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, expressed in a protein 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 Nakazumi 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 mullberry 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, photorelief, 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 1998.8 Later, Lethal 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,5,13-15 and other countries13,14,16–21 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.14,26,29 The previously reported GDLD-causing mutations in TACSTD2 are presented in Table 1.

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The \textit{TACSTD2} gene product is a multimodule transmembrane glycoprotein of 323 amino acids. The domains of the protein include an epidermal growth factor (EGF)-like repeat, a thyroglobulin type 1A (TY) repeat, a transmembrane domain (TM), and a phosphatidylinositol (PIP2)-binding site.\textsuperscript{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.\textsuperscript{30,31} With respect to GDLD, it

\begin{table}[h]
\centering
\begin{tabular}{cccccc}
\hline
No. & Gene Location\textsuperscript{*} & cDNA Location\textsuperscript{*} & Effect on Protein & Country & Ref. \\
\hline
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 & c.493.494 & 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 \\
11 & g.1173T>C & c.557T>C & p.L186P & Japan & 26 \\
12 & g.1177delC & c.561delC & p.P188fs & Europe & 14 \\
13 & g.1255C>T & c.619C>T & p.Q207X & Japan & 10 \\
14 & g.1248delA & c.632delA & p.S210fs & Japan & 10 \\
15 & g.1269delA & c.653delA & p.V217fs & Turkey & 21 \\
16 & g.1388.1389insT & c.772.773insT & p.L258fs & Vietnam & 19 \\
18 & delATCTATTACCTG & delATCTATTACCTG & delIYYL & Vietnam & 19 \\
19 & g.1427delA & c.811delA & p.M270fs & Tunisia & 14 \\
\hline
\end{tabular}
\caption{GDLD-Associated Mutations in \textit{TACSTD2}}
\end{table}

\textsuperscript{*} 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 NT032977, NM002353, and NP0023441.

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\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Geographic origin of Iranian GDLD patients. The geographic origins of the patients are shown with dots (●) within the provinces. The numbers indicate the \textit{TACSTD2} sequence variations associated with disease: 1, C66X; 2, F114C; 3, L186P; 4, E227K.}
\end{figure}
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.\textsuperscript{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.\textsuperscript{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 chemistry (Big Dye kit and the Prism 3700 sequencer; Applied Biosystems) and PCR with primers M1S1-Fa and M1S1-Rb were performed with a touchdown protocol. Amplification was sometimes performed in a single reaction with primers M1S1-Ra (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 sequencer; Applied Biosystems [ABI], Foster City, CA). Sequences were analyzed on com-

**Table 2. Clinical Features of Probands of Iranian GDLD Pedigrees**

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

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

\textsuperscript{†} Unless otherwise indicated, vascularization was prior to surgery.

\textsuperscript{‡} Including keratectomy, excimer laser keratomileusis, and lamellar and penetrating keratoplasty. Patients sometimes refused reoperation because of dissatisfaction with results of the previous surgery.

\textsuperscript{¶} Minimum time elapsed between surgery and recurrence.

\textsuperscript{¶} 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-
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 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 thyrogbulin type 1A domain, the corresponding position is always 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 thyrogbulin 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 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 the TY domain of TACSTD2. Phenylalanine at position 227 is completely conserved in TACSTD2 and related TACSTD1 proteins of all species thus far sequenced (Table 4). Furthermore, in various proteins containing a thyrogbulin type 1A domain, the corresponding position is always 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 thyrogbulin type 1A domains (Fig. 3 and Ref. 34).

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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 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 was not observed among the chromosomes of a GDLD patient from nearby Turkey. The c.653delA mutation recently reported in a GDLD patient from nearby Turkey was 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.

**DISCUSSION**

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. Four of these affect initiation of protein synthesis. The function of the region between the TY and TM domains is unknown, but the mutations 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. Four of these affect initiation of protein synthesis. The function of the region between the TY and TM domains is unknown, but the mutations 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. Four of these affect initiation of protein synthesis. The function of the region between the TY and TM domains is unknown, but the mutations are generally expected to have global detrimental effects on protein function.
in the TY domain may affect its role as a protease inhibitor and thus affect amyloid deposition.\textsuperscript{35,36} 

Mutation C66X causes very early protein truncation during protein synthesis. This nonsense codon may also result in reduced levels of mRNA.\textsuperscript{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 TASCTD2 may affect the phenotypic features of patients with GDLD.

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**References**


