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Case Report

Comprehensive analysis of clinical phenotype and genetic characteristics of retinoblastoma caused by *RB1* gene mutation: a case series

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Abstract: This study aimed to summarize the clinical and genetic characteristics of retinoblastoma associated with newly identified RB1 gene mutations. We retrospectively analyzed 15 pediatric patients diagnosed with retinoblastoma caused by RB1 mutations. A total of 25 affected eyes were examined (8 males, 7 females). The age at diagnosis ranged from 7 to 36 months (mean 16.00 ± 8.61 months). Bilateral involvement was observed in 10 patients, and unilateral in 5. Thirteen patients presented with leukocoria, while 2 were diagnosed during routine physical examinations due to vision loss. None of the patients had a family history of retinoblastoma. Whole-exome sequencing revealed heterozygous RB1 mutations in 14 cases and a mosaic mutation in one case. Five novel mutations not previously reported in the literature were identified: c.608-1G>A, c.1818T>A, c.962dupA, c.2086A>T, and c.574A>T. All patients received treatment, including intra-arterial chemotherapy, cryotherapy, photocoagulation, systemic chemotherapy, and/or enucleation. The follow-up duration ranged from 12 to 132 months, with a mean of 39.20 ± 24.07 months. Genetic testing remains a valuable tool for confirming RB1 mutations. Expanding the RB1 mutation spectrum may facilitate early diagnosis, personalized treatment, and informed genetic counseling for affected children.

Keywords: Retinoblastoma, RB1 gene mutation, clinical phenotype, genetic characteristics

Introduction

Retinoblastoma (RB) is the most common malignant intraocular tumor in children. It was first described by Benedict in 1929, and the concept of RB1 gene mutation as its genetic basis was introduced by Knudson in 1971. The incidence of RB is approximately 1 in 20,000 to 1 in 15,000 live births [1]. In China, there are approximately 1,100 new cases annually, with 84% presenting at an advanced or high-risk stage [2, 3]. RB can be unilateral or bilateral and may be associated with intracranial tumors, a condition referred to as trilateral RB. Without timely and effective treatment, RB can metastasize via the optic nerve or hematogenous routes to distant sites such as the bone marrow, potentially leading to death. RB typically manifests before the age of six, with bilateral cases often diagnosed before one year of age [1, 4, 5]. A significant proportion of patients have a family history of the disease.

This study retrospectively reviews cases of pediatric RB associated with newly identified RB1 mutations, aiming to summarize the clinical and genetic features and enhance pediatric ophthalmologists' understanding of the disease.

Material and methods

We retrospectively analyzed the clinical and genetic data of 15 children with RB who were admitted to Fujian Medical University Union Hospital and the Xiamen Branch of the Children's Hospital of Fudan University between January 1, 2022, and March 31, 2024. The

Table 1. Clinical profiles of 15 children diagnosed with retinoblastoma

Characteristics	Total (n=15)	Unilateral (n=4)	Bilateral (n=11)
Gender			
Male	8 (53.3%)	3 (75.0%)	5 (45.5%)
Female	7 (46.7%)	1 (25.0%)	6 (54.5%)
Age at Diagnosis (months)			
Median age	16 ± 8.61	21 ± 2.66	17 ± 8.05
≤12	9 (60.0%)	2 (50.0%)	6 (54.5%)
>12	6 (40.0%)	2 (50.0%)	5 (45.5%)
Treatment methods			
Enucleation	3 (20.0%)	1 (25.0%)	2 (18.2%)
No enucleation	12 (80.0%)	3 (75.0%)	9 (81.8%)

study was approved by the Ethics Committee of the Pediatric Hospital affiliated with Fudan University (EC-023-063), and all patients were diagnosed by a senior chief physician.

Diagnostic criteria for RB were based on established guidelines [6], including: (1) Clinical signs and symptoms involving the anterior chamber, iris, lens, vitreous, retina, or systemic manifestations; (2) Radiological evidence of optic nerve or extraocular muscle involvement; (3) Histopathological confirmation, particularly in cases of unilateral enucleation for bilateral disease.

Genetic testing

Peripheral blood samples (2 mL) were collected from each child and their parents using anticoagulated tubes after obtaining informed consent. Samples were sent to the University Institute of Medical Laboratory for DNA extraction and whole-exome sequencing. Variant pathogenicity was interpreted following guidelines from the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP), with reference to standards from the ClinGen Sequence Variant Interpretation Working Group and the British Society for Genetic Medicine (ACGS).

Follow-up and imaging

Patients underwent follow-up every 3 to 6 months, which included fundus examinations under general anesthesia, imaging with the Optos Panoramic 200 scanning laser ophthalmoscope, and local fundus examinations. Cranial and orbital magnetic resonance imaging (MRI) with contrast was performed based on clinical status (e.g., disease stability or recurrence).

Statistical analysis

Data were analyzed using SPSS version 22.0. Normally distributed continuous variables were expressed as mean ± standard deviation (SD), non-normally distributed data as median and interquartile range [M (Q1, Q3)], and categorical data as frequency (n) and percentage (%).

Results

Patient demographics and clinical presentation

In this study, 15 children (8 males and 7 females) with a total of 25 affected eyes were included. The age at diagnosis ranged from 6 to 36 months, with a mean of 16.00 ± 8.61 months. Among them, 10 patients had bilateral involvement and 5 had unilateral disease. Thirteen children presented with leukocoria (white pupil), while 2 were diagnosed due to vision loss detected during routine physical examinations. None of the patients had a family history of RB.

Tumor classification and treatment

According to the International Intraocular Retinoblastoma Classification, tumor staging revealed stage B in one eye, stage C in seven eyes, stage D in ten eyes, and stage E in seven eyes. In two bilaterally affected cases, one eye required enucleation due to disease severity, while the contralateral eye was preserved. The remaining 13 patients underwent two or more eye-preserving treatments, including superselective intra-arterial chemotherapy, cryotherapy, photocoagulation, and systemic chemotherapy.

Follow-up and imaging findings

The follow-up period ranged from 12 to 132 months, with a mean duration of 39.20 ± 24.07 months (**Table 1**). Representative imaging results are shown in **Figure 1**. RetCam

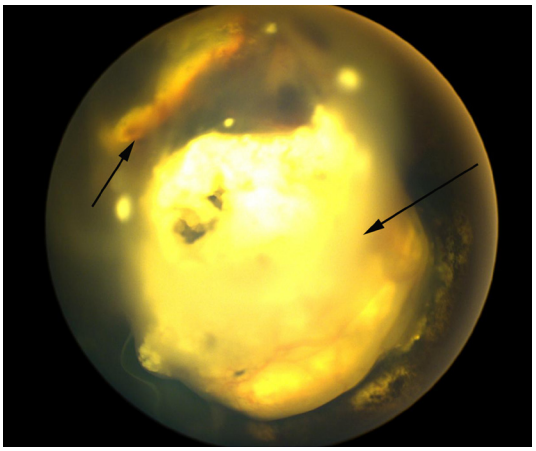

Figure 1. Retcam shows a huge tumor in the vitreous cavity, with no visible optic papilla. Partial calcification can be seen in the tumor body, but neovascularization can still be seen crawling. Arrows indicated the tumor site.

images demonstrated large intraocular tumors occupying the vitreous cavity, with no visible optic disc. Partial calcification was observed within the tumor, along with visible neovascularization on the retinal surface.

Genetic mutation analysis

Genetic analysis confirmed RB1 mutations in all 15 patients. Fourteen patients (93.33%) carried heterozygous mutations, while one patient (6.67%) had a mosaic mutation. These mutations were located across eight exons (6, 8, 10, 14, 16, 18, 19, 20) and four introns (6, 14, 23, 24). At the base level, point mutations were predominant. At the amino acid level, the most common mutation type was nonsense mutation (n=9), followed by splice-site mutations (n=3), frameshift mutations (n=2), and a single missense mutation (n=1). According to ACMG guidelines, all variants were classified as pathogenic. Ten were previously reported mutations, while five were novel variants not reported in the literature: c.608-1G>A, c.1818T>A, c.962dupA, c.2086A>T, and c.574A>T (**Table 2**).

Discussion

The RB1 gene, located on the long arm of chromosome 13 (13q14), spans approximately 180 kb and contains 27 exons, producing a 4.7 kb mRNA transcript. Its protein product, a ~110 kDa nuclear phosphoprotein (pRb), plays a crucial role in cell cycle regulation. Inactivation of RB1 - whether through germline or somatic alterations - is the direct cause of RB.

Based on nucleotide changes, RB1 mutations are typically categorized as point mutations (substitutions), small deletions, insertions, or complex mutations [7]. At the amino acid level, frameshift and nonsense mutations are most frequently observed [6, 8, 9].

In recent years, advances in molecular genetic technologies have significantly improved the diagnosis and management of RB in China. Early identification of RB1 mutations allows for timely diagnosis and targeted genetic counseling. A study in the Turkish population reported a germline RB1 mutation detection rate of 41.9% among 219 individuals with RB, with 51.5% diagnosed before 12 months of age and 32.4% harboring de novo mutations [10]. However, the RB1 variant database in the Chinese population remains incomplete. As of March 31, 2024, the ClinVar database includes over 1,000 pathogenic or likely pathogenic RB1 variants, of which approximately 255 have been reported in China [8, 11-14].

In our study, all 15 cases harbored germline RB1 mutations, consistent with previous findings that 10-12% of unilateral sporadic RB cases carry germline alterations [15]. Fourteen were heterozygous mutations, and one was a mosaic variant, verified through Sanger sequencing and pedigree analysis. Given that germline mutations carry a 50% chance of transmission to offspring, this emphasizes the importance of genetic screening for familial risk.

Among the five novel RB1 variants identified in our cohort: Case 2 exhibited a duplication of base A at position c.962, resulting in a frameshift and a premature stop codon at residue 321. Case 4 carried a c.2086A>T substitution in exon 20, generating a stop codon at residue 696. Case 3 showed a c.1818T>A mutation, leading to a stop codon at residue 606. Case 5 had a c.574A>T mutation, resulting in a premature stop at residue 192.

All four variants resulted in nonsense mutations, disrupting protein function.

Table 2. Asummary of *RB1* mutations in the 15 retinoblastoma patients

Patient ID	Location	Mutational type	Mutation	Change in protein	Laterality	Status
1	Intron6	Splicing	C.608-1G>A	/	Unilateral	Novel
2	Exon19	Nonsense	C.1818T>A	p.Tyr606*	Bilateral	Novel
3	Exon10	Nonsense	C.962dupA	P.Tyr321*	Bilateral	Novel
4	Exon20	Nonsense	C.2086A>T	P.Arg696*	Bilateral	Novel
5	Exon6	Nonsense	C.574A>T	P.Lys192*	Unilateral	Novel
6	Intron14	Splicing	C.1390-1G>A	/	Bilateral	Novel
7	Exon10	Nonsense	C.958C>T	P.Arg320*	Bilateral	Reported
8	Exon14	Nonsense	C1363C>T	P.Arg455*	Bilateral	Reported
9	Exon16	Frameshift	C.1450-1451delAT	P.Met484Valfs*8	Unilateral	Reported
10	Intron24	Frameshift	C.2520+3_2520+6del	/	Bilateral	Reported
11	Intron23	Splicing	C.2489+1G>T	/	Bilateral	Reported
12	Exon8	Nonsense	C.751C>T	P.Arg251*	Unilateral	Reported
13	Exon18	Nonsense	C.1735C>T	P.Arg579*	Bilatera	Reported
14	Exon18	Nonsense	C.1735C>T	P.Arg579*	Bilatera	Reported
15	Exon20	Missense	C.1981C>T	P.Arg661Trp	Unilateral	Reported

Note: * indicate nonsense and frameshift.

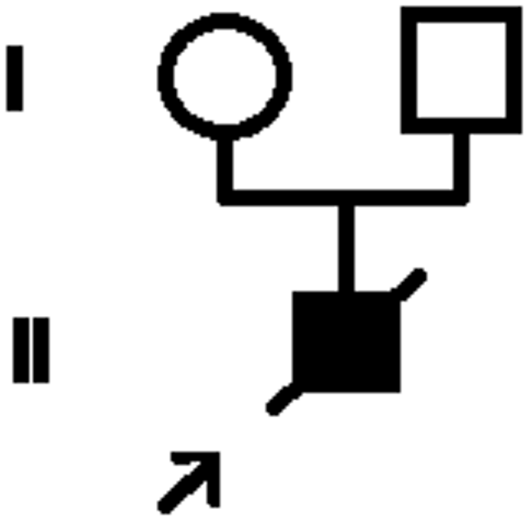

Figure 2. Retinoblastoma pedigree of Case 6. The black arrow indicates a proband with unilateral illness.

Previous studies in Vietnamese RB patients reported 41 distinct RB1 mutations, including novel missense variants in exons 6 (c.601G>C; p.A201P) and 22 (c.2264T>C; p.F755S), further supporting the genetic heterogeneity of RB [16].

Although intronic mutations are less well studied, we identified four such variants, including one not previously reported. Prior reports have indicated that patients diagnosed before one year of age typically present with bilateral RB and heterozygous mutations [17]. However, only about 2% of patients present with unilateral RB and advanced disease within the first year. In such cases, RB1 expression levels are often difficult to detect, possibly due to MYCN amplification or low-level mosaic germline mutations [2, 11].

In our cohort, Case 1 carried a c.608-1G>A mosaic mutation. The patient was diagnosed with stage E unilateral RB at 7 months of age. Despite undergoing six cycles of systemic chemotherapy and one intra-arterial intervention, the tumor persisted with total retinal detachment and optic disc involvement, ultimately requiring enucleation (**Figure 1**). The child remained recurrence-free during a three-year postoperative follow-up.

Case 6 presented a unique phenotype: early onset, high-grade unilateral RB with a detectable RB1 mutation of mosaic origin. This rare combination warrants further clinical attention. Moreover, the long-term risk of secondary malignancies in patients with low-level mosaicism remains unclear, underscoring the need for close surveillance (**Figures 2**, **3**).

Chai et al. [12] reviewed 44 RB1 mutations in Chinese patients and confirmed their distribution across all 27 exons, with no significant difference compared to other populations. Additional studies have identified mutations in up to 25 exons and in the promoter region [18].

