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Autosomal Recessive Cutis Laxa Syndrome

A Case Report

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Congenital cutis laxa (CCL) is a rare, genetically heterogeneous connective tissue disorder, manifested by loose, hanging skin, giving the appearance of premature aging. We report a 6-year-old female child with autosomal recessive CCL type III, to assess possible correlations between clinical, ultrastructural, cellular and biochemical features. Morphological aberrations of the elastic and collagen tissue, increased collagen I mRNA expression associated with increased protein synthesis and increased collagenase gene expression of the cutis laxa fibroblasts could be established. Our results suggest that CCL is not only a disease of the elastic fibers of the connective tissue but also of the collagen fibers, with a central role of the fibroblast.
Key words: collagen I; collagenase; elastic tissue.

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Congenital cutis laxa (CCL) is a rare inherited disorder of connective tissue, manifested by loose, hanging skin, giving the appearance of premature aging. These changes may be present at birth or may develop during infancy. At least three distinct patterns of inheritance are clearly established for this syndrome: autosomal dominant CCL, autosomal recessive CCL and an X-linked dominant CCL (1, 2). Patients with an X-linked trait were diagnosed as in fact affected with occipital horn syndrome, formerly Ehlers-Danlos syndrome type IX, a disorder of copper transport (3). Concerning the dominant forms, the changes are mild and largely confined to the skin. The course is mostly benign. In contrast, in the recessive form of CCL, which is usually present at birth, the skin lesions are severe and pulmonary and cardiovascular involvement is common. In this type of the condition, these complications often lead to death during childhood.

The ultrastructural aberrations of the elastic tissue allow to distinguish several types (I, II, and III) according to various depositions of elastic fiber components (4). There are, however, still unclassified cases with widespread aberrations of elastic tissue. Irregularities in collagen morphology are occasionally reported in CLL with little evidence of abnormal collagen metabolism.

We report a female child with autosomal recessive CCL type III to assess possible correlations between clinical, ultrastructural, cellular and biochemical features.
Morphological aberrations of the elastic and collagen tissues, increased collagen I mRNA expression associated with increased protein synthesis and increased collagenase gene expression could be established.

CASE REPORT

Case history

The patient, a 6-year-old female child, was first seen in our clinic when she was 4 months old. The most recent examination was performed in 1995. At birth it was noted that she had lax skin involving the whole body. In early infancy she suffered from repeated chest infections.

At the age of 6 years the skin features had progressed. The clinical picture was characterized by inelastic, loose and pendulous skin, which produced a wrinkled, prematurely aged, bloodhound-like appearance (Figs. 1a, b). The skin was warm and velvety. Wound healing was without problems. There were no hernias, no articular hypermobility and no arachnodactyly. The voice was husky. Laryngoscopy showed the vocal cords to be markedly elongated and slack. A pansystolic murmur was heard in the precordial area. Other physical findings were unchanged and the development was excellent.

Family history

No other members of the family were known to have cutaneous laxity. There was no consanguinity. Her brother and her sister were healthy.

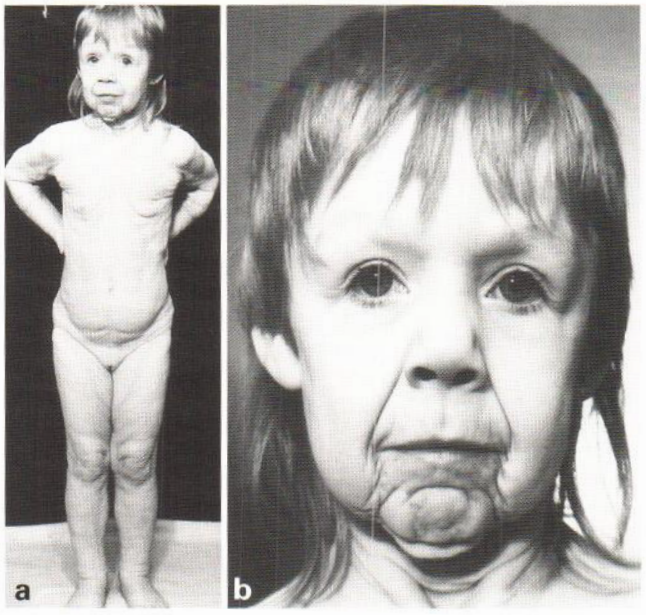

Fig. 1a,b. Our patient at the age of 6 years. Her skin hung in loose folds, giving her the appearance of an old woman. Her nose was hooked, with everted nostrils.

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Laboratory results

Blood and urine routine chemistry, serum electrophoresis, serum copper and ceruloplasmin, elastase inhibitors like α_1-antitrypsin and α_2-macroglobulin were in normal ranges. Bleeding and coagulation time were normal.

