Spectrum of Clinical Signs and Genetic Characterization of Gelatinous Drop-Like Corneal Dystrophy in a Colombian Family

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Purpose: To describe the clinical signs of gelatinous drop-like corneal dystrophy (GDLD) in a consanguineous Colombian family and determine the underlying genetic cause.

Methods: We performed ocular examination of available family members and bidirectionally Sanger sequenced the GDLD-associated gene, TACSTD2. In one individual, the presence of subepithelial amyloid was confirmed with biopsy.

Results: The parents were consanguineous and 5 of their 10 children had GDLD. Typical mulberry subepithelial deposits with subepithelial vascularization were present in 3 individuals; 2 individuals only had mild polymorphic anterior stromal opacity. We identified a homozygous TACSTD2 missense mutation, c.551A>G, p.(Tyr184Cys), in the affected family members. Both parents were heterozygous for the mutation, and unaffected siblings were either heterozygous or homozygous wild-type for this allele. In the Colombian population, this mutation has a minor allele frequency of 0.53%.

Conclusion: The clinical presentation of GDLD in this family was variable and does not solely support an age-dependent progression of the phenotype, suggesting that environmental or other genetic factors can modify phenotypic expression. The relatively high prevalence of this mutation in the Colombian population suggests that other individuals may have undiagnosed subclinical disease.

Key Words: gelatinous drop-like corneal dystrophy, primary familial amyloidosis of the cornea, hereditary corneal dystrophies, corneal amyloidosis

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Gelatinous drop-like corneal dystrophy (GDLD; MIM 204870) was first described in Japanese patients in 1914.[1] It is a rare autosomal recessive disease with features consistent with bilateral corneal amyloidosis, leading to pain and severe loss of sight.[2,3] The majority of reports are from Japan, where the prevalence is estimated to be 1:300,000 individuals.[4] The first case report from outside Japan was from the United States, where it was termed primary familial amyloidosis of the cornea.[5] In Latin America, clinical reports of GDLD cases have been described from Mexico, with 13 cases observed over a 44-year period, and a single case from Brazil.[6–8]

The phenotype of GDLD can gradually develop over time. In the first decade, there may only be subepithelial lesions that are similar to band-shaped corneal degeneration, or multiple small grayish epithelial nodules that cause severe photophobia, tearing, and foreign body sensation. Over time, the nodules increase in number and coalesce to give a mulberry appearance. Finally, in the third decade, there is secondary stromal opacification and neovascularization with severe visual loss.[9–11] The histopathological features include subepithelial and anterior stromal amyloid material deposit with loss of the Bowman layer. The amyloid deposits stain with Congo red and show birefringence and dichroism under polarized light.[12] Physiologically, there is increased permeability of the epithelial cell junction.[13] Biallelic mutations in *TACSTD2* (previously *M1S1* or *TROP2*), located on chromosome 1p32 cause GDLD (MIM 137290).[14] *TACSTD2* encodes tumor-associated calcium signal transducer 2 protein that, in association with claudins 1 and 7, functions as a transmembrane glycoprotein to maintain the corneal epithelial barrier. Loss of this barrier function is associated with accumulation of amyloid in the cornea.[15]

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The purpose of this study is to report the clinical features of a consanguineous family with GDLD from Colombia and establish the genetic cause of disease.

MATERIALS AND METHODS

All participants or their parents provided informed consent for this study. The research was approved by the Ethics Committee of the Medical Research Institute of the University of Antioquia and adhered to the tenets of the Declaration of Helsinki. A consanguineous family affected with GDLD was examined (Fig. 1). A family history and pedigree was constructed and a clinical ophthalmological examination was performed. A therapeutic superficial keratectomy was performed for 1 patient (individual II:10), and the material was processed for histology. A 10-mL peripheral venous blood sample was taken and DNA extracted (DNeasy; Qiagen).