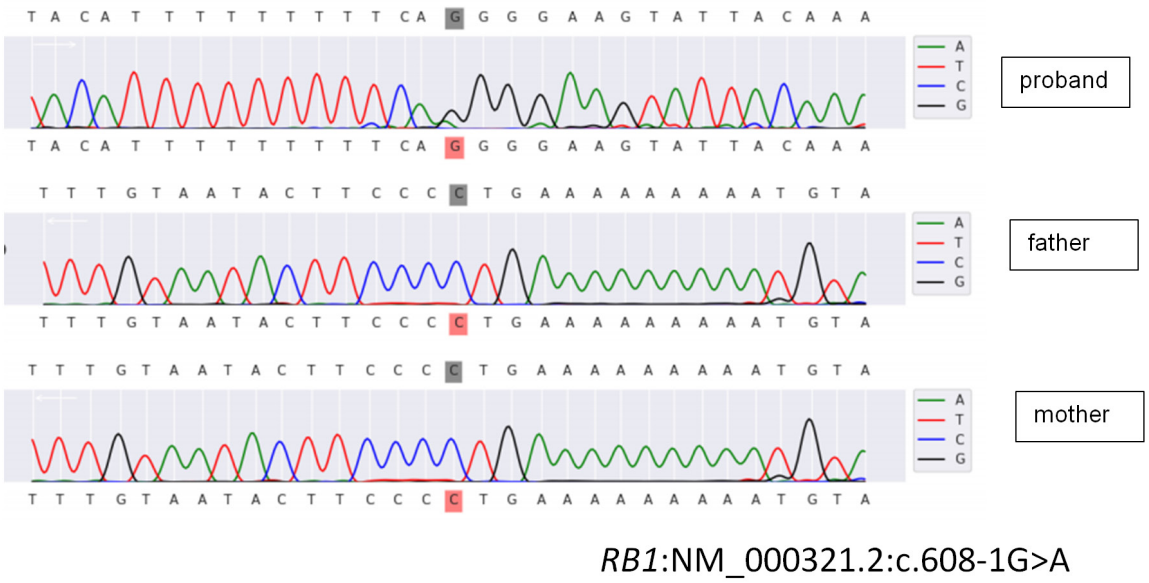

Figure 3. Genetic analysis of the *RB1* gene in Case 6 shows that the family (father, mother, proband) has one trace chimeric mutation. Arrows indicate the location of the mutation.

In our study, mutations were also identified in exons 8 and 18, consistent with global reports. Notably, among the five novel variants, the proportion of complex mutations appeared relatively higher in Chinese patients, although further population-based studies are needed to confirm this trend.

Conclusion

This study provides a comprehensive analysis of the genotype-phenotype correlations in 15 children with RB1 mutations, highlighting the genetic heterogeneity and clinical diversity of RB. Our findings expand the mutation spectrum of the RB1 gene in the Chinese population and underscore the utility of molecular testing in early diagnosis, individualized treatment planning, and genetic counseling. Future studies should further investigate how specific mutation types influence clinical presentation, treatment response, and long-term prognosis in pediatric RB.

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Disclosure of conflict of interest

None.

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References

[1] Cruz-Gálvez CC, Ordaz-Favila JC, Villar-Calvo VM, Cancino-Marentes ME and Bosch-Canto V. Retinoblastoma: review and new insights. Front Oncol 2022; 12: 963780.

[2] Luo Y, Zhou C, He F, Fan J, Wen X, Ding Y, Han Y, Ding J, Jin M, Liu Z, Wang S, Han M, Yuan H, Sun H, Xiao Y, Wu L, Wang J, Li Y, Yang H, Yu J, Gong J, Xu Y, Wen Y, Gao Z, Mei L, Ye J, Liu H, Chen Z, Xue S, Liu R, Chen H, Lu W, Liao H, Guo Q, Cui J, Zhu D, Lu F, Tang S, Wu Y, Yangkyi T, Guanghong Z, Wubuli M, Huiyu G, Wang X, He Y, Sheng X, Wang Q, Tan J, Liang J, Sun X,

