European Journal of Ophthalmology / Vol. 17 no. 3, 2007 / pp. 424-429

SHORT COMMUNICATION

Lattice corneal dystrophy type II: Clinical, pathologic, and molecular study in a Spanish family

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PURPOSE. *To report a family with lattice corneal dystrophy type II (LCD II) associated with systemic amyloidosis type V.*
METHODS. *A 69-year-old woman presented a LCD II and marked dermachalasis. A lower blepharoplasty was performed. Two years later a penetrating keratoplasty was performed in her left eye. Three children of the patient were studied. Subtle manifestations of LCD were identified in two of them. Pathologic study of the excised skin and corneal button was made. DNA from peripheral blood was obtained, and was subjected to amplification of exon 5 of the gelsolin.*
RESULTS. *Pathologic examination of the skin of blepharoplasty specimen demonstrated the presence of amyloid. Microscopic examination of the corneal button showed the presence of amyloid deposits beneath the normal-appearing Bowman layer and also within the stroma. Immunostaining for S-100 protein did not demonstrate a significant relationship between amyloid deposits and corneal nerves. Electron microscopic evaluation demonstrated the presence of amyloid fibrils. No clear relationship was found between amyloid deposits and corneal nerves. These findings confirm LCD type II or Meretoja syndrome. A mutation analysis of the gelsolin gene demonstrated the presence of G to A transition at nucleotide 654. Two children with manifestations of LCD also showed the identical mutation in gelsolin gene.*
CONCLUSIONS. *A new family with Meretoja syndrome is reported. This is the first documented family with Meretoja syndrome in Spain and in the Mediterranean countries. The molecular study shows the same mutation of reported families from Finland, Japan, the United States, and the United Kingdom. (Eur J Ophthalmol 2007; 17: 424-9)*

KEY WORDS. *Corneal lattice dystrophy type II, Meretoja syndrome, Amyloidosis type V, Mutation, Gelsolin*

Accepted: December 19, 2006

INTRODUCTION

The lattice corneal dystrophies (LCD) are characterized by the accumulation of amyloid within the cornea. Types I and III have a pattern of recurrent corneal erosions and gradual development of central anterior lattice lines and no systemic features (1, 2). In type II LCD, the corneal features are subtly different. The lattice lines are fine, being maximal in the periphery. The erosions occur less frequently and visual acuity is preserved until the sixth decade (1, 2). LCD type II is associated with systemic amyloidosis type V (Meretoja syndrome/Finnish type) (3, 4). This is an autosomal dominant systemic disease that appears in early adulthood and predominantly affects the cornea, skin, and cranial nerves (3). Amyloid deposition corresponds to a degradation product of gelsolin. Gel-

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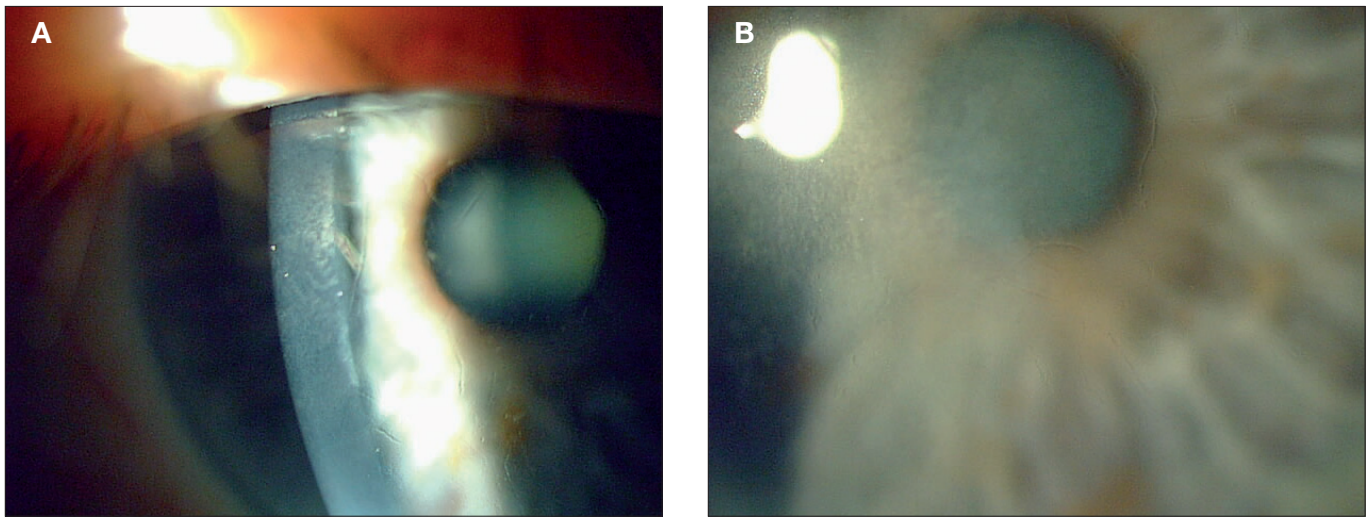