The single coding exon of *TACSTD2* was amplified by polymerase chain reaction (PCR) using KapaRobust polymerase (Kapa Biosystems) with primers F: 5′-CCTGCAGACCATCC-CAGAC-3′ and R: 5′-CAGGAAGCGTGACTCACTTG-3′.[14] PCR products were purified using a MultiScreen $PCR_{\mu 96}$ plate (Millipore) and bidirectionally Sanger sequenced. Sequences were aligned and compared with the reference sequence using DNAstar package software version 8.0.2 (Lasergene). Variants were annotated in accordance with Ensembl transcript ID ENST00000371225.

Two different bioinformatics tools were applied to predict the effect of amino acid substitution on *TACSTD2* function: SIFT (v4.0.3; http://sift.jcvi.org/),[16] which uses sequence homology to make predictions, and PolyPhen-2 (v2.0.23; http://genetics.bwh.harvard.edu/pph2/),[17] which uses both sequence-based and structure-based predictive features. *TACSTD2* variants were checked for their frequency in the Exome Aggregation Consortium database (ExAC; http://exac.broadinstitute.org/about), which includes exome data from 63,358 individuals of varying ethnicity (Table 1).

RESULTS

The family consisted of consanguineous parents from Caldas in Colombia (Fig. 1). The parents had a normal ophthalmic examination. The couple had 10 male children, of whom II:1 and II:2 were unavailable for evaluation but had no reported ophthalmic symptoms. Individuals II:6, II:7, and II:11 were clinically unaffected. A maternal uncle was reported to have died blind with opaque corneas of unknown etiology. The son of II:3 (III:1) had no ophthalmic symptoms. Patient II:5 had a well-differentiated rectal mucinous adenocarcinoma diagnosed at age 22 years, but no other extraocular phenotype was noted in any patient.

Clinical Findings

All affected individuals had photophobia, foreign body sensation, and blurred vision. There was a significant variation in the phenotype between affected individuals. The proband (II: 10) had photophobia and ocular discomfort since the first year of life. Corneal opacities were noted at 3 years of age, with a visual acuity of count fingers right eye and light perception left eye. By age 10, there were dense central corneal opacities with an irregular friable surface with peripheral superficial neovascularization (Fig. 2A). He had a left superficial keratotomy with an amniotic membrane onlay graft and temporal tarsorrhaphy. After 6 months, the

