Germline Cancer Predisposition Panel

Test Description

The MolQ Germline Cancer Predisposition Panel include genes associated with both common and rare hereditary cancers.

Clinician

Clinician Name: Dr Amit Verma
Medical Facility: Dr AV Institute of Personalized Cancer Therapy and Research
Pathologist: Not Provided

Patient Demographic

Name: Mr. Ahmed Ibrahim
Sex: Male
Date of Birth/Age: 28 years
Disease: Bilateral Schwannoma and Meningioma

Specimen

Booking ID: 012303040256
Sample Type: Blood
Date of Collection: 04-03-2023
Date of Booking: 04-03-2023

CLINICAL SYNOPSIS

Ahmed Ibrahim, is a case of bilateral schwannoma and meningioma. He has been evaluated for pathogenic variations in the genes listed in Appendix 2.

RESULTS

Pathogenic variant causative of the reported phenotype was detected.

Gene (Transcript)#	Location	Variant	Zygosity	Disease (OMIM/Ref‡)	Inheritance	Classification$
NF2 (+) (ENST00000338641.10)	Exon 11	c.1080del (p.leu361CysfsTer3)	Heterozygous	Neurofibromatosis, type 2	Autosomal Dominant	Pathogenic

ADDITIONAL FINDINGS: VARIANT(S) OF UNCERTAIN SIGNIFICANCE (VUS) DETECTED

Gene (Transcript)#	Location	Variant	Zygosity	Disease (OMIM/Ref‡)	Inheritance	Classification$
PTCH2 (-) (ENST00000372192.4)	Exon 22	c. 3505dup (p.Leu1169ProfsTer11)	Heterozygous	Basal cell nevus syndrome/ ‡Schwannoma	Autosomal Dominant/ -	Uncertain Significance

$Genetic test results are reported based on the recommendations of American College of Medical Genetics.[1]

CLINICAL CORRELATION AND VARIANT INTERPRETATION

NF2 **p.leu361CysfsTer3**
Overall depth: 404X

Variant description: A heterozygous single base pair deletion in exon 11 of the ***NF2*** gene (**chr22:g.29671906del; Depth: 404x**) that results in a frameshift and premature truncation of the protein 3 amino acids downstream to codon 361 **(p.Leu361CysfsTer3; ENST00000338641.10**) was detected (Table). The observed variation lies in the Ezrin/radixin/moesin, alpha-helical domain of the MERL_HUMAN protein[2]. The p.Leu361CysfsTer3 variant has not been reported in the 1000 genomes, gnomAD and reference laboratory internal databases. The *in-silico* prediction# of the variant is damaging by Mutation Taster2 tool. The reference region is conserved across species.

OMIM Phenotype: Neurofibromatosis, type 2 (OMIM#101000)[3] is caused by mutations in the ***NF2*** gene (OMIM*607379)[4]. The central or type II form of neurofibromatosis (NF2) is an autosomal dominant multiple neoplasia syndrome characterized by tumors of the eighth cranial nerve (usually bilateral), **meningiomas of the brain**, and **schwannomas** of the dorsal roots of the spinal cord, glioma and ependymoma etc.

Based on the above evidence[$], ***this NF2 variation is classified as a pathogenic variant and has to be carefully correlated with the clinical symptoms.***

PTCH2 **p.Leu1169ProfsTer11**
Overall depth: 265X

Variant description: A heterozygous single base pair duplication in exon 22 of the ***PTCH2*** gene (**chr1:g.44822528dup; Depth: 265x**) that results in a frameshift and premature truncation of the protein 11 amino acids downstream to codon 1169 (**p.Leu1169ProfsTer11; ENST00000372192.4**) was detected (Table). The **p.Leu1169ProfsTer11** variant has not been reported in the 1000 genomes database and has a minor allele frequency of 0.01% and 0.009% in the gnomAD and reference laboratory internal databases, respectively. The *in-silico* prediction[#] of the variant is damaging by Mutation Taster2 tool. The reference region is conserved across mammals.

OMIM Phenotype: Basal cell nevus syndrome (OMIM#109400)[5] is caused by mutations in the ***PTCH2*** gene (OMIM*603673)[6]. This disorder is characterized by incomplete penetrance and symptoms including abnormal cervical vertebrae, glaucoma, hamartomatous stomach polyps, **medulloblastoma**, ovarian carcinoma, ovarian fibromata, etc.

