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Spectrum of mutations in the *RB1* gene in Vietnamese patients with retinoblastoma

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Purpose: Retinoblastoma (RB) is a rare childhood malignant disorder caused by the biallelic inactivation of the *RB1* gene. Early diagnosis and identification of carriers of heritable mutations in *RB1* can improve disease outcome and management. In this study, we present the spectrum of mutations in the *RB1* gene in Vietnamese patients with RB.

Methods: Tumor RNA from 50 probands with RB, including 12 bilateral and 38 unilateral cases, was extracted. cDNA, after reverse transcription, was sequenced to identify the RNA mutation of the *RB1* gene. At the genomic DNA level, mutational analysis of all *RB1* exons, exon–intron boundaries, and the promoter region was conducted using PCR and direct sequencing. Multiplex ligation-dependent probe amplification (MLPA) analysis was performed for patients for whom the first two results were negative. For patients for whom either the sequencing or MLPA results were positive for a tumor mutation, patients' and their parents' blood DNA was analyzed to determine the germline mutation.

Results: Forty-one different kinds of *RB1* tumor mutations were identified in 41 probands (82.0%), including 11 of 12 bilateral cases (91.7%) and 30 of 38 unilateral cases (78.9%). The majority of the detected mutations were nonsense (15 different kinds), followed by frameshift (11 kinds), and splice site mutations (nine kinds). Each splice site mutation was confirmed to create a deletion of the corresponding exon with RNA sequencing. The single promoter mutation c.-197G>A was reported previously; however, both missense mutations identified in exon 6 (c.601G>C: p.A201P) and exon 22 (c.2264T>C: p.F755S) were novel. Gross deletions were detected with MLPA in three probands. The detection rate of germline mutations in bilateral and unilateral cases with mutations were 81.8% and 30.0%, respectively. Only one father out of the 20 parents tested was positive for a germline mutation.

Conclusions: Mutations in the *RB1* gene in Vietnamese patients were heterogeneous and highly prevalent with pathogenic truncated mutations. With advancement in therapeutics, early detection of RB is important for eye salvation.

Retinoblastoma (RB) is the most common intraocular neoplasm of childhood with an estimated incidence of 1/15,000 to 1/20,000 live births [1], and is responsible for 5% of blindness and 1% of overall childhood cancer deaths, respectively [2]. The majority of RB cases are diagnosed by 3 years of age with the most common presenting sign of leukocoria, followed by strabismus and proptosis [3].

Most clinical phenotypes of RB can be explained by hereditary or non-hereditary mutational inactivation of the *RB1* (Gene ID: 5925; OMIM 614041) gene [4]. Hereditary disease, caused by a constitutional mutation of *RB1*, predisposes to RB and other cancers later in life, and is transmitted as an autosomal dominant trait with high penetrance (90%) [5]. The non-hereditary form of RB is initiated by acquisition of two somatic mutations in the *RB1* gene of retinal progenitor cells [6]. Genetic testing to identify *RB1* mutations is not only essential for defining hereditary and non-hereditary forms of the disease but also critical for developing strategic care and management of patients with RB post-surgery and in screening for mutation carriers in related family members [1,7]. Identification of carriers of *RB1* mutations can initiate important genetic counseling related to family planning and to the strategic reduction of risk for development of other types of cancers later in life. Moreover, in some special cases, due to the risk of developing RB during pregnancy, children who carry mutations can be purposely delivered prematurely to initiate early treatment of potential macular tumors [8].

Because of the immense disparities that exist between developed and developing countries in access to and quality

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of healthcare systems, mortality and morbidity rates related to RB are likewise disturbingly divergent in these separate settings. The mortality rates of RB in Asia are estimated to be around ten-fold higher than in Europe, Canada, and the United States (approximately 40% versus 3–5%) [9]. In addition, in developed countries, detection and clinical management of RB often occur much earlier than in developing countries, leading to higher rates of successful treatment and preservation of patients' vision in the former. Ideally, patients at high risk for RB should undergo clinical screening for tumors using imaging techniques beginning in the first year of life. Because of the complex nature and expensive cost of these screening procedures, determination of genetic carrier status and risk stratification are important in avoiding such unnecessary clinical screenings. Most previously published studies of RB in Vietnam focused on the clinical presentation and application of imaging techniques to the diagnosis of RB. In contrast, the aim of the present study was to identify the spectrum of mutations in the *RB1* gene in 50 unrelated Vietnamese patients with RB, and to demonstrate the importance of genetic testing in disease management of patients with RB in Vietnam.