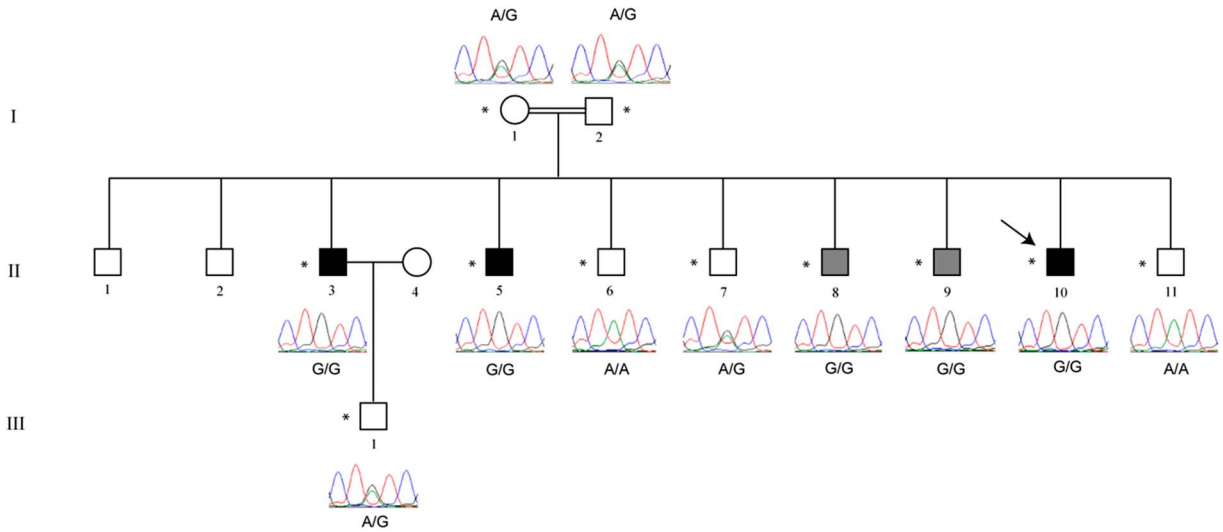

FIGURE 1. Pedigree of a Colombian family with GDLD and segregation of a homozygous *TACSTD2* missense mutation. The closed symbols represent the individuals diagnosed with GDLD, and open symbols indicate unaffected individuals. The proband (II:10) is marked with an arrow. The letters A and G represent the genotypes for each individual on the complementary strand; A is the wild-type allele and G is the mutated allele. Asterisk indicates patient has been clinically examined and a DNA sample obtained. No DNA sample was available for patients II:1 and II:2. Individuals II:3, II:5, and II:10 had a severe phenotype (shown in black), and II:8 and II:9 had a milder phenotype with preserved vision (shown in grey).

TABLE 1. Summary of Published Pathogenic Mutations in TACSTD2

Nucleotide Change	Protein Change	Ethnicity	SIFT Score (1–0)	Polyphen2 Score (0–1)	ExAC Frequency	Reference
c.2T>G	p.(M1R)	Indian	Damaging (0)	Possibly damaging (0.651)	NP	Ren et al (2002)
c.84dupG	p.(A29Gfs*66)	Chinese			NP	Zhang et al (2007)
c.198C>A	p.(C66X)	Iranian			NP	Alavi et al (2007)
c.250A>T	p.(K84X)	Japanese			NP	Murakami et al (2004)
c.322T>C	p.(108R)	Japanese	Damaging (0)	Probably damaging (0.999)	NP	Murakami et al (2004)
c.341T>G	p.(F114C)	Iranian	Damaging (0)	Probably damaging (0.999)	NP	Alavi et al (2007)
c.352C>G	p.(Q118E)	Indian	Damaging (0)	Probably damaging (0.994)	NP	Ren et al (2002)
c.352C>T	p.(Q118X)	Japanese, Chinese			NP	Tsujikawa et al[4] (1998), Tsujikawa et al,[14] Yoshida et al (2002), Tian et al[18]
c.354 G>C	p.(Q118H)	Chinese	Damaging (0)	Probably damaging (0.999)	NP	Zhang and Yao (2010)
c.355T>A	p.(C119S)	Tunisian	Damaging (0)	Probably damaging (0.999)	NP	Ren et al (2002)
c.356G>A	p.(119y)	Indian	Damaging (0)	Probably damaging (0.999)	NP	Paliwal et al (2010)
c.481delC	p.(R161Gfs*15)	Chinese			NP	Zhang et al (2007)
c.493_494insCCACCGCC	p.(G165Afs*15)	Indian			NP	Ren et al (2002)
c.509C>A	p.(S170X)	Japanese			NP	Tsujikawa et al[14]
c.519dupC	p.(A174Rfs*43)	Estonian			NP	Tasa et al (2001)
c.526_576del51	p.(L176_A192del)	Chinese			NP	Jing et al (2009)
c.551A>G	p.(Y184C)	Chinese, Colombian	Damaging (0)	Probably damaging (0.994)	1/107,098 (0.000009337)	Tian et al[18] this study
c.557T>C	p.(L186P)	Japanese, Iranian	Damaging (0)	Probably damaging (0.997)	NP	Taniguchi et al (2005), Alavi et al (2007)
c.564delC	p.(K189Sfs*82)	European			2/105,576 (0.00001894)	Ren et al (2002)
c.581T>A	p.(V194E)	Indian	Tolerated (0.09)	Probably damaging (0.960)	NP	Ren et al (2002)
c.619C>T	p.(Q207X)	Japanese			NP	Tsujikawa et al[14]
c.632delA	p.(Q211Rfs*60)	Japanese			NP	Tsujikawa et al[14]
c.653delA	p.(D218Vfs*53)	Turkish			1/110,474 (0.000009052)	Markoff et al (2006)
c.675C>A	p.(Y225X)	Japanese			NP	Nakatsukasa et al[15] (2010)
c.679 G>A	p.(E227K)	Iranian	Damaging (0)	Probably damaging (0.991)	NP	Alavi et al (2007)
c.772_783delinsT	p.(I258X)	Vietnamese			NP	Ha et al (2003)
c.812delA	p.(K272Sfs*26)	Tunisian			NP	Ren et al (2002)
c.840_841insTCA TCATCGCCGGCCTCAT	p.(I281Sfs*102)	Japanese			NP	Nakatsukasa et al[15] (2011)

