Cutis Laxa Arising from Frameshift Mutations in Exon 30 of the Elastin Gene (ELN)*

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Congenital cutis laxa, a rare syndrome with marked skin laxity and pulmonary and cardiovascular compromise, is due to defective elastic fiber formation. In several cases, skin fibroblast tropoelastin production is markedly reduced yet reversed in vitro by transforming growth factor-β treatment. We previously showed that this reversal was due to elastin mRNA stabilization in one cell strain, and here this behavior was confirmed in skin fibroblasts from two generations of a second family. cDNA sequencing and heteroduplex analysis of elastin gene transcripts from three fibroblast strains in two kindreds now identify two frameshift mutations (2012G and 2039AC) in elastin gene exon 30, thus leading to missense C termini. No other mutations were present in the ELN cDNA sequences of all three affected individuals. Transcripts from both alleles in each kindred were unstable and responsive to transforming growth factor-β. Exons 22, 23, 26A, and 32 were always absent. Since exon 30 underwent alternative splicing in fibroblasts, we speculate that a differential splicing pattern could conceivably lead to phenotypic rescue. These two dominant-acting, apparently de novo mutations in the elastin gene appear to be responsible for qualitative and quantitative defects in elastin, resulting in the cutis laxa phenotype.

Elastic fibers are the extracellular matrix structures responsible for the properties of resilience and elastic recoil in all elastic tissues (1, 2). There are two morphological elements in elastic fibers: the microfibrillar component and the amorphous component. The microfibrillar component is made up of 10–12-nm microfibrils that are composed of at least seven different glycoproteins, including the two genetically distinct fibrillins, whose genes are the loci for Marfan’s syndrome and congenital contractual arachnodactyly (3, 4). Elastin is also present in the amorphous component as a cross-linked complex of hydrophobic proteins synthesized from the single copy, multi-exon elastin gene (ELN) by extensive alternate usage of several exons (1, 5–7).

There are several inherited disorders characterized by aberrant elastin synthesis or degradation. Abnormal elastic fibers are seen in Menke’s syndrome due to altered copper transport resulting in decreased activity of lysyl oxidase (8), whereas elastic fibers are prematurely degraded due to unregulated elastase activity in patients with α1-antitrypsin deficiency (9) and some forms of atrophoderma (10). Pseudoxanithoma elasticum and Buschke-Ollendorff syndrome are examples of heritable skin diseases in which increased deposition of cutaneous or vascular elastin has been demonstrated (11, 12). In contrast, decreased or aberrant deposition of elastic fibers in certain tissues is characteristic of Marfan’s syndrome (13), supravalvular aortic stenosis (SVAS) (14–16), and cutis laxa (6, 17).

In some diseases of elastic tissue, mutations in genes for structural proteins have been demonstrated. Currently, there are at least three dominant disorders, Marfan’s syndrome (18–20), ectopia lentis (19, 20), and congenital contractual arachnodactyly (19), that are caused by mutations in fibrillin genes. Most patients with Williams syndrome that have SVAS are heterozygous for deletions of ELN and presumably other contiguous genes on chromosome 7q (21, 22). However, only in patients with SVAS have disruptions or point mutations within ELN itself been described (15, 16, 23). The connective tissue features of SVAS and Williams syndrome are consistent with, but not yet shown to be due to, functional hemizygosity at ELN.

We have previously shown in one cutis laxa cell strain that transcript instability was the basis of a defect in ELN mRNA accumulation and tropoelastin production (24). TGF-β was able to increase mRNA stability markedly and to stimulate production of immunoreactive tropoelastin protein in this cell strain. Since the metabolic and ultrastructural defect was confined to elastin, we hypothesized that a structural defect in the ELN transcript could be responsible for decreased mRNA stability. In this report, we describe heterozygosity for a frameshift mutation (2012G) in ELN in this cutis laxa patient and a similar mutation in two generations of a second cutis laxa family (2039AC), which we propose to be responsible for defects in tropoelastin production.

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†††††† The abbreviations used are: SVAS, supravalvular aortic stenosis; TGF-β, transforming growth factor-β; UTR, untranslated region; RT-PCR, reverse transcription-polymerase chain reaction; bp, base pair(s); ORF, open reading frame.
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Clinical Summary—Patient K.T. was the 3.8-kg full-term male product of an uncomplicated pregnancy, labor, and delivery to a 23-year-old G₁P₁ Caucasian mother. The parents denied consanguinity. Loose skin, stridor, and feeding difficulties were apparent from birth. Additional clinical findings ascertained during infancy included moderate subcutaneous edema with flabby subcutaneous structures, redundant mitral and tricuspid valves, mild dilatation of the proximal aorta and great vessels, and umbilical and inguinal hernias. He underwent inguinal herniorrhaphies at ages 7 months, 3 years, and 14 years.

At age 17 years, his height and weight are at the 75th percentile. His physical exam is significant for an aged appearance: smooth, loose skin lacking elastic recoil; tortuous, pulsatile external carotid arteries; and a hoarse voice. He complains of fatigue, dyspnea on exertion, and shortness of breath. Chest x-ray and electrocardiogram is relatively unremarkable except for minimal aortic root dilatation; an electrocardiogram reveals mild right ventricular hypertrophy. Pulmonary function testing shows reduced expiratory flow, suggestive of fixed or collapsible upper airway obstruction.

Histologic, ultrastructural, and biochemical analyses of skin and cultured fibroblasts from the patient have been reported previously (17, 24). Briefly, dermal collagen fibers appear normal, but elastic fibers appear fragmented with a paucity of amorphous elastin in the matrix. Tropoelastin production in cultured fibroblasts from this patient was the lowest of six cutis laxa patients studied (17), and an apparent nonspecific increase in type VI collagen production was also noted (25).

Limited clinical information is available on the second family at this time. The female proband (WM) was ascertained in 1965 with classical cutaneous features of cutis laxa at 2 years. This individual gave birth to an affected son (WS) in 1991, and skin biopsies were cultured from both affected individuals in 1993 by Drs. J. Uitto and E. Tan (Jefferson Medical College). The father and maternal parents were reportedly unaffected, and the patients have been lost to follow-up.

Tissue Culture—Skin fibroblasts were grown from skin biopsies obtained after appropriate consent. Normal human skin fibroblasts were cultured from an affected son (WS) in 1991, and skin biopsies were cultured from both affected individuals in 1993 by Drs. J. Uitto and E. Tan (Jefferson Medical College). The father and maternal parents were reportedly unaffected, and the patients have been lost to follow-up.

cDNA Synthesis—Confluent cultured cells were washed twice in phosphate-buffered saline and lysed in 2 ml of 4 M guanidine isothiocyanate containing 0.1 M β-mercaptoethanol. DNA was sheared by three passages through a 22-gauge needle, and RNA was isolated by extraction in acid phenol/chloroform (26). Isolated RNA was stored at −70 °C. cDNA was synthesized using 1–3 μg of total cellular RNA with 200 units of Moloney murine leukemia virus reverse transcriptase and 0.75 μM gene-specific oligonucleotide primers in a total volume of 20 μl for 60 min at 37 °C.

PCR—Eleven pairs of overlapping primers were designed to amplify the entire coding and untranslated regions of ELN mRNA. Primers were constructed according to the cdna sequences for the ELN coding region (GenBank™ accession number M36860) (27) and 3′-UTR were constructed according to the cDNA sequences for the psi experiment. The primers for the elastin coding region G2P1 Caucasian mother. The parents denied consanguinity. Loose skin, stridor, and feeding difficulties were apparent from birth. Additional clinical findings ascertained during infancy included moderate subcutaneous edema with flabby subcutaneous structures, redundant mitral and tricuspid valves, mild dilatation of the proximal aorta and great vessels, and umbilical and inguinal hernias. He underwent inguinal herniorrhaphies at ages 7 months, 3 years, and 14 years.