METHODS

Patients: Samples for the study were collected from 50 unrelated patients with RB, diagnosed by using standard ophthalmologic and histological criteria, at the Eye Hospital of Ho Chi Minh City, Vietnam, between November 2014 and April 2016. Samples included 46 tumor and blood-matched samples (11 bilateral and 35 unilateral) and four tumor-only samples (one bilateral and three unilateral). Because RB is a childhood disease, written informed consents for mutation analyses were obtained from the next of kin, caretakers, or guardians on behalf of the children enrolled in this study. The study was adhered to the ARVO statement on human subjects and was approved by the ethics committee of the University of Medicine and Pharmacy at Ho Chi Minh City.

DNA and RNA isolation: Tumor samples: Tumor-rich areas of archived formalin-fixed, paraffin-embedded (FFPE) tissues containing at least 50% tumor cells were marked on a hematoxylin and eosin slide by a pathologist, before manual microdissection of a corresponding unstained FFPE tissue section using a 21G needle. DNA was extracted using the ReliaPrep™ FFPE gDNA Miniprep System kit (Promega, Madison, WI).

Blood samples: Fresh peripheral blood samples were collected from patients for the purposes of the study. Total genomic DNA was isolated from EDTA-treated blood using the ReliaPrep™ Blood gDNA Miniprep System (Promega).

RNA extraction: RNA was extracted from fresh tumor tissues using the RNeasy Mini Kit (Qiagen, Germantown, MD) following the manufacturer's protocol.

PCR and Sanger sequencing:

Reverse transcription PCR—Approximately 3–4 µg of RNA were reverse-transcribed into single-stranded cDNA using the PrimeScript™ 1st strand cDNA Synthesis Kit (Takara Bio, Shiga, Japan). All primers used in this study were newly designed. Five sets of overlapping primers were then used to amplify the 2,787 bp RNA coding region of *RB1* (Supplementary Table 1, Appendix 1). Exons 1–5 were amplified using a touchdown PCR protocol: initial denaturation at 95 °C for 5 min, and 15 cycles of 95 °C for 30 s, 60 °C for 30 s (with a decrease of 1 °C per cycle), 72 °C for 90 s, followed by 30 cycles of 95 °C for 30 s, 58 °C for 30 s and 72 °C for 90 s, and a final elongation of 72 °C for 10 min. PCR amplification of exons 5–27 involved initial denaturation at 95 °C for 5 min, then 45 cycles of 95 °C for 30 s, 58 °C for 30 s, 72 °C for 90 s, with a final elongation of 72 °C for 5 min. PCR products were checked for size and purity using 2% agarose gel electrophoresis.

Genomic PCR—Twenty-two sets of primers (Supplementary Table 2, Appendix 2) were used to amplify all 27 exons, their flanking regions, and the promoter of *RB1* in separate 25 µl reactions consisting of 1X PCR Buffer, 1.5 mM $MgCl_2$, 200 µM each dNTP, 0.5 U Taq Hot Start Polymerase (Takara Bio), 0.1 µM each forward and reverse primer, and 25–50 ng of genomic DNA. PCR involved initial denaturation at 98 °C for 5 min followed by 40 cycles of 98 °C for 10 s, 60 °C for 30 s, and 72 °C for 2.5 min, with a final elongation of 72 °C for 10 min. PCR products were checked for size and purity using 1.5% agarose gel electrophoresis.