Mutations were annotated according to Ensembl transcript ID ENST00000371225. Predictions of pathogenicity were scored using SIFT, with a score of likely pathogenicity from 1 to 0 based on sequence homology, and Polyphen2, with a score from 0 to 1 based on homology and structural features. The frequency of the mutation in the control population was determined using ExAC.

NP, not present (in database).

visual acuity had improved to 20/200 right eye and 20/400 left eye, with a reduction in opacity (Fig. 2B). Histopathology of the excised material showed a stratified squamous corneal epithelium, with preserved polarity and maturation, but with diffuse deposition of an amorphous eosinophilic material in the stroma that stained positive with Congo red under polarized light showing apple-green birefringence, consistent with amyloid (see Figure, Supplemental Digital Content 1, http://links.lww.com/ICO/A421). A similar mulberry appearance with peripheral superficial neovascularization was noted in individuals II:3 aged 25 years (VA 20/100 BE) and II:5 aged 23 years (VA count fingers OD and hand movements OS) (Fig. 2C, F). However, in individuals II:8 and II:9 aged 14 and 11 years, the visual acuity was preserved (II:8 20/30 OD, 20/25 OS; II:9 20/20 BE). In both these individuals, the corneal changes were less severe with only polymorphic

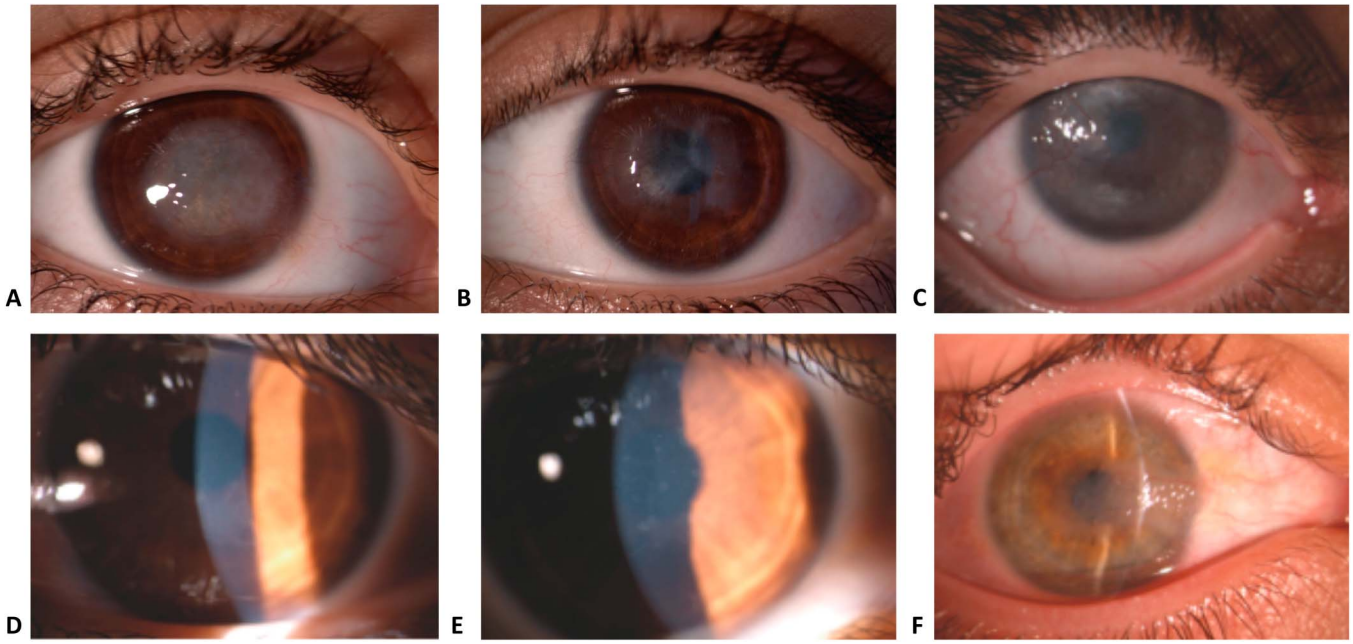

FIGURE 2. Differential phenotypic expression of GDLD in affected individuals. A, Individual II:10, aged 10 years (right eye), with a typical mulberry central lesion with superficial neovascularization in the peripheral nasal aspect of the cornea, and (B) (Left eye) 6 months after the superficial keratectomy. C, Individual II:5 aged 23 years (right eye) showing mainly peripheral lesions, some of which are raised with a mulberry appearance accompanied by extensive and diffuse corneal superficial neovascularization. D, Individual II:8 aged 14 years (left eye) and (E) (right eye) with grey central and paracentral subepithelial band-shaped opacities. F, Individual II:3 aged 25 years (right eye) with polymorphic white-yellowish subepithelial corneal and conjunctival lesions with a mulberry appearance and extensive superficial neovascularization.

grey subepithelial band-shaped opacities without neovascularization, with late epithelial stain after instillation of fluorescein (Fig. 2D, E). In addition, 2 affected individuals (II:3 and II:8) also showed perilimbal deposits suggestive of amyloid material (Fig. 2F).

Genetic Analysis of TACSTD2

