Blueprint Genetics

Hypothyroidism and Resistance to Thyroid Hormone Panel Plus

REFERRING HEALTHCARE PROFESSIONAL

NAME **HOSPITAL**

PATIENT

NAME **DOB** **AGE** **GENDER** **ORDER ID**
25 Male

CUSTOMER SAMPLE ID

PRIMARY SAMPLE TYPE **SAMPLE COLLECTION DATE**
DNA

SUMMARY OF RESULTS

TEST RESULTS

Patient is heterozygous for *THRB* c.1286G>A, p.(Arg429Gln), which is 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
THRB	3:24164475	NM_000461.4	c.1286G>A, p.(Arg429Gln)	missense_variant	HET	**Pathogenic**
	ID	**gnomAD AC/AN**	**POLYPHEN**	**SIFT**	**MUTTASTER**	
		0/0	probably damaging	deleterious	disease causing	
	OMIM	**PHENOTYPE**		**INHERITANCE**	**COMMENT**	
		Thyroid hormone resistance		AD,AR	-	

SEQUENCING PERFORMANCE METRICS

PANEL	GENES	EXONS / REGIONS	BASES	BASES > 20X	MEDIAN COVERAGE	PERCENT > 20X
Hypothyroidism and Resistance to Thyroid Hormone Panel	21	260	56597	56597	338	100

TARGET REGION AND GENE LIST

The Blueprint Genetics Hypothyroidism and Resistance to Thyroid Hormone Panel (version 3, Mar 01, 2018) Plus Analysis includes sequence analysis and copy number variation analysis of the following genes: DUOX2*, DUOXA2, FOXE1, GNAS, HESX1, IGSF1, NKX2-1, NKX2-5, PAX8, POU1F1, PROP1, SECISBP2, SLC16A2, SLC26A4, SLC5A5, TG, THRA, THRB, TPO, TSHB and TSHR. This panel targets protein coding exons, exon-intron boundaries (± 20 bps) and selected non-coding, deep intronic variants (listed in Appendix 5). This panel should be used to detect single nucleotide variants and small insertions and deletions (INDELs) up to 220 bps and copy number variations defined as single exon or larger deletions and duplications. This panel should not be used for the detection of repeat expansion disorders or diseases caused by mitochondrial DNA (mtDNA) mutations. The test does not recognize balanced translocations or complex inversions, and it may not detect low-level mosaicism.

*Some, or all, of the gene is duplicated in the genome. Read more: https://blueprintgenetics.com/pseudogene/
The sensitivity to detect variants may be limited in genes marked with an asterisk (*).

STATEMENT

CLINICAL HISTORY

Patient is a 25-year-old male with thyroid hormone resistance syndrome; normal TSH and raised T3/T4.

CLINICAL REPORT

Sequence analysis using the Blueprint Genetics (BpG) Hypothyroidism and Resistance to Thyroid Hormone Panel identified a heterozygous missense variant c.1286G>A, p.(Arg429Gln) in *THRB*.

There are no individuals with *THRB* c.1286G>A, p.(Arg429Gln) in the Genome Aggregation Database (gnomAD, n>120,000 exomes and >15,000 genomes). *In silico* tools PolyPhen, SIFT, and MutationTaster predict the variant as deleterious. *THRB* c.1286G>A, p.(Arg429Gln) is a well-known pathogenic variant that has been reported in several patients affected with either generalized or selective pituitary resistance to thyroid hormone (PMID: 8040303, 7528740, 11167935, 15815068, 21703645, 25040256). The variant has also been detected in clinical testing and is listed as a pathogenic variant in the ClinVar database (Variation ID: 548123). In functional studies, the p.(Arg429Gln) mutation does not seem to impair ligand binding (PMID: 7528740), and transcription through positive TH responsive element (TRE) occurs almost normally, but inhibition of transcription through negative TRE is defective because corepressor release is impaired (PMID: 7528740, 9605924). Homodimer formation by the mutant receptor has also been shown to be impaired, particularly with an everted repeat TRE configuration (PMID: 7838159, 7528740). In mice, introduction of the *THRB* p.(Arg429Gln) mutation into the genome leads to elevated serum TH and inappropriately normal thyroid-stimulating hormone levels, as well as a selective defect in TH-mediated negative regulation of hepatic (*Gsta*), cardiac (*Mhy7*), and pituitary (*Cga* and *Tshb*) genes, but preserved TH action on both peripheral and central genes that are positively regulated by TH (PMID: 19439650).

