Homozygous Missense Mutation in Fibulin-5 in an Iranian Autosomal Recessive Cutis Laxa Pedigree and Associated Haplotype

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Cutis laxa is a rare group of inherited and acquired disorders characterized by loose and redundant skin with reduced elasticity. Mutations in the elastin coding gene have been shown to cause autosomal dominant cutis laxa in three families. A homozygous mutation in the fibulin-5 coding gene was discovered in a Turkish pedigree showing recessive inheritance, and a different mutation in this gene was found in the heterozygous state in a sporadic case of the disease. Here, we report the third case of a mutation in the fibulin-5 coding gene in a recessive Iranian cutis laxa pedigree. The mutation is the same as previously reported in the Turkish pedigree, further confirming that it is causative of disease. A haplotype consisting of seven intragenic sequence variations common to both pedigrees is described for the mutation-carrying fibulin-5 allele.

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INTRODUCTION

Cutis laxa is a heterogeneous group of rare inherited and acquired connective tissue disorders, whose common feature is occurrence of loose and redundant skin with decreased elasticity (Uitto and Pulkkinen, 2002). This phenotype and other characteristics are ultimately due to fragmentation and paucity of elastic fibers (Hashimoto and Kanzaki, 1975; Kitano *et al.*, 1989). Hereditary cutis laxa is inherited in both autosomal dominant and autosomal recessive modes (Beighton, 1972; Agha *et al.*, 1978). Autosomal dominant cutis laxa (OMIM 123700) is relatively benign, and has been shown in three families to be due to mutations in the elastin coding gene (*ELN*) (Tassabehji *et al.*, 1998; Zhang *et al.*, 1999). Three types of autosomal recessive cutis laxa with distinct phenotypic features have been described (Agha *et al.*, 1978; Genevieve *et al.*, 2004). Autosomal recessive type I cutis laxa (OMIM 219100) shows the most severe phenotype and has the poorest prognosis. In addition to the skin, internal organs enriched in elastic fibers, such as the lung and arteries, are affected. In 2002, it was reported that a homozygous missense mutation in the *FBLN5* gene coding fibulin-5 was responsible for cutis laxa type I in a large Turkish pedigree (Loeys *et al.*, 2002). *FBLN5* was considered a strong candidate gene because the phenotypic abnormalities observed in fibulin-5 knockout mouse models were very similar to those associated with autosomal recessive cutis laxa type I (Nakamura *et al.*, 2002; Yanagisawa *et al.*, 2002). Fibulin-5 is an integrin ligand with six EGF-like domains. In adults it is found mainly in tissues that contain abundant elastic fibers, including the lung and the skin. The mutation found in the Turkish family was deemed disease-causing because of segregation with the disease phenotype, absence among healthy individuals of the population, conservation of the coded amino acid across species and across other human fibulins, and predicted structural and functional consequences on the protein (Loeys *et al.*, 2002). After this initial report, a tandem 22 kb duplication within the *FBLN5* gene in the heterozygous state was reported in a sporadic case of cutis laxa (Markova *et al.*, 2003). It was shown that the mutated fibulin-5 mRNA and protein were overexpressed compared to wild-type forms, suggesting a transcriptional, processing, and/or stability effect on the mRNA and thus accounting for the dominant-negative effect of this mutation. The missense mutation in the Turkish pedigree was suggested to affect protein folding and cause instability, thus precluding the dominant-negative effect in carriers. Four cutis laxa patients have been found not to have mutations in the fibulin-5 or elastin coding genes, suggesting further genetic heterogeneity of the disease (Markova *et al.*, 2003). In this investigation, we sought disease-causing mutations in an Iranian cutis laxa pedigree.

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Abbreviations: ELN, elastin coding gene

