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A novel GJA3 mutation causing autosomal dominant congenital perinuclear cataracts

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Abstract

Objective To identify the cause of congenital perinuclear cataracts in a Chinese family and its underlying mechanism.

Methods Family history and clinical data were recorded, and candidate genes were amplified by polymerase chain reaction (PCR) and screened for mutations using direct bidirectional DNA sequencing. The *GJA3* gene was acquired from a human lens cDNA library, and the *GJA3* mutant was generated by PCR-based site-directed mutagenesis. Connexin localization and gap junction formation were assessed by fluorescence microscopy, and hemichannel functions were analyzed by dye uptake assay.

Results Gene sequencing showed one base pair substitution at position 671 of the GJA3 gene's coding region (c.671A > G), leading to the conversion of the 224th amino acid of the Connexin 46 protein (Cx46), expressed by the *GJA3* gene, from histidine to arginine (p.H224R). In stable transfectants, the formation of gap junctions was detected in both wild-type Cx46 (wtCx46) and mutant Cx46H224R transfected HeLa cells, where the Cx46H224R transfected cells exhibited a much higher Propidium Iodide (PI) loading speed than the wtCx46 cells.

Conclusion This study was the first to identify the c. 671A > G mutation of the *GJA3* gene (p.H224R in Cx46), which leads to the generation of congenital perinuclear cataracts. We suggest that the H224R missense mutation of Cx46 may cause alterations in the activity of the hemichannel, leading to cataract development.

Keywords Congenital cataract, Connexin46, *GJA3*, Hemichannels

Introduction

A congenital cataract (CC) constitutes one of the predominant etiologies of visual debilitation and blindness in pediatric populations, with an incidence approximated to be within the range of 0.01%—0.06% of live births [1]. Most congenital cataracts require surgical treatment [2]. More than 22% of CC cases in infants can be attributed to an inherited genetic mutation, the most common of which is autosomal-dominant inheritance [3].

Autosomal dominant congenital cataracts (ADCCs) exhibit a high degree of clinical and genetic heterogeneity. Upwards of 40 causative genes have been correlated with ADCCs, and the quantity of identified genes is undergoing continual augmentation [4]. Approximately one-half of the identified ADCCs are caused by crystallin genes, while another one-quarter are connexin genes [5, 6].

The lens is comprised of two cell types: epithelial cells that form a single layer along the anterior surface and fiber cells that form the bulk of the organ. Three connexins have been identified in the lens: Connexin43 (Cx43), Connexin46 (Cx46), and Connexin50 (Cx50). Cx43 is expressed in lens epithelial cells, while Cx46 and Cx50

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are the two most abundant connexins in lens fiber cells [7, 8]. To date, mutations of the genes encoding Cx46 and Cx50 (*GJA3* and *GJA8*) have been identified in members of human families with ADCCs of various different phenotypes [9]. Since Mackay D discovered the c.fs380 and p.N63S mutations of the *GJA3* gene for the first time in 1999 [10], at least 53 mutations in *GJA3* in families with ADCCs have been reported [11].

Connexins, serving as the core molecular components, self-assemble into pairs of hemichannels called connexons. These connexons merge to create functional gap junctions, with each connexon consisting of six connexin molecules [12]. In the mature lens, after the organelles are eliminated, homeostasis is maintained by factors supplied through gap junctions and the lens microcirculation system [13]. Gap junctions facilitate intercellular communication by linking the cytoplasm of adjacent cells. They play a crucial role in the normal transport of signal molecules, nutrients, and metabolites between cells, a process essential for maintaining lens transparency [14, 15]. However, several studies suggest that not only gap junctions but also connexin hemichannels play an important role in lens homeostasis and transparency [16–18].

In this study, a novel *GJA3* mutation, the c.671A > G (p.H224R in Cx46), was identified for the first time to cause congenital perinuclear cataracts. Moreover, the mechanism of cataract formation in patients harboring the Cx46H224R mutation was examined in our experiment. The functional study showed that this mutation increased the permeability of hemichannels, which may provide a molecular mechanism in CC formation.

Methods

Ethics statement

This study was conducted in accordance with the ethical guidelines of the Declaration of Helsinki and was approved by the Institutional Review Board of the Second Affiliated Hospital of the Zhejiang University School of Medicine in Hangzhou, China. Written informed consent was obtained from all participants or their pediatric legal guardians after providing a detailed explanation of the study goals.

Clinical evaluation and examination

A Chinese family affected by ADCCs was recruited at the Second Affiliated Hospital of Zhejiang University (Hangzhou, China). Medical history of the family was recorded, clinical examinations were conducted, and phenotypes were documented using slit-lamp photography.

Genomic DNA preparation

Peripheral blood (5 ml) was collected from the proband and family members into ethylenediaminetetraacetic acid (EDTA) tubes. Genomic DNA was extracted from peripheral blood leukocytes using the the blood DNA extraction kit(Simgen®). The process included cell lysis, protein digestion with proteinase K, DNA binding to the column, washing to remove impurities, and elution with buffer to obtain purified DNA.

