

# Lipoid proteinosis and epilepsy: Molecular analysis

Mehmet Tecellioglu<sup>1</sup>, Ozden Kamisli<sup>1</sup>, Ceren Acar<sup>2</sup>, Hatice Mergen<sup>3</sup>, Mert Sozen<sup>2</sup>, Tugce Karaduman<sup>3</sup>, Gulbahar Sarac<sup>4</sup>, Mehmet Fatih Erbay<sup>5</sup>

<sup>1</sup>Inonu University Faculty of Medicine, Department of Neurology, Malatya, Turkey

<sup>2</sup>Inonu University Faculty of Medicine, Department of Biology and Genetics, Malatya, Turkey

<sup>3</sup>Hacettepe University Faculty of Medicine, Department of Biology Ankara, Turkey

<sup>4</sup>Inonu University Faculty of Medicine, Department of Dermatology, Malatya, Turkey

<sup>5</sup>Inonu University Faculty of Medicine, Department of Radiology, Malatya, Turkey

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## Abstract

**Aim:** Lipoid proteinosis (LP) is also known as Urbach-Wiethe disease which is a rare autosomal recessive disorder characterized by intracellular accumulation of hyaline material in skin and mucosae. LP has typical neurological, dermatological and radiological findings. The pathogenesis of disease is unknown. In literature several cases have been reported up to date. Mutations in extracellular matrix protein 1 (ECM1) gene have been found to cause the disease. We evaluated the molecular features of a family diagnosed with LP and evaluate the known and novel mutations of LP.

**Material and Methods:** Genomic DNA was extracted from peripheral blood of the patients and family members including clinically normal parents and two siblings of the two affected subjects by using a commercial DNA extraction kit. Polymerase chain reaction was performed for all 10 exons of ECM1 gene by using the primers defined.

**Results:** All of the exons of the ECM1 gene were sequenced and this revealed a 2-bp deletion in exon 7 of the ECM1 gene in both patients and both parents. Patients have the homozygous 2-bp deletions (c735del TG) and the parents and two healthy siblings showed heterozygous 2-bp deletion.

**Conclusions:** Our patients found to be homozygous for the deletion in ECM1 gene. In order to understand the molecular pathology of the disease in detail, further functional analysis of the mutations should be performed.

**Keywords:** Lipoid Proteinosis; Ecm1; Genetics; Epilepsy.

## INTRODUCTION

Lipoid proteinosis (LP) or Urbach–Wiethe disease is a rare autosomal recessive disorder characterized by the intracellular accumulation of hyaline material in the skin and mucosa (1,2). LP has typical neurological, dermatological, and radiological findings (3). The pathogenesis of LP is unknown. Symptoms usually begin at birth or in early infancy (4). The most common symptoms are voice changes, hoarseness, dysphagia, epileptic seizures, and neuropsychiatric symptoms (2). Histologically, widespread intercellular deposits of periodic acid-Schiff (PAS)-positive hyaline material accumulate in the dermis (5).

Numerous cases of LP have been reported (6-9). It is caused by mutations in the extracellular matrix protein 1 (ECM1) gene, which is located on 1q21.2 (3). ECM1 takes

part in various biological processes, including epidermal differentiation, the regulation of angiogenesis and dermal collagen, and proteoglycan binding (10). It is believed that that the mutations that cause LP most commonly occur in exons 6 and 7 of the ECM1 gene (11).

There have been several case reports of LP in Turkey, where consanguineous marriage is still common, but few molecular studies. We examined the molecular features of a family diagnosed with LP and evaluated the known and novel LP mutations.

## MATERIAL and METHODS

### Subjects

This study examined the clinical, molecular, and radiological findings of two siblings and their family members with skin manifestations and epileptic seizures, followed at the Department of Medicine Neurology, Inonu

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Corresponding Author: Mehmet Tecellioglu, Inonu University Faculty of Medicine, Department of Neurology, Malatya, Turkey

E-mail: mehmettecelli@hotmail.com

University, Turkey. The molecular analyses were performed at the Department of Molecular Biology and Genetics, Inonu University and the Department of Biology, Hacettepe University. The study was performed in accordance with the tenets of the Declaration of Helsinki and was approved by our local ethics committee. All participants were fully informed regarding the study protocol and provided written consent before the study commenced.

