Gene 675 (2018) 9–14

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Short communication

A novel missense mutation of *CRYGS* underlies congenital cataract in a Chinese family

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ARTICLE INFO

Keywords:
Congenital cataract
Whole exome sequencing
CRYGS
γS-crystallin

ABSTRACT

Congenital cataract is a clinically and genetically heterogeneous disease. In this study, we examined a five-generation Chinese family with autosomal dominant nuclear congenital cataracts by whole exome sequencing. A novel heterozygous missense mutation c.199T > A, p.(Tyr67Asn) in *CRYGS* was identified in this family. The p.(Tyr67Asn) substitution was predicted to decrease the local hydrophobicity and affect the three-dimensional structure of γS-crystallin, and resulted in a portion of mutant protein translocation from the cytoplasm to cell membrane. Our observations expand the mutation spectrum of *CRYGS* and provide further evidence for the genetic basis and molecular mechanism of congenital cataract.

1. Introduction

Congenital cataract (CC) is one of the most common causes of severe visual impairment and blindness during infancy or early childhood with a global prevalence of 1–15 in 10,000 live births. Autosomal dominant, autosomal recessive, and X-linked inheritance patterns have been reported, indicating some degree of genetic heterogeneity in CC (Apple et al., 2000; Gilbert and Foster, 2001). It may be isolated or associated with other ophthalmic abnormalities and syndromes (Trumler, 2011). Approximately 8.3–25% of CC is hereditary, and > 41 genes have been linked to nonsyndromic forms of CC, include genes coding for crystallins, intermediate filament proteins, cytoskeleton proteins, gap junction proteins, lens membrane proteins, and transcription factors (Hejtmancik, 2008; Ma et al., 2016; Javadiyan et al., 2017; Patel et al., 2017).

Crystallins are the major cytoplasmic proteins of the lens and their stability and appropriate interactions are critical for lens transparency. The mammalian lens is a protein-packed gel, as the major structural proteins of the lens, crystallins are divided into α, β, and γ families. α-Crystallins make up roughly 40%, β-crystallins 35%, and γ-crystallins 25% of total crystallins in the mature human lens (Yi et al., 2011). The smallest and simplest members of the crystallins family are the γ-crystallins. The human lens has three major γ-crystallins—γC, γD, and γS. The γC and γD are synthesized during embryogenesis, while γS is synthesized postnatally. During development, the relative expression of γS decreases along with a concomitant increase of α- and β-crystallins (Yi et al., 2011; Vendra et al., 2016). The human *CRYGS* gene is located at chromosome 3q26.3-qter, and it encodes γS-crystallin, a member of the βγ-crystallin superfamily. γS-crystallin comprises 178 amino acid residues and it shares a common two-domain structure composed of four Greek key motifs. γS-crystallin is not only a lens structural protein, but it is also likely to participate in epithelial cell proliferation, apoptosis, and migration. As a result, it plays an important role in maintaining lens transparency (Vendra et al., 2016).

In this study, we identified the disease-causing variant in a five-generation Chinese family with autosomal dominant CC by whole exome sequencing. The expression pattern and intracellular localization of wild and mutant γS-crystallin were investigated to explore the

Abbreviations: CADD, Combined annotation dependent depletion; CC, Congenital cataract; CRYGS, crystallin gamma S; DAPI, 4′,6-diamidino-2-phenylindole; ECL, Enhanced chemiluminescence; FATHMM, Functional analysis through Hidden Markov models; LOD, Logarithm of the odds; MT, Mutant type; NCBI, National center for biotechnology information; PolyPhen2, Polymorphism Phenotyping v2; PROVEAN, Protein variation effect analyzer; PVDF, Polyvinylidene difluoride; REVEL, Rare exome variant ensemble learner; RFLP, Restriction fragment length polymorphism; RIPA, Radioimmunoprecipitation assay; SDS, Sodium dodecyl sulfate; SIFT, Sorting intolerant from tolerant; STR, Short tandem repeat; TBS, Tris buffered saline; WT, Wild type

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https://doi.org/10.1016/j.gene.2018.06.100
Received 27 April 2018; Received in revised form 12 June 2018; Accepted 27 June 2018
Available online 28 June 2018
0378-1119/

