Four Mutations (Three Novel, One Founder) in *TACSTD2* among Iranian GDLD Patients

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Purpose. To perform a mutation screening of *TACSTD2* in 13 Iranian Gelatinous Drop-like Corneal Dystrophy (GDLD) pedigrees. To assess genotype–phenotype correlations. To determine intragenic SNP haplotypes associated with the mutations, so as to gain information on their origin.

Methods. The coding region of *TACSTD2* was sequenced in the probands of 13 unrelated Iranian GDLD pedigrees. Variations were assessed in other available affected and unaffected family members and in unrelated normal control subjects by restriction fragment length polymorphism (RFLP). The variations were classified as being associated with disease if they segregated with the disease phenotype in the families, were not observed in 100 control individuals, disrupted protein expression, or affected conserved positions in the coded protein. Three intragenic single-nucleotide polymorphisms (SNPs) were used to define haplotypes associated with putative disease-causing mutations.

Results. The probands were each homozygous for one of four putative disease-causing variations observed in *TACSTD2*: C66X, F114C, L186P, and E227K. Three of these are novel. E227K was found in 10 of the Iranian patients. There were some phenotypic differences among different patients carrying this mutation—for example, with respect to age at onset. Genotyping of intragenic SNPs identified four haplotypes. C66X, F114C, and L186P were each associated with a haplotype common among control chromosomes, whereas all E227K alleles were associated with a haplotype not found among the control chromosomes.

Conclusions. Although mutations in *TACSTD2* among Iranian patients with GDLD were heterogeneous, E227K was found to be a common mutation. It is suggested that E227K may be a founder mutation in this population. Based on positions of known mutations in *TACSTD2*, significance of the thyroglobulin domain of the *TACSTD2* protein in the pathogenesis of GDLD is suggested. (*Invest Ophthalmol Vis Sci.* 2007;48: 4490–4497) DOI:10.1167/iovs.07-0264

Gelatinous drop-like corneal dystrophy (GDLD, corneal familial subepithelial amyloidosis; OMIM 204870; Online Mendelian Inheritance in Man; http://www.ncbi.nlm.nih.gov/Omim/ provided in the public domain by the National Center for Biotechnology Information, Bethesda, MD) is a rare inherited disease first described by Nakaizumi in 1914.[1] It is characterized by the deposition of amyloid material in the subepithelial space of the cornea. GDLD is one of several forms of corneal dystrophies which are accompanied by amyloidosis, the others being Avellino corneal dystrophy (ACD) and different types of lattice corneal dystrophy (LCD). Clinical symptoms of GDLD most often manifest within the first decade of life. Nodular depositions in the central cornea which appear in the early stage of disease later increase in number and depth and coalesce, usually to create a protruding whitish-yellow mulberry appearance.[2–4] Other forms of coalescence have also been reported.[5] Neovascularization of the subepithelial and superficial stroma may appear in advanced stages of the disease.[6] Affected individuals experience lacrimation, photophobia, foreign body sensation, and blurred vision. Eventually, gelatinous masses severely impair visual acuity and penetrating or lamellar keratoplasty, photoablation, or keratectomy is prescribed. Unfortunately, symptoms generally recur within a few years after intervention, and repeated keratoplasties are often performed.[7]

GDLD is inherited in an autosomal recessive fashion.[8,9] The disease has most often been reported in the Japanese population, in which its incidence is estimated at 1 in 300,000.[8] A locus on the short arm of chromosome 1 was linked to the disease by homozygosity mapping of patients of this population in 1998.[6] Later, Membrane component, chromosome 1, Surface marker 1 (*M1S1*), originally identified as the gene encoding gastrointestinal tumor–associated antigen and also known as *GA733-1* and *TROP2*, was identified as the causative gene at this locus.[10] The official name of the gene is now *TACSTD2* (tumor associated calcium signal transducer 2).[11] A founder mutation, Q118X, was found in this gene among Japanese patients with GDLD.[8] In addition to Japan, cases of GDLD from India,[12–14] Tunisia,[3,13–15] and other countries[13,14,16–21] have also been reported. Putative disease-causing mutations in *TACSTD2* were found in almost all cases in which mutation screening of the gene was performed.[10,14,18–28] In total, 19 different GDLD-causing alterations in *TACSTD2* have been reported to date.[10,14,18–21,25,26] However, three unrelated GDLD pedigrees have been identified wherein mutations in *TACSTD2* were not found, suggesting genetic heterogeneity for the disease.[14,28,29] The previously reported GDLD-causing mutations in *TACSTD2* are presented in Table 1.

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TABLE 1. GDLD-Associated Mutations in *TACSTD2*

No.	Gene Location*	cDNA Location*	Effect on Protein	Country	Ref.
1	g.618T>G	c.2T>G	p.M1R	India	14
2	g.866A>T	c.250A>T	p.K84X	Japan	25
3	g.938T>C	c.322T>C	p.C108R	Japan	25
4	g.968C>G	c.352C>G	p.Q118E	India	14
5	g.968C>T	c.352C>T	p.Q118X	Japan	10
6	g.971T>A	c.355T>A	p.C119S	Tunisia	14
7	g.1109_1110 insCCACCGCC	c.493_494 insCCACCGCC	p.A164fs	India	14
8	g.1125C>A	c.509C>A	p.S170X	Japan	10
9	g.1136_1137insC	c.520_521insC	p.D174fs	Estonia	18
10	g.1167A>G	c.551A>G	p.Y184C	China	20
11	g.1173T>C	c.557T>C	p.L186P	Japan	26
12	g.1177delC	c.561delC	p.P188fs	Europe	14
13	g.1197T>A	c.581T>A	p.V194E	India	14
14	g.1235C>T	c.619C>T	p.Q207X	Japan	10
15	g.1248delA	c.632delA	p.S210fs	Japan	10
16	g.1269delA	c.653delA	p.V217fs	Turkey	21
17	g.1388_1389insT	c.772_773insT	p.I258fs	Vietnam	19
18	g.1388_1399 delATCTATTACCTG	c.772_783 delATCTATTACCTG	p.L257_p.D262 delIYYL	Vietnam	19
19	g.1427delA	c.811delA	p.M270fs	Tunisia	14

* Gene and cDNA locations are given as directly reported in the corresponding reference. If not reported in the reference, they are derived from reference sequences based on information provided in the articles. Reference sequences used were NT_032977, NM_002353, and NP_002344.1.