#### PCR amplification and genomic DNA mutation screening

Genomic DNA was extracted from the peripheral blood of the family members, including the clinically normal parents and siblings of the two affected subjects, using a commercial DNA extraction kit (PureLink™ Genomic DNA Mini Kit). The polymerase chain reaction (PCR) was performed for all 10 exons of the ECM1 gene using previously reported primers (12). The amplification consisted of 94°C for 3 min followed by 35 cycles of 94°C for 30 s, 58°C for 30 s, and 72°C for 30 s, with a final 5 min at 72°C. The PCR products were analyzed by 1.5% agarose gel electrophoresis and visualized under ultraviolet light by ethidium bromide staining. Before the sequence analysis, the PCR products were subject to enzyme-based purification using 4 µL of PCR product, 0.4 µL Exonuclease I (10 U/µL), and 0.4 µL shrimp alkaline phosphatase (1 U/µL) at 37°C for 20 min, 80°C for 20 min, and 94°C for 2 min. The primers used for PCR were also used for the sequencing reactions. Treated samples were sequenced directly using Big Dye labeling in an ABI 310 genetic analyzer (Applied Biosystems). The sequencing reaction was performed for 25 cycles at 96°C for 10 s, 50°C for 5 s, and 60°C for 4 min. Possible mutations were confirmed by bidirectional sequencing or restriction endonuclease digestion using 15 µL PCR product, 2 µL buffer, 2.5 µL H<sub>2</sub>O, and 0.5 µL enzyme, which were incubated overnight at 37°C.

## RESULTS

#### Clinical findings

Our first patient was a 39-year-old woman who had a history of a hoarse voice and itchy, blistering skin eruptions on her body and face. Her symptoms started at 1 year of age. She had generalized tonic-clonic seizures for about 10 years, which were more frequent while sleeping. She takes valproate 1,000 mg/day and carbamazepine 800 mg/day. The seizure frequency is now 1–2 per month. The patient's neurological examination was within normal limits, while neuropsychological tests revealed a mild cognitive decline.

The second patient was a 35-year-old woman with similar voice and skin complaints and epileptic seizures. Her seizures were focal motor seizures with loss of awareness. She also has oroalimentary automatisms. The seizures began at the age of 25 years and occurred 1–2 times per week. She was taking lamotrigine 200 mg/day, but we increased the dose to 300 mg/day and added levetiracetam. The seizure frequency decreased to once every 3–4 months and neuropsychological tests were normal.

The two patients were sisters and both were started on antidepressant treatment for depression.

Among five siblings, the two girls had symptoms, while the other two sisters, a brother, and both parents were healthy. The parents were first cousins. Figure 1 shows the family pedigree of LP.

