·Basic Research·

A novel p.R890C mutation in *EPHA2* gene associated with progressive childhood posterior cataract in a Chinese family

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Abstract

• AIM: To identify the genetic defect in a Chinese family with bilateral progressive childhood posterior cataract.

• METHODS: A two-generation family was recruited in this study. Family history and clinical data were recorded. All reported candidate genes associated with congenital posterior cataract were screened by direct DNA sequencing.

• RESULTS: All affected individuals presented posterior opacities in the lens. Direct sequencing of the candidate genes showed a heterozygous c. 2668C>T variation in *EPHA2* gene, which resulted in the replacement of arginine by cysteine at codon 890 (p. R890C). This mutation was found in two affected individuals, but was not observed in 200 normal controls.

• CONCLUSION: We report a novel mutation (p. R890C) in the *EPHA2* receptor tyrosine kinase gene. The finding expands the mutation spectrum of *EPHA2* in association with posterior cataract.

• KEYWORDS: *EPHA2*; gene mutation; posterior cataract

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INTRODUCTION

Congenital cataract (CC), which is characterized by any opacity of the lens presented at birth or shortly thereafter, is one of the major causes of visual impairment and blindness in childhood worldwide. Its prevalence is up to 6 in 10 000 live births, causing about 10% of childhood blindness worldwide [1]. Congenital cataract may occur as an isolated, nonsyndromic disease or as a multisystem syndrome, it is clinically and genetically heterogeneous with about 25% congenital cataracts are inherited [2]. It can be inherited as autosomal dominant traits, autosomal recessive traits and X-linked inheritance, but the most common mode is autosomal dominant traits.

To date, more than 40 loci and 26 genes have been reported to be linked with different forms of congenital cataract [3]. Among these genes, *αB-crystallin (CRYAB)* [4,5], *βA1-crystallin (CRYBA1)* [3], *βB2-crystallin (CRYBB2)* [6], *connexin 50 (GJA8)* [7], *paired-like homeodomain 3 (PITX3)* [8-10], *chromatin modifying protein 4B (CHMP4B)* [11], and *Eph-receptor type-A2 (EPHA2)* have been reported to be associated with congenital posterior cataract [12,13].

In the present study, we investigated a Chinese family with autosomal dominant progressive childhood posterior cataract and found a novel missense mutation in *EPHA2* which cosegregated with the disease in the family. To the best of our knowledge this is the first report associating this mutation in *EPHA2* with progressive childhood cataract.

SUBJECTS AND METHODS

Family data We studied a two-generation Chinese family with CC. The proband's parents refused to participate in the research, so only the proband and her sister were recruited in our study (Figure 1). Informed consents were obtained from all participants according to the Declaration of Helsinki. The patients participating in this study were diagnosed in the Eye Center of the Second Affiliated Hospital of Medical College, Zhejiang University. Complete ophthalmologic examinations were performed on all participants, including visual acuity,

slit lamp, and fundus examination. The phenotypes were documented by slit lamp photography.

Genomic DNA preparation Blood specimens (5mL) were collected in Vacutainer tubes (Becton-Dickinson, Franklin Lakes, NJ, USA) containing EDTA. We extracted genomic DNA in the peripheral blood leukocytes from all participants (including 2 patients and 200 unrelated control participants with no family history of CC) using a Simgen Blood DNA mini kit (Simgen, Hangzhou, China).

Genetic analysis We used the functional candidate gene analysis approach. Gene specific PCR primers for *CRYAB*[4,5], *CRYBB2*[6], *CRYBA3/A1*[3], *GJA8*[7], *PITX3*[8,9], *CHMP4B*[10], and *EPHA2* [12,13] were used as previous reports. PCR was performed in a volume of 25μL in a DNA Thermal Cycler (Perkin Elmer, Norwalk, CT, USA). PCR cycling conditions were as follows: 5 minutes at 95℃, followed by 10 cycles of touchdown PCR with 1℃ reduction per cycle from 60℃ to 50℃, followed by 30 cycles with denaturation at 95℃ for 30 seconds, annealing at 55℃ for 30 seconds, and extension at 72℃ for 45 seconds with a final extension step at 72℃ for 10 minutes. PCR products were isolated by electrophoresis on 1% agarose gels and sequenced using the BigDye Terminator Cycle sequencing kit V 3.1 (ABI Applied Biosystems; Sangon Co., China) on an ABI PRISM 3730 Sequence Analyzer (ABI), according to the manufacturer's instructions.

