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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 (2012AG 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 contractural 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 Menkes' 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 α 1-antitrypsin deficiency (9) and some forms of atrophoderma (10). Pseudoxanthoma 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)¹ (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 contractural 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 (2012AG) 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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¹ 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.

EXPERIMENTAL PROCEDURES

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 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 subglottic stenosis with floppy airway 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. His echocardiogram 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 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 (GM4390) were obtained from the NIGMS Human Genetic Mutant Cell Repository (Camden, NJ). Cells were grown to confluence in Dulbecco's modified Eagle's medium (Life Technologies, Inc.) supplemented with 10% fetal bovine serum (Atlanta Biologicals, Inc., Norcross, GA).

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 mM 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 mM 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 complete 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 (accession number M17282) (28). Primers designated F are based on the sense strand sequence, and primers designated R are based on the complementary strand sequence. The primers for the elastin coding region were as follows (numbering from the transcription start site): 96F, 5'-ATCGATCCTGCTGTCCATCCTCCA-3'; 419R, 5'-ACACTCCTAAGCCACCAACT-3'; 397F, 5'-AGGAGTTGGTGGCTTAGGAG-3'; 728R, 5'-GCAGTTTCCCTGTGGTGTAG-3'; 709F, 5'-CTGCACCACAGGGAAA-CTGC-3'; 1064R, 5'-CTCCTGGGACCAACTACT-3'; 992F, 5'-AAGTATGGAGCTGCTGCAGG-3'; 1410R, 5'-ACTCCGTACTTGGCAGCCTT-3'; 1271F, 5'-GGTGTGGAGTCGGAGGTAT-3'; 1798R, 5'-AACAC-CAGCACCACCACTCAA-3'; 1761F, 5'-GTGCTGGTGTTCCTGGACTT-3'; and 2292R, 5'-AGCAGTAGCACCACGTTGA-3'. The primers for the elastin 3'-UTR were as follows (numbering from the 3'-UTR): He3'UTR15F, 5'-CTGACTCAGACCTCATCAA-3'; He3'UTR328R, 5'-CAGGAAGATAAGAGCACCAG-3'; He3'UTR302F, 5'-CTACACGCTGTGCTCTTAT-3'; He3'UTR741R, 5'-GACAGGTCACCAAGGTTGAT-3'; He3'UTR721F, 5'-CATCAACCTGGTTGACCTGT-3'; He3'UTR1092R, 5'-TTCTACTGGGGATACAGCTC-3'; He3'UTR1029F, 5'-TTGTGTCTCGCTGTGATAGA-3'; and He3'UTR1255R, 5'-CCAACAGTTGAAGGCAGATT-3'. The primers for the elastin 5'-UTR were as follows (numbering from the transcription start site): -243F He-promoter, 5'-GTGTGTGCGTGTGTGTGTC-3'; and +155R He-intron I, 5'-CTTGA-GCGTCTAGTACCTG-3'. The primers for introns 29 and 30 were as follows: 212F in intron 29, 5'-GGAGTCTAATGCTCAGCTGT-3'; and 489R in intron 30, 5'-CACCTTGGCTACTAGAGTG-3'.

PCRs were carried out in a Perkin-Elmer minicycler in 50- μl volumes using 1–2 μl of reverse transcription products, a 0.2 μM concentration of each primer, a 0.4 mM concentration of each dNTP, and 2.5 units of *Taq* polymerase (Promega, Madison, WI). The reaction cycles consisted of an initial denaturation at 95°C for 3 min followed by 95°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 1 min per cycle for 30 cycles.

Direct DNA Sequencing—PCR products amplified by different F and R primer combinations from both cutis laxa and control cell strains were separated by ethidium bromide-1% agarose gel electrophoresis. Amplimers of the expected sizes were excised and purified using Prep-A-Gene DNA purification systems (Bio-Rad). Sequencing was performed using an ABI PRISM™ 377 DNA Sequencer (Perkin-Elmer) or manual sequencing using the double-stranded DNA cycle sequencing system (Life Technologies, Inc.). For mutation confirmation, three different RT-PCR products were independently sequenced.

Cloning of RT-PCR Products—RT-PCR products of ~3 kilobases synthesized with primers 96F and He3'UTR741R were separated by electrophoresis, purified, and cloned into the pNoTA vector using the PRIME PCR CLONER™ cloning system (5 Prime → 3 Prime, Inc., Boulder, CO) according to manufacturer's instructions. A total of 31 white colonies was picked from overnight cultures and propagated in LB medium for plasmid minipreps. The plasmid DNAs were digested with *Bam*HI, and positive clones with correctly sized inserts (2854 bp) were identified by gel electrophoresis.

Restriction Endonuclease Digestion—The 2012 Δ G deletion in exon 30 of K.T. created a novel *Alw*26I restriction site. RT-PCR products amplified by primers 11 and 12 (476 bp) from K.T. fibroblasts, a control cell strain, and genomic PCR products amplified by primers 23 and 24 (277 bp) from the proband, his parents, and 65 unaffected individuals were digested with *Alw*26I at 37°C for 60 min. The 2039 Δ C deletion abolished a *Pf*MI site in the same PCR product of the mutant allele in WM and WS. DNAs from an additional 48 individuals were also screened for the same mutation. The digests were electrophoresed through an 8% polyacrylamide gel and visualized by ethidium bromide/UV fluorescence.

Heteroduplex Assay—PCR was performed with primers 1761F and 2292R in the presence of 0.3 μCi of [^{32}P]dCTP for 30 cycles. Equal amounts of PCR products from normal and cutis laxa fibroblasts were mixed, incubated at 95°C for 2 min, and gradually (20–30 min) cooled to 37°C . After adding 1 μl of loading buffer to 5 μl of sample, the samples were separated by electrophoresis on a 0.5 $\times$ Mutation Detection Enhancement gel (FMC Corp. BioProducts, Rockland, ME).

RNA Stability—Elastin production and mRNA stability studies were carried out as described previously (17, 24). RNA was isolated and purified using the QIAshredder™ and the RNeasy™ mini kit (QIAGEN Inc., Chatsworth, CA) according to the manufacturer's instructions.

RESULTS

A Frameshift Mutation of *ELN* in Cutis Laxa Strain K.T.—A single base deletion (2012 Δ G) in exon 30 of *ELN* was initially identified by direct sequencing of the RT-PCR products from patient fibroblasts (Fig. 1). This mutation created a novel *Alw*26I recognition site ([N]₅GAGAC); heterozygosity for the deletion was confirmed in the RT-PCR product by the presence of diagnostic 244- and 132-bp bands after digestion of the 476-bp PCR product with *Alw*26I (Fig. 2A). Furthermore, heterozygosity for the mutation was confirmed at the genomic DNA level in the patient, but the polymorphism was absent in DNA from both parents (Fig. 2B) and 65 unrelated control samples (data not shown).

Upon sequencing the entire *ELN* coding region as well as 240 bp of the 5'-UTR and 1.2 kilobases of the 3'-UTR, no other sequence variations could be found between the two cloned patient alleles and controls. Both alleles contained A at position 1313, the site of an A/G (Ser⁴²²/Gly) polymorphism (29). Several other presumably less significant base changes were found in the 1.2-kilobase *ELN* 3'-UTR of both patient and control strains that differed from or complemented GenBank™ entry M17282 (28). These included seven base changes (T¹⁷⁴ to A, T¹⁹⁶ to C, C⁷³⁷ to G, A¹¹⁹⁵ to C, C¹²¹⁶ to A, T¹²⁴⁵ to A, and T¹²⁵⁸ to A), four insertions (C²³³, C⁷⁷⁶, C⁷⁸⁸, and C⁸⁶¹), three deletions (A³³⁵, A⁷²⁴, and A¹¹³⁷), and 15 newly identified residues (G⁸⁵⁷, C⁸⁶¹, C⁸⁶³, C⁸⁷⁶, C⁹²⁴, T⁹²⁵, C⁹²⁶, G⁹³⁹, C⁹⁴⁶, C⁹⁶²,

FIG. 1. A deletion (2012ΔG) in *ELN* in a cutis laxa patient. RT-PCR products from both patient (K.T.) and normal fibroblasts were generated by primers 1761F and 2292R (see "Experimental Procedures") and subjected to automated fluorescent sequencing. The location of the deletion is indicated by the arrow.

