

## GENETICS OF STRUCTURAL SKIN DISORDERS

# Heritable Collagen Disorders: The Paradigm of the Ehlers–Danlos Syndrome

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## INTRODUCTION

The heritable disorders called Ehlers–Danlos syndrome (EDS) are as disparate as they are fascinating. Their discovery and description has been an intimate part of the growth of our understanding of matrix biology. Joint hypermobility, skin extensibility, abnormal scarring, and tissue friability are the hallmark diagnostic features; however, McKusick (1956) recognized that EDS is under-recognized because when the physical signs are not “classic” the diagnosis may be elusive. The medical and scientific history of EDS can be seen in three phases: clinical characterization, biochemical and molecular genetic analysis, and the use of high throughput genomic analysis to extend the phenotypes.

## CREATING THE CLINICAL SPECTRUM OF EDS

Hippocrates described the features of this disorder in 400 BC; however, the first medical description of some of the characteristics is credited to van Meekeren (1682). Tschernogubow's presentation in 1892 seems to have been largely overlooked in Western Europe, probably because it was written in Russian. Weber (1936) is credited with christening the disorder as EDS in 1936 after Ehlers (1901) and Danlos (1908), two dermatologists who separately described affected patients in 1901 and 1908, respectively. The same year, Georg Sack, a German physician, described a condition that Barabas later identified as the arterial type. McKusick (1956) provided the first

synthesis of the clinical literature on the multisystemic and variable nature of EDS in his 1956 hallmark work on heritable connective tissue disorders. Over a decade later, Barabas (1967) proposed at least three distinct subtypes: classical, varicose, and arterial on the basis of his experience with 27 affected individuals. He suggested that the clinical subtypes reflected discrete etiologies, not simply variable expressions of one disorder. A short time later, Peter Beighton published a series of papers from a landmark clinical investigation of 100 individuals with EDS. He recognized and expanded Barabas' work to a classification of five types (Beighton *et al.*, 1969). The first group included individuals similar to those initially described with skin hyperextensibility and fragility, with abnormal scarring and bruising, and striking joint hypermobility (the gravis form). The second was similar but the skin findings were less dramatic (mitis). A hypermobility form in which the joint findings were striking almost to the exclusion of skin changes was the third type. The Sack-Barabas group (the fourth type) had very severe vascular fragility and was at risk of arterial rupture and bowel rupture. The fifth group was thought to be X-linked and was characterized by joint hypermobility and intramuscular hemorrhage (Table 1).

## THE FIRST PHASE OF BIOCHEMICAL CHARACTERIZATION OF EDS

During the early phase of clinical characterization, clues to the molecular

basis of any of the forms of EDS were sparse, with the exception that light and electron microscopy studies identified abnormalities in the structure of collagens in the dermal matrix (Wechsler and Fisher, 1964; Julkunen *et al.*, 1970). The dam broke in the early 1970s as a result of tissue analysis to assess interactions/cross-links between collagen molecules, animal studies, and use of cultured cells from selected individuals to examine collagen production. Detailed knowledge of the structure of cross-links and the awareness of their importance in tissue integrity provided the background in which the study of sisters with hypermobility, soft extensible skin, scoliosis recalcitrant to surgical intervention, and ocular globe fragility (Krane *et al.*, 1972; Steinmann *et al.*, 1975) led to the identification of lysyl hydroxylase deficiency. In these sisters, hydroxylated complex cross-links were diminished and the virtual absence of hydroxylation of lysyl residues in triple helical collagen molecules resulted from deficiency of lysyl hydroxylase. Once the gene (*PLOD1*) was isolated, sequence analysis demonstrated inactivating mutations, the most common of which was a 7-exon duplication mediated by Alu–Alu recombination (Heikkinen *et al.*, 1997). This was one of the very early demonstrations of the role of repetitive elements in the genome as mediators of deletions or duplications. This disorder, a recessively inherited condition, was called EDS type VI and was the first established true disorder of collagen biosynthesis and structure in humans.

