Mutation Analysis of ECM1 Gene in Two Related Iranian Patients Affected by Lipoid Proteinosis

Saeid Morovvati¹, Paniz Farshadyeganeh², Mojdeh Hamidizadeh², Ziba Morovvati³, Samaneh Doost Mohammadi⁴

¹ Human Genetic Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran
² School of Basic Sciences, Tehran Medical Branch, Islamic Azad University, Tehran, Iran
³ Department of Medical Genetics, Faculty of Advanced Medical Technologies, Golestan University of Medical Sciences, Gorgan, Iran
⁴ Faculty of Basic Sciences, Islamic Azad University of Zanjan, Zanjan, Iran

Received: 17 May 2017; Accepted: 18 Dec. 2017

Abstract- Lipoid proteinosis (LP) is a rare autosomal recessive disorder caused by malfunction mutations in extracellular matrix protein 1 gene (ECM1) with common features such as hoarseness of the voice, infiltration of the skin and mucosa, and varying degrees of skin scars. We studied two LP patients. Clinical and genetic examination and genetic counseling were carried out, and their family pedigree was drawn. Two different variants were found in exon 6 of ECM1 gene in both patients: a homozygous deletion of a nucleotide T at position 507 and a missense variant at nucleotide 389 which the first was a pathogenic mutation and the other one was a non-pathogenic variant.

Keywords: Extracellular matrix protein 1 gene; Lipoid proteinosis; Pathogenic and non-pathogenic; Mutations

Introduction

Lipoid Proteinosis (LP) (OMIM 247100), also known as Urbach-Wiethe disease, is known with features such as hoarseness of the voice, beaded papules on the edges of the upper eyelids, pock-like or acneiform scars on the face and extremities, infiltration of the skin and mucosa, thickening of the sub-lingual frenulum resulted in reduced tongue movement (1,2). There are also some neurological disorders such as epilepsy, mental retardation, and impaired memory and neuropsychiatric manifestations like paranoia, mistrustfulness, suspicion, and aggressive manner (3,4). According to Horve L et al., “the various manifestations are attributed to the infiltration of hyaline-like material.” (5) Although most appeared symptoms of this disorder are related to dermatology, actually it is not just a skin disorder, and it is a dermatogenetic disorder (6).

LP is a rare autosomal recessive disorder discovered by Urbach and Wiethe in 1929, and initially named “lipoidosis cutis et mucosae” which is caused by malfunction mutations in extracellular matrix protein 1 gene (ECM1) (7,8). ECM1 is a glycoprotein that is coded by an ECM1 gene on chromosome 1q21. The ECM1 gene has three known splice variants, and it has several important biological functions such as its role in extracellular matrix formation, cell adhesion, cell signaling, and regulation of tissue differentiation and maturation (5,9). In this article, we described genetic and clinical features of two LP patients from two related families.

Case Report

Initially, the patients were under clinical examination, and genetic counseling and their family pedigree were drawn. Informed consent for genetic studies of all participants was obtained. Blood samples were taken from two LP patients and their parents. DNAs were extracted by the standard phenol-chloroform method. For amplification, primers were designed of all exons of the ECM1 gene (Table 1). For all exons, the PCR mixture (25 µl) contained 0.3 µl Taq DNA polymerase, 0.6 µl mgcl₂, 1 µl dNTPs, 2.5 µl PCR buffer 10X, 9 µl ddH2O, 2 µl of each primer, and 2 µl extracted DNA. DNA denaturation was performed for 4 min at 95°C, followed by 30 cycles of amplification at 95°C for 45 s, 61°C for 45 s, and 72°C for 45 s, and a final extension step at 72°C for 7 min. PCR
products were analyzed by 1.5% agarose gel electrophoresis and stained with ethidium bromide. Then we performed DNA sequencing for all PCR products with BigDye terminator chemistry (Applied Biosystems, Foster City, CA) and the ABI PRISM 377 genetic sequencer (Applera Corporation, Norwalk, CT).

Table 1. Primers used for PCR amplification of ECM1 gene

<table>
<thead>
<tr>
<th>Exon</th>
<th>Forward primer</th>
<th>Revise primer</th>
<th>Annealing temperature (°C)</th>
<th>PCR product size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exon 1</td>
<td>AACCGTAACAGCCACCAGAC</td>
<td>CAGAAAAAGGAAAGCAAAAAGC</td>
<td>61</td>
<td>291</td>
</tr>
<tr>
<td>Exon 2, 3</td>
<td>ATACAGAGGGCGCATCTCGTG</td>
<td>CAACTCCTCCTGAAAAAGGA</td>
<td>61</td>
<td>597</td>
</tr>
<tr>
<td>Exon 4, 5</td>
<td>TCAAGCACCCTCCAGGGTTC</td>
<td>GCAAAAGAAGAACCTGGAAAGC</td>
<td>61</td>
<td>649</td>
</tr>
<tr>
<td>Exon 6</td>
<td>TCTCTCCACCCAGAGATC</td>
<td>GAAGGCAGAAAGTGGAAAGG</td>
<td>61</td>
<td>650</td>
</tr>
<tr>
<td>Exon 7</td>
<td>CCTGGCTCTGGAGGACC</td>
<td>GTCCATCCATGTGGATACGCAC</td>
<td>61</td>
<td>714</td>
</tr>
<tr>
<td>Exon 8, 9</td>
<td>TCTCCACAGGCAAGACATC</td>
<td>CAGAAAAGTCTTCCACCTGACCTG</td>
<td>61</td>
<td>804</td>
</tr>
<tr>
<td>Exon 10</td>
<td>AGGGAAACGAGGAGAGAG</td>
<td>CACCCACAGCAACACACCAC</td>
<td>61</td>
<td>554</td>
</tr>
</tbody>
</table>

