A novel TACSTD2 gene mutation in a Turkish family with a gelatinous drop-like corneal dystrophy

Arseni Markoff,¹ Nadia Bogdanova,² Constantin E. Uhlig,³ Markus Groppe,³,⁴ Jürgen Horst,² Ingo Kennerknecht²

(The first two authors contributed equally to this publication)

¹Institute of Medical Biochemistry, Zentrum für Molekularbiologie der Entzündung (ZMBE); ²Institute of Human Genetics, ³University Eye Hospital, University Clinics Münster and Westfalian Wilhelms-University of Münster, Münster, Germany; ⁴Worthing and Southlands Hospitals, Department of Ophthalmology, United Kingdom

Purpose: To identify the molecular defect causing gelatinous drop-like corneal dystrophy in a Turkish family and assign affected and carriership status.

Methods: Visual activity of affected family members was measured using Snellen optotypes. To identify the molecular defect, mutation analysis of the TACSTD2 (MIS1) gene was performed.

Results: We report on a new TACSTD2 mutation, c.653delA, in a Turkish family. The identified molecular defect cosegregates with the disease among affected members of the family and is not found in 100 unaffected individuals of various ethnic origin.

Conclusions: A few TACSTD2 gene mutations in the homozygous or compound heterozygous state have been described as causative for this abnormality, mainly in several Japanese families. The newly identified mutation is predicted to generate a shortened protein product, thereby completely altering the COOH-terminal region and deleting the transmembrane domain, required for anchoring at cell membranes and the phosphatidylinositol2-binding site.

Gelatinous drop-like corneal dystrophy (GDLD, OMIM 204870) is an abnormality, characterized by subepithelial deposits of amyloid and severe impairment of visual acuity leading to blindness. GDLD is an autosomal recessive disorder, described primarily in Japanese patients with an estimated prevalence of 1 in 30,000 [1-5]. It occurs also in Vietnamese [6], Indian [7,8], and Tunisian [8-10] families. A few cases have been reported in European and North American patients [8,11-13]. Early clinical symptoms of GDLD include blurred vision, severe photophobia, and ocular foreign body sensation. As the disease progresses, raised gelatinous masses accumulate on the cornea, which impair severely the visual acuity. Repeated lamellar or perforating keratoplasties is prescribed in a variety of human epithelial cells and carcinomas, and its product is a type I transmembrane protein with several predicted glycosylation sites. The 40 kDa TACSTD2 protein contains an N-terminal export signal sequence, an epidermal growth factor (EGF)-like repeat, a thyroglobulin repeat and a phosphatidylinositol (PIP2)-binding site, harboring phosphorylatable serine and threonine residues near the COOH-terminus [15]. The single transmembrane segment extends from position 275 to 298 of the amino acid sequence. The role of TACSTD2 is not well understood, although it has been previously suggested to function as a cell-cell adhesion receptor in cancer cells [18]. The gene structure is rather indicative of a function in cell signal transduction, which is corroborated by the observation that GDLD-associated mutations in TACSTD2 result in several orders of magnitude higher epithelial permeability and pronounced cell junction defects as compared to control samples [19]. About 20 different mutations in GDLD patients are reported to date, and these molecular changes are scattered across the whole gene sequence. The majority of reported mutations result in a truncated protein product, thus leading to elimination of the COOH-terminal transmembrane domain and PIP2 binding site. The Q118X mutation has a very high frequency among Japanese patients (90%) and is considered a GDLD founder mutation for the Japanese population [20].

Here we report on a new TACSTD2 gene mutation, c.653delA, which we identified in a family originating from southeast Turkey. To our knowledge this is the first GDLD mutation found in a family from this area.
METHODS

Case report: A 13-year-old girl was diagnosed with bilateral gelatinous drop-like corneal amyloidosis. Over the next 12 years, she underwent different treatment regimes, including three corneal graft procedures on both eyes, a corneal scraping and an excimer laser keratomileusis on her left eye. Conservative treatment with systemic or local steroids did not prevent the development of numerous gelatinous drop-like lesions. Visual acuity measured with Snellen optotypes decreased to 0.2 right eye and 0.05 left eye.

