Toronto Cancer Hospital

Division of Tumor Sequencing and Diagnostics

123 Main St. West,
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Draft PATHOLOGY LABORATORY RESULTS

Patient Info:

Name:
DOB:
Sex:

Patient Information:

Physician Name:
Health Card:
MRN #:
Clinic: Health Sciences North (Sudbury)

Procedure Date	Accession Date	Report Date
2025-05-16 20:45:00	2025-05-18 06:53:58	2025-05-19 07:29:17

Report Details

Genome Reference - GRCh38
Sequencing Range - Whole genome sequencing (WGS), Fusion analysis

Referral Reason - Clinical

Analysis Location - London Health Sciences Centre (London)

Table of findings:

Gene	Information	Interpretation
TNFRSF14	11 c.461G>C p.Gly154Ala heterozygous	Variant of uncertain clinical significance

Summary: One variant 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: One variant of uncertain clinical significance was detected in the sample.

Variant 1 of 1

Gene	Variant	Amino	Zygosity
TNFRSF14	c.461G>C	p.Gly154Ala	heterozygous

Variant of uncertain clinical significance

The c.2556099G>C variant occurs in chromosome chr1 , within the TNFRSF14 gene, and it causes c.461G>C change at position 154 in exon 11 , causing the mutation p.Gly154Ala . This mutation has been identified in 30 families. It has a population frequency of 3.90e-04 (188 alleles in 481776 total alleles tested), indicating it is a rare variant in the general population. It causes an amino acid substitution, which replaces glycine with alanine . ClinVar and other genomic databases report the TNFRSF14 c.461G>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: VCV007428002).

Supporting studies and case reports can be found in the scientific literature. Relevant PubMed references include: 555711469, 331379101, 323383718, 615225776, 649792533, 257203218, 297160652 .

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: IL7R, total. FOXQ1, total. KALRN, total. IRF4, total. KDM5C, total. CDH11, total. HDAC6, total. TNFRSF14, total. GABRG1, total. PHOX2B, total. WWTR1, total. NCOR1, total. RPL5, total. CHEK1, total. COL2A1, total. 15 total genes tested.

mRNA ref sequences tested NM_...: NM_002185.5 NM_033260.4 NM_001388419.1 NM_002460.4 NM_004187.5 NM_001797.4 NM_006044.4 NM_003820.4 NM_173536.4 NM_003924.4 NM_015472.6 NM_006311.4 NM_000969.5 NM_001114122.3 NM_001844.5

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

Amplified DNA was sequenced with Whole genome sequencing (WGS) and was analyzed using Fusion 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. In-

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terpretations reflect current knowledge and may be updated as new evidence emerges.

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Report Electronically Verified and Signed by: