Blueprint Genetics

Hereditary Melanoma and Skin Cancer Panel Plus

REFERRING HEALTHCARE PROFESSIONAL

NAME **HOSPITAL**

PATIENT

NAME	DOB	AGE	GENDER	ORDER ID
		33	Female	

PRIMARY SAMPLE TYPE	SAMPLE COLLECTION DATE	CUSTOMER SAMPLE ID
DNA		

SUMMARY OF RESULTS

TEST RESULTS

Patient is heterozygous for *PTCH1* c.2412dupT, p.(Ile805Tyrfs*24), which is likely pathogenic.

Del/Dup (CNV) analysis did not detect any known disease-causing copy number variation or novel or rare deletion/duplication that was considered deleterious.

VARIANT TABLE: GENETIC ALTERATIONS

GENE	POS	TRANSCRIPT	NOMENCLATURE	CONSEQUENCE	GENOTYPE	CLASSIFICATION
PTCH1	9:98229545	NM_000264.3	c.2412dupT, p.(Ile805Tyrfs*24)	frameshift	HET	**Likely pathogenic**
	ID	**EXAC AC/AN**	**POLYPHEN**	**SIFT**	**MUTTASTER**	
	.	0/0	N/A	N/A	N/A	
	OMIM	**PHENOTYPE**		**INHERITANCE**	**COMMENT**	
		Basal cell nevus syndrome		AD	-	

SEQUENCING PERFORMANCE METRICS OS-SEQ

PANEL	GENES	EXONS	BASES	BASES > 15X	MEDIAN COVERAGE	PERCENT > 15X
Hereditary Melanoma and Skin Cancer Panel	19	309	58304	58304	306	100

TARGET REGION AND GENE LIST

Blueprint Genetics Hereditary Melanoma and Skin Cancer Panel (version 1, March 9, 2016) consists of sequence analysis of genes associated with melanoma and skin cancer: BAP1, BRCA1*, BRCA2, CDK4, CDKN2A, DDB2, ERCC2, ERCC3, ERCC4, ERCC5, MITF, PTCH1, PTEN*, RB1, SUFU, TP53, WRN*, XPA and XPC. The panel is targeting all protein coding exons and exon-intron boundaries of all target genes. It also covers a number of mutations located outside these coding regions. This test covers the majority of melanoma and skin cancer mutations known to date and it should be used to detect single nucleotide substitutions and small insertions and deletions (INDELs). In addition, the Hereditary Melanoma and Skin Cancer Panel includes OS-Seq Del/Dup (CNV) Analysis (version 1, updated November 15, 2016) for the same genes as listed above. It should be used to diagnose deletions and duplications (e.g. copy number variants) in protein-coding regions of the genes included in the panel. Detection limit of the test varies through the genome from one to six exons depending on exon size, sequencing coverage and sequence content.

* Some regions of the gene are duplicated in the genome leading to limited sensitivity within the regions (link to duplicated regions): http://blueprintgenetics.com/pseudogene/. Thus, low-quality variants are filtered out from the duplicated regions and only high-quality variants confirmed by other methods are reported out.

The test does not recognise balanced translocations or complex inversions, and it may not detect low-level mosaicism. The exact boundaries of the copy number

aberration cannot be determined with this test. The test should not be used for analysis of sequence repeats or for diagnosis of disorders caused by mutations in the mitochondrial DNA.

STATEMENT

CLINICAL HISTORY

Patient is a 33-year-old female with clinical diagnosis of Gorlin syndrome - basal cell carcinoma, gross motor delays and bifid ribs. Patient's mother is also apparently affected. The analysis was requested to be limited to the following genes: *PTCH1* and *SUFU*.

CLINICAL REPORT

Sequence analysis using the Blueprint Genetics (BpG) Hereditary Melanoma and Skin Cancer Panel identified a heterozygous frameshift variant in the *PTCH1* gene: c.2412dupT, p.(Ile805Tyrfs*24). The one base pair duplication generates a frameshift leading to a premature stop codon in the new reading frame (829 out of 1447 aa). It is predicted to cause loss of normal protein function either through protein truncation or nonsense-mediated mRNA decay from the other allele. It has not been observed in large reference population cohorts (Exome Aggregation Consortium (ExAC) or Genome Aggregation Database (gnomAD)). According to our knowledge, the variant has not previously been reported in the literature or databases. However, other frameshift and nonsense variants have been reported in near-by codons in the HGMD and LOVD databases in association with basal cell nevus syndrome.

