Subset of retinoblastoma tumours is associated with *BRCA1/2* mutations

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ABSTRACT

Background We investigated the potential association between pathogenic BRCA1/2 variants and retinoblastoma pathogenicity.

Methods In this single-centre, retrospective case series, we performed hereditary cancer panel tests using blood samples for patients with retinoblastoma diagnosed between March 2017 and October 2021. Bioinformatics prediction tools were then used to conduct in silico pathogenicity assessments for patients with *BRCA1/2* family variants, in addition to the American College of Medical Genetics and Genomics (ACMG) variant classification. One patient with a germline *BRCA1* variant was analysed with whole-genome sequencing (WGS), mutational signature analysis and methylation analysis for *RB1* and *BRCA* using the patient's tumour and blood samples.

Results Of 30 retinoblastoma patients who underwent panel sequencing, six (20%) were found to carry germline variants in the BRCA1/2 or BRIP1 genes. Among these six patients, two had pathogenic or likely pathogenic variants as per the ACMG variant classification. Additionally, three patients showed potential pathogenic BRCA1/2 family variants through further analysis with alternative bioinformatics prediction tools. In the WGS analysis of a tumour from a patient with a germline likely pathogenic *BRCA1* variant in one allele, we observed the loss of one RB1 allele due to a large deletion. No somatic non-synonymous mutations or frameshift indels were detected in the RB1 locus of the remaining allele. This sample also showed *BRCA1* gene promoter hypermethylation in the tumour, indicating additional epigenetic silencing.

Conclusion This study demonstrated that some retinoblastoma patients harboured germline *BRCA1/2* family variants, which may be associated with the development of retinoblastoma along with *RB1* mutations.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ RB1 mutation is the causal mutation for most hereditary retinoblastoma patients.

WHAT THIS STUDY ADDS

⇒ Some of the retinoblastoma patients carry BRCA1/2 family gene variants, and they may be linked with the development of retinoblastoma.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Further studies should aim at retinoblastoma patients with BRCA1/2 variants in a larger population to clarify the relationship between the two.

INTRODUCTION

Retinoblastoma is the most common primary intraocular malignancy in children. The initiating genetic event of retinoblastoma is known to be inactivation of the *RB1* tumour suppressor gene. In germinal or heritable retinoblastomas, the first *RB1* gene is mutated in essentially all cells and the second *RB1* gene is mutated in retinal cells that develop into retinoblastoma. Germinal retinoblastoma accounts for approximately 45% of patients with bilateral (80%), unilateral (15%) or trilateral (5%) tumours, whereas the remaining 55% of patients lack germline *RB1* mutation.[1] Nearly 3% of non-hereditary tumours have active *RB1* alleles (RB^+/RB^+), and approximately half of RB^+/RB^+ tumours exhibit *MYCN* oncogene amplification.[2] The pathogenesis of retinoblastoma patients without inactivation of both *RB1* alleles remains unclear. Some of the less common alterations in gene copy numbers reported in retinoblastoma include gains in the oncogenes *MDM4*, *KIF14*, *MYCN*, *DEK* and *E2F3*, and loss of the tumour suppressor gene *CDH11*.[3]

BRCA1/2 gene mutations are associated with familial breast and ovarian cancers, and 13%–40% of sporadic malignancies are associated with loss of *BRCA1* expression.[4 5] These genes encode components of the DNA damage response, and mutations in these genes reduce the ability to repair DNA damage and increase the potential for gene mutations.[6] A recent study reported that patients with prostate cancer with coinactivation of *BRCA2* and *RB1* were more likely to have tumours with aggressive phenotypes and worse prognosis.[7]

In the present study, we assessed the pathogenicity of *BRCA* family genes with bioinformatics tools and analysed the clinical characteristics of patients with retinoblastoma with and without *BRCA* family variants based on the finding that approximately 20% of patients with retinoblastoma harboured germline variants of *BRCA1/2* or BRCA1-interacting helicase 1 (*BRIP1*) genes in a hereditary cancer panel sequencing test. Additional whole-genome sequencing (WGS) and promoter methylation status analyses were performed using both blood and tumour samples from a patient harbouring a germline *BRCA1* variant.

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METHODS

Study approval

This study was a single-centre, retrospective observational case series. It adhered to the tenets of the Declaration of Helsinki and received approval from the Institutional Review Board of Severance Hospital (IRB no. 4-2021-1675). Informed consent was obtained from participants or their guardians before enrolling them in this study.

Study participants

We identified 30 patients with retinoblastoma who underwent the hereditary cancer panel screen with blood samples at Severance Hospital from March 2017 to October 2021 and then conducted a retrospective chart review of demographic and clinical data for these patients. Clinical information with imaging results was collected from the medical records. The demographic and clinical data included age, sex, laterality, Reese-Ellsworth (RE) and International intraocular retinoblastoma classification (IIRC) categories, mode and number of treatments (systemic chemotherapy, intra-arterial chemotherapy, intravitreal injection, transpupillary thermotherapy, laser photocoagulation, external cryotherapy, vitrectomy, external-beam radiotherapy and enucleation) and prognostic factors including recurrence and metastasis. Demographic and clinical characteristics of patients with BRCA1/2 or BRIP1 variant were compared with those without BRCA1/2 or BRIP1 variant.

