Novel compound heterozygous mutations in the *CHST6* gene cause macular corneal dystrophy in a Han Chinese family

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Background: Macular corneal dystrophy (MCD), a rare autosomal recessive disorder, is caused by pathogenic mutations in the carbohydrate sulfotransferase 6 gene (*CHST6*) and is characterized by bilateral progressive stromal clouding and vision loss. Corneal transplantation is often necessary. This study aimed to identify disease-causing mutations in a Han-Chinese MCD patient.

Methods: A 37-year-old female diagnosed with MCD was recruited. The clinical materials were observed and described, and peripheral blood sample was extracted. Whole exome sequencing (WES) and Sanger sequencing were used to reveal genetic defects. The pathogenicity of identified mutations was assessed using *in silico* analysis.

Results: The patient had typical features of MCD, including decreased vision, multiple irregular gray-white corneal opacities, and corneal thinning. A novel nonsense mutation c.544C>T (p.Gln182Ter) and a validated missense mutation c.631C>G (p.Arg211Gly) were identified in the *CHST6* gene coding region, both classified as "pathogenic" following the American College of Medical Genetics and Genomics standards and guidelines.

Conclusions: This study reports a Han-Chinese MCD patient with a novel nonsense mutation c.544C>T (p.Gln182Ter) and a recurrent missense mutation c.631C>G (p.Arg211Gly), which expand the spectrum of genetic mutations. The results of this study extend genotype-phenotype correlations between the *CHST6* gene mutations and MCD clinical findings, contributing to a more accurate diagnosis and the development of potential gene-targeted MCD therapies.

Keywords: Carbohydrate sulfotransferase 6 gene (*CHST6*); compound heterozygous mutations; Han Chinese family; macular corneal dystrophy (MCD)

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Introduction

Macular corneal dystrophy (MCD, MIM 217800), a rare autosomal recessive corneal disease, is characterized by bilateral progressive scattered stromal clouding, irregular gray-white corneal opacities, and central corneal thinning. The late stage of vision loss requires corneal transplants (1-3). The prevalence of MCD is estimated to be 9.7/1,000,000, with different distribution frequencies worldwide. The prevalence is 1.2/1,000,000 and 7.6/100,000 in America and Iceland, respectively (4,5). The relatively common occurrence in India, Saudi Arabia and Iceland is associated with the high frequency of consanguineous marriage, without involvement of specific environmental or systemic factors (4,5). The onset is usually in the first decade and presents with central superficial irregular whitish corneal opacities. These opacities progressively expand to the corneal periphery, and even to the entire stroma with corneal thinning, resulting in severe vision impairment in middle age (2,6-8). Therefore, early diagnosis and treatment are necessary for MCD. The histopathological characteristics of MCD are typically glycosaminoglycan accumulation in different corneal layers, including the epithelium, Bowman's membrane, the stroma, Descemet's membrane, the endothelium, and keratocytes (3,7,9). The different onset ages, disease severity, and non-coding region mutations support that the gene expression may be regulated by epigenetic factor(s) (2,7,9).

In 2000, coding mutations in the carbohydrate sulfotransferase 6 gene (*CHST6*, MIM 605294) and rearrangements in the upstream region of the *CHST6* gene were reported as being responsible for MCD (10,11). The gene encodes corneal glucosamine N-acetyl-6-sulfotransferase (C-GlcNAc6ST), which contains an N-terminal with a short cytosolic tail, a transmembrane domain, and a large Golgi luminal C-terminal domain, belonging to the carbohydrate sulfotransferase family. The C-terminal domain has sulfate donor 3'-phosphoadenosine-5'-phosphosulfate (PAPS) binding sites, a catalytic module and an area that determines carbohydrate specificity *in vivo* (12-14). The C-GlcNAc6ST functions in the formation of sulfated keratan sulfate (KS), which is transported to the extracellular matrix where it forms some components of the corneal stroma (15). *CHST6* mutants lead to accumulation of poorly sulfated or unsulfated KS, resulting in corneal fibril organization malformations and progressive opacification (14,16). Up to now, at least 193 *CHST6* gene mutations, including missense, nonsense, deletion, insertion/duplication, deletion-insertion, and complex rearrangement, have been reported in different patients with MCD (1,14,17,18). The homozygous or compound heterozygous mutations in *CHST6* gene might be the potential early MCD diagnosis markers (2,3,8-17).

We present the following article in accordance with the MDAR reporting checklist (available at http://dx.doi.org/10.21037/atm-20-7178).

Methods

Subjects and clinical evaluation

A 37-year-old female diagnosed with MCD was recruited from the Third Xiangya Hospital, Central South University (Changsha, China). The study was conducted in accord with the Declaration of the Helsinki (as revised in 2013) and approved by the Institutional Review Board of the Third Xiangya Hospital, Central South University, Changsha, China (No.: 2018-S400). Written informed consent was obtained from the female patient. Two ophthalmologists evaluated the patient who underwent a comprehensive ocular examination, including best-corrected visual acuity (BCVA) tests, slit-lamp, confocal microscopy, and optical coherence tomography (OCT) examinations. The clinical diagnosis for MCD was based on the typical corneal features (1). The genomic DNA (gDNA) was isolated from peripheral blood sample of the patient using a phenol-chloroform extraction procedure, as previously described (19-21).

