Indian J Med Res 143, March 2016, pp 303-307
DOI:10.4103/0971-5916.182620

Extracellular matrix protein 1 gene (*ECM1*) mutations in nine Iranian families with lipoid proteinosis

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Received June 24, 2013

***Background & objectives*: Lipoid proteinosis (LP) is an autosomal recessive disease. Clinical characteristics of this disease are hoarse voice, scarring of the skin, brain calcifications, and eyelid papules (moniliform blepharosis). Mutations in the *ECM1* gene on 1q21.2 are responsible for this disease. This study was conducted to investigate the mutation spectrum of *ECM1* gene in nine Iranian families having at least one LP patient diagnosed clinically.**

***Methods*: The entire *ECM*1 gene was screened using PCR and direct sequencing in nine Iranian families with 12 suspected LP patients who were referred to the clinic, along with their parents and siblings. Thirty healthy individuals were included as controls.**

***Results*: In only one patient a homozygous G>A transition at nucleotide c.806 in exon 7 was detected. A *G*>A substitution at nucleotide 1243 in exon 8 that changes glycine (GGT) to serine (AGT) was observed in most of our patients. Furthermore, in one patient there was a change in the sequence of intron 8, the A>T transition in nucleotide 4307. In addition, in two cases (one patient and one healthy mother with affected child) there was a C (4249) deletion in intron 8.**

***Interpretation & conclusions*: Our results indicate that although mutation in *ECM*1gene is responsible for lipoid proteinosis, it is likely that this is not the only gene causing this disease and probably other genes may be involved in the pathogenesis of the LP disease.**

Key words Disease - *ECM*1gene - lipoid proteinosis - mutation - pathogenic - sequencing

Lipoid proteinosis (LP) is a rare autosomal recessive disorder characterized by a hoarse voice, infiltration and scarring of the skin and mucous membranes, and brain calcifications. Skin scars are atrophic or acneiform and may follow trauma. There are often 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[1,2]. More than 300 cases have been reported in the world literature[3]. Pathogenic mutations have been found in the extracellular matrix protein 1 gene (*ECM1*). ECM1 is a glycoprotein which is expressed in skin and other tissues[4,5]. The ECM1 protein has important physiological and biological roles in epidermal differentiation, binding of dermal collagens and proteoglycans, and regulation of angiogenesis[6].

More than 40 pathogenic mutations have been reported in this gene including missense, nonsense, frame shift or splice site mutations with the majority occurring in exons 6 and 7[7]. Here we report mutation spectrum of *ECM1* gene in Iranian patients clinically suspected to have LP disease from nine families.

Material & Methods

In total, nine families having at least one LP patient diagnosed clinically in Hazrat Rasool Hospital, Tehran, Iran during 2010-2012 were enrolled in this study. Following informed written consent, peripheral blood (n=30) samples (2 ml) from 12 suspected LP patients, their parents and siblings (if possible) and peripheral blood samples from 30 healthy individuals (as a control group aged 18-30 yr, volunteers) were collected. The study protocol was approved by the Ethics Committee of Hazrat Rasool Hospital.

PCR for ECM1 detection: The genomic DNA was extracted from peripheral blood samples of all the cases and controls by Diatom DNA Prep 200 kit [Isogen Lab, Russia]. The primers used for amplification of *ECM1* gene exons (1-10) and flanking region were chosen as described before[8]. The PCR mixture included 2 μM primer, 400 μM of each dNTP (BIORON, Germany), Taq DNA polymerase 1× reaction buffer with 1mM $MgCl_2$ and 2 unit Taq polymerase (5 U/μl), (BIORON, Germany). PCR conditions for all reactions were 94°C for 5 min; 35 cycles with denaturation at 94°C for 45 sec, annealing for 45 sec at (different for each primer) and elongation at 72°C for 45 sec; one cycle at 72°C for 5 min; and a final hold at 4°C. Amplified segments were analyzed by electrophoresis on a 1 per cent agarose gel, stained with ethidium bromide, and observed under ultraviolet light. The amplicons were sequenced directly in an ABI 310 genetic analyzer (Applied Bio systems, US).

Statistical analysis: Statistical analysis was performed using the SPSS software V16.0 (SPSS, Inc., Chicago, IL). The difference in genotype frequencies between controls and patients was determined using chi-square test.

Results & Discussion

In total, nine families including 12 clinically diagnosed LP patients and 18 possible carries (parents and siblings) were studied. All patients except of family 3, were from consanguineous marriages. There were nine female (75%) and three male (25%) patient. The youngest patient was one month old and the oldest was 17 yr old (the median age 4 yr).

The most common features of the patients were hoarseness, multiple beaded papules on the eyelid margins (moniliform blepharosis) and infiltrated plaques on the elbows and the knees (hyperkeratosis). The less common features were skin scaring, thickening and/or enlargement of the tongue, thickening of the mucosae of the vocal cords and subglottic region, skin-coloured papules on the nose, verrucous plaques on the knees, elbows and hands, neuropathological abnormality, atrophic alopencia plaques, yellowish papules and ice-pick scars on the forehead and along the rims of the eyelids, paranoid behaviour and xerostomia.

