition of cross-link formation in both collagen and elastin. β-APN, the toxic agent, is released *in vivo* by an amidase or protease from the precursor, β-(γ-glutamyl)aminopropionitrile, present in the legumes, and is a potent and irreversible inhibitor of lysyl oxidase, with I_{50} values of 3–5 μM (for a review, see Kagan [660]).

Lathyrism has been reviewed by Levene and Gross [661] and Barrow et al. [662]. Geiger et al. [663], by feeding growing rats with sweet pea, were the first to describe dramatic malformations of mesenchymal tissues, termed osteolathyrism as opposed to neurolathyrism [664], which included kyphoscoliosis, exostosis of the long bones, marked periosteal new bone formation, weakening of the tendinous and ligamentous attachments and of the epiphyseal plates, skin, cartilage, and healing wounds, dislocation of joints, loss of teeth, and herniae. Later, dissecting, diffuse, or saccular aneurysms of the aorta were reported (angiolathyrism) [665], and teratogenic effects such as cleft palate [666] and ectopia cordis and gastroschisis [667] were observed in 1968 and 1971, respectively. In 1954/1955, β-APN was found to be the toxic factor in *Lathyrus odoratus* causing osteolathyrism [668]. Using chick embryos, Levene and Gross [661] showed that both tissue fragility and the amount of extractable collagen were dose- and time-dependent and speculated that normal intermolecular cross-linking was affected by the lathyrogenic agent. Martin and co-workers reported that the interference by β-APN in collagen cross-linking, and in the biosynthesis of desmosine and isodesmosine, was through the specific inhibition of lysyl oxidase, the enzyme used in the first step of cross-linking of collagen and elastin [669]. Lathyrogenic compounds were shown to fall into four major groups, in order of diminishing potency: nitriles > ureides > hydrazides > hydrazines [670].

β-APN has proved to be a useful agent to administer to growing animals, or to add to organ and cell cultures, to block collagen cross-linking and thus allow the extraction in large amounts of newly formed collagen which is amenable to characterization. It is also required in the preparation of biosynthetically labeled substrate for the assay of lysyl oxidase [418]. The successful use of β-APN in the prevention of esophageal strictures after lye injury and in overcoming the restrictive effects of peritendinous adhesions in animal experiments [671] led to human clinical trials. Results with β-APN in the treatment of patients with ureteral strictures and tendon adhesions [672] indicate that the physical properties of scar tissue may be amenable to biochemical control. However, the therapeutic value of β-APN as an antifibrotic agent is, unfortunately, outweighed by its damaging systemic side effects [673].

Copper Deficiency

Because copper is a cofactor of lysyl oxidase, its dietarily induced deficiency has effects that strongly resemble those of lathyrism. Common to both conditions are the following: (1) the disease is readily inducible only in the young; (2) vascular rupture is the cause of mortality; (3) aortic tensile strength is reduced, and there is a decrease in aortic elastin; (4) elastic fiber alterations are the cardinal histopathologic

and ultrastructural vascular lesions; (5) bone deformities occur in both conditions; (6) increased solubility of elastin and collagen are due to deficient cross-linking. Lysyl oxidase activity is not detectable, or is grossly reduced, in extracts of tissues from copper-deficient animals, a fact that originally suggested copper as the naturally occurring cofactor for the enzyme. Indeed, copper-deficiency states enabled the early isolation and characterization of tropoelastin [674].

D-Penicillamine

D-penicillamine (β, β-dimethylcysteine), according to the conventional view, inhibits collagen cross-linking by interacting with the lysine-derived aldehydes, rendering them unavailable for cross-link formation, although there is evidence that its principal effect might be to block the synthesis of polyfunctional cross-links from bifunctional Schiff base precursors [675]. D-Penicillamine acts preferentially on collagen cross-linking in soft tissues, while the hydroxylysine-derived aldehydes present in bone are much less affected. At high concentrations, it also chelates copper and reduces the activity of lysyl oxidase [675] (see also Footnote 9).

The overall effectiveness of the drug in reducing the structural stability of collagen *in vivo* is well-documented in animal experiments. It has also been used to reverse collagen accumulation in patients with hepatic fibrosis and progressive systemic sclerosis, but side effects, reviewed by Steinmann et al. [676], have prevented its use in such high concentrations as in animal experiments. Side effects of penicillamine in the treatment of cystinuria and Wilson disease have been reported and include symptoms reminiscent of EDS [676] and pseudoxanthoma elasticum [677] as well as ultrastructural changes in dermal collagen and elastin [678].