Mutation screening

Mutation screening was performed using the candidate gene analysis approach after amplifying candidate genes associated with the family's phenotype via PCR. Gene-specific PCR primers flanking each exon and intron–exon junction were designed for genes related to perinuclear cataract, including *CRYAA*, *CRYAB*, *CRYBA3/A1*, *CRYBB1*, *CRYBB2*, *CRYGC*, *CRYGD*, *CRYGS*, *GJA3*, *GJA8*, and *MIP*. Details of the PCR and sequencing strategy are described in our previous study [19]. PCR products were sequenced using an ABI3730 Automated Sequencer (PE Biosystems, Foster City, CA), and the data were compared with sequences from the NCBI GenBank (GJA3: NM_021954.4). Sequencing of GJA3 gene was taken on all the family members. Negative controls included genomic DNA from 100 healthy Chinese individuals. The *GJA3* sequence from Homo sapiens was obtained from the NCBI and UCSC websites. Additionally, the online bioinformatics software SIFT and PROVEAN (http://provean.jcvi.org/index.php) were used to predict whether the amino acid substitution p.H224R in Cx46 could have an impact on the biological function.

Plasmids, cell culture, and transfections

The wild-type Cx46 (wtCx46) -EGFP plasmid was constructed using the *GJA3* cDNA sequence, which was amplified by PCR from a human lens cDNA library (provided by Graeme Wistow from the NIH as a gift) and cloned into pEGFP-N1. The mutant Cx46H224R-EGFP plasmid was generated using the QuikChange site-directed mutagenesis kit (Stratagene, UK). Human epithelial carcinoma cells (HeLa, ATCC) were cultured in DMEM (Gibco, Carlsbad, CA, USA) supplemented with 10% Fetal Bovine Serum (FBS). Transient transfections of HeLa cells were performed using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. HeLa cell monoclonal lines stably expressing human wtCx46 and mutant Cx46H224R were identified based on their resistance to geneticin (G418; 1 mg/mL). G418 (0.5 mg/mL) was used during cell culture to maintain the stability of target protein expressions. We conducted three rounds of transient transfection experiments, followed by selection procedures to successfully obtain three stable transfected clones. Each group conducted the following experiments

to eliminate potential inaccuracies in the data that could be associated with the use of a single clone.

Fluorescence imaging

Fluorescence images were captured when the cultivated cells reached 80% to 90% confluence. Stably transfected cells expressing GFP-tagged connexins were plated on four-well chamber slides (Lab Tek; Nalge Nunc International, Naperville, IL, USA). Distribution of Cx46 protein and gap junction formation were monitored based on tagged GFP signaling using a confocal laser scanning microscope (Zeiss LSM 510, Zurich, Switzerland).

Dye uptake experiments

Two cell populations (wtCx46 and Cx46H224R) were seeded separately into 35-mm collagen-coated glass-bottom culture dishes at 50% confluence for investigating hemichannel function. The cells were washed twice in Ca^{2+}-free HBSS (Gibco), and dye loading tests were conducted by incubating each set of cells in Ca^{2+}-free HBSS (Gibco), HBSS containing 1.2 mmol/L Ca^{2+}, or HBSS containing a specific hemichannel blocker, flufenamic acid (FFA, 300 mmol/L, Sigma, Saint Louis, MO, USA) [20]. The incubation temperature of cells used was 37℃.

Dynamic detection of stained cells was performed using an inverted fluorescent microscope (IX71, Olympus, Tokyo, Japan) for 30 min after adding 0.1% propidium iodide (PI, Sigma) to all solutions. We selected adherent eGFP-positive cells for statistical analysis. The cells, characterized by loss of normal shape or detachment from neighboring cells, were excluded from analysis. The total numbers of living cells and dye-uptake live cells were counted in thirty visual fields at the 4-min time point. Dye uptake experiments were repeated three times. Results are presented as percentages of uptake cells and expressed as means ± standard deviations (SDs). Statistical analysis was conducted using the Two-Way ANOVA.

Results

Clinical evaluation

We investigated a three-generation Chinese family with congenital perinuclear cataracts (Fig. 1A). Lens opacity was bilateral in all affected individuals. The proband, a 35-year-old, exhibited perinuclear granular opacities in the lens (Fig. 1B, C). Her clinical presentation closely resembled that of her brother (II:5). The affected member I:1, the mother of the proband, presented with perinuclear granular opacities accompanied by mild cortical and nuclear opacities. Her 7-year-old boy (III:3) showed mild granular opacities in the perinucleus. Prior to surgery, affected members had visual acuity ranging from 20/200 to 20/50. Following surgery, all patients achieved distance visual acuity of 20/25 to 20/20. Details of patients information is shown in

