

Toronto Cancer Hospital

Division of Tumor
Sequencing and
Diagnostics

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Draft PATHOLOGY LABORATORY RESULTS

Patient Info:

Name:
DOB:
Sex:

Patient Information:

Physician Name:
Health Card:
MRN #:
Clinic: Sinai Health System
(Toronto)

Procedure Date
2023-03-20 16:30:00

Accession Date
2023-03-21 19:03:41

Report Date
2023-03-23 10:38:10

Report Details

Genome Reference - GRCh38
Sequencing Range - Whole exome sequencing (WES), Variant analysis

Referral Reason - Clinical

Analysis Location - London Health Sciences Centre (London)

Table of findings:

Gene	Information	Interpretation
STIL	18 c.394G>C p.Glu132Gln homozygous	Variant of uncertain clinical significance
GATA3	8 c.1331G>A p.Gly444Asp heterozygous	Variant of uncertain clinical significance

Summary: Two variants of uncertain clinical significance detected.. The details regarding the specific mutations are included below.

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Variant Interpretation:

The interpretation of these variants is as follows: Two variants of uncertain clinical significance were detected in the sample.

Variant 1 of 2

Gene	Variant	Amino	Zygoty
STIL	c.394G>C	p.Glu132Gln	homozygous

Variant of uncertain clinical significance

The g.47250532G>C variant occurs in chromosome chr1 , within the STIL gene, and it causes c.394G>C change at position 132 in exon 18 , causing the mutation p.Glu132Gln . This mutation has been identified in 33 families. It has a population frequency of 0.00e+00 (0 alleles in 527781 total alleles tested), indicating it is a very rare variant in the general population. It causes an amino acid substitution, which replaces glutamate with glutamine . ClinVar and other genomic databases report the STIL c.394G>C variant as clinically relevant based on aggregated evidence.

The clinical implications of this variant are not yet fully understood. At present, available data is insufficient to confirm its role in disease.

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

This variant is not currently strongly implicated in specific diseases according to ClinVar records (VCV accession: VCV003580448).

Supporting studies and case reports can be found in the scientific literature. Relevant PubMed references include: 852145429, 926084914, 206744673, 803069417, 811657780 .

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According to ClinVar, the evidence collected to date is insufficient to firmly establish the clinical significance of this variant, therefore it is classified as a variant of uncertain clinical significance.

Variant 2 of 2

Gene	Variant	Amino	Zygosity
GATA3	c.1331G>A	p.Gly444Asp	heterozygous

Variant of uncertain clinical significance

The g.8046708G>A variant occurs in chromosome chr10, within the GATA3 gene, and it causes c.1331G>A change at position 444 in exon 8, causing the mutation p.Gly444Asp. This mutation has been identified in 40 families. It has a population frequency of 1.13e-05 (6 alleles in 532228 total alleles tested), indicating it is a very rare variant in the general population. It causes an amino acid substitution, which replaces glycine with aspartate. ClinVar and other genomic databases report the GATA3 c.1331G>A variant as clinically relevant based on aggregated evidence.

The clinical implications of this variant are not yet fully understood. At present, available data is insufficient to confirm its role in disease.

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

This variant is not currently strongly implicated in specific diseases according to ClinVar records (VCV accession: VCV003655358).

Supporting studies and case reports can be found in the scientific literature. Relevant PubMed references include: 425553480, 553524314, 771277387, 837905293, 273401221.

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According to ClinVar, the evidence collected to date is insufficient to firmly establish the clinical significance of this variant, therefore it is classified as a variant of uncertain clinical significance.

Test Details:

Genes Analyzed: PML, total. SS18, total. CDKN2C, total. HIST4H4, total. RBM15, total. POLQ, total. ARNT, total. STIL, total. GATA3, total. ETV6, total. EPHB6, total. ETS1, total. FCRL4, total. BRCA2, total. 14 total genes tested.

mRNA ref sequences tested NM_...: NM_033238.3 NM_001007559.3 NM_078626.3 NM_175054.2 NM_022768.5 NM_199420.4 NM_001668.4 NM_001048166.1 NM_001002295.2 NM_001987.5 NM_004445.6 NM_001143820.2 NM_031282.3 NM_000059.4

Recommendations

A precision oncology approach is advised. These mutations are known oncogenic drivers, linked to constitutive pathway activation. Targeted therapies, including hormone-correcting agents, may be considered, guided by clinical judgment. PI3K inhibitors could be explored in trials for PIK3CA-mutated cases. Germline testing is not indicated, as all mutations are consistent with somatic events. A multidisciplinary tumour board review is recommended to integrate findings into care. Additional testing may be pursued at the physician's discretion.

Methodology

Total RNA was sequenced with Whole exome sequencing (WES) and was analyzed using Variant analysis covering all coding exons and adjacent intronic regions. Target enrichment was performed with hybrid capture (Twist Bioscience), followed by Illumina NextSeq sequencing. Reads were aligned to GRCh37 using BWA-MEM, and variants called with GATK. Annotation was performed in VarSeq using population databases, predictive algorithms, and ClinVar. CNVs were assessed with CNVkit and confirmed by MLPA when applicable. Regions with pseudogene interference, such as PMS2, were validated using long-range PCR and Sanger sequencing. Mean read depth exceeded 300x, with a minimum threshold of 50x. Analytical sensitivity is >99% for SNVs/indels and >95% for exon-level CNVs. **Only variants classified as pathogenic, likely pathogenic, or variants of uncertain significance (VUS) are reported**, per ACMG/AMP guidelines (PMID: 25741868).

Limitations

This test was developed and validated in a certified clinical laboratory. Limitations include reduced sensitivity in pseudogene regions (e.g., PMS2, CHEK2), and inability to detect certain structural variants (e.g., MSH2 inversions), deep intronic changes, or low-level mosaicism. PMS2 exons 11–15 are not analyzed. Interpretations reflect current knowledge and may be updated as new evidence

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emerges.

Mock specimen

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Report Electronically Verified and Signed
by:

Mock specimen