Fig. 1 - *Biomicroscopy.* **(A)** *Fine lattice lines in the cornea of the right eye. They extended into the deep stroma and epithelium. Clear stroma between lines.* **(B)** *Fine lattice lines and central corneal haze in left eye.*

solin is a protein implicated in severing, capping, and nucleating actin filaments as part of cell locomotion and actin scavenging. Amyloidosis type V was first described in Finland (3) where it occurs with high frequency (5-7). Rare isolated cases have been reported in other countries such as Switzerland, Czechia, Holland, Denmark, the United States, Japan, and more recently in the United Kingdom (4, 6-16). Similar clinical features to those described in 1969 by Meretoja (3) in Finland were previously described in Czechia in three sisters (8). No series of patients have been reported in Mediterranean countries. The disease is caused by a point mutation in the gelsolin gene located in chromosome 9. Two mutations have been shown to cause amyloidosis type V. In families from Finland, Japan, the United Kingdom, and the United States, a transition at nucleotide 654 converts aspartic acid to asparagines (12, 16). A second mutation at nucleotide 654 converts aspartic acid to tyrosine, and has been detected in Dutch and Czech families (12, 15).
The aim of this study is to report the clinical, pathologic, and molecular features of a kindred with Meretoja syndrome. This is the first pedigree family documented in Spain.

MATERIALS AND METHODS

Patients

A 69-year-old woman was referred to our hospital for evaluation of corneal dystrophy. The patient did not have

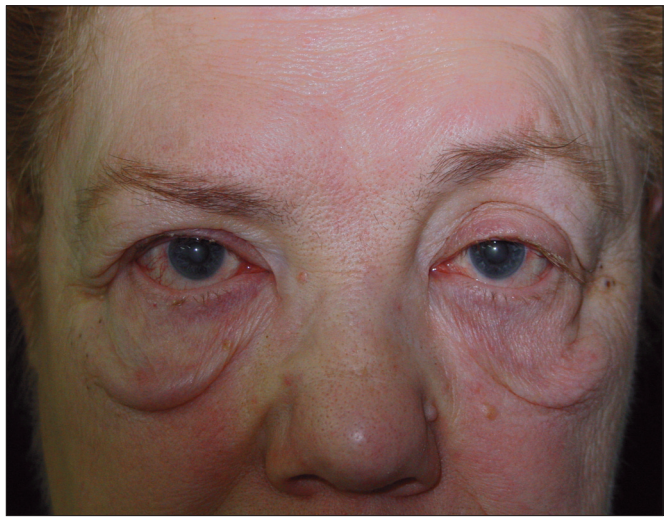

Fig. 2 - *Cutis laxa and marked dermachalasis in both lower eyelids, drooping of the face, and right brow ptosis by right frontal muscle weakness. Left eyelid ptosis.*

previous history of recurrent erosions. Visual acuity was 20/100 in right eye and 20/200 in left eye. On slit lamp examination, typical features of LCD were detected in both corneas. Fine lattice lines were more frequently found in the periphery of the corneas. They extended into the deep stroma and epithelium. Central corneal haze was more remarkable in the left eye (Fig. 1, A and B). No signs of dry eye were present at this moment. Intraocular pressure

was 18 mmHg in both eyes. The patient also presented cutis laxa and marked dermachalasis in both lower eyelids, drooping of the face, and right brow ptosis by right frontal muscle weakness (Fig. 2). Discrete left eyelid ptosis was also evident. The remaining ophthalmologic examination was unremarkable. Systemic evaluation only revealed no symptomatic cardiomegaly. With the exception of facial musculature weakness, no evidence of cranial nerve involvement was observable. Family history was unremarkable. The patient was not related to families with Meretoja syndrome from other countries. A lower blepharoplasty was performed for cosmetic reasons. The excised cutaneous tissue from the eyelid was sent to the Pathology department for microscopic evaluation. Two years later, a follow-up evaluation demonstrated an impairment of blurred vision. Fine lattice lines were more prominent and central haze was more extensive in the left eye. A nuclear cataract was also present. Penetrating keratoplasty and phacoemulsification with intraocular lens implantation were performed. The excised corneal button was sent to the Pathology department for microscopic evaluation.

