Characterization and Functional Annotation of Germline RB1 Variants from Peripheral Blood Mononuclear Cells in Retinoblastoma Patients

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INTRODUCTION

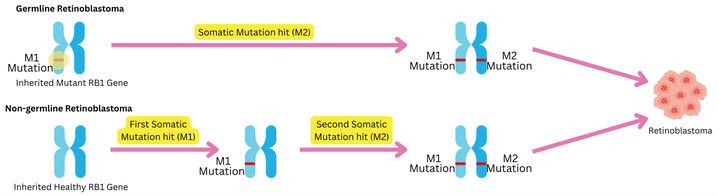
Retinoblastoma is the most common intraocular cancer in children, affecting approximately 1 in 16,000 to 18,000 live births worldwide. It arises from biallelic inactivation of the RB1 tumor suppressor gene, which regulates the G1 to S phase transition in the cell cycle.

RB1 acts as a molecular “brake” by binding and inhibiting E2F, preventing uncontrolled cell division. When both alleles are mutated, this control is lost, allowing unregulated proliferation and tumor formation.

About 40% of cases are heritable, caused by a germline mutation in one RB1 allele followed by a somatic “second hit.” The remaining 60% are sporadic, involving two somatic mutations.

Understanding the germline mutation spectrum of RB1 is essential for early diagnosis, genetic counseling, and long-term surveillance. Identifying pathogenic germline mutations also supports risk assessment in relatives, enabling earlier intervention and informed decision-making for family planning and clinical management.

Figure 1. Schematic comparison of germline and non-germline retinoblastoma, highlighting the difference in mutational events required for tumor development: one somatic “second hit” in germline cases versus two somatic mutations in non-germline cases.



RESEARCH AIM

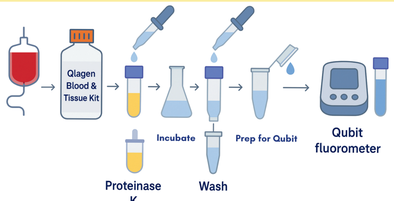
1. To survey and characterize the CHLA patient cohort to better understand the distribution and frequency of germline mutations within a larger population of retinoblastoma cases.
2. To analyze and functionally annotate germline RB1 variants detected in retinoblastoma patients, with the goal of assessing their potential clinical impact.

METHODS

Sample Collection & gDNA Extraction

Peripheral blood samples from seven retinoblastoma patients with known germline RB1 mutations were collected in the operating room prior to this study and processed. Genomic DNA was extracted using the Qiagen Blood & Tissue Kit and quantified with a Qubit fluorometer.

Figure 2. Overview of gDNA extraction from processed blood samples using Qiagen kit and Qubit quantification.



Primer Design & Sequencing Data

Primers targeting three specific RB1 mutations were designed using Primer-BLAST. Sanger sequencing had been previously performed on the samples; available sequencing data was used to confirm the presence of pathogenic variants.

Figure 3. Primer Pairs Designed for c.958C>T and c.1996dup Mutations Using Primer-BLAST

Primer pair 1

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	GTCATTCAGGTTGGGAAGGGT	Plus	21	66	86	59.92	52.38	3.00	0.00
Reverse primer	CACTCTCCACACTCACACTCA	Minus	21	255	235	59.32	52.38	1.00	1.00
Product length	190								

Primer pair 2

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	GATGACCTACCTGAGGGGAGTGA	Plus	23	3	25	62.55	56.52	5.00	3.00
Reverse primer	CTCCTGAGACCTCCTCTGACCTC	Minus	23	138	116	62.83	60.87	4.00	2.00
Product length	136								

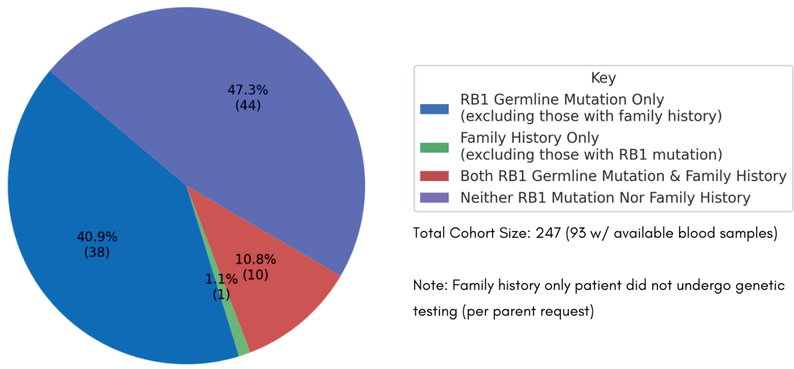
METHODS

Variant Annotation & Analysis

Identified variants were classified using ClinVar, LOVD, and AlphaMissense into one of three categories: pathogenic, benign, or variant of uncertain significance (VUS). Relevant literature cited within each database was reviewed to assess potential functional impact. Results were compiled in a variant annotation table.

RESULTS

Figure 4. Breakdown of RB Patients with Available Blood Samples (n = 93)



Total Cohort Size: 247 (93 w/ available blood samples)

Note: Family history only patient did not undergo genetic testing (per parent request)

Figure 5. Germline RB1 variants from patient cohort (n = 247) mapped across RB1 protein domains. Variants were identified through prior clinical testing at CHLA using NGS, Sanger sequencing, or MLPA, and compiled to visualize the overall mutation spectrum. A single primer set does not and can not capture all mutations shown. 36 unique mutations are mapped.