The *TACSTD2* gene product is a multimodule transmembrane glycoprotein of 323 amino acids. The domains of the protein include an epidermal growth factor (EFG)–like repeat, a thyroglobulin type 1A (TY) repeat, a transmembrane domain (TM), and a phosphatidylinositol (PIP2)-binding site.[10] It has been suggested that the coded protein functions as a cell–cell adhesion receptor in cancer cells and as a calcium signal transducer.[30,31] With respect to GDLD, it

FIGURE 1. Geographic origin of Iranian GDLD patients. The geographic origins of the patients are shown with dots (●) within the provinces. The numbers indicate the *TACSTD2* sequence variations associated with disease: 1, C66X; 2, F114C; 3, L186P; 4, E227K.

has been shown that the corneal epithelium of affected eyes has notably increased permeability, and it has been suggested that this may be a direct consequence of the abnormal TACSTD2 protein expressed in the tissue.[32,33] The increased permeability is likely to be relevant to the pathogenesis of the disease.

Among Middle Eastern countries, one GDLD pedigree from Turkey has been described.[21] Herein, we report the results of mutation screening of *TACSTD2* in 13 Iranian GDLD pedigrees. Four putative disease-causing mutations in *TACSTD2*, three of which are novel, were identified in the 13 pedigrees. An intragenic SNP haplotype associated with a common Iranian *TACSTD2* mutation is presented, and the possibility of its being a founder mutation is considered.

Materials and Methods

This research was performed in accordance with the Declaration of Helsinki and with the approval of the ethics board of the International Institute of Genetic Engineering and Biotechnology in Iran. Thirteen Iranian GDLD pedigrees were identified. All families consented to participate after being informed of the nature of the research. In total, 41 individuals belonging to these families were studied: 13 probands (one from each family), 8 additional affected individuals, and 20 unaffected individuals. The probands were first diagnosed with GDLD in the years between 1987 and 2005. Diagnosis was made by corneal specialists at the Farabi Hospital (associated with Tehran University of Medical Sciences) and the Labbafi-Nejhad Hospital (associated with Shaheed Beheshti University of Medical Sciences and Health Services) in Tehran. The hospitals are national reference centers, and patients from throughout the country are referred to them. Diagnosis of GDLD was based on classic clinical appearance and slit-lamp biomicroscopy. Individuals whose corneal surfaces had a mulberry-like appearance were considered to be affected. The diagnosis in all but two cases (probands of pedigrees 100-3 and 100-6) was confirmed with histopathology by staining for amyloid with congo red. Ocular, medical, and family histories were obtained for each available family member. Nine of the probands were offspring of consanguineous marriages. Both parents in the four remaining pedigrees came from small isolated villages wherein blood relationships between individuals are highly likely. One hundred ethnically matched unrelated healthy control individuals without a family history of eye diseases (self reported) were also recruited for the study.

The single exon of *TACSTD2* was amplified from the DNA of the 13 probands. Amplification was usually performed by two polymerase chain reactions (PCR) with primers M1S1-Fa (5′-GAGTATAAGAGCCG-GAGGGAG-3′) and M1S1-Ra (5′-CATCGCCGATATCCACGTCAC-3′), and M1S1-Fb (5′-CTGAGCCTACGCTGCGATGAG-3′) and M1S1-Rb (5′-GGATCTATTAAACCTGGTGTGTG-3′). There was a 244-nucleotide overlap in the two amplified products. Together, they provided the sequence of the entire 972-nucleotide coding region, and 109 nucleotides upstream, and 139 nucleotides downstream of the coding region. Amplification was sometimes performed in a single reaction with primers M1S1-Fa and M1S1-Rb. PCR with primers M1S1-Fa and M1S1-Ra and PCR with primers M1S1-Fa and M1S1-Rb were performed with a touchdown protocol.

The amplified products were sequenced in both forward and reverse directions with the PCR primers using dye termination chemistry (Big Dye kit and the Prism 3700 squencer; Applied Biosystems [ABI], Foster City, CA). Sequences were analyzed on com-

Table 2. Clinical Features of Probands of Iranian GDLD Pedigrees

Pedigree	Age at Onset (y)	Present Age (y)	Relative Severity in Two Eyes*	Type of Amyloidosis†	Site of Amyloidosis	Vascularization‡	Surgeries§ (*n*)	Recurrence‖	Visual Acuity¶
100-1	9	50		Mulberry-like	Central	After keratoplasty			
100-2	2	36	R > L	Mulberry-like	Central	No	R: 3 L: 2	8 mo	R: 3/10 L: 9/10
100-3	7	46	R > L	Mulberry-like	Central	Yes	R: 5 L: 4	2 y	R: 30 cm L: 3 m
100-4	< 1 (4 mo)	29	L > R	Mulberry-like	Central	No	R: 4 L: 3	~1 y	R: 2/10 L: 1/10
100-5	6	18	R > L	Mulberry-like	Central	No	R: 7 L: 7	1 y	R: 5/10 L: 5/10
100-6	1	21	L > R	Mulberry-like	Central	Yes	R: 2 L: 0	2 mo	R: 2 m L: 3 m
100-7	18	38	L > R	Mulberry-like	Paracentral	After keratoplasty	R: 2 L: 2	1 y	R: 1/10 L: 1/10
100-8	15	24	L > R	Mulberry-like	Paracentral	No	R: 1 L: 2	1 y	R: 4/10 L: 1/10
100-9	10	43	R > L	Mulberry-like	Central	After keratoplasty	R: 3 L: 3	4 mo	R: 0.5m L: 0.5m
100-10	9	37	L > R	Mulberry-like	Central	Yes	R: 3 L: 1	2 mo	R: 2 m L: 2 m
100-11	20	40	L > R	Mulberry-like	Central	No	R: 2 L: 1	1 mo	R: 1 m L: 1 m
100-12	~4	50		Mulberry-like	Central	Yes	R: 2 L: 3	~1 y	R: 3 m L: 1/10
100-13	< 1 (6 mo)	10	R > L	Mulberry-like	Central	No	R: 1 L: 0		R: 2 m L: 2 m

Unknown clinical features are left blank. R, right eye; L, left eye.