Zhang J, Ji X, Jin L, Zhao J, Yang X, Jia R and Fan X. Contemporary update of retinoblastoma in China: three-decade changes in epidemiology, clinical features, treatments, and outcomes. Am J Ophthalmol 2022; 236: 193-203.
[3] Tan RJD. Clinical features, treatment, and outcomes of retinoblastoma in China. Asian J Oncol 2022; 8: 127-135.
[4] Atima MO, Idakwo U, Komolafe O, Eisuke S, Shintaro N, Balogun EO, Dingwoke EJ, Orugun AJ, Ukumobe KO, Pam JD and Aladiuba A. Presentation pattern and survival rate of retinoblastoma following chemotherapy: a prospective study. BMC Pediatr 2023; 23: 538.
[5] Chahin S, Morse M, Qaddoumi I, Phipps S, Crabtree VM, Brennan RC, Wilson MW, Rodriguez-Galindo C, Russell KM, Parris K, Goode K and Willard VW. An exploratory study of sleep habits in school-aged survivors of retinoblastoma. Sleep Med 2023; 103: 123-130.
[6] Lan X, Xu W, Tang X, Ye H, Song X, Lin L, Ren X, Yu G, Zhang H and Wu S. Spectrum of RB1 germline mutations and clinical features in unrelated Chinese patients with retinoblastoma. Front Genet 2020; 11: 142.
[7] Yao Y, Gu X, Xu X, Ge S and Jia R. Novel insights into RB1 mutation. Cancer Lett 2022; 547: 215870.
[8] Li L, Li H, Zhang J, Gan H, Liu R, Hu X, Pang P and Li B. Five novel RB1 gene mutations and genotype-phenotype correlations in Chinese children with retinoblastoma. Int Ophthalmol 2022; 42: 3421-3430.
[9] Zou Y, Li J, Hua P, Liang T, Ji X and Zhao P. Spectrum of germline mutations in RB1 in Chinese patients with retinoblastoma: application of targeted next-generation sequencing. Mol Vis 2021; 27: 1-16.
[10] Akdeniz Odemis D, Kebudi R, Bayramova J, Kilic Erciyas S, Kuru Turkcan G, Tuncer SB, Sukruoglu Erdogan O, Celik B, Kurt Gultaslar B, Buyukkapu Bay S, Tuncer S and Yazici H. RB1 gene mutations and genetic spectrum in retinoblastoma cases. Medicine (Baltimore) 2023; 102: e35068.
[11] Davies HR, Broad KD, Onadim Z, Price EA, Zou X, Sheriff I, Karaa EK, Scheimberg I, Reddy MA, Sagoo MS, Ohnuma SI and Nik-Zainal S. Whole-genome sequencing of retinoblastoma reveals the diversity of rearrangements disrupting RB1 and uncovers a treatment-related mutational signature. Cancers (Basel) 2021; 13: 754.
[12] Chai P, Luo Y, Yu J, Li Y, Yang J, Zhuang A, Fan J, Han M and Jia R. Clinical characteristics and germline mutation spectrum of RB1 in Chinese patients with retinoblastoma: a dual-center study of 145 patients. Exp Eye Res 2021; 205: 108456.
[13] Wu S, Zou X, Sun Z, Zhu T, Wei X and Sui R. Unilateral retinocytoma associated with a variant in the RB1 gene. Mol Genet Genomic Med 2020; 8: e1156.
[14] Yi XQ, Qian J, Guo J and Xue K. Clinical features of patients with retinocytoma. Zhonghua Yan Ke Za Zhi 2021; 57: 526-530.
[15] Mehyar M, Mosallam M, Tbakhi A, Saab A, Sultan I, Deebajah R, Jaradat I, AlJabari R, Mohammad M, AlNawaiseh I, Al-Hussaini M and Yousef YA. Impact of RB1 gene mutation type in retinoblastoma patients on clinical presentation and management outcome. Hematol Oncol Stem Cell Ther 2020; 13: 152-159.
[16] Kiet NC, Khuong LT, Minh DD; Nguyen The Vinh; Quan NHM, Xinh PT, Trang NNC, Luan NT, Khai NM and Vu HA. Spectrum of mutations in the RB1 gene in Vietnamese patients with retinoblastoma. Mol Vis 2019; 25: 215-221.
[17] Chen Z, Moran K, Richards-Yutz J, Toorens E, Gerhart D, Ganguly T, Shields CL and Ganguly A. Enhanced sensitivity for detection of low-level germline mosaic RB1 mutations in sporadic retinoblastoma cases using deep semiconductor sequencing. Hum Mutat 2014; 35: 384-391.
[18] Rojanaporn D, Chitphuk S, Iemwimangsa N, Chareonsirisuthigul T, Saengwimol D, Aroonroch R, Anurathathapan U, Hongeng S and Kaewkhaw R. Germline RB1 mutation in retinoblastoma patients: detection methods and implication in tumor focality. Transl Vis Sci Technol 2022; 11: 30.