OPEN

Citation: Human Genome Variation (2015) **2**, 15047; doi:10.1038/hgv.2015.47
Official journal of the Japan Society of Human Genetics 2054-345X/15

www.nature.com/hgv

DATA REPORT

Novel *TACSTD2* mutation in gelatinous drop-like corneal dystrophy

Passara Jongkhajornpong[1], Kaevalin Lekhanont[1], Mayumi Ueta[2,3], Koji Kitazawa[2], Satoshi Kawasaki[4] and Shigeru Kinoshita[3]

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.

Human Genome Variation (2015) **2,** 15047; doi:10.1038/hgv.2015.47; published online 26 November 2015

GDLD is a rare autosomal recessive hereditary disease. It was first reported in 1914, by Nakaizumi[1–3] 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 Vietnam[11] 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

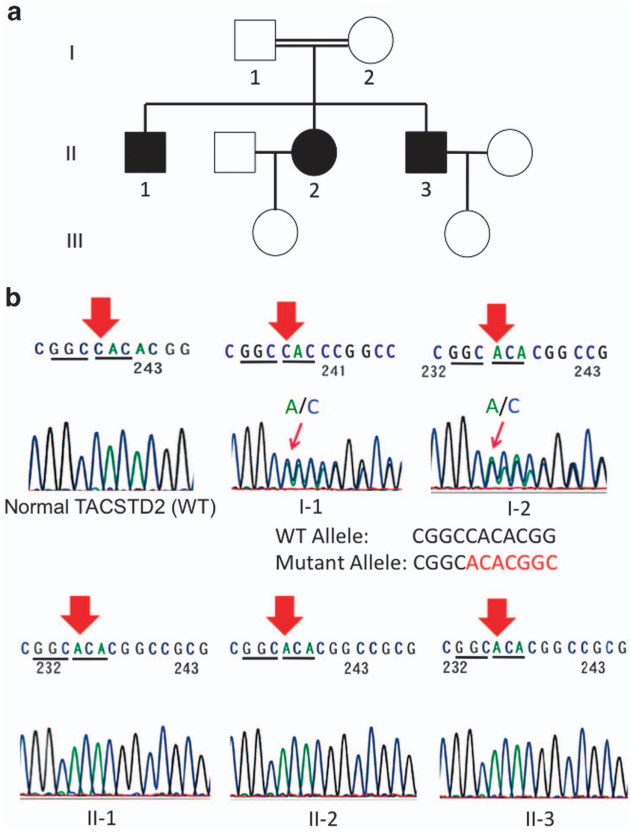

Figure 1. Family tree of a gelatinous drop-like corneal dystrophy (GDLD) consanguineous Thai family (**a**). Diagrams illustrating the nucleotide sequences of the tumor-associated calcium signal transducer 2 (*TACSTD2*) genes of all family members compared with the normal *TACSTD2* gene (wild type, WT) (**b**). Arrows indicate the seventy-ninth nucleotide of the *TACSTD2* gene, which exhibited a heterozygous deletion in both parents and a homozygous deletion in all three probands.

[1]Department of Ophthalmology, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand; [2]Department of Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, Japan; [3]Department of Frontier Medical Science and Technology for Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, Japan and [4]Department of Ophthalmology, Osaka University, Osaka, Japan.
Correspondence: P Jongkhajornpong (passaraj@yahoo.com)
Received 2 June 2015; revised 30 September 2015; accepted 1 October 2015

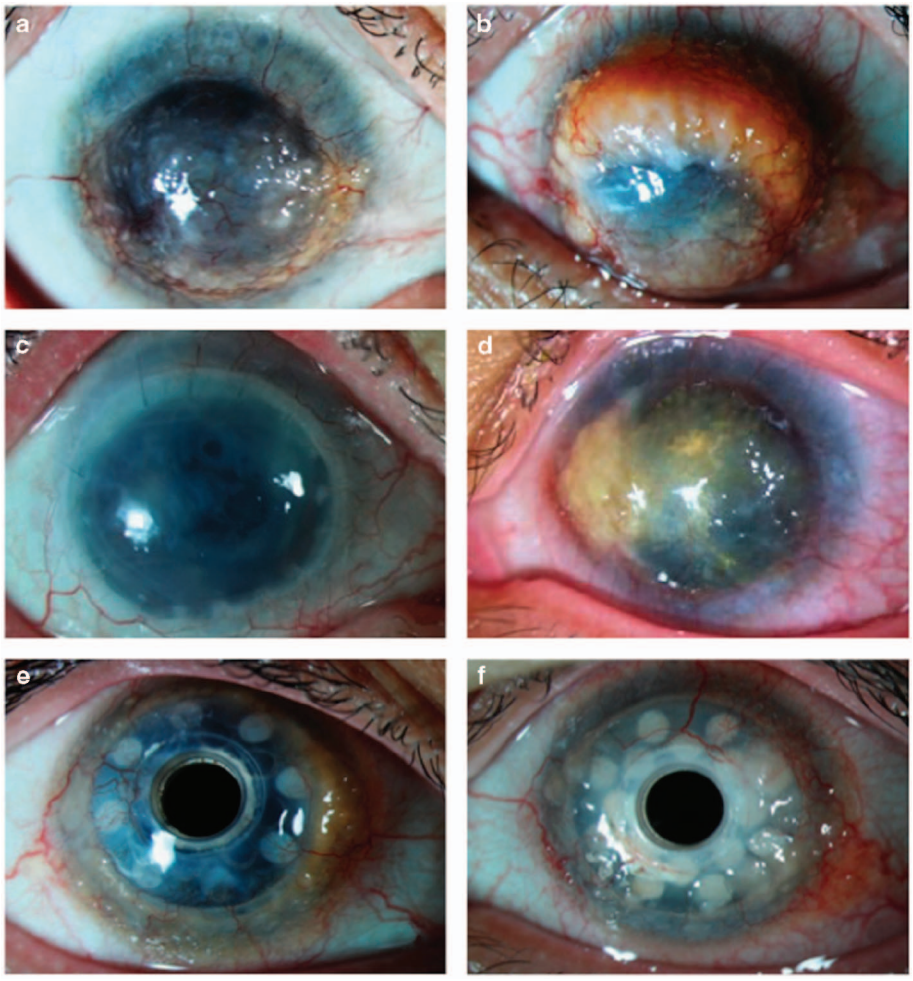

Figure 2. Slit-lamp examination of the three gelatinous drop-like corneal dystrophy (GDLD) patients. Proband 1: recurrent mulberry type in the right eye (**a**) and kumquat-like type in the left eye (**b**), Proband 2: post penetrating keratoplasty (PKP) in the right eye (**c**) and recurrent mulberry-type amyloid deposit in the left eye (**d**), Proband 3: post Boston keratoprosthesis (KPro) implantation in the right eye (**e**) and the left eye (**f**).

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′-CGTGACTCACTTGGGTCTGGGACG-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′-AGTGCAACCAGACGTCGGTGTGCT-3′ and intR: 5′-TCTGAGACGTGTTCTGCCGCAGCT-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

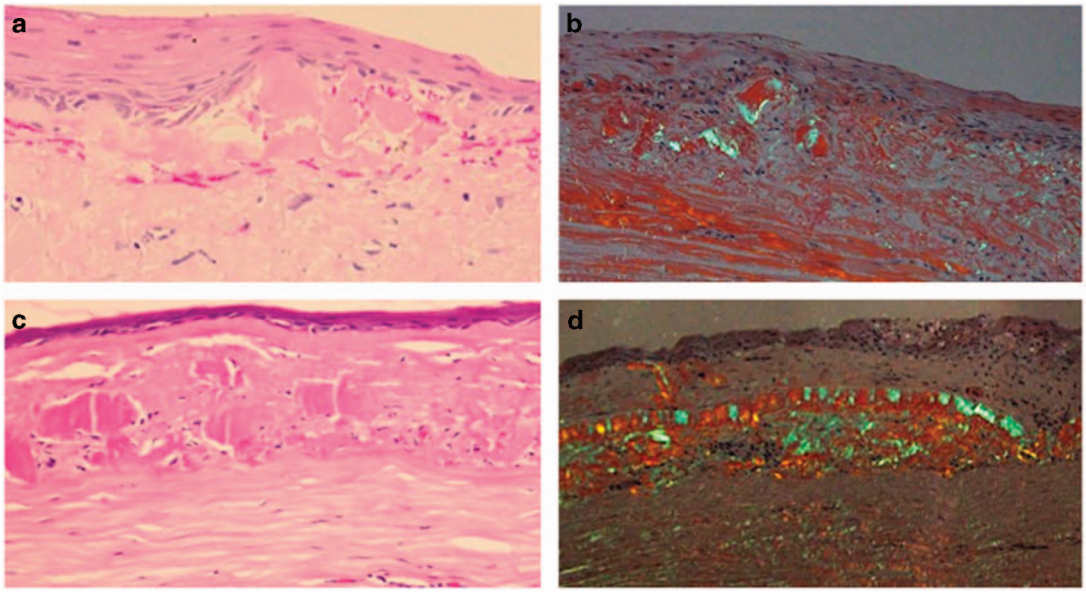

Figure 3. Histopathology images of the corneal tissues. Hematoxylin and eosin staining revealed multiple amyloid nodules deposited in the subepithelium and the anterior stroma in both proband 1 (**a**) and proband 3 (**c**). Using Congo red staining, an apple-green birefringence was observed under polarized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