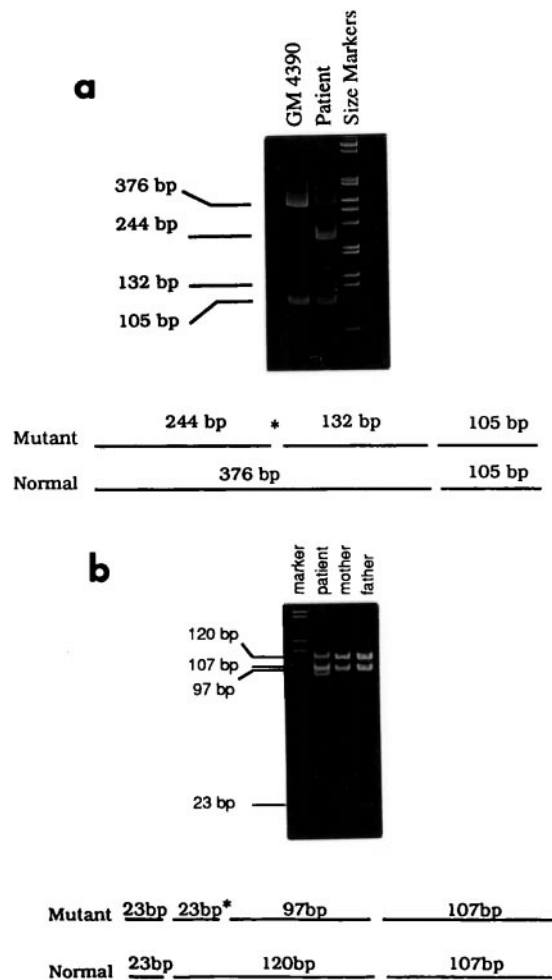
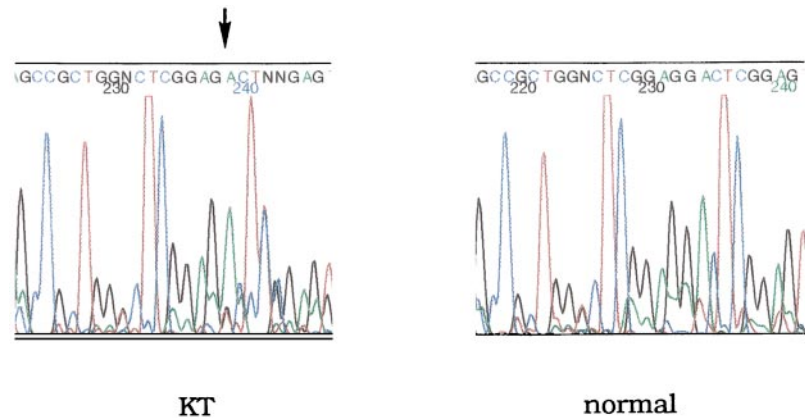


FIG. 2. Confirmation of heterozygosity for the *ELN* mutation in strain K.T. A, RT-PCR products from patient (K.T.) and control (GM4390) fibroblast mRNAs were digested with *Alw26I* and separated by 8% polyacrylamide gel electrophoresis. The mutation creates a novel *Alw26I* site (*), thus further cleaving the normal 376-bp fragment into 244 and 132 bp. B, genomic DNAs from the patient and his unaffected parents were amplified by PCR with primers 212F and 489R (see "Experimental Procedures"), digested with *Alw26I*, and separated by 8% polyacrylamide gel electrophoresis. The mutation creates a novel *Alw26I* site (*), thus further cleaving the normal 120-bp fragment into 97 and 23 bp.

C⁹⁶³, C¹⁰¹⁸, A¹²²⁷, A¹²²⁸, and C¹²³¹). Although these *ELN* 3'-UTR sequences differ from the original GenBank™ 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-β (Fig. 4). Elastin mRNA only accumulated in these strains in the presence of TGF-β, and it rapidly degraded as soon as TGF-β 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 C²⁰³⁹ (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 *Pf1MI* (Fig. 5B). 50% of WS and WM DNAs were resistant to enzyme digestion, whereas DNAs from 50 other unrelated individuals were fully susceptible to *Pf1MI* enzyme digestion at the exon 30 locus.