Hereditary cancer panel sequencing

Genomic DNA extracted from the individual's sample was used for library preparation and target capture using a customised hereditary cancer panel assessing 60 candidate genes (online supplemental table 1). Massive parallel sequencing was performed on the MiSeq System (Illumina). BaseSpace (Illumina) and NextGENE (SoftGenetics) software were used for quality control and sequence analysis, and the results were cross checked with a custom analysis pipeline. A customised analysis pipeline was used for copy number analysis. GRCh37 (hg19) was used as the reference sequence for mapping and variant calling. The Online Mendelian Inheritance in Man (http://www.omim.org), Human Gene Mutation Database (HGMD), ClinVar, dbSNP, 1000 Genome, Exome Aggregation Consortium (ExAC), Exome Sequencing Project (ESP) and Korean Reference Genome Database (KRGDB) databases were used for variant analysis and annotation. Variant classification followed the standards and guidelines established by the American College of Medical Genetics (ACMG).[8 9] All pathogenic and likely pathogenic variants were further confirmed by Sanger sequencing.

Whole-genome sequencing

The tumour sample was obtained by aspiration biopsy of vitreous using a 30-gauge needle in a patient with late-onset retinoblastoma exhibiting extensive vitreous seeding. Genomic DNA was extracted from the tumour sample and its matched peripheral blood sample. The amount of genomic DNA obtained from the aspirated sample was low. Therefore, we performed WGS of matched tumour-normal samples using the Illumina platform with Illumina TruSeq Nano DNA library/TruSeq DNA PCR-free library to generate a minimal 30×read depth. The Illumina platform generated raw images and base calling through integrated software (real-time analysis), and the binary base call data were converted into FASTQ using the Illumina package bcal2fastq2 (V.2.20.0). After the matched tumour-normal samples were sequenced, the raw sequence data in FASTQ format were trimmed to remove adapter sequences and then aligned against human reference genome UCSC assembly hg19 (original GRCh37 from NCBI, February 2009) for further analysis (iSAAC-04.18.11.09). Somatic single-nucleotide variants, short indels and structural variants, copy number aberrations and/or allelic imbalances were identified using Strelka (V.2.9.10) and Manta (V.1.5.0) softwares. Filtering criteria were applied to reduce the false-positive rate, and population variants and variants with quality warnings were identified. Tumour purity and ploidy were estimated using Sequenza algorithms.[10] The series of bioinformatics procedures is now organised as CancerVision (Genome Insight Inc).

Mutational signature analysis

Mutational signatures by linear decomposition were extracted using Mutalisk (http://mutalisk.org/).[11] Briefly, the relative contributions of mutational signatures were calculated by refitting mutational signatures from the Catalogue of Somatic Mutations in Cancer (COSMIC) database, including COSMIC signatures 1, 3, 5, 8 and 12 (https://cancer.sanger.ac.uk/cosmic/signatures). COSMIC signature 3 is associated with insufficient homologous recombination, and *BRCA1*-null cancers frequently display signature 3.

METHYLATION ANALYSIS OF *RB1* AND *BRCA1*

DNA methylation analysis of the late-onset retinoblastoma sample was performed using Infinium Human MethylationEPIC BeadChip (Illumina), and methylation levels were calculated using the R package methylumi (http://www.bioconductor.org/packages/release/bioc/html/methylumi.html). As reported previously, *RB1* and *BRCA1* promoters were defined as regions 5 kb upstream and 1 kb downstream from the transcriptional start site.[12] Twenty-one and 41 CpG loci were identified in *RB1* and *BRCA1* promoter regions, respectively. We used 80 cases of uveal melanoma from The Cancer Genome Atlas as references for this analysis. Hypermethylation of the probes in the promoter regions was defined as previously reported and the analysis pipeline of the report was used.[12] The gene was considered hypermethylated when there were more than four outlier probes for a specific promoter region. The results of whole genome sequencing and analysis of the RB1 and BRCA1 gene promoter methylation status have been provided in a separate supplemental file (.xlsx).

In silico pathogenicity assessment of variants

We evaluated the potential pathogenicity of *BRCA1/2* and *BRIP1* variants using various data prediction tools. Variant type, impact of the variants, sorting intolerant from tolerant (SIFT),[13] PolyPhen-2[14] and combined annotation-dependent depletion_Phred[15] scores were calculated using the annotation tools in Ensembl Variant Effect Predictor.[16] The PON-P2[17] score was calculated using a web tool (http://structure.bmc.lu.se/PON-P2/). The FATHMM-XF[18] score was calculated using a web server (http://fathmm.biocompute.org.uk/fathmm-xf/). The Exome Aggregation Consortium (ExAC),[19] Genome Aggregation Database (gnomAD),[20] and Korean Reference Genome Database (KRGDB)[21] were used for reference.

STATISTICAL ANALYSIS

Values are presented as mean±SD. Data were compared by applying independent t-tests, and non-parametric measures were used when sample sizes were too small. Differences were considered as significant at $p<0.05$. All statistical analyses were performed using IBM SPSS V.26 (SPSS, Chicago, Illinois).

