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Original Article

Human pituitary homeobox-3 gene in congenital cataract in a Chinese family

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Abstract: Objectives: Congenital cataract is the common cause of world blindness. It is generally inherited as an autosomal recessive trait and has various phenotypes. This study aimed to explore the gene responsible for autosomal recessive congenital cataract in a Chinese family, and to investigate the functional and cellular consequences of the mutation. Methods: A four-generation Chinese family with autosomal recessive congenital cataract was included in the study. A genome wide scan and linkage analysis were performed in the chromosomal region of Pituitary homeobox 3 (PITX3) to identify the linked region of the genome. And sequence analysis of PITX3 gene was also investigated using BigDye Terminator mix 3.0 and SeqScape Software 2.5. Results: The genome wide scan and linkage analysis identified a disease-haplotype interva. The maximum logarithm of odds LOD score was (Z_{max}) 3.11 at marker D10S1693 (θ_{max}=0.00), flanked by D10S1680 and D10S467, which included the PITX3 gene. Sequencing revealed a splice site mutation, G→A, at D10S1680 and D10S467, which co-segregated with all the affected members of this family. Conclusions: The 543delG is a novel mutation in PITX3 causing an autosomal recessive congenital cataract.

Keywords: Autosomal recessive congenital cataract, pituitary homeobox 3, Chinese family

Introduction

Congenital cataract (CC), the clouding of the lens resulting in blurred vision, is present at birth or within three months after birth. It can be: unilateral or bilateral, progressive, stationary or total or partial [1, 2]. Since congenital cataracts are among the leading causes of blindness in children, diagnosis and early treatment is very important to minimize amblyopia, and to improve visual recovery. Many of these crystalline opacities detected by clinical examination do not progress and may be visually insignificant in some case, however, others come to produce great visual impairment [3, 4]. Congenital Cataract is responsible for the blindness of a 10 to 38% of children in the world, 0.004% newborns suffers from congenital cataracts [5-7].

It is known that different types of congenital cataracts are assigned to a genetic locus and caused by a specific mutation [8, 9]. There has been considerable progress in identifying the molecular basis of human genetic cataract. Many genes are involved, such as water-soluble transparent lens proteins (crystalline), gap junction proteins (connexins), integral membrane proteins (aquaporins), diverse cytoskeletal protein and sequence-specific DNA-binding factors [10].

Sequence-specific DNA-binding factors play an important role in embryonic development of the lens, including the interaction between the embryonic external ectoderm and optic vesicle. This interaction is essential for normal lens induction [11]. And mutations in sequence-specific DNA-binding factors several genes have been reported in autosomal recessive congenital cataract, such as paired box gene 6 (PAX6), forkhead box E3 (FOX-E3), eyes absent homolog 1 (EYA1) and Pituitary homeobox 3 (PITX3) (12-16). Here, we report a novel PITX3 mutation, 543delG, in a Chinese family with autosomal recessive congenital cataract.

Table 1. PCR Primers and conditions

Set	Forward primer	Reverse primer	Annealing temp (°C)	Product size (bp)
Exon 1	cctggtctgccataaagtga	attctcgacctgttcccaag	60	237
Exon 2	acgcagccccagctttac	aagccagcgcatattctcc	60	296
Exon 3	gtgcaggacataacagcttc	gagcagaggctggaggttg	60	374
Exon 4	ctctagccacctcatctcg	aggcataagggcaggacac	60	808

