A Novel Nonsense Mutation(c.1499C>G) in CRB1 Caused Leber Congenital Amaurosis-8 in a Chinese Family and Literature Review

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Abstract

Background:Leber’s congenital amaurosis (LCA) is a severe hereditary retinopathy disease that is characterized by early and severe reduction of vision,nystagmus,sluggish or absent pupillary responses. To date, the pathogenesis of LCA remains unclear,and the majority cases are caused by autosomal recessive inheritance. In this study, we explored the mutation in the Crumbs homologue 1(CRB1) gene in a Chinese family with LCA.

Methods:We conducted comprehensive ocular examinations and collected 5 ml of blood samples from members of a Chinese family with LCA. The pathogenic gene was identified by capturing and sequencing the related genes of ocular diseases.

Results: We found a nonsense mutation(c.1499C>G) in the 6th exon of CRB1 in a Chinese family with LCA,which predicted a change of the protein p.S500X,may lead to loss of gene function.

Conclusions: This study reported a novel mutation(c.1499C>G,p.S500X) of the CRB1 gene occurred in a Chinese family with LCA, thus expanding the spectrum of CRB1 mutations causing LCA. And we summarize the 76 mutations reported so far in CRB1 that caused LCA8.

1. Introduction

Since Theodore Leber first described Leber’s congenital amaurosis (LCA) 152 years ago(in 1869), We have obtained a great deal of information about LCA both in terms of clinical characteristics and molecular genetics.LCA, a rare but important juvenile retinal dystrophy, is an inherited retinal disorder most often diagnosed in infancy in the first 6 months of life and characterized by the presence of nystagmus, poor visual acuity (VA), and a severely reduced or nondetectable electroretinogram[1, 2]. In the worldwide,the prevalence of LCA is 1/81000 to 1 /30000 in newborn babies.Though the incidence is low, it also causes blindness in 20% of school-age children and accounts for approximately 5% of all hereditary retinopathy[3, 4]. LCA is currently described into 21 types according to the pathogenic genes, with autosomal recessive inheritance as the dominant. LCA8 is caused by homozygous or compound heterozygous mutation in the CRB1 gene (604210) on chromosome 1q31.

2. Clinical Manifestation

This study was performed in agreeent with the declaration of Helsinki. It was reviewed by the research unit's professional ethics committee and informed consent was obtained and signed by the investigator.

The proband(figure 1,A,2),a 2-years-old girl came to the hospital on account of her parents complained that she could not accurately grasp things. Her both eyes are performance as a horizontal pendulum nystagmus and was unable to comply with the detailed eye examination. Sequencing chromatograms:the proband show a homozygous mutation in CRB1 gene: nucleotide 1499 changed from cytosine C to guanine G (c.1499C>G) homozygous mutation(figure 1,B) .Under the guidance of the paediatrician, the opportunity for examination was obtained through oral anesthesia.On examination,her eyes are in normal position, the cornea and lens are clear, fundoscopy showed the color of the optic disc in both eyes was light, and the blood vessels from both eyes were thin and narrow(figure 1,C). The pigmentation of the retina at the posterior pole was peppery and salt-like, and the macular area was a mass of lesions with a lot of pigmentation. Her parents and sister underwent detailed eye examinations(the results show in Table 1), including binocular corrected visual acuity, slit lamp examination, fundus photography, macular and optic disc OCT scanning, electroretinogram(ERG),which showed normal results(figure 1,D).

Table 1. Clinical examination data

Patient	Gender	Age	Substitution	UCVA		CVA		Corneal optical refraction	Nystagmus	Globe Retraction
				OD	OS	OD	OS			
Ⅹ:5	M	34		1.0	1.0	1.0	1.0	Normal	-	-
Ⅹ:8	F	28		0.6	0.7	1.0	1.0	Normal	-	-
Ⅹ:1	F	5		0.6	0.6	0.8	0.8	Normal	-	-
Ⅹ:2	F	2	p.S500X	unable	unable	unable	unable	Normal	+	-

Features of LCA8 and unaffected relatives. UCVA uncorrected visual acuity, CVA corrected visual acuity, LCA Leber's Congenital amaurosis. OD right eye, OS left eye.

For LCA, the criteria are: signs of blindness or severe visual impairment from birth or within the first year of life, an ERG reduction of more than 50%, and congenital nystagmus[5]. Fundus examinations could reveal diagnostic clues, including peripheral pigmentary retinopathy, central maculopathy with or without bull's eye pattern, or even macular atrophy. And, indispensable, molecular confirmation is needed.

In our study, the proband's eye examinations and genetic tests were consistent with the diagnosis of LCA. The homozygous mutation in the 6th exon of CRB1: nucleotide 1499 changed from cytosine C to guanine G (c.1499C>G), resulting in nonsense mutation of amino acids (p.S500X) which hasn't been reported before. Through genealogical analysis, the proband's parents and sister had heterozygous variation at this site. According to the ACMG (American College of Medical Genetics and Genomics) guidelines, the mutation was preliminarily determined to be pathogenic: PVS1+PM2+PM3_Supporting(hom). PVS1: This mutation is a zero-effect mutation (nonsense mutation), which may lead to loss of gene function; PM2: The frequency in the database of normal population is -, which is low-frequency variation; PM3_Supporting(HOM) : This mutation is a homozygous rare variant. No correlation of this locus was reported in the literature database. No pathogenicity analysis results were found in ClinVar database. Our study expanding the spectrum of CRB1 mutations causing LCA.

We use ScanProsite tool (<https://prosite.expasy.org/scanprosite/>) to check the secondary structure of CRB1 protein, the nonsense mutation (c.1499C>G, p.S500X) is in *Laminin G domain profile* 485-670: score = 32.931. L. Yang, et al. also reported a nonsense mutation (c.1576C>T, p.R526X) in this domain[6]. The *Laminin G* is an around 180 amino acid long domain found in a large and diverse set of extracellular proteins. It often occurs in multiple copies probably serving as general protein interaction domains that bind the target proteins and other macromolecules, such as carbohydrates. In most proteins, the precise function of the laminin G domain is unknown. A large number of ligands in the G domain of laminin has been reported, including heparin, sulfatides, integrins, dystroglycan, nidogen, and fibulin. In neurexin the G domain is known to bind neurexophilins, α -latrotoxin and neuroligins[7, 8].

Another anatomical feature of LCA includes decreased thickness in different layers, especially in the outer nuclear layer (ONL), loss of integrity in the ellipsoid zone, and disorganized macular atrophy[9]. Unfortunately, the proband we reported was too young to cooperate with optical coherence tomography (OCT) and ERG examination, so we could not analyze the clinical features of these two aspects.

3. Lca Caused By Crb1

In 2004, Hanein, S., et al.[10] reported a comprehensive mutational analysis of the all known genes in 179 unrelated LCA patients, including 52 familial and 127 sporadic cases. The result showed that mutations were identified in 47.5% patients. GUCY2D appeared to account for most LCA cases of our series (21.2%), followed by CRB1 (10%), RPE65 (6.1%), RPGRIP1 (4.5%), AIPL1 (3.4%), TULP1 (1.7%), and CRX (0.6%). Three years later, Francesca Simonelli, et al.[11] analyzed 95 patients in Italian with LCA. They identified some novel variants which occurred more frequently in the RPE65

(8.4%), CRB1 (7.4%), and GUCY2D (5.2%) genes. Through a detailed ophthalmic evaluation of patients with the mutation, they found that CRB1 mutations were associated with reduced retinal thickness and a coarsely laminated retina (by OCT). In London, Henderson, R.H., et al. acquired DNA samples from 250 probands with LCA/early-childhood-onset retinal dystrophy (EORD). They analysed using the LCA chip and twenty-one probands were found to have mutations in CRB1 [12]. Corton, M., et al. enrolled 404 Spanish cases in study, 114 of which suffered from LCA and 290 from EORP (early-onset RP). Their study revealed that 11% of Spanish patients carried mutations in CRB1, ranging from 9% of EORP to 14% of LCA cases. And more than three quarters of the mutations identified have been first described in their study [13].

Liping Yang et al. [6] through 18 cases presenting with LCA to identify disease-causing mutations. They report compound heterozygous mutations of the CRB1 gene which included three novel heterozygous mutations: c.3059delT (p.M1020SfsX1), c.3460T>A (p.C1154S), and c.4207G>C (p.E1403Q). Hosono, K., et al reports the mutations of LCA and inherited retinal dystrophy (IRD) associated genes in 34 Japanese families, which is the first to conduct a next generation sequencing (NGS) based molecular diagnosis of a large Japanese LCA cohort, achieved a detection rate of approximately 56%. Their results show that the most frequently mutated genes were CRB1, NMNAT1, and RPGRIP1 [14]. In recently, Zhu, L., et al. [15] enrolled 37 patients with strictly defined LCA in a cohort of IRD in ten years (2009–2019). Their results revealed that CRB1 gene occupied a greater proportion (27%) associated LCA in the western Chinese population.

CRB1 mutation is a common cause of LCA, and related mutations include missense mutation, nonsense mutation, insertion, deletion and splicing. The following Table 2 lists the mutations in LCA caused by CRB1 which including mutation types, sites, corresponding amino acid changes and regions in recent years. These results are for readers' verification and reference.

Table 2. Summary of CRB1 mutations caused LCA

		Exon	Mutation type	DNA change	Amino acid change	Region	Reference
Ex1	Missense	2T→C	M1T	Japanese	Hosono, et al [14]		
Ex1	Splicing	70 + 2T > A	Aberrant splicing	Chinese	Zhu, L., et al.[15]		
Ex2	Nonsense	107C→G	S36X	Pakistan	McKibbin,M.,e,a[16]		
Ex2	Nonsense	424G→T	G142X	uncertain	Beryozkin A,et.al.[17]		
Ex2	Nonsense	471C > A	C157X	Chinese	Zhu, L., et al.[15]		
Ex2	Insertion	481dupG	A161G fs*8	Spanish	Corton,M,et.al. [13]		
Ex2	Deletion	498_506del	1167_G169del	England	Ahmed, S,et.al. [18]		
Ex2	Deletion	613_619del	I205D fs*13	Spanish	Corton,M,et.al. [13]		
Ex2	Missense	614T→C	I205T	England	Henderson,et.al. [12]		
Ex3	Missense	664G > A	E222K	Chinese	Li, L., et.al.[19]		
Ex3	Insertion	668dupT	L223Ffs*4	Japanese	Hosono, et.al [14]		
Ex3	Insertion	733dupG	A245Gfs*16	Japanese	Hosono, et al [14]		
Ex3	Missense	866C→T	T289M	Italian	Simonelli,et al.[11]		
Ex3	Missense	998G > A	G333D	Korea	Moon,S, e.a.[20]		
Ex6	Deletion	1334_1740del	C445Yfs*8	Japanese	Hosono, et al [14]		

Ex6	Missense	1405T > G	C469G	Chinese	Zhu, L., et al.[15]
Ex6	Missense	1429G→A	G477R	Chinese	L,Yang, e.a. [6]
Ex6	Nonsense	1499C>G	S500X	Chinese	this study
Ex6	Insertion	1567dupC	L523Pfs*28	Japanese	Hosono, et al [14]
Ex6	Nonsense	1576C→T	R526X	Chinese	L,Yang, e.a. [6, 14]
Ex6	Missense	1604T→C	L535P	Spanish	Corton,M,et.al. [13]
Ex6	Missense	1678C > G	H560D	Chinese	Zhu, L., et al.[15]
Ex6	Missense	1690G→T	D564Y	Spanish	Corton,M,et.al. [13]
Ex6	Missense	1750G→T	D584N	uncertain	Hanein,S.,et al. [10]
Ex6	Missense	1831T→C	S611P	Chinese	L,Yang, e.a. [6]
Ex6	Missense	1841G > T	G614V	Chinese	Chen, Y., et al.[21]
Ex6	Deletion	1842delT	G614G fs*6	uncertain	Beryozkin A,et.al.[17]
Ex6	Missense	1903T > C	S635P	Chinese	Li, L., et al.[19]
Ex6	Missense	2107G >T	E703X	Iran	Saberi, M.e.a.[22]
Ex6	Missense	2128G > C	E710Q	uncertain	Hanein,S.,et al. [10]
Ex6	Splicing	2128+1G > A	Aberrant splicing	Iran	Saberi, M.e.a.[22]
Ex7	Missense	2222T > C	M741T	uncertain	Hanein,S.,et al. [10]
Ex7	Deletion	2227delG	V743S fs*11	Spanish	Corton,M,et.al. [13]
Ex7	Missense	2234 C→T	T745M	Chinese	L,Yang, e.a. [6]
Ex7	Deletion	2244_47delATC	S749del	Spanish	Corton,M,et.al. [13]
Ex7	Insertion	2276_2279dupCTTA	S758S fsX33	Iran	Saberi, M.e.a.[22]
Ex7	Missense	2290C→T	R764C	uncertain	Hanein,S.,et al. [10]
Ex7	Missense	2309 G→T	G770V	Spanish	Corton,M,et.al. [13]
Ex7	Missense	2401A→T	K801X	Italian	Simonelli,et al.[11]
Ex7	Nonsense	2479G→T	G827X	uncertain	Hanein,S.,et al. [10]
Ex7	Nonsense	2536G→T	G846X	Hungarian	Vamos,R.,et al.[23]
Ex7	Missense	2548G→A	G850S	England	Henderson,et.al. [12]
Ex7	Missense	2555T→C	I852T	uncertain	Hanein,S.,et al. [10]
Ex7	Deletion	2676 delG*	K892NfsX95*	England	Henderson,et.al. [12]