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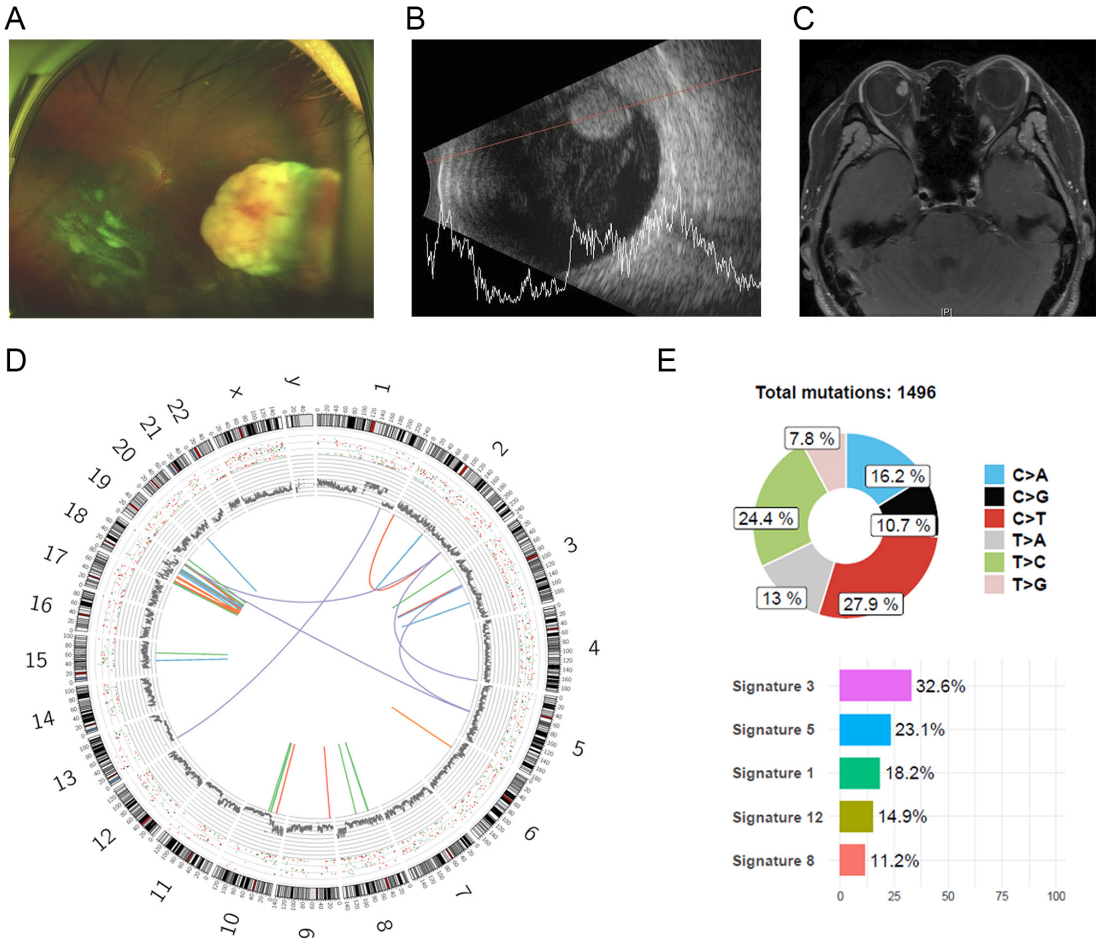

Figure 1 Clinical findings, whole-genome sequencing and mutational signature analysis of an 18-year-old patient with retinoblastoma (patient 5) with a *BRCA1* mutation and without an *RB1* mutation. (A) Wide-field fundus photo of the patient's eye with extensive vitreous seeding and whitish protruding mass. (B) B-scan ultrasonography of the patient's eye showing a well-defined mass with high echogenicity. (C) MRI of the patient's eye with a relatively hyperintense mass (7.74 mm × 6.62 mm) in the T1-weighted image. (D) Results of whole-genome sequencing. (E) Results of mutational signature analysis.

RESULTS

Next-generation sequencing of retinoblastoma in a atient harbouring germline *BRCA1* variant

An 18-year-old woman presented with a large whitish retinal mass with vitreous seeding of her right eye (figure 1). The mass was visualised as a well-defined hyperechoic tumour in B-scan ultrasonography. The mass was 7.74×6.62 mm and relatively hyperintense in T1-weighted MRI and was a low-signal mass in T2-weighted MRI, which was consistent with the diagnosis of retinoblastoma. The patient's peripheral blood sample was subjected to the hereditary cancer panel test targeting 60 genes (including *APC*, *ATM*, *BRCA1/2*, *NF1/2* and *PTEN* (online supplemental table 1)), which identified *BRCA1* (c.5339T>C), *AXIN1* (c.2218G>A) and *ATM* (c.323C>G) variants but no *RB1* mutation. Aspiration biopsy was performed at the vitreous cavity using a 30-gauge needle, and the sample was pathologically confirmed as retinoblastoma with SurePath liquid-based cytology.

The patient's tumour and peripheral blood samples were subjected to WGS. WGS of the germline cells detected the c.5339T>C p.(L1780P) variant in one *BRCA1* allele, which was consistent with the results of the hereditary cancer panel test but did not detect the *RB1* germline variant. In the WGS tumour analysis, the purity and ploidy were 0.8 and 2.4, respectively. One RB1 allele was lost in the tumour due to a large deletion, but no somatic non-synonymous mutations or frameshift indels were detected in the RB1 locus of the remaining allele.