TGF-β 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-β 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-β-treated and untreated K.T. fibroblasts were analyzed under semi-quantitative conditions. Amplified products were digested with *Alw26I*. The intensity of the 244-bp mutant allele fragment relative to the normal 376-bp fragment did not change after TGF-β 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

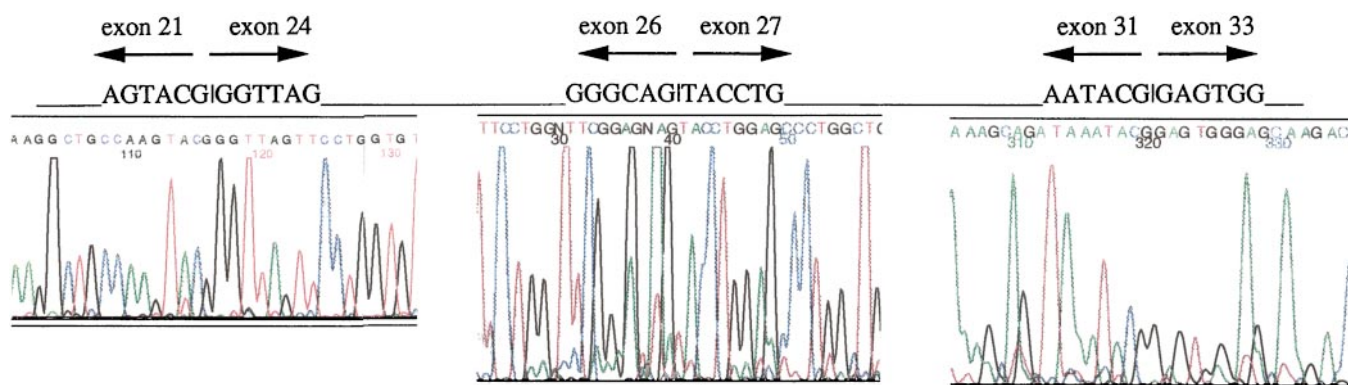


FIG. 3. Normal *ELN* mRNA splicing pattern in cutis laxa skin fibroblasts. The primary structure of the coding region was determined by direct cDNA sequencing. Exons 22, 23, 26A, and 32 were spliced out of the *ELN* mRNA from both cutis laxa (K.T.) and control fibroblasts.

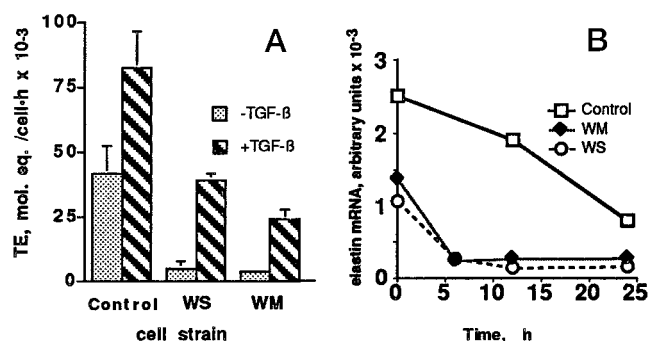


FIG. 4. TGF- β reversal of the elastin deficit in WM and WS is related to transcript instability. A, fibroblasts from the control strain (GM4390), WS, and WM were analyzed for tropoelastin (TE) production (9) in the presence and absence of 10 ng/ml recombinant TGF- β 2 (a gift of Genzyme Corp., Cambridge, MA). Under basal (5% newborn calf serum) conditions, tropoelastin production (expressed as molecular equivalents (mol. eq.)/cell/h $\pm$ S.E.) was near the base-line limits of the enzyme-linked immunosorbent assay in the cutis laxa strains, whereas production rose to nearly normal levels in the presence of TGF- β . B, cells pretreated with TGF- β 2 for 48 h as described above were put into fresh medium lacking TGF- β and containing 75 μ M 5,6-dichlorobenzimidazole riboside to block transcription. RNA was isolated immediately, 6, 12, and 24 h after treatment, and elastin transcripts were quantified in relation to cyclophilin transcripts by Northern blot hybridization. Consistent with the production data, elastin mRNA levels were lower in cutis laxa strains WM and WS and undetectable in the absence of TGF- β (data not shown). Elastin mRNA levels fell rapidly to base-line levels by 6 h. In control fibroblasts, the $t_{1/2}$ of elastin mRNA was >15 h.