Screening the *TACSTD2* gene identified a single variant, c.551A>G, p.(Y184C) (rs190800473), which fully segregated with the disease in the family. All affected individuals were homozygous for the mutation, both unaffected parents (I:1 and I:2), 1 healthy sibling (II:7), and the son of affected individual II:3 (III:1) were heterozygous for the mutation. All remaining unaffected family members were homozygous wild-type (Fig. 1).

The same mutation has previously been identified in a compound heterozygous state in an affected individual in combination with the Japanese founder mutation c.352C>T, p.(Q118X), and is therefore considered to be disease causing.[18] This is the first report of the mutation in the homozygous state. The *TACSTD2* gene is present only in mammals, birds, and reptiles. The nonsynonymous variant, p.(Y184C), alters a highly conserved residue (see Figure, Supplemental Digital Content 2, http://links.lww.com/ICO/A422). Using SIFT and Polyphen2 prediction tools, the p.(Y184C) substitution is predicted to be Damaging (0) and Probably Damaging (0.994), respectively.

The variant was identified only once in the heterozygous state out of 107,098 alleles in the ExAC database; thus, this variant is extremely rare (MAF = 0.000009337). The ExAC data set combines exome-sequencing data from large-scale sequencing projects worldwide; the single occurrence of the mutation was detected in an individual of Colombian origin sequenced as part of the 1000 Genomes Project. In total, 93 Colombian individuals were sequenced; therefore, the variant has an MAF of 0.53% in this specific population.

DISCUSSION

This is the first time, to our knowledge, that GDLD has been genetically solved in patients of Latin American origin (Table 1). The causative homozygous *TACSTD2* mutation identified in all affected family members has only previously been identified in the compound heterozygous state in a Chinese patient.[18]

The identification of 5 affected individuals in this consanguineous family helps to define the phenotypic spectrum associated with the p.(Y184C) *TACSTD2* mutation, which shows clinical variability between affected individuals. Two affected individuals (II:8 and II:9), aged 14 and 11 years, had a mild phenotype with an appearance similar to band-shaped keratopathy, characteristic of early GDLD.[11,13] The remaining 3 affected family members (II:3, II:5, and II:10), aged 25, 23, and 10 years, had a more characteristic phenotype with a mulberry appearance and secondary stromal neovascularization of the cornea, associated with severe photophobia, discomfort, and blindness. These differences suggest that environmental or other genetic factors contribute

