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A novel mutation in *ABCB*6 causes autosomal dominant coloboma in a Chinese family

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*ABCB*6 基因在一常染色体显性遗传脉络膜缺损家系中的突变筛查

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摘要
目的:对一个常染色体显性遗传的先天性脉络膜缺损家系进行 *ABCB*6 基因的突变筛查,明确致病基因。
方法:近来有报道 *ABCB*6 基因突变可导致先天性脉络膜缺损,我们搜集了一个中国汉族先天性脉络膜缺损家系,采集家系成员及一百位正常对照人群的静脉血 5mL,使用 PCR 产物直接测序对 *ABCB*6 基因进行突变筛查。
结果:在该家系中我们发现了一个新突变(c. 1380c>a),该突变在家系中与疾病表型共分离,并且在 100 名正常对照中均未发现该突变。
结论:我们的研究结果扩大了 *ABCB*6 基因的突变谱,进一步确认了该基因在眼组织缺损发病中发挥了重要作用。
关键词:眼组织缺损;*ABCB*6 基因;错义突变;眼球发育

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Abstract
• AIM: To screen mutations in the *ABCB* 6 gene in a Chinese family with autosomal dominant coloboma.
• METHODS: Recently *ABCB* 6 mutations have been reported to be associated with isolated coloboma. We collected 5 mL of blood samples from members of a Chinese family with coloboma and 100 normal controls. Mutations in *ABCB* 6 were determined by sequencing polymerase chain reaction (PCR) products.
• RESULTS: We identified a novel mutation (c. 1380c>a) in the Chinese family. The mutation co-segregated with the disease phenotype in the patients, while it was not detected in other relatives or in the 100 normal controls.
• CONCLUSION: Our results expand the spectrum of *ABCB* 6 mutations causing ocular coloboma, and further confirm the role of *ABCB* 6 in the pathogenesis of ocular coloboma.
• KEYWORDS: ocular coloboma; *ABCB* 6 gene; missense mutation; ocular development
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INTRODUCTION
Coloboma is due to delay in closure of the optic fissure and may affect the iris, choroid, retina and/or optic disc[1]. As developmental ocular anomaly, it is frequently associated with microphthalmia or anophthalmia. Approximately 10% of childhood blindness is caused by severe colobomatous malformations. There are more than 27 genes that were implicated in syndromes involving coloboma[2,3], and *PAX*6, *SHH*, *GDF*3, *RBP* and *YAP*1 have been shown by linkage and mutational screening to cause isolated coloboma[4-9]. Recently, *ABCB*6 was reported by Wang *et al*[2] as a new pathogenic gene causing ocular coloboma. After that there were a few articles reporting variants in *ABCB*6 which were associated with ocular coloboma[10,11].
In our study we present a previously unreported mutation (c. 1380c>a) in the 7th exon of *ABCB*6 in a Chinese family with chorioretinal coloboma. Our data expands the spectrum of *ABCB*6 mutations causing coloboma, and further confirm the role of *ABCB*6 in the pathogenesis of coloboma.

SUBJECTS AND METHODS
Clinical data and 5 mL blood samples were collected from a Chinese Han family with chorioretinal coloboma. All the patients were diagnosed as isolated coloboma clinically. The Institutional Review Board approved the project and investigators followed the principles of the Declaration of Helsinki. Informed consent was obtained from each person.
Human genomic DNA was isolated using the DNA Isolation Kits for Mammalian Blood according to the manufacturer's

Table 1　Primers used to amplify the exons of *ABCB*6

Exons	Forward primers	Reverse primers	Product length
1	gagtccaacaccgagcattc	cctaaagcctggaagcagtg	941
2	cagtccccggccctattat	tgtggtgtcatgcacctgta	385
3–5	ctgggagctgtaaccccata	cggggtctgttctcttcctc	1020
6	tggttccagtctgttgcttg	caatccacagcctcccatag	337
7–9	tgtgtacatggcaggtagtgg	aggccccctttttccttgt	812
10–13	gtcaccaggctcttcggtag	ggttccctctccaagaggtc	921
14	ctgggtgacggagtgagatt	gacagccagcccttatcatt	250
15,16	cctcttatcccacgtgcttc	gttctaggtggggacagtgc	571
17,18	tccagttctcaagcccaaac	accagcccacagagaggac	489
19	gtcctctctgtgggctggt	taagccagggaaaggagaca	259

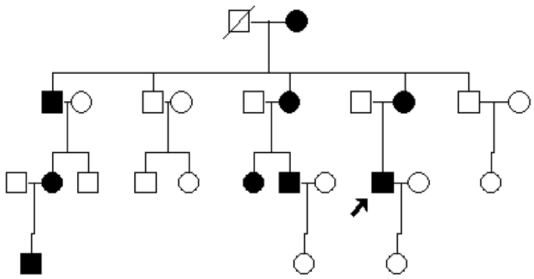

Figure 1　Pedigree of the Chinese family with chorioretinal coloboma　The squares and circles represent males and females, respectively. The shaded symbols signify the affected individuals, a diagonal line symbol indicates a deceased family member, and the arrow indicates the proband.