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RESULTS

Size analysis of *FBLN5* proximal microsatellite markers in the proband and her parents was consistent with linkage of the cutis laxa phenotype with *FBLN5* (not shown). Linkage to *ELN* consistent with recessive inheritance was not observed (not shown). As such, each of the exons and flanking intronic sequences of *FBLN5* were amplified and sequenced. The proband carried a homozygous n.679T>C mutation in exon 7 of the *FBLN5* gene (nucleotide numbering uses the convention wherein A of ATG start codon in cDNA is annotated as nucleotide +1), resulting in a serine to proline substitution (p.S227P) in the fourth EGF-like domain of fibulin-5 protein (Figure 1). This is the same mutation as previously reported in the Turkish autosomal recessive cutis laxa pedigree (Loeys *et al.*, 2002). (The mutation in the Turkish pedigree was reported as T998C in exon 5, causing amino-acid substitution S227P. The authors of the article have reported in a personal communication that the mutation was misnumbered and was in fact the same n.679T>C alteration reported here.) After identification of the mutation in the proband, exon 7 was sequenced in her parents (IV: 1, IV: 2), maternal grandfather (III: 2) and paternal grandfather (III: 3), who were brothers, and an unaffected sibling (V: 3) (Figure 2). The parents and grandparents all carried the same mutation in the heterozygous state, indicating that the mutation had been stable through at least eight meiotic events. Both alleles of the unaffected sibling were wild type. The n.679T>C nucleotide change creates a *Hae*III restriction enzyme recognition site. *Hae*III digestion of PCR-amplified exon 7 amplicons indicated the absence of the site in the DNA of 50 control individuals from the North-West region of Iran, which includes Hamadan (not shown).

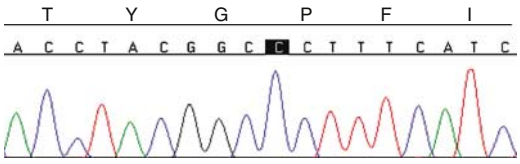

Figure 1. DNA sequence analysis of the region surrounding the n.679T>C mutation in proband. The site of mutated nucleotide and coded amino acids are indicated.

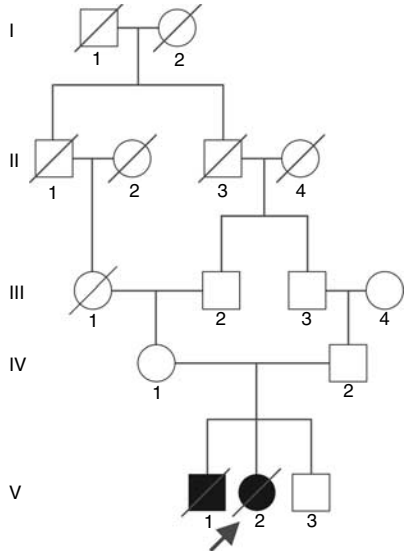

Figure 2. Pedigree of the cutis laxa family of Hamadan, Iran. The full pedigree consists of 106 individuals.

In addition to the putative disease-causing mutation, the DNA of the proband showed seven additional sequence variations as compared to the *FBLN5* genomic sequence NT_026437. One variation caused a synonymous codon change and six were intronic. The variation that caused a synonymous change was n.945T>C (rs2430347) in exon 9, which changed the coding of isoleucine 315 from ATT to ATC. The first intronic variation was IVS9+137delGTGTGTGT in intron 9, which caused a change in the number of a two-nucleotide repeat (17GT>13GT). The second, third, and fourth intronic variations, IVS9+154G>A, IVS9+167T>G (rs7148155), and IVS9-218A>G (rs2430342), were also in intron 9. The remaining two intronic variations, IVS10+68G>A (rs2430341) and IVS10-45A>G (rs929608), were both in intron 10. The first three intron 9 variations were all within a sequence of tandem GT repeats. The sequence after IVS9+137 in the reference sequence was 5′-GTGTGTGTGT GTGTGTGTGTGTGTGTGTGTGT-3′ and the sequence in the proband's DNA was 5′-GTGTGTGTATGTGTGTGTGTG GGTGT-3′. (This set of three changes is indicated by $X_{IVS9+137}$ in the haplotype in the Discussion.) All the variations were homozygous in the proband. None of these variations were predicted to affect splicing. All *FBLN5* sequence variations found in the DNA of the proband of the Iranian pedigree were also found in the homozygous state upon sequencing of genomic DNA of an affected member of the previously reported Turkish pedigree.

