DATA REPORT

Novel TACSTD2 mutation in gelatinous drop-like corneal dystrophy

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We identified a novel mutation in the tumor-associated calcium signal transducer 2 (TACSTD2) gene in a consanguineous Thai family with gelatinous drop-like corneal dystrophy (GDLD). All affected family members presented with an intense amyloid substance deposited on the cornea, which required surgical management. Genetic analysis of these individuals revealed a homozygous mutation c.79delC, in the TACSTD2 gene. Both parents of these individuals were unaffected and showed heterozygous mutations in the TACSTD2 gene. The mutation produced a truncated protein sequence that might be the cause of GDLD.

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GDLD is a rare autosomal recessive hereditary disease. It was first reported in 1914, by Nakaizumi1–3 and most of the subsequent reports of GDLD have originated from Japan. People with GDLD exhibit a wide variety of clinical presentations, and GDLD has been classified into four types: (1) typical mulberry type, (2) kumquat-like type, (3) band-keratopathy type and (4) stromal-opacity type.4 The onset of GDLD occurs in the first or second decade of life, and patients usually require surgical treatment in the third decade of life. In 1999, Tsujikawa et al.5 were the first to identify TACSTD2 as the pathologic gene of GDLD. Various mutations have been discovered in the TACSTD2 gene in various populations.6–15 To date, there are 28 reported mutations in the TACSTD2 gene. For Southeast Asian populations, one TACSTD2 mutation has been reported in Vietnam11 and, to the best of our knowledge, we are the first to investigate the genetic mutation of the TACSTD2 gene in Thailand.

Three GDLD probands (two males and one female) from the same consanguineous family (pedigree shown in Figure 1) were recruited for this study. The family was from Chiang Mai, which is a province in northern Thailand. There was no associated systemic disease or history of corneal trauma in the family, and the ocular examination in the parents and all offspring of the probands appeared normal. The clinical history, presentation and pathologic data for each proband were as follows:

Proband 1 (II-1) is a 39-year-old male with bilateral corneal opacity and horizontal nystagmus. His best spectacle-corrected visual acuity was counting fingers at a distance of 1 foot with both eyes. The onset of his symptoms occurred in the first decade of life. He had previously undergone three times of penetrating keratoplasties (PKP) on the right eye and twice on the left eye at another hospital. Ocular examination revealed multiple gray-to-yellowish nodules deposited in the corneal stroma in both the graft and host cornea (Figure 2a, b). We performed another PKP on his right eye and type 1 Boston keratoprosthesis (KPro) implantation (Massachusetts Eye and Ear Infirmary, Boston, MA, USA) on his left eye. Graft rejection was detected 4 months after the PKP operation. Graft failure subsequently occurred. The visual outcome of Boston KPro implantation was limited to counting fingers at a...
distance of 2 feet with his left eye owing to severe underlying amblyopia.

Proband 2 (II-2) is a 33-year-old female with a recurrent amyloid deposit who had previously undergone a PKP of her right eye once and of her left eye twice at another hospital. A mulberry-type amyloid deposit was found during the ocular examination (Figure 2d). We performed type 1 Boston KPro implantation on her right eye. Six years post operation, a severe Pseudomonas infection occurred in her right eye. The Boston KPro was removed and replaced with a corneal graft, which ultimately failed after 1 year (Figure 2b).

Proband 3 (II-3) is a 28-year-old male with a bilateral mulberry-type amyloid deposit. For treatment, PKP was performed on his right eye. Recurrence of the yellowish amyloid deposit subsequently occurred. Therefore, we performed a type 1 Boston KPro implantation as the secondary treatment on his right eye and as the primary treatment on his left eye (Figure 2e, f).

Histopathology of the corneal tissue showed drops and bands of amorphous material that were deposited at the subepithelial and anterior corneal stroma (Figure 3a, c for proband 1 and proband 3, respectively) and were positive for Congo red staining with birefringence consistent with amyloid (Figure 3b, d for proband 1 and proband 3, respectively).

Mutational analyses were performed on all of the family members (the three probands and both parents) after obtaining informed consent. Ten subjects without corneal amyloid deposit were recruited as a control group. Genomic DNA was extracted from 8 ml of whole peripheral blood using the PAXgeneBlood DNA Kit (QIAGEN GmbH, Hilden, Germany). DNA templates were quantified to be 150–300 ng before use in the reaction. PCR was performed using the following primer pair to cover the entire TACSTD2 gene: F; 5′-ATGTGTCACCCAAATACCAGTGGG-3′ and R; 5′-CGTGACTCACTTGCTGGGGACG-3′. After purification of the PCR products, the products were bi-directionally sequenced using the primers with a cycle sequencing kit (BigDye Terminator Cycle Sequencing Kit; Life Technologies, Carlsbad, CA, USA) according to the manufacturer’s instructions. After ethanol precipitation, the products were electrophoresed using an automated capillary sequencer (3130xl Genetic Analyzer; Life Technologies). The sequencing data were validated by PCR using a primer pair (intF: 5′-AGTGCAACCACAGACGTGTTGCT-3′ and intR: 5′-TCTGAGACGTGGGTCTCCTGCCAGC-3′) that spanned the site of the identified mutation.

By using direct DNA sequencing, homozygous c.79delC mutation (p.His27ThrfsTer15) in the TACSTD2 gene was found in all three patients (Figure 1). Heterozygous c.79delC mutation was confirmed in both of the parents who were unaffected.

In this report, we discovered a novel homozygous c.79delC mutation in the TACSTD2 gene of three Thai GDLD patients that is not present in the wild-type TACSTD2 gene (Figure 1) of Thai controls or in the Exome Aggregation Consortium database, which includes data from 60,706 unrelated individuals.16 The autosomal
resistant and a low expression of Claudin 1 and 7.\(^{18,19}\)

Regarding the relationship between genotypes and phenotypes, all patients in the present study carried the same mutation; however, they presented with different ocular findings, both inter-individually and intra-individually. Proband 1 presented with a mulberry-type amyloid deposit in his right eye and a kumquat-like type in his left eye (Figure 2). The onset of the disease in proband 1 was much earlier than that of his brother and sister, who initially experienced the symptoms in their second decade of life. Similar to the previous study of Tsujikawa et al.,\(^{20}\) we observed high phenotypic variability in a single family with GDLD.

In conclusion, we investigated the clinical features, histopathology and genetics of three GDLD patients from Thailand and discovered a unique mutation, c.79delC, in the TACSTD2 gene. This frameshift mutation produced a truncated TACSTD2 protein that might cause the severe clinical presentation, which was phenotypically heterogeneous and required surgical management.

**HGV DATABASE**

The relevant data from this Data Report are hosted at the Human Genome Variation Database at http://dx.doi.org/10.6084/m9.v741.

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**COMPETING INTERESTS**

The authors declare no conflict of interest.

Figure 3. Histopathology images of the corneal tissues. Hematoxylin and eosin staining revealed multiple amyloid nodules deposited in the subepithelium and the anterior stroma in both proband 1 (a) and proband 3 (c). Using Congo red staining, an apple-green birefringence was observed under polarized light in both proband 1 (b) and proband 3 (d).
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