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A novel *MIP* mutation in familial congenital nuclear cataracts

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ABSTRACT

We screened 60 known genes which are involved in inherited cataract in a pregnant woman with a four-generation family history of autosomal dominant congenital nuclear cataract through next-generation sequencing (NGS) and identified a heterozygous mutation, c.508dupC (p.L170fs), in the major intrinsic protein (MIP) gene. This mutation results in a frame-shift in MIP and has not been previously reported. The correlation of the mutation with disease was validated by Sanger sequencing of DNA from the other affected or unaffected members of the family. Therefore, our data expand the mutation spectrum of MIP mutation, and suggest that NGS is an accurate, rapid, and cost-effective method in the genetic diagnosis of congenital nuclear cataract.

1. Introduction

Congenital cataracts are a clinical disorder of opacity of the crystalline lens, usually presenting at birth or during infancy, childhood or adolescence [Zhou et al., 2013]. They are the main cause of blindness in children all over the world, and approximately one-quarter to one-third of congenital cataract cases are inherited. To date, at least 60 genes are involved in genetic congenital cataracts and this number continues to increase. These genes – including crystalline genes, lens-specific connexin genes, aquaporin, cytoskeletal structural protein genes, and developmental regulator genes– play an important role in the development of lenses at an early stage of fetal development and evolve throughout life to maintain the structure and function of the lenses [Santana and Waiswo, 2011].

Nuclear cataracts, the most common type of hereditary congenital cataract, are characterized by the opacification limited to the embryonic and/or fetal nuclei of the lens [Hejtmancik, 2008; Huang and He, 2010]. Congenital nuclear cataracts are related to at least 17 genes and can be inherited by autosomal dominant (AD), autosomal recessive (AR) or X-linked transmission. AD is the most frequent mode of inheritance with higher penetrance [Kon et al., 2013; Bermejo and Martinez-Frias, 1998]. Moreover, different mutations can cause similar cataract patterns and the same mutation can lead to different phenotypes. Because of the extensive genetic and phenotypic heterogeneity of congenital nuclear cataracts, an accurate molecular diagnosis based on Sanger sequencing is time-consuming and costly. Hence, next-generation sequencing (NGS) technology provides an advanced method for detecting the pathogenetic mutation in genetic congenital nuclear cataracts.

In this study, four generations of a Chinese family with congenital nuclear cataracts were examined. NGS was performed to detect 60 genes which were associated with congenital cataracts. A novel, heterozygous duplication mutation in the *MIP* gene was detected in this family. This seems to be the first report of a duplication mutation in *MIP* causing nuclear cataracts in a Chinese population.

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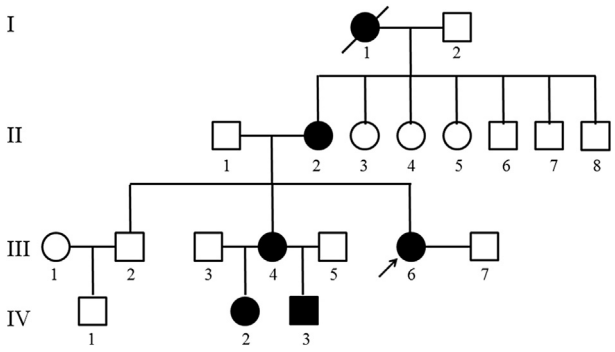

Fig. 1. Pedigree of the family with autosomal dominant congenital nuclear cataracts. Solid boxes and circles indicate affected individuals. The proband is marked with a black arrow.

2. Methods

2.1. Patient identification

A four-generation Chinese family with congenital nuclear cataracts was examined at the Henan Provincial People's Hospital, Henan, China. There were six affected (five female and one male) and 14 unaffected (four female and 10 male) people in the family. The pedigree of the family is shown in Fig. 1. The proband was a 26-year-old woman (III-6) who came to hospital for genetic counseling. She was diagnosed with a nuclear cataract months after birth and received surgery at age 4. Clinical examinations showed that she had poor eyesight. This study was approved by the Ethics Committee of Henan Provincial People's Hospital and all subjects signed the informed consent form.

2.2. Next-generation sequencing

Genomic DNA from the family members was extracted from peripheral blood lymphocytes using the Qiagen genomic DNA isolation kit (Qiagen, Hilden, Germany). The DNA sample of the proband (III-6) was quantified by agarose gel electrophoresis and NanoDrop (Thermo). Libraries were prepared following standard Illumina protocol. In sum, 3 mg of genomic DNA was fragmented by nebulization. Then the DNA was repaired and an adenine was ligated to the 3′ end of the fragments. After that, Illumina adapters were ligated. Next, the 350–400 bp product was selected for further PCR amplification, and each DNA fragment was tagged with a unique index. At the end, the final product was validated using the Agilent Bioanalyzer.

