Gelatinous Drop–Like Corneal Dystrophy

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Abstract: Gelatinous drop–like corneal dystrophy (GDLD) is a rare autosomal recessive disorder, clinically characterized by grayish corneal deposits of amyloid and by severely impaired visual acuity. Most patients require corneal transplantation. We identified the gene responsible for GDLD, tumor-associated calcium signal transducer 2 (TACSTD2), by positional cloning and detected 4 disease-causing mutations in Japanese patients with GDLD. During the positional cloning process, strong linkage disequilibrium was observed between GDLD and some markers in the critical region. More than 90% of GDLD patients possessed the same haplotype with a Q118X mutation in TACSTD2. This may be the result of a founder effect and reflects that most GDLD patients are Japanese. TACSTD2 deleterious mutations resulted in destabilized tight junction proteins, including claudins, ZO-1, and occludin. These findings may explain why the corneal epithelial barrier function is impaired in GDLD patients.

Key Words: gelatinous drop–like corneal dystrophy, positional cloning, Q118X mutation, TACSTD2, tight junction

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Gelatinous drop–like corneal dystrophy (GDLD; OMIM: 204870) is a relatively rare corneal dystrophy disease with an autosomal recessive trait. GDLD was first reported in 1914 by Nakaizumi in Japan.1 The incidence is reportedly 1 in 300,000 in Japan; however, in other countries, GDLD is a very rare disease.2 It is characterized by the deposition of amyloid material in the subepithelial space of the cornea, which causes blurred vision and photophobia from the first decade of life. Eventually, raised gray gelatinous masses severely impair visual acuity, and lamellar or penetrating keratoplasty is needed. Immunohistochemical studies have revealed that amyloid deposits contain the lactoferrin protein, which had been considered a disease-causing gene. However, this candidate approach was not successful. Thus, we undertook a positional cloning approach to identify the gene responsible for GDLD.

LINKAGE ANALYSIS

To identify the gene responsible for GDLD, we used a strategy of conventional positional cloning, called “homozygosity mapping.”3 For this method, the samples must be obtained from consanguineous families; thus, the total number of samples may be small. In our study, we could map the locus from only 10 Japanese GDLD families.4 After examining 63 markers distributed throughout the genome, we found that all 13 affected members and none of the 11 unaffected members from the 10 consanguineous GDLD families studied were homozygous at the D1S220 locus on the short arm of chromosome 1. This homozygosity occurs in consanguineous families because the same chromosomal region was delivered from both the paternal and maternal ancestors. Using additional markers flanking the D1S220 locus to confirm linkage, we found that all of the affected individuals were homozygous for 2 additional markers (D1S2831 and D1S2741) on the proximal side of D1S220 and for 2 markers on the distal side (D1S2869 and D1S2650). From these 5 markers, linkage analysis revealed no recombination, with LOD scores of 4.40 to 9.80; the maximum score of 9.80 was obtained at the D1S2741 locus. Haplotype analysis indicated that 3 patients were heterozygous at the D1S2890 locus proximal to D1S220, and 2 patients were heterozygous at the D1S2801 locus distal to D1S220. These cases defined a critical region for GDLD, within a 2.6-cM interval between D1S2890 and D1S2801. However, this 2.6-cM interval was still too large to perform positional cloning. We observed that 8 of 10 disease chromosomes (80%) carried the 247-bp allele at the D1S220 marker locus, whereas only 5% of the unaffected control chromosomes carried this allele. A significant linkage disequilibrium between GDLD and the D1S220 locus ($\chi^2 = 36.24; P < 0.001$) was observed. We also found that D1S2648 ($\chi^2 = 12.90; P = 0.032$) and D1S2752 ($\chi^2 = 19.77; P = 0.012$) showed significant linkage disequilibrium, indicating that the most critical region lay between them.

