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A novel missense mutation in exon 7 of the *ECM1* gene in an Iranian lipoid proteinosis patient

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ABSTRACT. Lipoid proteinosis (LP) is a rare autosomal recessive disorder. Classical clinical features include warty skin infiltration, papules on the eyelids, skin scarring, as well as extracutaneous abnormalities such as hoarseness of the voice, epilepsy, and neuropsychiatric abnormalities. A defect in the ECM1 gene is responsible for this disease. A 21-year-old female patient from consanguineous parents (first cousins) was referred to our clinic with many symptoms of LP, such as hoarse voice from infancy, diffuse acneiform scars on her face, and hyperkeratosis on her knees and elbows. The entire ECM1 gene was screened using PCR and sequencing. A novel missense mutation was found in exon 7 of this patient. We report a novel missense mutation in exon 7 of the ECM1 gene found in an Iranian LP patient that causes a C269Y amino acid exchange.

Key words: Lipoid proteinosis; Autosomal recessive; ECM1 gene; Mutation; Sequencing; PCR

INTRODUCTION

Lipoid proteinosis (LP), also known as hyalinosis cutis et mucosae, was first described by Urbach and Wiethe in 1929 (Urbach and Wiethe, 1929).

The pattern of its inheritance is autosomal recessive. The clinical characteristics of this disease are hoarse voice, infiltration and scarring of the skin and mucous membranes, and brain calcifications. Skin scars are atrophic or acneiform and may follow trauma. Often, there are warty papules and plaques on the elbows, hands, and knees, as well as eyelid papules known as moniliform blepharosis. Mucous membranes often have a cobbled, hard texture, and tongue movement is reduced (Muda et al., 1995).

Molecular genetic studies of LP patients have revealed that mutations in the ECM1 gene located on chromosome 1q21.2 are responsible for this disease (Oz et al., 2002).

The ECM1 gene encodes the glycoprotein extracellular matrix protein 1 (Hamada et al., 2002).

The ECM1 protein has important physiological and biological roles in epidermal differentiation, binding of dermal collagens and proteoglycans, and regulation of angiogenesis (Han et al., 2001).

To date, more than 40 pathogenic mutations have been reported, including missense, nonsense, frame shift, or splice site mutations, with the majority occurring in exons 6 and 7 (Wang et al., 2006).

Here, we report a novel mutation found in exon 7 of an Iranian LP patient.

MATERIAL AND METHODS

Patient

The patient was a 21-year-old female with LP from consanguineous parents (first cousins). She had suffered from a hoarse voice since infancy and had diffuse acneiform scars on her face. Hyperkeratosis of the knees and elbows was observed. The movement of her tongue was limited because of a thickened epithelium. She did not exhibit other common symptoms of LP (Figure 1A and B). The parents and siblings of this patient were not affected.

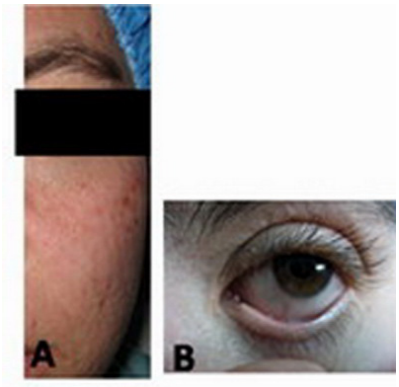

Figure 1. Acneiform scars on the face of the patient (**A**) and moniliform blepharosis (**B**).

DNA extraction

A genomic DNA sample of the patient and her parents was extracted from peripheral blood by Diatom DNA Prep 200 (Isogen Lab Ltd., Russia).

Genotyping

The primers that were used for the amplification of ECM1 gene exons (1-10) and flanking regions are shown in Table 1 (Hamada et al., 2003).

Table 1. Intronic-genomic primers used for PCR amplification of ECM1.

Exon No.	Forward primer (5'→3')	Reverse primer (5'→3')	Annealing temperature (°C)	Product size (bp)
1	agctgggactgagtcatggc	taaaggctccactggcctag	62	416
2/3	tcctacactcttgatctcca	ggtgtcaacaggatccatag	61	622
4/5	cagtgaccctccaggtttct	cagagcccaccgtcttgtct	62	484
6	agccttgagaagcaggagga	agtgaacgggacctgaggtt	61	671
7	ttatctgcctgcccagtgtc	acatggatggatggactggc	57	548
8	cacatcaacagttgcctcct	ggcatcttctggcatcagat	61	499
9	agttgcctagtccttcccca	aggccaggtcagagtgaaga	60	408

The PCR mixture included 2 µM primer, 400 µM of each dNTP (BIORON, Germany), Taq DNA polymerase 1X reaction buffer with 1 mM $MgCl_2$, and 2 U Taq polymerase (5 U/µL, BIORON).

The amplified segments were analyzed by electrophoresis on a 1% agarose gel, stained with ethidium bromide, observed under ultraviolet light, and sequenced directly in an ABI 310 Genetic Analyzer (Applied Biosystems, USA).

