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RAPID COMMUNICATION

Variations in *EXD3* caused congenital cataracts in three Chinese families

Congenital cataract is a clinically and genetically heterogeneous disease characterized by any opacity of the lens presenting at birth or in the first year of life, with an incidence of 1–6/10,000 live births in developed countries and 5–15/10,000 live births in developing countries. Congenital cataract accounts for 7.4%–15.5% of all childhood blindness, and it occurs as either a syndromic disease or an isolated (non-syndromic) disease with or without other ocular malformations, such as microcornea, microphthalmia, persistent fetal vascularization, glaucoma, or retinal dystrophies. Whereas autosomal dominant inheritance is most common, autosomal recessive and X-linked inheritance have also been reported, indicating a degree of genetic heterogeneity in congenital cataract.[1,2] To date, various cataract-associated loci have been detected, with over 453 genes associated with cataracts reported prior to October 1, 2021 (http://cat-map.wustl.edu/). The pathogenic variation detection rate in familial cases was approximately 75%, indicating that the genetic basis for approximately one-fourth of familial patients is still unknown.[1] In recent years, there has been a shift from traditional genetic tests (e.g., Sanger sequencing) to genomic tests (comparative genomic hybridization (CGH)), gene panels, and next-generation sequencing (NGS) technologies) for many diseases with complex inheritance and genotypes, especially rare Mendelian diseases. NGS technologies, such as whole-exome sequencing (WES) and whole-genome sequencing (WGS), are important for identifying pathogenic variations, especially for discovering new pathogenic genes in Mendelian diseases with genetic heterogeneity. With the development of NGS technologies, more disease-causing genes associated with congenital cataract will be discovered. In this study, we identified a novel association between a missense variation in *EXD3* and congenital cataract in three Chinese families by combining NGS technologies and linkage analysis.

Three families with autosomal dominant congenital cataract lacking mutations in known cataract-related genes were recruited via targeted panel sequencing at the Fourth Affiliated Hospital of China Medical University. Clinical information and blood specimens were obtained from 42 total family members, including 25 affected individuals. Family 1 has three generations of 27 individuals, including 13 affected individuals, six unaffected individuals, and eight spouses (Fig. S1). All individuals were examined, and all affected members had evidence of bilateral posterior polar cataract. In addition to posterior polar cataract, the affected twins had snowflake-like opacity in the lens nucleus and spot-like opacity in the cortical area (Fig. 1A–C). Family 2 has five generations of 17 affected individuals, 9 unaffected individuals, and 10 spouses (Fig. S2). All individuals were examined, and all affected family members had bilateral posterior polar cataract (Fig. 1D, E). Family 3 has three generations of eight members, with four affected individuals, one unaffected individual, and three spouses (Fig. S3). All family members were examined, and bilateral progressive posterior subcapsular opacity was seen in all affected individuals (Fig. 1F).

To investigate the genetic basis underlying congenital cataract, WES/WGS was performed for the three Chinese families (Table S1). Multipoint parametric linkage analysis using WES/WGS genotypes (six individuals in Family 1 and five members of Family 2) was performed to narrow down the genomic area with possible linkage with the congenital cataract. Family 1 and Family 2 each showed two linkage peaks: 9q34.11–q34.3 and 21p11.1–q21.2 in Family 1, and 1q41 and 9q34.13–q34.3 in Family 2. Maximal logarithm of the odds (LOD) scores of 3.68 and 3.61 were obtained by two-point linkage for marker STR6.3 in Family 1 and STR14.4 in Family 2 at $\theta = 0.0$, showing definitive evidence of linkage to 9q34.11–q34.3 (Table S2, S3). Furthermore, haplotype analysis in two recombinants (II 5 of Family 1 and IV 4 of Family 2) identified a critical region within an interval between STR14.14 and the chromosome terminus in Family 1 and between STR14.2 and the chromosome