Sequencing—PCR products were purified enzymatically using the ExoSAP IT™ PCR Product Cleanup Reagent (Thermo Scientific, Waltham, MA) for removal of excess primers and dNTPs before Sanger sequencing using the BigDye Terminator v3.1 Kit and the ABI 3500 Genetic Analyzer (Applied Biosystems, Foster City, CA). PCR fragments were sequenced and analyzed in both directions. The primers used for sequencing are listed in Appendix 1 and Appendix 2. Sequences were compared to the reference sequence of the *RB1* gene (GenBank accession number: NG_009009).

Blood DNA sequencing—DNA from the blood of patients with RB tumors found to contain potential mutations was PCR-amplified and sequenced to determine whether the mutations were somatic or germline. Only regions

Table 1. Clinical characteristics of RB patients.

Characteristics	Unilateral (n=38)	Bilateral (n=12)
Mean age (months)	26.6	18.3
Gender		
Male (n=30)	23	7
Female (n=20)	15	5
Side of RB		
Right eye	20	n/a
Left eye	18	n/a
Somatic mutation (n=50)		
Mutation (n. and %)	30 (78.9%)	11 (91.7%)
No mutation (n. and %)	8 (21.1%)	1 (8.3%)
Germline mutation (n=41)		
Mutation (n. and %)	9 (30.0%)	9 (81.8%)
No mutation (n. and %)	21 (70.0%)	2 (18.2%)

corresponding to the location of mutations found in the tumor were amplified and sequenced. If a germline mutation was confirmed, the patient's parents were counseled and tested for their carrier status.

MLPA analysis: Multiplex ligation-dependent probe amplification (MLPA) analysis was performed for probands with no detectable mutation after screening with the sequencing techniques. MLPA was performed using the SALSA MLPA P047 RB1 probemix (MRC-Holland, Amsterdam, the Netherlands) and 75 ng of DNA following the manufacturer's protocol. MLPA products were run on an Applied Biosystems 3500 Genetic Analyzer under standard settings. The results were visualized and analyzed using Coffalyser.Net software (MRC-Holland) according to the manufacturer's instructions.

RESULTS

Clinical characteristics of patients: All 50 patients with RB were diagnosed with advanced stage (group D or E) disease, 12 with bilateral and 38 with unilateral tumors, and underwent primary enucleation without any treatment before enucleation. Age at diagnosis ranged from 1 to 66 months, with an average age of 18.3 months for bilateral cases and 26.6 months for unilateral cases. In the unilateral cases, the distribution of left and right ocular lesions was similar (18 versus 20, respectively). A summary of cases and their clinical characteristics is shown in Table 1. These data are similar to other studies in which the bilateral cases were smaller in proportion to the unilateral cases, and were diagnosed earlier [10-12]. In the present study, the male to female ratio in general, unilateral, and bilateral RB was 30/20, 23/15, and 7/5, respectively. In the unilateral cases, the distribution of left and right ocular lesions was similar (18/20).

Somatic mutation spectrum of RB1: A total of 41 different somatic alterations in *RB1* were identified, including point mutations and chromosomal rearrangements (Figure 1 and Supplementary Table 3, Appendix 3). The majority of the point mutations, detected with Sanger sequencing, were nonsense mutations (15/41, 36.6%) followed by frameshift (11/41, 26.8%), splicing (9/41, 22.0%), missense (2/41, 4.9%), and promoter (1/41, 2.4%) mutations. Every splicing mutation created the corresponding exon deletion on RNA mutational analysis. MLPA detected three gross deletions (3/41, 7.3%), including deletion of exon 7, deletion of exon 17, and deletion of exons 6 to 10. Twenty-eight (56.0%) tumors carried biallelic mutations in *RB1* gene.

Germline mutations in RB1: Among 41 patients with mutations with matched tumor and blood samples, germline mutations were identified in nine out of the 30 (30.0%) unilateral RBs and nine out of the 11 (81.8%) bilateral RBs. There were nine mutations in coding regions and nine splice site mutations leading to a premature stop codon on RNA transcripts (Table 2). Ten parent-couples of patients who carried germline mutations agreed to undergo carrier status analysis. Genetic transmission was found in only one male parent out of the 20 subjects involving a heterozygous splice site mutation (father of patient ID RB-Y55). This individual refused to undergo an eye examination.