* All patients were affected bilaterally. The eye with more severe phenotypes, based on self-report by patients, is indicated.

† Before surgery; based on classification in Reference 5.

‡ Unless otherwise indicated, vascularization was prior to surgery.

§ Including keratectomy, excimer laser keratomileusis, and lamellar and penetrating keratoplasty. Patients sometimes refused reoperation because of dissatisfaction with results of the previous surgery.

‖ Minimum time elapsed between surgery and recurrence.

¶ Most recent recording.

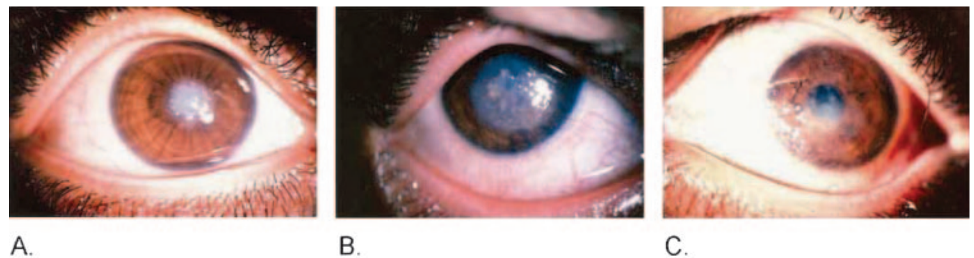
puter (Sequencher software; Gene Codes Corp., Ann Arbor, MI). Sequence variations and numbering were assessed by comparison with reference sequences available at the National Center for Biotechnology Information (NCBI; http://www.ncbi.nlm.nih.gov): NT_032977, NM_002353, and NP_002344.1. Predicted effects of variant sequences on splicing were determined by comparison with known canonical splice-site motifs (http://www.fruitfly.org/seq_tools/splice.html). For determination of the extent of conservation of amino acids altered due to nucleotide variations found in *TACSTD2*, the amino acid sequences of 11 TACSTD2 and related TACSTD1 proteins from eight species were obtained from NCBI (http://www.ncbi.nlm.nih.gov/BLAST/Blast.cgi) and aligned using ClustalW software (http://www.ebi.ac.uk/clustalw). Similarly, to assess conservation of amino acids altered within the thyroglobulin type 1A domain of *TACSTD2*, 18 such domains from 13 proteins were aligned.

Five of the sequence variations found in *TACSTD2* were assessed in available affected and nonaffected family members by restriction enzyme digestion and fragment length polymorphism (RFLP). They were also assessed in the 100 unrelated control individuals by the same procedure. Variations found in more than 1% of the chromosomes of our control cohort were considered polymorphisms. Core haplotypes defined by two intragenic polymorphisms and one rare variation in *TACSTD2* were assessed in the probands and control individuals using PLINK (provided in the public domain by the Psychiatric and Neurodevelopmental Genetics Unit, Harvard Medical School, Boston, MA, at http://pngu.mgh.harvard.edu/~purcell/plink).

Results

Ten of the 13 Iranian GDLD pedigrees were from northern Iran and three came from provinces in central Iran (Fig. 1). The clinical features of the probands are presented in Table 2. Typical slit lamp photographs and histologic sections of the corneas of patients are presented in Figure 2. Diagnosis of GDLD was based on clinical and histologic features such as those evident in this figure.

Seven sequence variations were identified in the *TACSTD2* gene of the Iranian patients with GDLD (Table 3). They were all observed in the homozygous state. Five of the variations resulted in amino acid changes, one created a stop codon, and one was in the $5'$ noncoding region. c.198C>A causing C66X was found in one patient and is a novel variation. It was assessed to be associated with GDLD, as it creates a stop codon early in the coding region, within the EGF-like domain. The protein product of the mutated allele is expected to be the shortest truncated TACSTD2 reported to date. Previously, mutation K84X, coding a slightly longer truncated product, was reported as one of two disease-causing mutations in *TACSTD2* in a Japanese patient with GDLD[25] c.341T>G causing F114C, c.557T>C causing L186P, and c.679G>A causing E227K were also associated with disease. L186P found in one Iranian patient has been described as a disease-causing mutation in a Japanese patient.[26] This mutation produces an amino acid change at a conserved position in the region between the TY and TM domains of the protein, wherein several disease-asso-



