Baylor College of Medicine

MEDICAL GENETICS LABORATORIES

2450 Holcombe Blvd - Houston, TX 77021 - 1-800-411-4363
Fax: 713-798-2787 - www.bcmgeneticlabs.org - genetictest@bcm.edu

CANCER GENETICS LABORATORY

Patient Name:	**D TEST4**				
Date of Birth:		Lab Number:		Baylor College of Medicine	
Gender:		Family #:		Tel No. :	713-798-6555
Hospital/MR #:	123	Date Collected:		Fax No:	713-798-2787
Accession #:	NA	Date Received:		CC:	
Sample Type:	TISSUE	Date Reported:		CC:	
Test Code:	9600	Date Ordered:			
Indication:					

Cancer Exome Sequencing

Tumor Report

Summary of Results:

This patient is enrolled in the BASIC3 study (Study ID:). Whole exome sequencing done on a representative sample of an specimen ( ) and paired normal peripheral blood specimen ( ) showed the following findings:

1. SOMATIC FRAMESHIFT MUTATION IN THE TSC2 [TUBEROUS SCLEROSIS 2] GENE.
2. SOMATIC MISSENSE MUTATION IN THE TP53 [TUMOR PROTEIN p53] GENE.
3. SOMATIC MUTATIONS IN OTHER NON-CANCER GENES OF UNKNOWN CLINICAL SIGNIFICANCE.

Please see report on Constitutional Exome Interpretation ( ) for reporting of variants of unknown significance in cancer susceptibility genes, as well as other germline variants of potential clinical significance.

INTERPRETATION:

1. Actionable Mutations

No Category 1 mutations were identified.

2. Tumor (Somatic) Mutations in Targeted Pathways and Cancer Genes

Whole exome sequencing on the tumor specimen identified a 2-bp deletion (c.2764_2765del) in exon 25 of the TSC2 [TUBEROUS SCLEROSIS 2] gene leading to a frameshift mutation (p.L922fs) and a premature stop codon in the TSC2 protein. The mutation is present at a variant allele fraction of 75%, implying the wild-type TSC2 gene to have been lost in the tumor cells, and has been confirmed using Sanger sequencing. The TSC2 protein in complex with TSC1 (TSC2:TSC1) is the principal cellular inhibitor of mTOR [mammalian target of rapamycin] signaling (PMID: 20146692, 22500797). Negative regulation of mTOR is exerted specifically by TSC2 through its GTPase activity in the C-terminal Rap/ran-GAP domain (PMID: 20146692, 20182617), a domain that is predicted to be absent in any truncated TSC2 protein resulting from the frameshift mutation described here. Germline inactivating mutations in TSC2 (and TSC1) with resultant mTOR hyperactivation is well-recognized to underlie the pathogenesis of the tuberous sclerosis complex (PMID: 20146692), and mTOR signaling inhibitors are approved for the treatment of CNS and kidney tumors in such patients (PMID: 23659703). Although somatic TSC2 mutations are rare in human malignancies, and never been reported in osteosarcomas (COSMIC database), given the key role of TSC2 in mTOR inhibition, it is possible that the TSC2 mutation in this osteosarcoma could lead to activated mTOR signaling. Although not approved for use in this specific tumor type, several mTOR inhibitors are approved in other cancers for targeting aberrant mTOR activation (PMID: 23659703).
It should also be noted that constitutively active mTOR signaling resulting from TSC2/TSC1 mutations is counteracted in cells by wild-type TP53 signaling through negative feedback mechanisms (PMID: 20182617), and induction of TP53-mediated cell death (PMID: 20605525); however neither of these negative regulatory actions are likely effective in this tumor with a mutant TP53 (see below).

Gene	RefSeq ID	Mutation (Genomic)	Mutation (Protein)	Type	% Mutation	Pathway	Protein Domain
TSC2	NM_000548	c.2764_2765del	p.L922fs	Frameshift indel	75.56	mTOR signaling	None

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3. Tumor (Somatic) Mutations in Cancer Genes