DISCUSSION

This is the first comprehensive study to describe the spectrum of somatic and germline mutations in *RB1* in Vietnamese patients with RB. To the best of our knowledge, only one previous study investigated the genetics of RB tumors in Vietnam; however, that analysis was limited to investigation of germline mutations in *RB1* [13]. In that study, Nguyen et al. reported a high frequency of germline mutations in unilateral (four out of nine) and bilateral (21 out of 25) cases [13].

In the present study, among the somatic mutations in *RB1*, nonsense and frameshift mutations were the most common ones likely causing truncation of the pRB protein. Similar results have been reported in other studies [14,15]. The mutation p.R358X in exon 11 was detected in four cases, suggesting that it is recurrent in Vietnamese patients with RB. This finding differs from that of a Chinese study of patients with RB in which the p.R579X in exon 18 was considered the most recurrent nonsense mutation [6]. Splice site mutations that disrupt RNA splicing play an important role in generating inactive pRB protein [16-18]. Accordingly, we found nine intronic mutations, all of which were associated with deletion of the involved exons, as revealed by sequencing of matched RNAs. All exon deletions induced shifts in the reading frame, and finally, introduction of a stop codon that would be expected to translate to truncation of pRB proteins without a C-terminal.

Almost all mutations detected in this study have been previously reported, except the two missense mutations identified in exon 6 (c.601G>C: p.A201P) and exon 22 (c.2264T>C: p.F755S). The first novel missense mutation c.601G>C (p.A201P) was found in the tumor and blood of a 21-month-old male infant with unilateral RB. The PolyPhen-2 score of this mutation is 1.0, suggesting a pathogenic ability; however, a functional study is needed to clarify this possibility. The other novel missense mutation, c.2264T>C (p.F755S), was found only in the tumor sample of a 42-month-old female child with unilateral RB. Codon 755 has been reported previously with the pathogenic p.F755N mutation [8]. In general, missense mutations contribute to only a small percentage of RB cases, and are usually related to low penetrance [19].

The cDNA screening analysis used in this study has some advantages. First, it can identify abnormalities that may occur during transcription. For example, in the tumor sample from patient RB-Y2, cDNA sequencing revealed substitution of exon 23 by a small segment of intron 22, but when the tumor

