Macular corneal dystrophy types I and II are caused by distinct mutations in the \textit{CHST6} gene in Iceland

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**Purpose:** To identify \textit{CHST6} mutations in five additional Icelandic cases of macular corneal dystrophy (MCD) type I and in four families with MCD type II from Iceland.

**Methods:** Genomic DNA was extracted from blood leukocytes of patients with MCD, their healthy family members, and from control individuals. \textit{CHST6} mutations were determined by PCR-sequencing. Immunophenotypes of MCD were determined by measuring antigenic keratan sulfate (AgKS) levels in serum and by an immunohistochemical study on corneal tissue.

**Results:** Five additional cases of MCD type I and four families with MCD type II from Iceland were studied. A homozygous p.A128V mutation in the coding region of the \textit{CHST6} gene was identified in four of the five MCD type I cases. The other person with MCD type I was a compound heterozygote for p.A128V and a frameshift p.V66fs resulting from a 10-base pair insertion (c.15_16insATGCTGTGCG). Four of five individuals with MCD type II were compound heterozygotes for p.A128V and p.V329L, thus sharing the same p.A128V mutation as MCD type I. One patient with MCD type II was homozygous for p.V329L. The p.V329L mutation was only found in MCD type II patients. An analysis of the upstream region of \textit{CHST6} disclosed no upstream deletion or replacements in Icelandic patients with MCD type II.

**Conclusions:** The findings fit the haplotype analysis that we reported previously in Icelandic MCD families and indicate that different mutations in \textit{CHST6} cause MCD type I and type II in Iceland.

Macular corneal dystrophy (MCD) is an autosomal recessive, genetically determined disease characterized by spotted corneal opacities which progressively increase and eventually lead to severe bilateral visual impairment. The disorder is subdivided into three immunophenotypes, MCD types I, IA, and II, based on the reactivity of the patient’s serum and corneal tissue to an antibody that recognizes sulfated keratan sulfate (KS) [1-3]. In MCD type I, antigenic KS (AgKS) is undetectable in both serum and corneal tissue. In MCD type II, AgKS is present in normal or subnormal levels in serum and is evident within the corneal stroma. In MCD type IA, AgKS is absent in the serum and in most of the cornea but can be detected in the keratocytes.

After identifying the locus for MCD on chromosome 16 [4], mapping of the locus was further refined [5,6]. Mutations in \textit{CHST6}, which encodes corneal N-acetyl glucosamine-6-O-sulfotransferase (C-GlcNac6ST), an enzyme that transfers sulfate to position 6 of GlcNAc residues and that participates in the biosynthesis of KS proteoglycan in the cornea, were finally identified as the cause of MCD types I and II [7]. Numerous missense mutations, insertions, and nonsense mutations in \textit{CHST6} have been reported in patients with MCD type I [7-10]. Deletions and/or rearrangements in the upstream region as well as missense mutations have been reported in patients with MCD type II [7,9,10]. An absence or deficiency of C-GlcNac6ST activity is thought to result in the formation of unsulfated KS, leading to a loss of corneal transparency in persons with MCD [11].

While rare in the United States and many other countries, MCD is common in Iceland, and accounts for about one-third of all corneal grafts performed there [12]. Previously we reported three distinct mutations in the \textit{CHST6} coding region in six Icelandic families with MCD type I [8]. To further characterize the role of \textit{CHST6} in Icelandic families with MCD we analyzed this gene in four families with MCD type II and in five more cases of MCD type I from Iceland.

**METHODS**

*Family data and diagnostic criteria:* This study was approved by Duke University Internal Review Board and Landakot Hospital Ethics Committee in Iceland, and conformed to the tenets of the declaration of Helsinki. Written informed consent was obtained from all participants. Five additional cases with MCD type I, together with four families with MCD type II (Figure 1) from Iceland were studied. In all instances MCD was diagnosed on a combination of the clinical presentations and the typical histopathologic features in corneal tissue following a penetrating keratoplasty in one or both eyes. In an earlier study using an anti-KS monoclonal antibody (5-D-4; ICN Biomedical, Irvine, CA) serum AgKS levels were determined on these cases with an enzyme-linked immuno-sorbent assay (ELISA) and excised pathologic corneal tissue was...
evaluated immunohistochemically using the same antibody [1]. Genomic DNA extracted from the blood of normal Icelandic individuals was used as control samples.

**Mutation analysis in CHST6:** Peripheral blood was collected from each participant, genomic DNA was extracted from leukocytes, and the coding region of CHST6 was amplified by the polymerase chain reaction (PCR) using three pairs of primers and conditions as previously described [8]. The nucleotide sequences were then compared with the CHST6 cDNA and with the sequences of normal controls using the SeqWeb web-based sequence analysis program (Accelrys, San Diego, CA).

The upstream region of CHST6 was screened for deletions or rearrangements according to a modification of the method of Akama et al. [7]. PCR products were then electrophoresed on 2% agarose gels, and analyzed using the BioChem Image Acquisition and Analysis Software (UVP BioImaging Systems, Upland, CA).