The chest X-ray revealed a dilatation of the ascending aorta. Ectasia of the ascending aorta associated with stenosis of the aortic valve could be diagnosed. The X-ray of the left hand established normal ossification responding to the patient's age group. By abdominal sonography no organ aberrations could be found. The caryotype was normal: 46 XX.

Light microscopy

Skin biopsies were obtained at the age of 6 years and processed routinely for light microscopy, performing hematoxylin-eosin, Verhoeff van Gieson, PAS and Weigert's elastic fibers stains.

Light microscopy showed the epidermis with normal structure, but decreased rete ridges when stained with hematoxylin-eosin (Fig. 2). The corium was reduced. Large septations of conjunctive tissue were expanding into the superior fatty tissue. The collagen bundles were stained homogeneously and basophilic. Using Verhoeff van Gieson stain (results not shown) enlargement and homogeneity of collagen fibers could be observed.

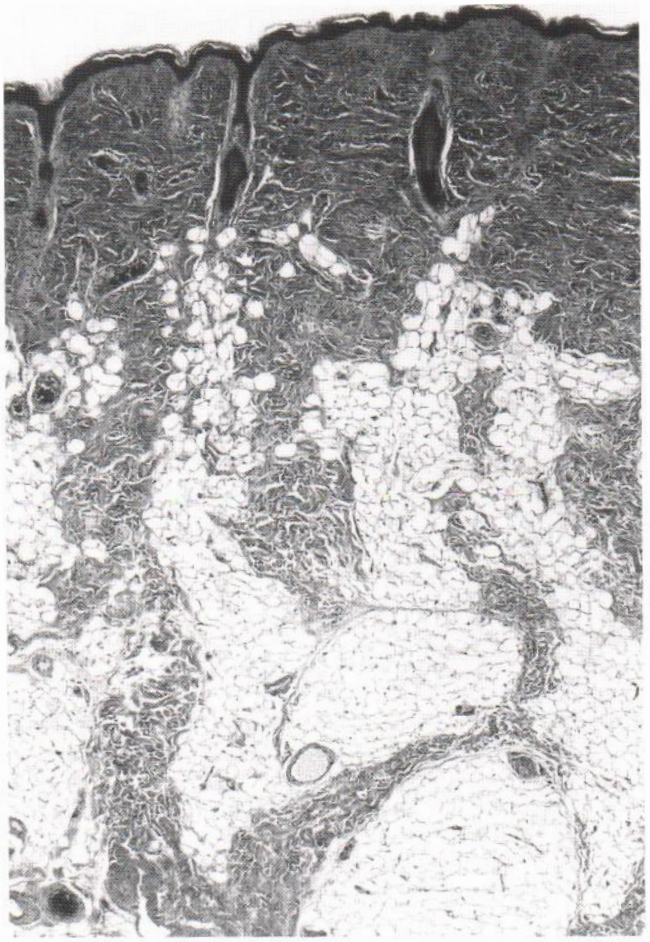

Fig. 2. Morphology of cutis laxa skin (hematoxylin-eosin stains). The epidermis represents normal structure with decreased rete ridges. The corium is reduced. Large septations of conjunctive tissue are expanding into the superior fatty tissue (×4,5).

The Weigert's elastic fibers technique showed a rarefication of elastic fibers with granular degeneration in the mid- and lower reticular dermis. Many of the fibers were short, fragmented and plump. Some of the elastic fibers were thickened in their midportion. Intact elastic fibers could not be observed. Dustlike orceinophilic granules were scattered in the dermis (results not shown).

Electron microscopy

Skin biopsies were prefixed for 2 h at room temperature in 3% glutaraldehyde, buffered with 0.1 M cacodylate, pH 7.4, and partially oxidized by adding hydrogen peroxide. The samples were cut into pieces of ca. 1 mm^3 and further fixed in 3% glutaraldehyde without hydrogen peroxide. After washing with buffer, specimens were postfixed for 1 h at 4°C in 1% osmium tetraoxide in cacodylate buffer. After being washed in water, the specimens were dehydrated through graded ethanol solutions, transferred into propylene oxide, and embedded in Epoxy resin (glycidether 100, formerly Epon 812). Semithin sections and ultrathin sections were cut with an ultramicrotome Reichert OmU3. Semithin sections were treated with methylene blue. Ultrathin sections were treated with uranyl acetate and lead citrate and examined with an electron microscope (Philips EM 400)(5).

Ultrastructurally (Fig. 3), rare and fragmented elastic fibers are situated between dense bundles of collagen fibers. Elastic fibers mainly consist of microfibrils. Few and small elastin deposits are clumped within the scaffold of elastotubules. The extreme decrease of elastin is especially pronounced in the mid- and lower dermis. Elastin and elastotubuli, however, are closely associated and not deposited independently, as in the case of the autosomal recessive form of CCL type II. The ultrastructural features are typical for the autosomal recessive type III.

