Ontario Cancer Hospital

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Division of Tumor Sequencing and Diagnostics

CLINICAL LABORATORY RESULTS

Report electronically signed by: _____

Patient Info.

First Name: Redacted
Last Name:
DOB:
Sex:
Health Card:
Medical Record #:
Sample tested: Amplified DNA Methylation analysis Referral Reason:
Referring Physician:

Dates

Collected – 2022-06-02 02:35:00
Assessed – 2022-06-03 18:29:38
Reported – 2022-06-04 11:26:50

Results:

One likely pathogenic variant detected.

Summary of Results:

Gene	Exon	Base	Amino Acid	Zygosity	Interpretation	
TNFAIP3	11	c.1413T>A	p.Ser471Arg	heterozygous	Likely pathogenic	

Genes Analyzed: 8 total SS18, ACVR1, HSP90AA1, TNFAIP3, RBL1, MXRA5, NCOA4, ASPSCR1,

Test Details:

Findings:

The interpretation of these variants is as follows: One likely pathogenic variant was detected in the sample. **Variant 1 of 1 TNFAIP3 (c.1413T>A p.Ser471Arg)**
The c.1413T>A variant occurs at position 471 and is located in exon 11 of the TNFAIP3 gene, within chromosome chr6 . It causes an amino acid substitution, which replaces serine with arginine . ClinVar and other genomic databases report the TNFAIP3 c.1413T>A variant as clinically relevant based on aggregated evidence.

This variant is considered likely pathogenic. It has been associated with deleterious effects on protein function and may contribute to disease in affected individuals.

The affected nucleotide lies within a region that is highly conserved across vertebrate species, which suggests functional importance and evolutionary constraint.

This variant is implicated in oncogenesis and other disease processes according to ClinVar records (VCV accession: VCV004216583). Supporting studies and case reports can be found in the scientific literature. Relevant PubMed references include: 555318228, 677905266, 271152895, 570482712, 348221633 . In accordance with existing evidence, this variant is therefore classified as a likely pathogenic variant.

Recommendations

We recommend a precision oncology approach. These variants are associated with constitutive pathway activation and are well-established drivers of tumourigenesis. Targeted therapies should be evaluated based on these molecular findings. Specifically, pharmaceutical treatment is also recommended to correct hormone imbalances that may be caused by these mutations. However, the clinician's advice takes precedence. Additionally, PI3K inhibitors could be explored in clinical trials for the PIK3CA-mutated context. Further germline testing is not indicated at this time, as all three mutations are consistent with somatic oncogenic events. Multidisciplinary tumour board review is advised to integrate molecular findings into the patient's treatment plan. Further genetic testing may be required and completed at a physician's discretion.

Methodology

Genomic DNA was extracted and analyzed using a custom-designed targeted sequencing panel encompassing all coding exons and at least 20 base pairs of flanking intronic regions for the specified genes. Target enrichment was performed using hybrid capture technology (Twist Bioscience), followed by paired-end sequencing on the Illumina NextSeq platform. Sequencing reads were aligned to the GRCh37/hg19 human genome reference using BWA-MEM, and variant calling was performed using GATK (Broad Institute). Annotation and interpretation of variants were conducted using VarSeq (Golden Helix), incorporating population frequency databases, in silico prediction tools, and ClinVar. Exon-level copy number variations were evaluated using CNVkit and confirmed by MLPA (MRC Holland) when applicable. Regions with known pseudogene interference, such as PMS2, were validated with long-range PCR and Sanger sequencing. The average read depth across

all targeted regions exceeded 300x, with a minimum depth threshold of 50x. The analytical sensitivity for single nucleotide variants and small indels is >99%, and for exon-level CNVs, >95%. **Only variants classified as pathogenic, likely pathogenic, or variants of uncertain significance (VUS) are reported**, according to ACMG/AMP guidelines (PMID: 25741868).

Limitations

This test was developed and validated by a certified clinical laboratory. Limitations include reduced sensitivity in regions with pseudogenes (e.g., PMS2, CHEK2), and inability to detect certain structural variants (e.g., MSH2 inversion), deep intronic changes, or low-level mosaicism. PMS2 exons 11–15 are not assessed due to pseudogene interference. Variant interpretation reflects current scientific knowledge and may evolve over time.