RB1 Gene Analysis in Hereditary Retinoblastoma

Clinical Features:
Hereditary Retinoblastoma is primarily characterized by a high risk of retinoblastoma in childhood. The lifetime risk for developing retinoblastoma is greater than 90% in most individuals with a germline pathogenic variant in *RB1*.[1] Frequently, individuals with hereditary retinoblastoma will develop bilateral tumors in infancy.[1] The risk of pineoblastoma in patients who have had bilateral retinoblastoma ranges from 2% in individuals who did not receive radiation treatment to up to 15% in those who did. [2–6] At least 50% of patients with pathogenic variants in *RB1* present with leukocoria, strabismus, or precursor lesions called retinomas.[5,7] Approximately 10% of families with a pathogenic variant in *RB1* demonstrate low penetrance with reduced expressivity (often diagnosed only unilaterally), sometimes showing a parent-of-origin effect on cancer risk.[8–10]

Second primary cancers are a significant concern for morbidity and mortality for childhood survivors of hereditary retinoblastoma. The cumulative incidence of a second primary malignancy 40 years after a diagnosis of retinoblastoma is 28%, increasing to greater than a 50% incidence at 50 years after diagnosis in individuals who received external beam radiation therapy. [11,12] Common second primary cancers observed in patients with pathogenic variants in *RB1* include soft tissue sarcoma (such as leiomyosarcoma or rhabdomyosarcoma), osteosarcoma, melanoma, lung cancer, and bladder cancer.[11,13,14]

Inheritance Pattern:
Hereditary Retinoblastoma is inherited in an autosomal dominant manner. Approximately 90% of pathogenic *RB1* variants are *de novo* (new).[15,16]

Test Methods:
Genomic DNA is extracted from the submitted specimen. For skin punch biopsies, fibroblasts are cultured and used for DNA extraction. This DNA is enriched for the complete coding regions and splice site junctions of the genes on this panel using a proprietary targeted capture system developed by GeneDx for next generation sequencing with CNV calling (NGS-CNV). The enriched targets are simultaneously sequenced with paired-end reads on an Illumina platform. Bi-directional sequence reads are assembled and aligned to reference sequences based on NCBI RefSeq transcripts and human genome build GRCh37/UCSC hg19. After gene specific filtering, data are analyzed to identify sequence variants and most deletions and duplications involving coding exons. Alternative sequencing or copy number detection methods are used to analyze regions with inadequate sequence or copy number data by next generation sequencing (NGS). Reported clinically significant variants are confirmed by an appropriate method. Sequence and copy number variants are reported according to the

Human Genome Variation Society (HGVS) or International System for Human Cytogenetic Nomenclature (ISCN) guidelines, respectively. Reportable variants include pathogenic variants, likely pathogenic variants and variants of uncertain significance. Likely benign and benign variants, if present, are not routinely reported but are available upon request.

Test Sensitivity:

The clinical sensitivity of sequencing and deletion/duplication analysis of *RB1* depends in part on the patient's clinical phenotype and family history. In general, the sensitivity is highest for individuals with features suggestive of Hereditary Retinoblastoma as outlined above. Sequencing and deletion/duplication analysis is expected to identify pathogenic variants in close to 100% of individuals with bilateral and/or familial retinoblastoma and in 10-15% of individuals with non-familial unilateral retinoblastoma. Large rearrangements account for approximately 10-15% of pathogenic variants.[16–18]

Genetic testing using the methods applied at GeneDx is expected to be highly accurate. Normal findings do not rule out the diagnosis of a genetic disorder since some genetic abnormalities may be undetectable by this test. The methods used cannot reliably detect deletions of 20bp to 250bp in size, or insertions of 10bp to 250 bp in size. Sequencing cannot detect low-level mosaicism. The copy number assessment methods used with this test cannot reliably detect mosaicism and cannot identify balanced chromosome aberrations. Rarely, incidental findings of large chromosomal rearrangements outside the gene of interest may be identified. Regions of certain genes have inherent sequence properties (for example: repeat, homology, or pseudogene regions, high GC content, rare polymorphisms) that yield suboptimal data, potentially impairing accuracy of the results. False negatives may also occur in the setting of bone marrow transplantation, recent blood transfusion, or suboptimal DNA quality. In individuals with active or chronic hematologic neoplasms or conditions, there is a possibility that testing may detect an acquired somatic variant, resulting in a false positive result. As the ability to detect genetic variants and naming conventions can differ among laboratories, rare false negative results may occur when no positive control is provided for testing of a specific variant identified at another laboratory. The chance of a false positive or false negative result due to laboratory errors incurred during any phase of testing cannot be completely excluded. Interpretations are made with the assumption that any clinical information provided, including family relationships, are accurate. Consultation with a genetics professional is recommended for interpretation of results.

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