CENTRAL EUROPEAN INSTITUTE OF TECHNOLOGY

GERMLINE EXOME ANALYSIS REPORT

PATIENT

Patient ID: LK0302
Diagnosis: NUT midline carcinoma
Specimen type: Peripheral blood
Date of collection: 23.1.2023

METHODOLOGY*

Method used: Whole-exome sequencing
Target enrichment: KAPA HyperExome
Sequencing device: NextSeq 500
Date of sequencing: 13.2.2023
Report issued: 17.2.2023

*Details regarding the methodology are listed on page 2.

RESULTS

No variants with known or potential clinical significance in genes associated with hereditary cancer-predisposing syndromes were found.

Variant classification:
Pathogenic (class 5)
Likely pathogenic (class 4)
Variant of uncertain significance (VUS, class 3)
Likely benign (class 2)
Benign (class 1)

DFP – disease-associated and/or functional polymorphism (according to the HGMD database)

A classification created according to Richards *et al.*: Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015 May;17(5):405-24.

Laboratory analysis: Mgr. Kateřina Koželková
Bioinformatic processing: Ing. Robin Jugas
Report preparation: Mgr. Petra Pokorná | petra.vesela@ceitec.muni.cz / Pokorna.Petra3@fnbrno.cz| Phone: +420 721 754 144
Revised by: prof. RNDr. Ondřej Slabý, Ph.D. | oslaby@med.muni.cz | Phone: +420 5 4949 1331
CEITEC Masaryk University, Kamenice 753/5, Brno 625 00, Czech Republic

METHODOLOGY

Library preparation and sequencing

DNA for whole-exome sequencing was extracted from peripheral blood using the QIAmp DNA Micro Kit (Qiagen, Germany). Sequencing libraries were prepared using KAPA HyperExome Kit (Roche, Switzerland). Sequencing was carried out on the NextSeq 500 device using NextSeq 500/550 Mid Output Kit v2,5 (150 cycles) (Illumina, CA, USA).

Patient-specific information regarding the analysis

The sequencing was performed on the 13th of February, 2023. 97% of the target regions were covered at least 20 times.

Bioinformatic workflow

Data processing:	BWA (alignment to GRCh38), Picard (marking duplicates), Samtools (sorting/indexing)
Quality Control:	Qualimap, PicardTools CollectHsMetrics, FastQC (aggregated in MultiQC)
Variant Calling:	GATK HaplotypeCaller, VarDict, Strelka (union approach)
Annotation:	Ensembl Variant Effect Predictor + in-house annotation scripts for Gene, Transcript assignment, Variant Consequence, Population databases (1000 Genomes, GnomAD, ExAC), Clinical databases (dbSNP, CancerGeneCensus, COSMIC, HGMD, NHLBI ESP, TruSight, ClinVar, MD Anderson, Foundation One CDx), Protein structure predictors (SIFT, PolyPhen2)

Limitations

Mutations outside of the coding regions, in other genes, copy number changes, and changes that cannot be detected at present technical possibilities and current level of knowledge cannot be excluded.

Not all detected variants are reported. Reported variants were pre-selected based on their known or potential significance in the disease. All cancer-associated variants found are listed in the protocol supplement.

VARIANT CLASSIFICATION SYSTEM

The classification system used in this report is based on recommendations of the American College of Medical Genetics and Genomics (ACMG), initially published in 2015 by Richards *et al.* (PMID: 25741868). Gene selection is focused solely on cancer-associated genes if not specified otherwise. Additionally, 72 genes defined by ACMG that should be evaluated in individuals undergoing clinical exome/genome sequencing based on the medical actionability of the associated condition were evaluated as well (for more details, see Miller *et al.*, 2022; PMID: 35802134). Any potential incidental/secondary findings in such genes are reported and consulted directly with a clinical geneticist.

Pathogenic – the variant has sufficient evidence to classify as pathogenic (capable of causing disease). Targeted testing of at-risk family members and appropriate changes in medical management (i.e., high-risk surveillance) for pathogenic mutation carriers are recommended. Pathogenic variants are always included in results reports.

Likely pathogenic – the variant has strong evidence in favor of pathogenicity. Targeted testing of at-risk family members and appropriate changes in medical management (i.e., high-risk surveillance) for carriers are recommended. Likely pathogenic variants are always included in results reports.

Variant of uncertain significance (VUS) – the variant has limited and/or conflicting evidence regarding pathogenicity. VUS in cancer-associated genes is typically included in results reports, however, can be omitted for heterozygous variants in genes associated with autosomal recessive disorders that predict rather benign by available prediction algorithms.

Likely benign – the variant has strong evidence against pathogenicity. Likely benign variants are not included in the results reports.

Benign – the variant has very strong evidence against pathogenicity. Likely benign variants are not included in the results reports.

Disease-associated and/or functional polymorphism – this category was defined by the HGMD database and describes variants that are either (i) disease-associated and of likely functional significance, or (ii) of clear functional significance even though no associated clinical phenotype may have been identified to date.