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Washington, D. C.

1973

Reprinted from the Transactions of the Association of American Physicians
vol. lxxxvi, 1973

PROCOLLAGEN PEPTIDASE DEFICIENCY IN A FORM OF THE EHLERS-DANLOS SYNDROME

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The Ehlers-Danlos syndrome is a heterogeneous group of disorders which are characterized by stretchable, fragile skin and hypermobile joints. McKusick has classified this group of disorders into seven subgroups[1] (Table 1). Four types are inherited in an autosomal dominant pattern and are distinguished by the clinical distribution and severity of the disease. A fifth subtype is inherited in an X-linked recessive pattern. The sixth subtype has hydroxylysine deficient collagen, due to a deficiency of the enzyme lysyl hydroxylase and is inherited in an autosomal recessive pattern.[2-4]

We are reporting in this paper on a seventh subtype of the Ehlers-Danlos syndrome found in three Caucasian females ages 3, 16 and 32 years. Similar clinical and biochemical findings were observed in all three patients. In the past this condition has been called arthrochalasis multiplex congenita.[1]

TABLE 1. HETEROGENEITY IN THE EHLERS-DANLOS SYNDROME

1. E-D Syndrome Type I	- Gravis Type
2. E-D Syndrome Type II	- Mitis Type
3. E-D Syndrome Type III	- Benign Hypermobile Type
4. E-D Syndrome Type IV	- Ecchymotic, Arterial or Sachs Type
5. E-D Syndrome Type V	- X-Linked Recessive Form
6. E-D Syndrome Type VI	- Ocular Type, Lysyl Hydroxylase Deficency
7. E-D Syndrome Type VII	- Arthrochalasis Multiplex Congenita and Procollagen Peptidase Deficiency

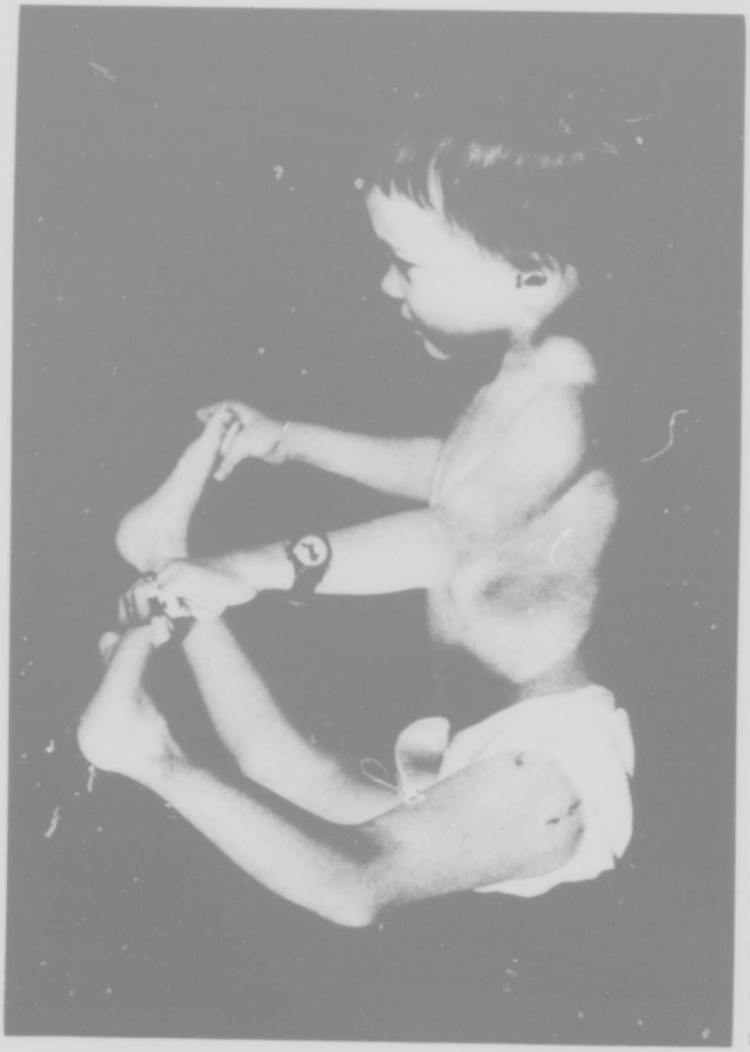

FIG. 1. One of the patients with Ehlers-Danlos syndrome type VII demonstrating the marked joint hypermobility and joint subluxations (Johns Hopkins Hospital photo.)

