Screening of *RB1* gene mutations in Chinese patients with retinoblastoma and preliminary exploration of genotype–phenotype correlations

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**Purpose:** Retinoblastoma (RB) sets the paradigm for hereditary cancer syndromes, for which medical care can change depending on the results of genetic testing. In this study, we screened constitutional mutations in the *RB1* gene via a method combining DNA sequencing and multiplex ligation-dependent probe amplification (MLPA), and performed a preliminary exploration of genotype–phenotype correlations.

**Methods:** The peripheral blood of 85 retinoblastoma probands, including 39 bilateral and 46 unilateral, was collected, and genomic DNA was extracted. DNA sequencing was conducted first. MLPA analysis was applied for patients with bilateral RB with negative sequencing results and unilateral probands whose age at diagnosis was less than 1 year old.

**Results:** Thirty-four distinct mutations were identified in 40 (47.1%) of the 85 probands (36 bilateral and four unilateral), of which 20% (8/40) was identified by MLPA. The total detection rate in bilateral cases was 92.3% (36/39). Of the total mutations identified, 77.5% (31/40) probands with a mean age of 10.7 months at diagnosis had null mutations, and 22.5% (9/40) with a mean age of 13.5 months at diagnosis had in-frame mutations. Of the 31 probands with null mutations, bilateral RB accounted for 96.8% (30/31). Of the nine probands with in-frame mutations, 66.7% had bilateral RB. There were seven new mutations of *RB1* identified in this report, including six null mutations and one missense mutation. Clinical staging of the tumor did not show obvious differences between patients with null mutations and in-frame mutations.

**Conclusions:** Our results confirm that the type of mutation is related to age of onset and the laterality, but not staging of the retinoblastoma tumor. MLPA is a reliable method for detecting gross deletion or duplication of the *RB1* gene. The combination of sequencing and MLPA improves the clinical diagnosis of RB.

Retinoblastoma (RB; OMIM 180200) is the most frequent primary intraocular malignant tumor in children, probably arising from cone precursor cells [1]. RB mainly affects children under 6 years old, with an incidence rate of 1 case per 15,000 to 20,000 live births [2,3]. According to the “two-hit” hypothesis, RB includes hereditary and nonhereditary forms, resulting from the mutation of both alleles of the *RB1* gene (Gene ID: 5925) [4,5]. Approximately 40% of the cases (including all bilateral and 15% of unilateral RB) are heritable, carrying a germ-line *RB1* mutation transmitted as an autosomal dominant trait with 90% penetrance [6,7]. The other 60% of cases are non-heritable RB (85% unilateral RB), caused by the inactivation of both alleles in the developing retina [8].

The *RB1* gene was the first tumor-suppressor gene found, located in 13q14.2, and the whole DNA length is 183 kb. Mutations in the *RB1* gene are highly heterogeneous and scattered in the promoter and the 27 coding exons. To date, more than 1,600 distinct mutations, ranging from small mutations to large deletions, have been registered in the *RB1* Gene Mutation Database. In developed countries, *RB1* gene testing has already been applied as a routine examination in RB probands [9,10], since the detection of *RB1* mutations provides evidence for genetic counseling and clinical management. However, molecular diagnosis is still in its early stages in China. A search of the PubMed and Chinese databases, including CNKI (China National Knowledge Infrastructure) and WanFang data, revealed several studies regarding *RB1* alterations in Chinese patients with RB [11-21]. However, these studies mainly focus on pedigree or have a small sample size, with no definitive analysis of mutational characteristics and the correlation between genotype and phenotype. Therefore, our cooperative group, which is based on encouraging treatment outcomes of patients with RB reported in a
previous article [6], developed the molecular detection project to further enhance the level of clinical management of retinoblastoma. We have already reported several RB1 alterations by using DNA sequencing with a detection rate of 78.6% in bilateral RB [22], which had not been capable of screening all RB1 variations. Here, we demonstrate that combining DNA sequencing and multiplex ligation-dependent probe amplification (MLPA) for detecting the mutation spectrum of RB1 allows for the preliminary exploration of genotype–phenotype correlations.

METHODS

Patients: This study recruited a total of 85 unrelated RB probands, including 37 (43.5%) boys and 48 (56.5%) girls, who were diagnosed between January 2012 and October 2013 in a Chinese cooperative group consisting of the Children’s Hospital of Fudan University and the Eye & ENT Hospital of Fudan University. The genetic testing results, using Sanger sequencing, of 35 of these patients were reported in a previous paper published in Chinese [22], but new findings emerged using the complementary MLPA method. The combined results are discussed in the present paper. The patients included 39 cases of bilateral RB and 46 cases of unilateral RB. Clinical features, including laterality, age at diagnosis, and International Classification of Intraocular Retinoblastoma (ICRB) staging at diagnosis (Appendix 1), were recorded. After the study protocol was approved by the Institutional Review Board of Fudan University, informed consent for genetic testing was obtained from the guardians of children affected with RB. The study protocol was in accordance with the provisions of the Declaration of Helsinki and the ARVO (the Association for Research in Vision and Ophthalmology) statement on human subjects. Peripheral blood samples (3 ml) were collected from the probands in EDTA (EDTA) anticoagulant tubes. Blood samples were stored at −20 °C until DNA was extracted.

RB1 gene sequencing: Genomic DNA was isolated from peripheral blood leukocytes using the QIAamp DNA Blood Mini Kit (Qiagen, Düsseldorf, Germany), according to the manufacturer’s instructions. Extracted DNA was measured with spectrophotometry (NanoDrop 2000; Thermo Scientific, Wilmington, DE) to ensure a concentration of 40 ng/µl.

Primers were designed according to a previous study by Abouzeid et al., with some modifications, using the Oligo software (National Biosciences, Plymouth, MN) [23]. Twenty-four pairs of primers were generated, which covered all coding exons and at least 50 bp flanking intronic sequences of each exon. PCR reactions were performed in a thermal cycler (ABI 2700), in a total volume of 10 µl containing 40 ng of genomic DNA, 2 picomoles of each primer, 0.2 µl of Taq DNA polymerase, and 1 µl of 10× buffer. Dimethyl sulfoxide (DMSO, 10%) was added to the exon 1 amplification system. Reactions were performed for the first 14 cycles of denaturation at 95 °C for 30 s, annealing at a specific temperature gradient (65 °C, −0.5 °C per cycle) for 30 s, extension at 72 °C for 40 s; the second 20 cycles of denaturation at 95 °C for 30 s, annealing at 58 °C for 30 s, extension at 72 °C for 40 s, and a final extension step at 72 °C for 5 min. The PCR products were qualified with agarose gel electrophoresis.

After the unincorporated deoxynucleoside triphosphates and primers were removed using Exonuclease I and shrimp alkaline phosphatase, the PCR products were directly sequenced using the ABI BigDye Terminator v 3.1 Sequencing Standard Kit (Carlsbad, CA) and run on an ABI 3500 Genetic Analyzer. The sequence data were analyzed by comparison to the consensus sequence of the RB1 gene (NM_000321), using Mutation Surveyor (V 4.0) software (SoftGenetics; State College, PA). Additional information was obtained from the literature, the RB1 Gene Database (LOVD, 2010), the Human Gene Mutation Database (HGMD, 2007), and the Thousand Genomes Project (Thousand Genome Consortium, 2010). The functional consequences of the mutations were predicted by bioinformatics tools, SIFT, and PolyPhen-2.

Multiplex ligation-dependent probe amplification analysis: MLPA analysis was applied in bilateral probands who did not show germ-line mutations in DNA sequencing and in unilateral probands whose guardians demanded additional genetic examinations, considering the earlier age at diagnosis, which was less than 1 year old, although the DNA sequencing results had been negative. Gross deletion and duplication of RB1 were examined using methylation-specific (MS)-MLPA analysis, the commercially available SALSA MLPA P047 RB1 probemix (MRC-Holland, Amsterdam, The Netherlands). This P047-C1 RB1 probemix contains probes for 26 of the 27 RB1 exons and 13 reference probes. This method was used to detect loss of heterozygosity (LOH) status of the RB1 gene located on 13q14. The procedure was based on the manufacturer’s protocol. Each MS-MLPA reaction was subjected to further analysis on an ABI 3730 automated sequencer (Applied Biosystems). The data were analyzed with GeneMarker v1.91 (SoftGenetics) according to the manufacturer’s instructions. The relative value of probes compared to the reference control was calculated. Threshold ratios for deletion and duplication were set at 0.75 and 1.35, respectively. The peak ratios for all target loci in a 1:1 ratio indicated normal copy numbers. The reduced or amplified
signal of probes with <0.75 or >1.35 indicated loss or gain, respectively, of the copy number in these probe loci.

Statistical analysis: Data were analyzed using SPSS software (version 16.0, Chicago, IL). Statistical analysis was performed with one-way ANOVA, the chi-square test, and Fisher’s exact test. \( p < 0.05 \) was considered to indicate statistical significance.

RESULTS

Patients included 39 (45.9%) bilateral and 46 (54.1%) unilateral cases, none of whom had a family history of RB. The age at diagnosis ranged from 1 to 72 months, with a median age of 11.1 months for patients with bilateral RB and 27.1 months for patients with unilateral RB. By combining the two different approaches, we discovered 34 distinct mutations (47.1%) in 40 probands (36 bilateral and four unilateral), of which 20% (8/40) was identified by MLPA. The ability to identify a germ-line mutation from blood samples of patients with bilateral RB was 92.3% (36/39). Of the total mutations identified (Table 1) in 40 patients, eight (20%) had gross deletions involving multiple exons or the whole gene, 17 (42.5%) had nonsense mutations, four (10%) had small deletions or insertions, two (5%) had splice mutations, and nine (22.5%) of patients had missense mutations, all of which were heterozygous mutations, except one homozygous missense mutation, c.2455C>G. The details of each group are as follows.

Null mutation: A null mutation is a pathogenic mutational type, which refers to those with mRNA harboring a premature translational-termination codon, including whole allele deletions, deletions involving one or more exons, and splice mutations, small frame-shifting intraexonic insertions and deletions, and nonsense mutations [24,25]. Our group identified 25 distinct null mutations in 31 (36.5%) of the 85 patients (Table 1). Of these mutations, the c.1735C>T mutation was a recurrent nonsense mutation identified in three patients. Six null mutations had not been reported before. The 31 patients with RB with an average age of 10.7 months at diagnosis included one patient (3.2%) with unilateral RB and 30 patients (96.8%) with bilateral RB (Table 2). Of the 61 eyes studied in this group, 20 (32.8%) presented with group E ICRB followed by groups D, A/B, and C ICRB, and one (6.7%) presented with extraocular extension (Table 3).

Negative genetic testing results: This group represented patients with negative genetic testing results. There were 45 cases, including three (6.7%) bilateral and 42 (93.3%) unilateral. The mean age at diagnosis was 27.3 months. Of the 48 eyes, 23 (47.9%) presented with group E ICRB, followed by groups D/C, B, and A ICRB. Sixteen of the 48 (33.3%) presented with extraocular extension (Table 3).

DISCUSSION

Genotype–phenotype correlations and implications for genetic counseling: In our study, all mutations were classified based on the prediction whether the transcripts harbored a premature translational-termination codon. Transcripts from alleles of the RB1 gene with null mutations have been shown to be subject to degradation by nonsense-mediated decay (NMD) [25-27], resulting in no detectable protein. Therefore, a null mutation is a comparatively more severe mutation than an in-frame mutation. Of the 31 probands with null mutations, 96.8% had bilateral RB, while only 66.7% of the probands with in-frame mutations developed bilateral RB (Table 2). Early onset retinoblastoma was seen more with null mutations (average age at diagnosis was 10.7 months) than in-frame mutations (average age at diagnosis was 13.5 months). These results are consistent with reports from Canada and India [24,28], and confirm that null mutations tend to the severe phenotype (early onset and bilateral RB). However, ICRB staging, an additional clinical feature, did not seem to be correlated with genotype. Group E was the most frequent staging at diagnosis in our study, which had no remarkable differences between patients with null mutations and in-frame mutations. We believe that tumor staging might be more related to access to care rather than the type of mutation.

