Mini review

Ehlers–Danlos syndrome: A showcase of conditions that lead to understanding matrix biology

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Abstract

The Ehlers–Danlos syndromes (EDS) are genetically and clinically diverse disorders in which affected individuals share a number of physical characteristics, including joint hypermobility, skin extensibility, and tissue friability. Clinical investigations opened the door to identifying the biochemical and molecular etiologies of this diverse but overlapping group of disorders. In this article, we provide an overview of how these disorders inform our understanding of matrix biology, including the role of collagens (types I, III and V), proteoglycans and other proteins.

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1. Introduction

As clinical scientists, it is our intent that clinical, biochemical, and genetic analysis of people with heritable disorders will lead to a therapeutic or curative pathway. But, in the back of our minds is always the hope that this patient, this family, holds the key to understanding fundamental biological processes.

The Ehlers–Danlos syndromes (EDS) are genetically and clinically diverse disorders in which affected individuals share a number of physical characteristics, including joint hypermobility, skin extensibility, and tissue friability (see Table 1). There is an intimate relationship between the recognition of clinical heterogeneity and genetic discovery — in essence, without detailed clinical investigations, the genetic diversity of EDS would have gone unrecognized. Study of Ehlers–Danlos syndromes and other heritable connective tissue disorders has taken advantage of opportunistic research to elucidate insights on the role of collagens, proteoglycans and other proteins in the biology of the extracellular matrix.

2. Creating the clinical spectrum of EDS

The first medical description of some EDS characteristics is credited to van Meekeren (McKusick, 1956; van Meek'ren, 1682). Tscherne's presentation in 1892 (Tscherne, 1892) was overlooked in western Europe, perhaps because it was presented in Russian. Parkes-
Weber suggested that the syndromic presentation of increased skin extensibility, joint hypermobility, abnormal scar formation, and easy bruising be considered as Ehlers-Danlos syndrome (Weber, 1936) to recognize the contributions of Edvard Ehlers and Henri-Alexandre Danlos, two dermatologists who separately described affected patients in 1901 (Ehlers, 1901) and 1908 (Danlos, 1908). Georg Sack, a German physician, provided an early description of what was later called vascular EDS (Sack, 1936; Barabas, 1967). McKusick...
provided a synthesis of the clinical literature on EDS in his 1956 work on heritable connective tissue disorders (McKusick, 1956). Over a decade later, Barabas distinguished three subtypes of EDS that he named as classical, varicose, and arterial (Barabas, 1967), which he thought reflected discrete etiologies and not variable expression within one disorder. Beighton's name became irrevocably linked to EDS when he published a series of papers from a landmark clinical investigation that expanded Barabas' classification to include 5 types — gravis, mitis, hypermorphic, vascular, and an apparently X-linked form (Beighton et al., 1969).

By the mid-1990s, there had been a profusion of single case reports that tried to define additional types of EDS. The clinical uncertainty led to a gathering of clinicians and geneticists and the generation of clinical and biochemical criteria for the diagnosis of different forms of EDS (Beighton et al., 1998). This new classification substituted a "descriptive" nomenclature for the previous Roman numeral "types". One underlying assumption was that most if not all of these types of EDS were a consequence of alterations in collagen genes or in genes that encoded collagen modifiers. With ongoing identification of mutations in new genes that lead to EDS-related phenotypes, this classification is showing its age and in sore need of revision. 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. A newly formed consortium that grew out of the first international meeting on EDS, convened in Ghent in 2012, will take on the task of updating the classification system.

3. Defects in type I collagen in different forms of EDS

Early clues to the molecular basis of forms of Ehlers Danlos syndrome were limited to light and electron microscopy studies that identified abnormal structure of collagen fibrils and fibers in the dermal matrix (Wechsler and Fisher, 1964; Wechsler and Fisher, 1964; Julkunen et al., 1970). An emerging appreciation of the structure and heterogeneity of collagens, as well as appearance of animal models, provided the background in which the first biochemical approaches yielded abnormalities. Lysyl hydroxylase (PLOD1) deficiency explained a clinical picture of hypermobility, soft extensible skin, recalcitrant scoliosis, and ocular globe fragility with apparent recessive inheritance (Krane et al., 1972; Steinmann et al., 1975). This disorder, EDS type VI (kyphoscoliotic type), was the first characterized disorder of collagen biosynthesis and structure in humans and established the paradigm by which types of EDS could be characterized.