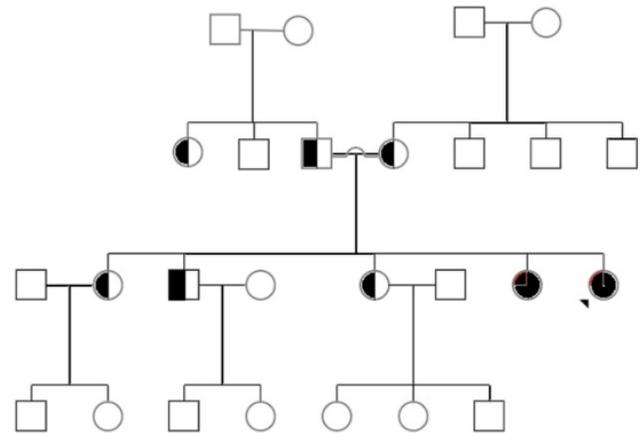


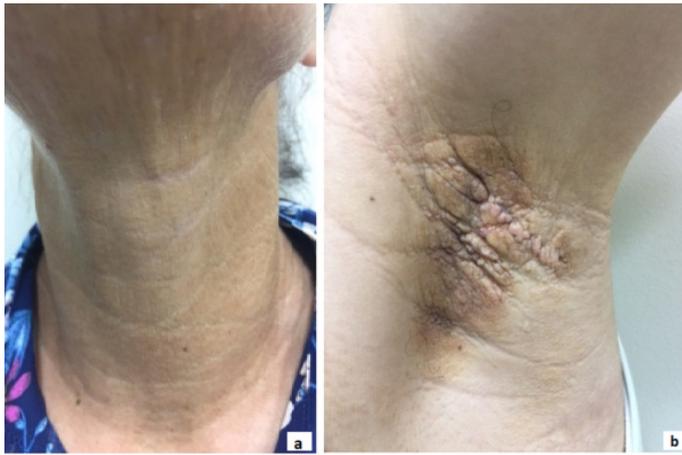
Figure 1. Pedigree of the LP family

#### Dermatological findings

Both patients showed similar findings on the dermatological examination. The face was covered with widespread, tiny yellowish papules and a few white atrophic scars (Figure 2). Face and neck wrinkles were prominent, and 2–3 mm, linearly arranged, transparent papules were observed along both upper eyelid margins (Figure 3a). There were many varicelliform scars on the anterior and posterior parts of the trunk and a 15-cm plaque formed by coalescence of approximately 3-cm yellowish papules was observed in both axillary regions (Figure 3b). Mucocutaneous examination of the oral cavity revealed tiny papules on the upper and lower lips, along with a whitish, sclerotic frenulum (Figure 4); mucocutaneous examination of the genitalia was within normal limits. Histopathologically, a punch biopsy taken from a trunk lesion showed a mildly hyperkeratotic epidermis with edema and accumulation of eosinophilic hyaline material in the dermis. There was PAS-positive hyaline material around vessels and skin appendages.



Figure 2. Photograph shows skin-coloured lesions of patients



**Figure 3a.** Photograph of the patients transparent papules along the both upper eyelid margins-3b. Photograph of the yellowish papules in bilateral axillary regions



**Figure 4.** Photograph of the eyelid and sclerotic frenulum of the patients

**Radiological findings**

Axial cranial tomography images showed symmetrical calcifications in the bilateral amygdala and uncus. Brain magnetic resonance imaging showed hypointense lesions in the medial temporal area on axial T1- and T2-weighted images, and hyperintense lesions in the lateral ventricle frontal horns bilaterally in the flair images (Figure 5-6).

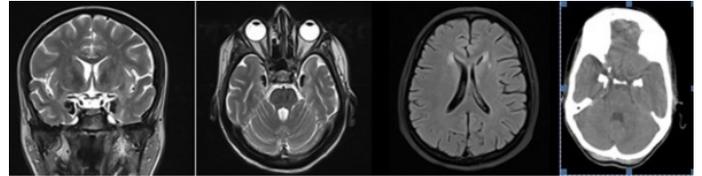
**EEG and video-EEG study**

Waking and sleep electroencephalography (EEG) were normal in the first case. In the second case, we observed one stereotypical seizure during long-term video-EEG monitoring. There were epileptiepileptic form discharges in the left temporo-occipital region on interictal EEG and the ictal activity originated from the left fronto-temporal area (Figure 7).

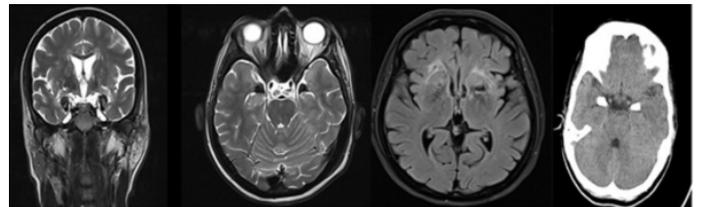
**Molecular analysis**

All of the exons of the ECM1 gene were sequenced and this revealed a 2-bp deletion in exon 7 of the ECM1 gene in both patients and both parents. The mutation was confirmed by bidirectional sequencing and restriction fragment length polymorphism (RFLP) analysis. Both patients were homozygous. Both parents, the two sisters,

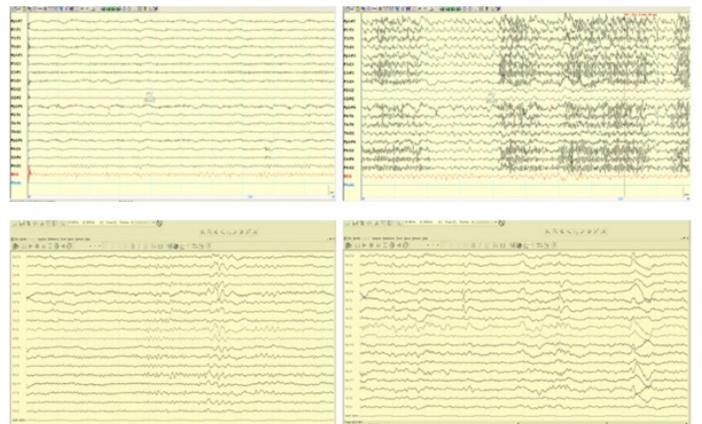
the brother, and an aunt were heterozygous, while an uncle was normal in the RFLP analysis.



