Rapid Detection of M1S1 Mutations by the Protein Truncation Test

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PURPOSE. To determine a method of rapid detection of M1S1 gene mutations in patients with gelatinous droplike corneal dystrophy.

METHODS. Forty-one patients from 35 families with gelatinous drop-like corneal dystrophy were studied. The entire coding region of the M1S1 gene was screened using the protein truncation test (PTT), with a polymerase chain reaction fragment amplified from genomic DNA serving as a template of in vitro translation.

RESULTS. Homozygous or compound heterozygous mutations were detected in all patients by a single reaction of the PTT. This result matched those obtained using the polymerase chain reaction–restriction fragment length polymorphism and direct sequence analyses. The Q118X mutation was present in 63 of the 70 alleles, accounting for 90% of the disease-associated chromosomes in Japanese patients.

CONCLUSIONS. The PTT is useful for detecting mutations in the M1S1 gene. This technique showed that the Q118X mutation is a founder mutation in Japanese patients with gelatinous droplike corneal dystrophy, and it reflects the linkage disequilibrium reported previously. (Invest Ophthalmol Vis Sci. 2000;41:2466–2468)

Gelatinous droplike corneal dystrophy (GDLD) is an autosomal recessive disorder characterized clinically by grayish corneal amyloid deposits that cause severe visual impairment.1 Recently, we successfully identified the gene responsible for GDLD, Membrane component, chromosome 1, surface marker 1 (M1S1), by positional cloning methods and detected four disease-causing mutations in Japanese patients with GDLD.2 It is possible to detect three of these mutations, but not the fourth, using the polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) method. The M1S1 gene has a single exon, but its 1.8-kb length is too long to be analyzed by a single reaction of single-strand conformation polymorphism or direct-sequence analysis. Therefore, we divided this region into several fragments and analyzed each separately; however, this was an inconvenient and time-consuming process. For rapid and convenient screening, we used the protein truncation test (PTT) to detect mutations in the M1S1 gene. PTT had been used to screen many genes related to disease, including familial adenomatous polyposis,3 hereditary breast and ovarian cancer,4,5 and Duchenne’s muscular dystrophy.6

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Figure 1 is a schematic diagram of the PTT. Briefly, the coding region of the gene was amplified by PCR, using a sense primer tailed by a T7 promoter sequence. The PCR product was then used as a template for in vitro translation testing. Synthesized protein was analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). The shorter product from the mutated allele was distinguished from the full-length product of the normal allele. We report the first use of PTT in ophthalmology.

MATERIALS AND METHODS

Patients

We studied 41 patients from 35 families with GDLD. We previously reported on 26 of these patients from 20 families who were homozygotes or compound heterozygotes for the Q118X, 632delA, Q207X, or S170X mutations,2 and they were reanalyzed in the present study. Twenty milliliters of peripheral blood was drawn from each participant. Genomic DNA was extracted from the leukocytes using a DNA extraction kit (Stratagene, La Jolla, CA). All patients provided written informed consent, and procedures followed the tenets of the Declaration of Helsinki.

PCR for PTT

Double-stranded DNA (1.1 kb) containing the entire coding region of the M1S1 gene was obtained from genomic DNA by PCR using primer M1S1T7F that contained the T7 promoter sequence, the Kozac consensus sequence, and the ATG-initiation codon (GGAATTC-TAATACGACTCACTATAGGG-AACAG-CCACC-ATG-GGTTTCCTCCGGCCACC) and the M1S1R (GGAATTCAGGAATCAGGAAGCGTGACTCA). The ATG-initiation codon was in frame and upstream of the natural translation

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initiation. PCR was performed in a 20-μl reaction mixture containing 50 ng genomic DNA, 10 picomoles of each primer, MgCl₂ containing reaction buffer (Takara, Tokyo, Japan), 250 μM dNTPs, and 1.0 U polymerase (EX Taq; Takara). Samples were amplified in 35 cycles of 30 seconds each at 94°C for denaturing, 30 seconds at 60°C for annealing, and 60 seconds at 72°C for extension, in a thermocycler (GeneAmp 9600; Perkin-Elmer, Foster City, CA).