Sequencing of PCR products revealed a homozygous G>A transition at nucleotide c.806 in exon 7 which changes a cysteine residue to tyrosine (TGC→TAG) at amino acid 269 (C269Y) was detected in one family (family 6)[9]. The *G*>A substitution at nucleotide 1243 in exon 8 that changes glycine (GGT) to serine (AGT) was observed, patient 1 of family 1 (Table). The genotype of this patient was AA at this position and her parents' genotypes were AG and AA (Fig. 1). In the same patient there was a change in the sequence of intron 8 that changes the A>T transition in nucleotide 4307 (Fig. 2). In two families (one patient in family 2 and one healthy mother in family 3 with affected child) there was a C (4249) deletion in intron 8 (Fig. 3). The patient was a 23 yr old girl with no history of other affected family members. The summary of the sequencing results of all the families is presented in the Table. Some of the common features of patients are shown in Fig. 4.

Loss of function mutations in *ECM*1 gene is responsible for LP disease. Though this disease occurs worldwide, but seems to be more common in some populations, such as South Africa where all patients had homozygosity for a nonsense mutation in exon 7 of the *ECM1* gene and haplotype analysis using markers from a 9.98 Mb region around the ECM1 locus

Table. The summary of sequencing result of all 9 families

	Consanguineous marriage	Genotype at 1234 position in exon 8	Genotype at 4307 position in intron 8	Deletion at 4249 position in intron 8	homozygous G>A transition at nucleotide c.806 in exon 7
Family 1 members					
Healthy father	Yes	AG	TT	No change	
Healthy mother	first cousin	AA	AA	No change	
Affected daughter (20 yr) (patient 1)		AA	AT	No change	
Family 2 members					
Healthy father	Yes	AG	No change	No change	
Healthy mother		GG	No change	No change	
Affected daughter (23 yr) (patient 2)		AG	No change	Yes	
Family 3 members					
Healthy father	No	AG	No change	No change	
Healthy mother		AG	No change	Yes	
Healthy sister		AG	No change	No change	
Affected daughter (27 yr) (patient 3)		AA	No change	No change	
Family 4					
Healthy father	Yes	AA	No change	No change	
Healthy mother		AA	No change	No change	
Affected son (6 yr) (patient 4)		AA	No change	No change	
Family 5					
Healthy father	Yes	GG	No change	No change	
Healthy mother		GG	No change	No change	
Affected daughter (8 yr) (patient 5)		GG	No change	No change	
Affected daughter*		GG	No change	No change	
Family 6					
Healthy father	Yes	AG	No change	No change	AG
Healthy mother	first cousin	AG	No change	No change	AG
Affected daughter (21 yr) (patient 6)		GG	No change	No change	AA
Family 7					
Affected father (45 yr) (patient 7)					
Healthy mother	Yes	GG	No change	No change	
Affected daughter (20 yr) (patient 8)		GG	No change	No change	
Affected son (22 yr) (patient 9)		GG	No change	No change	
		GG	No change	No change	
Family 8					
Healthy father	Yes	AG	No change	No change	
Healthy mother		GG	No change	No change	
Affected daughter (42 yr) (patient 10)		GG	No change	No change	
Family 9					
Healthy father	Yes	AG	No change	No change	
Healthy mother		GG	No change	No change	
Affected daughter (21 yr) (patient 11)		GG	No change	No change	

*Phenotypes not available

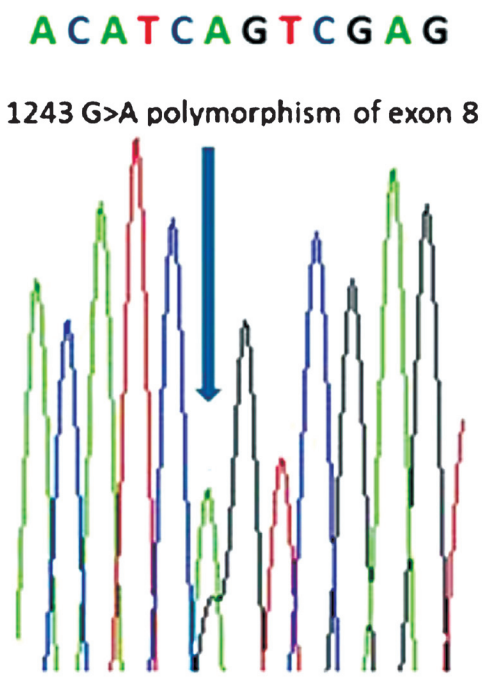

Fig. 1. 1243 *G*>A polymorphism of exon 8. Sequencing result of the 20 yr old female patient from family 1 with beaded papules on the eyelid. *G*>A substitution at nucleotide 1243 in exon 8 that changes glycine (GGT) to serine (AGT) is shown with arrow.

