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COMMENTARIES

A New Look at a Rare Old Disease

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Commentary on: Giunta C, Elçioglu NH, Albrecht B, Eich G, Chambaz C, Janecke AR, Yeowell H, Weis M, Eyre DR, Kraenzlin M, Steinmann B. Spondylocheiro dysplastic form of the Ehlers-Danlos syndrome--an autosomal-recessive entity caused by mutations in the zinc transporter gene SLC39A13. *Am J Hum Genet*. 2008 Jun;82(6):1290-305.

As emphasized by B. Korf (1), although current "excitement [in human genetics] has focused on the identification of genes that contribute to the risk of common diseases...much can [still] be learned from the study of rare, 'single-gene' disorders." Among these rare disorders is Ehlers-Danlos syndrome (EDS). One form of EDS (EDS VI) is caused by mutations in a lysyl hydroxylase gene (*PLOD1*) that result in deficient generation of hydroxylysine in collagens. In an interesting new report by Gunta *et al*., (2) individuals with a disorder that clinically resembles EDS VI have mutations not in *PLOD1* but unexpectedly in a gene that encodes a cellular zinc transporter. The resultant high concentrations of zinc can inhibit lysyl hydroxylase and other collagen hydroxylase activities. Patients with the disorder have phenotypes that include a skeletal dysplasia. This study beautifully illustrates how mutations in distinct genetic pathways can lead to similar, although not identical, clinical and biochemical phenotypes.

EDS comprises a group of inherited disorders of connective tissue that are clinically, biochemically and genetically heterogeneous (3). In general, the major manifestations of EDS affect "soft" connective tissues and result in such abnormalities as hypermobile joints, hyperextensible and fragile skin, and rupture of blood vessels and internal organs. In one form of EDS, EDS VI, kyphoscoliosis is a prominent feature. Nearly four decades ago, a family was described with two sisters who had marked muscular hypotonia as infants and subsequently developed severe kyphoscoliosis, hyperextensible and subluxed joints, thin, velvety skin and abnormal scars (4). Their mother and father and an older sister were clinically unaffected. Amino acid analysis of skin collagen from the two affected girls was unremarkable except for a marked decrease in the content of 5-hydroxylysine (Hyl) to approximately 5-10% of controls. It was known at the time these observations were made that 4-hydroxyproline (4-Hyp) and Hyl were not as such incorporated into precursor procollagen (also termed "protocollagen") chains but that specific Pro and Lys residues were enzymatically hydroxylated, catalyzed by specific prolyl and lysyl hydroxylases, in growing nascent chains before formation of triple helical molecules. From studies in rat and chick skin fibroblasts it was known that the Pro and Lys hydroxylases have similar substrate requirements: protocollagen, 2-oxyglutarate (α-ketoglutarate), ascorbic acid, ferrous iron and molecular oxygen; for each Pro or Lys residue hydroxylated, one molecule of 2-oxyglutarate is converted to succinate and CO_2. It is pertinent to note with regard to subsequent discussion that the underhydroxylated collagen used as substrate is obtained by labeling protocollagen in chick embryo tibiae incubated with α,α'-dipyridyl, an iron chelator. Lys-hydroxylase assays in fibroblasts from affected members of the family described above showed decreased activity (10% control). Some time later, after

the collagen hydroxylase genes were cloned, it was shown that in the form of EDS (EDS VI) characterized by decreased Hyl content of skin and other type I collagens the disorder was caused by mutations in one of three Lys hydroxylase genes, *PLOD1*, that encodes LH1 (EC 1.14.11.4; procollagen-lysine, 2-oxoglutarate 5-dioxygenase) that normally hydroxylates Lys residues in the -Y- position of the -Gly-X-Y- tripeptide repeat in the collagen triple helical domain (3;6). Hyl functions in inter- and intramolecular pyridinium crosslinking of collagen and as a site for O-linked glycosylation. Analysis of urinary ratios of lysyl pyridinoline (LP)/hydroxylysyl (HP) pyridinoline crosslinks is a useful procedure for screening individuals with possible EDS VI (7). In 2003, it was shown that mutations in *PLOD2* (which encodes LH2) cause Bruck syndrome, an autosomal recessive disorder characterized by short stature, osteoporosis and joint contractures (8). LH2 hydroxylates lysyl residues in the telopeptide (not the triple helical) domain of type I collagen. As predicted, levels of pyridinoline crosslinks are decreased in bone in patients with Bruck syndrome; in contrast, levels of LH2 in fibroblasts cultured from the dermis of patients with systemic sclerosis (scleroderma) are higher than controls and pyridinoline crosslinks are increased in collagen deposited by the scleroderma fibroblasts.