Ex8	Splicing	2677-2A	Aberrant splicing	Chinese	Lin Li, e.a.,[24]
Ex8	Deletion	2678-2682del5bp	CCAAC S893S fs*14	uncertain	Beryozkin A,et.al.[17]
Ex8	Nonsense	2688T	A C896X	Spanish	Corton,M,et.al. [13]
Ex8	Missense	2714G > A	R905Q	Chinese	Zhu, L., et al.[15]
Ex9	Missense	2843G	A C948Y	Polish	Skorczyk, et al [25]
Ex9	Missense	2843G	T C948F	uncertain	Hanein,S.,et al. [10]
Ex9	Splicing	2853_2854insT	A 952fsX972	uncertain	Hanein,S.,et al. [10]
Ex9	Missense	2945C > A	T982K	Chinese	Zhu, L., et al.[15]
Ex9	Missense	3002 T	A I1001N	Spanish	Corton,M,et.al. [13]
Ex9	Missense	3017C > A	S1006Y	Chinese	Zhu, L., et al.[15]
Ex9	Missense	3023T > G	L1008X	Chinese	Zhu, L., et al.[15]
Ex9	Deletion	3059delT	M1020SfsX1	Chinese	L,Yang, e.a. [6]
Ex9	Missense	3068T	G L1023R	Japanese	Hosono, et al [14]
Ex9	Missense	3074G	T S1025I	uncertain	Hanein,S.,et al. [10]
Ex9	Nonsense	3152 G	A W1051X	Spanish	Corton,M,et.al. [13]
Ex9	Missense	3218T > A	L1073Q	Chinese	Zhu, L., et al.[15]
Ex9	Missense	3221T	C L1074S	Chinese	Lin Li, e.a.,[16]
Ex9	Missense	3290T > A	L1097Q	Chinese	Zhu, L., et al.[15]
Ex9	Missense	3299 T	C I1100T	Spanish	Corton,M,et.al. [13]
Ex9	Missense	3307 G	A G1103R	Italian	Simonelli,et al.[11]
Ex9	Missense	3320T	G L1107R	uncertain	Hanein,S.,et al. [10]
Ex9	Deletion	3345delT	G1115fsX1140	uncertain	Hanein,S.,et al. [10]
Ex9	Missense	3466G	T D1156Y	uncertain	A I Hollander,e.a.[26]
Ex9	Missense	3482A	G Y1161C	Spanish	Corton,M,et.al. [13]
Ex9	Insertion	3542dupG	C1181WfsX12*	England	Henderson,et.al. [12]
Ex11	Nonsense	3879G	A W1293X	uncertain	Hanein,S.,et al. [10]
Ex11	Missense	3961T	A C1321G	uncertain	Hanein,S.,et al. [10]
Ex11	Deletion	3988delG	E1330fsX1340	uncertain	Hanein,S.,et al. [10]
Ex11	Deletion	4000delG	V1334W fs*7	Spanish	Corton,M,et.al. [13]

Ex12 Splicing	4005 + 1G > A	Aberrant splicing	Chinese	Zhu, L., et al.[15]
Ex12 Splicing	4013+1 G→T	Aberrant splicing	uncertain	A I Hollander,e.a.[21]
Ex12 Deletion	4121_4130del	R1374fsX1397	uncertain	Hanein,S,et al. [10]

Table 3.Types and proportion of CRB1 mutations caused LCA8

Types of mutations	Missense	Deletion	Nonsense	Insertion	Splicing
count	41	13	10	6	6
percentage	53.9%	17.1%	13.2%	7.9%	7.9%

