



Mutations in corneal carbohydrate sulfotransferase 6 gene (CHST6) cause macular corneal dystrophy in Iceland

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Abstract

Purpose: Macular corneal dystrophy (MCD) is subdivided into three immunophenotypes (MCD types I, IA and II). Recently, mutations in the carbohydrate sulfotransferase 6 gene (CHST6) were identified to cause MCD. The purpose of this study was to examine CHST6 for mutations in Icelandic patients with MCD type I.

Methods: Genomic DNA was extracted from leukocytes in the peripheral blood and the coding region of CHST6 was examined for mutations by polymerase chain reaction (PCR) and direct sequencing.

Results: Mutation analysis of the CHST6 coding region identified three different mutations in sixteen Icelandic patients with MCD type I. Eleven patients with MCD type I were homozygous for a C1075T mutation. One patient with MCD type I was found to be a compound heterozygous for C1075T and G1189C mutations. One family with MCD type I contained a 10 base pair insertion (ATGCTGTGCG) between nucleotides 707 and 708. In this family, two affected siblings had a homozygous insertion while both their affected mother and their affected maternal aunt had a heterozygous insertion and a heterozygous

C1075T mutation.

Conclusions: Three different nucleotide changes were identified in the coding region of CHST6 in sixteen Icelandic patients with MCD type I. All three of these alterations are predicted to affect the translated protein and each of them corresponded to a particular disease haplotype that we had previously reported in this population.

Introduction

Macular corneal dystrophy (MCD; OMIM entry [217800](#)) is an autosomal recessive disorder that is clinically characterized by progressive corneal stromal clouding in both eyes [1,2]. While MCD is rare in most parts of United States and other countries, it is the most common condition necessitating penetrating keratoplasty in Iceland [3,4]. Although clinically indistinguishable, cases of MCD can be subdivided into three immunophenotypes (MCD types I, IA, and II) based on the immunohistochemical reactivity of the corneal tissue to an anti-keratan sulfate antibody and the antigenic keratan sulfate (aKS) levels in cornea and serum [4-6]. In MCD type I neither cornea nor serum contain appreciable levels of aKS, while in MCD type II aKS is present in cornea and serum [4,5]. MCD type IA has no detectable aKS in serum or in most of the corneal stroma but the accumulations within the keratocytes react with the anti-keratan sulfate antibody [6]. We previously mapped the MCD type I gene to human chromosome 16 (16q22) and suggested that MCD type II may be allelic with MCD type I [7-10]. Recently, a carbohydrate sulfotransferase 6 gene (CHST6) was identified within the MCD critical region and mutations in CHST6 have been shown to be the fundamental defect in MCD [11]. Akama et al. [11] identified several mutations that may lead to an inactivation of N-acetylglucosamine-6-sulfotransferase within the coding region of CHST6 in patients with MCD type I. Homozygous mutations in the coding region of CHST6 were not detected in patients with MCD type II, but two DNA rearrangements in the upstream region of CHST6, which may include a gene regulatory element that affects transcription of CHST6, were found in MCD type II.

The aim of this study was to examine the CHST6 gene for mutations in Icelandic families with MCD type I.

Methods

Family data and diagnostic criteria

The protocol and participant consent forms were approved by Duke University Institutional Review Board and Landakot Hospital Ethics Committee. After appropriate informed consent was obtained, sixteen individuals with MCD and their family members from six Icelandic families were included in this study. The diagnosis of MCD type I was based on a

combination of the clinical presentation, the typical histopathologic features of the dystrophy in corneal tissue obtained after penetrating keratoplasty, and the absence of detectable aKS levels in serum and corneal tissue. These features were reviewed in an earlier publication [4]. The disease haplotypes of the sixteen affected individuals were previously reported [9,10].

Mutation analysis in the CHST6 gene

Peripheral blood was collected from each participant and genomic DNA was extracted from leukocytes as previously described [12]. Three pairs of primers were designed to amplify the whole coding region of CHST6 by PCR so that the PCR products were well overlapped. The primer pairs used were as follows: for the 5'-coding region, CK71h-intrn (5'-GCCCTAACCGCTGCGCTCTC-3') and CK71h-R166P (5'-GGCTTGCACACGGCCTCGCT-3'); for the middle coding region, CK71h-F1041 (5'-GACGTGTTTGATGCCTATCTGCCTTG-3') and CK71h-R1674 (5'-CGGCGCGCACCAGGTCCA-3'); for the 3'-coding region, CK71h-F1355 (5'-CTCCCGGGAGCAGACAGCCAA-3') and CK71h-R1953 (5'-CTCCCGGGCCTAGCGCCT-3'). The forward primer (CK71h-intrn) for the 5' coding region was located in the adjacent intron just upstream of the start codon. The backward primer (CK71h-R1953) for the 3' coding region was in the non-coding area immediately downstream of the stop codon. Each PCR was carried out in a 20 µl reaction mixture consisting of 20 mM Tris HCl pH 8.4, 50 mM KCl, 0.2 mM dNTPs, 1.5 mM MgCl₂, 0.4 µM of each primer, 1 unit of DNA polymerase Platinum Taq (Gibco Life Technologies, Grand Island, NY), and 60 ng of genomic DNA. Amplification was performed in a DNA Thermal Cycler (MJ Research, Waltham, MA). The cycling program started with an initial denaturing step of 2 min at 96 °C, followed by 35 cycles of 96 °C for 30 s, 57 °C for 30 s, and 72 °C for 45 s, with a final extension step at 72 °C for 5 min. The PCR products were then purified using the QIAquick PCR purification kit (Qiagen, Valencia, CA) and directly sequenced on both strands. Sequencing was carried out in an automatic fluorescent DNA sequencer ABI Prism 377 (Applied Biosystems, Foster City, CA). Nucleotide sequences were compared with the CHST6 cDNA and the sequences of normal control individuals. The mutations identified in this report were named according to the Nomenclature Systems for Human Gene Mutations [13]. Nucleotide +1 is the first nucleotide of the CHST6 cDNA sequence (GenBank accession number [AF219990](#)). For amino acid numbering, the codon for the initiator methionine starting at nucleotide 693 of the CHST6 cDNA is numbered as codon 1.