Whole exome sequencing (WES)

WES of the proband was performed by BGI Genomics, BGI-Shenzhen (Shenzhen, China), as previously described (22,23). The quantified gDNA sample was randomly sheared into fragments by Covaris E220 (Covaris, Inc.) and achieved library fragments between 150 bp and 250 bp. The DNA fragments were repaired by A-tailing reactions and then ligated with adapters. Ligation-mediated polymerase chain reaction (PCR) was used to amplify the fragments, and enrichment was performed after the purification and the hybridization to the exome array. The captured products were amplified by circularization. The qualified captured library was then loaded on the BGISEQ-500 platform. High-throughput sequencing was performed to ensure the average sequencing coverage.

Variants analysis

Raw data from the sequencing platform were further filtered to obtain clean reads by a standard data-filtering process. The qualified clean reads were aligned to the human reference genome sequence by the Burrows-Wheeler Aligner (BWA) software (v0.7.15). Picard tools (v2.5.0, https://broadinstitute.github.io/picard/) and Genome Analysis Toolkit (GATK, v3.7, https://www.broadinstitute.org/gatk/guide/best-practices) were used for marking and removing duplicate reads, Indel realignment, base recalibration and variant calling. The single nucleotide polymorphism effect (SnpEff, https://pcingola.github.io/SnpEff/) tool was applied for variant annotation and prediction, including 1000 Genomes Project, the Single Nucleotide Polymorphism Database (dbSNP) build 141, the National Heart, Lung, and Blood Institute-Exome Sequencing Project 6500 (NHLBI-ESP6500), and the 2,471 Chinese controls of the BGI in-house databases. The possible effects on protein function or structure of missense and nonsense variants were predicted by MutationTaster (http://www.mutationtaster.org/), Protein Variation Effect Analyzer (PROVEAN, http://provean.jcvi.org/index.php), and the Polymorphism Phenotyping v2 (PolyPhen-2, http://genetics.bwh.harvard.edu/pph2/). The American College of Medical Genetics and Genomics (ACMG) guidelines for interpreting gene variants were used to classify the variants as "pathogenic", "likely pathogenic", "uncertain significance", "likely benign", and "benign" (24,25).

Sanger sequencing

For the potential pathogenic variants identified by WES in the patient, Sanger sequencing was performed subsequently. Primers were designed for candidate variants with Primer3 (http://frodo.wi.mit.edu/primer3/) according to the sequences of the human reference genome (http://genome.ucsc.edu/). The primers were then synthesized by Sangon Biotech (Shanghai) Co., Ltd. Primer sequences were designed as: 5'-GTGTGCAAGCCACTGTGC-3' and 5'-GTGGCTACGGCACACCTC-3'. The sequencing results were analyzed by using Chromas software (v2.01, Technelysium Pty Ltd., South Brisbane, Australia).

Conservative analysis and structure modeling

Basic Local Alignment Search Tool of National Center for Biotechnology Information (NCBI BLAST, http://blast.st-va.ncbi.nlm.nih.gov/Blast.cgi) executed multiple sequence alignments for ten different species. The online CPHmodels-3.2 (http://www.cbs.dtu.dk/services/CPHmodels/) and RaptorX tool (http://raptorx.uchicago.edu) predicted wild-type and mutant protein structures. PyMOL software (v2.3, Schrödinger, LLC, Portland, USA) was used to show the three-dimensional structures (19).

Statistical analysis

Pie charts were used for categorical data to show the percentage of various mutations in each group or the percentage of patients with *CHST6* nonsense mutations in different continents and countries.

Results

Clinical data

The patient complained of bilateral blurred vision, discomfort, tearing, and photophobia as being present for more than twenty years. The patient's visual acuity was 0.06 in the right eye and 0.04 in the left eye. Vision was not significantly improved with prescription lens glasses. A slit-lamp examination detected multiple, irregular, gray-white opacities from the center of the cornea to the peripheral limbus in the patient (*Figure 1A*,*B*). Confocal microscopy examinations revealed the hyper-reflectivity was present in the basal epithelium and the anterior stroma of cornea (*Figure 1C*,*D*,*E*,*F*). Corneal images of OCT showed the subepithelial and anterior stromal deposits resulting in the thinning of the cornea (*Figure 2A*,*B*,*C*,*D*).

WES and identification of pathogenic mutations

Approximately 126.34 million clean reads and 116.27 million total effective reads were obtained, with 99.91% of the reads mapped to the human reference genome. The mean sequencing depth on target regions was 120.93. The fraction of the target regions covered by 10× or more was 98.98%. A total of 98,802 SNPs and 14,863 Indels were detected in the proband. The compound heterozygous variants (c.544C>T and c.631C>G) in the *CHST6* gene, a disease-causing gene for MCD, were prosecuted as the disease-causing factors in the proband, which were absent in 2,471 controls. The c.544C>T variant resulted in a premature stop codon in codon 182 (p.Gln182Ter). The c.631C>G variant led to an arginine-to-glycine substitution (p.Arg211Gly). The

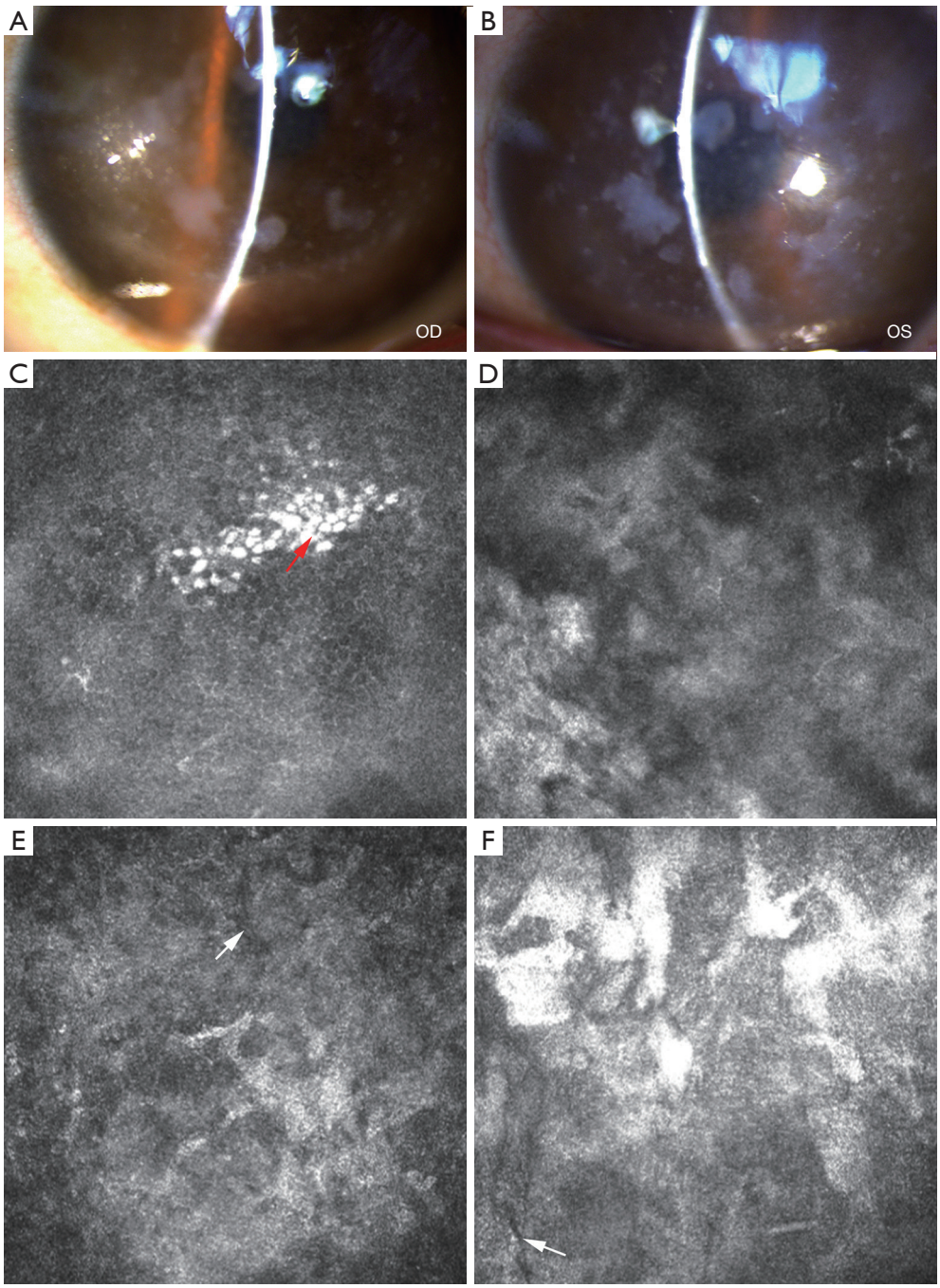

Figure 1 Slit-lamp and confocal microscopy photographs of the patient with macular corneal dystrophy. (A,B) Slit-lamp examination of the patient showed multiple irregular borders, gray-white opacities involving the center of the cornea, which extended to the limbus. (C) The confocal image at a depth of 39 μm. The basal epithelial layer appears to have numerous hyper-reflective cells (red arrow). (D) The confocal image at a depth of 50 μm. In the Bowman's layer, subbasal nerves with increased background were observed. (E) The confocal image at a depth of 83 μm. Hyper-reflective cells of the corneal stroma with multiple, small, black striae (white arrow). (F) The confocal image at a depth of 169 μm. The dark striae of the corneal stroma became large and wide along with the increased depth (white arrow).

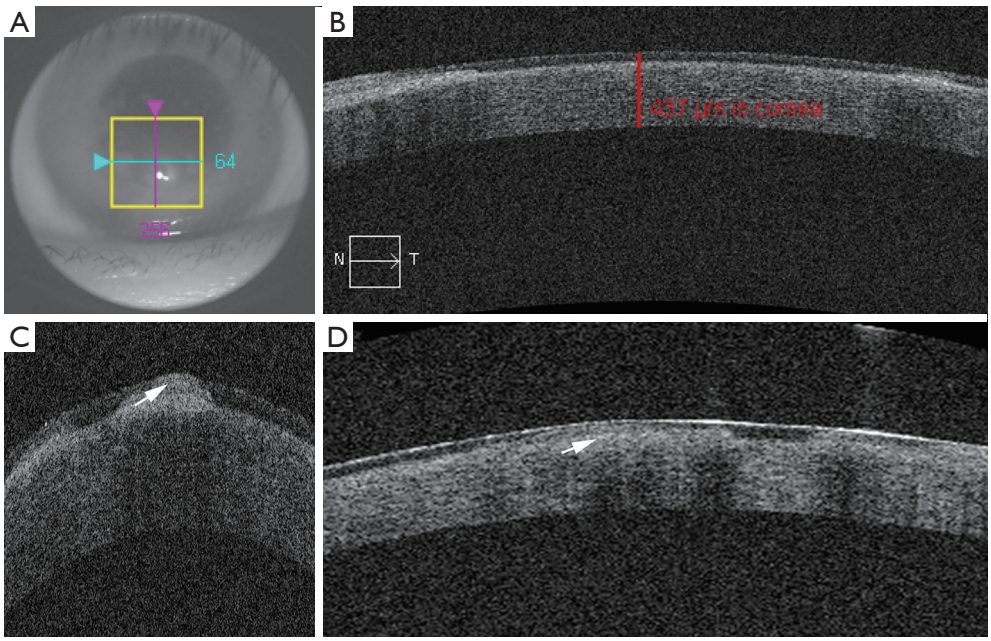

Figure 2 High-definition optical coherence tomography corneal imaging of the patient. (A) High-definition image of the right eye. (B) The central corneal thickness was 437 μm. (C) Large irregular subepithelial deposits (white arrow). (D) Diffuse deposits of hyper-reflectivity in the anterior stroma (white arrow).

prediction analyses of MutationTaster showed that the two variants, c.544C>T and c.631C>G, were disease causing with the score near or equal to 1. The variant, c.631C>G, was predicted to be deleterious by PROVEAN (score: −6.73), damaging by SIFT (score: 0.001), and probably damaging by PolyPhen-2 (score: 1.000, specificity: 1.00, sensitivity: 0.00). The variants were classified as "pathogenic" mutations using the ACMG standards and guidelines for variant interpretation. The *CHST6* compound heterozygous variants in the proband were further confirmed by Sanger sequencing (*Figure 3A*,*B*,*C*). Amino acid sequence analysis of the sulfotransferases of different species detected that arginine at mutant site (p.Arg211) was highly conserved (*Figure 3D*). Structural modeling showed the conformational alteration (*Figure 3E*).

Discussion

MCD is a stromal corneal dystrophy first described by ophthalmologist Oskar Fehr in 1904 (26). It is characterized by minute irregular gray-white fleck-like opacities deposited in the cornea during the first decade of life. It develops into diffuse multiple nodular opacities and results in severe vision loss over time (7,27). Early diagnosis and following ophthalmologic evaluation, especially in children, should be beneficial for the patients in treatment and management (28).

In this study, a Han-Chinese patient with MCD is described, which has a novel nonsense mutation c.544C>T (p.Gln182Ter) and a validated missense mutation c.631C>G (p.Arg211Gly) in the *CHST6* gene detected by both WES and Sanger sequencing. Compound heterozygous mutations may induce the production of truncated and single amino acid altered proteins and result in a typical MCD phenotype. Corneal phenotypes evaluated by slit-lamp, confocal microscopy, and OCT showed no obvious differences between the patient of this study and other previously reported MCD cases, regardless of the mutations involved (9).

The *CHST6* gene, located at chromosome 16q23.1, contains 3 exons and encodes a 395-amino acid protein with a molecular weight of 44 kDa (1,13). To date, various *CHST6* gene mutations in MCD patients have been reported in different ethnic populations (*Figure 4*), which suggests strong allelic heterogeneity. In this study, a novel *CHST6* nonsense mutation (p.Gln182Ter) in the female patient was identified, which is located in the critical sulfotransferase domain and may generate a truncated and dysfunctional protein product. Nonsense mutations