Computational algorithms Computational methods have been shown to be effective in predicting whether a specific amino acid substitution of a protein sequence is deleterious or neutral to the function of the protein. Two methods were used: Polymorphism Phenotyping-2 (PolyPhen-2), and Grantham score difference (Align-GVGD).

PolyPhen-2 takes into account the evolutionary conservation of the amino acid subjected to the mutation and the physicochemical characteristics of the wild-type and mutated amino acid residue and the consequence of the amino acid change for the structural properties of the protein [14]. Align-GVGD can be used to predict the transactivation activity of each missense substitution [15]. Grantham Variation (GV) measures the degree of biochemical variation among amino acids found at a given position in the multiple sequence alignment: Grantham Deviation (GD) reflects the 'biochemical distance' of the mutant amino acid from the observed amino acid at a particular position (given by GV). A value of GV=0 corresponds to a residue that is invariant in the alignment, a value of GV of 60-65 is the upper limit of conservative variation across species, and a value of GV>100 is indicative of positions that are under little functional constraint. A value of GD=0 corresponds to a missense substitution that is within the cross-species range of variation at its position in the protein; at invariant positions (GV=0); GD=60-65 is the upper limit of a conservative missense substitution [16].

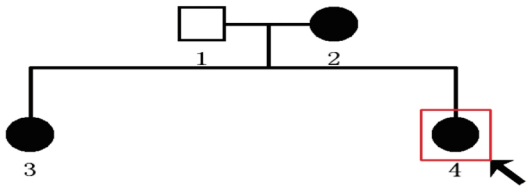

Figure 1 Pedigree of inherited cataract The filled symbols represent the affected individuals. The arrow indicates the proband.

RESULTS

Clinical Findings The transmission patterns of the two-generation Chinese family is autosomal dominant (Figure 1). Only the proband and her sister participated in the study. A detailed medical history was obtained, the performance of the proband and her sister was similar, they had bilateral cataract and noticed their vision impairments in their 10-year-old, and then their visual acuity decreased gradually. We found that opacification affected the posterior pole through slit-lamp examination (Figure 2). No other ocular or related systemic abnormalities were found in the family.

Mutation Analysis By directly sequencing all candidate genes for congenital posterior cataract, a single base substitution in the nucleotide 2668 in *EPHA2* (c.2668C>T, Figure 3) was found in the proband and her sister, which resulted in an amino acid change in the exon of 15 from arginine to cysteine at codon 890 (p. R890C). In addition, this heterozygous mutation was not found in 200 normal controls.

Multiple –sequence Alignment and Mutation Analysis Using the NCBI websites, a multiple sequence alignment showed that the arginine at position 890 of human EPHA2 protein (Homo sapiens, NP_004422.2) was conserved in various species (Figure 4) including: Pantroglodytes (XP_513064.3), Macaca mulatta (NP_001035768.1), Canis lupus familiaris (XP_544546.2), Bos Taurus (NP_001192660.1), Mus musculus (NP_034269.2), Rattus norvegicus (NP_001102447.1), Gallus gallus (XP_001234814.2), and Danio rerio (NP_571490.1)

Computational Analysis Computational protein analysis of R890C EPHA2 revealed the following results: PolyPhen-2 analysis produced a score of 1.000 (sensitivity: 0.00; specificity: 1.00), which is predicted to be "probably damaging". Align-GVGD showed a score of GV0.00, GD179.53, which belongs to class C65 and means "most

