December 2010

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Journal of Dermatological Case Reports

Homozygous frame shift mutation in ECM1 gene in two siblings with lipoid proteinosis

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Key words:

extracellular matrix protein 1, ECM1, gene, genodermatosis, lipoid proteinosis, mutation

Abstract

Background: The extracellular matrix protein 1 (ECM1) is a glycoprotein, expressed in skin and other tissues. Loss-of-function mutation in ECM1 causes a rare autosomal recessive disorder called lipoid proteinosis. Lipoid proteinosis is presented by varying degrees of skin scars, beaded papules along the eyelid margins, variable signs of hoarseness of voice and respiratory disorders. More than 250 cases of this disorder have been described in the literature, but occurrence of lipoid proteinosis in siblings is very rare. This study was designed to investigate the possible mutation causing lipoid proteinosis in a Pakistani family and to elaborate the scope of possible genetic changes, causing the genodermatosis in Pakistan.

Main observations: In this study, two siblings (12 and 9-years sisters) were presented with scaly itchy lesions on whole body, hoarse voice and macroglossia. Their deceased father had similar clinical manifestations but mother and younger brother were unaffected. Blood samples from clinically affected and unaffected family members were collected with informed consent. The coding region of ECM1 gene containing 10 exons were amplified and sequenced.

Both the affected siblings were shown to have homozygous frame shift mutation by deletion of the nucleotide T at 507, codon 169, exon 6. This resulted in a frame shift from codon 169 and appearance of a premature stop codon at 177, causing formation of a mutated protein (176 amino acids) instead of normal ECM1 protein (540 amino acids).

Conclusion: A case of homozygous 62-bp insertion in ECM1 gene causing lipoid proteinosis has been reported in another Pakistani family. The current study presents a homozygous frame shift mutation supporting an unusual function of ECM1 protein and broadens the spectrum of disease-linked mutations in this rare case of genodermatosis in this region.

Background

The extracellular matrix protein 1 (ECM1) is a secreted glycoprotein, expressed in skin and other tissues. Loss-of-function mutation in ECM1 gene causes a rare, autosomal recessive disorder, known as lipoid proteinosis (LP).[1] LP, also known as hyalinosis cutis et mucosae or Urbach-Wiethe disease (OMIM: 24700) was first described in 1929.[2] It is characterized by varying degrees of hoarseness of voice and skin and mucosal derangements. Associated findings include epilepsy, mild mental retardation, respiratory tract obstruction, abnormal dentition and ocular abnormalities.[3] Histologically, there is widespread deposition of hyaline-like material and disruption or duplication of basement membrane around blood vessels and at the dermal-epidermal junction.

The pathophysiology of LP has been shown to result from loss-of-function mutations in the ECM1 gene located on chromosome 1q21.[3-5] The ECM1 protein has important physiological and biological roles in epidermal differentiation, binding of dermal collagens and proteoglycans and angiogenesis. The precise function of ECM1 is still unknown.[6] More than 20 pathogenic mutations have been reported so far, are, mostly nonsense, missense or splice site mutations with the majority occurring in exon 6 or 7 of the ECM1 gene

encoding a glycoprotein. Although over 250 cases have been reported in the literature, the occurrence of the disease in siblings is very rare.[7] In this study, ECM1 gene mutation in two siblings with LP from Sindh province of Pakistan was analyzed to understand the spectrum of mutation in this case of genodermatosis.

Objective

The aim of this study is to investigate the possible mutation in ECM1 gene which causes LP in Pakistani family and to elaborate the scope of possible genetic changes, causing the genodermatosis in Pakistan.

Subjects & Methods

Subjects

The patients were two sisters of 12 and 9 years of age suffering from LP, from Karachi, Pakistan. Both the patients were presented with scaly itchy lesions on the whole body, hoarse voice and macroglossia. These lesions started at the age of 7 months in elder sister and 2½ years in the younger. Their hoarseness/ low pitch voice was so severe that it was difficult to hear them. On examination they had eczema, with multiple infections and scars involving face, chest and back. Both sisters had characteristic features of lipoid proteinosis such as multiple beaded papules along the eyelids, fissured lips and slight macroglossia with a hard woody tongue having a homogeneous look (Fig. 1). According to the mother, her husband died at the age of 35 years with similar skin problem and hoarseness whereas their only brother was normal (Fig. 2). Routine investigations (Blood CP, SUCE & LFT) along with Abdomen USG and CT scan were normal. However, buccal mucosa biopsy with PAS stain was suggestive of this condition.