At this time, ophthalmologic examination was performed on the three children of the patient. Informed consent was obtained from each family member, and peripheral blood was also obtained from each of them. The pedigree is shown in Figure 3.

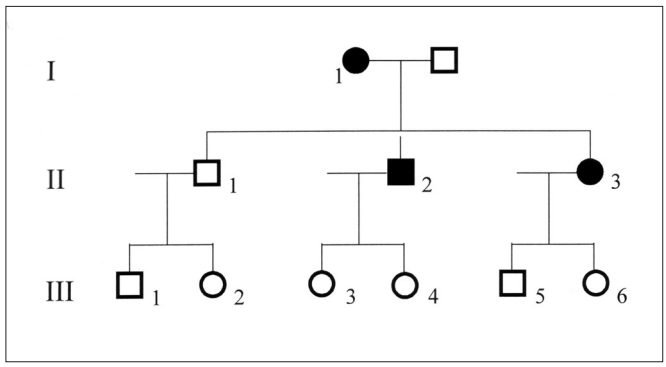

Fig. 3 - *Pedigree of index case and the three children. I-1, affected mother; II-1, a 43-year-old man; II-2, a 41-year-old man; and II-3, a 38-year-old woman. II-2 and II-3 present the clinical features of lattice corneal dystrophy type II in biomicroscopy study.*

Pathologic study

Skin of blepharoplasty and corneal button were fixed in formalin and embedded in paraffin according to standard procedures. The paraffin blocks were sectioned at a thickness of 3 µm, dried for 16 h at 56º. They were dewaxed in xylene, and rehydrated through a graded ethanol series. Hematoxylin-eosin and Congo red stains were performed. Immunohistochemical stains for S-100 protein were performed. Diaminobenzidine chromogen was used as substrate, and sections were counterstained with hematoxylin. For electron microscopic evaluation, small fragments of the corneal button were fixed in glutaraldehyde, dehydrated, and embedded in epoxy resin.

Molecular analysis

DNA from peripheral blood was obtained from the index case, as well as from three additional family members, and was subjected to amplification of exon 5 of the gelsolin gene (GSN gene, OMIM 137350) by polymerase chain reaction (PCR) and DNA sequencing. For PCR amplification, one primer pair was used to amplify exon 5 of the GSN gene (F-5'-aagcacgtggtacccaac-3' and R-5'-gcccaggtccaggatgaa-3'). PCR amplification was performed in 20 µL reaction volumes that contained 100 ng of DNA, 75 mM Tris HCl, 1.5 mM $MgCl_2$, 50 mM KCl, 20 mM $(NH_4)_2SO_4$, 0.2 µM of each primer, 0.2 mM of each dNTP, and 1 unit of Taq DNA polymerase (BIOTOOLS, B&M Labs, S.A., Spain). PCR was carried out under the following conditions: an initial 5 min denaturation at 94 ºC followed by 35 cycles of 1 min at 94 ºC, 1 min at 55 ºC, 1 min at 72 ºC, and a final extension of 10 min at 72 ºC. For DNA sequencing, PCR products were first purified using the MinElute PCR Purification Kit (Qiagen GmbH, Hilden, Germany) and were bidirectionally sequenced using the original primer pair and Applied Biosystems Cycle Sequencing kit (Applied Biosystems Inc., Santa Clara, CA). Samples were analyzed on the ABI Prism 3100-Avant instrument, using standard run parameters. The separation matrix used was POP-6 using 1X TBE with EDTA running buffer (Applied Biosystems Inc.).

RESULTS

Clinical data

The evolution of the blepharoplasty was satisfactory and the patient followed periodic controls in the ophthalmologic unit. Blepharoplasty improved the facial cosmetic