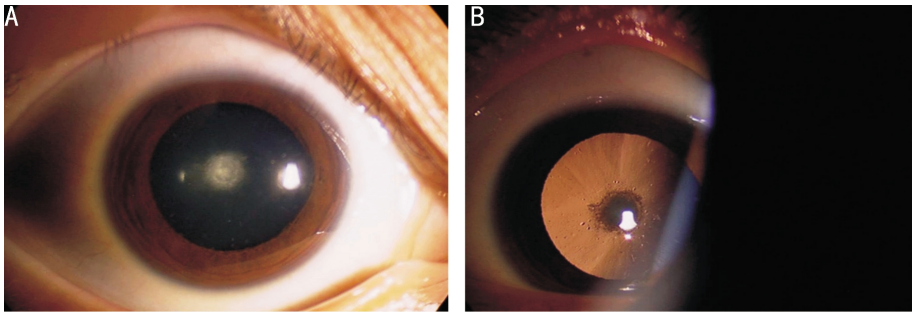

Figure 2 Slit-lamp photograph of the proband´s right eye It shows cataract characterized as a posterior sub-capsular opacity of the lens. A: Slit-lamp photograph using diffuse illumination; B: Slit-lamp photograph using retroillumination.

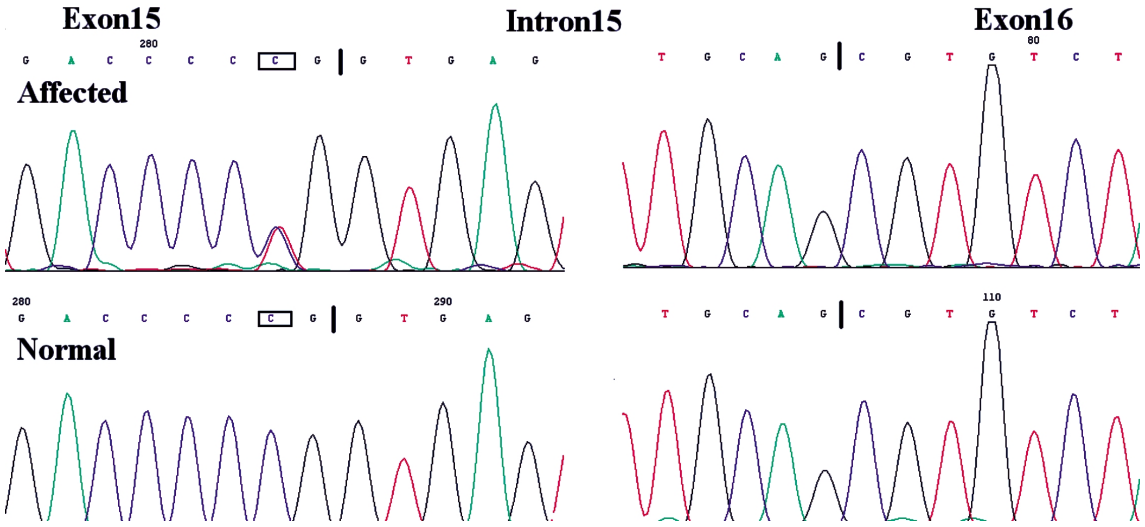

Figure 3 Partial DNA sequence chromatograms of *EPHA2* from one affected and one normal individual Forward sequence analysis of the affected and normal individuals in this family, showing a heterozygous mutation (c.2668C>T) in the exon15 of *EPHA2* (indicated by a box). The black vertical line denotes the exon and intron donor splice site.

Homo spaiens(human)	DPRVSIRLPSTSG
Pan troglodytes	DPRVSIRLPSTSG
Macaca mulatta(monkey)	DPRVSIRLPSTSG
Canis lupus familiaris(dog)	DPRVSIRLPSTSG
Bos Taurus(bovin)	DPRVSIRLPSTSG
Mus musculus(norvegicus)	DPRVSIRLPSTSG
Rattus norvegicus	DPRVSIRLPSTSG
Gallus gallus(chicken)	DPRVSIRLPSTSG
Danio rerio(zebrafish)	DPRVSIRLPSTSG

Figure 4 Multiple-sequence alignment in *EPHA2* from different species It reveals that codon 890, where the mutation (p. R890C) occurs is conserved (highlighted in red).

likely to interfere with function."