**Figure 5.** First patient's radiological findings: bilaterally temporal calcifications and hyperintense lesions in lateral ventricles frontal horns



**Figure 6.** Second patient's radiological findings: bilaterally temporal calcifications and hyperintense lesions in lateral ventricles frontal horns



**Figure 7.** EEG findings of the patient

**DISCUSSION**

LP is a heterogeneous clinical syndrome with many neurological, psychiatric, dermatological, and otorhinolaryngological symptoms (2). It is a rare autosomal recessive disorder, in which the clinical findings usually manifest in early infancy as voice changes due to hyaline deposition in the vocal cords (4). Dermatological symptoms and signs are the most common features, such as papules, nodules, and cicatrix (13). Histopathologically, there is extensive dermal and mucosal accumulation of hyaline (14). Neuropsychiatric symptoms and epilepsy are common extracutaneous symptoms due to brain involvement (4,15-16).

LP results from mutations of the ECM1 gene located on chromosome 1q21. The ECM1 gene has 10 exons (10). Known mutations lead to low or absent mRNA or protein expression (5,11). A few missense mutations have been reported in LP (5). The ECM1 mutation is the only known cause of LP. Hamada et al. described the first six ECM1

mutations (12). Currently, about 58 different pathogenic ECM1 mutations have been reported in LP, including distinct nonsense, missense, and splice site mutations, and large and small deletions or insertions (11). Approximately 50% of the mutations are found in exons 6 and 7 (5). The majority of LP patients have homozygous ECM1 mutations, although heterozygous mutation genotypes are also seen (11,17). A recent study reported five novel mutations from Egypt (11).

Seizures are the most common neurological symptom of LP. The exact incidence of seizures/epilepsy in LP is not known. The seizures may be resistant to antiepileptic drugs (14,18-19). Clinically, both of our patients had drug-resistant epilepsy: one experienced only focal seizures with impaired awareness, and the other had focal and secondary generalized tonic-clonic seizures. Our patients' neuroimaging results showed calcifications and atrophy in the bilateral medial temporal areas. Although the mechanism of the epilepsy is not known, calcifications in cortical areas can lead to seizures due to abnormal electrical activity between neurons (13). ECM1 function is abnormal in LP, so impaired interactions between ECM1 and matrix metalloproteinase 9 could also be related to the epilepsy and neuropsychiatric symptoms (20).

Both of our patients had the same mutation and both had epilepsy: it is necessary to determine the relationship between the mutations and symptoms. Although bilateral mesial temporal calcifications may be the cause of seizures, not all patients with calcifications have epilepsy (16). In their series, Oguz-Akarsu et al. found that 57% of the patients had epileptic seizures (16). Patients with exon 7 mutations do not have a mild epilepsy phenotype (12). Oguz-Akarsu et al. pointed that a compound heterozygous mutation in LP may be associated with more seizures than homozygous mutations of the ECM1 gene (16).

## CONCLUSION

Our patients were both homozygous for the deletion in the ECM1 gene. To understand the detailed molecular pathology of LP, further functional analysis of the mutations should be performed.

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*Mehmet Tecelioglu ORCID: 0000-0002-8381-9630*

*Ozden Kamisli ORCID: 0000-0003-1114-7860*

*Ceren Acar ORCID: 0000-0003-1842-9203*

*Hatice Mergen ORCID: 0000-0002-7980-8197*

*Mert Sozen ORCID: 0000-0002-1672-2206*

*Tugce Karaduman ORCID: 0000-0003-0728-0968*

*Gulbahar Sarac ORCID: 0000-0002-7246-6382*

*Mehmet Fatih Erbay ORCID: 0000-0002-1596-3147*

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