Interestingly, cases with negative genetic testing results, which were mostly non-hereditary (42 unilateral and 3 bilateral probands), seemed to present with more serious clinical staging at diagnosis, with 16 (33.3%) extraocular extension tumors. The finding that advanced tumors tend to occur in non-hereditary cases might be explained by two hypotheses. First, most of the hereditary cases were bilateral RB, and the growth of the retinoblastoma tumors in the two eyes was not completely synchronous. The first-discovered eye disease in bilateral probands likely contributed to early diagnosis of
| Mutation Group | Mutational Type | cDNA Change | Protein Location | Prediction (SIFT/ PolyPhen-2) | Status |
|---------------|----------------|-------------|-----------------|-----------------------------|--------|
| Null mutations | Nonsense | c.179T>A | p.L60X | Exon 2 | Novel, pathogenic |
| | Nonsense | c.224G>A | p.W75X | Exon 2 | Pathogenic |
| | Nonsense | c.751C>T | p.R251X | Exon 8 | Pathogenic |
| | Nonsense | c.963C>G | p.Y321X | Exon 10 | Pathogenic |
| | Nonsense | c.967G>T | p.E323X | Exon 10 | Pathogenic |
| | Nonsense | c.1072C>T | p.R358X | Exon 11 | Pathogenic |
| | Nonsense | c.1183C>T | p.Q395X | Exon 12 | Pathogenic |
| | Nonsense | c.1333C>T | p.R445X | Exon 14 | Pathogenic |
| | Nonsense | c.1363C>T | p.R455X | Exon 14 | Pathogenic |
| | Nonsense | c.1399C>T | p.R467X | Exon 15 | Pathogenic |
| | Nonsense | c.1424_170del | p.E48YfsX52 | - | Novel, pathogenic |
| | Frameshift | c.1954_1959dupA | p.V654MfsX14 | Exon 19 | Novel, pathogenic |
| | Frameshift | c.1947delG | p.L649fsX9 | Exon 19 | Novel, pathogenic |
| Splice | c.607+1G>C | - | Exon 6 | Pathogenic |
| | Splice | c.2490–1G>A | - | Exon 25 | Novel, Pathogenic |
| Gross deletion | - | Whole gene deletion (5) | - | Pathogenic |
| Gross deletion | - | Del exons 7–17 | - | Pathogenic |
| Gross deletion | - | Del exons 7–9 | - | Pathogenic |
| Gross deletion | - | Del exons 13–16 | - | Pathogenic |
| In-frame mutations | Missense | c.920C>T | p.T307I | Exon 9 | Tolerated/benign, pathogenic |
| | Missense | c.584G>T | p.W195L | Exon 6 | Damaging/probably damaging, pathogenic |
| | Missense | c.1468G>A | p.A490T | Exon 16 | Damaging/probably damaging |
| | Missense | c.1861C>A | p.R621S | Exon 19 | Tolerated/benign |
| | Missense | c.2455C>G | p.L819V | Exon 23 | Tolerated/benign |
| | Missense | c.1346G>A | p.G449E | Exon 14 | Damaging/probably damaging |

Note: Status includes pathogenic, probable pathogenic, and unknown significant based on MAF 0.001 (A)
| mutation group | mutational type | cDNA change | protein | location | prediction (SIFT/ PolyPhen-2) | status # |
|----------------|----------------|-------------|---------|---------|-------------------------------|---------|
| missense       | c.1981C>T      | p.R661W     | Exon 20 | damaging/probably damaging    | pathogenic |
| missense       | c.1332G>C      | p.Q444H     | Exon 13 | damaging/probably damaging    | probably pathogenic somatic |
| missense       | c.1994T>C      | p.L665P     | Exon 20 | damaging/probably damaging    | pathogenic |

* Arabic numbers in the parentheses show the occurrence times for each mutation. # Interpretation of mutations which refer to HGMD, LOVD and thousand genomes. Novel, pathogenic: Sequence variation is previously unreported and is of the type which is expected to cause the disorder; Unknown significant: Sequence variation is a missense mutation that alters an amino acid that is not evolutionarily conserved predicted by bioinformatics tools but is of the type which may or may not be causative of the disorder. MAF: minor allele frequency supported by 1000 genome database. #: Reported in our previous study published in Chinese;
another eye disease. Second, the growth processes of retinoblastoma tumors might have certain differences between patients with and without germ-line \(RB1\) mutation, resulting in faster tumor growth in probands with negative genetic testing. A recent study reported that a previously unrecognized subtype of retinoblastoma tumor with intact \(RB1\) alleles had high-level \(v\)-myc avian myelocytomatosis viral oncogene neuroblastoma derived homolog (MYCN) oncogene amplification, which might initiate retinoblastoma with intact \(RB1\) alleles [29]. This suggests that the underlying mechanism involved in the initiation or development of retinoblastoma tumor could be investigated in future studies.

