Abstract

Objective
Macular Corneal Dystrophy (MCD) is a rare autosomal recessive disorder affecting the stroma of cornea. Most cases of MCD are caused by mutations in CHST6 gene. The aim of this study was to determine mutations in the carbohydrate sulfotransferase 6 gene (CHST6) through genetic analysis of 7 Iranian patients with MCD.

Materials & Methods
We screened the CHST6 gene to determine the range of pathogenic mutations. Genomic DNA was extracted from peripheral blood leukocytes. The coding regions of the CHST6 gene were amplified using three pairs of primers, and directly sequenced in the final step.

Results
Four mutations were found to affect the translated protein and each of them corresponded to a particular disease haplotype that has been previously reported.

Keywords: Macular Corneal Dystrophy (MCD), Iranian Patients, Carbohydrate Sulfotransferase 6 Gene (CHST6)

Introduction
Corneal dystrophies are a heterogeneous group of disorders that may lead to severe visual impairment (1). Macular Corneal Dystrophy (MCD) is an autosomal recessive disorder that is clinically characterized by progressive corneal stroma haze in both eyes (1, 2). Initially, patients have diffuse, fine superficial clouding in the corneal stroma. In the course of time, the opacities extend through the entire thickness of the cornea and involve the central and surrounding cornea. The involved corneal stroma is often thinner than normal (3). The prevalence of MCD varies immensely in different parts of the world but the condition is rare in most populations. In some countries, MCD accounts for 10-75% of corneal dystrophies which require corneal grafting (4).

MCD is divided 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 sulphated keratan sulfate (KS). However, these subtypes are clinically indistinguishable from each other (5-7). By identifying the locus of MCD on chromosome 16 (8) and fine mapping the gene (9, 10), mutations in the carbohydrate sulfotransferase gene (CHST6) encoding corneal N-acetylglucosamine-6-O-sulfotransferase (C-GlcNAc-6-ST) were identified as the cause of MCD types I and II (11). While mutations of the coding region of CHST6 were found in MCD types I,
IA, and II (12-14), DNA rearrangements in the upstream region of CHST6 that could influence gene regulatory elements affecting transcription of CHST6 have been reported in MCD type II. The aim of this study was to screen CHST6 mutations in 7 Iranian patients from apparently unrelated families diagnosed with MCD.

Materials & Methods
Seven individuals with MCD from unrelated Iranian families, as well as 20 age-matched healthy control subjects, were included in this study. The age of the patients varied between 15 and 50 years of age with a mean of 28 years. An informed written consent was obtained from each participant for clinical and molecular genetic studies. Patients were referred by corneal specialists from Farabi Eye Hospital. On slit lamp examination, all patients had typical signs of the disease as characterized by whitish, nodular lesions or spots in the superficial stroma. In some patients, progression of disease was noted as the coalescence of the multiple spots into larger nodules and a diffuse stromal opacity including the peripheral cornea. Most patients had lost full vision and needed penetrating keratoplasty. Loss of vision and photophobia were common signs among the patients. In 7 patients who had undergone penetrating keratoplasty, diagnosis was confirmed histologically. Screening results showed 4 different mutations (table 1). Different mutations have been reported in MCD patients (11-22). These 4 mutations have also been reported in previous studies: H249R (15) P31L (20), R127C (15) and D203Y (11). This study confirmed the findings of another study which evaluated these mutations in Iran (23).

Our data regarding the CHST6 gene among 7 Iranian patients confirmed that present mutations in this gene were causative of MCD. We suggest the screening of these mutations in Iranian patients.

Table 1. The CHST6 mutations and related amino acid substitution

<table>
<thead>
<tr>
<th>Family</th>
<th>Number of patients in family</th>
<th>Nucleotide change</th>
<th>Type of change</th>
<th>Amino acid change</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>3</td>
<td>1438A&gt;G†</td>
<td>Homozygous</td>
<td>H249R</td>
</tr>
<tr>
<td>B</td>
<td>2</td>
<td>784C&gt;T†</td>
<td>Homozygous</td>
<td>P31L</td>
</tr>
<tr>
<td>C</td>
<td>1</td>
<td>1071C&gt;T†</td>
<td>Homozygous</td>
<td>R127C</td>
</tr>
<tr>
<td>H</td>
<td>1</td>
<td>1299G&gt;T†</td>
<td>Heterozygous</td>
<td>D203Y</td>
</tr>
</tbody>
</table>

† Reported mutations: H249R (15) P31L (20), R127C (15) and D203Y (11)
**Discussion**

Macular Corneal Dystrophy (MCD), a disease affecting the stroma of cornea, usually becomes symptomatic in the first decade of life. In this disease, fuzziness is developed in the cornea and the attacks of pain progress into the later years of the patient’s life. MCD is an autosomal recessive disease caused by abnormal configuration of keratin sulfate. Most cases of MCD are caused by mutations in CHST6 gene. The mutations reported in this study seem to be prevalent in Iran. By screening all the exons of the gene, new mutations might be discovered (23).

In this study, Sequencing analysis revealed four missense mutations (p.D203Y, R127C, p.P31L and p.H249R) of CHST6 in the Iranian patients with MCD. The p.R127C mutation is located between the 5’-PSB and the 3’-PB domain. The p.D203Y mutation is located in the RX7S sequence for the 3’-PB domain. The 203 and 249 mutations have been previously reported (11,15), but the amino acid changes at these positions are different from our study and the previous unique study performed in Iran (23). The 203 mutation causes the replacement of aspartic acid by tyrosine (p.D203Y) while in Japanese patients with MCD, a change at nucleotide position 609 (c.609C>A) causes the replacement of aspartic acid with glutamic acid at the same protein position (p.D203E)(11). In Iranian subjects, a change at nucleotide position leads to the replacement of histidine by arginine at position 249 of the protein (p.H249R) while in Indian patients with MCD, replacement of histidine by cysteine (p.H249C) has been reported (15).

**References**