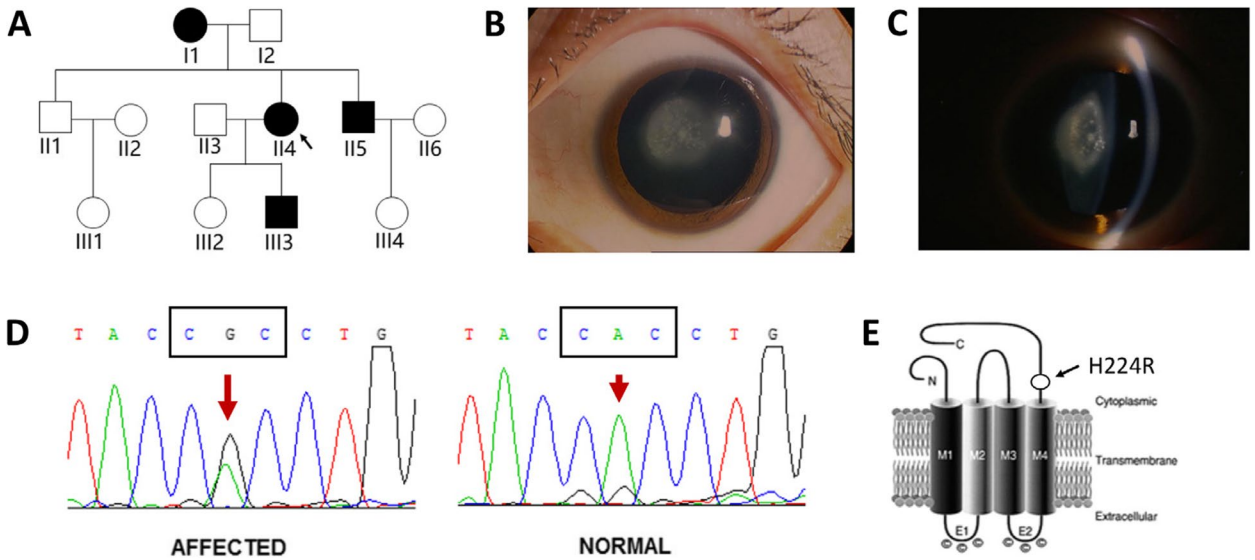

Fig. 1 Family data, cataract phenotype, and mutation sequencing of Cx46. **A** Pedigree of the proband, indicating autosomal dominant inheritance. Black symbols denote individuals who have been clinically diagnosed and genetically confirmed to have congenital cataract. The arrow indicates the proband. **B** Diffuse illumination of the left eye of the proband (II4: F, 35 years old). **C** Slit lamp photograph of the lens of the proband showing perinuclear granular opacities. **D** Partial DNA sequence of *GJA3* from one affected individual and one unaffected individual. The black box indicates the codon of the mutant amino acid. The red arrow indicates a heterozygous mutation (c.671A > G). **E** Topology of connexin. The black arrow indicates that H224 is located in the cytoplasmic carboxyl-terminal region of the Cx46 protein

Table 1 Ophthalmic medical information of patients with congenital cataract in the studied family

Patient	Gender	Age of diagnosis	Diagnosis	Cataract phenotype	Surgery	Preoperative corrected visual acuity		Postoperative corrected visual acuity	
						OD	OS	OD	OS
I1	Female	62	Congenital cataract and age-related cataract	Perinuclear opacities with mild cortical and nuclear opacities	Yes	20/200	20/200	20/25	20/25
II4	Female	35	Congenital cataract	Perinuclear cataract	Yes	20/100	20/125	20/20	20/25
II5	Male	33	Congenital cataract	Perinuclear cataract	Yes	20/100	20/80	20/20	20/20
III3	Male	7	Congenital cataract	Perinuclear cataract	Yes	20/50	20/50	20/20	20/20

Table 1. Besides cataracts, there was no family history of ocular or systemic abnormalities.

Genetic analysis

Gene sequencing revealed a conversion of adenine at base 671 of the *GJA3* gene's coding region to guanine (c.671A > G) in studied family members. This mutation resulted in the substitution of histidine with arginine (p.H224R) at the 224th amino acid position of the Cx46 protein encoded by the *GJA3* gene. This variation was present in all affected individuals (Fig. 1D) but was absent in unaffected family members and in 100 unrelated healthy Chinese Han individuals serving as controls. We presents a comparative analysis of the H224R substitution in the GJA3 gene, utilizing SIFT and PROVEAN predictions. The H224R substitution in the GJA3 gene, where histidine (H) at position 224 is replaced by arginine (R), is classified as novel. Both SIFT and PROVEAN predictions classify this substitution as harmful, with SIFT scoring it as "Damaging" (2.84) and PROVEAN deeming it "Deleterious" (−7.38), emphasizing the potential adverse effects of this novel substitution on GJA3's function.

Function analysis

Cellular distributions of the GFP-tagged wtCx46 and Cx46H224R proteins were determined based on their GFP fluorescence. In HeLa cells, both wtCx46 and Cx46H224R were localized to the plasma membrane of cultured cells, as observed in both the wild-type and mutant groups following stable transfection with wtCx46-EGFP and Cx46H224R-EGFP, respectively (Fig. 2A, B). These images primarily illustrate that the Cx46 mutation did not alter their plasma membrane localization, and indicate that the H224R mutation had no effect on Cx46 protein trafficking.