DISCUSSION

In the present study, we identified a novel p.R890C substitution in EPHA2 associated with autosomal dominant progressive childhood posterior sub-capsular cataract. The substitution was presented in the proband and her sister, but was absent in 200 ethnically matched control samples. Moreover, by the PolyPhen and Align-GVGD programs, we evaluated the possible effect of this amino acid substitution (R890C) on EphA2 protein. These programs were used to predict whether an amino acid substitution would alter protein structure and function. Because of the isolated predictive value of these programs can be increased by their combination [17], it was believed that the R890C mutation alters the structure of Epha protein and may contribute to the disease. Taken together, these results strongly suggest that variation in *EPHA2* is a causative mutation.

The EphA2 belongs to the subfamily of receptor tyrosine kinases. The extracelluar domains of Eph receptors interact with membrane-bound ligands, ephrins, located on the surfaces of adjacent cells [18]. Through Eph-ephrin signaling system, multiple developmental processes can be modulated, such as patterning of nervous, skeletal and vascular systems [13]. EphA2 (originally called epithelial cell kinase), which was first cloned from HeLa cells [19], was enriched in epithelial cell. Recent studies have detected the expression of *EPHA2*

Table 1 Known mutations and SNPs in EPHA2 in association with cataracts

DNA change	Coding change	Phenotype	Origin	Exon/Intron	Reference
rs7548209 (G/C)		Age-related cortical	China	5′-region	Tan *et al*[21]
c.2162G>A	p.R721Q	Age-related cortical	USA	Ex13	Jun *et al*[23]
c.2353G>A	p.A785T	Nuclear	Pakistan	Ex14	Kaul *et al*[22]
c.2819C>T	p.T940I	Posterior polar	China	EX17	Zhang *et al*[13]
g.IVS16-9G>A	p.D943PfsX71	Total	Australia	IVS16/Ex17	Zhang *et al*[13]
c.2842G>T	p.G948W	Posterior polar	USA	Ex17	Shiels *et al*[12]
c.2915-2916delTG	V972GfsX39	Posterior polar	UK	Ex17	Zhang *et al*[13]
rs7543472(T/C),		Age-related cortical	Italy	3′-region	Shiels *et al*[12]
rs11260867 (C/G)		Cortical and/or nuclear	India	3′-region	Sundaresan *et al*[20]
rs477558 (G/A)		Age-related cortical	China	3′-region	Tan *et al*[21]

gene in human lens epithelial cell line and human anterior lens capsule tissues [13]. Moreover, mutations and SNPs in the *EPHA2* gene have been reported to be associated with congenital cataract and age-related cortical and posterior cataract in humans and mice [20-23] (Table 1), but the precise role of the Epha2 in the lens is unknown.

Receptors in the Eph subfamily typically have a juxtamembrane domain, a tyrosine kinase domain, a SAM domain and a PDZ-binding motif [24-26]. Previous reported mutations are mostly located in the SAM domain, which may contribute to oligomerization and clustering of Eph-ephrin complex and meditate protein-protein interactions between an Ephs and regulatory proteins [13]. The 2669G>A is a common SNP (rs142789236), which will be carried by normal people. This nucleotide variation results in substitution of arginine by histidine in 890 amino acid site. Arginine and histidine belong to basic amino acids and are similarly charged under physiological state. These may explain why this change of amino acid would not affect the protein's structure or function. However, the mutation (c. 2668C>T, p.R890C) we identified in this research could be considered as an underlying pathogenicity of congenital cataract despite it located near a known SNP. On the one hand, cysteine is a polar amino acid and its magnitude of charge is different from arginine. This may change the protein's tertiary structure and disturb the proper folding of SAM domain, which is crucial for interaction between *EPHA2* and ligand. On the other hand, arginine at codon 890 is a conserved amino acid residue among species and its substitution was predicted as probable damaging to protein's function by multiple programs. Consequently, this mutation may contribute to the process of cataract forming. Yet the precise mechanism is still unclear and further investigation will be needed.

In conclusion, we have identified a progressive childhood posterior cataract associated with the R890C mutation of *EPHA2* in a Chinese family. This is the first report to relate this mutation to progressive childhood posterior cataracts. This study highlights the physiologic importance of *EPHA2* in normal lens and improves our understanding of the mechanism underlying human cataract formation.

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