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

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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.
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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 $mgcl_2$, 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

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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

Exon	Forward primer	Revise primer	Annealing temperature (°C)	PCR product size (bp)
Exon 1	AACCGTAACAGCCACCAGAC	CAGAAAAGGAAAGCCAAACG	61	291
Exon 2, 3	ATACAGAGGGGCATCTCGTGT	CAACTCCCTGCTGAAAAGGA	61	597
Exon 4, 5	TCAGTGACCCTCCAGGTTTC	TGCACAAAGAAGAACTCAGCAG	61	649
Exon 6	TCTCCTCCACCCCAGATTC	GAAGGCAGGAATGTGGAAAG	61	650
Exon 7	CCTGGGTCTGGAGGAACC	GTCCATCCATTGTGTTTGTCAC	61	714
Exon 8, 9	TTCCCAAGCCCACACATC	CAGAAAAGTCTTCACTCGACCTG	61	804
Exon 10	AGGGGAACGAGGGAGAGAG	CACACCACAGACAAACACACC	61	554

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 an 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.

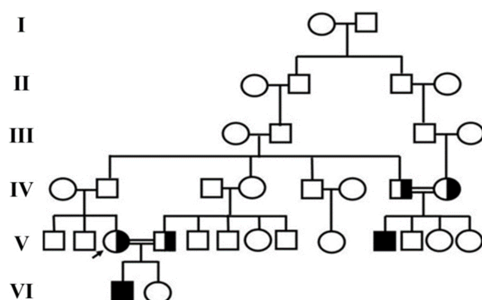

Figure 1. Pedigree of the family: the filled box shows the affected children, and the half-filled box shows the heterozygote parents

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).

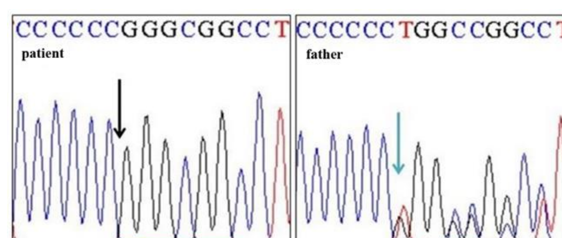

Figure 2. Direct sequencing of PCR products amplified from exon 6 of *ECM1* gene. The patient shows a homozygous mutation c.507delT of *ECM1* in exon 6 (black arrow), and his father as carrier shows heterozygous mutation of the same position (blue arrow)

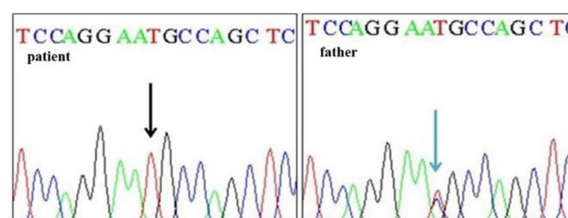

Figure 3. Direct sequencing of PCR products amplified from exon 6 of *ECM1* gene. The patient shows a homozygous mutation c.389C>T of *ECM1* in exon 6 (black arrow) and his father as carrier shows heterozygous mutation of the same position (blue arrow)

Discussion

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

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.

Table 2. Computational in-silico analysis of variations found in this study

Gene	Ensemble transcript ID / Genbank transcript ID	Variation	Population database: ExAC MAF	1000Gp MAF	dbSNP	Mutation taster	Predictive tools: Fathmm	SIFT	Provean	Poly phen	Mutation assessor	CADD phred
ECM1	ENST00000369047 NM_004425	c.507delT	0	0	No reported	Disease causing	NA°	damaging	NA°	NA°	NA°	23.9
		c.389C>T p.T130M	0.3412	0.2238	Rs3737240	Poly morphism	Tolerated	Tolerated	Natural	Benign	Low	0.135

*Not available, because the tool is specific for prediction of missense variations only

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.

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References

1. Dinakaran S, Desai SP, Palmer IR, Parsons MA. Lipoid proteinosis: clinical features and electron microscopic study. Eye (Lond) 2001;15:666-8.
2. Chan I, Liu L, Hamada T, Sethuraman G, McGrath JA. The molecular basis of lipoid proteinosis: mutations in extracellular matrix protein 1. Exp Dermatol 2007;16:881-

90.

3. Kleinert R, Cervós-Navarro J, Kleinert G, Walter GF, Steiner H. Predominantly cerebral manifestation in Urbach-Wiethe's syndrome (lipoid proteinosis cutis et mucosae): a clinical and pathomorphological study. Clin Neuropathol 1987;6:43-5.
4. Friedman L, Mathews RD, Swanepoel PD. Radiographic and computed tomographic findings in lipid proteinosis. A case report. S Afr Med J 1984;65:734-5.
5. Horev H, Potikha T, Ayalon V, Molho-Pessach V, Ingber A, Gany MA, et al. A novel splice-site mutation in ECM-1 gene in a consanguineous family with lipoid proteinosis. Exp Dermatol 2005;14:891-7.
6. Urbach E, Wiethe C. Lipoidosis cutis et mucosae. Virchows Arch A Pathol Pathol Anat 1929;273:285-319.
7. Hamada T, McLean WH, Ramsay M, Ashton GH, Nanda A, Jenkins T, et al. Lipoid proteinosis maps to 1q21 and is caused by mutations in the extracellular matrix protein 1 gene (*ECM1*). Hum Mol Genet 2002;11:833-40.
8. Hamada T. Lipoid proteinosis. Clin Exp Dermatol 2002:27:624-9.
9. Liu W, Xu W, Yang X, Lian S. A novel missense mutation of the ECM1 gene in a Chinese patient with lipoid proteinosis. Clin Exp Dermatol 2012;37:28-30.
10. Mongiat M, Fu J, Oldershaw R, Greenhalgh R, Gown AM, Iozzo RV. Perlecan protein core interacts with extracellular matrix protein 1 (ECM1), a glycoprotein involved in bone formation and angiogenesis. J Biol Chem 2003;278:17491-9.
11. Smits P, Ni J, Feng P, Wauters J, Van Hul W, Boutaibi ME, et al. The human extracellular matrix gene 1 (ECM1): genomic structure, cDNA cloning, expression pattern, and chromosomal localization. Genomics 1997;45:487-95.
12. Hamada T, Wessagowit V, South AP, Ashton GH, Chan I, Oyama N, et al. Extracellular matrix protein 1 gene (ECM1) mutations in lipoid proteinosis and genotype-phenotype correlation. J Invest Dermatol 2003;120:345-50.