**PTT Analysis**

An in vitro translation reaction was performed using a commercial system (TNT T7 Quick Coupled Transcription/Translation System; Promega, Madison, WI). A 25-μl reaction mixture containing 300 ng PCR products, 20 μl TNT Quick Master Mix, and 1 μl ³⁵S-methionine was incubated at 30°C for 90 minutes. A 5-μl aliquot of each reaction mixture was loaded onto an 18% SDS-polyacrylamide gel. Electrophoresis was performed at 30 mA for 2 hours, and the gel was fixed with acetic acid-methanol-water (10:30:60), dried, and visualized using the BAS 1000 system (Fujifilm, Tokyo, Japan) or autoradiography. When band shifts were observed, nucleotide alterations of the corresponding positions were detected by RFLP or direct-sequence analysis, as described previously.²

**RESULTS**

Figure 2 shows the PTT results from the M1S1 gene. In 35 Japanese families with GDLD, 31 had truncated products cor-

![Figure 1](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933219/)

**FIGURE 1.** Diagram of PTT illustrating mutation of a glutamine codon (CAG) to a stop codon (TAG).

![Figure 2](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933219/)

**FIGURE 2.** Protein truncation test results. Lane 1, homozygote of the Q118X mutation; lane 2, homozygote of the 632delA mutation; lane 3, homozygote of the Q207X mutation; lane 4, homozygote of the S170X mutation; lane 5, compound heterozygote of the Q118X mutation and the 632delA mutation; lane 6, heterozygote of the Q118X mutation (carrier); and lane 7, normal control. M, molecular weight marker.
responding to 20 kDa detected by PTT (Fig. 2, lane 1). Normal 46-kDa products were not detected. A homozygous Q118X mutation was detected in all patients by PCR-RFLP and sequence analysis. In one family, abnormal 35-kDa products were detected, and normal product was not detected (Fig. 2, lane 2). PCR-RFLP and direct-sequence analysis revealed that these patients had a homozygous 632delA mutation. In another patient, a compound heterozygote of Q118X and 632delA mutations, abnormal 20- and 35-kDa products were detected (Fig. 2, lane 5). PTT also detected homozygous Q207X (Fig. 2, lane 3) and S170X (Fig. 2, lane 4) mutations. Table 1 summarizes the mutations detected and their frequencies. The Q118X mutation was found in 90% of affected chromosomes.

### DISCUSSION

PTT offers two advantages over other mutation-detection methods. A single PTT reaction allows analysis of a large (2–3 kb) gene fragment, and PTT detects mutations of pathologic interest (i.e., those that result in a truncated protein). Phenotypically silent mutations (polymorphism) or questionable mutations (missense mutations) are not detected. For example, most of the Japanese population has a D216E polymorphism in the M1S1 gene (data not shown). However, this polymorphism was not detected by PTT in this study.

The M1S1 gene codes 323 amino acids and consists of a single exon. Because there is no intron on the gene, the entire coding region can be amplified by a single PCR reaction from genomic DNA, and reverse transcription from mRNA is therefore unnecessary. Considering that GDLD has an autosomal recessive trait, dysfunction of the M1S1 gene may lead to a GDLD phenotype. In fact, all the reported disease-causing mutations are nonsense or frame-shift mutations. These features are ideal for PTT. Truncated products were detected in all patients, and full-length products were not detected by PTT. Each of four nonsense or frame-shift mutations was detected under homozygous and heterozygous (carrier or compound heterozygote) conditions. These results exactly matched the results of direct-sequence and PCR-RFLP analysis, and all reactions using this method could be performed in 1 day. PTT is extremely useful for detecting mutations in the M1S1 gene.

We present the first example of the application of PTT to ophthalmic diseases. GDLD is ideal for screening by PTT; however, PTT also may be useful in detecting mutations in other ophthalmic diseases—for example, one type of autosomal dominant retinitis pigmentosa (RP1). Recently, the gene responsible for RP1 was identified. All eight identified disease-causing mutations are nonsense or frame-shift mutations on exon 4 of the RP1 gene. Exon 4 is large (6 kb), and the presence of some polymorphisms in this exon makes screening difficult using single-strand conformation polymorphism analysis. PTT from the genomic PCR product of exon 4 enables rapid and convenient screening, similar to the screening of exon 15 in patients with the APC gene in familial adenomatous polyposis. For other genes that do not consist of a single exon or do not contain a large exon, illegitimate transcript analysis by modified reverse transcription-PCR from lymphocyte could be used to obtain a large open reading frame, even if the gene expresses specifically in ocular tissue.

PTT may be useful in screening protein-truncating mutations in ophthalmic diseases.

This is the second report of a search for mutations in the M1S1 gene in patients with GDLD. All 15 families newly analyzed in this study had the homozygous Q118X mutation. Our previous results of haplotype analysis using nearby polymorphic markers in other patients indicated that this Q118X mutation is a Japanese founder mutation and reflects linkage disequilibrium. It also explained that most patients are in Japan 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 not only an interesting phenomenon in Japanese corneal dystrophy, but also is useful in the clinical genetic diagnosis of GDLD.

No mutations have been reported in patients with GDLD in other countries. Those patients may have a novel disease-causing mutation. For a first screening, PCR-RFLP for Q118X may be sufficient in Japan; however, this may not be the case in other countries. In our hands, the mutated M1S1 gene can be screened quickly and conveniently.

### References