Table 4.Numbers and proportion of CRB1 exon mutations caused LCA8

exon	Ex1	Ex2	Ex3	Ex4	Ex5	Ex 6	Ex 7	Ex8	Ex9	Ex10	Ex11	Ex12
count	2	7	5	0	0	17	13	4	21	0	4	3
percentage	2.6%	9.2%	6.6%	0	0	22.4%	17.1%	5.3%	27.6%	0	5.3%	3.9%

Up to now, a total of 76 certain CRB1 mutations caused LCA.Furthermore,it has been reported that mutations in the CRB1 are responsible for 7.4%-27% of LCA in different populations.The pathogenic mutations were mainly missense, deletion and nonsense mutations, including insertion and splicing, which reflected the richness of mutation types(Table 3). The mutation sites of LCA8 were mainly concentrated in exon 6, 7 and 9 of CRB1, and the clear pathogenic sites were found in all exons except exon 4, 5 and 10, indicating the universality of mutation regions(Table 4). The reported cases involved more than 10 countries and regions, including China, England, Japan, Spain and Italy, which also showed that the global coverage of LCA caused by CRB1 is extensive.

Other diseases of retinal dystrophy caused by CRB1 mutations:

In addition to LCA,mutations in CRB1 are associated with several other diseases of retinal dystrophy:Rosa Riveiro-Alvarez, et.al.[27] reported early-onset RP phenotype Spanish family which was caused by the CRB1 p.Cys948Tyr (c.2843G>A) mutation.Two CRB1 missense mutations, c.C3991T:p.R1331C and c.C4142T:p.P1381L, were reported illustrate a novel presentation of a macular dystrophy caused by CRB1 mutations by Stephen H. Tsang et,al.[28].Arif O. Khan et,al uncovered a homozygous CRB1 mutation (c.80G→T [p.Cys27Phe]) in three siblings with childhood cone-rod dystrophy and macular cystic degeneration in a family[29].Ajoy Vincent et,al reported biallelic mutations (p.Gly123Cys and p.Cys948Tyr, p.Ile167_Gly169del and p.Arg764Cys) in CRB1 in two families caused autosomal recessive Familial Foveal Retinoschisis,which maybe the mildest end of the spectrum of CRB1-related diseases[30].Benjamin K. Ghiam et,al reported a novel mutation (c.4014T > A)in CRB1 was related with retinal degeneration and may portend a poor prognosis for CME responsiveness to therapy[31].

4. Discussion

LCA is the earliest and most severe hereditary retinopathy, in which the function of cone-rod cells in both eyes is completely lost at birth or within one year after birth, leading to congenital blindness in infants. The majority cases are caused by autosomal recessive inheritance.Typical characteristics of LCA includes:early and severe reduction of vision associated with

nystagmus, photophobia, sluggish or absent pupillary responses, finger pressure on eyeballs; fundus appearance, ranging from normal, maculopathy, to typical RP-like abnormalities; and electroretinogram showed that A and B waves were flat and even severely reduced to non-detectable. It also can be accompanied by keratoconus, hyperopia, developmental delay and nervous system abnormalities et.al.[32]

In some cases/reports, there are many similar clinical features between LCA and early-onset RP and even the diagnosis is ambiguous[33]. Early-onset RP, usually, is considered as a relatively milder form, which patients do not have a congenital onset of visual impairment. We could distinguish the following phenotypes: LCA, early onset retinal degeneration; RP, presence of preservation of the para-arteriolar retinal pigment epithelium and Coats-like vasculopathy[34].

So far, 21 pathogenic genes associated with LCA have been reported. CRB1 belongs to LCA8. CRB1 gene maps to chromosome 1q31.3, is composed of 12 exons, the longest isoform consists of 1,406 amino acids. This gene encodes a protein which is similar to the Drosophila crumbs protein and localizes to the inner segment of mammalian photoreceptors. In Drosophila crumbs localizes to the stalk of the fly photoreceptor and may be a component of the molecular scaffold that controls proper development of polarity in the eye[35], and CRB1 has been found to be important in maintaining cellular polarity[36].