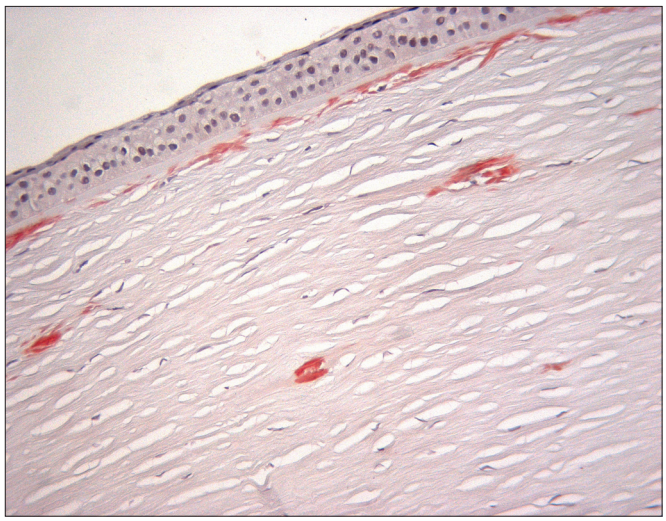

Fig. 4 - *Histology of the corneal button. Presence of amyloid deposits beneath the normal-appearing Bowman layer and in the stroma (Congo red ×400).*

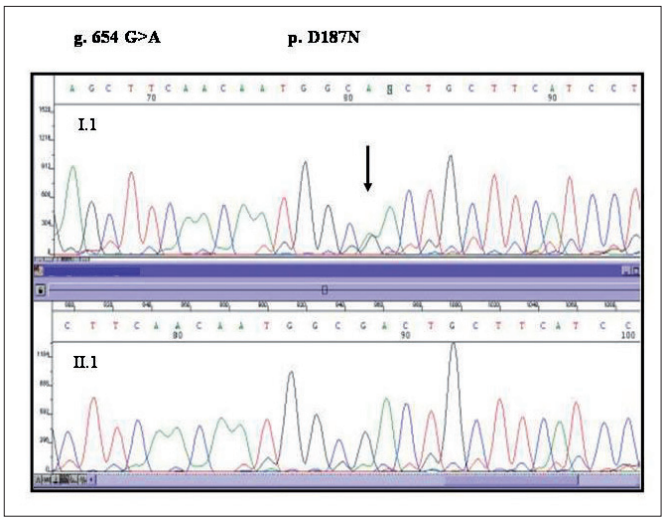

Fig. 5 - *Phenograph. Mutation in a G to A transition at nucleotide 654 by direct sequencing which converts aspartic acid to asparagine at amino acid residue 187. The same mutation appears in I-1, II-2, and II-3.*

appearance of the patient. After several follow-up controls, a discrete lower eyelid retraction was noted. Securely due to the dropping face to facial weakness and muscle atrophy. Treatment with artificial tears was administered as the patient referred symptoms of dry eye. Signs of keratitis sicca were not present. The keratoplasty developed satisfactorily. Epithelization of donor button occurred during the first week. No recurrent ulceration or neurotrophic epithelial defect was noted.

The patient's three children were a 43-year-old man (II-1), a 41-year-old man (II-2), and a 38-year-old woman (II-3). Screening of the three family members demonstrated a visual acuity of 20/20 in both eyes of each of them. No symptoms of visual loss or corneal erosions were referred by them. No clinical symptoms of systemic diseases were referred. Neither dermatochalasia nor drooping of the face was noticeable. Biomicroscopic examination showed typical features of peripheral fine lines of LCD in a son (II-2) and the daughter (II-3). Examination in the remaining son (II-1) was normal, suggesting that he was healthy. Intraocular pressure and the rest of the ophthalmologic exploration were normal in the three siblings.

Pathologic study

The skin of the blepharoplasty showed the presence of amyloid deposits in the dermis. Amyloid exhibited the typical amorphous and eosinophilic microscopic appearance with hematoxylin and eosin staining. It stained positively with the Congo red preparation and with this stain showed the characteristic apple-green birefringence under polarized light. Amyloid deposits were noticed around the vessels and cutaneous appendages. Microscopic examination of the corneal button showed the presence of amyloid deposits beneath the normal-appearing Bowman layer and also within the stroma (Fig. 4). Immunostaining for S-100 protein did not demonstrate a significant relationship between amyloid deposits and the corneal nerves. Electron microscopic evaluation demonstrated the presence of amyloid fibrils, characterized by random aggregates of rigid, non-branching rods measuring 8 to 10 nm in diameter. No clear relationship was found between amyloid deposits and the corneal nerves.

Molecular analysis

A mutation was observed in three individuals; the index case (I-1) and the two relatives who showed subtle manifestations of the disease (II-2 and II-3). The mutation consisted in a G to A transition at nucleotide 654 which converts aspartic acid to asparagine at amino acid residue 187. The mutation was not found in unaffected family member II-1 (Fig. 5). Generation III was not subjected to mutation analysis.

