A Novel Elastin Gene Mutation Resulting in an Autosomal Dominant Form of Cutis Laxa

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Background: Cutis laxa is an extremely rare disorder characterized by marked skin laxity. Few cases of cutis laxa have been described worldwide. Clinical presentation and mode of inheritance show considerable heterogeneity; autosomal dominant, autosomal recessive, and X-linked recessive forms have been reported. Only 3 mutations in the elastin gene have been described as the genetic cause of the autosomal dominant form of cutis laxa.

Observations: A 45-year-old woman and her 19-year-old son presented with inelastic, loose-hanging, and wrinkled skin that appeared prematurely aged and were clinically diagnosed as having cutis laxa. Mutational analysis of the elastin gene evidenced a novel mutation (2292delC) that predicts a frameshift in the coding region and causes translation to proceed into the 3'-untranslated region. This would replace the C-terminal amino acid of the normal elastin protein with a novel sequence.

Conclusion: This article is the fourth report of autosomal dominant cutis laxa to appear in the literature in which a mutation in the elastin gene has been correlated with the disease.

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CUTIS LAXA (ONLINE MENDELIAN INHERITANCE IN MAN codes 123700 and 219100) is a rare inherited or acquired connective tissue disorder characterized by lax, redundant, and inelastic skin. Histologically, elastic fibers are sparse and fragmented. These fibers are present in the extracellular matrix of several tissues including lung, large blood vessels, and dermis. They are basically composed of the protein elastin (ELN), which is responsible for imparting elasticity to these tissues and organs. Cutis laxa is one of the several disorders of the connective tissue that is characterized by aberrant synthesis, degradation, or destabilization of elastic fibers.

There are several forms of cutis laxa, and they all exhibit a considerable heterogeneity in their clinical manifestations. The disease may be hereditary or acquired, but all forms are very rare, and no precise data exist about their prevalence. The acquired form of cutis laxa is usually preceded by local or generalized inflammatory events. As for the hereditary forms, 2 autosomal recessive types and 1 autosomal dominant form of cutis laxa have been delineated. The previously defined X-linked form (also named Ehlers-Danlos syndrome type IX), caused by mutations in the ATP7A gene, is now classified within the group of copper deficiency syndromes and has been shown to be allelic with Menkes syndrome. The type I autosomal recessive form of cutis laxa is a perinatal form that presents pulmonary and other grave internal manifestations that lead to an early death. Type II, which is believed to be more frequent than type I, is called cutis laxa with joint laxity and developmental delay. The molecular defects underlying recessive form type I have been recently identified, but those underlying type II have yet to be characterized. It has been reported that mutations in the fibulin-5 gene (FBLN5), which plays an important role in normal elastic fiber development, causes autosomal recessive cutis laxa. In contrast, the autosomal dominant form is apparently free of systemic abnormalities; the onset of skin manifestations occurs between birth and puberty and is caused by mutations in the ELN gene, but molecular heterogeneity cannot be excluded.

Few families are known to have cutis laxa inherited as a dominant trait, and only 3 cases have been correlated to mutations in the ELN gene. Herein we re-
port 1 more case of autosomal dominant cutis laxa caused by a novel frameshift mutation (2292delC) in the ELN gene.

**METHODS**

**CLINICAL SUMMARY**

**Patient 1**

A 45-year-old woman, born to nonconsanguineous parents with no family history of cutis laxa, was clinically diagnosed as having cutis laxa at age 17 years. She presented with progressive cutaneous looseness and sagging, mainly on the face and neck. She also had deep perioral and ocular furrows and a prematurely aged appearance. She underwent 5 cosmetic surgical procedures. Cutaneous exploration showed loose skin, deep perioral wrinkles, and absence of infraocular fold as a consequence of the corrective surgery (Figure 1). Echocardiographic examination disclosed stenosis and regurgitation of the mitral valve, moderate aortic regurgitation, and a minor dilatation of the left auricula. She was diagnosed via spirometry and computed tomography (CT) as having pulmonary emphysema clinically attributed to a smoking habit.

**Patient 2**

A 19-year-old white man (son of patient 1) was diagnosed as having cutis laxa at age 1 year on the basis of cutaneous inelasticity. He had a history of infantile asthma and a language disorder. He was referred for a dermatology consult in 1996 and was found on physical examination to have loose, redundant skin of the face and neck with accentuated nasolabial folds and downsloping palpebral fissures. The skin recoiled slowly after stretching (Figure 2). The elastic fibers were diminished in size and number in a skin biopsy specimen. Pulmonary function test results disclosed an obstructive pattern. High-resolution pulmonary CT suggested panlobular emphysema. There was no evidence of cardiovascular or gastrointestinal involvement.