In the mouse retina, CRB1 is expressed in the inner segment of the photoreceptors and Muller cells to maintain adequate morphogenesis and polarity in retinal development[37]. Therefore, CRB1 gene mutations often lead to a variety of retinal dystrophy, including retinitis pigmentosa (RP), LCA, macular dystrophy and so on. Approximately 9-17% of LCA cases have been related to CRB1 mutations, especially which are higher in the Chinese population[38, 39]. A wide variety of visual acuity was noted in patients with mutations in CRB1, ranging from 20/30 to NLP[10, 40].

Among LCA, RPE65 mutations were almost always associated with normal macular thickness, as assessed by OCT, whereas CRB1 mutations were associated with reduced retinal thickness and a coarsely laminated retina. Fundus abnormalities were more heterogeneous in carriers of CRB1 mutations. In fact, some scholars observed salt-and-pepper retinal dystrophy in younger patients and subsequently massive spicular and not nummular pigmentation at the posterior pole, which was reported to be a phenotypic feature of carriers of CRB1 mutations[11]. Saloni Walia et.al.[41] through a multicenter retrospective observational study with 169 patients of LCA found that mutations in RPE65 (LCA-Type II) and CRB1 (LCA-8) may be associated with a relatively better VA in early life compared with other gene mutations. And, onset of the symptoms of LCA after the age of 1 year is also associated with an overall better VA prognosis.

5. Conclusions

LCA is one of the earliest and most severe forms of inherited IRD, the patients suffer from severe visual impairment during childhood, with their vision continuously deteriorating, the final outcome of which usually is complete loss of vision by their thirties or forties[42]. Therefore, it is very important to find an effective treatment. Albert M et.al. provided an entirely new dimension in ocular therapeutics for gene-therapy to LCA2, patients with LCA2 who received AAV2.hRPE65v2 by subretinal injection showed evidence of improvement in retinal function, in the pupillary light reflex, reduction in nystagmus. This clinical trial approaches to the treatment of LCA and possibly other forms of retinal degeneration[43].

Although much is still unknown about the pathogenesis of LCA. However, with the improvement of next-generation sequencing technology and the application of various molecular biological means, the research on corresponding cell functions, the identification of gene subtypes and the establishment of animal models have greatly promoted our understanding of LCA. These latest advances provide a steady stream of evidence for a better understanding and treatment of LCA in the future. And may be useful for faster gene diagnosis, prenatal testing, the development of potential gene therapies, and for improving the understanding of the molecular pathogenesis of LCA.

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Affiliated Hospital of Yunnan University. All experimental protocols were approved by the Affiliated Hospital of Yunnan University, and methods were carried out in accordance with relevant guidelines and regulations. All participants were informed about the purpose of the protocol and signed consent forms. The guardian (parent) of the patients consented to participation of the study.

Consent to publish

Written informed consent was obtained from the guardian (parent) of the patients, and they consented to publication of the study. The guardian (parent) of the patients consented for their medical information to be published.

Availability of data and materials

The relevant data were generated during this study and included in this article. And raw sequence data were not applicable to share in this article as no datasets were generated during the current study. The corresponding author Liwei Zhang (drzhangliwei@163.com) should be contacted if someone wants to request the data from this study.

Competing interests

The authors declare that they have no competing interests.

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Authors Contribution

Wenhua Duan and Taicheng Zhou carried out the experiments, and drafted the manuscript. Huawei Jiang and Minhui Zhang prepared the figure and tables. Liwei Zhang and Min Hu designed and funded this study. All authors read and approved the final manuscript. Dr. Min Hu (fudanhumin123@sina.com) and Dr. Liwei Zhang (drzhangliwei@163.com) are co-corresponding authors for this paper.

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Figures

Figure 1

Pedigree of LCA family with a CRB1 variant, sequencing chromatogram, and diagnostic fundus. **(A)** Pedigree of LCA family with a CRB1 variant. The proband is marked by an arrow, black symbols denote affected members, white symbols denote unaffected members, squares denote males, and circles denote females. **(B)** Sequencing chromatograms. Affected proband shows a homozygous mutation in CRB1 gene: nucleotide 1499 changed from cytosine C to guanine G (c.1499C>G) homozygous mutation, resulting in nonsense mutation of amino acids (p.S500X). **(C)** Diagnostic of the fundus. The proband shows pigmentation of the retina at the posterior pole was peppery and salt-like, and the macular area was a mass of lesions with a lot of pigmentation. **(D)** Her 5 years old sister's fundus shows normal.