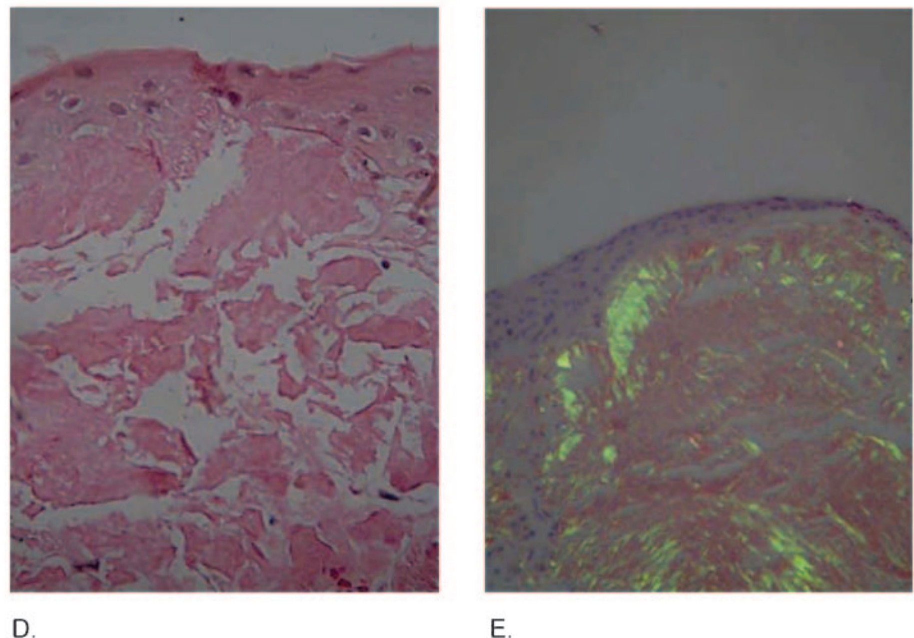


Figure 2. Slit lamp and histologic appearance of the corneas. (**A–C**) Slit lamp photographs of eyes of GDLD probands homozygous for the E227K mutation. Pictures of pedigrees 100-4 and 100-10 were taken before surgery, whereas picture of pedigree 100-7 was taken after surgery. (**A**) Pedigree 100-4: early mulberry-like amyloid deposition in the central cornea, without vascularization. (**B**) Pedigree 100-10: advanced stage mulberry-like amyloid deposition in the central cornea with significant vascularization. (**C**) Pedigree 100-7: advanced stage paracentral mulberry-like amyloid deposition with vascularization. (**D**, **E**) Histologic sections of the proband of pedigree 100-6. (**D**) Hematoxylin-eosin–stained section viewed with a Tungsten filter. The subepithelial and superficial stroma contained amorphous eosinophilic material. The overlying epithelium and Bowman's membrane were atrophied. (**E**) Congo red staining under polarized light. Typical *apple-green* birefringence in subepithelial and superficial stroma, indicative of amyloid, are shown. Magnification: (**D**) ×400; (**E**) ×100.

Table 3. *TACST2* Variations in Iranian Patients with GDLD

Gene Location*	cDNA Location*	Effect on Protein*	Patients† (*n*)	Pedigree ID	Percent of Variant Allele among Control Chromosomes‡	
g.563A>C	c.-54A>C	5′ Noncoding	10	100-1-100-10	8.6	(*Hpy*99I)
g.814C>A	**c.198C>A**	**p. C66X**	1	100-11	—	
g.957T>G	**c.341T>G**	**p. F114C**	1	100-12	0	(*Hpy*CH4V)
g.1057G>C	c.441G>C	p. E147D	10	100-1-100-10	16.5	(*Alu*I)
g.1173T>C	**c.557T>C**	**p. L186P**	1	100-13	—	
g.1264C>A	c.648C>A	p. D216E	10	100-1-100-10	0	(*Hpy*CH4IV)
g.1295G>A	**c.679G>A**	**p. E227K**	10	100-1-100-10	0	(*Taq*I)

* Reference sequences used were NT_032977, NM_002353, and NP_002344.1. Bold: variations thought to cause GDLD.
† All patients carried the variations in the homozygous state.
‡ The variations were assessed by RFLP in 100 control individuals. The restriction enzyme used for each variation is indicated in parenthesis. For analysis of variation c.341T>G, a 175-bp fragment was amplified using M1S1-Fm (5′-CCACGCTGACCTCCAAGTGTC-3′) and M1S1-Rm (5′-CACACCGACGTCTGGTTG**T**AC-3′). A nucleotide variation (shown in bold) was introduced into M1S1-Rm, so as to eliminate a cleavage site and facilitate interpretation of electrophoretic patterns.

ciated amino acid alterations have been reported (Table 4 and Ref. 14).

F114C observed in one Iranian patient, has not been previously reported. The only sibling of the proband, who was also affected, was unavailable, but the nucleotide change which caused the amino acid substitution was not observed in the DNA of 100 control individuals. F114C causes the nonconservative substitution of a polar for a nonpolar amino acid within the TY domain of TACSTD2. Phenylalanine at position 114 of the human protein is completely conserved in the TACSTD2 and related TACSTD1 proteins of all species thus far sequenced (Table 4). Furthermore, in various proteins containing a thyroglobulin type 1A domain, the corresponding position is always phenylalanine or tyrosine (Table 5). Position 114 is close to the highly conserved QC and CWCV motifs of the domain (Tables 4, 5, and Ref. 34). Creation of an additional cysteine residue at this position by mutation most likely affects the three disulfide bonds that normally occur in all thyroglobulin type 1A domains (Fig. 3 and Ref. 34).

E227K, which also has not been previously reported, was observed in 10 of the 13 Iranian patients with GDLD, whereas the causative nucleotide substitution was not seen in the 100 control individuals screened. Affected and nonaffected family members were available from five of the pedigrees carrying the variation. A representative pedigree is shown in Figure 4. In this and all other pedigrees, affected individuals carried the variation in the homozygous state, and nonaffected individuals carried one or two copies of the wild-type nucleotide (Fig. 4). Glutamic acid at position 227 is completely conserved in TACSTD2 and related proteins (Table 4). The variation identified results in a negatively charged amino acid to be substituted by a positively charged amino acid. These findings strongly suggest that the variation causes GDLD. E227K is positioned in the region between the TY and TM domains of TACSTD2.