Germline mutations in the ***PTCH2*** gene are reported in schwannoma patients.[7]

Based on the above evidence[$], ***this PTCH2 variation is classified as a variant of uncertain significance and has to be carefully correlated with the clinical symptoms.***

Due to lack of evidence associating loss of function variants in the ***PTCH2*** gene with schwannoma and meningioma predisposition, this variant is currently being classified as a variant of uncertain significance. Reclassification of the variant shall be considered based on the availability of additional evidence from literature and segregation of the variant in family members or presence of clinical symptoms/additional features associated with the ***PTCH2*** gene in the patient as well as family members.

RECOMMENDATIONS

- Validation of the variant(s) by Sanger sequencing is recommended to rule out false positives.
- **Clinical correlation for *PTCH2* associated features in the patient and/or family members is recommended.**
- **Targeted testing of at-risk family member especially first-degree relatives (children, siblings and parents) is recommended, as it could benefit other carriers in risk assessment and constituting measures for early detection, prevention and better management of disease.**
- **Sequencing the variant(s) in the other affected and unaffected members of the family is recommended to confirm the significance.**
- Genetic counselling is advised for interpretation on the consequences of the variant(s).

Jatinder Kaur, PhD
Head, Molecular Biology & Genomics

Dr. Gulshan Yadav, MD
Head, Pathology

REFERENCES

1. Richards S. 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., 17(5): 405-24, 2015.
2. https://www.ebi.ac.uk/interpro/protein/UniProt/P35240/
3. https://www.omim.org/entry/101000
4. https://www.omim.org/entry/607379
5. https://www.omim.org/entry/109400
6. https://www.omim.org/entry/603673
7. Min B.J. et al., Germline Mutations for Novel Candidate Predisposition Genes in Sporadic Schwannomatosis. Clin Orthop Relat Res., Nov;478(11):2442-2450, 2020.

APPENDIX 1: TEST METHODOLOGY

Method

Targeted gene sequencing: Selective capture and sequencing of the protein coding regions of the genome/genes is performed. Mutations identified in the exonic regions are generally actionable compared to variations that occur in non-coding regions. Targeted sequencing represents a cost-effective approach to detect variants present in multiple/large genes in an individual. In this comprehensive hereditary cancer panel, in addition to complete Coding segment (CDS) of 143 genes, promoter regions of relevant genes and critical other non-coding / coding pathogenic variants <100 bp documented in the ClinVar, HGMD, BRCA Exchange and LOVD databases mapping to the targeted genes are also included[1-4]. Additionally, there is an enhanced CNV coverage for better detection of CNVs. This panel provides a comprehensive and robust approach to identify SNV's, Indels and CNVs through single test.

DNA extracted from sample was used to perform targeted gene capture using a custom capture kit. The libraries were sequenced to mean >80- 100X coverage on Illumina sequencing platform. We follow the GATK best practices framework for identification of variants in the sample using Sentieon (v201808.07)[5]. The sequences obtained are aligned to human reference genome (GRCh38.p13) using Sentieon aligner[5,6] and analyzed using Sentieon for removing duplicates, recalibration and re-alignment of indels. Sentieon haplotype caller has been used to identify variants which are relevant to the clinical indication. Gene annotation of the variants is performed using VEP program[7] against the Ensemble release 99 human gene model[8]. Copy number variants (CNVs) are detected from targeted sequence data using the ExomeDepth (v1.1.10) method, a coverage based approach[9]. This algorithm detects rare CNVs based on comparison of the read-depths of the test data with the matched aggregate reference dataset and the overall sensitivity of CNV surveillance through ExomeDepth was found to be 97%[10]. In reference laboratory internal validation experiments on MLPA verified samples, >80-90% sensitivity was achieved for detecting CNVs .

Clinically relevant mutations were annotated using published variants in literature and a set of diseases databases - ClinVar, OMIM (updated on 11th May 2020), GWAS, HGMD (v2020.2), LOVD, BRCA Exchange and SwissVar[1-4, 11-13]. Common variants are filtered based on allele frequency in 1000Genome Phase 3, gnomAD (v3.1), EVS, dbSNP (v151), 1000 Japanese Genome and reference laboratory internal Indian population database[14-18]. Non-synonymous variants effect is calculated using multiple algorithms such as PolyPhen-2, SIFT, MutationTaster2 and LRT. Only non-synonymous, splice site and critical non-coding variants found in the hereditary cancer panel genes were used for clinical interpretation. Silent variations that do not result in any change in amino acid in the coding region are not reported.