**MUTATION DETECTION AND SEQUENCING**

Blood samples from the cutis laxa family, including the mother, her parents, and her affected son, were collected after appropriate written consent was obtained, and genomic DNA was extracted following the salting out procedure. Exons 30 to 34 of the ELN gene were amplified via polymerase chain reaction (PCR) using the conditions and primers described by Tassabehji et al. Three microliters of PCR product was combined with loading buffer then denatured and electrophoresed in a 12% non-denaturing acrylamide for 1.45 to 2.00 hours at 15°C using the Gene neph (Pharmacia Biotech, Uppsala, Sweden) single-strand conformation polymorphism analysis and following the manufacturer’s instructions. The different migrations were silver stained. The aberrant pattern detected was automatically sequenced using the d-Rhodamine Terminator Ready Reaction kit and an ABI 3100 automatic sequencer (Applied Biosystems, Foster City, Calif). To disclose the possibility of a false paternity, we studied the inheritance pattern of 13 polymorphic markers distributed in different chromosomes using the ABI PRISM Linkage Mapping Sets (Applied Biosystems) according to the recommended protocol.

**TISSUE CULTURE**

One punch biopsy specimen was taken from the upper arm of each patient and from a healthy control. Skin fibroblasts were obtained after appropriate consent. Fibroblast culture was routinely maintained at 37°C in a humidified 10% carbon dioxide/90% air atmosphere in Dulbecco modified Eagle medium (Life Technologies Inc, Barcelona, Spain) supplemented with 10% fetal bovine serum (Life Technologies Inc).

**REVERSE TRANSCRIPTASE PCR ANALYSIS**

Total RNA from the skin fibroblasts culture was isolated using the RNeasy kit (Qiagen, Hilden, Germany). Total RNA (1 µg) from each patient and from a control was reverse transcribed using a Taq Man Reverse Transcription kit (Applied Biosystems) according to manufacturer’s instructions. Complementary DNA (cDNA) from the patients and from a control was amplified by nested PCR in 2 steps using 3 primers designed from cDNA to amplify a fragment that included exon 33. The first step used a forward primer (5’-GCTGGGCTCAGGGAGCTCAGTC-3’) and an external reverse primer (5’-ATTTTCTCCTCGGCGCACA-3’) covering from exon 30 to 34 (241 base pairs). The second step used an internal reverse primer (5’-CACCTGGAAAAATGGGAGAC-3’) and the same forward primer (202 base pairs). Standard PCR conditions were used: 95°C for 5 minutes; 10 cycles at 94°C for 1 minute and 72°C for 1 minute; and 25 cycles at 94°C for 40 seconds, 52°C for 40 seconds, and 72°C for 40 seconds, with an extension of 7 minutes at 72°C. Products were used to perform the second reaction with an annealing temperature of 58°C. The obtained products were separated and visualized on a 2% agarose Tris-borate EDTA gel.
We identified a heterozygous frameshift mutation in the ELN gene in 2 generations of a nonconsanguineous family affected by autosomal dominant cutis laxa. We performed DNA mutation analysis only in the 3′ end (from exon 30 to 34) of the ELN gene because this is the region where mutations causing these disorders have been reported. An abnormal band was detected in both patients when exon 33 was screened (Figure 3A), and by sequencing the PCR product, we found a heterozygous single-base deletion of a cytosine (2292delC) compared with a control sample (Figure 3B). This change was not detected in 100 healthy control samples. We detected no aberrant pattern in the parents of patient 1, which suggests that a de novo mutation occurred. We ruled out the possibility of false paternity within the family by studying the inheritance of 13 polymorphic microsatellite markers distributed on several chromosomes.

To test our hypothesis that ELN is the disease-causing gene in this family, we performed cDNA studies. To obtain RNA, we grew skin fibroblasts in culture and by optical microscope evaluation observed that they did not differ significantly from control cells morphologically or from a proliferation point of view. Total RNA was isolated from the cultured skin fibroblasts of the mother, the son, and 1 control. Reverse transcriptase PCR was performed, and total cDNA was obtained. The presence of the mutant messenger RNA (mRNA) was confirmed by nested PCR and single-strand conformation polymorphism analysis. It was carried out using cDNA primers designed to amplify a fragment that would include exons from 30 to 34. This analysis showed that the mutated allele was also expressed (data not shown).

These results evidenced that a single-base deletion of a cytosine was identified at nucleotide position 2292 of ELN. The mutated allele was transcribed and the nucleotide change also detected in the mRNA of both patients. This deletion predicts a frameshift in the coding region, and thus it will cause translation to proceed into the 3′-untranslated region and create a novel protein that is 25 amino acids larger than the wild type (Figure 4).

