A Novel Mutation in the M1S1 Gene Responsible for Gelatinous Droplike Corneal Dystrophy

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PURPOSE. To identify the genetic defect in the M1S1 gene causing gelatinous droplike corneal dystrophy (GDLD) in an Estonian family.

METHODS. DNA was extracted from members of a GDLD-affected family and control persons. Polymerase chain reaction followed by direct sequencing was used to detect mutations in the M1S1 gene. Sequencing results were confirmed with restriction analysis.

RESULTS. Sequencing of the M1S1 gene revealed a novel mutation and a common polymorphism. All patients with GDLD were found to be homozygous for the insertion of nucleotide C in position 520 in M1S1. The mutation leads to formation of truncated protein. The mutation was excluded in 105 normal, unaffected individuals. Very close to the location where the mutation was identified in the M1S1 gene, a single-nucleotide polymorphism (518A/C) was found, changing aspartic acid to alanine at codon 173.

CONCLUSIONS. The data indicate that mutation ins520C in the M1S1 gene is the primary cause of GDLD in the family studied. (Invest Ophthalmol Vis Sci. 2001;42:2762–2764)

Gelatinous droplike corneal dystrophy (GDLD; On-line Mendelian Inheritance in Man number 204870), first described by Nakaizumi,1 is a form of primary amyloidosis of the cornea leading to blindness. Clinical manifestations usually appear in the first decade of life, and symptoms include foreign-body sensation, photophobia, lacrimation, and blurred vision. In later stages of the disease, accumulation of gelatinous masses in subepithelium and anterior stroma causes loss of vision, and in most cases recurrent lamellar keratoplasty is required.2 GDLD is an autosomal recessive disorder with highest frequency in the Japanese population (incidence 1:300,000).1,2 Recently, a gene responsible for GDLD was localized to a 2.6-centimorgan (cM) interval on chromosome 1p by linkage analysis.3 Focusing further studies on the critical localized to a 2.6-centimorgan (cM) interval on chromosome 1p region, M1S1 by linkage analysis.4 Focusing further studies on the critical

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Analysis of DNA from other family members revealed that the mother of the affected individuals was heterozygous for the mutation; the father's DNA was not available. All the patients' descendants were found to be homozygous for insertion of C at nucleotide 520. Among the 103 healthy people tested in the mutation screening, no carriers of the 520insC mutation were detected.

A common single nucleotide polymorphism (518A/C) was found in the \textit{MIS1} gene, very close to the position where the mutation was discovered. Nucleotide substitution A\textendash}C at position 518 of the \textit{MIS1} gene changes codon 173 from GAC to GCC, which replaces aspartic acid with alanine (Fig. 1).

Restriction enzyme analysis in all members of the GDLId-affected family and 103 unrelated control individuals confirmed the results obtained by direct sequencing. Because the mutation and polymorphism were very close to each other, three enzymes (\textit{Eco}47I, \textit{Hin}III, and \textit{Cfr}I) with overlapping restriction sites were used. The 520insC mutation in the \textit{MIS1} gene created a restriction site for enzyme \textit{Eco}47I in those individuals who had A at the position of 518. Restriction enzyme analysis in all members of the GDLId-affected family and 103 unrelated control individuals confirmed the results obtained by direct sequencing. Because the mutation and polymorphism were very close to each other, three enzymes (\textit{Eco}47I, \textit{Hin}III, and \textit{Cfr}I) with overlapping restriction sites were used. The 520insC mutation in the \textit{MIS1} gene created a restriction site for enzyme \textit{Eco}47I in those individuals who had A at the position of 518.