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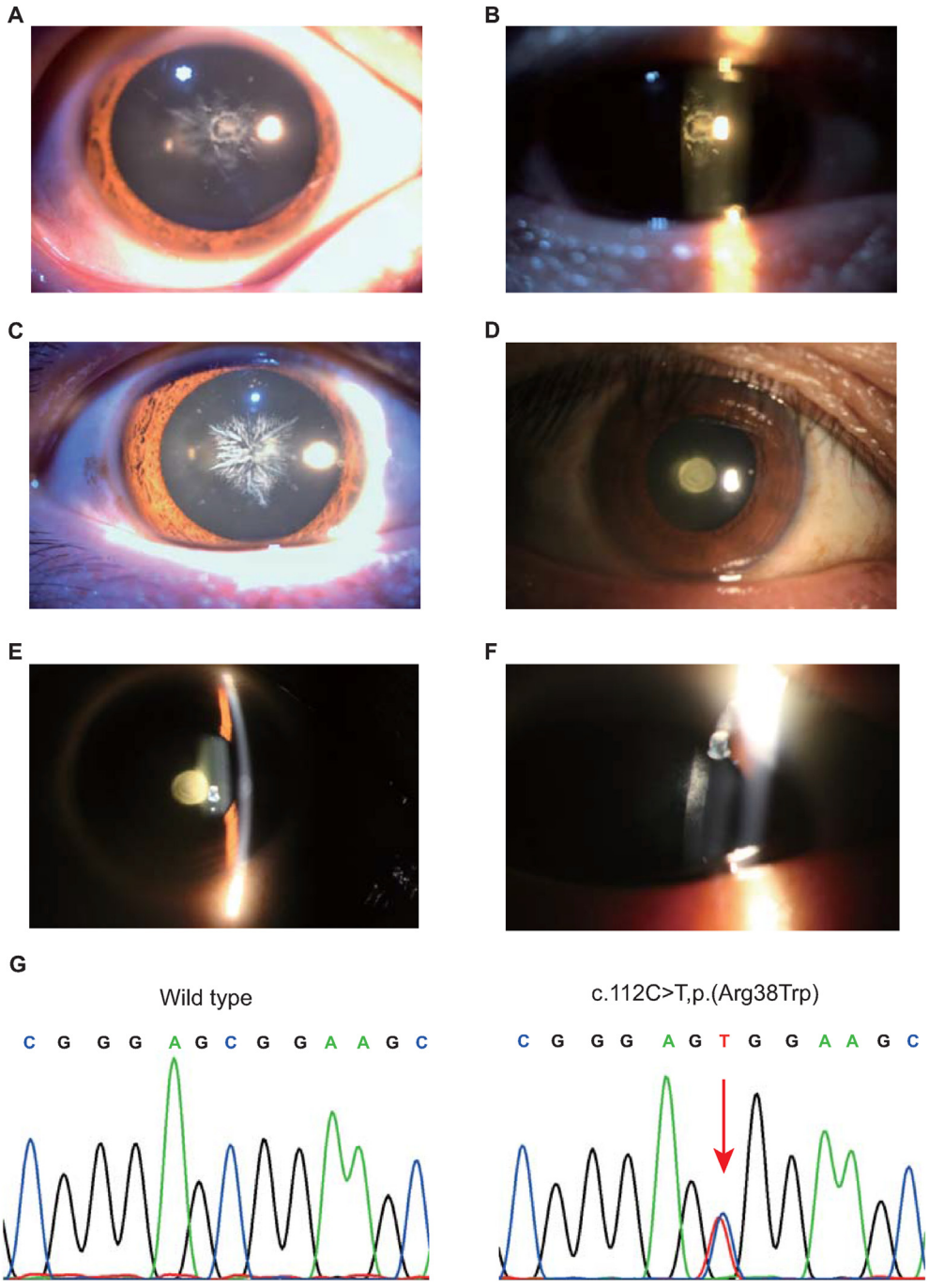

Figure 1 Clinical manifestations of the families with congenital cataract and sequence chromatogram of *EXD3*. **(A—C)** Lens pictures from the proband of Family 1 show opacity in the posterior pole of the lens, snowflake-like opacity in the lens nucleus, and spot-like opacity in the cortical area. **(D, E)** Lens picture from the proband of Family 2 showing opacity in the posterior pole of the lens. **(F)** Lens picture from the proband of Family 3 showing posterior subcapsular opacity of the lens. **(G)** Sequence chromatogram showing heterozygous *EXD3* c.112C > T, p.(Arg38Trp) in an affected individual. The varied base is marked by a red arrow.

terminus in Family 2, representing a linked genomic extent of 3.4 Mb (chr9:137,460,649—140,841,365) in Family 1 and 5.7 Mb (chr9:135,091,657—140,841,365) in Family 2. Moreover, additional linkage peaks at 21p11.1—q21.2 in Family 1 and 1q41 in Family 2 were excluded by haplotype analysis of all available family members with no shared haplotype observed at these loci (Fig. S4, 5). Next, the missense mutation c.112C > T, p.(Arg38Trp) in the *EXD3* locus on 9q34.3 was identified in all affected individuals and absent in all normal individuals with available DNA from both Family 1 and Family 2 (Fig. 1G). Interestingly, c.112C > T, p.(Arg38Trp) in *EXD3* was also found in the affected individuals in Family 3 (Fig. 1G). Except for *EXD3* c.112C > T, p.(Arg38Trp), no other suspicious copy number variants

(CNVs), single nucleotide variants (SNVs), insertion/deletion polymorphisms (InDels), or structural variants (SVs) were found at 9q34.3 or on other chromosome regions within these families. The patients in Families 1, 2, and 3 were found to have different haplotypes consisting of alleles at microsatellite markers close to the *EXD3* gene, suggesting that the *EXD3* c.112C > T, p.(Arg38Trp) mutation arose independently without a common ancient founder (Fig. S6).