recessive hereditary pattern of GDLD was confirmed by detection of heterozygous c.79delC mutation of the *TACSTD2* gene in both parents who were unaffected. This novel deletion (c.79delC) caused a frameshift mutation that resulted in an early stop codon. The resultant TACSTD2 molecule contained a threonine instead of a histidine at the 27th amino acid, with the new open reading frame encoding 14 amino acids (p.His27 Thrfs Ter15) before termination. The aberrant protein contained 41 amino acids. Generally, the TACSTD2 molecule is composed of five main regions: (1) a signal sequence (located at amino acid (aa) 1–26), (2) an epidermal growth factor-like repeat, (3) a thyroglobulin repeat, (4) a transmembrane domain (TM, located at aa 275–297) and (5) a phosphatidylinositol 4,5-bis phosphate (PIP2)-binding sequence.[5] It has been proposed that the transmembrane domain plays a role in anchoring the molecule to tight junction-related proteins via the AxxxG motif.[17] Furthermore, knocking out *TACSTD2* in human corneal and conjunctival epithelial cells has been shown to result in a significant decrease in transepithelial resistance and a low expression of Claudin 1 and 7.[18,19] Investigation of the truncated TACSTD2 molecule identified in this study revealed that four of the necessary functional domains of the molecule had disappeared, including the TM domain. We posited that the aberrant TACSTD2 molecule was unable to bind to the plasma membrane or function properly, thus resulting in the clinical appearance of GDLD.

The most frequently reported mutation in the *TACSTD2* gene in Japan is p.Gln118X, which accounts for 82.5% of all mutations in the *TACSTD2* gene.[5] However, p.Glu227Lys is reported to be the most common mutation among Iranian GDLD patients.[12] This phenomenon can be explained through a founder effect, as shown by analysis of polymorphic markers near the *TACSTD2* gene. A few transethnic mutations have been discovered, such as p.Gln118X in Japanese and Chinese populations, p.Leu186Pro in Japanese and Iranian populations and p.Ile258X 772_783del +772insT in Vietnamese and Chinese populations. Uncovering a unique *TACSTD2* mutation in people from these nations, including Thai GDLD patients is strong evidence of high genotypic heterogeneity of GDLD. As no other nucleotide variations were recognized in the *TACSTD2* region, we did not find a founder effect in Thai GDLD patients.

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.figshare.hgv.741.

ACKNOWLEDGEMENTS

We thank Dr Rangsima Aroonroch, Dr Varintorn Chuckpaiwong, Dr Pichaya Chuepanich, Dr Nutthida Nimvorapun and Dr Manachai Manasopon from Ramathibodi Hospital, Mahidol University, Bangkok, Thailand, for the tissue pathological reports and clinical data; Dr Morio Ueno from the Department of Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, Japan, for assisting in the DNA sequencing; the Exome Aggregation Consortium; and the groups that provided the exome variant data for comparison. A full list of contributing groups can be found at http://exac.broadinstitute.org/about. Ethics approval was obtained from the Institutional Review Board for Human Studies of Ramathibodi Hospital, Mahidol University, Bangkok, Thailand, and the Institutional Review Board for Human Studies of Kyoto Prefectural University of Medicine, Kyoto, Japan. This research project was supported by the Faculty of Medicine, Ramathibodi Hospital, Mahidol University.

COMPETING INTERESTS

The authors declare no conflict of interest.