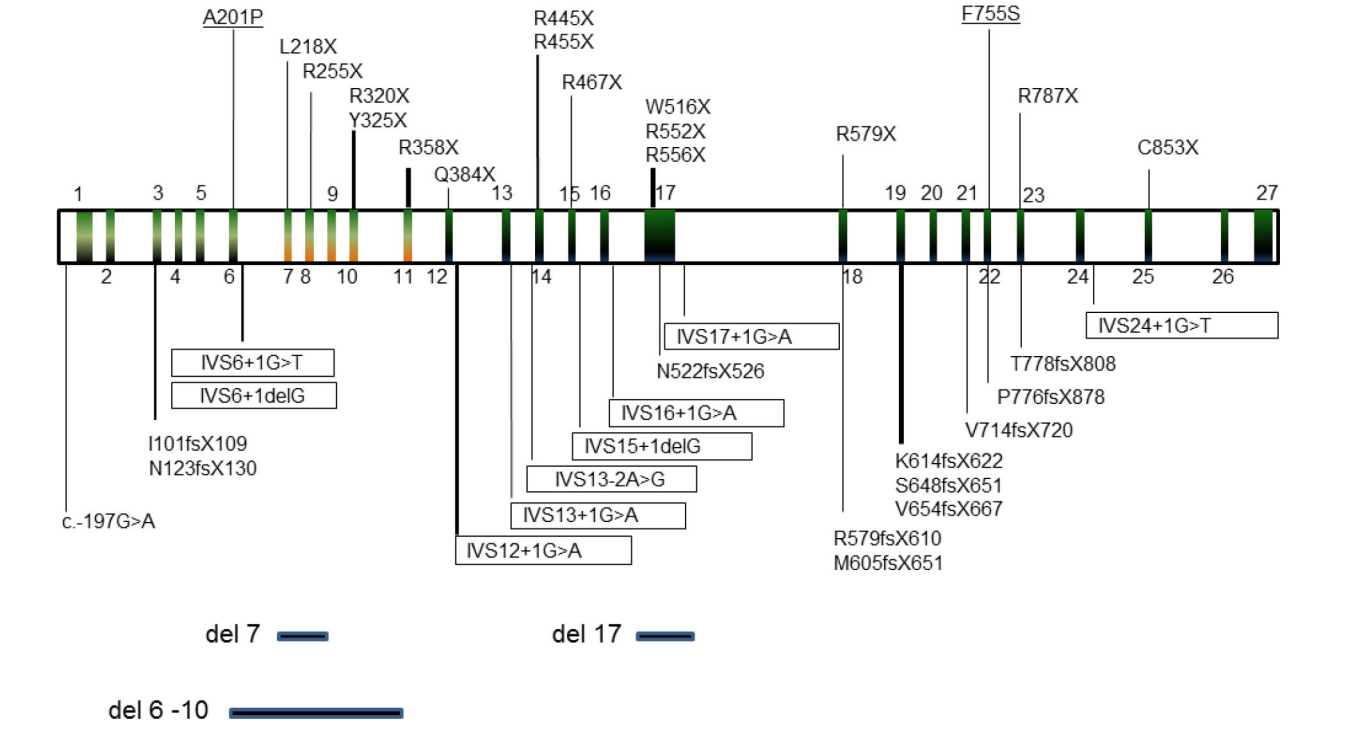

Figure 1. Distribution of mutations in *RB1* identified in this study. Gross deletions are represented as black lines. The underlined mutations are two missense novel mutations. Splicing mutations are indicated in squares. Nonsense mutations are above the coding sequence, while frameshift mutations are below.

Table 2. Germline mutations and parental carrier status of RB patients.

Patient ID	Patient	Father	Mother
RB-Y4 (Bilateral)	c.1399C>T	WT	WT
RB-Y5	c.1363C>T	ND	ND
RB-Y7 (Bilateral)	c.1150C>T	WT	WT
RB-Y13	c.2559delT	ND	ND
RB-Y14	IVS12+1G>A	WT	WT
RB-Y15 (Bilateral)	IVS12+1G>A	ND	ND
RB-Y16 (Bilateral)	IVS24+1G>T	WT	WT
RB-Y19	c.763C>T	ND	ND
RB-Y20	c.1940–1941delTT	ND	ND
RB-Y23 (Bilateral)	IVS13+1G>A	WT	WT
RB-Y26	c.601G>C	WT	WT
RB-Y30	c.1666C>T	ND	ND
RB-Y32 (Bilateral)	IVS6+1delG	WT	WT
RB-Y36 (Bilateral)	c.1841delA	WT	WT
RB-Y50	IVS12+1G>A	ND	ND
RB-Y53	IVS6+1G>T	ND	ND
RB-Y54 (Bilateral)	IVS15+1delG	WT	WT
RB-Y55 (Bilateral)	IVS13–2A>G	IVS13–2A>G (hetero-)	WT

WT, wild-type; ND, not done; hetero-, heterozygous

DNA was sequenced, we could not find any mutation in exons 22 to 24 and their flanking regions. Similar phenomena were observed in samples from patients RB-Y8, RB-Y27, RB-Y32, and RB-Y34 where mutations were detected in RNA, but not at the DNA level. One possible theory for this discordance in results is the damage of transcriptional machinery in cancer cells. Second, cDNA screening helps to predict the location of intronic mutations (Appendix 3). Curiously, cDNA sequencing showed wild-type *RB1* transcripts in samples from patients RB-Y1, RB-Y11, and RB-Y29, whereas gross deletions were detected in these samples using MLPA. It is possible that the wild-type transcripts could be downregulated in these samples due to genomic gross deletions. A well-designed real-time PCR can resolve this issue. Despite the advantages of combined RNA and DNA analyses in the present study, we did not investigate promoter methylation status and loss of heterozygosity. Consequently, the percentage of biallelic somatic mutations in this study was relatively low.