Total data generated (Gb)	0.4632
Total reads aligned (%)	99.99
Reads that passed alignment (%)	93.01
Data ≥ Q30 (%)	96.21

$The classification of the variations is done based on American College of Medical Genetics as described below[19]

Variant	A change in a gene. This could be disease causing (pathogenic) or not disease causing (benign).
Pathogenic	A disease causing variation in a gene which can explain the patient's symptoms has been detected. This usually means that a suspected disorder for which testing had been requested has been confirmed.
Likely Pathogenic	A variant which is very likely to contribute to the development of disease however, the scientific evidence is currently insufficient to prove this conclusively. Additional evidence is expected to confirm this assertion of pathogenicity.
Variant of Uncertain Significance	A variant has been detected, but it is difficult to classify it as either pathogenic (disease causing) or benign (non-disease causing) based on current available scientific evidence. Further testing of the patient or family members as recommended by your clinician may be needed. It is probable that their significance can be assessed only with time, subject to availability of scientific evidence.

#The transcript used for clinical reporting generally represents the canonical transcript, which is usually the longest coding transcript with strong/multiple supporting evidence. However, clinically relevant variants annotated in alternate complete coding transcripts could also be reported.
Variants annotated on incomplete and nonsense mediated decay transcripts will not be reported.
#The *in-silico* predictions are based on Variant Effect Predictor, Ensembl release 99 (SIFT version - 5.2.2; PolyPhen - 2.2.2); bdNSFPv4.0 (LRT version – December 5, 2019) and Mutation Taster2 (MT2). MutationTaster2 predictions are based on NCBI/Ensembl 66 build (GRCh38 genomic coordinates are converted to hg19 using UCSC LiftOver and mapped to MT2).
For any further technical queries please contact contact@molq.in.

DISCLAIMER

- Interpretation of variants in this report is performed to the best knowledge of the laboratory based on the information available at the time of reporting. The classification of variants can change over time and MolQ cannot be held responsible for this. Please feel free to contact MolQ Laboratory (contact@molq.in) in the future to determine if there have been any changes in the classification of any variations. Re-analysis of variants in previously issued reports in light of new evidence is not routinely performed, but may be considered upon request, provided the variant is covered in the current panel.
- The sensitivity of this assay to detect large deletions/duplications of more than 10 bp or copy number variations (CNV) is 80-90%. The CNVs detected have to be confirmed by alternate method.
- Due to inherent technology limitations of the assay, not all bases of the exome can be covered by this test. Accordingly, variants in regions of insufficient coverage may not be identified and/or interpreted. Therefore, it is possible that certain variants are present in one or more of the genes analyzed, but have not been detected. The variants not detected by the assay that was performed may/ may not impact the phenotype.
- It is also possible that a pathogenic variant is present in a gene that was not selected for analysis and/or interpretation in cases where insufficient phenotypic information is available.
- Genes with pseudogenes, paralog genes and genes with low complexity may have decreased sensitivity and specificity of variant detection and interpretation due to inability of the data and analysis tools to unambiguously determine the origin of the sequence data in such regions.
- The mutations have not been validated/confirmed by Sanger sequencing.
- Incidental or secondary findings (if any) that meet the ACMG guidelines[20] can be given upon request.
- The report shall be generated within turnaround time (TAT), however, such TAT may vary depending upon the complexity of test(s) requested. MolQ Laboratory under no circumstances will be liable for any delay beyond afore mentioned TAT.
- It is hereby clarified that the report(s) generated from the test(s) do not provide any diagnosis or opinion or recommends any cure in any manner. MolQ Laboratory hereby recommends the patient and/or the guardians of the patients, as the case may be, to take assistance of the clinician or a certified physician or doctor, to interpret the report(s) thus generated. MolQ Laboratory hereby disclaims all liability arising in connection with the report(s).
- In a very few cases genetic test may not show the correct results, e.g. because of the quality of the material provided to MolQ Laboratory. In case where any test provided by MolQ Laboratory fails for unforeseeable or unknown reasons that cannot be influenced by MolQ Laboratory in advance, MolQ Laboratory shall not be responsible for the incomplete, potentially misleading or even wrong result of any testing if such could not be recognized by MolQ Laboratory in advance.
- This is a laboratory developed test and the development and the performance characteristics of this test was determined by reference laboratory.