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C\textsuperscript{963}, C\textsuperscript{1018}, A\textsuperscript{1227}, A\textsuperscript{1228}, and C\textsuperscript{1231}. Although these ELN 3'-UTR sequences differ from the original GenBank\textsuperscript{TM} data, they were identical in our patient and control fibroblast mRNAs. Further sequencing of elastin cDNA revealed that exons 22, 23, 26A, and 32 were spliced out of transcripts from both control and K.T. fibroblasts (Fig. 3), a feature that has been reported previously in normal cells (30).

A Related Mutation in a Second Family Is Alternatively Spliced—A second set of fibroblast strains from a kindred with an affected mother (WM) and son (WS) showed characteristic low tropoelastin production with high induction of the protein by TGF-\(\beta\) (Fig. 4). Elastin mRNA only accumulated in these strains in the presence of TGF-\(\beta\), and it rapidly degraded as soon as TGF-\(\beta\) was withdrawn (Fig. 4B). Heteroduplex analysis revealed a novel band mobility in WM and WS using primer pairs that spanned the exon 30 region (data not shown). Eight full-length cDNA clones were derived from WS mRNA and completely sequenced. Three of the cDNA clones lacked exon 30 (Fig. 5A). Of the five cDNA clones containing this exon, four showed a deletion of C\textsuperscript{2039} (Fig. 5A), which predicts a frameshift mutation with consequences similar to those of strain K.T.

Heterozygosity of the mutation in WM and her son (WS) was established by direct sequencing of genomic DNA. To confirm the heterozygosity and to rule out a polymorphism, restriction digests were performed with \(PfI\) (Fig. 5B). 50% of WS and WM DNAs were resistant to enzyme digestion, whereas DNAs from 50 other unrelated individuals were fully susceptible to \(PfI\) enzyme digestion at the exon 30 locus.

TGF-\(\beta\) Does Not Change the Ratio of ELN mRNA Expressed from Two Alleles—As shown previously for K.T. (24) and above for WM and WS, TGF-\(\beta\) could in part restore tropoelastin expression in these cutis laxa fibroblasts by stabilizing ELN mRNA. To examine whether partial restoration of ELN mRNA stability was functioning through selective expression of ELN mRNA from the normal allele, RT-PCR products amplifying the region spanning nucleotides 1671–2292 from TGF-\(\beta\)-treated and untreated K.T. fibroblasts were analyzed under semi-quantitative conditions. Amplified products were digested with \(Alw\)\textsubscript{26I}. The intensity of the 244-bp mutant allele fragment relative to the normal 376-bp fragment did not change after TGF-\(\beta\) exposure (Fig. 6). Similar results were obtained with RNAs from WS and WM (data not shown). Transcripts of both alleles had equivalent instability.

Mutational Consequences—The two single base deletions in exon 30 each predict a frameshift in the coding region for the elastin carboxyl terminus. The predicted, truncated protein product would consist of 667 amino acids (Fig. 7). Since exon 32 was usually absent, the predicted open reading frame (ORF) of the mutant transcript would more frequently continue into the 3'-UTR to be translated as a missense structure of 713 amino acids lacking the distinctive carboxyl terminus of tropoelastin (Fig. 7). In the less likely event of inclusion of exon 32, the mutation would create a premature termination codon in exon 32, predicting a truncated, missense C terminus and a translation product of 667 amino acid residues. Among the 3'-UTR
sequence corrections/additions that were noted, only the T174 to A substitution would have functional significance in the translation of the mutant allele since it would create a novel stop codon (TAA174) in the shifted ORF. This new stop codon would be 25 amino acids downstream of the normal translation termination site.