Variations c.−54A>C in the 5′ noncoding region, c.441G>C causing E147D, and c.648C>A causing D216E are thought not to be associated with GDLD. The altered nucleotides at c.−54 (C) and c.441 (C) were observed in 8.6% and 16.5%, respectively, of the chromosomes of the 100 healthy control individuals. The relatively high frequency of the variant nucleotides (C at both positions) suggests they are unlikely to cause to the disease. The altered nucleotide at c.648 (A) was not found in any of the Iranian control individuals, but has been reported in the HapMap site (http://www.hapmap.org/cgi-perl/gbrowse/hapmap_B35/; rs 232835; Provided by the International HapMap Project, a worldwide consortium of scientists) at frequencies of 0.058, 0.080, 0.131, and 0.427 among European, Chinese, Japanese, and African individuals, respectively. It was also reported as a common polymorphism among the control cohort of a study on GDLD in the Japanese population.[23] It is thus assumed that the variation was not observed among the Iranian control subjects because it is very rare. E174D and D216E both result in the substitution of one negatively charged amino acid for another at nonconserved positions of TACSTD2 (not shown). At position 216 precisely, glutamic acid is found in many TACSTD2-related proteins.

The three variant positions c.−54A>C, c.441G>C, and c.648C>A, thought not to be associated with GDLD, were

Table 4. Alignment of Disease-Associated Amino Acid Variations

Variation	C66X	F114C*	L186P	E227K	Sequence ID†
TACST2-Human	VD CSTLTSKCLL	R FKAR*QC*NQTSV*CWCV*NSVG	RERYR LHPK	QKAAGEVDIGDAAYYFE RDI	NP_002344.1
TACST2-Rat	VD CSTLTSKCLL	R FKAR*QC*NQTSV*CWCV*NSVG	KERYK LHPS	QKGLRDVDIADAAYYFE RDI	NP_001009540.2
TACST2-Mouse	VD CSTLTSKCLL	R FKAR*QC*NQTSV*CWCV*NSVG	QERYK LHPS	QKGLRDVDIADAAYYFE RDI	NP_064431.2
TACST1-Chiken	VN CEILTSKCLL	L FKAK*QC*NGT-T*CWCV*NTAG	TSRYM LDGR	DKTPGDVDITDVAYYFE KDV	NP_001012582.1
TACST1-Rat	VI CSKLASKCLV	L FKAK*QC*NGTAT*CWCV*NTAG	ASRYM LNPK	QKTQDDVDIADVAYYFE KDV	NP_612550.1
TACST1-Pig	VI CSKLASKCLV	L FKAK*QC*NGTSM*CWCV*NTAG	TDRYQ LDPK	QKTLNEVDIADVAYYFE KDV	NP_999584.1
TACST1-Mouse	VI CSKLASKCLA	L FKAK*QC*NGTAT*CWCV*NTAG	TSRYK LNQK	QKTQDDVDIADVAYYFE KDV	NP_032558.2
TACST1-Human	VI CSKLAAKCLV	L FKAK*QC*NGTST*CWCV*NTAG	TTRYQ LDPK	QKTQNDVDIADVAYYFE KDV	NP_002345.1
TACST1-M.mulatta	VL CSKLAAKCLV	L FKAK*QC*NGTST*CWCV*NTAG	KTRYQ LDPK	QKTQNDVDIADVAYYFE KDV	NP_001035118.1
TACST1-Cow	VI CTKLATKCLV	L FKAK*QC*NGTST*CWCV*NTAG	TNRYQ LDPK	QKTQNDVDIADVAYYFE KDV	NP_001030367.1
TACST1-Xenopus	VD CTKLIPKCWL	V FKAR*QC*NNTDT*CWCV*NTAG	LNRYG LPEK	QKLPGEVDITDVGYYME KDI	AAN86618.1

Row showing sequence of human *TACSTD2*, and columns showing positions of amino acid changes are shaded.
* Italics: Conserved motifs QC and CWCV in the TY domain.
† Sequence ID numbers at NCBI (http://www.ncbi.nlm.nih.gov/BLAST/).

Table 5. Conservation of F114 in Thyroglobulin Type 1 Domains

Variation	F114C*	Seq ID†
TACD2_HUMAN	GR FKAR*QC*N-----QTSV*CWCV*NS-VG-	P09758
TACD1_HUMAN	GL FKAK*QC*N-----GTST*CWCV*NT-AGV	P16422
IBP1_HUMAN	GF YHSR*QC*ETSMDGEAGL*CWCV*YPWNGK	P08833
IBP2_HUMAN	GL YNLK*QC*KMSLNGQRGE*CWCV*NPNTGK	P18065
IBP3_HUMAN	GF YKKK*QC*RPSKGRKRGF*CWCV*DK-YGQ	P17936
IBP4_HUMAN	GN FHPK*QC*HPALDGQRGK*CWCV*DRKTGV	P22692
IBP5_HUMAN	GF YKRK*QC*KPSRGRKRGI*CWCV*DK-YGM	P24593
IBP6_HUMAN	GF YRKR*QC*RSSQGQRRGP*CWCV*DR-MGK	P24592
THYG2_HUMAN	GD YAPV*QC*D----VQQVQ*CWCV*D-AEGM	P01266
THYG4_HUMAN	GD YQAV*QC*QT-----EGP*CWCV*DA-QGK	P01266
THYG5_HUMAN	GS YEDV*QC*FS------GE*CWCV*NS-WGK	P01266
THYG6_HUMAN	GH FLPV*QC*------FNSE*CYCV*D-AEGQ	P01266
THYG10_HUMAN	-- FSPV*QC*D----QAQGS*CWCV*M-DSGE	P01266
NID2a_HUMAN	GN FLPL*QC*H----GSTGF*CWCV*D-PDGH	Q14112
NID2b_HUMAN	GH FIPL*QC*H----GKSDF*CWCV*D-KDGR	Q14112
TICN1_HUMAN	GY YKAT*QC*HGSTGQ----*CWCV*DK-YGN	Q08629
SAX_RANCA	-- YQPQ*QC*H----GSTGH*CWCV*N-AMGE	P31226
EQST_ACTEQ	GS YNPV*QC*W----PSTGY*CWCV*D-EGGV	P81439

Row showing sequence of human *TACSTD2*, and column showing residues corresponding to F114 in the human *TACSTD2* are shaded.