LIMITATIONS

- Genetic testing is an important part of the diagnostic process. However, genetic tests may not always give a definitive answer. In some cases, testing may not identify a genetic variant even though one exists. This may be due to limitations in current medical knowledge or testing technology. Accurate interpretation of test results may require knowing the true biological relationships in a family. Failing to accurately state the biological relationships in {my/my child's} family may result in incorrect interpretation of results, incorrect diagnoses, and/or inconclusive test results.
- Test results are interpreted in the context of clinical findings, family history and other laboratory data. Only variations in genes potentially related to the proband's medical condition are reported. Rare polymorphisms may lead to false negative or positive results. Misinterpretation of results may occur if the information provided is inaccurate or incomplete.
- Structural variants such as deletions/duplications (CNVs) reported through NGS assay needs to be confirmed by orthogonal method to rule out the possibility of false positives. Translocations, repeat expansions and chromosomal rearrangements are not detected through this assay.
- Genetic testing is highly accurate. Rarely, inaccurate results may occur for various reasons. These reasons include, but are not limited to mislabelled samples, inaccurate reporting of clinical/medical information, rare technical errors or unusual circumstances such as bone marrow transplantation, blood transfusion; or the presence of change(s) in such a small percentage of cells that may not be detectable by the test (mosaicism).
- The variant population allele frequencies and *in silico* predictions for GRCh38 version of the Human genome is obtained after lifting over the coordinates from hg19 genome build. The existing population allele frequencies (1000Genome, gnomAD-Exome) are currently available for hg19 genome version only. This might result in some discrepancies in variant annotation due to the complex changes in some regions of the genome.

REFERENCES

1. Landrum M. J. et al., ClinVar: public archive of interpretations of clinically relevant variants. Nucleic Acids Res., 44(D1):D862-8, 2015.
2. Stenson P. D. et al., The Human Gene Mutation Database: towards a comprehensive repository of inherited mutation data for medical research, genetic diagnosis and next-generation sequencing studies. Human Genet., 136(6): 665-677, 201.
3. Cline, Melissa S et al. BRCA Challenge: BRCA Exchange as a global resource for variants in BRCA1 and BRCA2. PLoS genetics vol. 14,12 e1007752. 26 Dec. 2018, doi:10.1371/journal.pgen.1007752.
4. Fokkema IF, Taschner PE, Schaafsma GC, et al., LOVD v.2.0: the next generation in gene variant databases. Hum Mutat., 32(5):557-63, 2011.
5. Freed D. et al., The Sentieon Genomics Tools-A fast and accurate solution to variant calling from next-generation sequence data. BioRxiv:115717, 2017.
6. Li H. et al., Fast and accurate long-read alignment with Burrows-Wheeler transform. Bioinformatics, 26(5):589-95, 2010.
7. McLaren W. et al., Deriving the consequences of genomic variants with the Ensembl API and SNP Effect Predictor. Bioinformatics, 26(16):2069-70, 2010.
8. Zerbino D. R. et al., Ensembl 2018. Nucleic Acids Res., 46(D1):D754-D761, 2018.
9. Plagnol V. et al., A robust model for read count data in exome sequencing experiments and implications for copy number variant calling. Bioinformatics, 28:2747-2754, 2012.
10. Ellingford JM, et al., Validation of copy number variation analysis for next-generation sequencing diagnostics. Eur J Hum Genet., 25(6):719-724, 2017.
11. McKusick V.A., Mendelian Inheritance in Man. A Catalog of Human Genes and Genetic Disorders. Baltimore: Johns Hopkins University Press (12th edition), 1998.
12. Welter D. et al., The NHGRI GWAS Catalog, a curated resource of SNP-trait associations. Nucleic Acids Res., 42:D1001-1006, 2014.
13. Mottaz A. et al., Easy retrieval of single amino-acid polymorphisms and phenotype information using SwissVar. Bioinformatics, 26(6): 851-852, 2010.
14. 1000 Genomes Project Consortium et al., A global reference for human genetic variation. Nature, 526(7571): 68-74, 2015.
15. Karczewski, K.J., Francioli, L.C., Tiao, G. et al. The mutational constraint spectrum quantified from variation in 141,456 humans. Nature 581, 434-443, 2020.
16. Exome Variant Server, NHLBI GO Exome Sequencing Project (ESP), Seattle, WA (URL: http://evs.gs.washington.edu/EVS/).
17. Sherry S. T. et al., dbSNP: the NCBI database of genetic variation. Nucleic Acids Res., 29(1):308-11, 2001.
18. Nagasaki, M. et al., Rare variant discovery by deep whole-genome sequencing of 1,070 Japanese individuals. Nat. Commun., 6:8018, 2015.
19. Richards S. *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., 17(5):405-24, 2015.
20. Kalia S.S. et al., Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2016 update (ACMG SF v2.0): a policy statement of the American College of Medical Genetics and Genomics. Genet Med., 19(2):249-255, 2017.