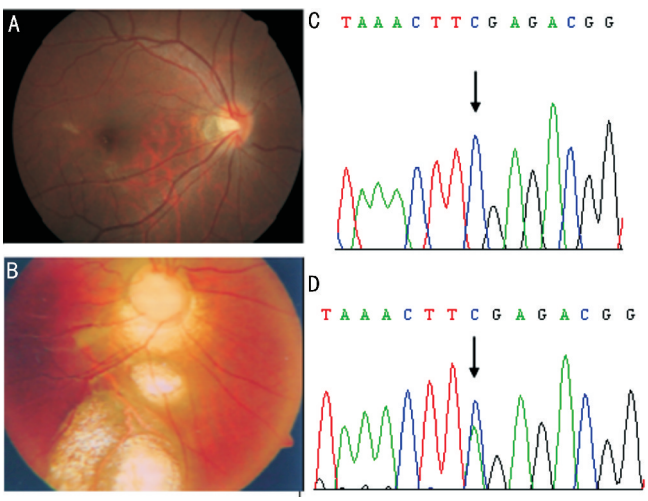

Figure 2　Fundus photographs of a normal individual (A) and a patient with coloboma (B), and their sequencing chromatograms [normal family member (C), affected individual (D)], showing a heterozygous mutation: c. 1380c>a.

instructions (Roche Diagnostics Corporation, Indianapolis, IN, USA). PCR-amplification of *ABCB*6's 19 exons and exon-intron boundaries was performed using primers listed in Table 1. DNA sequence analysis was determined by BigDye™ terminator cycle sequencing with an ABI-3130 Genetic Analyzer (ABI Corporation, Carlsbad, CA, USA).

RESULTS

The family included 9 patients and 16 normal individuals (Figure 1). All patients involved in the study were diagnosed as typical chorioretinal coloboma clinically without any systemic disease. The fundus photographs were shown below (Figure 2A, 2B).

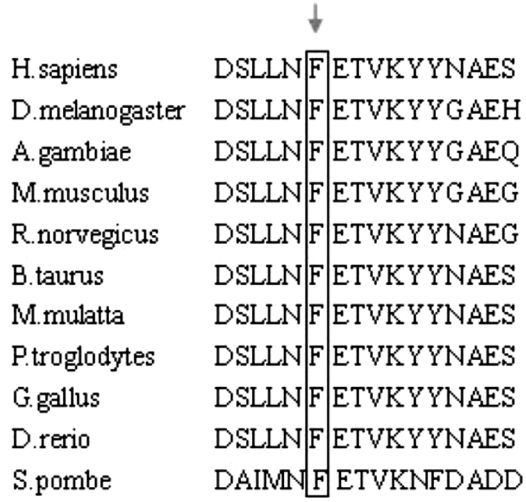

Figure 3　A partial sequence of *ABCB*6 was compared with other species' orthologs (http://www. ncbi. nlm. nih. gov/)
Arrows indicate the location of the mutation identified in the patients with ocular coloboma.

Sequencing of *ABCB*6 in the proband revealed a missense mutation in exon 7 (c. 1380c>a: Figure 2C, 2D), which resulted in a conservative substitution of Phe to Leu at codon 460 (p. F460L). The mutation was absent in the dbSNP database. Then it was confirmed and further extended to other family members. The mutation was detected in all patients, while it was not found in other unaffected relatives or in the 100 normal controls. Polyphen analysis predicted F460L substitution to be probably damaging with a score of 1 (score ranges from 0 to a positive number, where 0 is neutral, and a high positive number is damaging).

DISCUSSION

The closure of the optic fissure requires precisely coordinated sculpting and folding of the epithelial tissue which is controlled by a complex network of transcriptional factors, cell-cycle regulators, and diffusible signaling molecules[12-15].

As a pathogenic gene of ocular coloboma, it can be inferred that *ABCB*6 plays an important role in the network. *ABCB*6 is a member of the ATP-binding cassette (ABC) family which might work in heavy metal detoxification[16]. Being identified as close homologs of ATM1, previous studies suggested that *ABCB*6 may help transport Fe/S clusters from mitochondria to the cytosol, thereby helping to prevent iron accumulation and DNA damage in the organelle[17-21].

Here, we identified a novel mutation (p. F460L) in *ABCB*6. Comparison of *ABCB*6 with the same gene in other species showed that Phe460 is highly conserved (Figure 3), implying that the mutation led to biological function changing of the protein.

Metal ions have been shown to affect the development of the eye. Therefore, we can presume that defect of *ABCB*6's functions results in abnormal metal homeostasis, which is detrimental to proper eye develop.

Our results expand the spectrum of *ABCB*6 mutations causing ocular coloboma, and provide further evidence for *ABCB*6's involvement in ocular development.

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