WGS analysis of the tumour sample identified 1496 single-base substitutions, 194 indels and 175 breakpoints (online supplemental figure). Non-synonymous single-base substitutions were detected in 18 genes (*NADK*, *PRSS38*, *RRM2*, *NDUFS1*, *ALS2CL*, *PCDHB7*, *CFAP77*, *IRF7*, *OR10Q1*, *SYT7*, *SUDS3*, *ZFHX2*, *EXD1*, *ZNF106*, *SNX1*, *DPP8*, *GYS1* and *RPGR*). No frameshift indels or additional driver mutations were detected in the tumour sample. We also performed mutational signature analysis using the COSMIC V.3 compendium of mutational signatures. Mutational signatures (including COSMIC signature 3) that frequently occur in the *BRCA1*-mutated tumour were detected (online supplemental figure). Therefore, *BRCA1* appeared to have a role in the retinoblastoma tumorigenesis in this patient.

Assessment of CpG island methylation status in *RB1* and *BRCA1* promoter regions

We investigated the possibility of epigenetic silencing as WGS analysis showed that only single *RB1* and *BRCA1* alleles had mutations. The results of the methylation analysis are presented in figure 2. The *RB1* promotor is known to contain 21 CpG loci, and two of these CpG loci were hypermethylated in the

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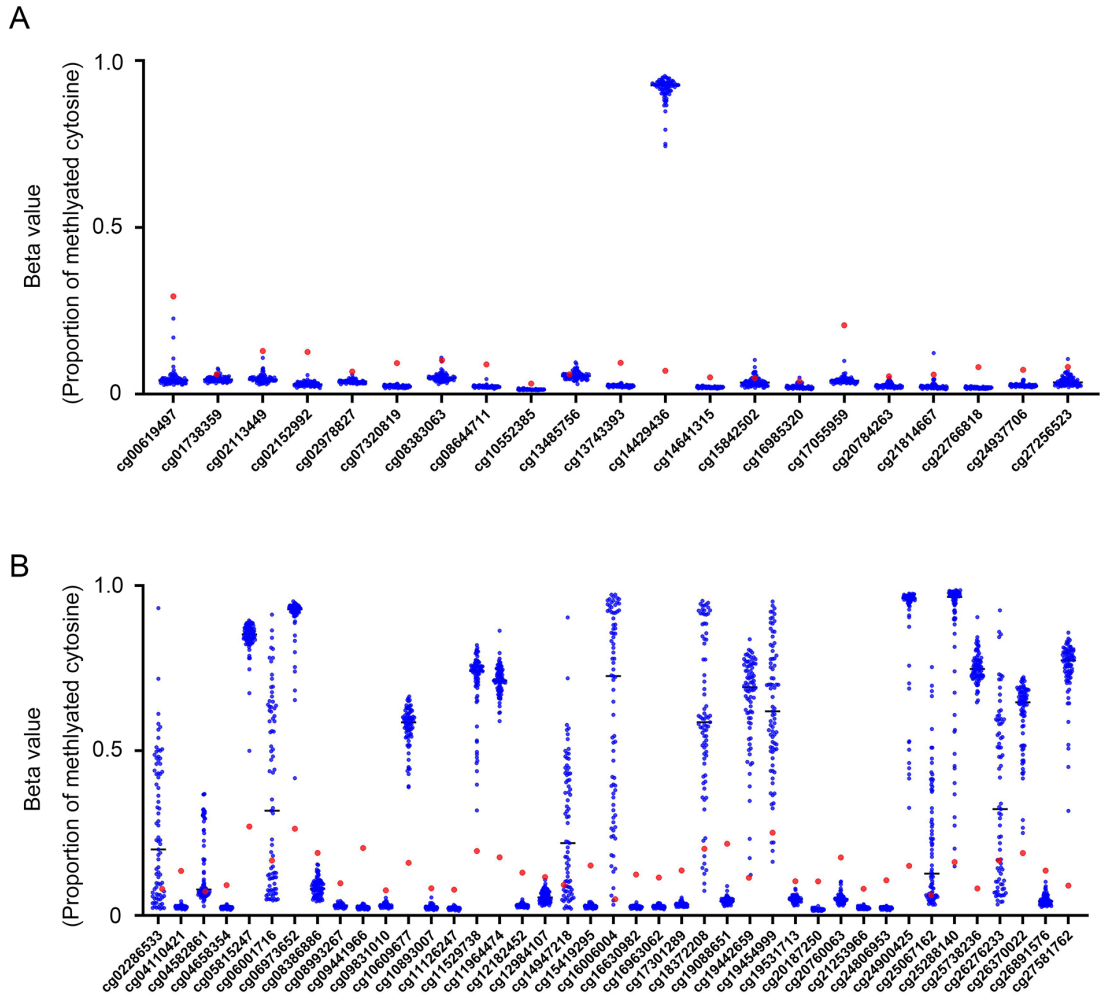

Figure 2 DNA methylation analysis of an 18-year-old patient with retinoblastoma (patient 5) with a *BRCA1* mutation and without an *RB1* mutation. (A) Methylation analysis of the *RB1* promoter region. Each item on the *x*-axis indicates a CpG island of the *RB1* promoter region. Red dots indicate the proportions of methylated cytosine residues, and blue dots indicate the proportions of 80 uveal melanoma cases (The Cancer Genome Atlas) used as reference. Two of these CpG loci were hypermethylated in the patient with retinoblastoma. (B) Methylation analysis of the *BRCA1* promoter region. Each item on the x-axis indicates a CpG island of the *BRCA1* promoter region. Red dots indicate the proportions of methylated cytosine residues, and blue dots indicate the proportions of 80 uveal melanoma cases (The Cancer Genome Atlas) used as reference. Six of these CpG loci were hypermethylated in the patient with retinoblastoma.

retinoblastoma sample. This indicates that although *RB1* exhibited a loss of heterozygosity (LOH), no biallelic inactivation might have occurred. The *BRCA1* promoter is known to contain 41 CpG loci, and 6 of these CpG loci were hypermethylated in the retinoblastoma sample. This suggests that additional epigenetic silencing occurred on the basal pathogenic mutation. The combined WGS and methylation status analysis results for this patient suggested that the retinoblastoma tumour was possibly caused by LOH of the *RB1* gene and *BRCA1* inactivation.