A hemichannel dye-loading assay was conducted to assess the impact of Cx46H224R on hemichannel activity. Cells were incubated in either Ca^{2+}-free HBSS (GIBCO), HBSS containing 1.2-mmol/L Ca^{2+}, or HBSS containing FFA for a 30-min dyeing process. PI dye uptake assays revealed distinct differences between wtCx46 and Cx46H224R cells. wtCx46 cells began absorbing the PI dye in the Ca^{2+}-free HBSS medium after 10 min of treatment, whereas Cx46H224R cells exhibited PI dye absorption as early as 4 min into treatment (Fig. 3A, B). We measured PI uptake at 4 min to eliminate the interference of apoptosis. Notably, after 4 min of treatment, no wtCx46 cells absorbed the PI dye during incubation in Ca^{2+}-free HBSS medium, while (76.3 ± 2.6)% of Cx46H224R cells did (Fig. 3C). This significant difference in the PI staining ratio between wtCx46 and mutant Cx46H224R cells underscores the increased PI loading speed in the mutant protein cells. Following incubation in HBSS containing 1.2 mmol/L Ca2 + for 30 min, very few wtCx46 (1.25 ± 1.86%) and Cx46H224R cells (1.01 ± 1.81%) absorbed the PI dye ($p > 0.05$). In the FFA-treated cells, only 0.48 ± 1.31% of the wtCx46 cells, 0.52 ± 1.50% of the Cx46H224R cells absorbed the PI dye ($p > 0.05$). In summary, these results demonstrate that the H224R mutation increases the activity of connexin hemichannels.

Discussion

The phenotypes of congenital cataracts are complex and variable, often overlapping, and exhibit genetic heterogeneity, making the study of their molecular genetics relatively challenging. According to previous literature reports, hereditary congenital nuclear cataracts are often associated with the GJA3/Cx4) [21–23], GJA8/Cx50 [24], MIP/AQP0 [25–27], CRYAB, CRYAA [28, 29], CRYBA3/A1 [30, 31], CRYBB2 [32], CRYGC [33, 34], and CRYGD [35, 36]. Connexins are membrane proteins that constitute the basic structure and function of gap junctions between cells [37, 38]. Cx46 plays a critical role in coupling of fiber cells. *GJA3* mutations can cause amounts of

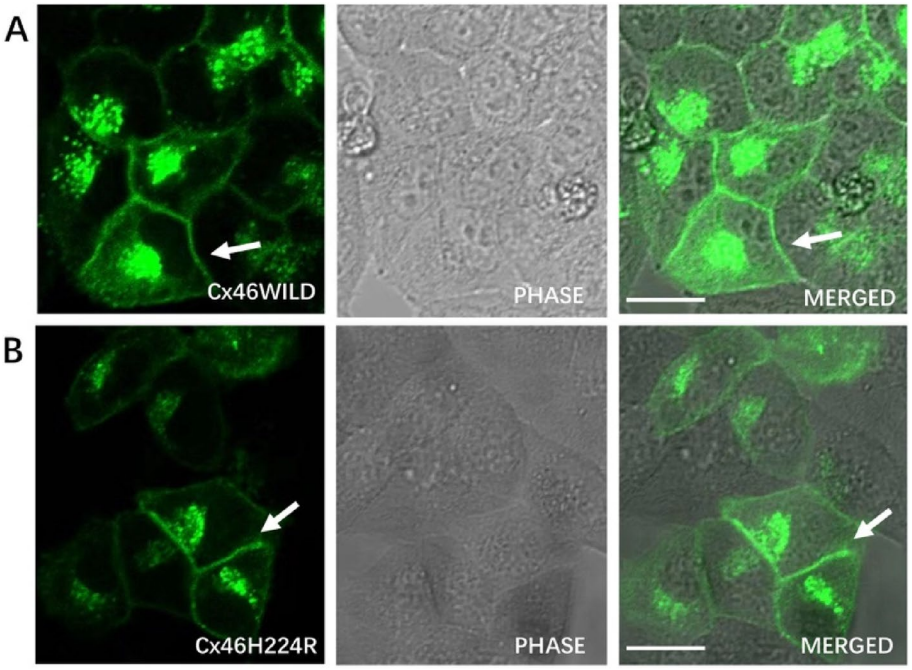

Fig. 2 Subcellular localization of wtCx46 (**A**) and Cx46H224R (**B**) in stably transfected HeLa cells. (the white arrows show the protein is located on the plasma membrane). A-B Scale bar: 20 μm