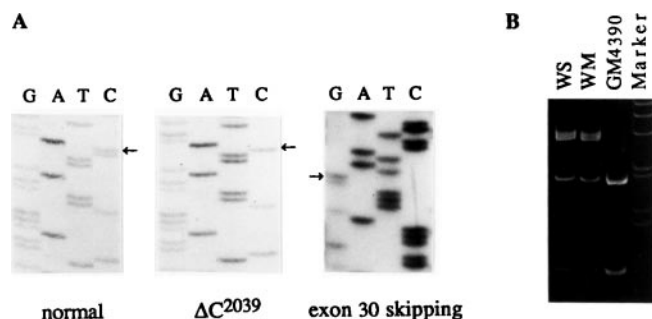


FIG. 5. Detection of a point deletion in cutis laxa strains from WM and WS. A, the left panel shows a segment of the normal sequence within exon 30; the middle panel (2039 Δ) illustrates the point deletion (GCCTTA $\rightarrow$ GCTTA); and the right panel shows the splice junction between exons 29 and 31. B, genomic DNAs from the two affected strains and GM4390 were amplified by PCR to yield a 277-bp product. Digestion with *Pf*MI produced quantitative cleavage of the product of control cells, whereas only 50% of WM and WS products were cleaved.

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

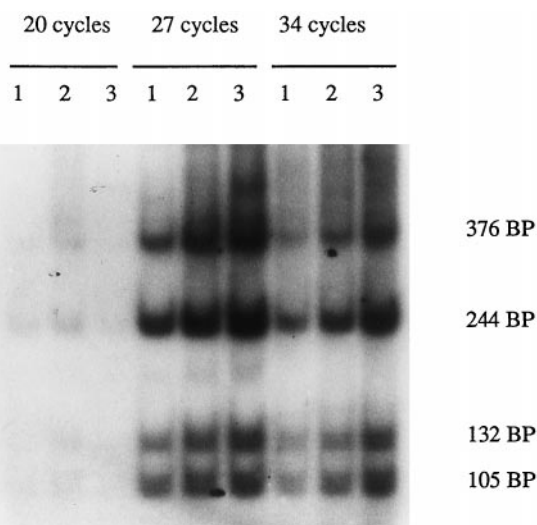


FIG. 6. Effect of TGF- β on the relative abundance of transcripts from the two *ELN* alleles in cutis laxa skin fibroblasts. A 481-bp cDNA region was amplified by RT-PCR using primers 11 and 12 and mRNAs from TGF- β -treated (K.T./TGF, lanes marked 1) and duplicate untreated (K.T., lanes marked 2 and 3) cells in the presence of 5 μ Ci of [³²P]dCTP for 20, 27, and 34 cycles. Equal aliquots were digested with *Alu*26I, separated by 8% polyacrylamide gel electrophoresis, and visualized by autoradiography. The sizes of the fragments are indicated. The cDNA restriction maps are shown in Fig. 2A.