A new and interesting twist in the collagen hydroxylation story is revealed in the study by Giunta *et al*. (2). These authors investigated six patients from two consanguineous families (one from North-Western Iraq and the other from South-Eastern Turkey), who had features of EDS VI (joint hypermobility, hyperextensible, thin, bruisable skin) as well as a skeletal dysplasia. The latter comprised platyspondyly, osteopenia, short stature and widened metaphyses, tapered fingers and a tendency to develop contractures of small joints. Because of the spine (spondylo) and hand (cheiro) features they termed this variant the spondylocheiro dysplastic form of EDS (SCD-EDS). In control adults and children, the LP/HP ratio is ~ 0.2 (0.12-0.25) whereas in patients with EDS VI and LH1 deficiency, the ratio is ~ 6 (> 4.0). In the affected subjects described by Giunta *et al*., the ratios of urinary LP/HP were all higher than controls (> 0.6, mean ~ 1) but not at the level seen in EDS VI with *PLOD1* mutations. Furthermore, they found, using purified urinary N-telopeptide of type I collagen and crosslinked C-telopeptide from type II collagen, that both collagens were underhydroxylated at the triple helical crosslinking sites. These results were consistent with analysis of skin biopsies and of collagens secreted by cultured fibroblasts. Using mass spectroscopy they found a generally decreased content of Hyl along the length of the whole α1 and α2 chains of type I collagen and surprisingly a modest decrease in 4-Pro content as well. Even the single Pro residue at residue 986 in α1(I) chains was underhydroxylated in the 3-position (3-Hyp) compared to controls, although not to the extent found in forms of osteogenesis imperfecta associated with deficiencies of CRTAP or prolyl 3-hydroxylase (see IBMS BoneKEy. 2006 Nov;3(11):10-13) (9;10). Clearly, deficiency of LH1 alone could not account for these observations and the possibilities of mutations in *PLOD1*, *PLOD2* and *PLOD3* were further excluded by detailed analysis. Activities of lysyl and prolyl hydroxylases measured in cell extracts in the presence of optimal concentrations of co-factors were also normal. The authors therefore postulated that concentrations of one of the several substrates common to all of the collagen hydroxylases might somehow be altered as an explanation for the biochemical findings. They therefore performed a genome-wide SNP scan and identified a region on chromosome 11p11.2-q13 and then fine-mapped it to an interval between markers D1S1779 and D11S4191, with a combined maximal LOD score of 5.3. Although several potential candidates were in this region, sequencing at the genomic and cDNA level revealed a mutation (a 9 bp in-frame deletion) only in the gene, *SLC39A13*. *SLC39A13* encodes the zinc transporter SLC39A13 (ZIP13), a member of the LIV-1 subfamily of Zip zinc transporters, eight trans-membrane domain proteins that can transport zinc from the extracellular

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space into cells or from lumens of organelles into the cytoplasmic compartment (11).

Over the past 50 years, studies of zinc homeostasis and metabolism focused on the role of metallothionein (12;13), an unusual protein discovered in Bert Vallee's laboratory, that tightly binds zinc as well as iron, cadmium and copper. The biological roles of zinc in catalytic functions in enzymes and structural functions in zinc finger proteins had been well documented (14). As reviewed elegantly by Eide (11) (Fig. 1),