Germline mutation of *RB1* and *BRCA1/2* family genes in patients with retinoblastoma

We screened an additional 29 patients (30 total, including the patient whose case is presented) with retinoblastoma for *BRCA1/2* mutations by reviewing their hereditary cancer panel sequencing results. There were 9/30 bilateral patients, and none of the 30 patients had a family history of retinoblastoma. All nine bilateral retinoblastoma patients carried germline RB1 variants, of which five were pathogenic, three were likely pathogenic and one was of uncertain significance (8/9 pathogenic/likely pathogenic Rb1 variants, 88.9%). Of 21 unilateral retinoblastoma patients, 6 carried RB1 variants, of which four were pathogenic and two were likely pathogenic (6/21, 28.6%).

Variants in *BRCA1/2* or *BRIP1* were detected in 6 of 30 (20%) patients (table 1). Three of these six patients also had a mutation in *RB1*. One patient (patient 4) had *RB1*, *BRCA1* and *BRCA2* mutations. Two of the six patients with *BRCA1/2* or *BRIP1* variants were found to have a pathogenic variant of *BRCA1* and *BRCA2* genes by ACMG variant classification (2/30, 6.7%). All other *BRCA1/2* or *BRIP1* mutations were variants of unknown significance (4/30, 13.3%). The potential for these variants to affect *BRCA1/2* or *BRIP1* gene status was investigated with in silico pathogenicity assessment using bioinformatics tools (table 2, online supplemental tables 2 and 3). Five of these six patients exhibited potential pathogenicity with at least one of the pathogenicity assessment tools (5/30, 16.7%).

Clinical characteristics of retinoblastoma in patients harbouring *BRCA1/2* or *BRIP1* variants

We reviewed the clinical findings, treatments and treatment outcomes of patients with retinoblastoma with and without *BRCA1/2* or *BRIP1* variants (table 3). There were no statistically

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Table 1 *BRCA* family gene variants in patients with retinoblastoma

Patient	Age (years, months)	Sex	Laterality	Family history	Gene	Accession	Nucleotide	Amino acid	Inheritance
1	5Y5M	F	Bilateral	No	*RB1*	NM_000321.2	c.409G>T	p.Glu137Ter	AD
					BRCA1	NM_007294.3	c.3448C>T	p.Pro1150Ser	AD
2	5Y	F	Unilateral	No	*RB1*	NM_000321.2	c.796C>T	p.Gln266Ter	AD
					BRCA2	NM_000059.3	c.6325G>A	p.Val2109Ile	AD,AR
					ALK	NM004304.4	c.941A>G	p.Glu314Gly	–
					FANCM	NM_020937.2	c.1741C>T	p.Arg581Cys	–
					RAD50	NM_005732.3	c.2209C>G	p.Gln737Glu	AR
3	3Y3M	F	Unilateral	No	*BRCA1*	NM_007294.3	c.2481A>C	p.Glu827Asp	AD
					CDKN2A	NM_058197.4	c.326C>T	p.Ala109Val	AD
4	1Y5M	M	Bilateral	No	*RB1*	NM_000321.2	c.1597G>T	p.Glu533Ter	AD
					BRCA2	NM_000059.3	c.7480C>T	p.Arg2494Ter	AD,AR
					BRCA1	NM_007294.3	c.4729T>C	p.Ser1577Pro	AD
					ATM	NM_000051.3	c.7516-9dup	–	AR
5	18Y	F	Unilateral	No	*BRCA1*	NM_007294.3	c.5339T>C	p.Leu1780Pro	AD
					AXIN1	NM_003502.3	c.2218G>A	p.Ala740Thr	AD
					ATM	NM_000051.3	c.323C>G	p.Ala108Gly	AR
6	1Y11M	M	Unilateral	No	*BRIP1*	NM_032043.2	c.1935+7T>C	–	AD, AR

M, months; Y, year.

significant differences in age at diagnosis, laterality, classification or treatment outcomes between patients with *BRCA1/2* or *BRIP1* variants and patients without these. Subset analysis of patients with no germline *RB1* mutation (n=15) showed no statistically significant differences in age at diagnosis, laterality, classification or treatment outcomes between three patients with *BRCA1* variants and those without, although patients with *BRCA1* variants had shorter follow-up duration than patients without *BRCA1* or *RB1* mutation (mean follow-up duration 3.00 and 9.25 months, respectively, p=0.031, Mann-Whitney's U test). One patient with germline *RB1* mutation and *BRCA1* variant had bilateral retinoblastoma and had one of the eye enucleated despite systemic and local therapy due to progression.