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

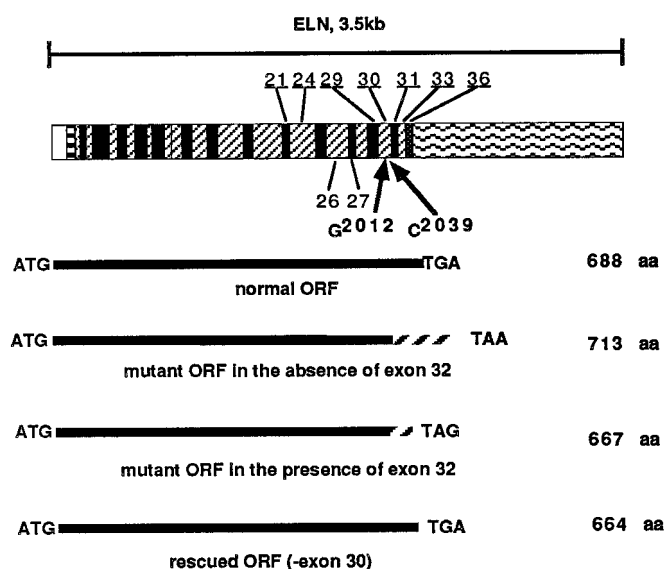


FIG. 7. Proposed effect of the *ELN* 2012ΔG and 2039ΔC mutations on translation. The organization of the domains in the transcript is based on cDNA sequence analysis of normal and mutant cell strains. In the normal transcript (top), the open box is the 5'-UTR; the striped box is the signal peptide; the hatched boxes are hydrophobic domains; the closed boxes are cross-link domains; the stippled box represents cysteine-containing C-terminal exon 36, and the wavy box is the 3'-UTR. Most domains are encoded by distinct exons, and exon numbering is shown to emphasize the consensus alternate splicing pattern present in skin fibroblasts. The positions of the 2012ΔG and 2039ΔC deletions are marked by arrows. The normal and three possible mutant ORFs are illustrated below this. Normal translation terminates at TGA in exon 36, producing a tropoelastin molecule of 688 amino acids (aa). Missense sequence following the frameshift is indicated by hatched areas in the two mutant ORFs. In the absence of exon 32 (as in skin fibroblasts), missense translation terminates at a stop codon (TAA) that is 75 bp 3' to exon 36, producing a defective protein of 713 amino acids. In the presence of exon 32, missense translation terminates at a premature stop codon (TAG) in exon 32, producing a defective protein of 667 amino acids. In transcripts lacking exon 30 (rescued ORF), the mutation is skipped, and a 664-residue tropoelastin is synthesized. kb, kilobases.

been described. However, another group has recently reported autosomal dominant cutis laxa associated with a point deletion in exon 32 (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 lineages. 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 to her son, WS. Both of the deletions would result in translation of missense protein sequence 3' to the mutation, and they predict subsequent loss of the functional carboxyl terminus in the tropoelastin molecule by missense and/or premature termination, depending on the more frequent splicing out or less frequent inclusion of exon 32 (Fig. 7). Thus, different tissues could produce defective elastin molecules with slightly different carboxyl termini, depending on the tissue-specific mRNA splicing pattern. Regardless of the status of exon 32 splicing, each of these point deletions dictates the loss of two conserved cysteine residues that create an intramolecular disulfide bridge and that are thought to be important, together with a basic tetrapeptide tail, for interaction of tropoelastin with other pro-

teins present in the elastic fibers of microfibrils (38, 39). Although little or no normal or mutant protein is expressed in fibroblast cultures, tissues *in vivo* (particularly under the influence of TGF- β) might express significant levels of either defective gene product and lead to a dominant-negative effect at the level of protein assembly. We may be able to resolve this issue with C terminus-specific antibodies when tissue becomes available.

Analysis of the mutation in WM and WS confirmed *ELN* as the cutis laxa locus. Both the similar location in exon 30 and the predicted translational consequences of 2012ΔG and 2039ΔC mutations are intriguing, and they may indicate a susceptible site for production of dominant-negative, viable *ELN* mutations. Exon 30 has recently been identified, at least in the rat, as a site for a *cis*-acting mRNA stability element.² More important, our finding that exon 30 is alternatively spliced may be indicative of a phenotypic rescue mechanism (40) whereby cells or tissues could escape the effects of the mutations. The same possibility would apply for the recent mutation described in exon 32 (37). The fact that all three affected individuals are not severely incapacitated suggests that pulmonary and cardiovascular elastic tissues must be relatively spared. In contrast to the predominant effect of SVAS in the aortic root, both of the cutis laxa mutations appear to have a largely cutaneous phenotype. This could be due to tissue-specific or developmentally regulated differential expression of the two alleles, increased frequency of alternative splicing of exon 30 (or 32) in non-cutaneous tissues, or differential consequences of mutant RNA and protein in different cell types.

The low frequency of polymorphisms in *ELN* makes analysis of allelic usage problematic. Transcripts from both normal and mutant alleles were equally unstable, and both transcripts were equally stabilized by TGF- β 2. 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 WM 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,

² M.-C. Zhang and W. C. Parks, submitted for publication.

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 that affects the elastin-rich aortic root (14–16). Complementing these findings, autosomal dominant cutis laxa appears to arise from dominant-negative effects at the level of both RNA stability and protein assembly, with the prospect of a novel mechanism for variable tissue consequences. 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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