The patient's family and normal controls provided informed consent and the study was conducted with approval from the Scientific and Ethics Committees of NIGEB.

RESULTS

Sequencing of the PCR products amplified from this patient revealed a homozygous G>A transition at nucleotide c.806 in exon 7, which changes a cysteine residue to tyrosine (TGC→TAC) at amino acid 269 (C269Y). No other changes or polymorphisms were found in the coding region of exon 7 in this patient. Both parents were heterozygous carriers, and the 25 normal controls were negative for this mutation (Figure 2). The family pedigree and the result of the sequence from exon 7 of the patient and the parents are shown in Figure 3.

DISCUSSION

LP is an autosomal recessive disorder. The disease has been reported in many countries worldwide, but has been seen more frequently in South Africa.

The loss of function in the ECM1 gene is responsible for this disease. ECM1 was

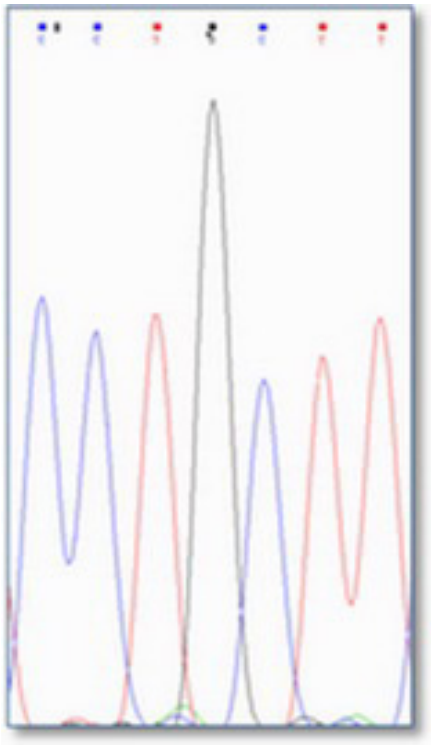

Figure 2. Sequence of an unrelated healthy control individual failed to disclose the presence of the mutation.

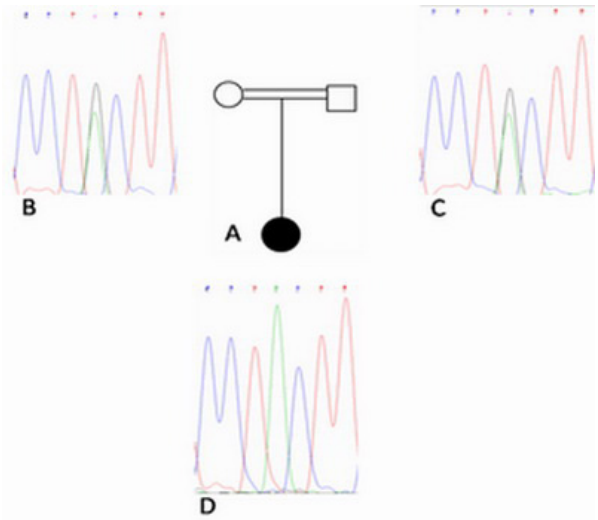

Figure 3. A. Pedigree of patient family. **B.** Heterozygous missense mutation G806A in the mother of the patient. **C.** Heterozygous missense mutation G806A in the father of the patient. **D.** Homozygous missense mutation G806A in the patient.

identified as a novel 85-kDa protein secreted by a mouse osteogenic stromal cell line in 1994 (Mathieu et al., 1994); in 1995, it was shown that the full-length cDNA is 1.9-kb long and contains a 1677-bp open reading frame that codes for a 559-amino acid protein (Bhalerao et al., 1995). The ECM1 gene appears to be expressed as a 1.8-kb transcript predominantly in the placenta and heart, while an additional 1.4-kb alternatively spliced message has been detected in the tonsils. The full-length human *ECM1* transcript contains 1838 bp and has an overall homology of 79.6% with the mouse *Ecm1* cDNA.

The ECM1 human gene contains 10 exons (Smits et al., 1997). To date, more than 40 distinct germline missense, nonsense, splice site, and small and large deletions and insertions have been reported (Van Hougenhouck-Tulleken et al., 2004; Horev et al., 2005; Chan et al., 2003, 2004, 2007).

Most of them were missense, nonsense, deletions/insertions, and splice site mutations. Missense mutations have rarely been reported in this gene.

Here, we found a novel missense mutation in exon 7 of an Iranian patient. The mutation in this exon can affect ECM1a or/and ECM1b. Hamada et al. (2002) found that the LP patients with mutations in exon 7 exhibited a less severe phenotype than those with mutations in other exons. Although we did not detect any skull radiologic sign or seizure in our patient history evaluation, the condition of this patient was like that of other LP patients that had no mutation in exon 7. Therefore, we cannot report that she exhibited the less severe phenotype.

This missense mutation changes a cysteine residue to tyrosine (TGC→TAC) at amino acid 269 (C269Y).