Icelandic control individuals

Genomic DNA extracted from the blood of fifty Icelandic individuals was used as control samples.

Results

Three distinct nucleotide changes were identified in the coding region of CHST6 in sixteen patients with MCD type I, including C1075T, G1189C, and a 10 base (ATGCTGTGCG) insertion between nucleotides 707 and 708 ([Figure 1](#)). Eleven patients with MCD type I from four families were homozygous for the C1075T mutation ([Table 1](#)). One patient with MCD type I was heterozygous for a G1189C and the C1075T polymorphism ([Table 1](#)). The ATGCTGTGCG insertion was identified in one family with four patients ([Figure 2](#)). Two patients in this family were homozygous for this insertion, while their affected mother and affected maternal aunt were heterozygous for the insertion and heterozygous for the C1075T mutation ([Figure 2](#)).

Four of the fifty control Icelandic individuals without MCD manifest a heterozygous C1075T mutation in CHST6. None of the controls were homozygous for C1075T. The G1189C nucleotide change and the 10 base pair insertion were not identified in any control individuals.

Discussion

In this study, we examined the CHST6 coding region for mutations in sixteen individuals with MCD type I from six Icelandic families. Sequencing analysis revealed three distinct alterations in the nucleotide sequence in the patients: C1075T, G1189C, and a 10 base insertion between nucleotides 707 and 708 of CHST6. The C1075T and G1189C nucleotide changes are missense mutations that modify the amino acid in the protein product. The C1075T nucleotide change converts alanine to valine at codon 128, while G1189C alters the amino acid from arginine to proline at codon 166 ([Table 1](#)). The 10 base pair insertion between nucleotides 707 and 708 affects the translated protein markedly so that only the first 6 amino acids are normal. The fact that none of the fifty controls were homozygous for the C1075T change in CHST6 adds weight to our interpretation that this is a true disease causing mutation. Because MCD is an autosomal recessive disease and is relatively common in Iceland the finding of a heterozygous C1075T alteration in four control individuals, who were probably carriers, is not surprising. The nucleotide changes identified in the Icelandic patients are different from those recently reported in CHST6 by Akama et al. in non-Icelandic patients with MCD [11].

While the C1075T alteration results in the relatively minor amino acid change from alanine to valine, such a mutation has been reported to cause other disorders [14-16]. For example, the A79V and A231V mutations in the presenilin-1 gene are two of several mutations that cause Alzheimer disease in the relatively homogeneous Dutch population [15,16]. While the CHST6 cDNA is predicted to encode a membrane protein consisting of 395 amino acids [11], an alanine to valine mutation resulting from the C1075T nucleotide change is predicted to be located in the catalytic domain based on a protein prediction program. A mild amino acid change, such as alanine to valine, in conserved amino acid residues may severely affect the protein function and cause disease [14-16].

In previous publications that included this Icelandic population, we have reported five different disease haplotypes [9,10]. Haplotypes 1,2 and 4 were found in individuals with MCD type I; haplotypes 3 and 5 occurred in MCD type II. A comparison of the mutations with the haplotypes revealed that each mutation corresponded to a specific different haplotype ([Table 1](#)). The major common ancestral founder haplotype (haplotype I) corresponds to the most common C1075T mutation identified in the current study ([Table 1](#)). Haplotypes 2 and 4 represent the ATGCTGTGCG insertion between nucleotides 707 and 708 and the G1189C change, respectively ([Table 1](#)).

In conclusion, our sequencing data of the CHST6 gene confirms the presence of distinct mutations in the CHST6 coding region in patients with MCD type I. Three distinct mutations seem to be responsible for MCD type I in Icelandic patients and each of them corresponds to a distinct disease haplotype that was reported previously in this population [9,10].

Acknowledgements

We would like to thank the families who participated in this study. Research Grant R01 EY 08249 from the National Eye Institute supported this study.

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[Typographical corrections](#)

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ISSN 1090-0535