DISCUSSION

To our knowledge, this is the first report on the potential association of *BRCA1/2* with retinoblastoma pathogenesis. Retinoblastoma is the first cancer in which the causal tumour suppressor gene mutation was revealed.[22] The RB1 protein is essential for the maintenance of chromosomal stability, and virtually all retinoblastoma tumours were thought to display biallelic inactivation of *RB1* genes.[23] However, a recent study reported that no *RB1* mutations were detected in 2.7% of non-hereditary retinoblastoma tumours.[2] In 52% of these $RB^{+/+}$ tumours, amplification of the *MYCN* oncogene ($RB^{+/+}$ $MYCN^{A}$) was identified as the potential driver of tumour initiation.[2] This result suggested that, regardless of the initiating mutation, epigenetics is the driver of tumour progression in retinoblastoma and E2F transcriptional targets may mediate this process.[24]

Our study presented that 2 of the 30 patients with retinoblastoma (6.7%) displayed *BRCA1/2* family variants that were likely pathogenic/pathogenic. Five of the 30 patients (16.7%) exhibited potential pathogenicity according to at least one of the computational predictive programmes. *BRCA1/2* mutations are detected in 13%–40% of patients with ovarian, breast or prostate cancers, which are known to be caused by these mutations.[4 5 25]

Table 2 Assessment of *BRCA* gene variants with respect to cancer gene diagnosis panel and the development of retinoblastoma

Patient	1	2	3	4		5	6
ACMG class	VOUS	VOUS	VOUS	Pathogenic	VOUS	Likely pathogenic	VOUS
Gene	*BRCA1*	*BRCA2*	*BRCA1*	*BRCA2*	*BRCA1*	*BRCA1*	*BRIP1*
Nucleotide	c.3448C>T	c.6325G>A	c.2481A>C	c.7480C>T	c.4729T>C	c.5339T>C	c.1935+7T>C
Inheritance	AD	AD,AR	AD	AD,AR	AD	AD	AD, AR
Mutation type	Missense_variant	Missense_variant	Missense_variant	Stop_gained	Missense_variant	Missense_variant	Splice_region_variant, intron_variant
Impact	Moderate	Moderate	Moderate	High	Moderate	Moderate	Low
Exon/Intron	Exon	Exon	Exon	Exon	Exon	Exon	Intron
SIFT	Deleterious (0)	Tolerated (0.73)	Deleterious (0.04)	–	Tolerated (0.21)	Deleterious (0)	–
Polyphen2	Possibly_damaging (0.873)	Benign (0.001)	Benign (0.16)	–	Probably_damaging (0.97)	Probably_damaging (1)	–
PON-P2 prediction	Unknown (0.399)	Neutral (0.168)	Unknown (0.299)	–	Unknown (0.5)	Pathogenic (0.797)	–
FATHMM-XF	Pathogenic (0.79693)	Benign (high conf.) (0.024698)	Benign (0.165983)	Benign (0.084482)	Benign (0.194545)	Pathogenic (0.771234)	Pathogenic (0.81587)
CADD_PHRED	24.4	0.009	4.886	41	17.38	25.6	5.496

ACMG, American College of Medical Genetics and Genomics ; SIFT, sorting intolerant from tolerant.

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Table 3 Clinical results for retinoblastoma patients with and without possibly pathogenic *BRCA1/2* or *BRIP1* variants

Demographic and clinical characteristics	Pathogenic *BRCA1/2* or *BRIP1* variant	No pathogenic *BRCA1/2* or *BRIP1* variant	P-value
Age at diagnosis (years)	4.40±7.64 1.00 (9.75)	2.00±3.08 1.00 (3.00)	0.594
Sex (%)			1.000
Male	2 (40.0)	11 (44.0)	
Female	3 (60.0)	14 (56.0)	
Bilaterality (%)	2 (40.0)	8 (32.0)	1.000
Reese-Ellsworth classification at diagnosis (%)			0.919
1	0 (0)	3 (9.1)	
2	0 (0)	1 (3.0)	
3	1 (14.3)	4 (12.1)	
4	1 (14.3)	2 (6.1)	
5	5 (71.4)	18 (57.6)	
IIRC classification at diagnosis			0.103
A	1 (14.3)	1 (3.0)	
B	0 (0)	5 (15.2)	
C	0 (0)	4 (12.1)	
D	4 (57.1)	5 (15.2)	
E	2 (28.6)	15 (45.5)	
Follow-up duration (months)	31.00±25.04 27.00 (38.00)	66.92±50.37 55.00 (26.00)	0.074
Total number of systemic chemotherapy treatments	9.60±5.60 8.00 (9.00)	8.22±3.40 7.00 (4.00)	0.954
Total number of intra-arterial chemotherapy treatments	2.50±1.05 2.50 (1.50)	3.71±2.37 3.00 (2.50)	0.274
Total number of intravitreal chemotherapy treatments	3.00±7.94 0.00 (0.00)	2.34±5.33 0.00 (1.50)	0.696
Additional treatment			
TTT	3 (42.9)	15 (45.5)	1.000
Laser photocoagulation	0 (0)	7 (21.2)	0.303
External cryotherapy	1 (14.3)	3 (9.1)	1.000
Vitrectomy	0 (0)	1 (3.0)	1.000
EBRT	0 (0)	0 (0)	–
Brachytherapy	0 (0)	1 (3.6)	1.000
Treatment outcomes			
Enucleation	1 (14.3)	7 (21.2)	1.000
Recurrence	0 (0)	3 (9.1)	1.000
Metastasis	0 (0)	0 (0)	–

EBRT, external-beam radiotherapy; IIRC, International Intraocular Retinoblastoma Classification.