Distribution of the \(RB1\) gene mutation: Our \(RB1\) mutational distribution results revealed that \(RB1\) mutations were scattered along the genomic sequence (Figure 1), lacking hot spots clustered in the important functional domains—A/B pocket regions. Richter et al. reported that 13 of 15 missense mutations of the \(RB1\) gene gathered in the A/B pocket and the intervening spacer region, confirming that in-frame mutations favored the critical regions [24]. However, only five of the nine missense mutations appeared in the A/B pocket domains in our study. All five missense mutations that appeared in the A/B pocket domains were identified in bilateral probands, while only one of the four missense mutations that appeared in other regions were identified in bilateral probands, suggesting that in-frame mutations in A/B pocket domains could be considered to have more complete penetrance. Mutations occurring in different regions along the \(RB1\) gene still need to be investigated in a study with an expanded sample size.

Incomplete detection rate: We reported a detection rate of 78.6% in our previous paper by using single DNA sequencing. Here, a method combining DNA sequencing and MLPA increased the detection rate to 92.3% in bilateral RB, which is consistent with an earlier study that reported that the \(RB1\) mutation detection rate was 92.6% via a combination of full sequencing and deletions/duplication analysis of \(RB1\) [30]. The remarkable enhancing of the effectiveness of detecting gross deletions attributable to MLPA was 20% (8/40), which is slightly higher than in previous studies [31]. However, \(RB1\) defects can also result from hypermethylation of the promoter region and different levels of mosaic mutations, as evidenced by reports that highly sensitive allele-specific PCR increased the sensitivity from 92.6% to 94.8% for detecting mosaic \(RB1\) mutations [30]. Detecting methylation and mosaic mutations of \(RB1\) would be interesting in future studies.

In conclusion, we developed the molecular detection project to optimize the clinical management of all patients with retinoblastoma. This study reports our results involving

| Groups                           | probands (no. and %) | mean age (months) | \(P\) value | laterality (no. and %) | \(P\) value |
|---|---|---|---|---|---|
|   |   |   |   | bilateral | unilateral |
| null mutation                  | 31 (34.5%)           | 10.7             |             | 30 (96.8%)         | 1 (3.2%)     | 0.008** |
| in-frame mutation              | 9 (10.6%)            | 13.5             | <0.001*     | 6 (66.7%)          | 3 (33.3%)    |         |
| negative genetic testing results | 45 (52.9%)          | 27.3             |             | 3 (6.67%)          | 42 (93.3%)   |         |
| total                          | 85                   | 19.8             |             | 39 (45.9%)         | 46 (54.1%)   |         |

*ANOVA was applied (\(F=22.97\)); there were significant difference between two groups (null versus in-frame, null versus neg, in-frame versus neg). **Chi-Square test was applied between null and in-frame (\(\chi^2=7.0251\)).

### Table 3. Clinical profile of retinoblastoma probands (staging).

| Groups                           | total no. of eyes | staging (no. and %) | A  | B  | C  | D  | E  | Extraocular |
|---|---|---|---|---|---|---|---|---|
|   |   |   |   |   |   |   |   |   |
| null mutation                  | 61 | 3 (4.9%) | 8 (13.1%) | 16 (26.2%) | 10 (26.4%) | 20 (32.8%) | 4 (6.6%) |
| in-frame mutation              | 15 | 1 (6.7%) | 1 (6.7%)  | 0           | 4 (26.7%)  | 8 (53.3%)  | 1 (6.7%)  |
| negative genetic testing results | 48 | 1 (2.1%) | 2 (4.2%)  | 3 (6.3%)   | 3 (6.3%)   | 23 (47.9%) | 16 (33.3%) |
| total                          | 124| 5 (4.0%) | 11 (8.9%) | 19 (15.3%) | 17 (13.8%) | 51 (41.1%) | 21 (16.9%) |

*Fisher’s exact was applied between null and in-frame (\(p=0.261\)); Additionally, mutations (including null and in-frame) were compared with negative testing. Chi-Square test was applied between the intraocular tumor (including staging A, B, C, D, E) and extra-ocular tumor (\(\chi^2=14.9696, p=0.000\)).
31 patients who had been identified with null mutations and nine patients identified with in-frame mutations. The different groups had different clinical outcomes, and the type of mutations was related to age of onset and laterality, but not related to staging of the tumor, which has implications for genetic counseling. In addition, 20% of the total mutations were detected with MLPA, which confirms that combining sequencing and MLPA is a convenient, reliable, and powerful method for evaluating RB1 gene mutations in patients with retinoblastoma.

APPENDIX I. RESULTS OF GENETIC TESTING AND CLINICAL FEATURES COVERING LATERALITY, AGE AT DIAGNOSIS, STAGING AT DIAGNOSIS.

To access the data, click or select the words “Appendix 1.”

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REFERENCES
1. Xu XL, Fang Y, Lee TC, Forrest D, Gregory-Evans C, Almeida D, Liu A, Jhanwar SC, Abramson DH, Cobrinik D. Retinoblastoma has properties of a cone precursor tumor and depends upon cone-specific MDM2 signaling. Cell 2009; 137:1018-31. [PMID: 19524506].
2. Stiller CA, Parkin DM. Geographic and ethnic variations in the incidence of childhood cancer. Br Med Bull 1996; 52:682-703. [PMID: 9039726].
3. Kivelä T. The epidemiological challenge of the most frequent eye cancer: retinoblastoma, an issue of birth and death. Br J Ophthalmol 2009; 93:1129-31. [PMID: 19704035].
4. Knudson AG. Mutation and cancer: statistical study of retinoblastoma. Proc Natl Acad Sci USA 1971; 68:820-9. [PMID: 5279523].
5. Friend SH, Bernards R, Rogelj S, Weinberg RA, Rapaport JM, Albert DM, Dryja TP. A human DNA segment with properties of the gene that predisposes to retinoblastoma and osteosarcoma. Nature 1986; 323:643-6. [PMID: 2877398].
6. Gao YJ, Qian J, Yue H, Yuan YF, Xue K, Yao YQ. Clinical characteristics and treatment outcome of children with intraocular retinoblastoma: a report from a Chinese cooperative group. Pediatr Blood Cancer 2011; 57:1113-6. [PMID: 21681922].
7. Bonanomi MT, Almeida MT, Cristofani LM, Odone FV. Retinoblastoma: a three-year-study at a Brazilian medical school hospital. Clinics 2009; 64:427-34. [PMID: 19488609].
8. Dimaras H, Kimani K, Dimba EA, Gronsdahl P, White A, Chan HS, Gallie BL. Retinoblastoma. Lancet 2012; 379:1436-46. [PMID: 22414599].
9. Garber JE, Offit K. Hereditary cancer predisposition syndromes. J Clin Oncol 2005; 23:276-92. [PMID: 15637391].
10. Robson ME, Storm CD, Weitzel J, Wollins DS, Offit K. American Society of Clinical Oncology policy statement update: genetic and genomic testing for cancer susceptibility. J Clin Oncol 2010; 28:893-901. [PMID: 20065170].
11. Choy KW, Pang CP, Yu CB, Wong HL, Ng JS, Fan DS, Lo KW, Chai JT, Wang J, Fu W, Lam DS. Loss of heterozygosity and mutations are the major mechanisms of RB1 gene inactivation in Chinese with sporadic retinoblastoma. Hum Mutat 2002; 20:408- [PMID: 12402348].

12. Chen CY, Xu CM, Du ZF, Chen XL, Ren GL, Zhang XN. A c.1363C>T (p.R455X) nonsense mutation of RB1 gene in a southern Chinese retinoblastoma pedigree. Genetic Testing and Molecular Biomarkers. 2010; 14:193-6. [PMID: 20059380].

13. Hung CC, Lin SY, Lee CN, Chen CP, Lin SP, Chao MC, Chiou SS, Su YN. Low penetrance of retinoblastoma for p.V654L mutation of the RB1 gene. BMC Genet 2011; 12:76- [PMID: 21615945].

14. Chen CP, Kao LY, Su YN, Chen M, Chang SD, Wang W. A 20.5-Mb germline deletion of 13q13.1→q14.3 and somatic mutations of the RB1 gene in an 8-year-old girl with unilateral retinoblastoma, developmental delay and mental retardation. Genet Couns 2011; 22:431-4. [PMID: 22303805].

15. Lau CS, Choy KW, Fan DS, Yu CB, Wong CY, Lam DS, Pang CP. Prenatal screening for retinoblastoma in Hong Kong. Hong Kong Med J 2008; 14:391- [PMID: 18840911].