Methods

Following informed consent and approval from the institutional ethical committee, DNA of both the patients and their clinically unaffected family members were extracted from peripheral blood by the standard phenol-chloroform DNA extraction method.[8] ECM1 gene was amplified by Polymerase Chain Reaction (PCR) using eight sets of primers (forward and reverse) encompassing all 10 exons as described earlier[9] and briefly given in Table 1.

For PCR amplification, the 50 µL PCR reaction volume was used containing 1xPCR buffer, 250 ng genomic DNA, 0.6 mM each primer, 1.5 mM MgCl2, 0.2 mM each dNTPs and 2.5 U Taq DNA polymerase. The amplification was performed by using standard protocol as described earlier.[9,10] In PCR, initial denaturation was performed at 95˚C for 5 minutes, followed by 35 cycles of 95˚C for 45 seconds, primer-specific annealing temperature for 45 seconds, and 60˚C for 45 seconds. PCR products (5 µL) were analyzed by 2.5% agarose gel electrophoresis. PCR products were then purified by using commercial kit (QIAquick PCR Purification Kit, Qiagen, Crawley, UK) and sequenced directly using Big Dye® Terminator v3.1 cycle sequencing kit in an ABI 310 genetic analyzer (Applied Biosystems, Foster City, CA, USA).

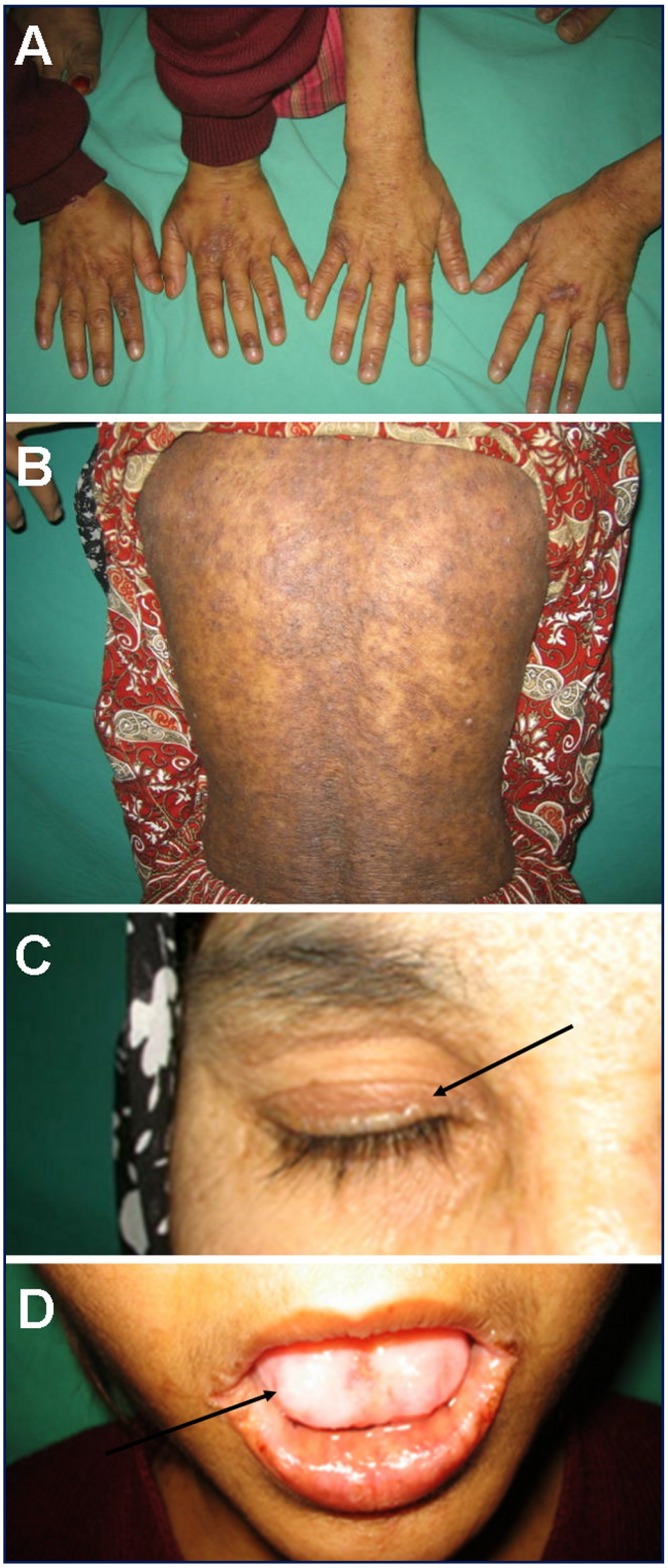

Figure 1

Lipoid proteinosis. Warty skin on the dorsal aspect of hands in both siblings (A), acneiform scars on the back (B), flesh-colored beaded papules on the edges of eyelids (C) and oral mucosa with yellow-white infiltrates.

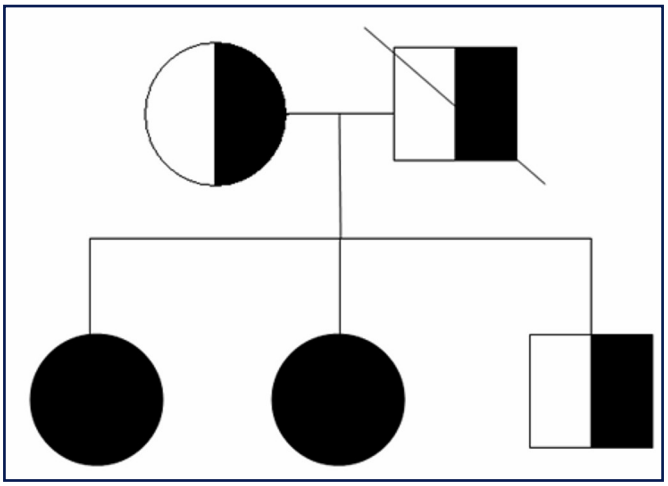

Figure 2