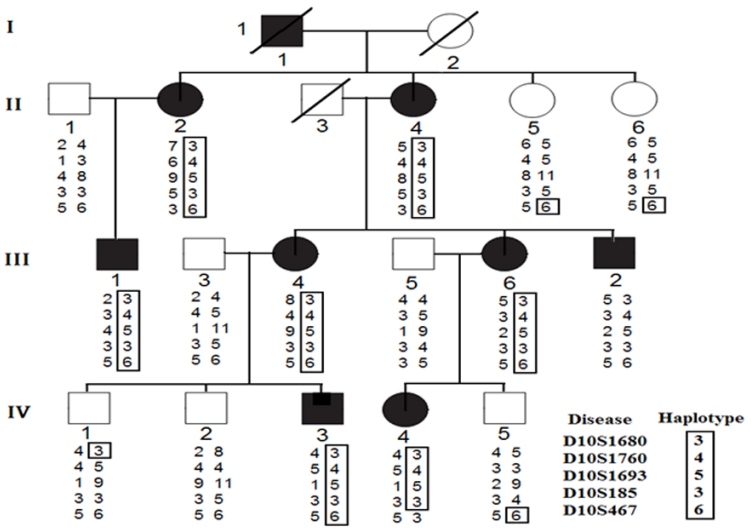

Figure 1. Pedigree and haplotype analysis of a Chinese family of five microsatellite markers listed in descending order (■: Affected males; ●: Affected females; □: Unaffected males; ○: Unaffected females).

Table 2. Two point z values for cataract phenotype and markers

Marker	CM	LOD at θ=						
		0.0	0.01	0.05	0.1	0.2	0.3	0.4
D10S1680	4.51	1.80	1.77	1.71	1.55	1.14	0.64	0.20
D10S1760	1.18	0.52	0.52	0.45	0.40	0.30	0.24	0.14
D10S1693	3.34	3.11	3.03	2.83	2.53	1.90	1.21	0.52
D10S185	0.00	0.81	0.81	0.74	0.65	0.47	0.27	0.09
D10S467	0.00	-4.44	-1.53	-0.79	-0.47	-0.20	-0.10	-0.07

Patients and methods

Patients

Thirteen family members, eight affected and five unaffected, from a four-generation Chinese family with autosomal recessive congenital cataract participated in this study and recieved a full ophthalmological examination at the Department of Ophthalmology in the Affiliated Xiamen Eye Center, Eye Institute of Xiamen University. All participants in this study had signed the consent for the use of collected data without disclosure of personal identity. And the study protocol was also approved by the local research ethics committee of the Eye Institute of Xiamen University Blood samples were collected from each of the thirteen family members. Furthermore, genomic DNA extraction from peripheral blood leukocyte for genetic analysis was carried out with the TIANamp Genomic DNA blood kit (Tiangen Biotech, Beijing, China).

Linkage analysis

Genomic DNA was extracted from 5 ml EDTA blood samples with Nucleon II DNA extraction kit (Tepnel Life Sciences, Manchester, UK). Genotype data of family members were collected GeneChip® Mapping 50K XbaI Array (Affymetrix, Inc., Santa Clara, CA, USA). Genotyping Console 3.0.2 software (Affymetrix) assigned genotypes. The initial linkage analysis of affymetrix genotype data was performed by ALOHOMORA_M (Mega-Chips) v0.33.0 software tool, while mendelian errors and the correct relationships within families were checked for by using the PedCheck and GRR programs. GENEHUNTER v2.1 was used to perform parametric and model-independent nonparametric linkage of the disease allele with a frequency of 0.0001 in the general population.

Significant logarithm of odds (LOD) score markers was calibrated by Marshfield genetic map and Genome Data Base (GDB). The analysis was performed using using commercially available analysis software (GeneMapper version 4.0, Applied Biosystems) on an ABI-Prism 3730 Genetic Analyzer (Applied Biosystems/Hitachi, Foster City, CA). Linkage analysis between two

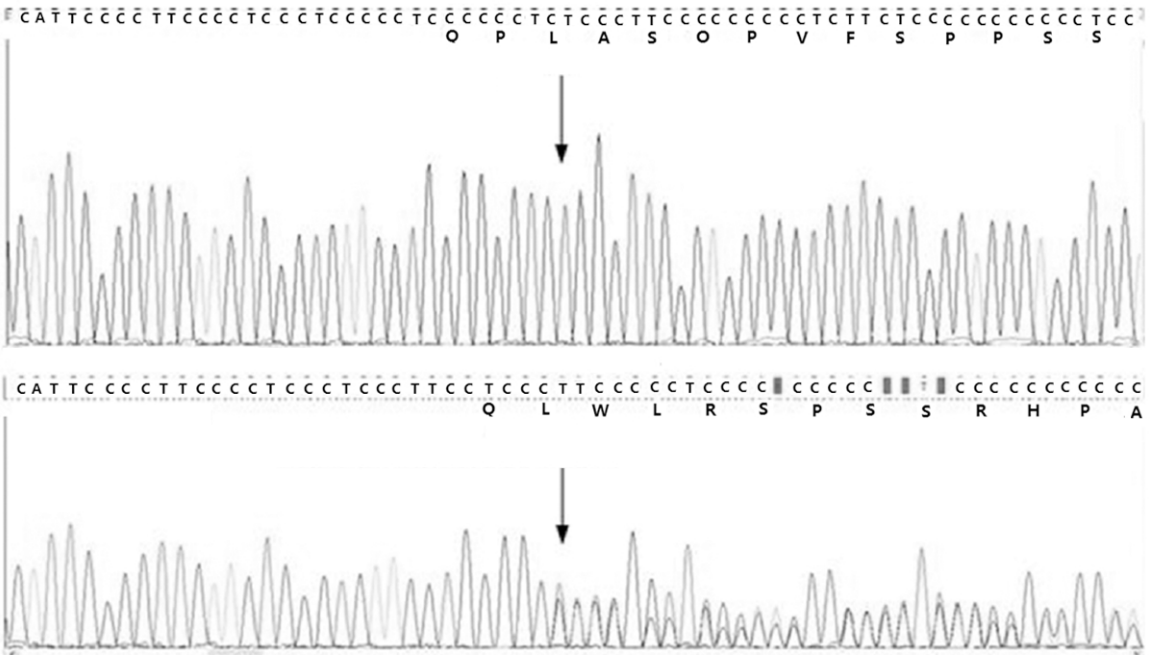

Figure 2. New mutation point of PITX3 in a Chinese family with autosomal recessive congenital cataract.

point was carried out by the the MLINK component of the LINKAGE program package version 5.10. Genealogical data and haplotypes was administered by the Cyrillic v2 software (Cyrillic Software).

Sequencing

Genomic DNA from all the family members was assessed by the detecting PCR products using an ABI PRISM 3100-Avant Genetic Analyzer (Applied Biosystems, Foster City, CA). PCR primers used to amplify the region and PCR conditions were shown in **Table 1**. The products were amplified with 0.5 units of HotStarTaq DNA polymerase (Qiagen, Valencia, CA) in the presence of Q solution (Qiagen, Valencia, CA). Amplification was performed under the following conditions: 95°C for 30 s, then 60°C for 30 s, and at 72°C for 45 s, followed by one cycle of final step at 72°C for 5 min. In order to observe the characterization of insertion, the gel-purified PCR products were sequenced using the cycle sequencing BigDye Terminator mix version 3.0 (PE Applied Biosystems, Foster City, CA) in the forward and reverse directions. Sequencing data collection was performed by the ABI DNA Sequence Analysis Software, version 5.2 (Applied Biosystems, Foster City, CA). And sequencing analysis was performed by the SeqScape Software 2.5 (Applied Biosystems, Foster City, CA).

Results

A family of four generations with autosomal recessive polar cataracts, including 13 members of the family tree (**Figure 1**), including eight affected and five unaffected individuals were genotyped with SNP markers with the GeneChip® Mapping 50K XbaI Array. Linkage analysis identified a disease risk haplotype on chromosome 10q region. In addition, microsatellite markers were used to restrict the region on chromosome 10q25 spans. Positive 2-point LOD score was obtained with markers D10S1693 ($Z=3.11$ at $\theta=0.00$), flanked by markers D10S467 and D10S1680 (**Table 2**).