Collagen I measurement

Skin fibroblast cultures were routinely obtained and maintained at 37 °C in a humidified 10% CO_2 air atmosphere in modified Eagle's medium containing HEPES buffer (25 mM, pH 7.6), 100 U/ml penicillin, 100 µg/ml streptomycin and 10% heat-inactivated fetal calf serum.

The determination of collagen type I synthesis was performed by a modification of the method of Sykes et al. (6). Fibroblasts were seeded in 6-well plates (10,000 cells/well) in DMEM supplemented with 10% FCS. After cultures reached confluence, the medium was removed and the monolayer was rinsed twice with PBS. In each well 2 ml serum-free DMEM supplemented with β-aminopropionitrile (100 µg/ml) was added. Afterwards the cultures were pulsed with L-[U-^{14}C] Proline, 1 µCi/well (Amersham) for 48 h. At the end of each experiment the medium was removed, transferred to a new tube and 1 ml of a pepsin solution (2 mg/ml 0.5 M acetic acid) was added. The digestion continued for 16 h at 20 °C. The clear solution was neutralised with 2.5 N NaOH. The sample was mixed with sample buffer (1:1; v/v) and incubated for 5 min at 100 °C before application to SDS-PAGE. The was performed in a Bio-Rad Mini Protean Chamber® using a gradient gel (4–15% acrylamide concentration). The determination

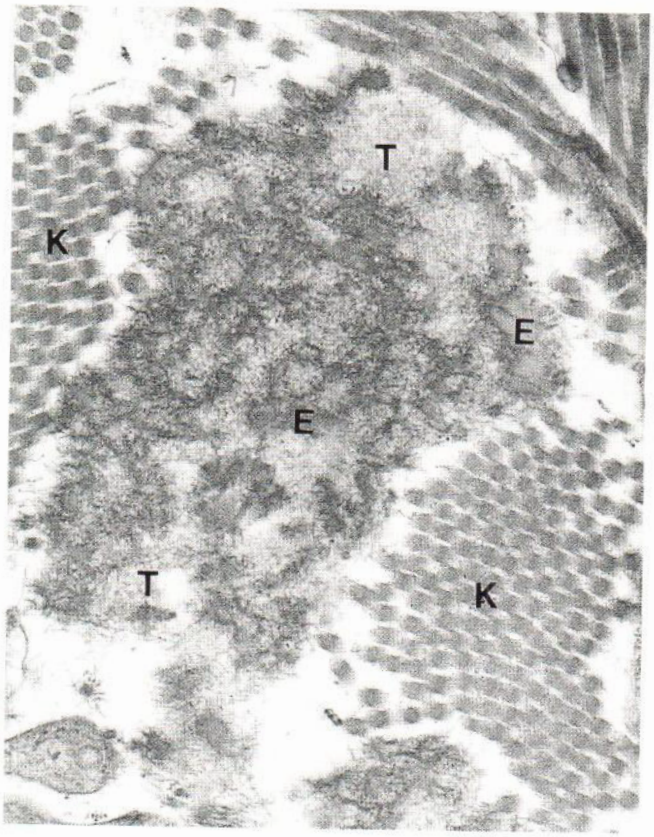

Fig. 3. Morphology of cutis laxa skin (electron microscopy). Elastic fibers are reduced in size and situated between large and dense collagen bundles (*K*). There is a large amount of microfibrillar component (elastotubuli *T*), with a paucity of elastin components (*E*) within the microfibrillar network (× 30 000).

of [^{14}C]proline-labelled collagen type I was performed using the Phospholmager®(Molecular Dynamics). Increased collagen I protein synthesis of cutis laxa skin fibroblasts in comparison to control fibroblasts of normal persons could be determined (Fig. 4).

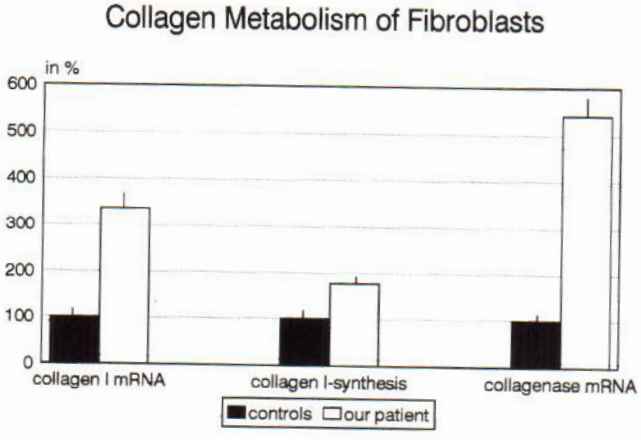

Fig. 4. Collagen metabolism in fibroblast strain of our patient with CCL. Determination of collagen I mRNA and collagenase mRNA by Northern blotting, determination of collagen I synthesis by interrupted gel electrophoresis. Results are shown as mean values of triplicates ± standard deviations in comparison to fibroblasts of normal controls ($n = 3$).