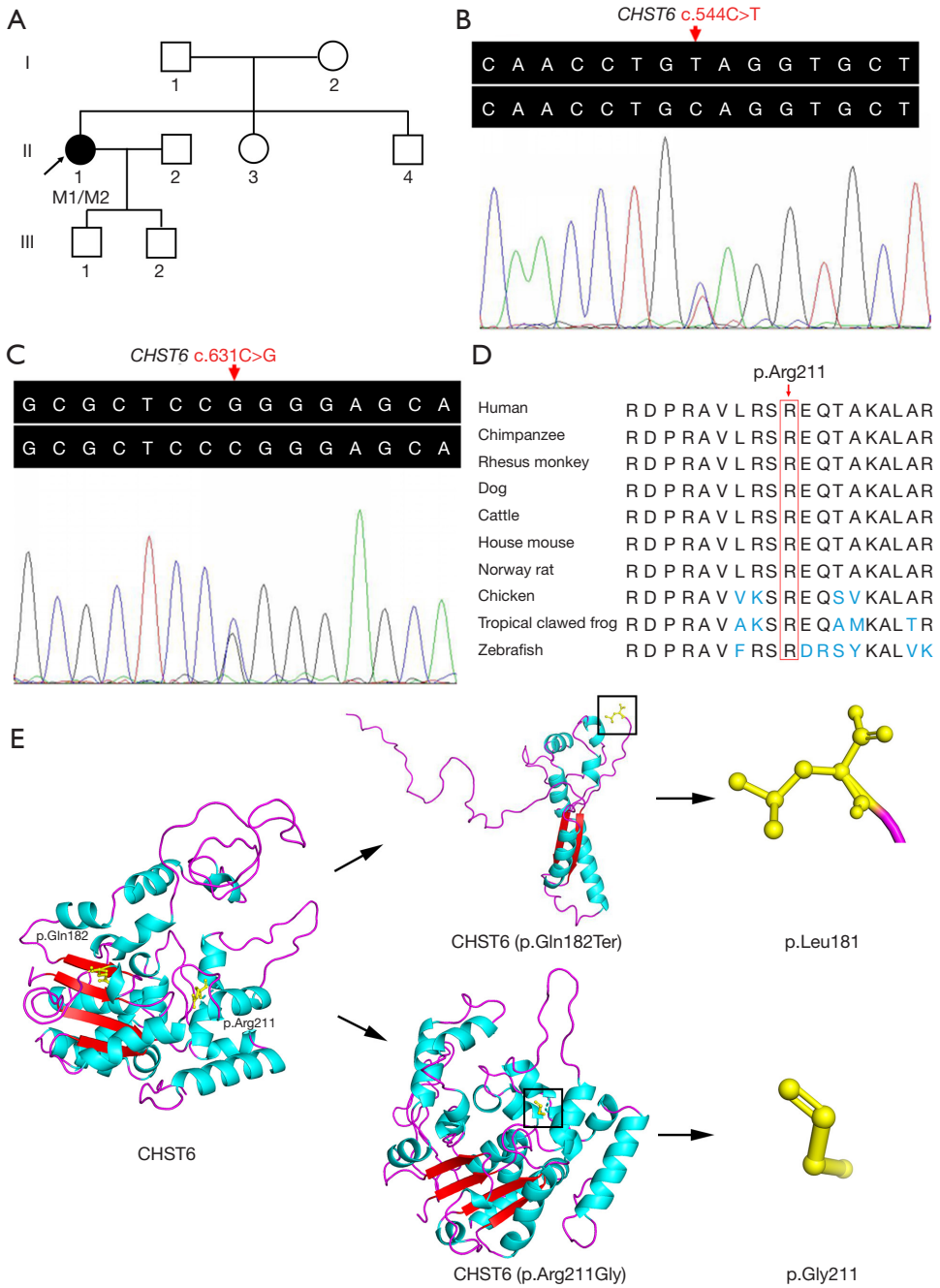

Figure 3 The identified variants of the *CHST6* gene in the MCD family. (A) Pedigree figure. The arrow indicates the proband. M1, the allele with the *CHST6* c.544C>T mutation; M2, the allele with the *CHST6* c.631C>G mutation. (B,C) Patient (II:1) with the compound heterozygous mutations c.544C>T and c.631C>G in the *CHST6* gene. (D) Conservation analysis of the CHST6 p.Arg211 amino acid residue. (E) Cartoon models of the CHST6 proteins. The wild-type glutamine at position 182, arginine at position 211, leucine at position 181 before the premature truncation, and mutated glycine at position 211 are shown in ball-and-stick models by the PyMOL based on the CPHmodels and RaptorX. *CHST6*, the carbohydrate sulfotransferase 6 gene; MCD, macular corneal dystrophy.