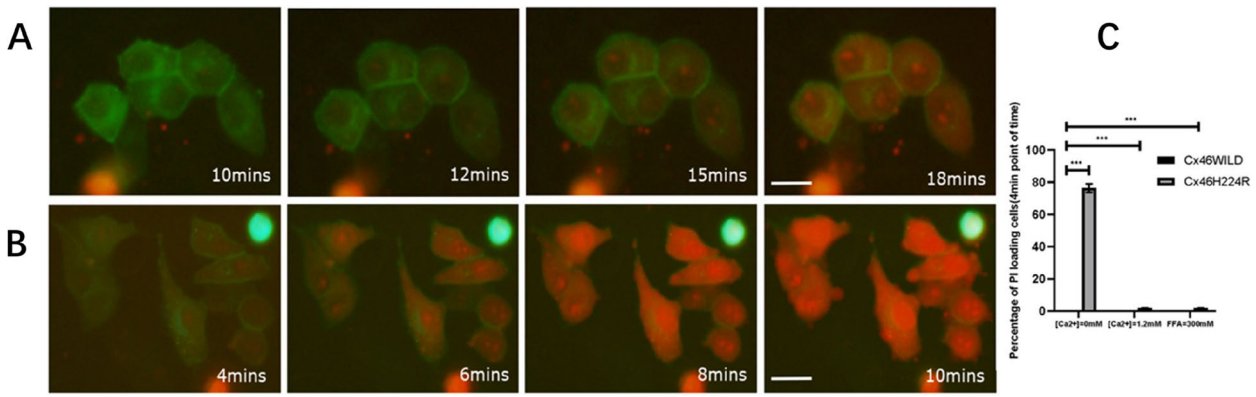

Fig. 3 PI dye uptake in HeLa cells stably transfected with wtCx46 and Cx46H224R. **A** PI dye uptake began in wtCx46 cells in Ca^{2+}-free HBSS after 10 min. **B** PI dye uptake began in Cx46H224R cells in Ca^{2+}-free HBSS after 4 min. A-B Scale bar: 20 μm. **C** Statistical analysis of the percentage of PI-loaded cells at the 4-min time point. Data are presented as mean ± SD. ($P < 0.001$, marked with ***)

congenital cataracts phenotypes, such as nuclear, perinuclear, Coppock-like, egg-like, and so on [39]. In our study, we identified a novel c.671 A > G mutation of *GJA3* in a congenital perinulear cataracts Chinese family, resulting in a missense mutation of H224R in Cx46.

Cx46 comprises two exons encoding a transmembrane protein of 435 amino acids, and all connexins are predicted to have the same topological structure. This structure includes four transmembrane helices (TM1-4) formed by highly conserved α helices, two extracellular loops (EC1 and EC2), an amino-terminal (NT) domain, a carboxyl terminus (CT), and a cytoplasmic loop (CL) [40]. Due to the different functions of each connexin domain, mutations occurring in different domains have different pathogenic mechanisms. The C-terminal of connexin is reported to be related to chemical regulation, protein phosphorylation gating, and interactions with other intracellular proteins [41, 42]. The Cx46 H224R mutation that we identified is located at the CT of the Cx46 cytoplasm, a common site of human

connexin mutations associated with diseases. In our study, Cx46H224R proteins were able to traffic in the plasma membrane of transfected HeLa cells.

Since Cx hemichannel opening can be regulated by the concentration of extracellular Ca^{2+} [43], previous studies have confirmed the hemichannel dye-loading assay as a method to quantify the effect of connexins on hemichannel activities [20, 44]. In our experiment, compared to wild-type hemichannels, the mutant hemichannels formed by Cx46H224R significantly accelerated the PI dye uptake process in a Ca^{2+}-free environment. Although the concentration of calcium in the human aqueous humor is typically 1.3 mM [45], calcium homeostasis in the central mature fibers of the lens appears to be more complex. The results suggest that the mutant hemichannels achieve an earlier open time point in a Ca2 + -free environment, indicating increased activity in the lens.

Hemichannels were once believed to function only when two such channels formed an intercellular channel (the gap junction) between closely apposed cells [46, 47]. However, not all hemichannels are destined to become components of a gap junction channel, and research has suggested that hemichannels play an important role in cellular processes, such as volume regulation [48], the influx/efflux of metabolically relevant solutes such as ATP [49], and cell death [50, 51]. Studies of cataract-linked connexin mutants suggest several ways that hemichannels contribute to the pathogenesis of cataracts [10]. Many of the cataract-associated Cx46 mutants do not form functional hemichannels (e.g., Cx46L11S, Cx46fs380) [52, 53]. Others exhibit a reduced ability to form them (e.g., Cx46G2D, Cx46D3Y, Cx46N63S) [20, 43, 52]. These mutants would lead to less than normal connexin hemichannel activity in the lens, contributing to cataracts. However, increased hemichannel activity has also been reported to be related to cataract formation. Enhanced hemichannel activity is considered to cause the loss of membrane potential, the activation of intracellular proteases, decreased metabolic activity, and even cell death [54]. Moreover, Hu reported that the *GJA3* G143R mutation increased membrane permeability mediated by Cx46 hemichannels, resulting from the enhancement of CaM-Cx46 interaction, which might be related to cataract formation [11]. Ren also suggested that the increased hemichannel function resulting from the *GJA3* G143R mutation would decrease the resistance of the cells to oxidative stress [9]. Interestingly, the *GJA3* G143R mutation caused the coppock-like cataract, which was a pulverulent disc-like opacity involving the embryonal and fetal nucleus [55]. This serves as a reminder that although the H224R mutation of *GJA3* in our study causes an enhanced activity of hemichannel similar to the *GJA3* G143 mutation, it still leads to varying phenotypes. This raises the question of whether such changes in hemichannel function caused by different mutations begin to play a role at different stages of lens development, which warrants further investigation.