* Italics: conserved motifs QC and CWCV in the TY domain.

† Sequence ID numbers at the Expasy server (http://www.expasy.org/sport/).

used to define intragenic haplotypes. Three haplotypes, -AGC-, -CGC-, and -ACC- were identified among the control subjects, and the most frequent of these was -AGC- (71.6%). Three of our putative disease-causing variations, C66X, F114C, and L186P, were found on this haplotype background. All alleles carrying the E227K mutation common among the Iranian patients were associated with a fourth haplotype -CCA-, not found among the control subjects.

Discussion

Four disease-associated mutations in *TACSTD2*, C66X, F114C, L186P, and E227K, were identified among the 13 Iranian GDLD pedigrees studied. Three of the mutations are novel. E227K was common among the Iranian patients, having been observed in probands of 10 of the pedigrees. All E227K mutated alleles identified were associated with the haplotype -CCA-, and this haplotype was not observed among the chromosomes of 100 Iranian control individuals. Furthermore, at least two mutation and/or recombination events between the haplotypes found among the control subjects would be required to create the -CCA- haplotype. The linkage of all observed E227K mutated alleles with the same haplotype and the rarity of that haplotype among Iranians suggest that E227K is a founder mutation in this population. In addition, the E227K mutation originally either occurred on a very rare haplotype background or was introduced into the population. The other three mutations of the Iranian patients were all associated with a haplotype (-AGC-) common among the Iranian control cohort. However, as the haplotype is expected to be common worldwide based on allele frequencies available at the HapMap site, the origin of the mutations cannot be assessed (not shown). These mutations were rare and, therefore, are expected to have been introduced more recently than E227K. The c.653delA mutation recently reported in a GDLD patient from nearby Turkey was not found among the Iranian patients.[21]

Mutations affecting initiation of protein synthesis or those creating early stop codons and frame shifts during translation are generally expected to have global detrimental effects on protein function. However, those causing amino acid alterations may be more informative with regard to the biochemical reason for development of a disease phenotype. With regards to GDLD, 8 of the 21 reported putative disease-causing mutations produce amino acid alterations (including mutations presented in this study and excluding M1R which affects initiation of protein synthesis). Four of these (C108R, F114C, Q118E, and C119S) change amino acids within the TY domain of TACSTD2 and the remaining four (Y184C, L186P, V194E, and E227K) change amino acids within a region between the TY and TM domains, signifying the importance of these regions in relation to TACSTD2 function. The TY domain is constituted by only 76 of the 323 amino acids of TACSTD2 (residues 70-145; http://ca.expasy.org/uniprot/P09758). The function of the region between the TY and TM domains is unknown, but the mutations

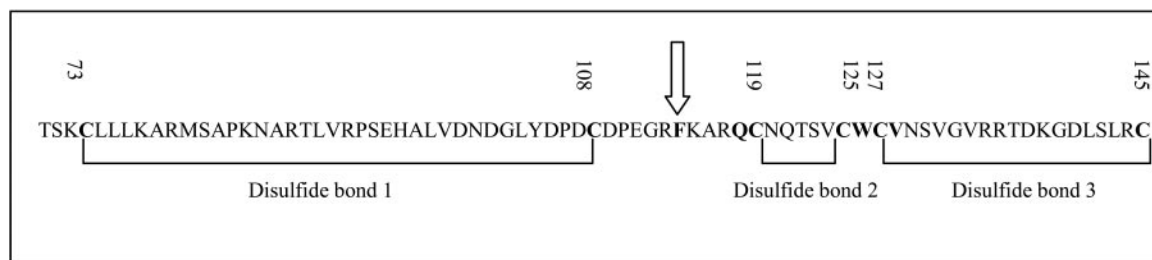


Figure 3. Conserved disulfide bonds of the thyroglobulin type 1A domain. The conserved disulfide bonds are shown within the framework of the TY domain of human TACSTD2. The amino acid numbers of the cysteines between which disulfide bonds are formed are indicated *above* the sequence. *Arrow*: phenylalanine, which is changed to cysteine by the mutation c.341T>G. *Bold*: QC and CWCV motifs.