Cattle with severe skin fragility (dermatosparaxis) provided insight into how an essentially insoluble molecule – collagen – could be synthesized and secreted from cells and, at the same time, opened the door to identification of a set of human collagen disorders. Affected animals failed to process a previously unrecognized amino-terminal precursor peptide from all three chains of type I collagen (Lenaers et al., 1971) so that collagen fibrillogenesis was impaired and skin integrity was compromised (Piérard and Lapière, 1976). These studies occurred in parallel with others of collagens produced by rat calvaria (Bellamy and Bornstein, 1971) and cultured fibroblast (Layman et al., 1971) that provided the details of procollagen structure and established the existence of the amino-terminal and carboxyl-terminal propeptides (Byers et al., 1975). Although there was no recognized human “dermatosparaxis”, the animal studies led to investigation of individuals with striking hypermobility. Skin from one individual born with bilateral hip dysplasia/dislocation and marked joint laxity contained α2(1) chains of type I collagen with an extension at the amino-terminal end (Lichtenstein et al., 1973; Steinmann et al., 1980). Initially interpreted to result from enzymatic deficiency, studies later showed that a woman had a heterozygous splice donor mutation in intron 6 of one COL1A2 allele that led to exon skipping and loss of both the propeptide cleavage site and the amino-terminal non-helical crosslink site (Steinmann et al., 1980). The loss of the substrate for the amino-terminal propeptidase (later shown to be encoded by ADAMTS2 (Colige et al., 1999) produced a set of dominantly inherited phenotypes (Byers et al., 1997) designated as EDS type VIIA (COL1A1) and EDS type VIIIB (COL1A2). The outcomes of mutations in the splice sites flanking exon 6 differ in detail but both can lead to the same phenotype. Interestingly, fewer individuals have COL1A1 mutations because donor site mutations lead to use of an out of frame cryptic site or intron inclusion, each of which leads to mRNA instability and an osteogenesis imperfecta phenotype (unpublished data). It took more than 20 years to identify individuals with the human equivalent of dermatosparaxis (EDS type VIIc) (Nusgens et al., 1992; Smith et al., 1992) shown to result from biallelic mutations in ADAMTS2 (Colige et al., 1999). Variation in the clinical picture reflected the presence of missense mutations rather than the earlier identified nonsense mutations (Colige et al., 2004; Malfait et al., 2004).

One of the most provocative proposals to come from studies of cells from animals with dermatosparaxis was that the amino-terminal propeptide of type I procollagen could control procollagen production via feedback inhibition (Wiestner et al., 1979). A variety of mechanisms have been suggested. In one, the cleaved propeptide would be taken up by the cell and act to regulate translation (Paglia et al., 1979). The amino terminal propeptide of proα1(1) chains contains a cysteine rich domain that may affect downstream TGFβs and BMPs to regulate developmental processes. This domain is also present in the chains encoded by the clade of collagens including COL1A1, COL2A1, COL3A1 and COL5A2, though not COL5A1. The precise function of the domain remains uncertain and may depend upon the specific protein, species, and tissue (McAlinden et al., 2005; Oganesian et al., 2006). When the amino terminal propeptide of proα1(1) was expressed in cells, protein phosphorylation (including SMAD3 and Akt) was increased and type I procollagen expression was decreased (Oganesian et al., 2006). This could not be replicated by addition of the peptide into the medium. The proposed model was that the amino-terminal propeptide of type I procollagen (and presumably others) could bind the TGFβ receptor in the secretory vesicles and counteract TGFβ signaling. This model awaits validation and is one of the few to incorporate the concept that the chordin-like domain could interact with TGFβ family members and influence collagen production. This line of investigation could provide clues to treatments for EDS type IV and for forms of osteogenesis imperfecta (OI), given the success in pursuing such a model in Marfan syndrome (Dietz et al., 2005).

Type I collagen has also been implicated in two other forms of EDS. Homozygosity or compound heterozygosity for COL1A2 null mutations results in a clinical presentation with polyvalvular cardiac involvement, moderate joint hypermobility, skin hyperextensibility, and limited bruising (Schwarze et al., 2004; Malfait et al., 2006). Cells from these individuals produced type I collagen molecules that contained only proα1(1) chains. Cells from another individual homozygous for a frame shift mutation in COL1A2 that resulted in complete failure of chain association had an intermediate OI phenotype (Plijilajemi et al., 1984). The different clinical outcome in these two instances one with mRNA instability and the other with protein instability – provides the unexpected insight that part of the pathogenesis in OI involves activation of the unfolded protein response. In contrast, the EDS phenotype that results from no proα2(1) chains reflects what appears to be a more limited response in the matrix. Type I collagen is also implicated in a clinical picture consistent with classical EDS with an uncharacteristic propensity to arterial complications, as was described in several individuals with substitutions of arginine by cysteine within the triple helical domain of proα1(1) chains (Nuytinck et al., 2000; Malfait et al., 2007). At other positions within the triple helical domain of proα1(1), substitutions of arginine by cysteine result in bone fragility with joint hypermobility (Cabrál et al., 2007). All told, however, mutations in type I collagen genes account for only a small fraction of patients with classical forms of EDS.
4. Type III collagen and vascular EDS