The molecular basis of cutis laxa remains elusive, but analyses of skin fibroblasts have confirmed defects in elastin production or gene expression in some of the cases.12,13 The ELN protein is responsible for tissue elasticity. Elastic fibers are composed of 2 morphologically and chemically distinct components. The amorphous component is quantitatively the most abundant, and it is made of ELN. The microfibrillar component is mostly composed of fibrilin and microfibril-associated glycoprotein.14 The ELN gene is a single-copy gene of 34 exons localized in humans at 7q11.2. Its product, tropoelastin, is a 72-kDa polypeptide with a primary structure of alternating hydrophobic and lysine-rich sequences. The hydrophobic domains are thought to be responsible for the elastic properties of the protein, while the lysine-rich sequences are where the tropoelastin molecules link covalently to form a highly insoluble network of elastic fibers.15 Only 3 cases of autosomal dominant cutis laxa have been reported in which the molecular defect has been described. In 2 of them, a single-base deletion in exon 30 (2012delG and 2039delC) leads to the loss of the C-

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terminal part of the molecule. Additionally, the stability of mRNA is decreased, and the production of tropoelastin is also affected.8 The other case is a frameshift mutation (2292delC) in exon 32 that predicts the replacement of 37 amino acids at the C-terminus of ELN by a novel sequence of 62 amino acids. The abnormal protein is synthesized, secreted, and incorporated into the elastic matrix, disrupting the normal architecture of elastic fibers.7

We report the fourth case of autosomal dominant cutis laxa arising from an ELN mutation. The clinical picture of our 2 patients was typical of this disorder in that they showed wrinkled and extensible skin with no elasticity and a few systemic manifestations including para-symptomatic emphysema in both patients and discrete cardiac abnormalities in the mother. The clinical manifestation in this family is similar to that described in the other 3 families with cutis laxa caused by ELN mutations (Table). The skin symptoms of the son first appeared at birth and of the mother, during puberty. No other members of the family presented clinical features for cutis laxa. However, to demonstrate that the mutation arose de novo in the mother, we evaluated her parents and ruled out the possibility of false paternity. By single-strand conformation polymorphism analysis we confirmed that neither her mother nor her father had the mutation and therefore that a de novo mutation first originated with her (patient 1) and was inherited in an autosomal dominant way by her son (patient 2).

Mutations in the ELN gene are not only responsible for autosomal dominant cutis laxa but also cause supravalvular aortic stenosis, which may occur as an isolated disease or as a part of a complex developmental disorder called Williams-Beuren syndrome (Online Mendelian Inheritance in Man code 194050), which is a microdeletional syndrome that involves the deletion of 1 complete copy of ELN. It is characterized by cardiovascular, neurobehavioral, facial, connective tissue, metabolic, and growth abnormalities. Point mutations responsible for supravalvular aortic stenosis are located in the 5’-end and middle region of ELN and result in premature termination mutations.10 These mutations in supravalvular aortic stenosis cases, and the deletion of a copy of the ELN gene in patients with Williams-Beuren syndrome, result in a functional haploinsufficiency of the ELN gene. By contrast, in autosomal dominant cutis laxa, the mutations described and the one detected by us are located in the 3’-end of the coding region of the ELN gene. They are all single-nucleotide deletions that do not truncate the protein but result in abnormal tropoelastin protein synthesis. It is hypothesized that this defective protein would interfere in 2 different ways: (1) in a dominant negative way that affects the deposition of normal elastin because the mutation alters the domain required for this deposition; or (2) in a gain-of-function mechanism that would make the mutant protein more susceptible to proteolytic degradation.10

Molecular analysis of our 2 patients showed that they share in heterozygosis a point deletion in exon 33 (2292delC). Messenger RNA studies confirmed that ELN was transcribed, and the mutation was also detected at this level. As the ELN mRNA of our patients was expressed, apparently stable, and in consonance with the work published by Tassabheji et al,7 we hypothesize that our mutation produces a tropoelastin protein larger than normal with an aberrant C-terminal domain. We cannot conclude in which way this mutant protein affects the elastic fiber structure once secreted to the extracellular matrix, but we believe that it is responsible for the symptoms in the present family.

It has been reported that while the hydrophobic sequences exhibit considerable variability without affecting functionality, the lysine-rich sequences and those encoded by exons 33 and 34 are very well conserved.17 On the basis of these observations, the dramatic effect that this point deletion located in exon 33 has in our 2 pa-

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<th>Clinical Manifestations</th>
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<th>Zhang et al.8 Patient W.M.</th>
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<td>Not reported</td>
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<td>Hoarse voice</td>
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<td>Repeated cosmetic procedures</td>
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tients is not surprising. It is worth reporting new cases of ELN mutations in autosomal dominant cutis laxa to better understand the molecular basis of this disease.

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REFERENCES