EXD3 encodes the exonuclease mut-7 homolog, which belongs to the mut-7 family and consists of a 3'—5' exonuclease domain and a Mut7-C RNase domain. Conservation analysis showed that the 38th amino acid of the exonuclease mut-7 homolog is highly conserved in primate species, including humans, chimpanzees, rhesus monkeys, marmosets, baboons, macaques, and orangutans (Fig. S7). The c.112C > T, p.(Arg38Trp) variant was annotated in a heterozygous state in three individuals (two Europeans and one South Asian) in the gnomAD V2.1.1 database and one individual (European) in the gnomAD V3.1.2 database, corresponding to allele frequencies of 0.0000196% and 0.0000066%, respectively. It was not annotated in East Asian individuals in any public genomic databases, including 1000Genomes, dbSNP, Exome Variant Server, Exome Aggregation Consortium, and our inhouse databases.

Caenorhabditis elegans Mut-7 is a human protein ortholog involved in Werner syndrome (WRN), a rare autosomal recessive segmental progeroid syndrome characterized by scleroderma-like skin changes, including bilateral cataracts.[3] Little is known about the function of the exonuclease Mut-7 homolog, which is presumed by structure similarity analysis to possess molecular functions, such as nucleic acid binding, 3'—5' exoribonuclease activity, hydrolase activity, and metal ion binding, possibly indicating its involvement in nucleobase-containing compound metabolic processes and nucleic acid phosphodiester bond hydrolysis. The protein is also required for 3'-end trimming of AGO1-bound microRNAs (miRNAs). MiRNAs are associated with lens development. Let 7b, let 7c, miR29a, miR29c, miR126, and miR551b are all increased or decreased in abundance in lens epithelial cells/lenses during late embryonic and postnatal development and in cataract. Among them, miR29a, miR29c, and miR126 were dramatically decreased in cataractous lens epithelial cells from Shumiya Cataract Rats.[4] Mutations in miR184 are responsible for congenital cataract and corneal abnormalities or EDICT syndrome, which is characterized by endothelial dystrophy, iris hypoplasia, congenital cataract, and stromal thinning. Knockout of *Bin3*, the target gene of miR184, can induce cataract in mice soon after birth. MiR204 is required for lens and retinal development via Meis2 targeting inhibiting the expression of Pax6, which is a causative gene of congenital cataract.[5] Additional studies will be needed to explore the role of exonuclease Mut-7 homolog in 3'-end trimming of AGO1-bound miRNAs, especially with regard to turnover and abundance of miRNAs involved in lens development.

We identified *EXD3* c.112C > T, p.(Arg38Trp) as a novel genetic cause of congenital cataract in three unrelated Chinese families by combining WGS/WES and linkage analysis. Our observations expand the genetic spectrum of congenital cataract and suggest that genetic linkage analysis by WGS/WES genotypes is helpful for identifying novel genes in Mendelian diseases with high genetic heterogeneity.

Ethics declaration

The study was approved by the ethics committee of China Medical University and performed in accordance with the Declaration of Helsinki, with all participants providing written informed consent.

Conflict of interests

The authors declare no conflict of interests.

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Data availability

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gendis.2022.05.025.

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Lihua Cao [a,b,*], Yunji Leng [b], Xinmiao Nie [a], Shuyang Zhao [a], Tianxiao Zhang [c,**]

[a] *College of Kinesiology, Shenyang Sport University, Shenyang, Liaoning 110102, China*
[b] *The Research Center for Medical Genomics, China Medical University, Shenyang, Liaoning 110122, China*
[c] *Department of Ophthalmology, The Fourth Affiliated Hospital of China Medical University, Key Laboratory of Lens Research Liaoning Province, Eye Hospital of China Medical University, Shenyang, Liaoning 110033, China*

*Corresponding author. College of Kinesiology, Shenyang Sport University, Shenyang, Liaoning 110102, China.

**Corresponding author. Department of Ophthalmology, The Fourth Affiliated Hospital of China Medical University, Shenyang, Liaoning 110033, China.
E-mail addresses: lhcao@cmu.edu.cn (L. Cao), txzhang@cmu.edu.cn (T. Zhang)

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