Thus, our observation that 16.7% of patients with retinoblastoma have *BRCA1/2* family variants with potential pathogenicity seems high enough to be explored further for its significance. However, caution might be needed in interpreting these results because South Asian populations were reported to have a higher prevalence of BRCA1/2 pathogenic variants (1 in 81, compared with 1 in 139 overall prevalence).[26]

The result that four of six patients carrying *BRCA1/2* family variants had variants of unknown significance according to the ACMG classification does not necessarily indicate that these four variants are pathogenic for retinoblastoma. A joint consensus recommendation of the ACMG and Association for Molecular Pathology states that additional evidence is required to support a gene's association with disease before any variant in the gene itself can be considered pathogenic for that disease.[8] This consensus recommendation also states that computational (in silico) predictive programmes can aid in the interpretation of sequence variants, including some of the tools used in this study, such as PolyPhen2 and SIFT.[8] In silico analyses for *BRCA1/2* family variants revealed that 5/30 (16.7%) patients had likely pathogenic variants according to at least one of the prediction tools.

RB1 is a gatekeeper gene whose inactivation can function as an important rate-limiting step in tumour initiation, and studies have investigated its association with other genes. *BRCA2* and *RB1* genes are located in close proximity on chromosome 13q, and LOH of the *BRCA2* and *RB1* loci is observed in approximately 30% of sporadic breast tumours.[27] A previous study demonstrated that osteosarcoma cells depleted of *RB1* display spontaneous DNA damage evidenced by increased γH2AX foci and elevated reactive oxygen species[28] that may lead to loss of *BRCA2* expression.[7] In prostate cancer cells, knockout/knockdown of *RB1* partially attenuated *BRCA2* expression, and LNCaP cells with *BRCA2* knockout exhibited partial loss of *RB1* expression, indicating a possible positive feed-forward loop between *BRCA2* and *RB1* in prostate cancer cells.[7]

A recent study reported the loss of both *BRCA2* and *RB1* genes in patients with prostate cancer. Patients with prostate cancer who lost a copy of *BRCA2* frequently lose a copy of the tumour suppressor *RB1* gene, and the coloss of both genes in early prostate cancer is sufficient to induce a distinct biology that is likely associated with worse prognosis.[7] Our study did not detect a statistically significant difference in retinoblastoma severity according to the RE or IIRC classification. Further studies are needed on *BRCA2* variants in retinoblastoma development and its effect on prognosis. Patients with prostate cancer with coloss of *BRCA* and *RB1* genes are routinely considered candidates for targeted therapies such as PARPi. Thus, patients with retinoblastoma with *BRCA* and *RB1* gene mutations also could be considered possible candidates for targeted PARPi therapies.[6]

BRCA1 germline mutation typically causes breast tumours with basal-like subtype, and *RB1* is frequently inactivated by gross gene disruption in *BRCA1* hereditary breast cancer and *BRCA1*-methylated sporadic basal-like breast cancer.[29] The *RB* locus is one of the most frequently lost loci in *BRCA1/p53* mouse breast tumours.[30] In our study, the patients with retinoblastoma with only *BRCA1* germline variant but no *RB1* mutation displayed mutational signatures related to *BRCA1*, suggesting the possibility of germline *BRCA1* mutation–derived *RB1* mutation or inactivation.

Retinoblastoma survivors carry a lifelong risk of developing new cancers. Breast cancer was one of the frequent events among moderately irradiated sites (third to seventh most common secondary primary cancer), with a standardised incidence ratio of 3.96 in a hereditary retinoblastoma survivor population.[31 32] Although genetic profiles of the breast cancers of retinoblastoma survivors have not been reported, *BRCA1/2* family variants that had a role in retinoblastoma pathogenesis also may have acted as a key mutation driving the secondary breast tumour progression.

The limitations of this study include the small number of patients and limited deep-sequencing data for patients with retinoblastoma with *BRCA1/2* family variants. Although this study included genetic analysis results of blood samples of patients with retinoblastoma, only one tumour sample of the patient with *BRCA1/2* variant and *RB1* mutation was analysed. Further research with a larger patient population is necessary to confirm the role of *BRCA1/2* and *RB1* coloss on retinoblastoma pathogenesis.