In conclusion, studies using lathyrogens have been instrumental in demonstrating that the ability of collagen and elastin fibers to function is primarily dependent on a system of covalent cross-links between the polypeptide chains of the respective proteins.

CONCLUDING REMARKS

Mutations that give rise to the several different phenotypes of EDS affect collagen structure (some cases of EDS I and II, EDS VIIA and VIIB), expression (a few cases of EDS I and III), maturation (EDS VI, VIIC, occipital horn syndrome), and fibrillogenesis (EDS I/II, VII), noncollagenous proteins, such as proteoglycans (progeroid form of EDS) and tenascin-X, and potentially many more proteins of the extracellular matrix in the vast majority of cases. The EDS phenotypes reflect both disturbances in the intermolecular cross-linking of collagen (EDS VI, VII, and occipital horn syndrome) and the lack of an appropriate scaffold (EDS IV, progeroid form of EDS) on which to build the major components of the skin. The precise phenotypic findings therefore depend on the nature of a mutation and the molecule in which it occurs. Despite such a wide genetic heterogeneity, the organism has only a limited repertoire for functional and morphological changes.

RECENT DEVELOPMENTS

EDS Types II and III

Clinical Features

Absence of the inferior labial and lingual frenula has been reported in 12 patients, four with EDS II and eight with EDS III, with a mean age of 29.7 years (range 15–45 years), and suggested to be a highly specific and sensitive marker for these disorders [679]. It will remain uncertain, however, whether this is a congenital anomaly or rather an acquired trait due to tissue fragility, until prospective, longitudinal observations have been performed and the natural history is known. It is further unknown whether this clinical finding also occurs in other types of EDS.

Vascular Type of EDS (EDS Type IV)

Genetics and Pathogenesis

Because EDS IV has hitherto been associated exclusively with dominant negative *COL3A1* mutations, it has been unclear whether any *COL3A1* haploinsufficiency mutation would cause an EDS IV-like phenotype differing in severity or symptomatic range from usual, or indeed any phenotype, and therefore have escaped analysis. This uncertainty was reinforced by the finding that heterozygous *Col3a1* knockout mice are phenotypically normal [334], although late onset signs would have been missed if they had occurred after the 18 months follow-up period.

Schwarze et al. [680] have now presented data on four patients with EDS IV with *COL3A1* haploinsufficiency mutations. Three of them had frameshift mutations that led to premature termination codons in exons 27 (1832delAA), 6 (413delC), and 9 (555delT), respectively, and to allele product instability. In the fourth patient, a point mutation introduced a premature stop codon in the most 3′ exon (4294C>T; Arg1432Ter), resulting in the synthesis of truncated proα1(III) chains that were not incorporated into procollagen III molecules. All four index patients had vascular aneurysms or ruptures, and the phenotype of family members presumed to be affected was reported to be within the range of "classical EDS IV" due to structurally abnormal collagen III. Surprisingly, biochemical evidence of reduced synthesis of procollagen III was limited to dermal fibroblasts from a single patient, procollagen III synthesis in cell strains from each of the other three patients being normal. The authors concluded that a 50% reduction in the amount of structurally normal procollagen III is as deleterious to the vascular system as the biosynthesis of collagen III, seven-eighths of which is structurally abnormal, that occurs as a consequence of the more than 200 other reported mutations. However, their conclusions have some limitations in that the probands had been ascertained because of "typical" EDS IV signs and symptoms and on average they, as well as their relatives presumed to be affected, were older than the reported median age of survival for EDS IV [256]. The probands may thus simply represent the more severe range of a milder phenotype characterized by the attainment of a greater age before symptomatic arterial events occur and by longer survival.