**Table 1. Classification of EDS**

Numerical type	Descriptive type	Genes	OMIM	Inheritance	Clinical features and notes
I (Gravis)	Classical <sup>1</sup>	<i>COL5A1</i>	130000	AD	Marked joint hypermobility, skin hyperextensibility, bruising, and abnormal scarring
II (Mitis)		<i>COL5A2</i>	130010		
III	Hypermobility <sup>2</sup>	<i>TNXB</i> Largely unknown	130020	AD	Marked joint hypermobility, minor skin findings
IV	Vascular	<i>COL3A1</i>	130050	AD	Thin translucent skin, marked bruising, small joint hypermobility, high risk for rupture of arteries, bowel, and gravid uterus
VIA	Kyphoscoliosis	<i>PLOD1</i>	225400	AR	Joint hypermobility and kyphoscoliosis recalcitrant to surgical intervention, risk for arterial rupture
VIB	Musculocontractural	<i>CHST14</i>	601776	AR	Congenital contractures of digits, dysmorphic features, kyphoscoliosis and hypermobility, hyperextensible thin skin, ocular involvement
VIIA	Arthrochalasia multiplex congenita	<i>COL1A1</i>	130060	AD	Marked joint hypermobility, bilateral congenital hip dislocation
VIIIB		<i>COL1A2</i>			
VIIC	Dermatosparaxis	<i>ADAMTS2</i>	225410	AR	Soft, very fragile skin with late onset skin redundancy, blue sclerae, joint hypermobility
VIII	Periodontitis	Probably heterogeneous; one locus at 12p13	130080	AD	Periodontal loss, joint hypermobility, soft skin with characteristic plaque on anterior tibial region
<i>Other</i>					
	Progeroid	<i>B4GALT7</i>	130070	AR	
	Cardiac valvular	<i>COL1A2</i>	225320	AR (null)	Cardiac valvular insufficiency, joint hypermobility, skin hyperextensibility
	<i>FKBP14</i> related	<i>FKBP14</i>	614557	AR	Marked kyphoscoliosis, hearing loss, myopathy, short stature, joint hypermobility
	Spondylocheiro dysplastic	<i>SLC39A13</i>	612350	AR	Spondyloepiphyseal dysplasia with mild short stature, hyperelastic thin skin with easy bruising, protuberant eyes, bluish sclerae, fine wrinkling on palms
	Tenascin-X deficient	<i>TNXB</i>	606408	AR	Joint hypermobility, hyperextensible and sleeve-like character to skin, marked bruising, normal scarring
	Periventricular heterotopia	<i>FLNA</i>	300537	XL	Periventricular heterotopia, joint hypermobility
<i>Poorly characterized, disputed, retired, or reclassified<sup>3</sup></i>					
V	X linked	Unknown	305200	XL	Joint hypermobility with muscle hemorrhage
VIB	Brittle Cornea syndrome	<i>ZNF469</i>	22920	AR	Blue sclerae, corneal rupture, keratoconus, hyperextensible skin, joint hypermobility
		<i>PRDM5</i>	614170		
IX	Occipital Horn syndrome	<i>ATP7A</i>	304150	XL	Hyperextensible skin and bruising, hernias and bladder diverticuli, joint hypermobility
X	Fibronectin deficient	Unknown	225310	?	One reported family with platelet dysfunction and mild features of EDS
XI	Familial Joint Laxity	Unknown	147900	AD	Retired

Abbreviations: AD, autosomal dominant; AR, autosomal recessive; EDS, Ehlers-Danlos syndrome; XL, X linked.

<sup>1</sup>Specific mutations in *COL1A1* (substitutions of cysteine for arginine residues within the triple helical domain of pro $\alpha$ 1(I) chains) have been recognized to cause a form of EDS reminiscent of classical type with joint hypermobility, skin hyperextensibility, bruising and abnormal scarring, and predisposition to aortic aneurysm.

<sup>2</sup>One family with a specific mutation in *COL3A1* is said to have only joint hypermobility with none of the other consequences seen in EDS type IV.