DISCUSSION

Amyloidosis type V, also known as Meretoja syndrome or gelsolin-related amyloidosis, is an autosomal dominant disease. The cardinal features of amyloidosis type V consist of a triad of 1) ophthalmic (LCD type II, glaucoma), 2) neurologic (cranial, peripheral, and autonomic neuropathies), and 3) dermatologic (amyloid deposition in the skin) manifestations (17).

In the present family, the corneal features were typical of LCD in the index case and two asymptomatic relatives. In these individuals, fine lines of lattice were predominantly located in the peripheral cornea. In LCD type II, the clinical corneal changes are late in onset, recurrent erosions are unusual, and visual outcome is favorable (1, 2). Only a few cases need keratoplasty (1, 2). The earliest clinical finding of LCD is usually identified during the third or fourth decade (5). The stroma between lines is initially clear. The fine lines can extend into deep stroma and epithelium, resulting in corneal opacity. With time, the opacities coalesce and a diffuse haze may develop in the anterior and mid stroma. Some cases manifest slowly progressive visual loss, which usually occurs during the sixth or seventh decades. Treatment of the patients with keratoplasty is sometimes followed by subsequent neurotrophic epithelial defects (10, 14), secondary to amyloid perineural deposits and cranial neuropathy of V cranial nerve. Altered corneal sensitivity has been documented in these patients (7, 15). No persistent epithelial defects were noticed in the index case. Glaucoma and pseudoexfoliation with or without glaucoma are common in Meretoja syndrome (6, 14, 18), but they were not found in this family.

The cranial nerves, particularly the facial, are frequently affected in cases of amyloidosis type V (5, 13). Bilateral facial palsies and muscular facial weakness have also been reported (10, 13). In the present family, the mother presented drooping of the face and right eyebrow ptosis presumably secondary to involvement of the VII cranial nerve or facial nerve and muscular facial weakness. With time, a discrete lower eyelid retraction was noted. Securely by the droop of the face due to the muscular atrophy. None of the remaining family members presented clinical manifestations secondary to cranial nerve involvement.

Amyloid deposition in the skin was demonstrable in the excised skin of the lower lid blepharoplasty. Amyloid deposits were noticed around the vessels and cutaneous appendages.

Systemic exploration of the index case demonstrated no symptomatic cardiomegaly. Renal insufficiency and cardiac involvement have been reported in patients with amyloidosis type V (14, 19). The first studies on renal changes were reported by Meretoja et al in 1972 (20). A review of the Spanish literature revealed a single patient with clinical features of Meretoja syndrome who underwent heart transplantation; 5 years later the patient had mild chronic renal failure and normal cardiac allograft function (19). Details about the patient's family history were not provided.

Pathologic evaluation of the cornea in patients with Meretoja syndrome has shown deposits of amyloid beneath the normal-appearing Bowman layer and focally within the stroma (21). Meretoja found amyloid deposits surrounding partially healthy corneal nerves (21). Confocal microscopic examination demonstrated diminished or absent nerves of the subbasal nerve plexus or basal epithelial nerve plexus (7). In the stroma, these authors noted undulating thin structures that could correspond to amyloid deposits or altered nerves. They also observed thick filaments. They attributed to these elements the morphologic basis of lattice lines, but did not note association with stromal nerves. A recent study with confocal microscopy has suggested that the degree of corneal nerve damage in patients with Meretoja syndrome correlates with the severity of clinical involvement (15). In the present case, no significant association between amyloid material and corneal nerves was seen, and the patient did not show epithelial neurotrophic defects in postoperative follow-up evaluation, suggesting that corneal nerves were not affected.

The molecular basis of Meretoja syndrome is well known. Two mutations in the gelsolin gene on chromosome 9q34 have been described. In the present report, we show the presence of a G to A transition at nucleotide 654 of the gene. This is the most common mutation found in this syndrome. This mutation has been described in families from Finland, Japan, the United States, and the United Kingdom (12, 16). The identification of a mutation in the gelsolin gene provides the correct diagnosis of Meretoja syndrome (16), which explained both the ocular and systemic disease. Secondary amyloidosis could be suggested if the mutation of codon 187 is not detectable (16).

In conclusion, we report the clinical, pathologic, and molecular findings in the first Spanish pedigree family with amyloidosis type V (Meretoja syndrome; gelsolin-related amyloidosis), in which genetic analysis has demonstrated

identical mutation to the families described in other countries. This type of corneal dystrophy may also be considered in late onset LCD in the Spanish population.

Proprietary interest: None.

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