Mutations in *THRB* gene (MIM #*190160*) are known to cause generalized thyroid hormone resistance (GRTH, MIM #*188570*), a syndrome characterized by high levels of circulating thyroid hormone (T3-T4), with normal or slightly elevated thyroid stimulating hormone (TSH). Selective pituitary resistance to thyroid hormone (PRTH) (MIM #145650) is characterized by resistance in the pituitary gland but not in peripheral tissues. Heterozygous mutations in the ligand-binding domain of THRB impair hormone binding and/or transcriptional activity and result in resistance to thyroid hormone (RTH). The mutant thyroid hormone receptor beta (TRb) interferes with the function of wild-type receptor, resulting in a dominant negative effect and dominant inheritance. Homozygous mutations in *THRB* are rare and result in a severe phenotype. Patients with RTH-b have a variable phenotype including goitre, tachycardia, raised energy expenditure, hyperactive behaviour, delayed bone age, and learning disabilities. Diffuse goitre and sinus tachycardia are the most common clinical findings. Symptoms are due to a combination of low TH action in predominantly TRb-expressing tissues (TRb1 in liver, kidney and thyroid, TRb2 in retina, cochlea, pituitary) and TH overexposure in TRa-expressing tissues (such as brain, bone and heart). Patients have elevated serum thyroid hormone levels and normal or elevated TSH levels and are clinically thyrotoxic. Thyroid hormone resistance is a notable example of a phenotype, which can be either completely recessive or completely dominant, even though the causative mutations are in the same gene. In most patients, treatment is not necessary (PMID: *23834164*). Tachycardia and tremor as symptoms of hyperthyroidism can be treated using beta adrenergic blockers. According to Visser WE et al. (PMID: *23834164*), in rare cases treatment with TA3, which has a higher affinity for TRb than for TRa, can be used to lower serum TSH and TH levels and thereby reduce clinical symptoms of hyperthyroidism.

Several alternatively spliced transcript variants encoding the same protein have been observed for this gene. Molecular genetic studies have shown different mutations, including point mutations, in-frame deletions and frameshift insertions, which are all localized to the genetic code for the ligand binding domain of the *THRB* gene. The mutations occur mostly within three clusters, termed “hot spots”. The clusters are CpG dinucleotide-rich sequences encoding amino acids 234–282, 310–353 and 438–461 (PMID *11427693*). The diagnosis of RTH is based on clinical and laboratory findings, and genetic analyses can confirm it. Patients with RTH may have various clinical symptoms typical of both hypothyroidism and hyperthyroidism.

Mutation nomenclature is based on GenBank accession NM_000461.4 (*THRB*) with nucleotide one being the first nucleotide of the translation initiation codon ATG.

CONCLUSION

Considering the current literature and the well-established role of *THRB* c.1286G>A, p.(Arg429Gln) as a disease-causing variant, we classify it as pathogenic. Genetic counseling and family member testing are recommended. Disease caused by *THRB* variants can be inherited in an autosomal dominant or recessive manner, and this variant has been associated with autosomal dominant disease. Thus, each child of an affected individual has a 50% chance of inheriting the variant. A proband with autosomal dominant disease may have the disorder as a result of a *de novo* event. BpG offers targeted variant testing for the family if requested.

CONFIRMATION

THRB c.1286G>A, p.(Arg429Gln) has been confirmed with bi-directional Sanger sequencing.