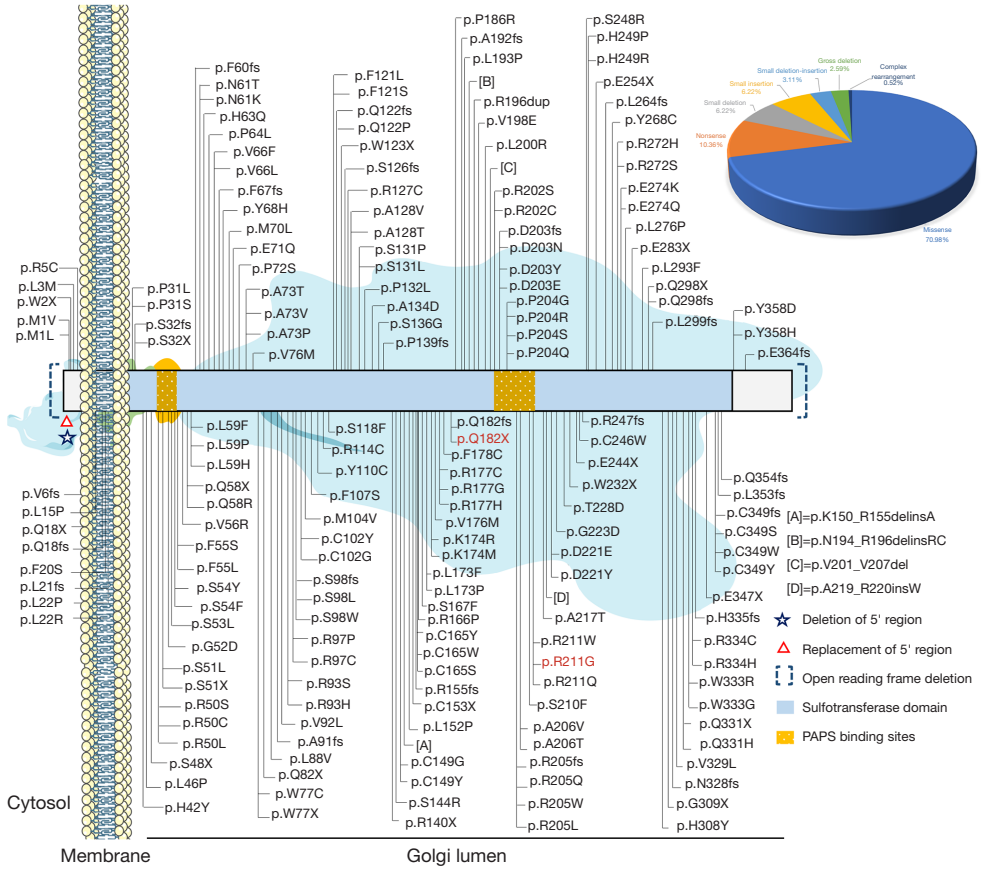

Figure 4 The mutations identified in the *CHST6* gene associated with MCD. The schematic diagram annotated with the sulfotransferase domain (residue 42-356) and PAPS binding sites (residue 49-55 and 202-210) and model structure of the CHST6 protein were shown (UniProtKB: Q9GZX3). Small deletion, insertion and deletion-insertion are defined as those changes involving 20 bp or less according to the definition in Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/ac/index.php). *CHST6*, the carbohydrate sulfotransferase 6 gene; MCD, macular corneal dystrophy; PAPS, 3'-phosphoadenosine-5'-phosphosulfate.

accounted for 10.36% of *CHST6* alterations (*Figure 4*), in which Asian patients comprised a great proportion. For example, ethnic Chinese and Indian sufferers occupied 53.13% and 17.19%, respectively (*Table 1*, *Figure 5*).

Genetic mutation type and position may influence *CHST6* functions, particularly conserved positions across either or both of carbohydrate sulfotransferases or within a critical domain important for enzyme activity (40). The c.631C>G (p.Arg211Gly) mutation, previously reported as unique in two other Chinese families, was identified as one of the disease-causing alleles in our patient (10,31). The identical nucleotide substitution observed in the other two families of the same ethnicity may indicate a founder effect (10,31). The c.631C>G variant leads to the arginine-to-glycine substitution, changing from a positively charged basic amino acid residue to a neutral amino acid. The c.632G>A (p.Arg211Gln) variant has been identified in ethnic Vietnamese, Chinese, and German pedigrees (3,10,44). The c.631C>T (p.Arg211Trp) variant has been found in ethnic Turkish and Japanese populations (16,45). This multiple-ethnicity presence supports a conclusion that amino acid position 211 may be a hot spot mutation position (*Table 2*).

Cornea deposition was detected by confocal microscopy and was consistent in several MCD patients (9,47). High-definition OCT showed some larger diffuse opacities and a hyper-reflective background located on the subepithelial. Multifocal hyper-reflective deposits were detected in the anterior stroma. The OCT results indicated that hyper-reflective deposits caused thickening of the subepithelial