REFERENCES

1 Weiss JS, Møller HU, Aldave AJ, Seitz B, Bredrup C, Kivela T *et al.* IC3D Classification of corneal dystrophies-edition 2. *Cornea* 2015; **34**: 117–159.
2 Nakaizumi G. A rare case of corneal dystrophy. *Acta Soc Ophthalmol Jpn* 1914; **18**: 949–950.
3 Kawano H, Fujiki K, Kanai A. Prevalence of gelatinous drop-like corneal dystrophy in Japan [in Japanese]. *Atarashii Ganka* 1992; **9**: 1879–1882.
4 Ide T, Nishida K, Maeda N, Tsujikawa M, Yamamoto S, Watanabe H *et al.* A spectrum of clinical manifestations of gelatinous drop-like corneal dystrophy in Japan. *Am J Ophthalmol* 2004; **137**: 1081–1084.
5 Tsujikawa M, Kurahashi H, Tanaka T, Nishida K, Shimomura Y, Tano Y *et al.* Identification of the gene responsible for gelatinous drop-like corneal dystrophy. *Nat Genet* 1999; **21**: 420–423.
6 Nakatsukasa M, Kawasaki S, Yamasaki K, Fukuoka H, Matsuda A, Nishida K *et al.* Two novel mutations of TACSTD2 found in three Japanese gelatinous drop-like corneal dystrophy families with their aberrant subcellular localization. *Mol Vis* 2011; **17**: 965–970.
7 Kinoshita S, Adachi W, Sotozono C, Nishida K, Yokoi N, Quantock AJ *et al.* Characteristics of the human ocular surface epithelium. *Prog Retin Eye Res* 2001; **20**: 639–673.
8 Ren Z, Lin PY, Klintworth GK, Iwata F, Munier FL, Schorderet D. Allelic and locus heterogeneity in autosomal recessive gelatinous drop-like corneal dystrophy. *Hum Genet* 2002; **110**: 568–577.
9 Markoff A, Bogdanova N, Uhlig CE, Groppe M, Horst J, Kennerknecht I. A novel TACSTD2 gene mutation in a Turkish family with a gelatinous drop-like corneal dystrophy. *Mol Vis* 2006; **12**: 1473–1476.
10 Tasa G, Kals J, Muru K, Juronen E, Piirsoo A, Veromann S *et al.* A novel mutation in the M1S1 gene responsible for gelatinous droplike corneal dystrophy. *Invest Ophthalmol Vis Sci* 2001; **42**: 2762–2764.
11 Ha NT, Chau HM, Cung le X, Thanh TK, Marukami A, Kanai A. A novel mutation of M1S1 gene found in a Vietnamese patient with gelatinous droplike corneal dystrophy. *Am J Ophthalmol* 2003; **135**: 390–393.
12 Alavi A, Elahi E, Tehrani MH, Amoli FA, Javadi MA, Rafati N *et al.* Four mutations (three novel, one founder) in TACSTD2 among Iranian GDLD patients. *Invest Ophthalmol Vis Sci* 2007; **48**: 4490–4497.
13 Jing Y, Liu C, Wang L. A novel TACSTD2 mutation identified in two Chinese brothers with gelatinous drop-like corneal dystrophy. *Mol Vis* 2009; **15**: 1580–1588.
14 Zhang B, Yao YF. Gelatinous drop-like corneal dystrophy with a novel mutation of TACSTD2 manifested in combination with spheroidal degeneration in a Chinese patient. *Mol Vis* 2010; **16**: 1570–1575.
15 Paliwal P, Gupta J, Tandon R, Sharma N, Titiyal JS, Kashyap S *et al.* Identification and characterization of a novel TACSTD2 mutation in gelatinous drop-like corneal dystrophy. *Mol Vis* 2010; **16**: 729–739.
16 Exome Aggregation Consortium (ExAC), Cambridge, MA, USA http://exac.broadinstitute.org. Accessed 29 September 2015; Updated on 29 October 2014.
17 Nakatsukasa M, Kawasaki S, Yamasaki K, Fukuoka H, Matsuda A, Tsujikawa M *et al.* Tumor-associated calcium signal transducer 2 is required for the proper subcellular localization of claudin 1 and 7: implications in the pathogenesis of gelatinous drop-like corneal dystrophy. *Am J Pathol* 2010; **177**: 1344–1355.
18 Kinoshita S, Kawasaki S, Kitazawa K, Shinomiya K. Establishment of a human conjunctival epithelial cell line lacking the functional TACSTD2 gene (an American Ophthalmological Society thesis). *Trans Am Ophthalmol Soc* 2012; **110**: 166–177.
19 Kitazawa K, Kawasaki S, Shinomiya K, Aoi K, Matsuda A, Funaki T *et al.* Establishment of a human corneal epithelial cell line lacking the functional TACSTD2 gene as an in vitro model for gelatinous drop-like dystrophy. *Invest Ophthalmol Vis Sci* 2013; **54**: 5701–5711.
20 Tsujikawa M, Maeda N, Tsujikawa K, Hori Y, Inoue T, Nishida K. Chromosomal sharing in atypical cases of gelatinous drop-like corneal dystrophy. *Jpn J Ophthalmol* 2010; **54**: 494–498.