STEP	DATE
Order date	Jun 08, 2018
Sample received	Jun 21, 2018

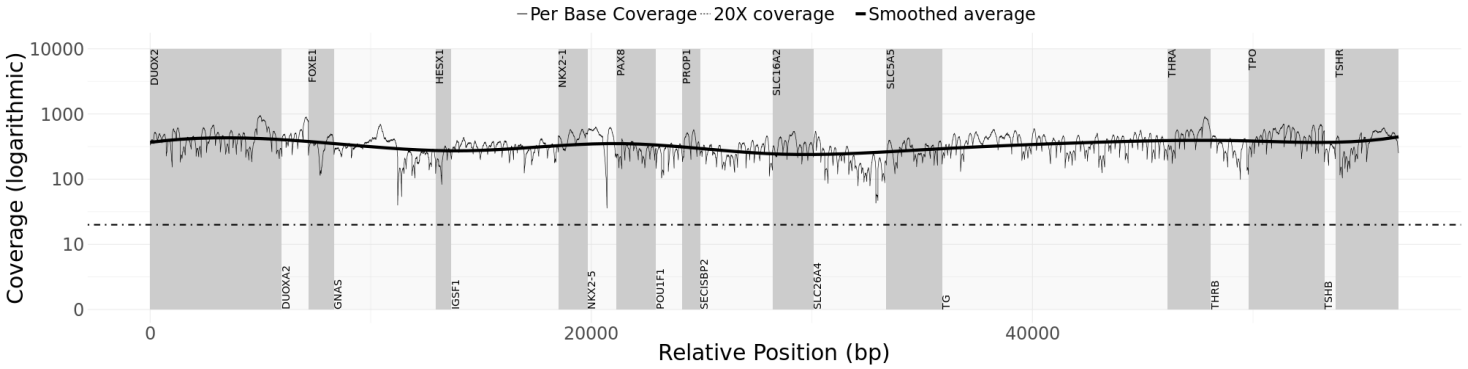
STEP	DATE
Reported	Jul 25, 2018

On Jul 25, 2018 the statement has been prepared by our geneticists and physicians, who have together evaluated the sequencing results:

Johanna Känsäkoski, Ph.D.
Geneticist

Juha Koskenvuo, MD, Ph.D.
Lab Director, Chief Medical Officer

Matti Hero, MD, Ph.D.
Clinical Consultant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20X coverage
Smoothed average
Coverage (logarithmic)
10000
1000
100
10
0
DUOX2
FOXE1
HESX1
NKX2-1
PAX8
PROP1
SLC16A2
SLC5A5
THRA
TPO
TSHR
DUOXA2
GNAS
IGSF1
NKX2-5
POU1F1
SECISBP2
SLC26A4
TG
THRB
TSHB
0
20000
40000
Relative Position (bp)