Pedigree of the family studied shows that both the parents are heterozygous carrier. Two sisters are homozygous patients and the only brother is unaffected (carrier).

Results

Direct sequencing of PCR products from the affected siblings with LP were shown to have homozygous frame shift mutation by deletion of a nucleotide T at 507 (c.507delT), codon 169 in exon 6 (Fig. 3). The mutation c.507delT followed a frame shift from codon 169 and the appearance of downstream premature stop codon, which resulted in a 176 amino acid protein truncation. The mutation was homozygous in both the affected siblings. The screening of control subjects did not disclose the presence of that mutation. The normal ECM1 protein contains 540 amino acids. The frame shift mutation has resulted in appearance of a mutated protein containing 176 amino acids (supplementary data).

Table 1. Genomic primers used for PCR amplification of ECM1.

ECM1 Exons	Primer Sequence (5'-3')	PCR Product Size (bp)	Annealing Temperature	MgCl2, mM
ECM1-1F	AGCTGGGACTGAAGTCATGGC	416	62°C	1.5
ECM1-1R	TAAAGGCTCCACTGGCCTAG			
ECM1-2/3F	TCCTACACTCTTGATCTCCA	622	59°C	1.5
ECM1-2/3R	GGTGTCAACAGGATCCATAG			
ECM1-4/5F	CAGTGACCCTCCAGGTTTCT	484	59°C	1.5
ECM1-4/5R	CAGAGCCCACCGTCTTGTCT			
ECM1-6F	AGCCTTGAGAAGCAGGAGGA	671	59°C	1.5
ECM1-6R	AGTGAACGGGACCTGAGGTT			
ECM1-7F	TTATCTGCCTGCCCAGTGTC	548	59°C	1.5
ECM1-7R	ACATGGATGGATGGACTGGC			
ECM1-8F	CACATCAACAGTTGCCTCCT	499	59°C	1.5
ECM1-8R	GGCATCTTCTGGCATCAGAT			
ECM1-9F	AGTTGCCTAGTCCTTCCCCA	408	60°C	1.5
ECM1-9R	AGGCCAGGTCAGAGTGAAGA			
ECM1-10F	AATCCAGCTGTGCAAGGCAG	469	62°C	1.5
ECM1-10R	GTAATGAGTGTTCAGATGGG			