Table 1 Nonsense mutations in the *CHST6* gene associated with MCD

Family number	Number of patients	Nucleotide change	Amino acid change	Mutation status	Geographical/ethnic distribution	References
1	1	c.6G>A/c.7C>A	p.W2X/p.L3M	Homozygote	Southern India	(29)
2	1	c.52C>T	p.Q18X	Homozygote	Indian	(30)
3	1	c.95C>A/c.613C>T	p.S32X/p.R205W	Compound heterozygote	Korean	(2)
4	1	c.95C>A/c.613C>T	p.S32X/p.R205W	Compound heterozygote	Chinese	(31)
5	1	c.143C>A	p.S48X	Homozygote	Iranian	(32)
6	1	c.152C>A	p.S51X	Homozygote	Chinese	(33)
7	3	c.172C>T	p.Q58X	Homozygote	French	(34)
8	1	c.172C>T/c.65T>G	p.Q58X/p.L22R	Compound heterozygote	North India	(35)
9	3	c.231G>A/c.217G>C	p.W77X/p.A73P	Compound heterozygote	United States	(36)
10	1	c.244C>T/c.44T>C	p.Q82X/p.L15P	Compound heterozygote	French	(34)
11	1	c.244C>T/c.632G>A	p.Q82X/p.R211Q	Compound heterozygote	Vietnamese	(3)
12	1	c.244C>T/c.632G>A	p.Q82X/p.R211Q	Compound heterozygote	Vietnamese	(37)
13	1	c.369G>A	p.W123X	Homozygote	Southern India	(29)
14	1	c.369G>A	p.W123X	Homozygote	Indian	(30)
15	1	c.369G>A	p.W123X	Heterozygote	North India	(35)
16	1	c.418C>T	p.R140X	Homozygote	Chinese	(31)
17	1	c.418C>T	p.R140X	Homozygote	United States	(38)
17	2	c.418C>T	p.R140X	Heterozygote	United States	(38)
18	1	c.418C>T/c.827T>C	p.R140X/p.L276P	Compound heterozygote	British	(39)
19	3	c.418C>T/c.613C>T	p.R140X/p.R205W	Compound heterozygote	Chinese	(40)
20	1	c.418C>T/c.814C>A	p.R140X/p.R272S	Compound heterozygote	Chinese	(31)
21	1	c.418C>T/c.1072T>C	p.R140X/p.Y358H	Compound heterozygote	Chinese	(40)
22	1	c.459C>A	p.C153X	Homozygote	Southern India	(29)
23	1	c.459C>A	p.C153X	Homozygote	Indian	(30)
24	1	c.544C>T/c.631C>G	p.Q182X/p.R211G	Compound heterozygote	Chinese	Present study
25	1	c.696G>A	p.W232X	Homozygote	Chinese	(33)
26	1	c.696G>A	p.W232X	Homozygote	Chinese	(33)
27	1	c.696G>A	p.W232X	Homozygote	Vietnamese	(3)
28	1	c.730G>T/c.613C>T	p.E244X/p.R205W	Compound heterozygote	Chinese	(31)
29	1	c.730G>T/c.1072T>C	p.E244X/p.Y358H	Compound heterozygote	Chinese	(31)
30	3	c.730G>T/c.1072T>C	p.E244X/p.Y358H	Compound heterozygote	Chinese	(40)
31	1	c.760G>T	p.E254X	Homozygote	Chinese	(31)
32	1	c.847G>T/c.304T>G	p.E283X/p.C102G	Compound heterozygote	Australia	(41)
33	2	c.892C>T	p.Q298X	Homozygote	Chinese	(31)

Table 1 (*continued*)

Table 1 (*continued*)

Family number	Number of patients	Nucleotide change	Amino acid change	Mutation status	Geographical/ethnic distribution	References
34	1	c.892C>T/c.62delinsGA	p.Q298X/p.L21fs	Compound heterozygote	Chinese	(17)
35	3	c.892C>T/c.183C>T	p.Q298X/p.N61K	Compound heterozygote	Chinese	(31)
36	1	c.892C>T/c.183C>T	p.Q298X/p.N61K	Compound heterozygote	Chinese	(31)
37	1	c.892C>T/c.218C>T	p.Q298X/p.A73V	Compound heterozygote	Chinese	(31)
38	1	c.892C>T/c.218C>T	p.Q298X/p.A73V	Compound heterozygote	Chinese	(31)
39	3	c.892C>T/c.1072T>C	p.Q298X/p.Y358H	Compound heterozygote	Chinese	(40)
40	3	c.892C>T/c.1072T>C	p.Q298X/p.Y358H	Compound heterozygote	Chinese	(42)
41	2	c.892C>T	p.Q298X	Heterozygote	Chinese	(40)
42	1	c.925G>T	p.G309X	Homozygote	Indian	(30)
43	2	c.991C>T	p.Q331X	Homozygote	American	(43)
44	1	c.1039G>T	p.E347X	Homozygote	Indian	(30)
45	1	c.1039G>T	p.E347X	Homozygote	Indian	(30)

CHST6, the carbohydrate sulfotransferase 6 gene; MCD, macular corneal dystrophy.