The patients were all the product of breech delivery with bilateral congenital hip dislocations recognized shortly after birth. The most striking feature of the disease is the marked generalized joint hypermobility and multiple joint subluxations (Fig. 1). All three patients had repeated subluxations of their hips, patellas, radial heads and feet (despite corrective surgery) and scoliosis. At the time of surgery for her hip dislocations, patient #1 was found to have both round ligaments rent in two and extremely fragile tissues with dissection easily performed manually. All three patients had a similar facies with a scooped out mid-facies, epicanthal folds and hypertelorism. They all had stretchable thin velvety skin. In two of the patients, wounds healed normally, while in the third,

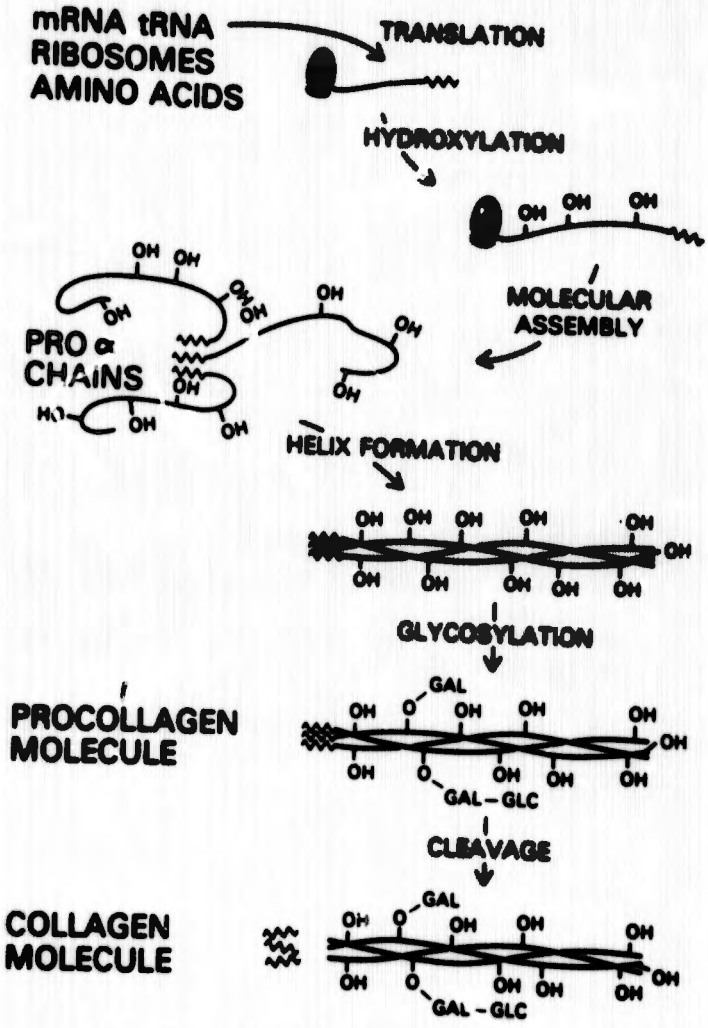

FIG. 2. A scheme of collagen biosynthesis.

wounds healed with the typical atrophic scars of the Ehlers-Danlos syndrome. All of the patients are short statured. The two older ones have heights of 4′ 10″ and 4′ 2″ and the third is below the third percentile.

The clinical features which distinguish this entity from other forms of the Ehlers-Danlos syndrome are marked joint hypermobility, multiple joint subluxations and short stature.

Studies on the biosynthesis of collagen are summarized in Figure 2. Precursor polypeptide chains (the proα chains) are synthesized on polyribosomes and three of the proα chains assemble into the procollagen molecule.[5-8] Hydroxylation of certain prolines and lysines and glycosylation of certain hydroxylysines occur during procollagen synthesis. Procollagen is converted to the collagen molecule by cleavage of the extra amino terminal peptides from the proα chains by a specific enzyme procollagen peptidase.[9,10] Following cleavage collagen molecules form fibers and undergo crosslinking.[11]