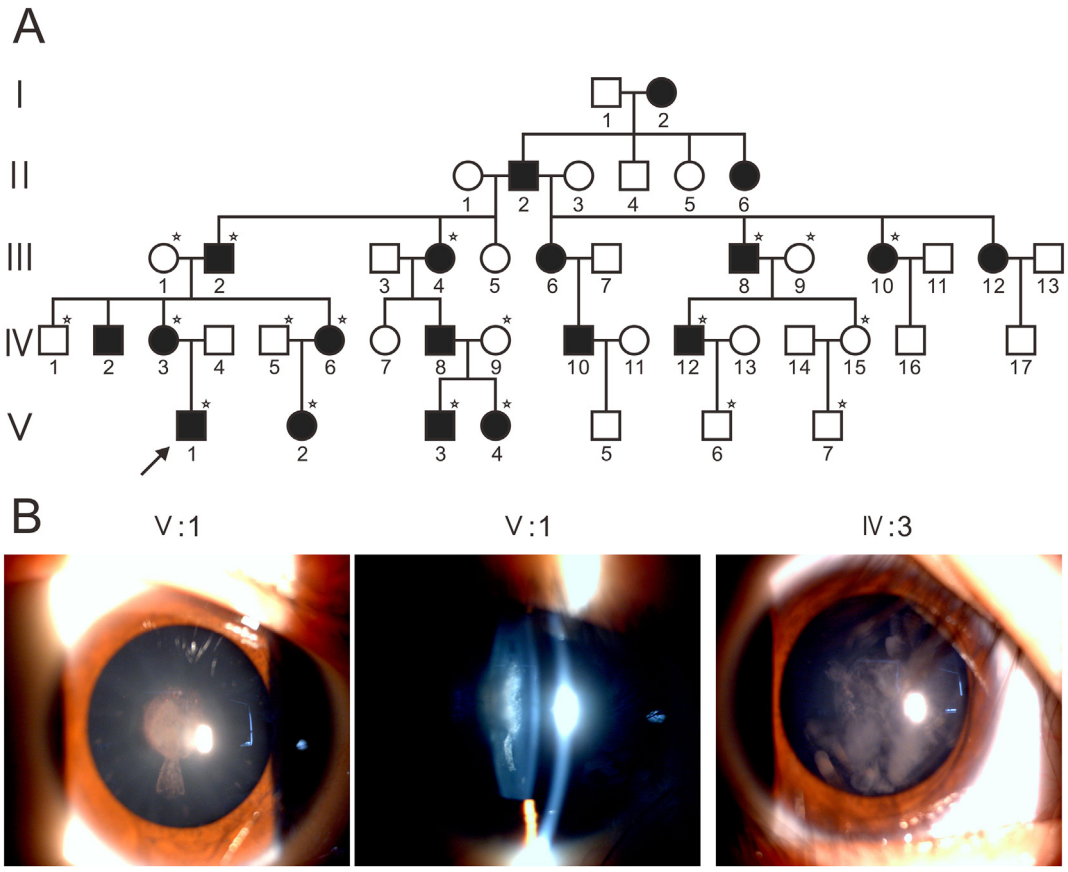

Fig. 1. Pedigree and phenotype of the family with CC.
A: Pedigree of the family with CC. Filled symbols represent affected individuals with CC; open symbols represent individuals with a normal lens phenotype. Circles and squares indicate females and males, respectively. The proband is marked by an arrow, asterisks indicate with DNA available. B: Phenotype of affected individuals. Lens pictures from the proband (V1; left and middle) and his mother (IV3; right) show the opacity located in the lens nucleus, and that the cortex was also involved with age.

pathogenic mechanism at the cellular level.

2. Subjects and methods

2.1. Clinical evaluation

A five-generation Chinese family with autosomal dominant CC was recruited at the Fourth Affiliated Hospital of China Medical University. Clinical information and blood specimens were obtained from 19 family members, including 11 patients with CC (Fig. 1A). All participants underwent ophthalmic examination, including visual acuity assessment, slit-lamp examination, and intraocular pressure measurement. In addition, a total of 120 unrelated control subjects with no family history of ophthalmic diseases were recruited. This study was approved by the China Medical University Institutional Review Board, and it was conducted in accordance with the principles of the Declaration of Helsinki, with all participants providing written informed consent.

2.2. Whole exome sequencing

Individuals III-8 and IV-6 were chosen for whole exome sequencing using the Illumina sequencing platform (WuXi NextCODE, Shanghai, China). Variants were initially annotated using wANNOVAR (http://wannovar.wglab.org/), the respective minor allele frequencies were assessed in the dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP), 1000genomes (http://www.1000genomes.org/), Exome Variant Server (http://evs.gs.washington.edu/EVS/), and Exome Aggregation Consortium database (http://exac.broadinstitute.org/), before visualization using the Integrative Genomics Viewer to facilitate the detection of false variants. Heterozygous variants with minor allele frequencies > 0.01 were filtered out. Missense or nonsense variants, indels overlapping exonic regions, and variants ± 2 bases around intron-exon junctions were defined as coding variants.

2.3. Sanger sequencing

Genomic DNA was extracted from peripheral venous blood using the Universal Genomic DNA Extraction Kit (TaKaRa, Japan) following the manufacturer's protocol. To validate coding variants screened by whole exome sequencing, Sanger sequencing was performed in three affected family members, suspected variants were then further validated by Sanger sequencing of all available family members, and variation in the *CRYGS* gene was confirmed by restriction fragment length polymorphism (RFLP) analysis in 120 unrelated normal controls.

2.4. Bioinformatics analysis

The γS-crystallin sequences were obtained from the NCBI Gene Database. Online tools PolyPhen2, SIFT, Mutation Taster, PROVEAN, and FATHMM were used to predict the pathogenicity of the variants. The hydrophobic properties of wild and mutant γS-crystallin were analyzed by ProtScale (https://web.expasy.org/protscale/). Three-dimensional models of wild and mutant γS-crystallin were generated using the I-TASSER online server (http://zhanglab.ccmb.med.umich.edu/I-TASSER/). Illustrations were prepared using PyMOL (http://www.pymol.org/).