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 vascular type (Pope et al., 1975). At first thought to be recessively inherited (Pope et al., 1977), subsequent investigation has shown this is a dominant disorder due to mutations in COL3A1 (Tsipouras et al., 1986; Pepin et al., 2000). A single example of bi-allelic mutations has been reported (Plancke et al., 2009). It was striking that some mutations resulted in almost complete failure of fibroblasts to secrete type III procollagen with accumulation of the protein in the rough endoplasmic reticulum (RER) (Holbrook and Byers, 1981). The mechanism by which these molecules were contained in the RER remains uncertain. Judging from studies of similar mutations in type I collagen genes (Chessler and Byers, 1993), there is no activation of the usual unfolded protein responses. It is not clear whether aggregates form or if the partially unfolded proteins are recognized by other components of the chaperone pathway that regulate exit of procollagen trimers from the RER.

Genotype–phenotype correlations have been difficult to identify with mutations in COL3A1 (Pepin et al., 2000). One striking example, however, is that heterozygosity for null mutations in COL3A1 results in an attenuated phenotype (Leistritz et al., 2011). The mechanisms by which mutations in COL3A1 lead to an increased incidence of clubfoot and congenital hip dislocation remains unknown but, along with the marked decrease in skin thickness, arterial wall thickness and arterial diameter, points to a role for type III procollagen/collagen as a developmental scaffold.

5. An unexpected role for type V collagen

EDS type I/II, the classical type, was more difficult to solve at the molecular level than might have been expected. The most striking alterations were in the large dermal collagen fibrils, composed largely of type I collagen (Vogel et al., 1979). Though many studies pointed to type I collagen genes as the culprit, linkage studies excluded them as candidates (Sokolov et al., 1991; Wordsworth et al., 1991). Two studies shaped the way forward. An X-9 translocation in a woman with EDS type I located the breakpoint on chromosome 9 in COL5A1 (Toriello et al., 1996) while, at the same time, linkage studies and sequencing of both COL5A1 and COL5A2 led to identification of mutations in individuals with EDS type I/II (Burrows et al., 1996; Nicholls et al., 1996; Wenstrup et al., 1996). The majority of mutations identified led to COL5A1 haploinsufficiency with reduction in the amount of type V collagen generated (Schwarze et al., 2000; Wenstrup et al., 2000; Malfait et al., 2005). A recent comprehensive study indicates that most, if not all, individuals with classical EDS have mutation in type V collagen genes (Symoens et al., 2012).

Type V collagen was long considered a minor skin collagen and its role in the extracellular matrix was unclear. It appeared to play a significant role in the unusual organization of collagen fibrils in the cornea (Birk et al., 1990; Linsenmayer et al., 1993; Birk, 2001). The critical role of type V collagen in nucleation of the large fibrils in skin and other tissues became apparent because homozygous COL5A1 knockout mice failed to survive embryogenesis and no large collagen fibrils were assembled in any tissue (Wenstrup et al., 2004).

6. Proteoglycans

Collagens in the extracellular space have a rich social life and interact with many neighbors, among which proteoglycans are particularly notable. Proteoglycans share a common structure in which the core proteins are modified by addition of a tetrasaccharide linker (xylosyl–galactosyl–galactosyl–glucuronosyl) to target serine residues in the peptide backbone. The proteins are then modified by the glycosaminoglycan disaccharides of heparin sulfate (N-acetylgalactosamine–glucuronic acid), chondroitin sulfate (N-acetylgalactosamine–glucuronic acid), and dermatan sulfate (N-acetylgalactosamine–iduronic acid) in which some of the N-acetylgalactosamine residues are sulfated. A progeroid type of EDS was proposed in the 1980s in which features of early aging appeared to result from defective glycosaminoglycan addition to several proteoglycan core proteins (Hernández et al., 1986; Quentin et al., 1990). This phenotype was ultimately shown to result from biallelic mutations in B4GALT7 (Okajima et al., 1999; Faiyaz-Ul-Haque et al., 2004), which encodes glycosyltransferase I, responsible for transfer of a galactose residue to the O-linked xylose on the core protein. The target matrix proteins are probably not limited to decorin and biglycan but the lack of post-translational modification presumably affects their function (Ameve and Young, 2002; Götte and Kresse, 2005; Seidler et al., 2006). Biallelic mutations in B3GALT6, which encodes the third enzyme in the pathway to build the tetrasaccharide linker (galactosyl-transferase II), result in a newly recognized disorder with some features of EDS in addition to progressive contractures, hypotonia, bone fragility and severe kyphoscoliosis (Malfait et al., 2013; Nakajima et al., 2013). To date, the mutations in both of the galactosyl-transferase genes have resulted in reduced enzyme function and at least some of the target proteins become well glycosylated. It is unclear if full loss of function would retain a similar phenotype.