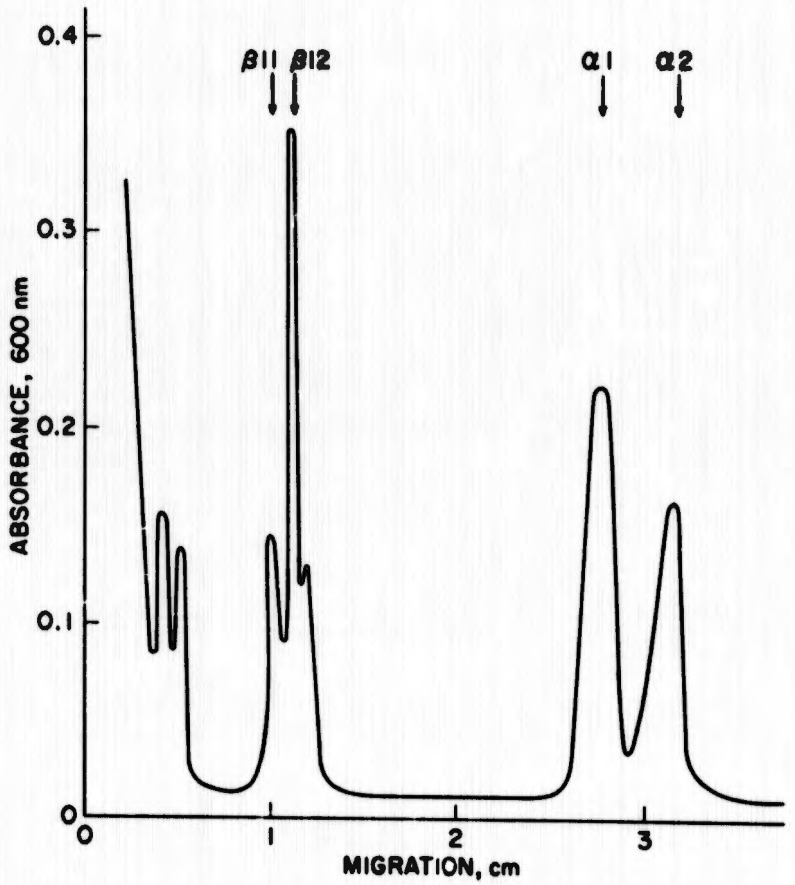

FIG. 3a. Densitometric scan of normal human collagen on 5% acrylamide SDS gel electrophoresis.

The characteristics of procollagen, due to the extra peptide, which allow it to be distinguished from collagen are a higher molecular weight, the presence of cysteine, an amino acid not present in collagen, an increase in the acidic amino acids serine, aspartic acid and glutamic acid and a relative decrease in glycine, hydroxyproline and hydroxylysine residues.[12,13] These differences allow procollagen to be distinguished from collagen by acrylamide gel electrophoresis, molecular sieve and ion exchange chromatography and amino acid analysis.

SDS acrylamide gel electrophoresis[14] was initially used to examine the collagen α chains and polymeric components in extracts of patients' and control tissues. Extracts of control tissues and tissues from patients with other forms of the Ehlers-Danlos syndrome were found to contain the expected $\alpha 1$ and $\alpha 2$ chains and crosslinked components (Fig. 3a). Extracts of tissues from these three patients had additional bands of higher molec-