Detection of collagen I mRNA and collagenase mRNA by Northern analysis

For the Northern blot analysis, total RNA was harvested from the cell cultures of fibroblasts according to the ULTRASPEC® isolating system (Biotex Incorporation). The RNA was dissolved in 10 × MOPS and denatured by heating at 65 °C for 10 min. After agarose gel electrophoresis (1% agarose, electrophoresis buffer: MOPS; 60 V, 4 h) the gel was washed with DEPC-H_2O. The transfer to positively charged nylon membranes was done by vacuum blotting with 20 × SSC for 1 h. The membranes were dried, incubated for 15 min at 120 °C and exposed to UV-light for 45 s. The membrane was prehybridized for 1 h with hering sperma DNA (100 µl/ml, H-^{Mix}Northern). The hybridization was performed at 60–70 °C with the appropriate [^{32}P]-labeled RNA-probe overnight. The human α1(I) collagen and collagenase probes were a kind gift of T.Krieg (University of Cologne, Germany). The quantification of mRNA steady state level was performed using the Phospholmager®.

We found increased levels of mRNA expression for the collagen I gene and for the collagenase gene (Fig. 4).

DISCUSSION

Characterization of the rare hereditary cutis laxa syndromes has been largely restricted to clinical observations (1, 2, 7), and occasional histologic and ultrastructural descriptions. The first case was described in 1885 (8). Concerning the congenital forms of cutis laxa there are widespread aberrations in quantity and morphology as well as organization of elastic fibers. In the dermis of our patient we could find rare and small elastic fibers with ultrastructural aberrations in their organization (Fig. 3). The variations in morphology of elastic fibers among skin samples from different patients with cutis laxa suggest that the biochemical and molecular genetic basis of the disorder is heterogeneous. It is possible that cutis laxa could result from mutations that affect the synthesis, stabilization, or degradation of elastic fibers. Defects may involve genes encoding elastin, elastases, elastase inhibitors and microfibrillar components. Various authors have described a reduced elastin gene expression in cutis laxa fibroblast strains earlier (9, 10). Up to now the basic defects leading to loss of elastin, elastic fibers and/or their function, respectively, are still unknown (11). Recently, posttranscriptional defects in elastin synthesis were suggested (12).

In patients with severe forms of CCL, irregularities in collagen morphology with little evidence of abnormal collagen metabolism could also be found (13). Small and separated collagen bundles, fibrils of varied diameters and loosely aggregated fibrils have been reported in cutis laxa skin (13). However, collagen metabolism in CCL has not been sufficiently studied and is poorly understood. There exist contradictory results about the synthesis of collagen I and III (13–16) in cutis laxa fibroblast strains. Increased synthesis of collagen VI has been observed in fibroblasts of an affected individual (17).

In our patient we found compact collagen bundles in the dermis. The ultrastructure of collagen fibrils, however, was normal. Furthermore, we could identify an increased collagen I mRNA expression associated with increased collagen I protein synthesis (Fig. 4), observed, usually, in patients with progressive scleroderma. To achieve a better understanding of

collagen metabolism in cutis laxa we investigated the gene expression of not only collagen but also collagenase, a metalloprotease with the unique ability to initiate collagen degradation, in skin fibroblast cultures from our patient. Interestingly, the expression of collagenase mRNA was also increased (Fig. 4) and may be related in the same way to the structural abnormality of dermal connective tissue in cutis laxa. Increased collagenase gene expression has also been described in senescent fibroblast strains, but in this case in association with decreased synthesis of the protein (18). Hatamochi et al. (14) obtained data in fibroblasts from patients with cutis laxa indicating that a reduced elastin expression is associated with increased collagenase expression. These fibroblasts appear to offer a unique model for the study of mutual control in the metabolism of different components of connective tissue.

Our results underline that CCL is not only a disease of the elastic fibers of the connective tissue but also shows abnormalities of collagen metabolism with a central role of the fibroblasts synthesizing all the components of the connective tissue.The elasticity of the skin depends on qualitative intact elastic fibers forming an ensemble that has physiological interactions with numerous other components of the connective tissue.

A causal therapy of CCL is not known. Early diagnosis is important for therapeutical management before damage of organs can occur. Plastic surgical procedures can be aesthetically and psychologically beneficial in children with CLL, though the results may be transient and therefore demand several repetitions (19). Genetic counselling is another important consequence of the correct diagnosis, which is still only achieved by a common approach combining clinical and morphological data.

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