POSITIONAL CLONING

To identify the gene responsible for GDLD, we subsequently isolated cosmids and bacterial artificial clones approximately covering the 400-kb critical region between D1S2752 and D1S220 and then performed DNA sequencing experiments using a shotgun cloning method (Fig. 1). Computer analysis of genomic DNA sequences indicated that 6 expressed sequence tags and a single known gene, tumor-associated calcium signal transducer 2 (TACSTD2; also known as MISI), consisting of a single exon, were located in the region. Northern blot analysis revealed that TACSTD2 was expressed in the cornea, as well as in the kidney, lung,
placenta, pancreas, and prostate gland, suggesting that this gene may be a candidate for harboring mutations responsible for GDLD. We amplified a 1.8-kb fragment, covering the entire coding region of TACSTD2, from genomic DNA (TACSTD2 is a single exon gene) isolated from members of 20 Japanese GDLD families and determined DNA sequences. All 26 affected members of these families were homozygotes or compound heterozygotes for the mutations shown in Table 1. The most commonly detected mutation was a C to T transition at nucleotide 352, replacing a glutamine at codon 118 with a stop codon (Q118X). Affected members from 16 of 20 GDLD families were homozygotes for this mutation. In addition, a patient from another family carried this mutation on one allele and a different mutation on the other. Thus, the Q118X mutation accounted for 82.5% (33 of 40) of the disease alleles present in our panel of GDLD families. All 33 alleles carried the major disease haplotype (Table 1), indicating that the Q118X mutation is a Japanese founder mutation and reflects linkage disequilibrium. This may explain why most GDLD patients are Japanese and few cases have been reported in other countries. In Japanese patients, 90% of the disease chromosomes have this major mutation. This allelic homogeneity is an interesting phenomenon in Japanese corneal dystrophies. In addition, we also reported allelic homogeneity as a result of the founder effect in other corneal dystrophies in Japan. 10,11

ATYPICAL CASES

In GDLD, clinical variability and atypical cases have been reported. 12 An important question is whether these atypical cases are caused by genetic background differences, including allelic or locus heterogeneities. To address this, we performed genetic analyses of 4 Japanese families who had bilateral corneal amyloidoses. 13 All families included a patient whose clinical features alone could not be used to diagnose GDLD. In 1 family, obvious clinical differences were observed between 2 members who had corneal amyloidosis. Members from 3 families had atypical amyloidosis that had not been initially diagnosed as GDLD (Fig. 2). Sequence analysis revealed that all the patients possessed a homozygous Q118X mutation in TACSTD2. There were no differences in the entire sequence of TACSTD2 in these patients compared with other GDLD patients. Moreover, the genotyping of polymorphic markers near the TACSTD2 gene revealed that these patients shared the same founder chromosome along with TACSTD2 (Fig. 3). Therefore, even in atypical cases, GDLD patients carry the same genetic background around TACSTD2.

FUNCTIONAL ANALYSIS

The identification of the responsible gene for GDLD enabled us to investigate the pathogenic mechanisms of GDLD using reverse genetic methods. The function of the encoded protein, TACSTD2, is not well understood, but several potential modification sites within the molecule have been suggested (Fig. 4). TACSTD2 contains an epidermal growth factor–like repeat and a thyroglobulin repeat. This structure suggests that TACSTD2 is a cell adhesion molecule.
Furthermore, we have observed that TACSTD2 can induce hemophilic binding (unpublished data). This is interesting because the corneal epithelium of GDLD patients has a significantly increased permeability for fluorescence. Additionally, the apical side of the corneal epithelium of GDLD exhibited loosened cell–cell junctions and an increased number of scarred cells compared with normal cornea. Recently, it was shown that TACSTD2 can bind to the proteins claudin-1 and claudin-7 and stabilize these in corneal cells. In the absence of TACSTD2 expression, there is a change in the subcellular localization of tight junction–related proteins, including claudin-1, claudin-4, claudin-7, ZO-1, and occludin, leading to impaired corneal epithelial barrier function. However, TACSTD2 has a cytoplasmic tail with a phosphatidylinositol 4,5-bisphosphate–binding consensus sequence. Thus, TACSTD2 is thought to be a calcium transducer, although how this function may play a role in GDLD remains unknown.

**CONCLUSIONS**

Using positional cloning, we have identified TACSTD2, the gene responsible for GDLD. The major mutation identified was Q118X in TACSTD2 for the majority of Japanese GDLD cases. Among the Japanese GDLD families, a founder effect was observed, and this likely explains why GDLD is so dominant among the Japanese population. Even in atypical cases, the founder chromosomal region was preserved.
REFERENCES