APPENDIX 2: GENE LIST WITH COVERAGE^

Gene	Percentage of coding region covered	Gene	Percentage of coding region covered	Gene	Percentage of coding region covered
ABRAXAS1	100.00	*AIP*	100.00	*ALK*	100.00
*APC**	100.00	*AR*	100.00	*ATM*	100.00
AXIN2	100.00	*BAP1*	100.00	*BARD1*	100.00
BLM	100.00	*BMPR1A*	100.00	*BRCA1*	100.00
BRCA2	100.00	*BRIP1*	100.00	*BUB1B*	100.00
CBL	100.00	*CD82*	100.00	*CDC73*	100.00
CDH1	100.00	*CDK12*	100.00	*CDK4*	100.00
CDKN1B	100.00	*CDKN1C*	100.00	*CDKN2A*	100.00
CEBPA	100.00	*CEP57*	100.00	*CHEK1*	100.00
CHEK2	100.00	*CTNNA1*	100.00	*CYLD*	100.00
DDB2	100.00	*DICER1*	100.00	*DIS3L2*	100.00
EGFR	100.00	*ELAC2*	100.00	*ENG*	100.00
EPCAM	100.00	*ERCC2*	100.00	*ERCC3*	100.00
ERCC4	100.00	*ERCC5*	100.00	*EXT1*	100.00
EXT2	100.00	*EZH2*	100.00	*FAN1*	100.00
FANCA	100.00	*FANCB*	100.00	*FANCC*	100.00
FANCD2	100.00	*FANCE*	100.00	*FANCF*	100.00
FANCG	100.00	*FANCI*	100.00	*FANCL*	100.00
FANCM	100.00	*FH*	100.00	*FLCN*	100.00
GALNT12	100.00	*GATA2*	100.00	*GPC3*	100.00
HOXB13	100.00	*HRAS*	100.00	*KIF1B*	100.00
KIT	100.00	*LZTR1*	100.00	*MAX*	100.00
MEN1	100.00	*MET*	100.00	*MITF*	100.00
MLH1	100.00	*MLH3*	100.00	*MRE11*	100.00
MSH2	100.00	*MSH3*	100.00	*MSH6*	100.00
MSR1	100.00	*MUTYH*	100.00	*MXI1*	100.00
NBN	100.00	*NF1*	100.00	*NF2*	100.00
NSD1	100.00	*NTHL1*	100.00	*PALB2*	100.00
PALLD	100.00	*PAX5*	100.00	*PDGFRA*	100.00
PHOX2B	100.00	*PMS1*	100.00	*PMS2*	100.00
POLD1	100.00	*POLE*	100.00	*POT1*	100.00
PPP2R2A	100.00	*PRF1*	100.00	*PRKAR1A*	100.00
PRSS1	100.00	*PTCH1*	100.00	*PTCH2*	100.00
PTEN	100.00	*RAD50*	100.00	*RAD518*	100.00
RAD51C	100.00	*RAD51D*	100.00	*RAD54L*	100.00
RB1	100.00	*RECQL*	100.00	*RECQL4*	100.00
RET	100.00	*RHBDF2*	100.00	*RINT1*	100.00
RNASEL	100.00	*RNF43*	100.00	*RUNX1*	100.00
SBDS	100.00	*SDHA*	100.00	*SDHAF2*	100.00
SDHB	100.00	*SDHC*	100.00	*SDHD*	100.00
SLC45A2	100.00	*SLX4*	100.00	*SMAD4*	100.00
SMARCA4	100.00	*SMARCB1*	100.00	*SMARCE1*	100.00
SRGAP1	100.00	*STK11*	100.00	*SUFU*	100.00
*TERT**	100.00	*TGFBR2*	100.00	*TMEM127*	100.00
TP53	100.00	*TSC1*	100.00	*TSC2*	100.00

Germline Cancer Predisposition Panel

TYR	100.00	*VHL*	100.00	*WRN*	100.00
WT1	100.00	*XPA*	100.00	*XPC*	100.00
XRCC1	100.00	*XRCC3*	100.00		

* Promoter regions of these genes are also analyzed. ^ In addition to complete CDS coverage in these genes, critical non-coding variants reported as pathogenic in clinical databases are also analyzed in this assay.