The amplified DNA was captured using biotinylated oligo-probes with a disease-related gene panel (MyGenostics GenCap Enrichment technologies). The capture experiment was conducted according to the manufacturer's protocol. As such, 1 μg DNA library was mixed with Buffer BL and a GenCap gene panel probe (MyGenostics, Beijing, China) to pre-hybridize. Then pre-warmed buffer HY (MyGenostics, Beijing, China) was added to the mixture to hybridize for 22 h. MyOne beads (Life Technology) were prepared and bonded with the target DNA which was eluted with a buffer and amplified sequentially. The PCR product was purified using SPRI beads (Beckman Coulter) according to the manufacturer's protocol. The enriched libraries were sequenced on an Illumina HiSeq 2000 sequencer for a paired-end read of 100 bp.

2.3. Bioinformatics analysis

Clean reads were aligned to each human reference genome using the BWA program and quality scores were recalibrated and realigned to reference using GATK software. Duplicated reads were removed using Sequence Alignment/Map tools (SAMtools)3, and only unique mapping reads were used for variation detection. SNVs were detected and genotyped with the GATK UnifiedGenotyper and indels were detected with GATK Indel GenotyperV2. Annotation of the variant was performed by an in-house bioinformatics tool with RefSeq (hg19, from UCSC) and UCSC annotation. The SNPs/indels were filtered if they showed up with >5% frequency in several databases such as dbSNP138, 1000 Genomes, and an in-house Asia database. The probable pathogenic mutations were then predicted according to the SIFT, PolyPhen and MutationTaster.

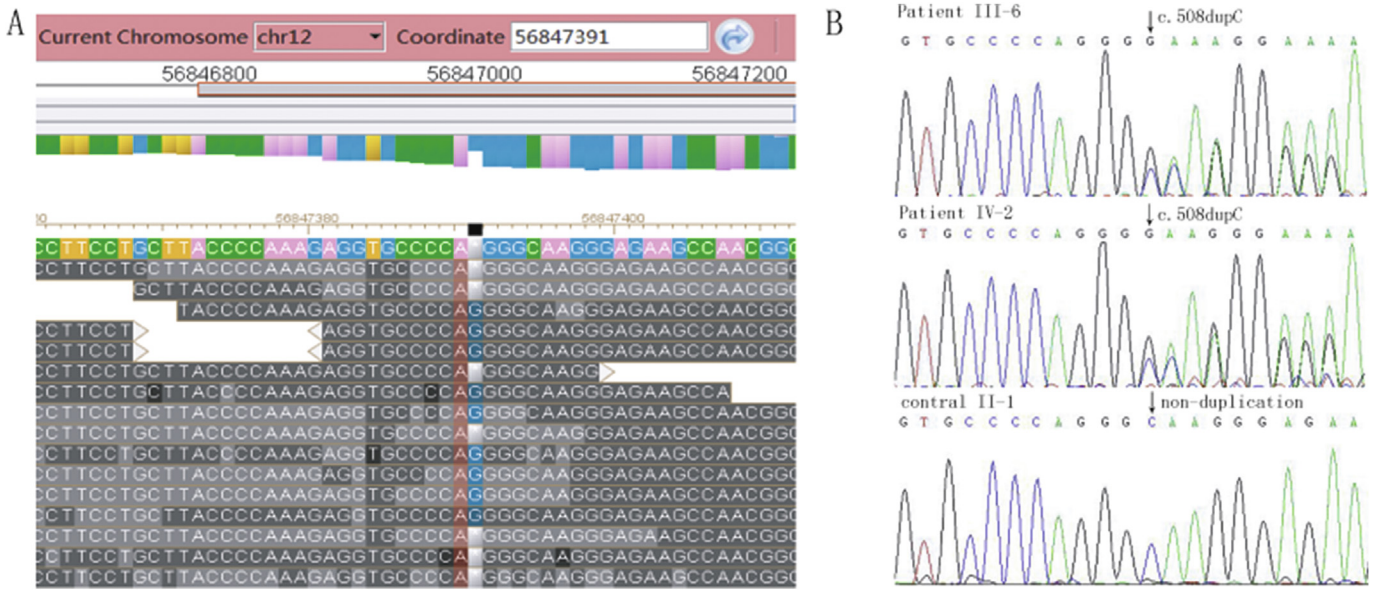

Fig. 2. Identification of mutations in the *MIP* gene. (A) The results of NGS showed a heterozygous c.508dupC (p.L170fs) of the proband's *MIP* gene. (B) Electropherogram analysis of *MIP* in the patient III-6 (proband) and IV-2 showing heterozygous c.508dupC (p.L170fs) of the *MIP* gene. Note that the mutation was not detected in unaffected family member II-2.