In our study, hemichannels formed by the Cx46H224R mutation could uptake the PI dye in a Ca^{2+}-free solution but not in Ca^{2+} or FFA solutions. Furthermore, mutant Cx46 protein transfected cells exhibited earlier and faster PI dye uptake than wtCx46 transfected cells. Our study indicated that mutant hemichannels were functional to some extent, potentially regulated by dynamic ranges of extracellular calcium in the lens. However, increased hemichannel activity might disrupt ion exchange and lens homeostasis, contributing to the pathogenesis of cataracts.

Previous study have shown that mutant Cx50(S276F) inhibits wild-type Cx50 function in a dominant negative manner when co-transfected, highlighting the complex effects of mutant/wild-type connexin co-expression on gap junction channel function [56]. This warrants further investigation into the mechanism of this dysfunction in our follow-up experiments. Additionally, research has shown that Cx46 and Cx50 connexins co-localize at gap junction plaques and can form mixed hexamers. Compared to homogeneous hemichannels, heteromeric hemichannels change several parameters, including the threshold for activation, rate of deactivation, unitary conductance, steady-state open probability, and mean open times at negative potentials, which may change the hemichannel activity [57]. Further investigation into gap junction protein formation, utilizing advanced imaging and refined culture models, is crucial for a comprehensive understanding of their channel functions. Therefore, further research is warranted to explore the underlying mechanisms of hemichannel activity enhancement and the function of mutant gap junctions in the future.

In conclusion, we have identified a novel c.671 A > G mutation of *GJA3* (p.H224R in Cx46) associated with congenital perinuclear cataracts in a Chinese family. Our study suggests that the H224R mutation of Cx46 could increase hemichannel activity, potentially disrupting cellular homeostasis and leading to cataract formation.

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Authors' contributions
YN.Z. concept and design, made the interpretation of the information, YN.Z. and NL.L. drafted the initial manuscript and prepared figures 1-3. YN.Z., W.W., JY.L. and carried out the collection of the reported studies and performed the experiments. K.Y., YN.Z. reviewed and revised the manuscript. All authors read and approved the final manuscript.

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Data availability
We have deposited the new DNA sequences into the International Nucleotide Sequence Database Collaboration (INSDC) repository at https://www.insdc.org/. We have provided a GenBank accession number for our nucleotide sequence: BankIt2931272 p.H224R PV241679. The data that support the findings of this study are not publicly available due to their containing information that could compromise the privacy of patient but are available from the corresponding author (YN.Z.) upon reasonable request.

Declarations

Ethics approval and consent to participate
This research followed the tenets of the Declaration of Helsinki, written informed consent was obtained from all the subjects after explanation of the nature and possible consequences of the study. This study was approved by the Institutional Review Board of the Second Affiliated Hospital of the Zhejiang University School of Medicine in Hangzhou, China.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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References

