MUTATIONS IN CORNEAL CARBOHYDRATE SULFOTRANSFERASE 6 GENE (CHST6) IN IRANIAN MACULAR CORNEAL DYSTROPHY (MCD) PATIENTS: A REPORT OF 7 PATIENTS FROM IRAN

ATAEI Mitra BSc [1],
ZARE MEHRJERDI Mohammad Ali MD [2],
YAZDI Amir Reza MD [3],
ZAMANI Akram MSc [4],
FARAJI Abolfazl BSc [1],
HOUSHMAND Massoud MD [5]

1.Department of Medical Genetics, National Institute of Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran
2.Associate Professor of Ophtalmology, Farabi Eye Research Center, Tehran University of Medical Sciences, Tehran, Iran
3.General Physician, Genetic Diagnostic Department, Special Medical Center, Tehran, Iran
4. Department of Medical Genetics, National Institute of Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran
5.Assistant Professor of Human Genetics, Department of Medical Genetics, National Institute of Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran

Corresponding Author:
Houshmand M. MD
Department of Medical Genetics, National Institute of Genetic Engineering and Biotechnology (NIGEB),
P.O.Box: 14155-6343
Tel: +98 21 4580 390,
Fax: +98 21 4580 399,
E-mail: massoudh@nrcgeb.ac.ir

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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-6-0-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 need penetrating keratoplasty. Loss of vision and photophobia were common signs among the patients. In 7 patients who had undergone penetrating keratoplasty, diagnosis was confirmed by histopathologic examination.

Examination of the pedigree of all patients confirmed the autosomal recessive pattern of the inheritance. None of the patients had raised intraocular pressure or posterior segment abnormalities.

For mutation analysis, peripheral blood (5 ml) was collected from each patient and genomic DNA was extracted by using the Diatom DNA extraction Kit (gene Fanavaran, Tehran, Iran). The *CHST6* gene is 126.9 kb in length and consists of 4 exons, of which only exon 4 contains the coding sequence of 1,189 bp. Three pairs of primers were used to amplify the entire coding region of *CHST6* as described previously (13). PCR products were purified by the Qia quick PCR purification kit (Qiagen, Valencia, CA) and directly sequenced on both strands. Sequencing was carried out in an automatic fluorescent DNA sequencer ABI 3700 (Macrogene, Korea), and the resulted data were compared with the published cDNA sequence of *CHST6* by Clustal-X software (version 1.8). The nucleotide +1 is the first nucleotide of the *CHST6* cDNA sequence (GenBank accession number AF2119990). For the enumeration of the amino acids, the codon for the initiator methionine, starting at nucleotide 693 of the *CHST6* cDNA, is numbered as codon 1.

Results

All patients received penetrating keratoplasty (PK) before or after DNA sampling. Corneal buttons were stained with alcian blue and hematoxylin-eosin and the MCD 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 patient.

Table 1. The CHST6 mutations and related amino acid substitution

amily	Number of patients in family	Nucleotide change	Type of change	Amino acid change
A	3	1438A>G†	Homozygous	H249R
B	2	784C>T†	Homozygous	P31L
C	1	1071C>T†	Homozygous	R127C
H	1	1299G>T†	Heterozygous	D203Y

† 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.P31Land 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 nucleotid position leads to the replacement of histidine by ariginine 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).

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