Results

Patient 1, a 7-year-old male, and patient 2, a 14-year-old male, were relative. As shown in the pedigree, in the six generations there are two male patients affected by LP (Figure 1). The clinical features of these two patients were hoarse voice that has been started since infancy, small nodules on the edges of the upper eyelids with a beaded appearance, pock-like scars particularly on the face and extremities, thickening of the skin on the elbows and knees region, relative skin fragility, a shortened tongue with a thickened sub-lingual frenulum that caused inability to fully protrude the tongue, white lesions on the oral mucosa, bilateral intracranial calcifications, and panic attacks. Although both patients had similar symptoms, patient 1 showed mild symptoms in comparison with patient 2.

![Figure 1](image1.png)

**Figure 1.** Pedigree of the family: the filled box shows the affected children, and the half-filled box shows the heterozygote parents

After clinical examination, a genomic analysis was performed by using DNA of two LP patients and their parents. A homozygous deletion of a nucleotide T at position 507 was found in both patients in exon 6 (c.507delT) and their parents were heterozygous for this mutation (Figure 2). In addition, we found a C>T transition at nucleotide 389 in exon 6 (c.389C>T) which patients were homozygous and their parents were heterozygous for it (Figure 3).

![Figure 2](image2.png)

**Figure 2.** Direct sequencing of PCR products amplified from exon 6 of ECM1 gene. The patient shows a homozygous mutation c.507delT of ECM1 in exon 6 (black arrow), and his father as carrier shows heterozygous mutation of the same position (blue arrow)

![Figure 3](image3.png)

**Figure 3.** Direct sequencing of PCR products amplified from exon 6 of ECM1 gene. The patient shows a homozygous mutation c.389C>T of ECM1 in exon 6 (black arrow) and his father as carrier shows heterozygous mutation of the same position (blue arrow)

Discussion

The ECM1 gene has three known splice variants, including ECM1a, ECM1b, and ECM1c which are respectively coding proteins with 540, 415, and 559 amino acids. ECM1b lacks exon 7, and ECM1c contains an additional exon which is within intron 5 (10,11). Furthermore, the ECM1a transcript has a more significant overall biological role in human skin and mucosae in comparison with the ECM1b transcript (12). According to this fact, we worked on ECM1a in this study and found
Mutation analysis of ECM1 gene

two different variants in ECM1 gene in each LP patient. The first variant was a homozygous deletion in exon 6 named as NM_004425.3 (ECM1_v001):c.507delT due to the Human Genome Variation Society (HGVS) nomenclature, which causes a frameshift mutation as p.(Arg171Glyfs*7). The result of this frameshift mutation in codon 171 was a premature stop codon at 177, which developed a truncated protein of 176 amino acids long, instead of the normal ECM1 protein which has 540 amino acids. Besides, because of nonsense-mediated mRNA decay, any protein products will be degraded. Hamada et al. has previously detected this mutation in two unrelated patients with LP. It is believed that the change happens due to slipped mispairing during replication because T nucleotide is surrounded by palindromic sequences of multiple C or G repeats (12). This frameshift mutation is a null variant in a gene where loss of function (LOF) is a known mechanism of disease. The variant was neither found in ExAC nor 1000 Genomes Project database. Multiple lines of computational evidence support a deleterious effect on the gene or gene product (Table 2). Based on the American College of Medical Genetics and Genomics (ACMG) standards and guidelines for the interpretation of sequence variants, these collective evidence support p.(Arg171Glyfs*7) as a recessive pathogenic variant for LP disease.

Another variant was a missense at nucleotide 389 in exon 6 named as NM_004425.3 (ECM1_v001):c.389C>T due to HGVS nomenclature. This variation converted threonine amino acid at position 130 to methionine. The p(Thr130Met) variant has previously been identified as a common missense polymorphism (12), has been recorded in dbSNP (rs3737240), and observed in the ExAC, and 1000 Genomes Project databases at a frequency of 34.12% and 22.38%, respectively. Multiple lines of computational in-silico evidence predict a benign impact on the resultant protein (Table 2). Furthermore, in our study, this variation is observed in cis with the pathogenic variant, which is supporting evidence of a variant to be interpreted as benign. Interestingly, Hamada et al. showed that one of the two unrelated patients with the first pathogenic mutation p (Arg171Glyfs*7) was homozygote for C in 389 and the other patient was homozygous for the T allele in this position. Moreover, missense variants in a gene for which primarily truncating variants are known to cause the disease also support the benign effect. Based on the ACMG standards and guidelines, the observed high allele frequency (>5%) is considered as stand-alone evidence to interpret p(Thr130Met) as a benign variant with respect to the disease.

So we come to the conclusion that the mutation p (Arg171Glyfs*7) is the causative pathogenic mutation responsible for the disease in patients of the studied Iranian pedigree.

Acknowledgments

We thank the staff of the Biogene Medical and Genetic Laboratory of Tehran for their assistance in this research.

References