The ultimate goal of RB therapy is to ensure a high survival rate while minimizing adverse events and recurrence of the disease. Promising new therapies for RB, including platforms for intraocular delivery of drugs, have been developed in recent years [20]. However, additional disease management may be gained from genetic testing and effective genetic counseling of family members. Approximately 15% of individuals with sporadic unilateral retinoblastoma carry a germline mutation in *RB1* [21,22], and these individuals can develop RB in the remaining eye and transmit the RB predisposition to their offspring. Germline mutations in the present study comprised 9/38 (24%) of the mutations

in the unilateral cases, which is higher, although perhaps not significantly, than the average 15% generally noted in other studies. Similar increased rates of germline mutations in Vietnamese patients were noted in another study [13], possibly underscoring the added benefit of genetic testing and counseling for this population. Moreover, it has been shown that mutational screening of *RB1* of relatives at risk for RB is more cost-effective compared to conventional ophthalmological examinations [23]. Prenatal diagnosis could also be offered to parents in the future based on the first successful report of preimplantation genetic testing [24]; this preventive intervention appears promising for future disease management in families at risk for having children with inherited RB. Because nonsense mutations comprise the majority of reported point mutations in RB, a gene therapy approach, designed to allow read-through of premature termination codons, offers possible future targeted therapy for RB [25]. Information on mutational profiles of patients with RB will further aid in development of targeted therapeutics, ensuring effective disease management and life-long follow-up.

In conclusion, this study is the first comprehensive investigation of somatic and germline mutations in Vietnamese patients with RB. We described a wide spectrum of mutations, including two novel mutations that appear not to have been described previously. A relatively high rate of germline mutations in this cohort of patients with RB indicates the benefit that would occur for implementation of genetic testing and genetic counseling of families of Vietnamese patients with RB.

APPENDIX 1. PRIMERS FOR RT-PCR AND CDNA SEQUENCING.

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

APPENDIX 2. PRIMERS FOR GENOMIC PCR AND SEQUENCING.

To access the data, click or select the words "Appendix 2."

APPENDIX 3. SPECTRUM OF MUTATIONS IN *RB1* GENE IDENTIFIED IN RB TUMORS FROM VIETNAMESE PATIENTS.

To access the data, click or select the words "Appendix 3."

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REFERENCES

1. Lin P, O'Brien JM. Frontiers in the management of retinoblastoma. Am J Ophthalmol 2009; 148:192-8. [PMID: 19477707].
2. Donaldson SS, Smith LM. Retinoblastoma: biology, presentation, and current management. Oncol Williston Park N 1989; 3:45-51. [PMID: 2701417].
3. Subramaniam S, Rahmat J, Rahman NA, Ramasamy S, Bhoo-Pathy N, Pin GP, Alagaratnam J. Presentation of retinoblastoma patients in Malaysia. Asian Pac J Cancer Prev APJCP 2014; 15:7863-7. [PMID: 25292078].
4. Fletcher O, Houlston RS. Architecture of inherited susceptibility to common cancer. Nat Rev Cancer 2010; 10:353-61. [PMID: 20414203].
5. Naumova A, Sapienza C. The genetics of retinoblastoma, revisited. Am J Hum Genet 1994; 54:264-73. [PMID: 8304343].
6. Wiggs J, Nordenskjöld M, Yandell D, Rapaport J, Grondin V, Janson M, Werelius B, Petersen R, Craft A, Riedel K. Prediction of the risk of hereditary retinoblastoma, using DNA polymorphisms within the retinoblastoma gene. N Engl J Med 1988; 318:151-7. [PMID: 2892131].
7. Bamne MN, Ghule PN, Jose J, Banavali SD, Kurkure PA, Amare Kadam PS. Constitutional and somatic RB1 mutation spectrum in nonfamilial unilateral and bilateral retinoblastoma in India. Genet Test 2005; 9:200-11. [PMID: 16225399].
8. Richter S, Vandezande 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].
9. 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].
10. He M, An Y, Gao Y, Qian X, Li G, Qian J. Screening of RB1 gene mutations in Chinese patients with retinoblastoma and preliminary exploration of genotype–phenotype correlations. Mol Vis 2014; 20:545-52. [PMID: 24791139].
11. Mohd Khalid MKN, Yakob Y, Md Yasin R, Wee Teik K, Siew CG, Rahmat J, Ramasamy S, Alagaratnam J. Spectrum of germ-line RB1 gene mutations in Malaysian patients with retinoblastoma. Mol Vis 2015; 21:1185-90. [PMID: 26539030].
12. Ottaviani D, Parma D, Giliberto F, Ferrer M, Fandino A, Davila MT, Chantada G, Szijan I. Spectrum of RB1 mutations in argentine patients: 20-years experience in the molecular diagnosis of retinoblastoma. Ophthalmic Genet 2013; 34:189-98. [PMID: 23301675].