DISCUSSION

This is only the third report of a disease-causing mutation in *FBLN5* in a cutis laxa patient. The mutation reported here, n.679T>C in exon 7 (S227P), was the same as the one previously reported in a Turkish pedigree, and the inheritance pattern of the disease in both families carrying the mutation was autosomal recessive. The finding of the variation in our pedigree and its absence in 50 Iranian control individuals further strengthens the original authors' arguments that the S227P alteration in fibulin-5 protein can cause cutis laxa. Recently, it was reported that sequence variations in *FBLN5* may be involved in age-related macular degeneration (Stone *et al.*, 2004). In that study, no variation in the fibulin-5 gene which caused an amino-acid change, was found among 429 control individuals from the United States. Individuals III: 2 and III: 3 in the Iranian pedigree (Figure 2), known by sequencing to be heterozygous for n.679T>C, are both over 80 years old. They would not submit to an eye examination, but both appeared to have no or minimal visual difficulties, making it unlikely that they had age-related macular

degeneration. It may be that some amino acid alterations affect fibulin 5 functions not relevant to retinal morphology and physiology. Alternatively, the genetic and environmental backgrounds of the individuals may affect the penetrance of the variations in relation to retinal function.

As all seven sequence variations found in the DNA of our proband were homozygous, the haplotype defined by these variations on the mutation-carrying *FBLN5* allele can be unambiguously described as $C_{n.945}$ $X_{IVS9+137}$ $G_{IVS9-218}$ $A_{IVS10+68}$ $G_{IVS10-45}$, where the subscripts indicate positions of nucleotides in the *FBLN5* gene sequence (NT_026437) and X refers to the altered GT tandem repeat sequence described above. As the same haplotype was found on the n.679T>C-carrying allele of the Turkish pedigree, it is probable that the mutation in the two pedigrees had a common origin. Identification of a haplotype associated with the fibulin-5 n.679T>C mutation will be useful in future investigations on the gene and can help identify the origin of the mutation. Diagnosis of carrier state is being offered to all young members of the Iranian pedigree.

MATERIALS AND METHODS

Subjects

This study was performed after approval from the Ethics Review Board of the National Institute for Genetic Engineering and Biotechnology (Iran) and with informed consent of pedigree members in accordance with The Declaration of Helsinki Principles. The proband of the study belongs to a highly inbred pedigree whose members have resided in Hamadan for at least five generations (Figure 2). The pedigree has been previously described (Pour-Jafari and Sarihi, 2004). The patient was originally identified at the age of 12, at which time she appeared prematurely aged. Her family reported changed skin features, predominantly laxity of the skin, from when she was 2 years old. They also reported umbilical hernia and breathing difficulties during childhood. Her clinical features were described in a Farsi article (Sarihi *et al.*, 2004). Emphysema, indications of supravalvular aortic stenosis, and a very hoarse voice were among the features described. Light microscopy of skin biopsy sections depicted normal dermis, but thin and atrophied epidermis. Orcein Giemsa staining showed reduced and abnormal elastic fibers. Her karyotype was normal (46,XX). The patient died at the age of 14 and genetic studies were performed on cells of a skin fibroblast cell line, which had been prepared.

The patient was diagnosed with cutis laxa before death. A male sibling with similar abnormal skin features had died because of pulmonary infections at the age of 2, and it was surmised that he was also afflicted with cutis laxa. The existence of two afflicted siblings who were children of a consanguineous marriage between phenotypically normal individuals in a highly inbred pedigree suggests an autosomal recessive mode of inheritance.

Linkage and mutation analysis

DNA of the proband was prepared from cultured skin fibroblast cells and DNA of available members of the pedigree was extracted from leukocytes. Linkage to fibulin-5 and *ELN*s was determined using microsatellite markers located within 2 cM of the genes. Amplicons containing the repeat sequences were amplified from the DNA of the proband and her parents. Each of the 11 exons of the *FBLN5* gene and flanking intronic sequences of the proband's DNA were amplified by the PCR. In all, 9,509 nucleotides were sequenced, including 167 nucleotides upstream of site of initiation of transcription, the entire 5′-untranslated, coding and 3′-untranslated regions, and 412 nucleotides downstream of the 3′-end of the gene. More limited sequencing was performed on the DNA of an affected member of the previously reported Turkish pedigree, which had kindly been put at our disposal.

Sequence variations were determined by comparison with the NCBI genomic DNA reference sequences for the *FBLN5* gene NT_026437 and the *FBLN5* mRNA NM_006329. The reference numbers (rs#) of variations registered in build 124 of the NIH SNP database (http://www.ncbi.nlm.nih.gov) are provided. Effects of sequence changes on splicing were predicted by comparison with known canonical splice site motifs (http://www.fruitfly.org/seq_tools/splice.html).

CONFLICT OF INTEREST

The authors state no conflict of interest.

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