**Mutation nomenclature:** The nomenclature of the mutations is provided according to Antonarakis et al. [13] and the current recommendation of the Human Genome Variation Society (HGVS). Accordingly, we refer to the A of the ATG-translation initiation codon of CHST6 as nucleotide +1. For amino acid numbering, the codon for the initiator methionine is codon 1.

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Figure 1. Pedigrees of five individuals from four Icelandic families with MCD type II, showing detected CHST6 changes and previously reported disease haplotypes between microsatellite markers D16S2624 and D16S518 in chromosome 16 (16q22). The presence or absence of the single nucleotide change c.768C>T in each haplotype is indicated. Family numbers and haplotypes are as designated in previous publications which document how the haplotypes were determined [5,6,8]. Haplotypes were not determined on all family members because of insufficient available DNA. Three haplotypes (1, 3, 5) were detected in the 5 patients with MCD type II. Haplotypes 1c and 1e are subgroups of haplotype 1, and haplotype 3a is a subgroup of haplotype 3. In the figure, N indicates a normal haplotypes in the same region, as the disease haplotypes. Filled circles show affected females, filled squares show affected males, empty circles indicate normal females, and empty squares show normal males.
In the previous report on MCD type I in Iceland [8] we designated the first nucleotide of the CHST6 cDNA sequence as documented by Akama et al. [7] (GenBank AF219990) as nucleotide +1. In the current study, the previously reported mutations have been renamed from the former documentation to conform to the recommended nomenclature.

### Table 1.

<table>
<thead>
<tr>
<th>MCD Type</th>
<th>Family number in family</th>
<th>Patients in family</th>
<th>Disease haplotype</th>
<th>DNA change</th>
<th>Protein change</th>
<th>Status of CHST6 change</th>
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<tr>
<td>I</td>
<td>1</td>
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<td>1</td>
<td>c.383C&gt;T</td>
<td>p.A128V</td>
<td>Homozygous</td>
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<tr>
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<td>4</td>
<td>1</td>
<td>c.383C&gt;T</td>
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<td>Homozygous</td>
</tr>
<tr>
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<td>1</td>
<td>1</td>
<td>c.383C&gt;T</td>
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<td>Homozygous</td>
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<tr>
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<td>5</td>
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<td>1</td>
<td>c.383C&gt;T</td>
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<tr>
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<td>3</td>
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<td>p.V329L</td>
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</tr>
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</table>

Summary of mutations identified in the coding region of CHST6 in Icelandic families with MCD type I and MCD type II. Haplotypes were determined as previously described [5,6]. Data for families 1, 2, 3, 5, 7, and 9 are from our previous publication [8], but the DNA changes have been altered to conform to the currently recommended nomenclature. Families 11-15 were isolated patients without family pedigrees, therefore, their haplotypes were not able to be determined (ND indicates not determined).

**RESULTS**

The pedigrees of the four Icelandic families with MCD type II are shown in Figure 1. Serum AgKS levels of the affected individuals with MCD type II ranged from 185 ng/ml to 343 ng/ml. Serum AgKS levels of the 5 MCD type I patients were all below 9 ng/ml. The missense mutation p.A128V...
(c.383C>T) and a frameshift (p.V6fs) that resulted from a 10-base pair insertion (c.1516insATGCTGTGCG) were found in the coding region of \textit{CHST6} in the MCD type I patients. Four of the five cases with MCD type I were homozygous for the p.A128V mutation (Table 1). One patient with MCD type I was a compound heterozygous for p.A128V and p.V6fs (Table 1).

In MCD type II families, two mutations, p.A128V and p.V329L (c.985G>C), were identified. Four of the five patients with MCD type II were compound heterozygous for p.A128V and p.V329L, and one patient was homozygous for p.V329L (Figure 1). No deletion or replacements were detected upstream of \textit{CHST6} in Icelandic patients with MCD type II. Moreover, a single nucleotide c.768C>T change was identified in all five patients with MCD type II (Figure 1). Patients homozygous for p.V329L were also homozygous for c.768C>T, while patients who were compound heterozygous for p.V329L and p.A128V were heterozygous for c.768C>T (Figure 1). In addition, four unaffected individuals from the MCD type II families were heterozygous for c.768C>T (Figure 1). The single nucleotide c.768C>T change was not found in 21 affected individuals with MCD type I (5 cases in the current study and 16 previously reported Icelandic patients) and is predicted to not alter the encoded amino acid sequence (p.A256A).

An analysis of fifty Icelandic control subjects failed to disclose the p.V329L mutation, but revealed a single heterozygous example of a c.768C>T nucleotide change (p.A256A). Moreover another control subject contained a previously unrecognized heterozygous c.828G>A (p.L276L) single nucleotide polymorphism (SNP).

Combined with the previous study, mutations identified in all Icelandic patients, including 10 cases with either MCD type I or type II from the current study and 16 cases with MCD types I from a previous report [8], are summarized in Table 1.

**DISCUSSION**

This genetic analysis of five previously unreported cases with MCD type I from Iceland is consistent with our previous observation that three mutations in \textit{CHST6} are responsible for Icelandic families with MCD type I, including p.A128V, p.R166P, and a frameshift p.V6fs, which results from a 10-base pair insertion (c.1516insATGCTGTGCG). Four subjects were homozygous for the p.A128V mutation and one patient was a compound heterozygous for p.A128V and the frameshift p.V6fs.

