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:
Client:
Clinician:

Type of Specimen:
Specimen ID:
Collected:
Received:
Reported:

RESULTS SUMMARY

Clinically significant alterations were identified in the following genes:

GNA11

Mutations in GNA11 occur frequently (~40-45%) in uveal melanoma and result in constitutive activation of signaling pathways downstream of G-protein-coupled receptors.

EIF1AX

EIF1AX mutations occur in up to 24% of uveal melanomas and it has been suggested that they carry a lower risk of metastasis than BAP1 or SF3B1 mutations.

BAP1

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, however, this test does not distinguish between somatic and germline mutations.

RESULTS

GNA11,BAP1,EIF1AX

Gene	Variant (DNA)	Variant (Protein)	Variant Type	Observed Variant Allele Frequency
BAP1	c.2057-4G>T	p.unknown	Splice site SNV	53.8%
GNA11	c.626A>T	p.Q209L	Missense	19.2%
EIF1AX	c.44G>A	p.G15D	Missense	34.2%

RESULTS INTERPRETATION

BAP1

The test identified a splice site mutation (c.2057-4G>T) at the border of exon 17 of BAP1. This mutation results in the potential disruption of a splice site (region within 10bp of the intron/exon junction) (p.unknown), which may alter the function/structure of the BAP1 protein. BAP1 inactivation and loss of expression have been associated with metastasis in uveal melanoma. This mutation is considered to be Tier II (a variant of potential clinical significance) with Level C evidence (based on the results of multiple small studies) (PMID 27993330). BAP1 mutations occur in approximately 40-45% of uveal melanomas (PMID 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 strongly correlated with other unfavorable prognostic characteristics, such as a Class 2 gene expression profile and monosomy 3 (PMID 2105595, 24970262). However, a Class 2 result has been shown to be a stronger predictor of metastasis compared to BAP1 mutations (PMID 27123562).

GNA11

The test identified a missense mutation (c.626A>T) in exon 5 of GNA11 which results in an amino acid change from glutamine (Q) to leucine (L) (p.Q209L). This mutation occurs within the ras-like domain of GNA11 and inactivates its GTPase activity, resulting in constitutive activation of the associated G-protein-coupled receptor and downstream pathways (PMID 21083380). This mutation is considered to be Tier II (a variant of potential clinical significance) with Level C Evidence (based on the results of multiple small studies) for its diagnostic significance (27993330).

RESULTS INTERPRETATION

EIF1AX

The test identified a missense mutation (c.44G>A) in exon 2 of EIF1AX, which results in an amino acid change from glycine (G) to aspartic acid (D) (p.G15D). Mutations in EIF1AX occur in the N-terminal tail of the protein and have not been shown to result in loss-of-function or expression of the protein (PMID 23793026). Instead, these mutations may affect the rate of translation and start codon recognition. In small, retrospective studies, EIF1AX mutations have been associated with a Class 1 gene expression profile, absence of ciliary body involvement, and a low risk of metastasis (PMIDs 27123562, 24970262, 23793026). This mutation is considered to be Tier II (a variant of potential clinical significance) with Level C Evidence (based on the results of multiple small studies) (PMID 27993330).

METHODOLOGY

Genomic DNA extracted from the submitted tissue sample was subjected to targeted amplification of specific genomic regions using an Ion Custom Ampliseq panel, and sequenced on an Ion GeneStudio S5 Prime instrument. Reads are aligned to the human reference sequence (GRCh37) using TMAP (Torrent Suite (5.16)) and variants are detected and annotated with Ion Reporter (5.18). 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 a positive percent agreement (PPA) of 100% and technical positive predictive value (TPPV) of 100% . PPA and TPPV were used for the UMSeq 7 target genes to characterize test performance during validation, since independent test results were used to confirm agreement. Variants indicated by <5.0% of aligned sequence reads are not detected. Frequent reportable variants have been validated according to New York State guidelines. 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 http://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: 49281308-49281421	exon 1 (p.L129)
EIF1AX	NM_001412.3	chrX: 20159723-20160042	exon 1 +/- 10bp
EIF1AX	NM_001412.3	chrX: 20156538-20156872	exon 2 +/- 10bp
GNA11	NM_002067.2	chr19:3118930-3119036	exon 5 (p.Q209)
GNA11	NM_002067.2	chr19: 3114958-3115053	exon 4 (p.R183)
GNAQ	NM_002072.3	chr9: 80409443-80409558	exon 5 (p.Q209)
GNAQ	NM_002072.3	chr9: 80412432-80412552	exon 4 (p.R183)
PLCB4	NM_000933.3	chr20: 9389729-9389853	exon 20 (p.D630)
SF3B1	NM_012433.2	chr2: 198267349-198267494	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

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. A modification was added to the Ion reporter workflow that allows for mutations greater than 40bp to be detected. The longest mutation that we have detected is 82bp. 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 at least 80% tumor tissue for FFPE samples. However, presence of tumor tissue is not confirmed prior to testing for FNABs. 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. Consultation with a Medical Geneticist and/or Genetic Counselor is recommended.

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

Sherri Borman, PhD, HCLD

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