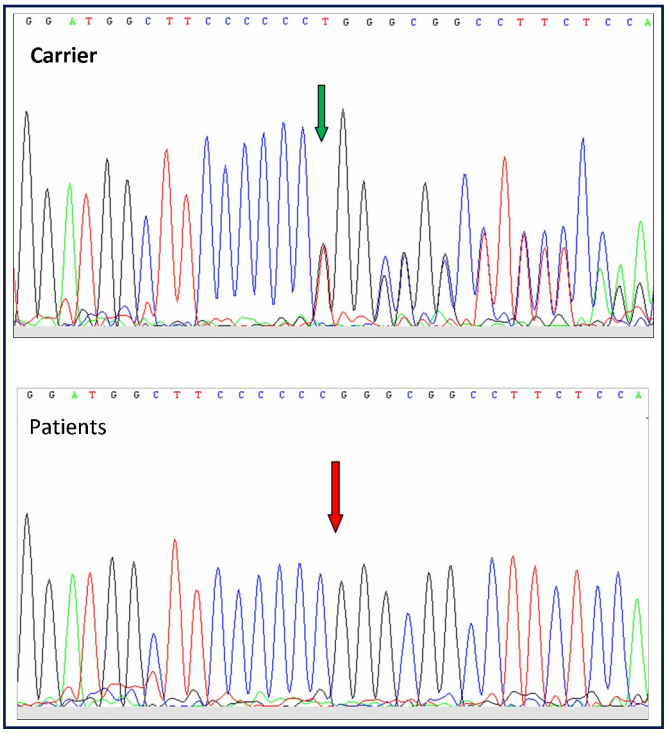

Figure 3

Direct sequencing of PCR products amplified from exon 6 of ECM1 gene. The patients show a homozygous mutation of c.507delT (red arrow) as compared to Carrier without deletion (green arrow).

Discussion

LP is a rare autosomal recessive disorder with heterogeneous clinical manifestations from varying degrees of skin infiltrations and scars, hoarseness of voice, respiratory distress and extracutaneous implications.[2] A number of genetic studies showed the involvement of mutations in ECM1 gene in this disorder which is located on chromosome 1q21.[2,9,11] It is found worldwide, but is more common in some countries, such as South Africa, where a founder effect has been demonstrated.[10] Most cases of LP involve loss-of-function mutations in ECM1. To date, 41 distinct germline missense, nonsense, splice site, small and large deletions and insertions, have been reported. Approximately 50% of the mutations cluster to exon 6 and 7 of the gene.[12] Almost all the mutations of ECM1 published in LP are supposed to lead to low or absent mRNA or protein expression except very few missense mutations.[13]

This study reports an unusual homozygous frame shift mutation in exon 6 in two sisters suffering from LP of a Pakistani family. This mutation in this region has not been reported hitherto. Both the patients were shown to have homozygous frame shift mutation by deletion of the T nucleotide at position 507, codon 169, exon 6 of ECM1 gene. This mutation resulted in a frame shift from codon 169 and the appearance of premature stop codon at 177. It results in the premature truncation of the normal ECM1 protein and the appearance of a mutated protein containing 176 amino acids instead of a normal protein that contains 540 amino acids.

A case of homozygous 62-bp insertion in ECM1 causing LP has already been reported in another Pakistani family.[6]

The current study presents a homozygous frame shift mutation supporting an unusual function of ECM1 protein and broadens the spectrum of disease-linked mutations in this rare case of genodermatosis in this region. The identification of this mutation in ECM1 gene may be helpful in understanding the better role of the gene in LP. The results of this study may also be used to establish a phenotype-genotype correlation, more precise and accurate prognosis and/or diagnosis, carrier screening for the transfer of LP with in families horizontally as well as vertically and also in making the effective strategies for genetic counseling in this regard.