<sup>3</sup>We have included these previously identified "types" of EDS for historical perspective. They are not included in most classifications.

Cattle with a curious and severe form of skin fragility provided the next step in the identification of human collagen disorders, as well as insight into the question of how an essentially insoluble molecule—collagen—could be synthesized and secreted from cells. The bovine disorder, dermatosparaxis, appeared to be inherited in an autosomal recessive manner, and biochemical studies demonstrated that affected animals failed to process an amino-terminal precursor peptide from all three chains of type I collagen (Lenaers *et al.*, 1971). As a consequence, collagen fibrillogenesis was impaired and skin integrity compromised (Piérard and Lapière, 1976). The animal study prompted investigation of tissues from a young woman born with bilateral hip dysplasia/dislocation and very marked joint laxity. Her skin contained  $\alpha 2(I)$  chains of type I collagen with an extension at the amino-terminal end (Lichtenstein *et al.*, 1973; Steinmann *et al.*, 1980). Although initially interpreted to result from enzymatic deficiency, studies later showed that the woman had a heterozygous splice donor mutation in intron 6 of one *COL1A2* allele that led to exon skipping and loss of the sequence that contained both the propeptide cleavage site and the amino-terminal non-helical cross-link site (Steinmann *et al.*, 1980). Consistent with this finding, autosomal dominant inheritance was soon recognized and mutations in the *COL1A1* gene that led to loss of the sequences of the homologous exon 6 were found in other affected individuals (Byers *et al.*, 1997). Although the outcomes of mutations in the preceding acceptor site and succeeding donor site differ in detail, both lead to the same phenotype. Fewer individuals have *COL1A1* mutations because *COL1A1* donor site mutations lead to use of a cryptic donor site or intron inclusion, each of which leads to a frame shift, premature termination codon, mRNA instability, and an osteogenesis imperfecta phenotype. More than 20 years after the basis of dermatosparaxis was explained, a human form of the disorder (then called EDS VIIC) was identified (Nusgens *et al.*, 1992; Smith *et al.*, 1992) and shown to result

from biallelic mutations in *ADAMTS2*, which encodes the procollagen 1 N-proteinase (Colige *et al.*, 1999). Variation in the clinical picture reflected the presence of missense mutations rather than the early identified nonsense mutations (Colige *et al.*, 2004).

The early biochemical discovery phase culminated with the demonstration that alteration in the amount of type III procollagen produced was the underlying cause of EDS type IV, the Sack Barabas type (Pope *et al.*, 1975). At first thought to be recessive because of its rarity, an isolated individual in a family, and apparent decrease in production of type III procollagen by cells from the parents (Pope *et al.*, 1977), subsequent analyses of the *COL3A1* gene has shown that this is a dominant disorder (Tsipouras *et al.*, 1986). A single example of bi-allelic mutations has been identified out of more than 600 families studied (Plancke *et al.*, 2009). Some mutations result in failure to secrete type III procollagen from fibroblasts and accumulation of the protein in the rough ER and marked alteration in dermal structure that led to the idea that type III collagen formed a scaffold on which type I fibrillogenesis occurred (Holbrook and Byers, 1981). A large clinical study proved it difficult to identify clear genotype phenotype correlations (Pepin *et al.*, 2000). Recent studies, however, make it clear that heterozygosity for a null mutation in *COL3A1* endows an individual with an extended natural history compared with the effects of missense and exon skipping mutations (Leistriz *et al.*, 2011).