This area covers the PITX3 homeobox gene between the markers D10S1760 and D10S1693. Sequence analysis of the gene showed that 1-bp deletion of G at nucleotide position 543 (543delG, shown in **Figure 2**), the affected family members with all autosomal recessive congenital cataract cosegregated. This led to a frameshift in codon 181 and probably produced an abnormal protein consisting of 127 additional residues. This mutation in PITX3 affected area outside the homeodomain in the COOH terminus of the protein and result mainly from the autosomal recessive congenital cataract. This change has not been observed in 100 healthy individuals.

Discussion

Autosomal recessive congenital cataract (arCC) is a clinically significant haze, which is located on the back of the lens and because of its proximity to the optical center of the eye, can have a significant effect on vision. The arCC has already been associated with the mutation described in five genes, such as Eph-receptor tyrosine kinase-type A2 (EPHA2) on 1p36, 11q22-Q22.3 on crystallin alpha-B protein (CRYAB), chromatin modifying protein 4B (CHMP4B) on chromosome 20p12, crystallin beta A1 (CRYBA1) on 17q and PITX3 on 10q [17-20]. The family members of pituitary homeobox, including PITX1, PITX2 and PITX3, are involved in the development of the eye, cornea, lens and retina [21]. Mutations in the PITX2 are associated with Rieger syndrome involved in the onset of glaucoma and mild craniofacial dysmorphism in humans [22]. In the aphakia mutant mice, two deletions in the promoter of the homeobox transcription factor Pitx3 led to loss of function and development of eye at the stage of arrested glass rod [23].

Furthermore, mutations in the human Pitx3 homologus gene might also lead to arCC. The first mutation was a recurrent 17-bp insertion (c.657_673dup17) in the COOH-terminal region, resulting in arCC along with highly variable anterior segment mesenchymal dysgenesis (ASMD) in some individuals. This mutation was found in a large family with anterior segment dysgenesis and ocular cortical cataracts [16]. Several recent studies have shown a recurrence of the mutation 17 bp insert same a number of families from different ethnic backgrounds with posterior polar congenital cataract have shown that in some cases affected the defects of the anterior segment [24]. The second mutation was a serine to asparagine substitution in the N-terminal region, 38G→A (S13N) [16]. Another deletion of a single nucleotide (650delG) 650delG in C-terminus was identified in two families with arCC and this mutation is predicted that in a shortening of the normal protein in the same place two amino acids upstream as lead a recurring theme of 17 bp insertion [24, 25]. Homozygous mutation in two brothers of consanguineous marriages 650delG to cause microphthalmia and congenital central nervous system has been found [25].

The Pitx3 mutant K111E, S13N and G219f have shown that patterns of DNA binding and transactivation activities have been change, and there is a partial functional loss in K111E, S13N and G219f with the G219fs form. G219fs the mutation was found in several families affected by arCC with anterior segment malformations in many members. These results suggest that the presence/severity of anterior vaginal segment defects in families affected with G219fs may be determined by secondary factors, which are located in the gene-specific expression and developing structures of the anterior segment, and the effect of this mutation can be determined [26]. Pitx3 is not only widely expressed in skeletal muscle under the control of a muscle and the developing lens, but also required for development of substantia nigra dopaminergic neurons of the substantia nigra in the brain. Furthermore, Pitx3 may be correlated with Parkinson's disease [27].

Here, we report a novel PITX3 mutation, 543delG, in a Chinese family with autosomal recessive congenital cataract. This mutation 1-bp deletion of G at nucleotide position 543 resulted in a reading frame in codon 181 may lead to the production of an aberrant protein consisting of 127 additional residues at the COOH-terminal region. This region is probably involved in complex protein-protein interactions, the specificity and effectiveness of the mediation homeoprotein [26]. This mutation has no effect on the homeodomain region of the protein, but stresses the importance of the COOH-terminal region, which have already been placed by the disease.

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Disclosure of conflict of interest

None.

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