The report by Schwarze et al. [680] gives rise to the following considerations in relation to the diagnosis of EDS IV: (1) The cornerstone of the diagnosis should remain suggestive clinical findings in the proband and similarly affected family members in conjunction with detailed medical histories; by this approach alone, however, sporadic cases are less likely to be detected. (2) In fibroblast strains with *COL3A1* haploinsufficiency, the relative proportions of collagens I and III secreted into the medium and retained by the cells do not constitute a reliable diagnostic indicator because they depend on such parameters of culture conditions as cell density [218,342]. It must also be stressed that the ratio of $[\alpha1(III)]_3$ molecules to combined α1(I) and α2(I) chains after limited proteolysis with pepsin provides a more accurate measure of synthesis of the two collagen types than may be achieved by determining the relative proportions of reduced

proα1(III) and proα1(I) chains, as done in the study under discussion [680]. Furthermore, subtle structural alterations in type III collagen are more readily detectable in $[\alpha1(III)]_3$ molecules than in proα1(III) chains. (3) Mutation analysis in individuals in whom the diagnosis of EDS IV has been made on clinical grounds has been successful in those whose cultured cells have synthesized a cohort of structurally abnormal procollagen III molecules. Now that a clinical phenotype due to haploinsufficiency has been recognized, direct genomic analysis is likely to be a more efficient way of identifying cell strains from individuals so afflicted. (4) Because the role of *COL3A1* mutations in other conditions such as isolated cerebral aneurysm [272] and abdominal aortic aneurysm [271] has been explored only at the cDNA level, non-expressed alleles or unstable mRNA products might not have been detected in a minority of individuals homozygous (or hemizygous) at known polymorphic sites; retesting of such individuals at the genomic level might therefore be warranted.

Kyphoscoliotic Type of EDS (EDS Type VI)

Clinical Features

Pregnancy in EDS VI. There remain no published reports on pregnancies of individuals with EDS VI. Two affected women are known to have delivered normally after 36 weeks without untoward consequences (R. Wenstrup, personal communication, 2001). Another affected woman with a moderately severe phenotype (case 55, see legend to Fig. 22) had an early abortion and later delivered a boy at term (B. Steinmann, personal observation).

Differential Diagnosis

Infants with Ullrich disease (MIM 254090; see Chapter 26, Part VI, this volume) may present with severe muscular hypotonia, marked joint laxity, Marfanoid proportions, and autosomal recessive inheritance, and thus may expand the differential diagnosis of EDS VI. Children with the autosomal recessively inherited Nevo syndrome (MIM 601451) have tall stature at birth, muscular hypotonia, joint laxity, kyphoscoliosis, a Marfanoid habitus, wrist drop, long spindle-shaped fingers, osteopenia, and dural ectasia, and may be confounded with EDS VI (B. Steinmann, personal observation).

Therapeutic Approaches

With the aim of local gene therapy, Rauma et al. [681] cloned human lysyl hydroxylase 1 cDNA into a recombinant adenoviral vector (Ad5RSV-LH). Transfection of human EDS VI fibroblasts from case 3 (see legend to Fig. 22) with the vector increased lysyl hydroxylase activity in these cells in a dose-dependent manner from residual values of 20% of normal to levels greater than in control cells. The adenoviral vector also successfully transfected rat fibroblasts *in vitro*, and intradermal injections of the vector in rats produced human lysyl hydroxylase 1 mRNA and elevated lysyl hydroxylase 1 activity *in vivo*. This study suggests the feasibility of gene replacement therapy to modify skin wound healing in EDS VI patients.

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APPENDIX: PATIENT SUPPORT GROUPS

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Fax: 323-651-3038
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Web site: http://www.ednf.org
Contact person: Mrs. Linda Neumann-Potash

ELECTRONIC-DATABASE INFORMATION

Online Mendelian Inheritance in Man (OMIM)
http://www.ncbi.nlm.nih.gov/omim

London Dysmorphology Data Base
http://genetics.ich.ucl.ac.uk/lddb/lddb.html

The Human Gene Mutation Database Cardiff (HGMD)
http://www.uwcm.ac.uk/uwcm/mg/hgmd0.html

Mutations/Polymorphisms in *COL1A1*
http://www.le.ac.uk/genetics/collagen/col1a1.html

Mutations/Polymorphisms in *COL1A2*
http://www.le.ac.uk/genetics/collagen/col1a2.html

Mutations/Polymorphisms in *COL3A1*
http://www.le.ac.uk/genetics/collagen/col3a1.html

Mutations/Polymorphisms in *COL5A1*
http://www.uwcm.ac.uk/uwcm/mg/search/131457.html

Mutations/Polymorphisms in *COL5A2*
http://www.uwcm.ac.uk/uwcm/mg/search/119064.html

Enzyme Nomenclature Database
http://www.expasy.ch/enzyme/

Online Resource
http://www.familyvillage.wisc.edu/lib_e-ds.htm

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