16. Zhang Q, Minoda K, Zeng R, Wu Z, Xiao X, Li S, Zhang F. Exon-by-exon screening for RB germline mutations using Heteroduplex-SSCP analysis. Yan Ke Xue Bao. 1997; 13:5-11. [PMID: 11189328].

17. Huang Q, Dryja TP, Yandell DW. Distinct Rb gene point mutations in families showing low penetrance of hereditary retinoblastoma. Chinese Journal of Medical Genetics. 1998; 15:139-42. [PMID: 9621119].

18. Huang Q, Dryja TP, Yandell DW. Gene diagnosis and genetic counselling of Rb gene mutations in retinoblastoma patients and their family members. Chinese Journal of Medical Genetics. 1998; 15:65-8. [PMID: 9531640].

19. Du C, Gallie BL. Detection of RB1 mutations by using quantitative fluorescent multiplex PCR. Chinese Journal of Medical Genetics. 1999; 16:386-9. [PMID: 10581351].

20. Du C, Jiang Y, Gallie BL. Low-penetrance retinoblastoma due to exons 24 and 25 deletions in the RB1 gene. Chinese Journal of Medical Genetics. 2002; 19:370-4. [PMID: 12362308].

21. Zhang XL, Fu WL, ZhaoHX, Zhou LX, Huang JF, Wang JH. Molecular studies of loss of heterozygosity in Chinese sporadic retinoblastoma patients. Clin Chim Acta 2005; 358:75-80. [PMID: 15921673].

22. He MY, An Y, Li G, Qian J, Gao YJ. Characteristics of RB1 gene mutations in Chinese patients with retinoblastoma. Chinese Journal of Medical Genetics. 2013; 30:509-12. [PMID: 24078560].

23. Abouzeid H, Schorderet DF, Balmer A, Munier FL. Germline mutations in retinoma patients: relevance to low-penetration and low-expressivity molecular basis. Mol Vis 2009; 15:771-7. [PMID: 19390654].

24. Richter S, Vandezaande K, Chen N, Zhang K, Sutherland J, Anderson J, Han L, Panton R, Branco P, Gallie B. Sensitive and efficient detection of RB1 gene mutations enhances care for families with retinoblastoma. Am J Hum Genet 2003; 72:253-69. [PMID: 12541220].

25. Hentze MW, Kulozik AE. A perfect message: RNA surveillance and nonsense-mediated decay. Cell 1999; 96:307-10. [PMID: 10025395].

26. Frischmeyer PA, Dietz HC. Nonsense-mediated mRNA decay in health and disease. Hum Mol Genet 1999; 8:1893-900. [PMID: 10469842].

27. Culbertson MR. RNA surveillance. Unforeseen consequences for gene expression, inherited genetic disorders and cancer. Trends Genet 1999; 15:74-80. [PMID: 10098411].

28. Ali MJ, Parsam VL, Honavar SG, Kannabiran C, Vemuganti GK, Reddy VA. RB1 gene mutations in retinoblastoma and its clinical correlation. Saudi J Ophthalmol 2010; 24:119-23. [PMID: 23960888].

29. Rushlow DE, Mol BM, Kennett JY, Yee S, Pajovic S, Theriault BL, Prigoda-Lee NL, Spencer C, Dimaras H, Corson TW, Pang R, Massey C, Godbout R, Jiang Z, Zackenhous E, Paton K, Moll AC, Houdayer C, Raizis A, Halliday W, Lam WL, Boutros PC, Lohmann D, Dorsman JC, Gallie BL. Characterisation of retinoblastomas without RB1 mutations: genomic, gene expression, and clinical studies. Lancet Oncol 2013; 14:327-34. [PMID: 23498719].

30. Rushlow D, Piovesan B, Zhang K, Prigoda-Lee NL, Marchong MN, Clark RD, Gallie BL. Detection of mosaic RB1 mutations in families with retinoblastoma. Hum Mutat 2009; 30:842-51. [PMID: 19280657].

31. Gerdes T, Kirchhoff M, Lind AM, Vestergaard LG, Kjaergaard SE. Multiplex ligation-dependent probe amplification (MLPA) in prenatal diagnosis-experience of a large series of rapid testing for aneuploidy of chromosomes 13, 18, 21, X, and Y. Prenat Diagn 2008; 28:1119-25. [PMID: 19003800].

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