Her younger sister was diagnosed with the same condition when she was 8 years old. Her symptoms were not as severe, and she had small gelatinous drops in the midperiphery of both corneas. Surgical treatment was not indicated because her visual function was still satisfactory and surgery was not expected to dramatically improve her vision. Seven years later the gelatinous drops had increased in volume (about 6.5 times altogether) and the patient suffered from severe photophobia, intermittent blurred vision, and a sensation of grittiness. An excimer laser keratomileusis might be a treatment option to reduce her symptoms.

Figure 1. Image of the right cornea of the older sister. The beginnings of gelatinous drop-like amyloidosis can be clearly seen in the periphery of the cornea. Within a few years, the gelatinous drops invaded the center of the cornea. This photograph was taken before microsurgical treatment.

Figure 2. Pedigree diagram of the Turkish GDLD family. Carriership and affected status of assayed family members is indicated. Relevant excerpts of the sequencing analysis electrophoregrams illustrate the normal sequence and the pathological change of the TACSTD2 gene.
Mutation analysis: The study complies with the ethical guidelines of the institutions involved. Informed consent was obtained from all subjects examined. DNA was extracted from peripheral whole blood using standard procedures [21]. Polymerase chain reaction (PCR) was performed on 50 ng of genomic DNA in a final volume of 25 μl using 1.5 units of Taq polymerase (Invitrogen, Karlsruhe, Germany) and 1.5 mM MgCl₂. The coding sequence of TACSTD2 was amplified in two overlapping formats, using primer pairs M1S1.F1/R1, 5'-CGG GTA GAG TAT AAG AGC CGG A-3'/5'-GAT CTG GAT GGT GGG CTG CTC GTA-3' and M1S1.F2/R2, 5'-CCA CCG CCG CCT TCA ACC A-3'/5'-GCG CCG AGG AAT CAG GAA GCG TGA-3'. Cycling conditions were as follows: denaturation at 94 °C for 45 s, annealing at 55 °C for 30 s, and extension at 68 °C for 1 min in 25 cycles. PCR products were purified using MultiScreen™ PCR plates (Millipore, Eschborn, Germany), according to the manufacturer’s instructions. Purified amplicons were directly sequenced in a 96-well plate format using the BigDye Terminator Cycle Sequencing Ready Reaction Kit (ABI/Perkin-Elmer, Weiterstadt, Germany), and sequencing reactions were analyzed on an ABI PRISM® 3700 DNA Analyzer (ABI/Perkin-Elmer, Weiterstadt, Germany).

RESULTS & DISCUSSION

Both sisters were diagnosed with bilateral gelatinous drop-like corneal amyloidosis, which indicated a familial pattern for the condition. Amyloid deposits had begun to form peripherally on the right cornea of the older sister at the time of diagnosis (Figure 1). DNA analysis identified mutation c.653delA in the patient and her affected sister. This TACSTD2 sequence alteration was also confirmed in a heterozygous state in the girl’s mother, father, and brother, as well as their uncle (father’s side; Figure 2). The mutation was not found in 100 healthy individuals of various ethnic origin, including those from the Middle East. The effect of c.653delA is a shift of the reading frame immediately after the TACSTD2 codon 258, causing a premature translation termination at position 310 of the protein sequence. Thus it is similar to the previously identified c.632delA, which has been shown to alter the subcellular localization of the protein, when expressed in cell culture [15]. Another similar mutation, an insertion of “C” at position 520 of the cDNA sequence, has been found in another Japanese family [22]. The deleterious effect of such TACSTD2 mutations (deletions or insertions) consists of the structural removal of the transmembrane domain and the PIP2-binding site from the COOH-terminus of the protein, which is likely necessary for proper subcellular localization. The whole variety of sequence alterations is observed among the few identified pathological molecular changes in TACSTD2, nonsense and missense nucleotide substitutions, single nucleotide deletions, and insertions or deletions/insertions (indels), such as the recently identified c.783del(ATCTATTACCTG)+c.772insT in a Vietnamese patient [6]. The general impression is that most of identified mutations so far would lead to a truncated protein product with altered subcellular localization.

To our knowledge, this is the first report of a TACSTD2 mutation in a family with GDLD from the Middle East. Locus heterogeneity for the disease has been previously suggested, but no TACSTD2 offending mutation has been found in a single GDLD family [9]. Our analysis confirms TACSTD2 mutation as the cause for the observed corneal dystrophy in a Turkish family, which is also cosanguineous, characteristic for the Anatolian ethnic region.

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REFERENCES