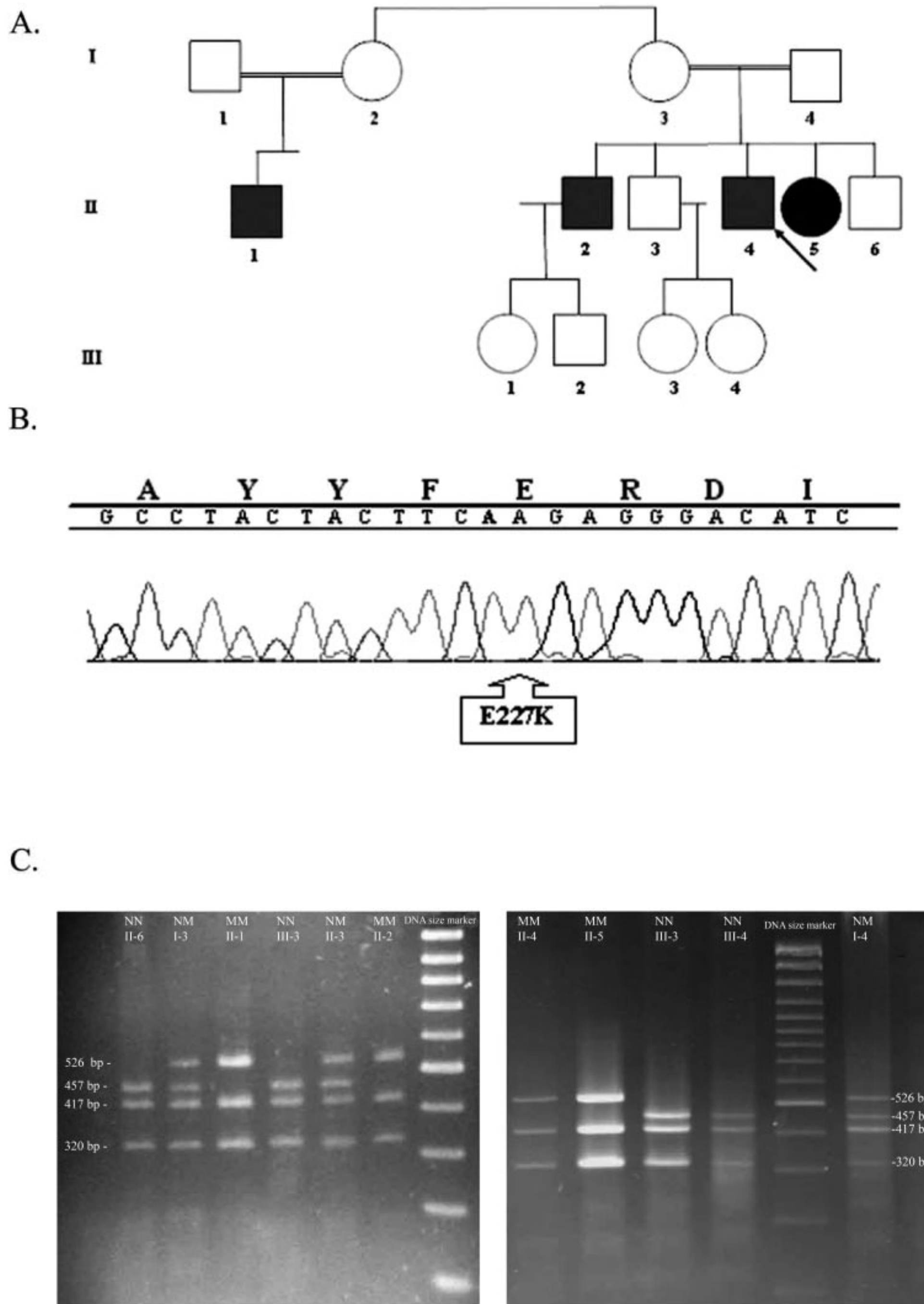


Figure 4. Pedigree 100-2 carrying the E227K variation. (**A**) The pedigree. (**B**) Sequence chromatogram of the proband showing a homozygous c.679G>A variation resulting in E227K. (**C**) RFLP patterns of *Taq*I digest of the PCR amplicon of pedigree members obtained with M1S1-Fa and M1S1-Rb primers. Nonaffected individuals are homozygous (NN) or heterozygous (NM) for the wild-type allele, whereas affected individuals were all homozygous for the variant allele (MM). *Taq*I digestion of the wild-type amplicon produced a 69-bp fragment which migrates out of the gel.

in the TY domain may affect its role as a protease inhibitor and thus affect amyloid deposition.[35,36]

Mutation C66X causes very early protein truncation during protein synthesis. This nonsense codon may also result in reduced levels of mRNA.[37] GDLD could be due to the absence of functional TASCTD2 and/or effects of the truncated protein. It is interesting that the patient carrying this mutation showed initial symptoms of disease only at the age of 20, later than all the other patients studied. Different individuals within the same or different pedigrees carrying the same E227K mutation presented various phenotypic features. For example, age at onset in different individuals carrying the E227K mutation ranged from 4 months to 7 years within the same pedigree (pedigree 100-4) and from 4 months to 18 years in different pedigrees (Table 2). Whereas amyloid deposition initially occurred centrally in the cornea of most of the E227K carrying patients, deposition was paracentral in the eyes of two patients (pedigrees 100-7 and 100-8; Table 2, Fig. 2A). These observations suggest that factors in addition to mutations in *TACSTD2* may affect the phenotypic features of patients with GDLD.