DISCUSSION

Cutis laxa is a relatively rare connective tissue disease characterized by genetic heterogeneity and clinical variability (6, 31–33). In all cases, the primary diagnostic feature is loose, hyperextensible skin with decreased resilience and elasticity, leading to a premature aged appearance. The skin changes are often accompanied by extracutaneous manifestations, including pulmonary emphysema, bladder diverticula, pulmonary artery stenosis, and pyloric stenosis. Histological examination of the skin in cutis laxa often reveals marked fragmentation or diminution of elastic fibers (6, 17, 32). This is a considerably different phenotype than that found in SVAS, a pathology arising from elastin mutations that lead to functional hemizygosity (15, 16, 23, 34, 35). Skin fibroblast cultures from many cutis laxa patients exhibit reduced ELN mRNA levels or tropoelastin production, but fibroblasts from other affected individuals exhibit normal levels of elastin production with abnormal elastic fiber morphology (17, 36). At the time of submission of this manuscript, the molecular basis for cutis laxa had not been elucidated.
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been described. However, another group has recently reported autosomal dominant cutis laxa associated with a point deletion in exon 30 (37). This exon was absent from the cDNAs examined in this study. Unlike the biochemical phenotype reported here, elastin mRNA is expressed and apparently stable in this strain, and the data from that report are consistent with a dominant-negative effect at the level of elastic fiber formation. The fibroblasts in this study produced insignificant amounts of elastin mRNA and protein in the absence of TGF-β stimulation.

We identified two single base deletions (2012ΔG and 2039ΔC) in the coding region of ELN cDNA from two cutis laxa kindreds. Analysis of the parental DNA indicated a de novo mutation in proband K.T., and limited familial information suggests that a de novo mutation in WM was passed from WM to WS, compound heterozygous mutation appears to exert a dominant effect on the cutis laxa phenotype. The molecular data make analysis of allelic usage problematic. Transcripts from both normal and mutant alleles were equally stabilized by TGF-β stimulation, but both transcripts were equally stabilized by TGF-β stimulation. Both alleles were otherwise identical throughout the coding region, 223 bp of the 5'-UTR, and 1.2 kilobases of the 3'-UTR (data not shown). A comparable increase in the amount of both normal and mutant transcripts by TGF-β stimulation suggests that at least the proximal regulatory regions of both alleles are intact, including the response sites for TGF-β (41) and insulin-like growth factor-1 (42) in the elastin promoter. We have previously shown that ELN transcription rates are normal in K.T. (24). In addition, since the point deletion was passed from WM to WS, compound heterozygosity could only have persisted in WS by donation of an independent mutation from his paternal ELN allele. These findings, together with the absence of the deletions in DNAs from both parents of K.T. and unrelated controls, argue that K.T. and probably WS are the origins of new dominant mutations that account for the cutis laxa phenotype. The molecular data predict that if mutant tropoelastin were synthesized, at least in skin, it would contain defective carboxyl termini. The quantitative and qualitative defects in tropoelastin production could readily account for the patients’ abnormal (cutaneous) phenotype. We have not determined the mechanism whereby the heterozygous mutation appears to exert a dominant effect on ELN mRNA stability. Nonsense-mediated mRNA decay is a protective cellular mechanism described for several genes; however, it acts in cis as a rule (43). Indeed, this mechanism appears to be operative in some forms of SVAS (16). A dominant, trans-acting effect might arise if mutant transcripts altered the availability of a rate-limiting factor for elastin mRNA stability. Elastin mRNA levels drop if secretion is perturbed (44), and translational or packaging defects may have a feedback effect on mRNA stability (45). To test these possibilities,
current studies are directed at examining the effects of introducing a mutant ELN cDNA into elastin-expressing cells.

In conclusion, we have found two point deletions in ELN from individuals with the classical cutaneous phenotype of congenital cutis laxa. These mutations result in frameshifts that disrupt the sequence of the conserved carboxyl terminus of tropoelastin and lead to marked transcript instability. We speculate that variable splicing of the mutated exon may account for reduced severity and selective tissue effects. The elastin mutations in SVAS appear to involve functional hemizygosity for reduced severity and selective tissue effects. The elastinulate that variable splicing of the mutated exon may account for reduced severity and selective tissue effects. Identification of additional elastin mutations in both dominant and recessive forms of cutis laxa will enable us to understand better the structural biology and regulation of this important extracellular matrix component.

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