EDS type I/II proved to be more difficult than expected to solve at the molecular level. Ultrastructural studies of the skin showed that there were dramatic alterations in the large dermal collagen fibrils, with variation in fibril diameter and aggregate formation (Vogel *et al.*, 1979). Despite this, linkage studies in families excluded type I collagen genes as candidates (Sokolov *et al.*, 1991; Wordsworth *et al.*, 1991). It was the finding of an X:9 chromosomal translocation in a woman with EDS type I and skin pigment alteration with identification of a breakpoint in one *COL5A1* allele that shed light upon

the molecular basis of the disorder (Toriello *et al.*, 1996). At the same time, linkage studies and sequencing of both *COL5A1* and *COL5A2* led to the identification of mutations in individuals with EDS type I/II (Burrows *et al.*, 1996; Nicholls *et al.*, 1996; Wenstrup *et al.*, 1996). Most affected individuals studied had mutations that led to instability of mRNA derived from one *COL5A1* allele (Schwarze *et al.*, 2000; Wenstrup *et al.*, 2000; Malfait *et al.*, 2005). A critical role of type V collagen in fibril nucleation was established in homozygous *COL5A1*-knockout mice that failed to survive embryogenesis because no large collagen fibrils were assembled (Wenstrup *et al.*, 2004).

Although often cited to suggest that only about half of affected individuals with EDS type I or EDS type II (the previous gravis and mitis type and future “classical type”) have mutations in type V collagen genes (Malfait *et al.*, 2005), a recent study using more consistent clinical diagnosis places this number closer to 90% (Symoens *et al.*, 2012). Although genetic studies failed to demonstrate that mutations in type I collagen genes could cause EDS type I/II (Sokolov *et al.*, 1991; Wordsworth *et al.*, 1991), biochemical analysis of type I collagens synthesized in culture succeeded. Several individuals with substitutions of arginine by cysteine within the triple helical domain of pro $\alpha 1(I)$  chains of type I collagen, encoded by *COL1A1*, had a clinical picture of EDS type I/II and also developed aortic aneurysms or dissection (Nuytinck *et al.*, 2000; Malfait *et al.*, 2007). In addition, homozygosity or compound heterozygosity for *COL1A2*-null mutations were found in several people who had a clinical presentation with polyvalvular cardiac involvement, moderate joint hypermobility, skin hyperextensibility, and limited bruising (Schwarze *et al.*, 2004; Malfait *et al.*, 2006). Nonetheless, mutations in type I collagen genes account for only a small fraction of patients with EDS type I/II.

By the mid 1990s there had been a profusion of single case reports that tried to define additional types of EDS, most of which relied largely on clinical differentiation in single systems and

were not substantiated by comprehensive genetic or biochemical studies. Few survived the later purge done to help stabilize a classification. Notable among the survivors, but not included in the later classification, is what became known as a progeroid type of EDS in which features of early aging accompanied significant hypermobility and appeared to result from defective glycosaminoglycan addition to several proteoglycans (Hernández *et al.*, 1986) (Quentin *et al.*, 1990), ultimately shown to result from mutations in *B4GALT7* (Okajima *et al.*, 1999) (Faiyaz-Ul-Haque *et al.*, 2004). The target matrix proteins are probably not limited to decorin and biglycan, but the lack of posttranslational modification presumably affects their function (Götte and Kresse, 2005).

The hope to restore order, similar to that brought by Beighton's studies of the 1960s, led to a gathering of interested clinicians and geneticists at Villefranche in 1997 and the creation of a new set of clinical and biochemical criteria for the diagnosis of EDS (Beighton *et al.*, 1998). This new classification abolished the previous Roman numerical system and substituted a "descriptive" nomenclature, while at the same time banishing some previous types to an "other" category. Created before genetic testing for many disorders became widespread, this classification is showing its age and is in sore need of revision. However, any static classification system may be challenged by the expanding molecular characterization of disorders with some features of EDS. The tensions among a purely clinical classification, a purely genetic classification, and a mixed classification may be difficult to resolve and could satisfy neither clinicians nor molecular geneticists in the long run.

