CASTLE BIOSCIENCES

DecisionDx-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)

Castle ID: uS000-0

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:

GNAQ

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

SF3B1

SF3B1 mutations occur in up to 24% of uveal melanomas and it has been suggested that they confer an intermediate risk of metastasis compared to EIF1AX and BAP1 mutations.

CYSLTR2

Mutations in CYSLTR2 occur in ~3% of uveal melanomas and increase the activity of the G-protein-coupled receptor, resulting in increased downstream signaling similar to that induced by GNAQ and GNA11 mutations.

RESULTS

GNAQ,SF3B1,CYSLTR2

Gene	Variant (DNA)	Variant (Protein)	Variant Type	Observed Variant Allele Frequency
GNAQ	c.626A>T	p.Q209L	Missense	41.3%
SF3B1	c.1866G>T	p.E622D	Missense	28.6%
CYSLTR2	c.461G>A	p.S154N	Missense	53.0%

RESULTS INTERPRETATION

GNAQ

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 inactivates 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 (based on the results of multiple small studies) (PMID 27993330).

SF3B1

The test identified a missense mutation (c.1866G>T) in exon 14 of SF3B1, which results in an amino change from glutamic acid (E) to aspartic acid (D) (p.E622D). SF3B1 is a component of splicing factor 3B and mutations in this gene have been shown to result in alternative splicing of several RNA transcripts (PMID 23861464). In small retrospective studies, SF3B1 mutations have been associated with younger patient age, positive PRAME expression, and an intermediate risk of metastasis (PMIDs 27123562, 26923342, 26933176). This mutation is considered to be Tier III (a variant of unknown clinical significance) (PMID 27993330).

RESULTS INTERPRETATION

CYSLTR2

The test identified a missense mutation (c.461G>A) in exon 5 of CYSLTR2, which results in an amino acid change from serine (S) to asparagine (N) (p.S154N). This mutation has been shown to increase activity of the G-protein coupled receptor CYSLTR2 and its downstream signaling, similar to the effects conferred by mutations in the genes GNAQ and GNA11 (PMID 27089179). This mutation is considered to be Tier III (a variant of unknown clinical significance) (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 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. 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.

Curtis McGuyer, MD, Lab Director

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