CASTLE BIOSCIENCES

Decision Dx·UMSeq

DecisionDx-UM*Seq* Report

7 Gene Sequencing Panel, Uveal Melanoma, Tumor

GNAQ (R183; Q209), ***GNA11*** (R183; Q209), ***CYSLTR2*** (L129), ***PLCB4*** (D630), ***SF3B1*** (R625), ***EIF1AX*** (exons 1-2), ***BAP1*** (all coding exons)

FINAL REPORT

Patient:
Sex:
DOB:
MRN:

Type of Specimen:
Ordering Physician:
Collected:
Received:
Reported:

RESULTS SUMMARY

Clinically significant alterations were identified in the following genes:

GNAQ, BAP1

Mutations in GNAQ occur frequently (~40-45%) in uveal melanoma and result in constitutive activation of signaling pathways downstream of G-protein-coupled receptors. BAP1 mutations occur in 40-45% of uveal melanoma tumors and are associated with an elevated risk of metastasis. In some uveal melanoma cases (~3-5%), a BAP1 mutation may be present in the patient's germline and thus be heritable (see results interpretation section below).

RESULTS

Gene	Variant (DNA)	Variant (Protein)	Variant Type	Observed Variant Allele Frequency
GNAQ	c.626A>T	p.Q209L	Missense Mutation	0.49
BAP1	c.588G>A	p.W196*	Missense Mutation	0.27

RESULTS INTERPRETATION

GNAQ

GNAQ c.626A>T (p.Q209L)

The test identified a missense mutation (c.626A>T) in exon 5 of GNAQ, which results in an amino acid change from glutamine (Q) to leucine (L) (p.Q209L). This mutation occurs within the ras-like domain of GNAQ and has been reported to inactivate its GTPase activity, resulting in constitutive activation of the associated G-protein-coupled receptor and downstream pathways (PMID 19078957). This mutation is considered to be Tier II (a variant of potential clinical significance) with Level C Evidence (multiple small published studies with some consensus). Mutations in GNAQ occur in approximately 40-45% of uveal melanomas (PMID 19078957, 21083380, 27123562) and are considered an early step in tumorigenesis of uveal melanomas. GNAQ is highly homologous to GNA11, and mutations in these genes are mutually exclusive. Approximately 85% of uveal melanomas will have a mutation in either GNAQ or GNA11 (PMID 21083380). GNAQ mutation status does not inform prognosis in uveal melanoma.

BAP1

BAP1 c.588G>A (p.W196*)

The test identified a nonsense mutation (c.588G>A) in exon 8 of BAP1, which is part of the BARD1-binding domain of BAP1. The mutation results in a premature termination codon (p.W196*), and it is expected to lead to nonsense mediated decay of the mRNA transcript (PMID 21051595). BAP1 inactivation and loss of expression have been associated with metastasis in uveal melanoma. This mutation is considered to be Tier II (variant of potential clinical significance) with Level C evidence (multiple small published studies with some consensus) (PMID 27993330). BAP1 mutations occur in approximately 40-45% of uveal melanomas (PMIDs 21051595, 27123562) and are not usually found in conjunction with SF3B1 or EIF1AX mutations. In uveal melanoma, retrospective studies have shown that BAP1 mutations are associated with an increased risk of metastasis (PMID 27123562) and are correlated with other unfavorable prognostic characteristics, such as a Class 2 gene expression profile and monosomy 3 (PMIDs 2105595, 24970262). However, a Class 2 gene expression result such as that derived from the Decision Dx-UM test, has been shown to be a stronger predictor of metastasis compared to BAP1 mutations (PMID 27123562).

This test does not determine whether the identified variant is of somatic or germline origin; variant allele frequency in a tumor derived sample is not an indicator of germline status. Germline variants in the BAP1 gene can be associated with BAP1 Tumor Predisposition Syndrome. If an inherited cancer syndrome is suspected in this patient, genetic counseling to determine the appropriateness of germline testing should be considered. Castle Biosciences does not provide germline BAP1 testing, but can provide additional resources upon request.

METHODOLOGY

Genomic DNA extracted from the submitted tissue sample was subjected to targeted amplification of specific genomic regions using TruSeq Custom Amplicon Low Input Library Kit, and sequenced on an Illumina MiSeq instrument. Reads are aligned to the human reference sequence (GRCh37) using BWA, Picard and Abra software packages with sequence changes identified with SAMtools, FreeBayes and Wheeljack. Observed variants within the reportable range are interpreted in the context of a single clinically relevant transcript, indicated below. Unless otherwise indicated, all reportable regions are sequenced at a minimum 200X coverage, with an average overall depth of ≥500x. This test has an analytic sensitivity of 1.00 and specificity of 1.00. Variants indicated by <5.0% of aligned sequence reads are not detected. Rare or new reportable variants are confirmed using an orthogonal technology. Variants classified as benign or likely benign are not validated or reported, but are available upon request. Variants are classified following the Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer (PMID 27993330) and ACMG/AMP's Standards and Guidelines for the Interpretation of Sequence Variants (PMID 25741868). Each PubMed ID (PMID) referenced herein is annotated to a specific,
scientific publication accessible at https://www.ncbi.nlm.nih.gov/pubmed by searching with the PMID number.

Interpretation of variants and assignment of significance, Tier, and Level of Evidence are performed using literature searches and several databases, including ExAC, ClinVar, and COSMIC. The most recently updated version of each database available at the time of reporting is used.

Gene	Transcript ID	Genomic position (start-end)	Region tested (specific variant if hotspot)
BAP1	NM_004656.3	chr3: 52436304-52443894	all coding exons +/- 10bp*
CYSLTR2	NM_020377.2	chr13: 49281306-49281475	exon 1 (p.L129)
EIF1AX	NM_001412.3	chrX: 20159733-20159758	exon 1 +/- 10bp
EIF1AX	NM_001412.3	chrX: 20156647-20156750	exon 2 +/- 10bp
GNA11	NM_002067.2	chr19:3118916-3119061	exon 5 (p.Q209)
GNA11	NM_002067.2	chr19: 3114986-3115080	exon 4 (p.R183)
GNAQ	NM_002072.3	chr9: 80409456-80409518	exon 4 (p.R183)
GNAQ	NM_002072.3	chr9: 80412466-80412574	exon 5 (p.Q209)
PLCB4	NM_000933.3	chr20: 9389724-9389838	exon 20 (p.D630)
SF3B1	NM_012433.2	chr2: 198267458-198267560	exon 14 (p.R625)

*For exon 10 of BAP1, the region analyzed includes 10 bp 5' and 8 bp 3' of the exon.

TESTING LIMITATIONS

Result Limitations: For this patient sample, the test may have reduced sensitivity in the following regions due to coverage <200X:

Sequence changes outside of the targeted regions will not be detected by this assay. Sensitivity may be reduced for large insertions or deletions which may disrupt sequence alignment or target enrichment. Sequence properties in some targets may disrupt the detection of some classes of mutations and yield sub-optimal data. This assay does not detect copy number changes. This report reflects the analysis of extracted DNA from a provided tissue sample that is expected to contain tumor tissue. However, presence of tumor tissue is not confirmed prior to testing. While mutations in *GNAQ*, *GNA11*, *CYSLTR2* or *PLCB4* have been reported in up to 98% of uveal melanoma tumors, failure to detect these mutations does not necessarily indicate absence of tumor tissue. Likewise, as mutations in these genes have been reported in other tumor types, their presence does not confirm the diagnosis of uveal melanoma. These results and interpretations are made within the limits of sample collection, methodology and current knowledge. They should be correlated by the referring physician with respect to the ongoing clinical situation of the patient.

For more information, please visit the Castle Biosciences Uveal Melanoma website @www.MyUvealMelanoma.com.

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