1. Shiels A, Hejtmancik JF. Inherited cataracts: Genetic mechanisms and pathways new and old. Exp Eye Res. 2021;209:108662.
2. Chen X, Xu J, Chen X, Yao K. Cataract: Advances in surgery and whether surgery remains the only treatment in the future. Adv Ophthalmol Pract Res. 2021;1:100008.
3. Sheeladevi S, Lawrenson JG, Fielder AR, Suttle CM. Global prevalence of childhood cataract: a systematic review. Eye (Lond). 2016;30(9):1160–9.
4. Berry V, Ionides A, Pontikos N, et al. The genetic landscape of crystallins in congenital cataract. Orphanet J Rare Dis. 2020;15(1):333.
5. Berthoud VM, Ngezahayo A. Focus on lens connexins. BMC Cell Biol. 2017;18(Suppl 1):6.
6. Mathias RT, White TW, Gong X. Lens gap junctions in growth, differentiation, and homeostasis. Physiol Rev. 2010;90:179–206.
7. Paul DL, Ebihara L, Takemoto LJ, Swenson KI, Goodenough DA. Connexin46, a novel lens gap junction protein, induces voltage-gated currents in the nonjunctional plasma membrane of Xenopus oocytes. J Cell Biol. 1991;115:1077–89.
8. White TW, Bruzzone R, Goodenough DA, Paul DL. Mouse Cx50, a functional member of the connexin family of gap junction proteins, is the lens fiber protein MP70. Mol Biol Cell. 1992;3:711–20.
9. Qiu Y, Zheng J, Chen S, Sun Y. Connexin Mutations and Hereditary Diseases. Int J Mol Sci. 2022;23:4255.
10. Mackay D, Ionides A, Kibar Z, et al. Connexin46 mutations in autosomal dominant congenital cataract. Am J Hum Genet. 1999;64:1357–64.
11. Shiels A, Bennett TM, Hejtmancik JF. Cat-Map: putting cataract on the map. Mol Vis. 2010;16:2007–15.
12. Evans WH, De Vuyst E, Leybaert L. The gap junction cellular internet: connexin hemichannels enter the signaling limelight. Biochem J. 2006;397:1–14.
13. Brennan L, Costello MJ, Hejtmancik JF, et al. Autophagy Requirements for Eye Lens Differentiation and Transparency. Cells. 2023;12(3):475.
14. Brink PR, Valiunas V, White TW. Lens Connexin Channels Show Differential Permeability to Signaling Molecules. Int J Mol Sci. 2020;21:6943.
15. Berthoud VM, Gao J, Minogue PJ, et al. Connexin Mutants Compromise the Lens Circulation and Cause Cataracts through Biomineralization. Int J Mol Sci. 2020;21:5822.
16. Ren Q, Riquelme MA, Xu J, et al. Cataract-Causing Mutation of Human Connexin 46 Impairs Gap Junction, but Increases Hemichannel Function and Cell Death. PLoS ONE. 2013;8:e74732.
17. Beyer EC, Berthoud VM. Connexin hemichannels in the lens. Front Physiol. 2014;5:20.
18. Hu Z, Riquelme MA, Wang B, et al. Cataract-associated connexin 46 mutation alters its interaction with calmodulin and function of hemichannels. J Biol Chem. 2018;293:2573–85.
19. Zhu Y, Shentu X, Wang W, et al. A Chinese family with progressive childhood cataracts and IVS3+1G>A CRYBA3/A1 mutations. Mol Vis. 2010;16:2347–53.
20. Yao K, Wang W, Zhu Y, et al. A novel GJA3 mutation associated with congenital nuclear pulverulent and posterior polar cataract in a Chinese family. Hum Mutat. 2011;32:1367–70.
21. Wang KJ, Zhu SQ. A novel p.F206I mutation in Cx46 associated wim autosomal dominant congenital cataract. Mol Vis. 2012;18:968–73.
22. Yao K, Wang B, Zhu Y, et al. A novel GJA3 mutation associated with congenital nuclear pulverulent and posterior polar cataract in a Chinese family. Hum Mutat. 2011;32(12):1367–70.
23. Ding X, Wang B, Luo Y, et al. A novel mutation in the connexin 46(GJA3) gene associated with congenital cataract in a Chinese pedigree. Mol Vis. 2011;17:1343–9.
24. He W, Li X, Chen J, et al. Genetic linkage analyses and Cx50 mutation detection in a large multiplex Chinese family with hereditary nuclear cataract. Ophthalmic Genet. 2011;32(1):48–53.
25. Yang G, Zhang G, Wh Q, et al. A novel mutation in the MIP gene is associated with autosomal dominant congenital nuclear cataract in a Chinese family. Mol Vis. 2011;17:1320–3.
26. Wang L, Chen X, Lu Y, et al. A novel mutation in gammaD-crystallin associated with autosomal dominant congenital cataract in a Chinese family. Mol Vis. 2011;17:804–9.
27. Wang KJ, Li SS, Yun B, et al. A novel mutation in MIP associated with congenital nuclear cataract in a Chinese family. Mol Vis. 2011;17:70–7.
28. Li FF, Yang M, Ma X, et al. Autosomal dominant congenital nuclear cataracts caused by a CRYAA gene mutation. Curr Eye Res. 2010;35(6):492–8.
29. Gu F, Luo W, Li X, et al. A novel mutation in AlphaA-crystallin (CRYAA) caused autosomal dominantcongenital cataract in a large Chinesefamily. Hum Mutat. 2008;29(5):769.
30. Yang G, Zhai X, Zhao J. A recurrent mutation in CRYBA1 is associated with an autosomal dominant congenital nuclear cataract disease in a Chinese family. Mol Vis. 2011;17:1559–63.
31. Yang Z, Su D, Li Q, et al. A novel T–>G splice site mutation of CRYBA1/A3 associated with autosomal dominant nuclear cataracts in a Chinese family. Mol Vis. 2012;18:1283–8.
32. Wang KJ, Wang BB, Zhang F, et al. Novel beta-crystallin gene mutations in Chinese families with nuclear cataracts. Arch Ophthalmol. 2011;129(3):337–43.
33. Guo Y, Su D, Li Q, et al. A nonsense mutation of CRYGCassociated with autosomal dominant congenital nuclear cataracts and microcornea in a Chinese pedigree. Mol Vis. 2012;18:1874–80.
34. Li XQ, Cai HC, Zhou SY, et al. A novel mutation impairing the tertiary structure and stability of gamma C-crystallin (CRYGC) leads to cataract formation in humans and zebrafish lens. Hum Mutat. 2012;33(2):391–401.
35. Wang B, Yu C, Xi YB, et al. A novel CRYGD mutation(p.Trp43Arg) causing autosomal dominant congenital cataract in a Chinese family. Hum Mutat. 2011;32(1):E1939-47.
36. Vendra VP, Balasubramanian D. Structural and aggregation behavior of the human gammaD-crystallin mutant E107A, associated with congenital nuclear cataract. Mol Vis. 2010;16:2822–8.
37. Quan Y, Du Y, Tong Y, Gu S, Jiang JX. Connexin Gap Junctions and Hemichannels in Modulating Lens Redox Homeostasis and Oxidative Stress in Cataractogenesis. Antioxidants (Basel). 2021;10:1374.
38. Myers JB, Haddad BG, O'Neill SE, et al. Structure of native lens connexin 46/50 intercellular channels by cryo-EM. Nature. 2018;564:372–7.
39. Hassan AY, Yousaf S, Levin MR, et al. Novel Homozygous Missense Variant in GJA3 Connexin Domain Causing Congenital Nuclear and Cortical Cataracts. International Journal of Molecular Science. 2022;23(1):240.