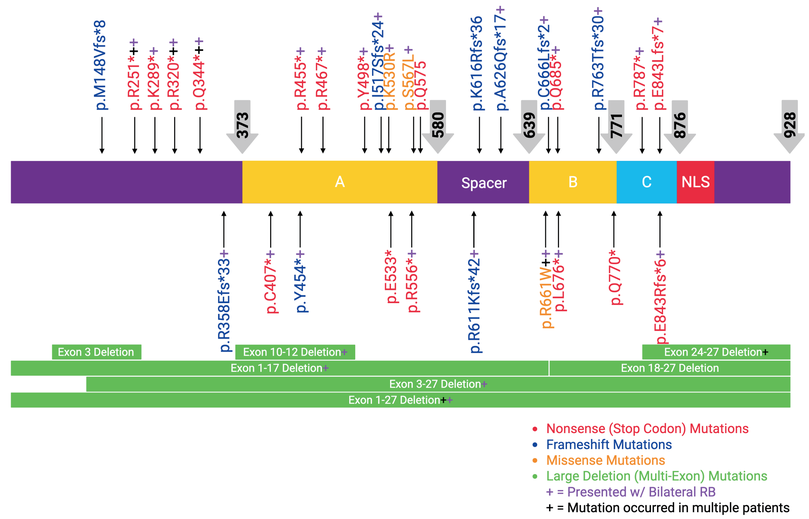


Figure 6. Distribution of bilateral and unilateral retinoblastoma by germline status. Patients with germline mutations showed a higher rate of bilateral disease compared to those with non-germline mutations. Data represents patients with available blood samples (n=93).

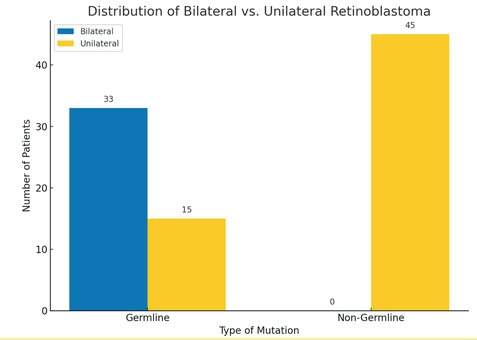


Table 1. Functional Annotation of RB1 Germline Variants Identified in 7 Patients with Available Blood Samples

DNA Change (c.)	Protein Change (p.)	ClinVar Result	AlphaMissense Score	Comments	Sequencing Method
c.1421+12_1421+32del21bp	p.(=) (no change)	Not Listed	Not Listed	Intronic variant in intron 15; reported in LOVD as splice-altering (r.spl); reported as casuing no change at the protein level by LOVD (p. (=))	NGS, Sanger, and MLPA (per CHLA RB1 panel protocol)
c.1362C>G	p.Tyr454	Pathogenic	Not Listed	Nonsense variant in exon 14; causes premature stop codon; classified as VUS in LOVD	NGS, Sanger, or MLPA (per CHLA RB1 panel protocol)
c.1215+1G>A	p.? (unknown)	Pathogenic	Not Listed	Canonical splice donor site variant (standard, most essential sequence required for splicing); reported pathogenic in ClinVar and LOVD; supported by PMID: 29568217, 33456302	NGS, Sanger, or MLPA (per CHLA RB1 panel protocol)
c.958C>T	p.Arg320	Pathogenic	Not Listed	Nonsense variant in exon 10; premature stop codon; reported pathogenic in ClinVar and LOVD; supported by multiple publications (PMIDs: 7704558, 28575107, 34294096, 33456302)	NGS, Sanger, or MLPA (per CHLA RB1 panel protocol)
Large 13q deletion (pending further testing)	p.? (unknown)	Not Listed	Not Listed	Suspected copy number loss affecting RB1; classified as pathogenic if confirmed to include 13q14 region; further testing pending	Pending further testing
c.1996dup	p.Cys666Leufs2	Pathogenic	Not Listed	Frameshift variant; introduces premature stop codon; absent from gnomAD; pathogenic in ClinVar and LOVD	NGS, Sanger, or MLPA (per CHLA RB1 panel protocol)
c.-198G>A	N/A (promoter)	Pathogenic	Not Listed	Promoter variant affecting transcription; listed as pathogenic in ClinVar and LOVD; supported by segregation and functional studies (PMIDs: 1881452, 30636860, 20447117); reduced penetrance noted — some carriers may not develop disease	NGS, Sanger, or MLPA (per CHLA RB1 panel protocol)

CONCLUSION

This study identified and functionally annotated germline RB1 variants from seven retinoblastoma patients, offering insight into the gene’s mutation spectrum. Most variants were classified as pathogenic, supporting their potential relevance in early diagnosis, genetic counseling, and long-term care. Ultimately, a major takeaway is that RB1 has no mutation hotspots — variants are distributed throughout the entire gene. This broad distribution also led to the identification of novel pathogenic mutations. Additionally, bilateral disease was observed almost exclusively in patients with germline mutations, reinforcing the clinical importance of early genetic screening.

Looking Ahead: Under the continued mentorship of Dr. Liya Xu, this project will expand to include functional annotation of the full patient cohort and investigate the two-hit hypothesis by mapping somatic hits in RB patients.

ACKNOWLEDGEMENTS

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References:

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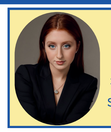

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