Exome sequencing on the tumor specimen also identified a c.742C>T missense mutation in exon 7 of the TP53 [TUMOR PROTEIN p53] gene that is predicted to change a conserved arginine residue in the TP53 DNA-binding domain to a tryptophan at codon 248 (p.R248W). This mutation is present in the tumor at a variant allele fraction of 29% and has been confirmed using Sanger sequencing. The TP53 tumor suppressor is the most commonly mutated gene in human cancers (PMID: 23360998, 17417627) and either TP53 or its upstream or downstream signaling proteins are mutated or inactivated in virtually all cancers (PMID: 19776747 and 23263379). TP53 is a master transcription factor that regulates the cellular response to diverse stresses and acts by coordinating target gene expression to induce cell cycle arrest, apoptosis, DNA repair, etc. The R248W mutation (COSM10656) is a common hotspot mutation in the TP53 DNA-binding domain that abolishes TP53-mediated tumor suppression (PMID: 17417627) and has been reported in a wide variety of cancers including osteosarcomas (COSMIC database). TP53 mutations are seen in 20-25% of osteosarcomas but in at least one study the mutations were found to have little impact on metastasis or clinical outcome (PMID: 15735124). Small molecules that bind specific TP53 mutant proteins and partially restore the transcriptional activity are currently in early phases of investigation (PMID: 21209413).

Gene	RefSeq ID	Mutation (Genomic)	Mutation (Protein)	Type	% Mutation	Pathway	Protein Domain
TP53	NM_000546	c.C742T	p.R248W	Missense	29.04	p53 signaling	DNA-binding

4. Tumor (Somatic) Mutations in Non-Cancer Genes

Eighteen somatic (tumor) mutations in genes not currently listed in the cancer gene census were identified. The significance of these mutations in this patient is uncertain. These mutations are reported in Table 4. Mutations in this category have NOT been confirmed by a different method. Confirmation of specific mutations is available upon request. More information about specific genes can be found at http://www.ncbi.nlm.nih.gov/gene.

Gene	RefSeq ID	Mutation (Genomic)	Mutation (Protein)	Type	% Mutation in Tumor	Pathway	Protein Domain
ADD2	NM_001185054	c.A2161T	p.K721X	Nonsense	36.79	Membrane-cytoskeleton interaction	None
ALPL	NM_000478	c.319_320insT	p.V107fs	Frameshift indel	25.52	alkaline phosphatase	alkaline phosphatase
C14orf79	NM_174891	c.G409T	p.V137F	Missense	32.43	Unknown	None
CYTH2	NM_004228	c.T1136C	p.F379S	Missense	57.79	membrane trafficking	None
DUOX1	NM_017434	c.T3256A	p.S1086T	Missense	5.75	dual oxidase	None
LMNB2	NM_032737	c.G1285A	p.G429R	Missense	73.2	nuclear envelope assembly	None

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MICU1	NM_001195519	c.C517G	p.L173V	Missense	44.52	mitochondrial calcium uptake	None
MUL1	NM_024544	c.G806A	p.R269H	Missense	50.61	SUMOylation	None
MXRA5	NM_015419	c.G6124A	p.V2042M	Missense	15.82	matrix remodeling	Immunoglobulin I-set domain
MYBPC2	NM_004533	c.T2068A	p.F690I	Missense	6.47	muscle contraction	Fibronectin type III domain
MYH1	NM_005963	c.C1133G	p.P378R	Missense	10.36	muscle contraction	myosin head
MYH7	NM_000257	c.G5071A	p.V1691M	Missense	43.93	cardiac muscle contraction	myosin tail
PCCA	NM_001178004	c.G40T	p.A14S	Missense	56.6	lipid metabolism	None
PKD1L2	NM_001076780	c.A772T	p.M258L	Missense	36.71	Unknown	None
PLG	NM_000301	c.C1217G	p.P406R	Missense	21.18	fibrinolysis	Kringle
SCN2A	NM_001040143	c.A4504G	p.K1502E	Missense	56.07	ion channel	None
TTN	NM_001256850	c.G53596A	p.D17866N	Missense	21.28	sarcomere organization	Fibronectin type III domain
ZBBX	NM_001199201	c.1418-1G>T	NM_024687	Splicing	5.59	Unknown	None

METHODOLOGY:

1. DNA is extracted from normal and tumor tissue sources**.
2. Whole exome sequencing (WES)*: for the paired-end pre-capture library procedure, both germline and tumor DNA are fragmented by sonicating genomic DNA and ligating to the Illumina multiplexing PE adapters. The adapter-ligated DNA is then PCR amplified using primers with sequencing barcodes (indexes). For target enrichment/exome capture procedure, the pre-capture library is enriched by hybridizing to biotin labeled VCRome 2.1 (reference 3) in-solution Exome Probes at 47°C for 64 - 72 hours. For massively parallel sequencing, the post-capture library DNA is subjected to sequence analysis on Illumina Hiseq platform for 100 bp paired-end reads. The following quality control metrics of the sequencing data are generally achieved: >70% of reads aligned to target, >95% target base covered at >20X, >85% target base covered at >40X, mean coverage of target bases >150X. SNP concordance of germline sequence to genotype array: >99%. This test may not provide detection of certain genes or portions of certain genes due to local sequence characteristics or the presence of closely related pseudogenes. Gross deletions or duplications, mutations from repetitive sequences may not be accurately identified by this methodology.
3. As a quality control measure, the individual's germline DNA is also analyzed by a high-density SNP-array (Illumina HumanOmni1-Quad array)**. The SNP data are compared with the WES data to ensure correct sample identification and to assess sequencing quality.