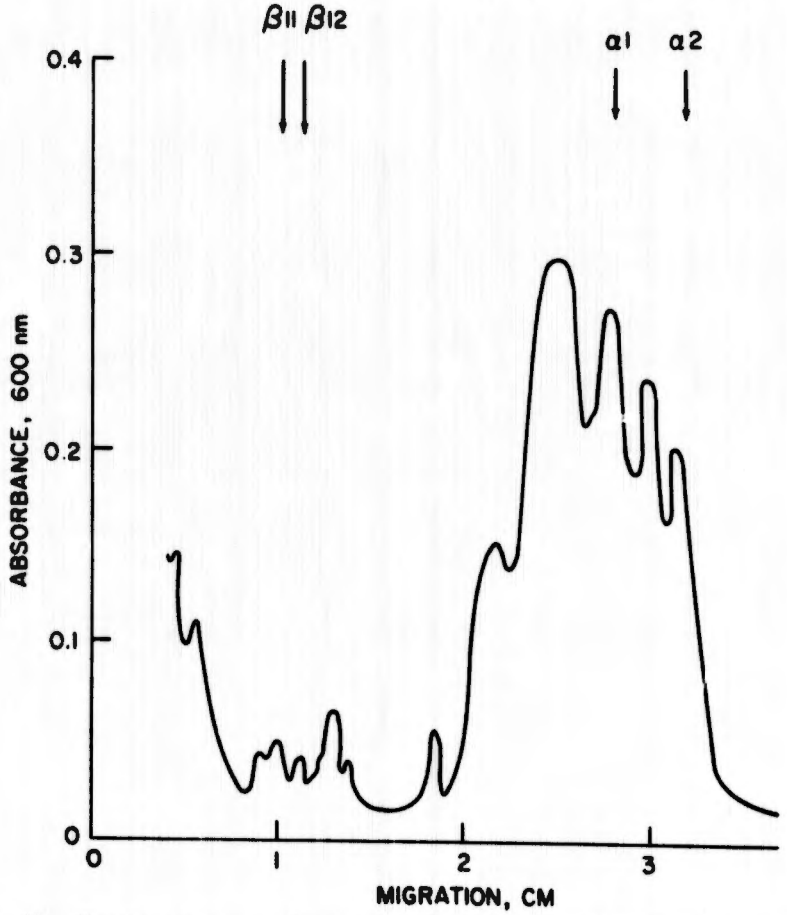

FIG. 3b. Densitometric scan of patient's collagen on 5% acrylamide SDS gel electrophoresis demonstrating the presence of proα chains.

ular weight than α chains which comigrated with authentic proα chains on acrylamide gel electrophoresis (Fig. 3b). On molecular sieve the α chains from the extracts of patients' tissues eluted ahead of normal collagen α chains, indicating a higher molecular weight. Sufficient material was obtained by molecular sieving for amino acid analyses. The amino acid composition of these α chains from the patients' extracts indicated a composition similar to procollagen with 5–7 half-cysteine residues/thousand. Thus, extracts of the skin and tendon of these patients contain procollagen which is not found in appreciable amounts in extracts of normal tissues or tissues from patients with other forms of the Ehlers-Danlos syndrome.

The activity of the enzyme procollagen peptidase was measured in cell culture media by determining the amount of procollagen substrate converted to collagen.[9] The procollagen peptidase activity in media from fibroblast cultures from the patients' skin contained 10 to 40% of the activity of control fibroblast cultures. Confluent cell cultures of skin fibroblasts from the patients contained normal activity of certain other enzymes (lysyl hydroxylase, prolyl hydroxylase and lysyl oxidase) which modify collagen.

We attribute the accumulation of procollagen in the tissues of these patients to the low level of procollagen peptidase. A defect in the conversion of procollagen to collagen has been found previously in cattle and sheep.[13,14]

In the dermatosparaxic cattle, in contrast to these patients, the most prominent clinical feature is easily torn skin, although a generalized connective tissue defect is present.[12] The phenotypic differences between the human disease may be due to an abnormality in a different isoenzyme of procollagen peptidase, with the defect in the animals affecting the isoenzyme most prominent in skin and that in the three human patients affecting an isoenzyme most prominent in tendon and ligaments. Further evidence for the presence of either several different enzymes or isoenzymes of procollagen peptidase is the presence of normal bone x-rays and no fracture history in these patients indicating that the defect is not expressed in bone. There is no indication of skeletal abnormalities in the dermatosparaxic animals. If this reasoning is correct, it is likely that there will be other human diseases, with different phenotypic features, attributable to a defect in procollagen peptidase.

Phenotypically these patients resemble the patients with lysyl hydroxylase deficiency although the basic biochemical defect is quite different. Preliminary studies indicate that the extractability of collagen from the tissues of these patients is increased; however, it is likely that both defects interfere with the crosslinking of collagen.[11]

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DISCUSSION

DR. BENJAMIN ALEXANDER (New York): For several years our laboratories have been very interested in the effects of collagen and various chemical alterations of the collagen molecule that are discussed in this report. Hemorrhagic phenomena are known to occur in this syndrome, and I am curious to know whether the procollagen demonstrable in the skin of these patients can agglutinate platelets such as is usually observed with normal collagen, or are the effects of the latter indeed indirectly referable to the procollagen peptidase?

DR. LICHTENSTEIN: I don't have any data for that subject. The one disorder where you really would expect an effect on platelet agglutination is in lysine hydroxylase deficiency, but we can't demonstrate the defect in that disorder. We have not done any studies for agglutination of these patients with procollagen peptidase deficiency, however.