PTCH1 (MIM *601309) encodes a protein patched 1 that functions as a receptor for sonic hedgehog, a secreted molecule implicated in the formation of embryonic structures and in tumorigenesis. *PTCH1* functions as a tumor suppressor. Germline mutations in *PTCH1* have been associated with basal cell nevus syndrome, also known as Gorlin syndrome (MIM #109400; GeneReviews NBK1151) and holoprosencephaly (MIM #610828). Nevoid basal cell carcinoma syndrome (NBCCS) is an autosomal dominant syndrome characterized by the development of multiple jaw keratocysts, frequently beginning in the second decade of life, and/or basal cell carcinomas (BCCs) usually from the third decade onward. The prevalence is estimated at one per 57 000. More than 100 clinical features have been described. These include recognizable appearance with macrocephaly, frontal bossing, coarse facial features, and facial milia; palmar/plantar pits; skeletal anomalies (e.g., bifid ribs, wedge-shaped vertebrae); ectopic calcification, particularly in the falx, ovarian fibromas and childhood medulloblastoma. NBCCS can be caused by germline mutations in either *PTCH1* or *SUFU*. *PTCH1* mutations have been found in 40–80% of NBCCS patients (PMID: 8840969, 12925203, 16909134). Majority of the mutations (>80%) lead to premature termination of the protein (PMID: 9425597, 12655573). The penetrance is high. To date, 329 mutations in *PTCH1* have been reported in HGMD (October 2016), of which 277 have been associated with NBCCS.

Mutation nomenclature is based on GenBank accession NM_000264.3 (*PTCH1*) with nucleotide one being the first nucleotide of the translation initiation codon ATG.

CONCLUSION

We classify the identified *PTCH1* c.2412dupT, p.(Ile805Tyrfs*24) as likely pathogenic and probable cause for patient's disease, considering the current evidence of the variant (established association between the gene and patient's phenotype, rarity in control populations and mutation type (frameshift)). However, additional information is still needed to confirm the pathogenicity of the variant, which could allow independent risk stratification based on this mutation. Genetic counseling and family member testing is recommended. Disease caused by *PTCH1* mutations is inherited in an autosomal dominant manner, thus each child of an affected individual has a 50% chance of inheriting the mutation. BpG offers mutation testing for the family if requested.

CONFIRMATION

PTCH1 c.2412dupT, p.(Ile805Tyrfs*24) was confirmed using bidirectional Sanger sequencing.

STEP	DATE
Order date	Apr 20, 2017
Sample received	Apr 20, 2017
Reported	May 11, 2017

On May 16, 2017 the statement has been prepared by our geneticists and physicians, who have together evaluated the sequencing results:



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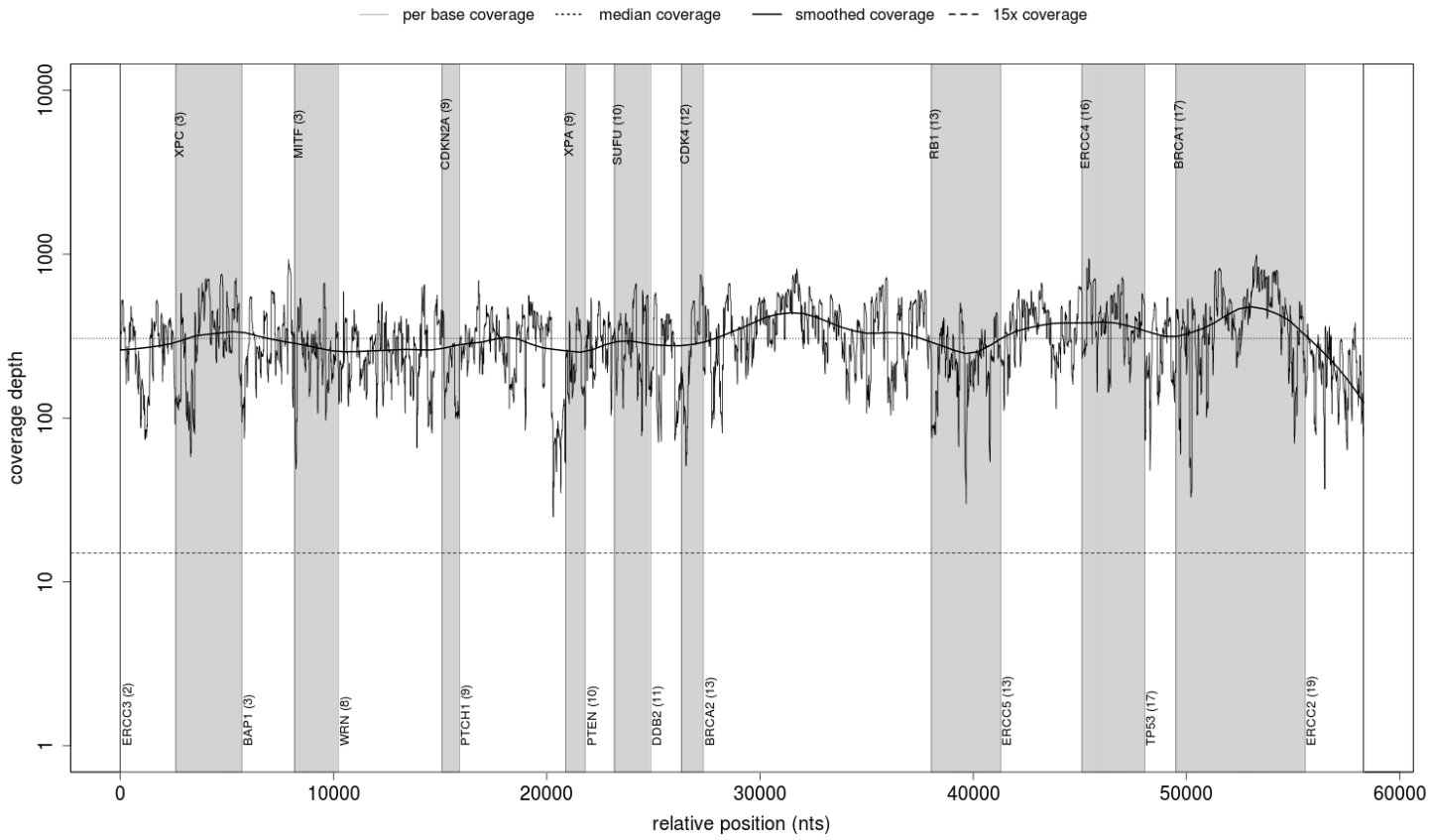
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median coverage
smoothed coverage
15x coverage
coverage depth
relative position (nts)
XPC (3)
MITF (3)
CDKN2A (9)
XPA (9)
SUFU (10)
CDK4 (12)
RB1 (13)
ERCC4 (16)
BRCA1 (17)
ERCC3 (2)
BAP1 (3)
WRN (8)
PTCH1 (9)
PTEN (10)
DDB2 (11)
BRCA2 (13)
ERCC5 (13)
TP53 (17)
ERCC2 (19)

APPENDIX 5: SUMMARY OF METHODS

OS-SEQ (SEQUENCE ANALYSIS)

