Hereditary Retinoblastoma (RB1) Sequencing and Deletion/Duplication

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Retinoblastoma is a malignant tumor of the retina that typically occurs in children under 5 years of age. Hereditary retinoblastoma, caused by a single germline pathogenic variant in the *RB1* gene, predisposes individuals to retinoblastoma and other nonocular tumors, including pinealoblastoma, osteosarcoma, soft tissue sarcoma, and melanoma.

Featured ARUP Testing

Hereditary Retinoblastoma (RB1) Sequencing and Deletion/Duplication 3005696

Method: Massively Parallel Sequencing

Recommended test for individuals with a suspected diagnosis or family history of heritable retinoblastoma

If a familial sequence variant has been previously identified, targeted sequencing for that variant may be appropriate; refer to the Laboratory Test Directory for additional information.

Disease Overview

Retinoblastoma should be **suspected** in children with any of the following[1]:

- Leukocoria (white pupil)
- Strabismus
- Change in eye appearance
- Reduced visual acuity

Hereditary retinoblastoma should be **suspected** in an individual with any of the following[1]:

- A diagnosis of retinoblastoma, including unilateral (unifocal and multifocal) and bilateral involvement
- A retinoma
- A family history of retinoblastoma

The diagnosis of hereditary retinoblastoma is **established** in an individual with both retinoblastoma/retinoma and a family history of retinoblastoma.[1]

- Approximately 6-8% of individuals with retinoblastoma have a chromosome deletion of 13q14, which can also be associated with developmental delay and birth defects.[1]

Genetics

Gene

RB1 (NM_000321)

Etiology

Retinoblastoma affects approximately 1/15,000 to 1/20,000 live births. Hereditary retinoblastoma accounts for approximately 10% of retinoblastoma cases.[1]

Inheritance

Autosomal dominant

Penetrance

Complete penetrance, except for fewer than 10% of families that show a "low-penetrance" phenotype with reduced expressivity.[1] See GeneReviews for additional details.

Test Interpretation

Contraindications for Ordering

- Should not be ordered to detect somatic variants associated with tumors/malignancy because sensitivity for mosaic variants is low with methodology used for germline assays
- Individuals with hematologic malignancy and/or a previous allogeneic bone marrow transplantation should not undergo molecular genetic testing on a peripheral blood specimen.
  - Testing of cultured fibroblasts is required for accurate interpretation of test results.

Methodology

This test is performed using the following sequence of steps:

- Selected genomic regions, primarily coding exons and exon-intron boundaries, from the targeted genes are isolated from extracted genomic DNA using a probe-based hybrid capture enrichment workflow.
- Enriched DNA is sequenced by massively parallel sequencing (MPS; also known as next generation sequencing [NGS]) followed by paired-end read alignment and variant calling using a custom bioinformatics pipeline. The pipeline includes an algorithm for detection of large (single exon-level or larger) deletions and duplications.
- Sanger sequencing is performed as necessary to fill in regions of low coverage and in certain situations, to confirm variant calls.
- Large deletion/duplication calls made using MPS are confirmed by an orthogonal exon-level microarray when sample quality and technical conditions allow.

Sensitivity/Specificity

Clinical Sensitivity

The clinical sensitivity is variable and dependent on phenotype. The table below details the probability of a germline pathogenic variant being present in a proband with retinoblastoma based on family history and tumor presentation.

Retinoblastoma Presentation	Family History	Probability That an *RB1* Germline PV Is Present (%)
Unilateral (unifocal)	Positive[a]	100
	Negative[b]	Approximately 14
Unilateral (multifocal)	Positive[a]	100
	Negative[b]	14-95
Bilateral	Positive[a]	100
	Negative[b]	Close to 100[c]

[a]Positive family history is defined as more than one affected family member.

[b]Negative family history is defined as only one affected individual in the family.

[c]*RB1* pathogenic variants are identified by conventional molecular testing in 90-97% of simplex cases with bilateral involvement; the remaining 5% may have translocations, deep intronic splice variants, or low-level mosaic pathogenic variants that may or may not be in the germline.

Source: Lohmann, 2018[1]

Analytic Sensitivity/Specificity

Variant Class	Analytic Sensitivity (PPA) Estimate[a] (%) and 95% Credibility Region	Analytic Specificity (NPA) Estimate (%)
SNVs	>99 (96.9-99.4)	>99.9
Deletions 1-10 bp[b]	93.8 (84.3-98.2)	>99.9
Insertions 1-10 bp[b]	94.8 (86.8-98.5)	>99.9
Exon-level[c] deletions	97.8 (90.3-99.8) [2 exons or larger] 62.5 (38.3-82.6) [single exon]	>99.9
Exon-level[c] duplications	83.3 (56.4-96.4) [3 exons or larger]	>99.9

[a]PPA values are derived from larger methods-based MPS and/or Sanger validations. These values do not apply to testing performed by multiplex ligation-dependent probe amplification (MLPA) unless otherwise indicated.

[b]Variants greater than 10 bp may be detected, but the analytic sensitivity may be reduced.

[c]In most cases, a single exon deletion or duplication is less than 450 bp and 3 exons span a genomic region larger than 700 bp.

bp, base pairs; NPA, negative percent agreement; PPA, positive percent agreement; SNVs, single nucleotide variants

Results

Result	Variant(s) Detected	Clinical Significance
Positive	One *RB1* pathogenic variant detected	Consistent with a diagnosis of hereditary retinoblastoma
Negative	No *RB1* pathogenic variants detected	Risk of hereditary retinoblastoma is reduced but not eliminated (less than 1% of individuals with retinoblastoma or retinoma and a negative germline result on blood have mosaicism)[1]
Uncertain	*RB1* variant(s) of unknown clinical significance detected	Uncertain; it is unknown whether variant is benign or pathogenic

Limitations

- A negative result does not exclude a heritable form of retinoblastoma.
- Diagnostic errors can occur due to rare sequence variations.
- Interpretation of this test result may be impacted if this patient has had an allogeneic stem cell transplantation.
- The following will not be evaluated:
  - Variants outside the coding regions and intron-exon boundaries of targeted gene(s)
  - Regulatory region and deep intronic variants
  - Breakpoints of large deletions/duplications
- The following may not be detected:
  - Deletions/duplications/insertions of any size by MPS
  - Large duplications less than 3 exons in size
  - Deletions/duplications in the following exon RB1 (NM_000321) 22
  - Noncoding transcripts
  - Some variants due to technical limitations in the presence of pseudogenes, repetitive, or homologous regions
  - Low-level somatic variants
- Exon *RB1* (NM_000321) 22 may have reduced sequencing sensitivity due to technical limitations of the assay.

References

1. Lohmann DR, Gallie BL. Retinoblastoma. In: Adam MP, Everman DB, Mirzaa GM, et al, eds. *GeneReviews*. University of Washington, Seattle. Updated Jul 2000; accessed Jun 2022.

Related Information

Hereditary Cancer Germline Genetic Testing

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