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure1.png}
\caption{Part of the sequence of the \textit{MIS1} gene. Sequences of (A) \textit{MIS1} obtained from a patient with GDLId who had an insertion of C at position of 520 of the gene. Nucleotide A is seen in the polymorphic locus of the \textit{MIS1} gene at position 518; (B) a wild-type allele from a control subject with nucleotide A in the polymorphic site of the \textit{MIS1} gene; and (C) a wild-type allele from a control subject with nucleotide C at the polymorphic site of the gene.}
\end{figure}

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\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure2.png}
\caption{Pedigree of GDLId-affected family. All genotyped patients with the disease were homozygous for the mutation ins520C (M/M). All carriers having the ins520C in only one chromosome (wt/M) had no clinical signs of GDLId. Genotypes for persons found to have wild-type alleles in both chromosomes are designated wt/wt.}
\end{figure}

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure3.png}
\caption{Restriction analysis of the \textit{MIS1} gene. (A) Amplified \textit{MIS1} gene digested with \textit{Eco}47I. The mutation ins520C creates a recognition sequence for \textit{Eco}47I, if there is a nucleotide A in the position of 518, amplified DNA (681 bp) is cut into 416 bp and 266 bp fragments by the restriction enzyme. Lane 1: homozygote for the haplotype 520insC/518A; lane 2: haplotype 520insC/518A in one chromosome; lane 3: haplotype 520insC/518A absent. (B) Amplified fragments of the \textit{MIS1} gene digested with \textit{Hin}III. Wild-type allele with 518A is cut by the enzyme. A DNA fragment of 264 bp indicates the presence of haplotype wt/518A. Lane 1: homozygote for the haplotype wt/518A; lane 2: haplotype wt/518A present in one chromosome; lane 3: haplotype wt/518A absent. (C) Amplified fragments of the \textit{MIS1} gene digested with \textit{Cfr}I. The presence of a 244 bp DNA fragment after digestion with \textit{Cfr}I allows identification of the haplotype wt/518C. Lane 1: homozygote for the haplotype wt/518C; lane 2: haplotype wt/518C present in one chromosome; lane 3: haplotype wt/518C absent.}
\end{figure}
endonuclease \textit{Hin} II was specific to wild-type allele with A at position 518. For \textit{Cfr} I an additional restriction site was present in DNA fragments amplified from individuals with the wild-type allele and nucleotide C at position 518 (Fig. 3).

Genotype frequencies for 518A/C polymorphism in the \textit{M1S1} gene were as follows: 81.5% of individuals from the control population were A/A homozygotes, 17.5% were A/C heterozygotes, and 1.0% were C/C homozygotes. All members of the family with GDLD who were available for molecular analysis had the 518A/A genotype.

\textbf{DISCUSSION}

This is the first report of a mutation analysis performed in white patients with GDLD. All patients with GDLD in Estonia were found to be homozygous for 520insC in the \textit{M1S1} gene and the mutation was well cosegregated with the phenotype in the GDLD pedigree, whereas no \textit{M1S1} mutations were found in the control population. Thus, the data suggest that ins502C in the \textit{M1S1} gene is responsible for GDLD in Estonia. The mutation found in Estonian patients has not been described in the Japanese population, where the disease has the highest frequency, and relatively many patients have been genotyped. It can be speculated that the mutation may also be found in other patients with GDLD among European descendants, because it was found in a family from Dago Island (off the west coast of Estonia), whose settlement had close connections with other countries around the Baltic Sea in the Middle Ages.

\textit{M1S1} is a cell surface phosphoglycoprotein and a substrate for protein kinase C, the Ca\textsuperscript{2+}-dependent protein kinase. The phosphorylation occurs on serine 303 in the cytoplasmic domain of the protein. The carboxyl-terminus of \textit{M1S1} possesses a phosphatidylinositol 4,5-bis phosphate (PIP-2)-binding consensus sequence, which regulates binding to plasma membrane or to other cytosolic proteins. The mutation detected by us, as well as other mutations described so far, leads to formation of truncated protein, resulting in loss of the transmembrane domain of \textit{M1S1}, the serine phosphorylation site, and the PIP2-binding site. The truncated gene product triggers process of amyloid formation in the cornea. The exact mechanism of amyloid deposition remains to be investigated. Although \textit{M1S1} is expressed at high levels by human multistratified epithelia, no amyloid formation in tissues other than cornea has been found in patients with GDLD.

A single-nucleotide polymorphism, 518A/C, in the \textit{M1S1} gene changes amino acid residue encoded by codon 173 from aspartic acid to alanine. Determining whether the change has any effect on biological properties of the protein is the object of our further studies. The close position of the 518A/C polymorphism and the ins502C mutation in the \textit{M1S1} gene must be considered when designing the molecular analysis method for mutation detection.

\textbf{References}