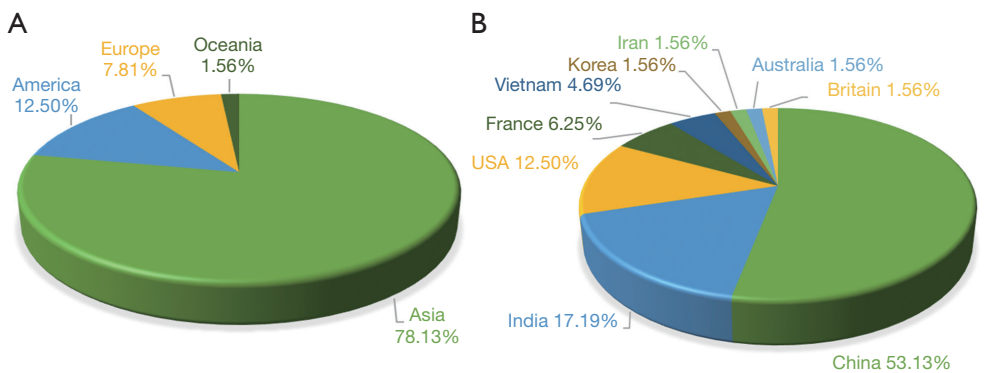

Figure 5 Distribution of *CHST6* nonsense mutations with MCD. A pie chart showing the percentage of MCD patients carrying the *CHST6* nonsense mutations in different continents (A) and countries (B). *CHST6*, the carbohydrate sulfotransferase 6 gene; MCD, macular corneal dystrophy.

and anterior stroma, which led to thinning of the cornea. Though MCD has been widely studied and various *CHST6* mutations have been reported, the limited phenotype-genotype relationship data seem to have little value (7,9). It has been reported that there may be more severe clinical phenotypes and earlier requirements of keratoplasty in ethnic German and French patients with *CHST6* nonsense and frameshift mutations (2,34,44). However, a few studies from Turkey, Egypt, and India have shown there are no consistent phenotypic differences between patients with missense mutations and truncating mutations (12,16,30). More large-scale evaluations of MCD genotype-phenotype associations are warranted.

Different modalities of treatment are suggested for visual restoration of MCD (4,41). Phototherapeutic keratectomy treats superficial and anterior corneal pathology (4,48). Penetrating keratoplasty may improve the BCVA of patients having corneal dystrophies without recurrence (44). Femtosecond laser-assisted lamellar keratectomy is reportedly successful in removing corneal opacities secondary to anterior

Table 2 Missense mutation in codon 211 of the *CHST6* gene in MCD patients

Family number	Number of patients	Nucleotide change	Amino acid change	Mutation status	Geographical/ethnic distribution	References
1	1	c.631C>T	p.R211W	Homozygote	NA	(11)
2	1	c.631C>T	p.R211W	Homozygote	Japanese	(45)
3	1	c.631C>T	p.R211W	Homozygote	Japanese	(45)
4	3	c.631C>T	p.R211W	Homozygote	Turkish	(16)
5	1	c.631C>T/c.530G>A	p.R211W/p.R177H	Compound heterozygote	Japanese	(45)
6	2	c.631C>T/c.649G>A	p.R211W/p.A217T	Compound heterozygote	Japanese	(45)
7	2	c.631C>G/c.445T>G	p.R211G/p.C149G	Compound heterozygote	Chinese	(31)
8	1	c.631C>G/c.544C>T	p.R211G/p.Q182X	Compound heterozygote	Han Chinese	Present study
9	2	c.631C>G/c.613C>T	p.R211G/p.R205W	Compound heterozygote	Chinese	(10)
10	1	c.631C>G/c.760G>T	p.R211G/p.E254X	Compound heterozygote	Chinese	(10)
11	3	c.631C>G	p.R211G	Heterozygote	Chinese	(10)
12	8	c.632G>A	p.R211Q	Homozygote	Vietnamese	(3)
13	1	c.632G>A	p.R211Q	Homozygote	Chinese	(10)
14	1	c.632G>A/c.244C>T	p.R211Q/p.Q82X	Compound heterozygote	Vietnamese	(3)
15	1	c.632G>A/c.244C>T	p.R211Q/p.Q82X	Compound heterozygote	Vietnamese	(37)
16	1	c.632G>A/c.1045_1046del	p.R211Q/p.C349fs	Compound heterozygote	German	(44)
17	1	c.632G>A/c.529C>G	p.R211Q/p.R177G	Compound heterozygote	Vietnamese	(46)

NA, not available; *CHST6*, the carbohydrate sulfotransferase 6 gene; MCD, macular corneal dystrophy.

corneal dystrophies (48). Newer therapy strategies such as gene transfer and gene editing have been evidenced to be effective in both *in vitro* and experimental models (4,14).

In summary, a novel nonsense mutation p.Gln182Ter and a recurrent missense mutation p.Arg211Gly in the *CHST6* gene were identified in a Han-Chinese patient with MCD. This finding expands the spectrum of genetic mutations. The discovery of additional *CHST6* mutations in diverse ethnic populations will help to further explicate genotype-phenotype associations. Additional functional investigations of specific mutations both *in vitro* and/or *in vivo*, such as in human corneas or constructed genetic-deficient animal models, will contribute to elucidate the underlying mechanisms in MCD.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Institutional Review Board of the Third Xiangya Hospital, Central South University, China (No.: 2018-S400). Written informed consent was obtained from the female patient.

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