The molecular basis for differences between the different immunophenotypes of MCD remains unexplained. In their analysis of patients with MCD type II, Akama et al. [7] discovered upstream rearrangements or deletions that presumably resulted in altered promoter function and reduced cornea cell-specific transcription of \textit{CHST6}. They identified a deletion that appeared to result from a chromosomal recombination between the highly similar sequences involving \textit{CHST5} and \textit{CHST6} and the surrounding genome. In an Italian family with MCD type II, a homozygous deletion in the region upstream of \textit{CHST6} was also detected [9]. A defect in the promoter region of \textit{CHST6} is expected to control C-GlcNAc6ST activity and hence might cause the milder immunophenotype of MCD characterized by the presence of significant AgKS in the serum and cornea. However, using the same PCR primers and methods, we failed to demonstrate a deletion or replacement upstream of \textit{CHST6} in Icelandic patients with neither MCD types I nor II based on an analysis using the method of Akama et al. [7]. This procedure does not exclude subtle upstream abnormalities but the lack of upstream abnormalities in MCD type II from Iceland and other families from other populations raises doubt in the idea that a \textit{CHST6} promoter defect is the sole explanation for MCD type II immunophenotype. Moreover, we have found such an upstream deletion in two individuals from one family with MCD type II who lacked mutations in the coding region of \textit{CHST6} and had no apparent deletion or insertion upstream of this gene using the Akama et al. [7] procedure.

In Icelandic families, all patients with MCD type II are associated with either compound p.A128V and p.V329L mutations or a homozygous p.V329L mutation. It is noteworthy that p.V329L and a single nucleotide change c.768C>T (p.A256A) were associated with MCD type II, but not with MCD type I. The c.768C>T polymorphism, which is only 217 base pairs apart from the p.V329L (c.985G>C) mutation, is unlikely to contribute to MCD in the Icelandic MCD type II families. Nevertheless, one can not conclude that the p.V329L or any other mutation is responsible for a particular immunophenotype of MCD, since the effect of different \textit{CHST6} mutations on GlcNAc6ST enzymatic activity remains unknown.

The four \textit{CHST6} mutations found in Icelandic MCD families (p.A128V, p.V6fs, p.R166P, and p.V329L) correspond with the previously reported five disease haplotypes in this population [5,6]. The most frequent mutation in Icelandic individuals (p.A128V), which corresponds to the previously reported ancestral haplotype 1, is shared by both MCD type I and MCD type II. Haplotypes 2 and 4, which are MCD type I specific, represent p.V6fs and p.R166P, respectively. The MCD type II specific haplotypes 3 and 5 are associated with the p.V329L mutation. Apart from p.R166P that has been reported in one Icelandic [8] and two French families with MCD type I [14], the three other \textit{CHST6} mutations identified in Icelandic MCD families have not been identified in other populations.

Consistent with an autosomal recessive inheritance, two coding region mutations in \textit{CHST6} have been identified on different chromosomes in all affected individuals with MCD in Iceland. Unaffected family members who are clinically and serologically indistinguishable from normal control individuals were found to be either disease carriers with only a heterozygous mutation on one chromosome or to have two normal copies of \textit{CHST6} (Figure 1). Thus far all Icelandic patients with MCD can be explained by the four mutations in the coding region of \textit{CHST6} mentioned above, in either homozygous or compound heterozygous forms.

Our mutation analysis clearly shows that the two immunophenotypes of MCD in the Icelandic population are
due to the effects of different mutations in CHST6. While this provides a molecular basis for the difference between MCD type I and II in the Icelandic families, it does not explain the reason for the difference between these two types of MCD. Because the serum and corneal tissue fails to react with the anti-KS monoclonal antibody (5D4) in MCD type I than in MCD type II. Such an assumption found support in an unusual American family where both MCD types I and II coexisted in a single sibship [15]. In that family, the sibling with the greater deficiency of AgKS (MCD type I) had homozygous c.418C>T (p.R140X) mutations in CHST6, which is predicted to generate a stop-codon and hence a truncated C-GlcNAc6ST. Siblings with the milder MCD type II were heterozygous for c.418C>T, c.993G>T (p.Q331H), and c.668G>A (p.G223D) nucleotide changes in CHST6 and would presumably still have some residual enzymatic function albeit defective. However, the molecular root for the different immunophenotypes is more complex as an investigation of MCD in Saudi Arabia disclosed identical CHST6 mutations in families with MCD type I, IA, and II [16]. Regardless of the immunophenotype and the wide variety of mutations reported in multiple populations throughout the world, both MCD type I and MCD type II apparently have similar clinical manifestations.

An understanding of the genetic basis of MCD and other inherited disorders of the cornea provides clinicians with the ability to make precise molecular diagnoses so that patients and their offspring can be properly counseled. The identification of causative mutations in genes such as CHST6 that result in corneal opacities is the first step toward understanding the role that such genes play in maintaining corneal clarity.

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REFERENCES


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