Sequencing. Total genomic DNA was extracted from the biological sample. DNA quality and quantity were assessed using a fluorometric electrophoresis method. Extracted total genomic DNA was mechanically fragmented and enzymatically end-repaired. DNA adapters were added using a ligation-based method and the sequencing library was amplified using PCR. Quality and quantity of the sequencing library DNA were assessed through electrophoresis and fluorometric analyses, respectively. A proprietary Oligonucleotide-Selective Sequencing (OS-Seq) method was used for capturing genomic targets and sequencing was performed using an Illumina sequencing system.
Data analysis. Raw sequence reads were filtered to exclude reads with ambiguous base calls and trimmed from the 3' ends based on base call quality and presence of adapter, poly-A or capture oligo sequences. The remaining high-quality reads were mapped to the human genome reference sequence (Hg19). Single nucleotide variants (SNVs) and short insertions and deletions (INDELs) were identified using a proprietary data analysis pipeline. The pathogenicity of the identified variants was predicted based on the biochemical properties of the codon change and the degree of evolutionary conservation using PolyPhen, SIFT and Mutation Taster. Identified variants were annotated using allelic frequencies from large population studies (e.g. 1000 Genomes Project and the Exome Aggregation Consortium), by matching with various genotype-phenotype association databases (e.g. ClinVar) as well as by searching from an in-house curated database of previously reported variants.
Interpretation. The clinical evaluation team assessed the pathogenicity of the identified SNV and INDEL variants by evaluating allele frequency, in silico predictions, the annotations from public variant databases and matches in the in-house mutation database and related medical literature. Information in the referral about the patient's phenotype was compared with experimental data in the relevant medical literature to link the identified variants to specific clinical phenotypes. Sequencing data was manually inspected to confirm the variant findings.
Confirmation. Novel SNV and INDEL variant(s) classified as pathogenic or likely pathogenic as well as variants of uncertain significance with quality score <500 were confirmed using direct Sanger sequencing of the PCR amplicons. Confirmation of recurrent pathogenic and likely pathogenic variants is initially performed for three consequent cases using Sanger sequencing and subsequently only, when variant quality so requires.
Reporting. Reporting was carried out using an HGNC-approved gene nomenclature and mutation nomenclature following the HGVS guidelines.
Notes. This laboratory-developed test has been independently validated by Blueprint Genetics. This test has not been cleared or approved by the FDA. A normal result by this test does not rule out the diagnosis of a disorder since some DNA abnormalities may be undetectable by this technology. Test results should always be interpreted in the context of clinical findings, family history, and other relevant data. Inaccurate, or incomplete information may lead to misinterpretation of the results.
Accreditation. This analysis has been performed in a CLIA-certified laboratory (#99D2092375), accredited by the College of American Pathologists (CAP #9257331) and by FINAS Finnish Accreditation Service, (laboratory no. T292), accreditation requirement SFS-EN ISO 15189:2013. All the tests are under the scope of the ISO 15189 accreditation.

DEL/DUP (CNV) ANALYSIS

Data analysis. Deletions and duplications (Del/Dups) were detected from the sequence analysis data using a proprietary bioinformatics pipeline, which processes aligned sequence reads provided by the Blueprint Genetics OS-Seq data analysis pipeline. The difference between observed and expected sequencing depth at the targeted genomic regions was calculated and regions were divided into segments with variable DNA copy number. Expected sequencing depth was obtained by using other samples processed in the same sequence analysis as a guiding reference. The sequence data was adjusted to account for the effects of varying guanine and cytosine content. Identified variants were annotated using data from our in-house curated and maintained database and public databases (1000 Genome Project, Database of Genomic Variants, ExAC and DECIPHER).
Interpretation. The clinical evaluation team assessed the pathogenicity of the identified Del/Dups by reviewing the variant annotations. Clinical relevance of the identified variants was evaluated by relating the findings to the information in the patient referral and reviewing the relevant literature and databases.
Confirmation. Del/Dup variant(s) classified as pathogenic or likely pathogenic were confirmed using a quantitative-PCR assay if they cover less than 10 target exons or the sum of on-target exons and off-target bins (200kb) is < 10 (at least one on-target exon is required).
Reporting. Reporting was done using an HGNC-approved gene nomenclature.
Notes. This laboratory-developed test has been independently validated by Blueprint Genetics. This test has not been cleared or approved by the FDA. A normal result by this test does not rule out the diagnosis of a disorder since some DNA abnormalities may be undetectable by this technology. Test results should always be interpreted in the context of clinical findings, family history, and other relevant data. Inaccurate, or incomplete information may lead to misinterpretation of the results.
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GLOSSARY OF USED ABBREVIATIONS:

POS = genomic position of the variant in the format of chromosome:position
ID = rsID in dbSNP
Transcript = GenBank accession for reference sequence used for variant nomenclature
Nomenclature = HGVS nomenclature for a variant in the nucleotide and the predicted effect of a variant in the protein level
ExAC AC/AN = allele count/allele number in the Exome Aggregation Consortium Database
AD = autosomal dominant
AR = autosomal recessive
OMIM = Online Mendelian Inheritance in Man®
ExAC = Exome Aggregation Consortium Database (>60,000 unrelated individuals)
het = heterozygous
hom = homozygous
Del/Dup = Deletion and Duplication
CNV = copy number variation

PolyPhen, SIFT and MutationTaster are in silico prediction tools used to evaluate the significance of identified amino acid changes.