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References

1. Nakaizumi G. A rare case of corneal dystrophy. *Acta Soc Ophthalmol Jpn*. 1914;18:949–950.

2. Mondino BJ, Rabb MF, Sugar J, Sundar R, Brown SI. Primary familial amyloidosis of the cornea. *Am J Ophthalmol*. 1981;92:732–736.
3. Weber FL, Babel J. Gelatinous drop-like dystrophy; a form of primary corneal amyloidosis. *Arch Ophthalmol*. 1980;98:144–148.
4. Ohnishi Y, Shinoda Y, Ishibashi T, Taniguchi Y. The origin of amyloid in gelatinous drop-like corneal dystrophy. *Curr Eye Res*. 1982;2:225–331.
5. Ide T, Nishida K, Maeda N, et al. A spectrum of clinical manifestations of gelatinous drop-like corneal dystrophy in Japan. *Am J Ophthalmol*. 2004;137:1081–1084.
6. Fujita S, Sameshima M, Hirashima S, Nakao K. Light and electron microscopic study of gelatinous drop-like corneal dystrophy with deeper stromal involvement [in Japanese]. *Nippon Ganka Gakkai Zasshi*. 1988;92:1744–1757.
7. Smolin G. Corneal dystrophies and degenerations. In: Smolin G, Thoft RA, eds. *Cornea*. 3d ed. Little, Brown, Boston, 1994, pp 499–533.
8. Tsujikawa M, Kurahashi H, Tanaka T, et al. Homozygosity mapping of a gene responsible for gelatinous drop-like corneal dystrophy to chromosome 1p. *Am J Hum Genet*. 1998;63:1073–1077.
9. Klintworth GK. The molecular genetics of the corneal dystrophies: current status. *Front Biosci*. 2003;8:687–713.
10. Tsujikawa M, Kurahashai H, Tanaka T, et al. Identification of the gene responsible for gelatinous corneal dystrophy. *Nat Genet*. 1999;21:420–423.
11. Calabrese G, Crescenzi C, Morizio E, Palka G, Guerra E, Alberti S. Assignment of TACSTD1 (alias TROP1, M4S1) to human chromosome 2p21 and refinement of mapping of TASCSTD2 (alias TROP2, M1S1) to human chromosome 1p32 by in situ hybridization *Cytogenet Cell Genet*. 2001;92:164–165.
12. Li S, Edward DP, Ratnakar KS, Reddy M, Tso MO. Clinohistopathological finding of gelatinous droplike corneal dystrophy among Asians. *Cornea*. 1996;15:355–362.
13. Klintworth GK, Summer JR, Obrian G, et al. Familial subepithelial corneal amyloidosis (gelatinous drop-like corneal dystrophy): exclusion of linkage to lactoferrin gene. *J Mol Vis*. 1998;4:31–38.
14. Ren Z, Lin P-Y, Klintworth GK, Iwata S, et al. Allelic and locus heterogeneity in autosomal recessive gelatinous drop-like corneal dystrophy. *Hum Genet*. 2002;110:568–577.
15. El Matri L, Bachtobji A, Ghorbal M, et al. Familial form of gelatin drop corneal dystrophy (in French). *J Fr Ophtalmol*. 1991;14:125–129.
16. Klintworth GK, Valnickova Z, Kielar RA, Baratz KH, Campbell RJ, Enghild JJ. Familial subepithelial corneal amyloidosis- a lactoferrin related amyloidosis. *Invest Ophthalmol Vis Sci*. 1997;38:2756–2763.
17. Gartry DS, Falcon MG, Cox RW. Primary gelatinous drop-like keratopathy. *Br J Ophthalmol*. 1989;73:661–664.
18. Tasa G, Kals J, Muru K, et al. A novel mutation in the M1S1 gene responsible for gelatinous droplike corneal dystrophy. *Invest Ophthalmol Vis Sci*. 2001;42:2762–2764.
19. Ha NT, Chau HM, Cung LX, et al. A novel mutation of M1S1 gene found in a Vietnamese patient with gelatinous droplike corneal dystrophy. *Am J Ophthalmol*. 2003;135:390–393.
20. Tian X, Fujiki K, Li Q, et al. Compound heterozygous mutations of M1S1 gene in gelatinous droplike corneal dystrophy. *Am J Ophthalmol*. 2004;137:567–569.
21. 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.
22. Ha NT, Fujiki K, Hotta Y, Nakayasu K, Kanai A. Q118X mutation of M1S1 gene caused gelatinous drop-like corneal dystrophy: the P501T of BIGH3 gene found in a family with gelatinous drop-like corneal dystrophy. *Am J Ophthalmol*. 2000;130:119–120.
23. Tsujikawa M, Tsujikawa K, Maeda N, et al. Rapid detection of *M1S1* mutations by the protein truncation test. *Invest Ophthalmol Vis Sci*. 2000;41:2466–2468.
24. Yoshida S, Kumano Y, Yoshida A, et al. Two brothers with gelatinous drop-like dystrophy at different stages of the disease: role of mutational analysis. *Am J Ophthalmol*. 2002;133:830–832.
25. Murakami A, Kimura S, Fujiki K, Fujimaki T, Kanai A. Mutations in the membrane component, chromosome 1, surface marker 1 (*M1S1*) gene in gelatinous drop-like corneal dystrophy. *Jpn J Ophthalmol*. 2004;48:317–320.
26. Taniguchi Y, Tsujaikawa M, Hibino S, et al. A novel missense mutation in a Japanese patient with gelatinous droplike corneal dystrophy. *Am J Ophthalmol*. 2005;139:186–188.
27. Klintworth GK. Advances in the molecular genetics of corneal dystrophies. *Am J Ophthalmol*. 1999;128:747–754.
28. Fujiki K, Nakayasu K, Kanai A. Corneal dystrophies in Japan. *J Hum Genet*. 2001;46:431–435.
29. Akhtar S, Bron AJ, Qin X, Creer RC, Guggenheim JA, Meek KM. Gelatinous drop-like corneal dystrophy in a child with developmental delay: clinicopathological features and exclusion of the M1S1 gene. *Eye*. 2005;19:198–204.
30. Zutter MM. Gastrointestinal carcinoma antigen GA733: target for immunodestruction and potential modifier of invasiveness and chemoresponsiveness. *J Natl Cancer Inst*. 1998;90:642–644.
31. Ripani E, Sacchetti A, Corda D, Alberti S. Human Trop-2 is a tumor associated calcium signal transducer. *Int J Cancer*. 1998;76:671–676.
32. Kinoshita S, Yokoi N, Komuro A. Barrier function of ocular surface epithelium. *Adv Corneal Res*. 1997;5:47–55.
33. Konoshita S, Nishida K, Dota A, et al. Epithelial barrier function and ultrastructure of gelatinous drop-like corneal dystrophy. *Cornea*. 2000;19:551–555.
34. Molina F, Bouanani M, Pau B, Granier C. Characterization of the type-1 repeat from thyroglobulin, a cysteine-rich module found in proteins from different families. *Eur J Biochem*. 1996;240:125–133.
35. Lenarcic B, Turk V. Thyroglobulin type-1 domains in equistatin inhibit both papain-like cysteine proteinases and cathespsin D. *J Biol Chem*. 1999;274:563–566.
36. Meh P, Pavšiè M, Turk V, Baici A, Lenarèiè B. Dual concentration-dependent activity of thyroglobulin type-1 domain of testican: specific inhibitor and substrate of cathepsin L. *Biol Chem*. 2005; 386:75–83.
37. Strubin M, Bert C, Mach B. Alternative splicing and alternative initiation of translation explain the four forms of the Ia antigen-associated invariant chain. *EMBO J*. 1986;5:3483–3488.