Hamada et al. (2002) also reported an insertion mutation in exon 8 and a deletion mutation in exon 7 in two Iranian LP cases. We believe this is the third Iranian LP case in which the disease was confirmed genetically, although the defect is a missense mutation in exon 7.

Hamada et al. (2002) identified homozygosity for a single-base pair deletion (A1019) in exon 7 of the ECM1 gene in affected individuals of a consanguineous Kuwaiti family. The mutation resulted in a premature stop codon 108 bp downstream (Hamada et al., 2002).

Hamada et al. (2002) also identified a homozygous 14-year-old Pakistani boy with a C-to-T transition at position 1036 in exon 7 of the ECM1 gene. He was also a product of consanguineous parentage. The mutation resulted in conversion of codon gln346 to a premature stop codon (Q346X).

Direct sequencing of exon 7 of ECM1 in affected South African patients by Hamada et al. (2002) revealed Q276X, Q346X, W359X, and 1019delA mutations in exon 7. They also reported 735delTG, 785delA, and 892delC mutations in exon 7; the combined data so far suggest that the majority of mutations occur in exon 7 (Hamada et al., 2002).

CONCLUSIONS

In summary, we report one LP patient with many classical features of LP in whom the ECM1 defect was caused by a novel missense mutation in exon 7. Our patient exhibited some of the common clinical symptoms of LP, such as hoarseness, acneiform scars, and limited tongue motion.

This patient, possessing a novel missense mutation in exon 7, belonged in the midportion category of signs and symptoms. It should be noted that during the treatment process, one session of resurfacing laser surgery with a CO_2 laser on both vocal cords was carried out, and her hoarseness improved when she was evaluated after 12 months.

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REFERENCES

Bhalerao J, Tylzanowski P, Filie JD, Kozak CA, et al. (1995). Molecular cloning, characterization, and genetic mapping of the cDNA coding for a novel secretory protein of mouse. Demonstration of alternative splicing in skin and cartilage. *J. Biol. Chem.* 270: 16385-16394.

Chan I, El-Zurghany A, Zendah B, Benghazil M, et al. (2003). Molecular basis of lipoid proteinosis in a Libyan family. *Clin. Exp. Dermatol.* 28: 545-548.

Chan I, Sethuraman G, Sharma VK, Bruning E, et al. (2004). Molecular basis of lipoid proteinosis in two Indian siblings. *J. Dermatol.* 31: 764-766.

Chan I, Liu L, Hamada T, Sethuraman G, et al. (2007). The molecular basis of lipoid proteinosis: mutations in extracellular matrix protein 1. *Exp. Dermatol.* 16: 881-890.

Hamada T, McLean WH, Ramsay M, Ashton GH, et al. (2002). Lipoid proteinosis maps to 1q21 and is caused by mutations in the extracellular matrix protein 1 gene (ECM1). *Hum. Mol. Genet.* 11: 833-840.

Hamada T, Wessagowit V, South AP, Ashton GH, et al. (2003). Extracellular matrix protein 1 gene (ECM1) mutations in lipoid proteinosis and genotype-phenotype correlation. *J. Invest. Dermatol.* 120: 345-350.

Han Z, Ni J, Smits P, Underhill CB, et al. (2001). Extracellular matrix protein 1 (ECM1) has angiogenic properties and is expressed by breast tumor cells. *FASEB J.* 15: 988-994.

Horev L, Potikha T, Ayalon S, Molho-Pessach V, et al. (2005). A novel splice-site mutation in ECM-1 gene in a consanguineous family with lipoid proteinosis. *Exp. Dermatol.* 14: 891-897.

Mathieu E, Meheus L, Raymackers J and Merregaert J (1994). Characterization of the osteogenic stromal cell line MN7: identification of secreted MN7 proteins using two-dimensional polyacrylamide gel electrophoresis, western blotting, and microsequencing. *J. Bone Miner. Res.* 9: 903-913.

Muda AO, Paradisi M, Angelo C, Mostaccioli S, et al. (1995). Lipoid proteinosis: clinical, histologic, and ultrastructural investigations. *Cutis* 56: 220-224.

Oz F, Kalekoglu N, Karakullukcu B, Ozturk O, et al. (2002). Lipoid proteinosis of the larynx. *J. Laryngol. Otol.* 116: 736-739.

Smits P, Ni J, Feng P, Wauters J, et al. (1997). The human extracellular matrix gene 1 (ECM1): genomic structure, cDNA cloning, expression pattern, and chromosomal localization. *Genomics* 45: 487-495.

Urbach E and Wiethe C (1929). Lipoidosis cutis et mucosae. *Virchows Arch. Pathol. Anat. Physiol.* 273: 285-319.

Van Hougenhouck-Tulleken W, Chan I, Hamada T, Thornton H, et al. (2004). Clinical and molecular characterization of lipoid proteinosis in Namaqualand, South Africa. *Br. J. Dermatol.* 151: 413-423.

Wang CY, Zhang PZ, Zhang FR, Liu J, et al. (2006). New compound heterozygous mutations in a Chinese family with lipoid proteinosis. *Br. J. Dermatol.* 155: 470-472.