A third EDS phenotype, the “musculocontractural” type (type VIB), results from mutations in CHST14, which encodes dermatan 4-O-sulfotransferase I (Malfait et al., 2010; Miyake et al., 2010). This enzyme sulfates some of the N-acetylgalactosamine entities in the dermatan sulfate side chains. The clinical features include blue sclerae, joint laxity when young with development of flexion contractures of the small joints, progressive and severe kyphoscoliosis, dystrophic scarring, and ocular involvement. In all the identified individuals the mutations are biallelic and predicted to lead to no functional enzyme mostly through mRNA instability.

These three defects in the same pathway have overlapping phenotypes, perhaps related to the extent to which enzyme activity is retained and the place in the pathway. In both B4GALT7 and B3GALT6, the mutations result in partial residual enzyme function and affect the addition of all glycosaminoglycan side chains to protein cores. In contrast, the mutations in CHST14 should leave no active enzyme and result in the side chain able to be built but not sulfated. The pleiotropic effect of the mutations attests to the broad distribution and important functions of the proteoglycan family and the overlap of clinical features with mutations in collagens and collagen modifying genes argues for the interactions of these proteins in the generation of phenotype.

7. Other proteins

The “post-Villefranche” era experienced a proliferation of EDS types distinguished by clinical and genetic grounds but not yet incorporated into a classification scheme. Deficiency of tenascin X was the first non-collagen or collagen-modifier implicated to cause a form of EDS (Burch et al., 1997). This etiology was first identified in a child with 21-hydroxylase deficiency and EDS due to a contiguous gene deletion that included the gene TNXB. Subsequent studies in mouse knock-outs (TNxΔ−/−) suggested that tenascin X likely plays a role in collagen deposition but not synthesis (Mao et al., 2002), which may differ through development (Egging et al., 2006). The exact function of tenascin X in the extracellular matrix has not yet been defined but may result from bridging interactions with fibrillar collagens (types I, III, V), fibril-associated collagens (XII, XIV), decorin and other matrix proteins (Lethias et al., 2006). Clinically, tenascin X deficient EDS is distinguished from classical EDS by autosomal recessive inheritance, normal scarring, profound joint hypermobility and striking bruising (Schalkwijk et al., 2001). The presence of hypermobility in heterozygous carriers of null mutations suggested TNXB as a candidate gene in EDS type III, the hypermobile type. Indeed, about 65% of carrier women had significant joint hypermobility (Zweers et al., 2003), but wider
screening identified mutations in TNXB in only a small subset (2.5%) of persons with hypermobile EDS.

Several additional forms of EDS were solved through astute observation of distinctive clinical features in consanguineous families coupled with genomic investigation. These include the spondylochondrodysplastic form due to mutations in SLC39A13 (Fukada et al., 2008; Giunta et al., 2008), the gene that encodes ZIP13, a zinc transporter that localizes to punctate vesicles. This condition is reminiscent of EDS type VI with additional features of a skeletal dysplasia and hand abnormalities. It has been proposed that loss of function of this transporter leads to sequestration of zinc within the vesicles and deficient Zn availability for a multitude of processes throughout the cell (Jeong et al., 2012). The exact mechanism by which this leads to EDS has yet to be determined. Another recessive form of EDS characterized by kyphoscoliosis, myopathy, and hearing impairment was recently found to result from mutations in FKBP14 (Baumann et al., 2012), a member of the prolyl cis-trans isomerase family. Several mechanism investigations have been performed to date, though it is possible that there is a role for aberrant folding of the fibrillar procollagen chains or of lysyl hydroxylases, based on study of a form of OI due to mutations in a protein family member, FKBP10 (Alany et al., 2010; Schwarze et al., 2013).

8. Unsolved forms of EDS

Though understanding of the molecular and biochemical causes of different types of EDS has greatly expanded, there are several types that have been clinically defined but for which the etiology remains unknown. 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 (periodontal type). 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 yet to be determined. There are a variety of challenges to dissecting the genetic causes of hypermobile EDS, including but not limited to inadequate clinical diagnostic criteria, clinical variability, seeming sex-related penetrance and likely genetic heterogeneity. As these, and previously unrecognized forms of EDS, are solved at the molecular level, it is likely that our understanding of matrix biology will continue to grow.

9. Final considerations

The genomic era promises to shed additional light on unsolved forms of Ehlers–Danlos syndrome. Identifying and understanding the clinical diversity, genetic etiology and pathophysiologic mechanisms of various forms of EDS will undoubtedly continue to expand the last 50 years’ work towards understanding the biology of the extracellular matrix and the role of the constituent macromolecules in human health and disease.

References