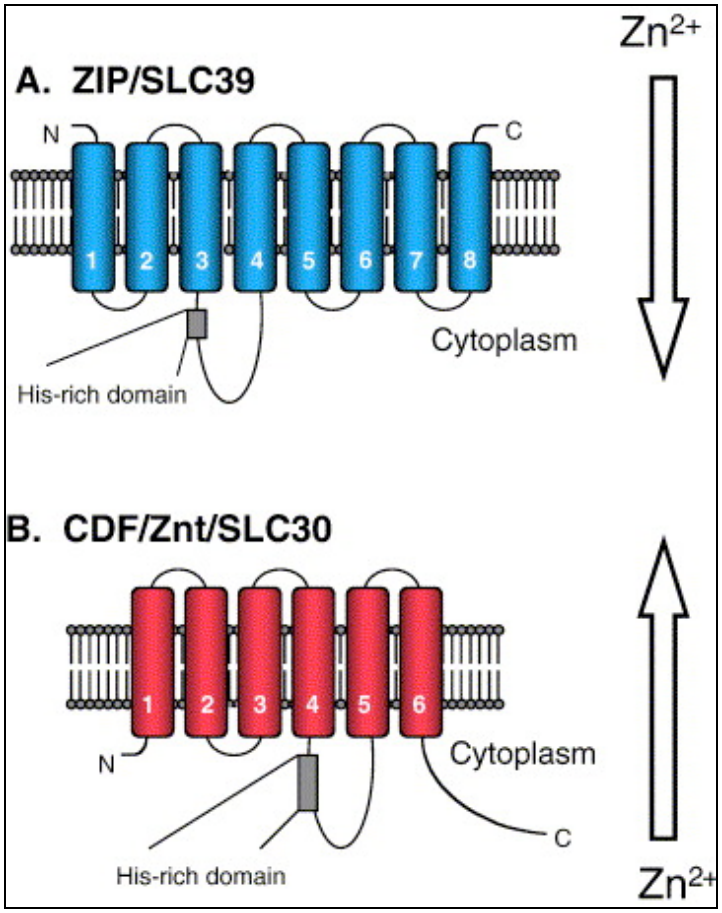

Fig 1. Model of the two types of metal ion transporters described by Eide (11). A. Members of the Zip family of transporters, with eight predicted transmembrane domains (blue), transport zinc and other metal ions from the ECF into the cell cytoplasm or from organelles such as the ER and Golgi into the cytoplasm. B. The CDF/Znt family transporters, with six predicted transmembrane domains (red) move metal ion substrates in the opposite direction, *i.e*., from the cytoplasm into intracellular organelles or to the outside of the cell. In the two families with SCD-EDS (2), the mutation is in the gene that encodes Zip13, *SLC39A13*. It is proposed that defective function of Zip13 results in high concentrations of zinc within the ER that inhibit the activity of lysyl- and prolyl-3 and prolyl-4 hydroxylases. (Reproduced from Eide (11), with permission from Elsevier).

over the past decade, understanding the cell biology of zinc changed dramatically with the discovery of the ZIP and CDF/Znt families of zinc cellular transporters. The ZIP family (Zrt-, Irt-like Proteins) is named after the Zert1 protein in yeast and the Irt1 protein in *Arabodopsis*. ZIPs, most with eight predicted transmembrane domains, transport zinc and other metal ion substrates from the ECF or lumen of intracellular organelles into the cytoplasm. CDF ("cation diffusion facilitator") proteins, most with six predicted transmembrane domains, transport zinc and other metal ion substrates from the cytoplasm into the lumen of intracellular organelles or to the ECF. Giunta *et al*. (2)

note that *SLC39A13* (ZIP13) and *SLC39A7* (ZIP7) are phylogenetically closely related transporters based on sequence alignments and that SLC39A7 localizes to ER and Golgi membranes and transports zinc to the cytosol. They postulate that the deletion in the highly conserved third transmembrane domain of *SLC39A13* impairs conformation and function and would result in increased concentration of zinc in the ER. Furthermore, they "suspect that an increased concentration of Zn^{2+} in the ER competes with Fe^{2+} for binding to lysyl hydroxylase, prolyl 4-hydroxylase and prolyl 3-hydroxylase, thus impairing hydroxylation of lysyl and prolyl residues." This suspicion is based on earlier studies of inhibition kinetics showing that zinc is an effective competitor for iron with respect to both PH1 and LH1 (15).

In the initial report of the findings in patients with what was later termed EDS VI, it was noted that the content of Hyl relative to Hyp in bone collagen was considerably higher than in collagen from skin (4). The kyphoscoliosis and presumable joint hypermobility and subluxations are probably accounted for by defective lysyl hydroxylation in collagens in soft tissues (ligaments, tendons and joint capsules) rather than that in bone. The patients with SCD-EDS did not have kyphoscoliosis in contrast to those with EDS VI, although they did have platyspondyly and 5/6 had vertebral osteopenia. In 2/5 patients with osteopenia who had DXA determinations, both children, total DXA T scores were low, -5.8 and - 3.8. The one adult among the six with *SLC39A13* mutations had no obvious osteopenia and a T score of + 1.1. Thus, whether or not the *SLC39A13* mutation has a major role in determining the function of bone matrix *per se* has yet to be established.

More work needs to be done to prove the hypotheses suggested by the authors. Nevertheless, their observations beautifully demonstrate the value of careful clinical observations of patients in conjunction with up-to-date biochemical and genetic technology. They further illustrate the importance of the continuous and long term dedication of scientists such as David Eyre, Heather Yeowell and Beat Steinmann, among the co-authors on the paper by Giupta *et al*., in studies of a rare hereditary disease.

Conflict of Interest: None reported.

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