Fig. 2. Sequencing result of the 20 yr old female patient from family 1 with atrophic scarring. The change in the sequence of intron 8 which substitute the A (4307) to T (shown with arrow) was detected in the patient.

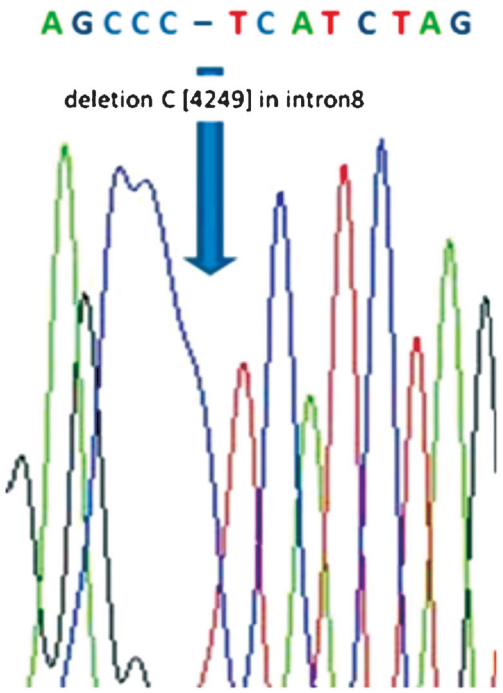

Fig. 3. Sequencing result of the 20 yr old female patient from family 2 with beaded papules on the eyelid. A deletion in the sequence of intron 8 in C (4249) position was detected in this patient.

confirmed the founder effect[10]. More than 41 distinct germ line missenese, nonsense, splice site, small and large deletions and insertions have been reported[11-16].

In many reports only a few cases or families have been analyzed. For example, Horev *et al*[12] and Syed *et al*[13] analyzed only one family[13], and the *ECM1* gene was amplified and sequenced in two siblings (18 and 24-year-old) of a Pakistani family. Both patients showed non-pathogenic missense and silent mutations in exons 6 and 8. We analyzed nine families and among them only in one family we reported a pathogenic substitution in exon 7 of *ECM1* gene[9], the rest of the patient had either no nucleotide changes or polymorphisms.

In a study by Hamada *et al*[8] heteroduplex analysis of amplified DNA from normal controls identified similar band shifts in several of the PCR products spanning exons 6 and 8. Sequencing revealed two point mutations, a C>T transition at nucleotide 389 in exon 6 that converts a threonine residue (ACG) to methionine (ATG), and a *G*>A substitution at nucleotide 1243 in exon 8 that changes glycine (GGT) to serine (AGT). For the 1243 G/A polymorphism, the major allele was

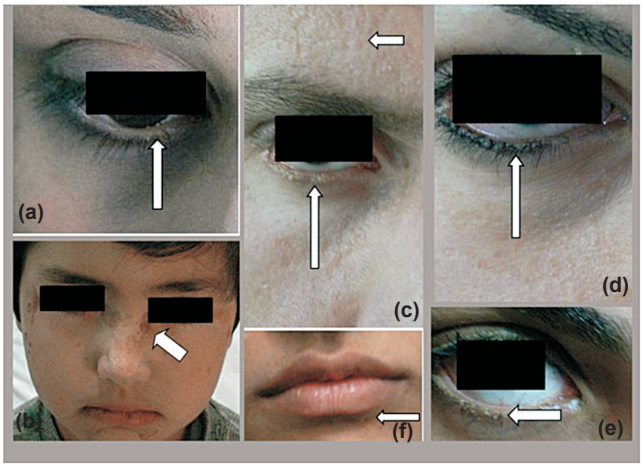

Fig. 4. Common features of some of the patients with lipoid proteinosis. **(a)** the patient from family 3 with beaded papules on the eyelid; **(b)** the patient from family 4 with atrophic scarring; **(c)** the first patient from family 7 with beaded papules on eyelid and atrophic scarring; **(d)** the second patient from family 7 with beaded papules on eyelid; **(e)** the patient from family 8 with beaded papules on eyelid; **(f)** the patient from family 8 with atrophic scarring.

G (53%) and the minor allele was A (47%)[8]. A *G*>A substitution at nucleotide 1243 in exon 8 that changes glycine (GGT) to serine (AGT) was observed in most of our patients.

The allele frequencies of G and A were 70 and 30 per cent among patients. The allele frequencies of G and A among their unaffected parents and siblings were 58 and 42 per cent and among healthy controls were 50 and 50 per cent, respectively. However, the frequency was not significantly different between patient group and unaffected parents and siblings group.

In conclusion, our findings show a few pathogenic *ECM1* mutations in suspected LP patients. However, it is possible that *ECM1* is not the only gene responsible for LP disease, and other genes may also be involved. Further studies need to be done to confirm these findings.

Conflicts of Interest: None.

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