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4. Data analysis**: The output data from Illumina Hiseq are converted from bcl file to FastQ file by Illumina CASAVA 1.8 software, and mapped by BWA program (Li & Durbin, 2009). The variant calls are performed using Atlas-SNP and Atlas-indel developed in-house by BCM HGSC. Variants detected in both normal and tumor tissues are filtered to leave only those present exclusively in the tumor tissue (somatic or acquired mutations). Germline variants in cancer susceptibility genes are also retained. The variant annotations are performed using in-house developed software: HGSC-SNP-anno and HGSC-indel-anno. Synonymous variants, intronic variants not affecting splicing site, non-coding RNA and 5'/3' UTR variants are excluded from interpretation unless they were previously reported as deleterious mutations.

5. Germline variants are interpreted according to ACMG guidelines (Richards et al, 2008) and patient phenotypes. Somatic mutations/variants are classified in five categories as follows**:

Category IA - Somatic sequence variants that have been established as diagnostic, prognostic and/or predictive of treatment response in the tumor type being tested, following published clinical practice guidelines or recommendation of the BCM Genomics Tumor Board.

Category IB - Germline sequence variants in cancer susceptibility genes that have been reported as pathogenic or likely pathogenic in HGMD, locus specific databases or peer reviewed literature.

Category 2 - Somatic sequence variants in genes that are members of cancer pathways, gene families, or functional groups/pathways that are targets of approved or investigational therapeutic agents.

Category 3 - Somatic sequence variants in consensus cancer genes as defined by the Wellcome Trust Sanger Institute Cancer Gene Census or the BCM Genomics Tumor Board.

Category 4 – Other somatic sequence variants not included in Categories I-III.

Variants in categories IA, IB, 2 and 3 are confirmed by an alternate method and confirmation is noted in the interpretation section for that variant if performed. It should be noted that the data interpretation are based on our current understanding of genes and variants at the time of reporting. Pathway information is derived from the Kyoto Encyclopedia of Genes and Genomes (KEGG) or the NCBI Entrez Gene database. Location of the variant within protein domains is based on Pfam data from the COSMIC database (None indicates that the variant does not involve a recognized protein domain, Unknown indicates that there is no available protein domain information).

References:

1. Illumina, Inc. (2011) Multiplexing Sample Preparation Guide (Part # 1005361 Rev. D). 2011.
2. Roche NimbleGen, Inc. (2010) NimbleGen SeqCap EZ Exome Library SR User's Guide (Version 2.2).
3. Bainbridge MN, Wang M, Wu Y, Newsham I, Muzny DM, Jefferies JL, Albert TJ, Burgess DL, Gibbs RA (2011). Targeted Enrichment beyond the Consensus Coding DNA Sequence Exome Reveals Exons with Higher Variant Densities. Genome Biology 12(7):R68.
4. Li H. and Durbin R. (2009) Fast and accurate short read alignment with Burrows-Wheeler Transform. Bioinformatics, 25:1754-60.
5. Richards CS, et al. Molecular Subcommittee of the ACMG Laboratory Quality Assurance Committee (2008). ACMG recommendations for standards for interpretation and reporting of sequence variations: Revisions 2007. Genet Med, 10, 294-300.
6. Kanehisa, M. and Goto, S.; KEGG: Kyoto Encyclopedia of Genes and Genomes. Nucleic Acids Res. 28, 27-30 (2000).
7. Maglott D, et al. Entrez Gene: gene-centered information at NCBI. Nucleic Acids Res. 2005; 33:D54–D58.

Christine M. Eng, M.D.
Medical Director

Angshumoy Roy, M.D., Ph.D.
Pediatric Molecular Pathologist

This test was developed and its performance determined by this laboratory. It has not been cleared or approved by U.S. Food and Drug Administration. Since FDA is not required for clinical use of this test, this laboratory has established and validated the test's accuracy and precision, pursuant to the requirement of CLIA '88. This laboratory is licensed and/or accredited under CLIA and CAP. (CAP# 2109314 / CLIA# 45D0660090 WGL:CAP# 8004250 / CLIA# 45D2027450). * performed at the Whole Genome Laboratory; ** performed at both the Medical Genetics Laboratories and the Whole Genome Laboratory