13. Nguyen HH, Nguyen HTT, Vu NP. Mutational screening of germline RB1 gene in Vietnamese patients with retinoblastoma reveals three novel mutations. Mol Vis 2018; 24:231-8. [PMID: 29568217].

14. Ahani A, Akbari MT, Saliminejad K, Behnam B, Akhondi MM, Vosoogh P, Ghassemi F, Naseripour M, Bahoush G, Khorshid HRK. Screening for large rearrangements of the RB1 gene in Iranian patients with retinoblastoma using multiplex ligation-dependent probe amplification. Mol Vis 2013; 19:454-62. [PMID: 23441118].

15. Nichols KE, Houseknecht MD, Godmilow L, Bunin G, Shields C, Meadows A, Ganguly A. Sensitive multistep clinical molecular screening of 180 unrelated individuals with retinoblastoma detects 36 novel mutations in the RB1 gene. Hum Mutat 2005; 25:566-74. [PMID: 15884040].

16. Parsam VL, Ali MJ, Honavar SG, Vemuganti GK, Kannabiran C. Splicing aberrations caused by constitutional RB1 gene mutations in retinoblastoma. J Biosci 2011; 36:281-7. [PMID: 21654082].

17. Schubert EL, Strong LC, Hansen MF. A splicing mutation in RB1 in low penetrance retinoblastoma. Hum Genet 1997; 100:557-63. [PMID: 9341870].

18. Zhang K, Nowak I, Rushlow D, Gallie BL, Lohmann DR. Patterns of missplicing caused by RB1 gene mutations in patients with retinoblastoma and association with phenotypic expression. Hum Mutat 2008; 29:475-84. [PMID: 18181215].

19. Valverde JR, Alonso J, Palacios I, Pestaña A. RB1 gene mutation up-date, a meta-analysis based on 932 reported mutations available in a searchable database. BMC Genet 2005; 6:53-[PMID: 16269091].

20. Mendoza PR, Grossniklaus HE. The Biology of Retinoblastoma. Prog Mol Biol Transl Sci 2015; 134:503-16. [PMID: 26310174].

21. Draper GJ, Sanders BM, Brownbill PA, Hawkins MM. Patterns of risk of hereditary retinoblastoma and applications to genetic counselling. Br J Cancer 1992; 66:211-9. [PMID: 1637670].

22. Lohmann DR, Gerick M, Brandt B, Oelschläger U, Lorenz B, Passarge E, Horsthemke B. Constitutional RB1-gene mutations in patients with isolated unilateral retinoblastoma. Am J Hum Genet 1997; 61:282-94. [PMID: 9311732].

23. Noorani HZ, Khan HN, Gallie BL, Detsky AS. Cost comparison of molecular versus conventional screening of relatives at risk for retinoblastoma. Am J Hum Genet 1996; 59:301-7. [PMID: 8755916].

24. Xu K, Rosenwaks Z, Beaverson K, Cholst I, Veeck L, Abramson DH. Preimplantation genetic diagnosis for retinoblastoma: the first reported liveborn. Am J Ophthalmol 2004; 137:18-23. [PMID: 14700639].

25. Wang X, Gregory-Evans CY. Nonsense suppression therapies in ocular genetic diseases. Cell Mol Life Sci CMLS 2015; 72:1931-8. [PMID: 25651836].

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