APPENDIX 5: SUMMARY OF THE TEST

PLUS ANALYSIS

Laboratory process: Total genomic DNA was extracted from the biological sample using a spin column method. DNA quality and quantity were assessed using electrophoretic methods. After assessment of DNA quality, qualified genomic DNA sample was randomly fragmented using non-contact, isothermal sonochemistry processing and purified with SPRI beads. DNA fragments were then end-repaired and sequencing adapters were ligated to both ends of the resulting fragments. Prepared DNA-Adapter libraries were size-selected with SPRI beads to ensure optimal template size and then amplified by ligation-mediated PCR (LM-PCR). The amplified sequencing library was purified using SPRI beads and a hybridization-capture method was applied for enrichment of whole exome and select non-coding regions (xGen Exome Research Panel with custom-designed capture probes, IDT). The enriched sequencing library was amplified by LM-PCR and purified using SPRI beads. The quality of the completed sequencing library was controlled by ensuring the correct template size and quantity and to eliminate the presence of leftover primer-dimers. Each captured library passing quality control was sequenced using the Illumina sequencing system with paired-end sequencing (150 by 150 bases). Sequencing-derived raw image files were processed using a base-calling software (Illumina) and the sequence data was transformed into FASTQ format.
Bioinformatics and quality control: The bioinformatics analysis began with quality control of raw sequence reads. Clean sequence reads of each sample were mapped to the human reference genome (GRCh37/hg19). Burrows-Wheeler Aligner (BWA-MEM) software was used for read alignment. Duplicate read marking, local realignment around indels, base quality score recalibration and variant calling were performed using GATK algorithms (Sentieon). The panel content was sliced from high-quality exome sequencing data acquired as presented above. The sequencing depth and coverage for the tested sample was calculated based on the alignments. The sequencing run included in-process reference sample(s) for quality control, which passed our thresholds for sensitivity and specificity. The patient's sample was subjected to thorough quality control measures as well, after which raw sequence reads were transformed into variants by a proprietary bioinformatics pipeline. Copy number variations (CNVs), defined as single exon or larger deletions or duplications (Del/Dups), were detected from the sequence analysis data using a proprietary bioinformatics pipeline, which processes aligned sequence reads. 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. The 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.
Interpretation: Our variant classification follows the Blueprint Genetics Variant Classification Schemes modified from the ACMG guideline 2015. Minor modifications were made to increase reproducibility of the variant classification and improve the clinical validity of the report. Likely benign and benign variants were not reported. The pathogenicity potential of the identified variants were assessed by considering the predicted consequence, the biochemical properties of the codon change, the degree of evolutionary conservation as well as the number of reference population databases and mutation databases such as, but not limited, to the 1000 Genomes Project, gnomAD, ClinVar and HGMD. For missense variants, *in silico* variant prediction tools such as SIFT, PolyPhen, MutationTaster were used to assist with variant classification. In addition, the clinical relevance of any identified CNVs was evaluated by reviewing the relevant literature and databases such as 1000 Genomes Project, Database of Genomic Variants, ExAC, DECIPHER. The clinical evaluation team assessed the pathogenicity of the identified variants by evaluating the information in the patient referral, reviewing the relevant literature and manually inspecting the sequencing data if needed. Reporting was carried out using HGNC-approved gene nomenclature and mutation nomenclature following the HGVS guidelines.
Confirmation: Pathogenic and likely pathogenic variants that established a molecular diagnosis were confirmed with bi-directional Sanger sequencing unless all of the following criteria were fulfilled: 1) the variant quality score (QS) was above the internal threshold for a true positive call, 2) an unambiguous manual curation of the variant region using IGV was concordant with the variant call and 3) previous Sanger confirmation of the same variant has been performed at least three times in our laboratory. Reported variants of uncertain significance were confirmed with bi-directional Sanger sequencing only if the QS was below our internally defined score for a true positive call. CNVs (Dels/Dups) were confirmed using a quantitative-PCR assay if they covered less than 10 target exons or were not confirmed at least three times previously at our laboratory.
Analytic validation: This laboratory-developed test has been independently validated by Blueprint Genetics. The sensitivity of this panel is expected to be in the same range as the validated whole exome sequencing laboratory assay used to generate the panel data (sensitivity for SNVs 99.65%, indels 1-50 bps 99.07%, one-exon deletion 92.3% and two exons CNV 100%, and specificity >99.9% for most variant types). A normal result does not rule out the diagnosis of a genetic disorder since some DNA abnormalities may be undetectable by the applied 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.
Regulation and accreditations: This test has not been cleared or approved by the FDA. 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.

NON-CODING VARIANTS COVERED BY THE PANEL:

NM_080425.2(GNAS):c.2242-11A>G
chr5:g.172672291-172672291
chr5:g.172672303-172672303
NM_006261.4(PROP1):c.343-11C>G
NM_024077.3(SECISBP2):c.1212+29G>A
NM_000441.1(SLC26A4):c.-103T>C
NM_000441.1(SLC26A4):c.-60A>G
NM_000441.1(SLC26A4):c.-4+1G>C
NM_000441.1(SLC26A4):c.-4+5G>A
NM_000441.1(SLC26A4):c.1264-12T>A
NM_000441.1(SLC26A4):c.1438-7dupT

GLOSSARY OF USED ABBREVIATIONS:

AD = autosomal dominant
AR = autosomal recessive
CNV = Copy Number Variation e.g. one exon or multiexon deletion or duplication
gnomAD = genome Aggregation Database (reference population database; >138,600 individuals)

gnomAD AC/AN = allele count/allele number in the genome Aggregation Database (gnomAD)
HEM = hemizygous
HET = heterozygous
HOM = homozygous
ID = rsID in dbSNP
MutationTaster = in silico prediction tools used to evaluate the significance of identified amino acid changes.
Nomenclature = HGVS nomenclature for a variant in the nucleotide and the predicted effect of a variant in the protein level
OMIM = Online Mendelian Inheritance in Man®
PolyPhen = *in silico* prediction tool used to evaluate the significance of amino acid changes.
POS = genomic position of the variant in the format of chromosome:position
SIFT = *in silico* prediction tool used to evaluate the significance of amino acid changes.