The "post-Villefranche" era has experienced a proliferation of EDS types distinguished by clinical and genetic grounds but not yet incorporated into a coherent classification. The elegant studies by Burch *et al.* (1997) of a child with 21-hydroxylase deficiency and a form of EDS led to identification of a new gene, the loss of function of which explained the connective tissue manifestations. The active 21-hydroxylase gene (*CYP21A*) is located centro-

meric to *TNXB*, which encodes tenascin X. A copy of the 3' exons of *TNXB* (called *TNXA*) are located on the centromeric side of *CYP21A* and a deletion mediated by the almost exact sequence identity between the two tenascin X genes led to 21-hydroxylase deficiency and loss of function of both copies of *TNXB*. A member of the matrix tenascin protein family, tenascin X interacts with collagens and other matrix macromolecules. Tenascin X-deficient EDS is distinguished from classical EDS by autosomal recessive inheritance, absence of abnormal scarring in the presence of profound joint hypermobility, very hyperextensible skin, and striking bruising (Schalkwijk *et al.*, 2001). The presence of significant hypermobility in heterozygous carriers of null mutations suggested *TNXB* as a candidate gene in EDS type III, the hypermobile type. Indeed, obligate heterozygotes demonstrated that about 80% of carrier women but only 20% of carrier men had significant joint hypermobility (Zweers *et al.*, 2003) but wider screening failed to document mutations at sufficient frequency to account for EDS type III. The genomic complexity of this region has proven a significant barrier to molecular diagnostics for this gene.

#### GENETIC AND GENOMIC ANALYSIS IDENTIFIES MORE TYPES OF EDS AND THEIR GENETIC BASES

Astute observation of distinctive clinical features in consanguineous families coupled with genomic searches led to recognition of several forms of EDS that fall outside the Villefranche classification system. These include the spondylocheiro dysplastic form that results from mutations in *SLC39A13* (Fukada *et al.*, 2008; Giunta *et al.*, 2008). The gene encodes an endoplasmic reticulum-located zinc transporter but additional studies suggested that transforming growth factor- $\beta$  signaling may also be defective, although the relationship to phenotype is not clear. Mutations in *CHST14* (Malfait *et al.*, 2010; Miyake *et al.*, 2010), which encodes dermatan-4-sulfotransferase 1, confirms the importance of this modification as a part of matrix stability. The

mutations result in a musculocontractural form of EDS. Very recently a new form of EDS characterized by kyphoscoliosis, myopathy, and hearing impairment was identified that results from biallelic mutations in *FKBP14* (Baumann *et al.*, 2012), a member of the prolyl *cis-trans* isomerase family. Mutations in a family member, *FKBP10*, result in a form of osteogenesis imperfecta (Alanay *et al.*, 2010), perhaps because of effects on folding of either the pro $\alpha$  chains of type I procollagen or of lysyl hydroxylases. This type of mechanism might help explain the clinical overlaps among one form of osteogenesis imperfecta, EDS type VI, Bruck syndrome, and this new form of EDS. The genetic etiology of another rare form of EDS associated with periventricular heterotopia was established by recognition of the similarity of the neurology features with that of periventricular heterotopia due to *FLNA* mutations (Sheen *et al.*, 2005). To date, this is the only confirmed X-linked form of EDS. Most reported individuals are female, consistent with an embryonic lethal effect in males.

#### FINAL CONSIDERATIONS

The genomic era promises to shed additional light on unsolved forms of EDS. For example, a form of EDS characterized by joint hypermobility, easy bruising, and early periodontal loss without significant inflammation was defined as EDS type VIII. This was shown not to result from mutations in *COL3A1* even though some features were shared. Linkage to a locus on the short arm of chromosome 12 (12p13) was identified in a large Swedish family but excluded in others, consistent with locus heterogeneity (Rahman *et al.*, 2003). To date, the molecular etiology of this form remains to be established. Similarly, the genetic etiology in the majority of persons affected with hypermobile EDS (type III) remains to be determined. There are a variety of challenges to dissecting the genetic causes of hypermobile EDS, including but not limited to the clinical variability, seeming sex-related penetrance, and likely genetic heterogeneity. Identifying and understanding the clinical

diversity, genetic etiology, and pathophysiological mechanisms of various forms of EDS will undoubtedly continue to expand the work of the last 50 years toward understanding the biology of the extracellular matrix and the role of the constituent macromolecules in human disease.

## CONFLICT OF INTEREST

The authors state no conflict of interest.

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