40. Gong X, Cheng C, Xia CH. Connexins in lens development and cataractogenesis. J Membr Biol. 2007;218(1–3):9–12.
41. Walter WJ, Zeilinger C, Bintig W, Kolb HA, Ngezahayo A. Phosphorylation in the C-terminus of the rat connexin46 (rCx46) and regulation of the conducting activity of the formed connexons. J Bioenerg Biomembr. 2008;40(4):397–405.
42. Xu X, Berthoud VM, Beyer EC, Ebihara L. Functional role of the carboxyl terminal domain of human connexin 50 in gap junctional channels. J Membr Biol. 2002;186(2):101–12.
43. Ebihara L, Liu X, Pal JD. Effect of external magnesium and calcium on human connexin46 hemichannels. Biophys J. 2003;84(1):277–86.
44. Zhu Y, Yu H, Wang W, et al. A Novel GJA8 Mutation (p.V44A) Causing Autosomal Dominant Congenital Cataract. PLoS ONE. 2014;9:e115406.
45. Duncan G, Jacob TJC. Calcium and the physiology of cataract. Ciba Found Symp. 1984;106:132–52.
46. Maurer P, Weingart R. Cell pairs isolated from adult guinea pig and rat hearts: effects of [Ca^{2+}] ion nexal membrane resistance. Pflugers Arch. 1987;409:394–402.
47. Bukauskas FF, Jordan K, Bukauskiene A, et al. Clustering of connexin43-enhanced green fluorescent protein gap junction channels and functional coupling in living cells. Proc Natl Acad Sci USA. 2000;97:2556–61.
48. Quist AP, Rhee SK, Lin H, Lal R. Physiological role of gap-junctional hemichannels. Extracellular calcium-dependent is osmotic volume regulation. J Cell Biol. 2000;148:1063–74.
49. Bruzzone S, Franco L, Guida L, et al. A self-restricted CD38-connexin43 cross-talk affects NAD+ and cyclic ADP-ribose metabolism and regulates intracellular calcium in 3T3 fibroblasts. J Biol Chem. 2001;276:48300–8.
50. Plotkin LI, Manolagas SC, Bellido T. Transduction of cell survival signals by connexin-43 hemichannels. J Biol Chem. 2002;277:8648–57.
51. Kalvelyte A, Imbrasaite A, Bukauskiene A, Verselis VK, Bukauskas FF. Connexins and apoptotic transformation. Biochem Pharmacol. 2003;66:1661–72.
52. Tong J, Sohn BC, Lam AT, et al. Properties of two cataract-associated mutations located in the NH2 terminus of connexin 46. Am J Physiol Cell Physiol. 2012;304(9):C823–32.
53. Minogue PJ, Gao J, Zoltoski RK, et al. Physiological and Optical Alterations Precede the Appearance of Cataracts in Cx46fs380 Mice. Invest Ophthalmol Vis Sci. 2017;58:4086–94.
54. Orrenius S, Zhivotovsky B, Nicotera P. Regulation of cell death: the calcium-apoptosis link. Nat Rev Mol Cell Biol. 2003;4:552–65.
55. Zhang L, Qu X, Su S, et al. A novel mutation in GJA3 associated with congenital Coppock-like cataract in a large Chinese family. Mol Vis. 2012;18:2114–8.
56. Liu Y, Qiao C, Wei T, et al. Mutant connexin 50 (S276F) inhibits channel and hemichannel functions inducing cataract. J Genet. 2015;94(2):221–9.
57. Ebihara L, Xu X, Oberti C, Beyer EC, Berthoud VM. Co-expression of lens connexins modifies hemi-gap-junctional channel behavior. Biophys J. 1999;76:198–206.

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