impact genetics inc.
advancing genetic diagnostics

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Patient name:	--	**Referring specialist:**	--
		Contact person:	--
Date of birth:	*Dec. 25, 2010*		
Medical record #:	*987654*	**Institution:**	--
			Address:
Patient history:	*Unilateral unifocal*		*Fax:*
Family history:	*Isolated case*		
Date of report:	*Dec. 7, 2012*		
Family #:	*15-0099*		
Patient #:	*0299*	**Copies to:**	*Name*
			Fax:

Test requested: *Retinoblastoma genetic test - proband*

Test results

The patient has heritable retinoblastoma and is at increased risk of other tumors.

Sample	Allele 1	Allele 2
Tumor	c.2134T>C (p.Cys712Arg)	c.21_137+140del
Blood	c.2134T>C (p.Cys712Arg)	*normal*
Methods Used	Multiplex ligation-dependent probe amplification (MLPA), sequence and allele-specific PCR analysis	

Interpretation

- Molecular analysis identified two *RB1* mutations in the DNA extracted from this patient's tumor sample:
  - a heterozygous c.2134T>C (p.Cys712Arg) missense change in *RB1* exon 21.
  - a heterozygous c.21_137+140del deletion encompassing 258 nucleotides within *RB1* exon 1 and intron 1.
- **Most significantly, c.2134T>C *RB1* mutation was also detected in the DNA extracted from this patient's blood sample, indicating that this patient has heritable/germline retinoblastoma.** The c.2134T>C change is a known reduced penetrance mutation (Ahmad et al.1999, PMID: 10617920). In families with reduced penetrance mutations, retinoblastoma develops in a reduced number of infants below the age of seven, rather than at a rate of 95-100% as found with most *RB1* mutations, and there is a high incidence of unilateral as opposed to bilateral retinoblastoma.

Guidance and recommendations

- Genetic counseling is recommended to discuss the implications of this test report.
- Individuals who inherit reduced penetrance mutations may or may not develop tumors, yet their children have a 50% risk to inherit the mutation.
- Based on our experience and knowledge of retinoblastoma, the patient's family members have the following risks of carrying this heritable *RB1* mutation. Genetic testing is available for all at risk relatives.

	Risk of carrying *RB1* mutation	Recommendation
Patient	100%	Examine regularly for developing tumors in the unaffected eye.
Parents	Over 5%	Important to test parents for c.2134T>C; one parent may be an unaffected carrier.
Siblings	Over 2.5%	Even if neither parent tests positive for the mutation, siblings should be tested for the c.2134T>C mutation (because of the risk of germline mosaicism in one parent).
Offspring	50%	The patient`s future children should be tested at or before birth for the c.2134T>C mutation.

These results and the interpretation, including guidance and supplemental information, were reviewed and approved by:

Electronically signed by Hilary Racher, PHD FCCMG DABMGG, Laboratory Director, on MMM DD, YYYY at <time>
Electronically signed by Brenda Gallie, MD FRCSC, Medical Director, on MMM DD, YYYY at <time>

Details of samples tested

Patient Sample	Sample #	Collected	Received	Authorized/Test Started
Blood	*##-####*	MMM DD, YYYY	MMM DD, YYYY	MMM DD, YYYY
Tumor	*##-####*	MMM DD, YYYY	MMM DD, YYYY	MMM DD, YYYY

Supplemental information

Test Methods (not all samples employ every method)**:**

- Sequence analysis of the *RB1* core promoter and of exons 1 through 27, including nearby flanking intronic regions
- Gross deletion/duplication analysis was performed using quantitative multiplex PCR (QM-PCR) or multiplex ligation-dependent probe amplification (MLPA, SALSA P047-D1 RB1 MRC Holland).
- Allele-specific PCR (AS-PCR) to detect even low levels of the eleven recurrent *RB1* CpG nonsense mutations
- Methylation-specific PCR analysis to detect methylation of the *RB1* promoter in tumors
- *MYCN* amplification and other genomic copy number changes in unilateral tumors with no identified *RB1* mutation
- Reverse-transcriptase PCR RNA analysis where indicated

Test method sensitivity and specificity: As of Jan 27, 2017, 2,525 clinical families have been tested.

	Mutations found	Samples tested	Sensitivity
Bilateral proband	837	864	96.9%
Unilateral proband with family history	38	41	92.7%
Tumor from unilateral proband	855	892	95.9%
Blood only from unilateral proband	130	728	17.9%*

*An estimated 15% of unilateral probands carry a germline (in blood) mutation.

Whenever possible, Impact Genetics banks DNA and RNA for future tests when a causative mutation is not found. Outside of human error, our *RB1* mutation detection strategy shows 100% specificity.

Reporting: Non-pathogenic (benign) variants may not be included on reports but are available upon request. Classification of DNA variants may change over time as new information becomes available and where possible, reports will be re-issued if appropriate.

Test method limitations: Very low level mosaic mutation carriers may not be detected by our methods. Our methods can detect mutant levels as low as 12.5% mutant DNA for most mutation types, and as low as 1% mutant DNA for eleven recurrent *RB1* mutations tested by AS-PCR. Most translocations or gross intronic re-arrangements cannot be detected by our methods. Deep intronic splice mutations cannot be detected by conventional DNA analysis, but may be detected by analysis of the *RB1* mRNA transcript by reverse-transcriptase PCR using a fresh blood sample.

Notation: Mutations are described using HGVS (ver. 2.0) guidelines and the RefSeq accession # NM_000321.2. For coding DNA sequences, the A of the ATG initiator codon is denoted as nucleotide 1.

General disclaimer: This test was developed and its performance characteristics determined by Impact Genetics. It has not been cleared or approved by the Food and Drug Administration. Each of Impact Genetics' molecular tests use a direct method of mutation detection and analysis is based on current knowledge of the genes. Characterization of a mutation in a family does not preclude the remote possibility that a second, unidentified mutation occurs in an individual patient. Moreover, it is possible for two relatives to have different gene mutations.

Additional Information for clinicians and patients regarding the test performed and retinoblastoma is available at impactgenetics.com.