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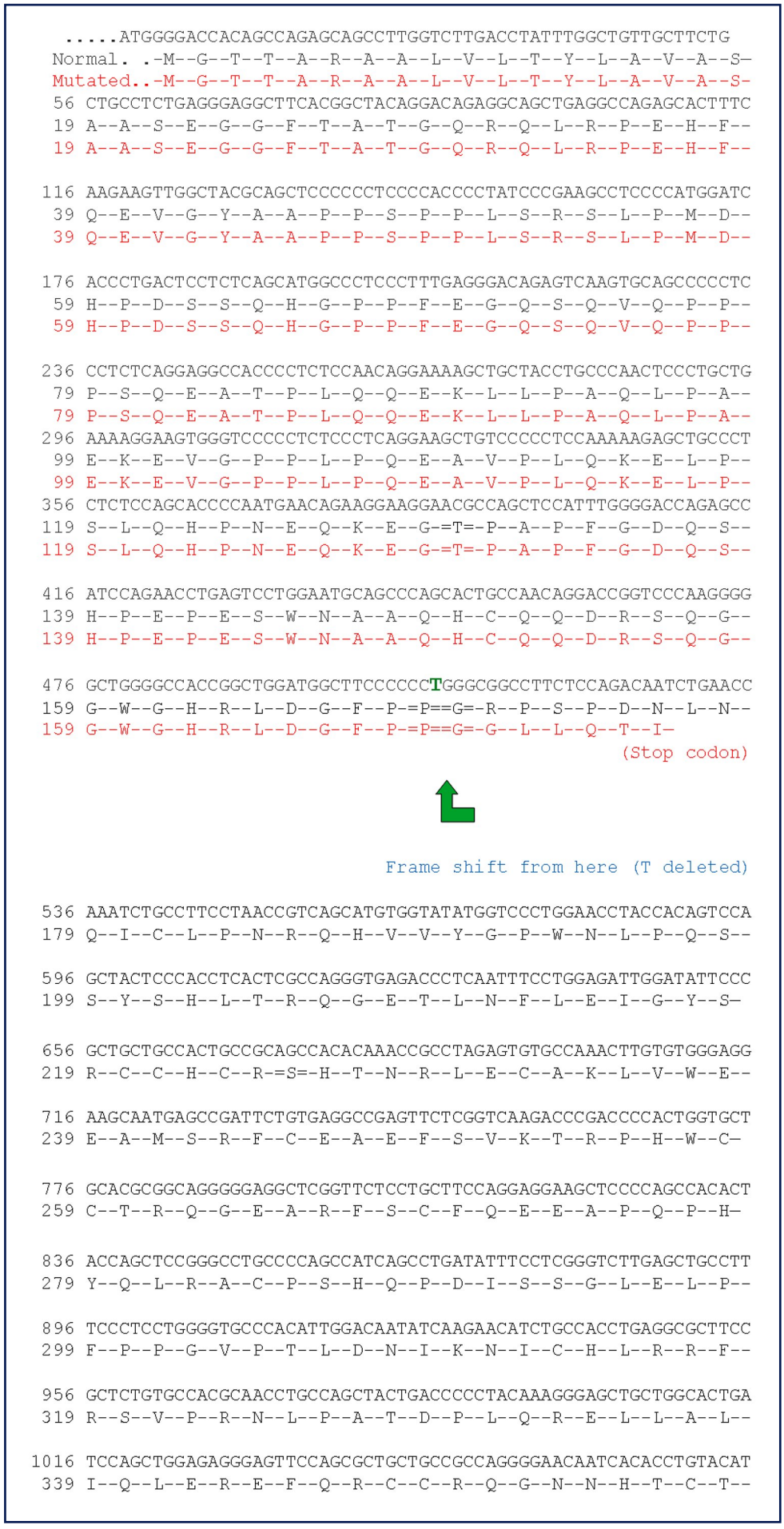

Figure 4

Complete ECM1 gene (normal and mutated) showing premature termination at 177.