to the phenotype. Because this family is consanguineous, affected individuals are likely to share an extended haplotype at the *TACSTD2* locus so genetic variants in the shared genomic interval are unlikely to contribute to the observed phenotypic variability. Similarly, Tsujikawa et al[19] described a homozygous Q118X mutation in 4 Japanese families with inter- and intrafamilial phenotypic variability. Previous reports have indicated that the phenotypic variability for this dystrophy is because of age-related progression over time, as the nodular subepithelial amyloid deposits gradually increase in number and coalesce during the first and second decades of life,[3,9,10] and it remains possible that the milder phenotype observed in some individuals in this family could progress in the next few years or decades. Two affected individuals (II:3 and II:8) also showed perilimbal deposits suggestive of amyloid material, although confirmation would require a histopathological examination.[20]

GDLD is normally associated with severe ocular morbidity and blindness. Visual rehabilitation is problematic, often requiring multiple surgical interventions, with a high rate of recurrence and continued visual decline. After superficial excision of affected material, lamellar or penetrating keratoplasty, temporary improvement can be achieved, but with recurrence at an average of 26 months.[21,22] Individual II:10 had a superficial keratotomy with an amniotic membrane graft that improved the visual acuity and gave a marked reduction in symptoms for 6 months, but further follow-up is required to confirm the duration of this effect.

Although most reports of GDLD are from East Asia, cases have also been reported from other regions.[5] To date, 28 different disease-causing mutations in the *TACSTD2* gene have been reported. The majority of mutations are predicted to produce truncated proteins, or proteins degraded by nonsense mediated decay, because of nucleotide substitutions that generate premature stop codons. The remainder are missense changes of conserved residues. The most frequently reported mutation is the c.352C>T, p.(Q118X) mutation, which is a founder mutation in the Japanese population, explaining the higher prevalence of GDLD in Japan.[14]

Figure 3 shows a schematic representation of pathogenic *TACSTD2* mutations, including the p.(Y184C) mutation, and their location in regards to the functional domains of the TACSTD2 protein. Given the range of previously described missense mutations, it is noteworthy that cysteine residues are often eliminated or created, which might point to a common mechanism of disruption of disulfide bonds in the extracellular domain of the protein. In the cornea, TACSTD2 is localized to epithelial cell junctions, and reduction of *TACSTD2* expression