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REFERENCES

1 Soliman SE, Racher H, Zhang C, *et al*. Genetics and molecular diagnostics in Retinoblastoma--an update. *Asia Pac J Ophthalmol (Phila)* 2017;6:197–207.
2 Rushlow DE, Mol BM, Kennett JY, *et al*. Characterisation of Retinoblastomas without Rb1 mutations: Genomic, gene expression, and clinical studies. *Lancet Oncol* 2013;14:327–34.
3 Thériault BL, Dimaras H, Gallie BL, *et al*. The Genomic landscape of Retinoblastoma: a review. *Clin Exp Ophthalmol* 2014;42:33–52.
4 Paul A, Paul S. The breast cancer susceptibility genes (BRCA) in breast and ovarian cancers. *Front Biosci* 2014;19:605.
5 Zhang S, Royer R, Li S, *et al*. Frequencies of Brca1 and Brca2 mutations among 1,342 Unselected patients with invasive ovarian cancer. *Gynecol Oncol* 2011;121:353–7.
6 Lozano R, Castro E, Aragón IM, *et al*. Genetic aberrations in DNA repair pathways: a cornerstone of precision oncology in prostate cancer. *Br J Cancer* 2021;124:552–63.
7 Chakraborty G, Armenia J, Mazzu YZ, *et al*. Significance of Brca2 and Rb1 Co-loss in aggressive prostate cancer progression. *Clin Cancer Res* 2020;26:2047–64.
8 Richards S, Aziz N, Bale S, *et al*. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American college of medical Genetics and Genomics and the Association for molecular pathology. *Genetics in Medicine* 2015;17:405–24.
9 den Dunnen JT, Dalgleish R, Maglott DR, *et al*. HGVS recommendations for the description of sequence variants: 2016 update. *Human Mutation* 2016;37:564–9.
10 Favero F, Joshi T, Marquard AM, *et al*. Sequenza: allele-specific copy number and Mutation profiles from tumor sequencing data. *Ann Oncol* 2015;26:64–70.
11 Lee J, Lee AJ, Lee J-K, *et al*. Mutalisk: a web-based somatic Mutation Analyis toolKit for Genomic, transcriptional and Epigenomic signatures. *Nucleic Acids Res* 2018;46:W102–8.
12 Nik-Zainal S, Davies H, Staaf J, *et al*. Landscape of somatic mutations in 560 breast cancer whole-genome sequences. *Nature* 2016;534:47–54.
13 Ng PC, Henikoff S. SIFT: predicting amino acid changes that affect protein function. *Nucleic Acids Res* 2003;31:3812–4.
14 Adzhubei IA, Schmidt S, Peshkin L, *et al*. A method and server for predicting damaging Missense mutations. *Nat Methods* 2010;7:248–9.
15 Rentzsch P, Witten D, Cooper GM, *et al*. CADD: predicting the Deleteriousness of variants throughout the human genome. *Nucleic Acids Res* 2019;47:D886–94.
16 McLaren W, Gil L, Hunt SE, *et al*. The Ensembl variant effect Predictor. *Genome Biol* 2016;17:122.
17 Niroula A, Urolagin S, Vihinen M. PON-P2: prediction method for fast and reliable identification of harmful variants. *PLoS One* 2015;10:e0117380.
18 Rogers MF, Shihab HA, Mort M, *et al*. FATHMM-XF: accurate prediction of pathogenic point mutations via extended features. *Bioinformatics* 2018;34:511–3.
19 Karczewski KJ, Weisburd B, Thomas B, *et al*. The Exac Browser: displaying reference data information from over 60 000 Exomes. *Nucleic Acids Res* 2017;45:D840–5.
20 Karczewski KJ, Francioli LC, Tiao G, *et al*. The mutational constraint spectrum quantified from variation in 141,456 humans. *Nature* 2020;581:434–43.
21 Jung KS, Hong K-W, Jo HY, *et al*. KRGDB: the large-scale variant database of 1722 Koreans based on whole genome sequencing. *Database* 2020;2020.
22 Knudson AG. Mutation and cancer: statistical study of Retinoblastoma. *Proc Natl Acad Sci U S A* 1971;68:820–3.
23 Dunn JM, Phillips RA, Becker AJ, *et al*. Identification of Germline and somatic mutations affecting the Retinoblastoma gene. *Science* 1988;241:1797–800.
24 Benavente CA, Dyer MA. Genetics and Epigenetics of human Retinoblastoma. *Annu Rev Pathol* 2015;10:547–62.
25 Messina C, Cattrini C, Soldato D, *et al*. BRCA mutations in prostate cancer: Prognostic and predictive implications. *J Oncol* 2020;2020:4986365.
26 Abul-Husn NS, Soper ER, Odgis JA, *et al*. Exome sequencing reveals a high prevalence of Brca1 and Brca2 founder variants in a diverse population-based Biobank. *Genome Med* 2019;12:2.
27 Cleton-Jansen AM, Collins N, Lakhani SR, *et al*. Loss of Heterozygosity in sporadic breast tumours at the Brca2 locus on Chromosome 13Q12-Q13. *Br J Cancer* 1995;72:1241–4.
28 Marshall AE, Roes MV, Passos DT, *et al*. Rb1 deletion in Retinoblastoma protein pathway-disrupted cells results in DNA damage and cancer progression. *Mol Cell Biol* 2019;39:e00105-19.
29 Jönsson G, Staaf J, Vallon-Christersson J, *et al*. The Retinoblastoma gene undergoes Rearrangements in Brca1-deficient basal-like breast cancer. *Cancer Res* 2012;72:4028–36.
30 Holstege H, van Beers E, Velds A, *et al*. Cross-species comparison of aCGH data from mouse and human Brca1- and Brca2-Mutated breast cancers. *BMC Cancer* 2010;10:455.
31 Zhao NO, Daewoo P, El-Hadad C, *et al*. Characteristics and survival outcomes of second primary cancers in long-term Retinoblastoma survivors. *Asia Pac J Ophthalmol (Phila)* 2021;10:366–72.
32 Kleinerman RA, Tucker MA, Tarone RE, *et al*. Risk of new cancers after radiotherapy in long-term survivors of Retinoblastoma: an extended follow-up. *J Clin Oncol* 2005;23:2272–9.

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