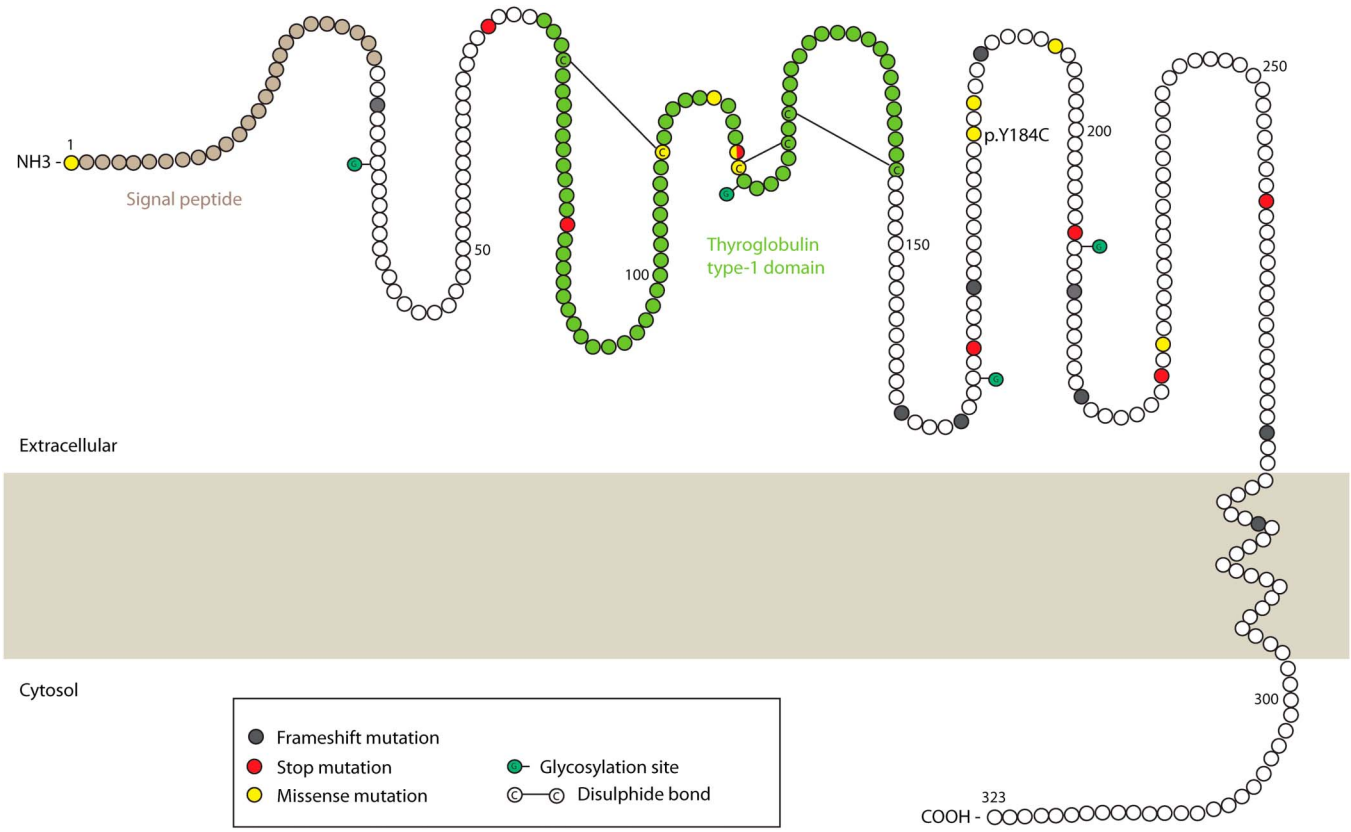

FIGURE 3. Topology diagram showing the location of pathogenic *TACSTD2* mutations. Amino acids are numbered at 50 residue intervals. Intracellular, cytosolic, and extracellular aspects of the protein are shown, with functional domains and residues (glycosylation sites and disulfide residues) highlighted. All published mutations are mapped to the protein, colored for functional consequences: missense (yellow), frameshift (grey), and stop (red) mutations.

has been shown to lead to altered expression and modification of intercellular proteins, including claudins 1, 4, and 7, ZO1, and occludin.[15] This impairs the function of the epithelial barrier, allowing the formation of amyloid deposits of lactoferrin, apolipoprotein, and transforming growth factor β.[23–26] There is evidence that amyloid fibers are formed by nonenzymatic posttranslational modifications of these proteins, including the formation of advanced glycation end products and the racemization of aspartyl residues that lead to the misfolding of DNA, resistance to degradation, and posterior accumulation.[25]

A single heterozygous carrier of the p.(Y184C) mutation was identified in the 1000 Genomes Project, which includes exome sequence data for 93 unrelated individuals from Colombia, suggesting a 0.53% frequency for the allele in this population. Based on this allele frequency, and excluding inbreeding, we estimated the prevalence of GDLD to be 1:35,714 in Colombia, which is 8.5 times the prevalence of GDLD in the Japanese population, for which a founder effect for the p.(Q118X) mutation has been reported. The family is originally from a village in the Department of Caldas in Colombia that has had a rapid expansion in population since its founding.[26]

This is the first clinical, genetic, and histopathological description of GDLD from Colombia. The clinical variability of the corneal changes in the affected family members helps define the phenotype of this disease. The most severely affected individual (II:5; 23 years of age) had a history of a colon adenocarcinoma. Although this association has not been highlighted in other reports, a higher expression of TACSTD2 has been reported to be associated with aggressive colorectal carcinoma.[27] It is noteworthy that the relatively high frequency of this allele in ethnically matched control subjects predicts a higher frequency of the disease in Colombia than has been reported in the Japanese population.

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