Blueprint Genetics

*Sample report as of June 1st, 2022. Regional differences may apply. For complete and up-to-date test methodology description, please see your report in Nucleus online portal. Accreditation and certification information available at **blueprintgenetics.com/certifications***

Congenital Adrenal Hyperplasia Panel Plus

REFERRING HEALTHCARE PROFESSIONAL

NAME **HOSPITAL**

PATIENT

NAME **DOB** **AGE** **GENDER** **ORDER ID**
29 Female

PRIMARY SAMPLE TYPE **SAMPLE COLLECTION DATE** **CUSTOMER SAMPLE ID**
Blood

SUMMARY OF RESULTS

PRIMARY FINDINGS

The patient is homozygous for *CYP21A2* c.844G>T, p.(Val282Leu), which is pathogenic.

Del/Dup (CNV) analysis

Negative.

PRIMARY FINDINGS: SEQUENCE ALTERATIONS

GENE	TRANSCRIPT	NOMENCLATURE	GENOTYPE	CONSEQUENCE	INHERITANCE	CLASSIFICATION
CYP21A2	NM_000500.7	c.844G>T, p.(Val282Leu)	HOM	missense_variant	AR	**Pathogenic**
	ID	**ASSEMBLY**	**POS**	**REF/ALT**		
	rs6471	GRCh37/hg19	6:32007887	G/T		
					PHENOTYPE	
	gnomAD AC/AN	**POLYPHEN**	**SIFT**	**MUTTASTER**	Adrenal hyperplasia, congenital, due to 21-hydroxylase deficiency, Hyperandrogenism, nonclassic, due to 21-hydroxylase deficiency	
	1508/273420	possibly damaging	tolerated	disease causing		

SEQUENCING PERFORMANCE METRICS

PANEL	GENES	EXONS / REGIONS	BASES	BASES > 20X	MEDIAN COVERAGE	PERCENT > 20X
Congenital Adrenal Hyperplasia Panel	12	136	27398	27398	262	100

TARGET REGION AND GENE LIST

The Blueprint Genetics Congenital Adrenal Hyperplasia Panel Plus Analysis includes sequence analysis

and copy number variation analysis of the following genes: *ARMC5, CYP11A1, CYP11B1*, CYP11B2*, CYP17A1, CYP21A2*, HSD3B2, PDE11A, PDE8B, POR, PRKAR1A and STAR.* 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) 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 29-year-old female who was found to have elevated 17-OH alpha hydroxyprogesterone serum levels during the first trimester of pregnancy. There is no family history of similar disease.

CLINICAL REPORT

Sequence analysis using the Blueprint Genetics (BpG) Congenital Adrenal Hyperplasia Panel identified a missense variant *CYP21A2* c.844G>T, p.(Val282Leu). Due to high sequence homology between the functional gene *CYP21A2* and its corresponding pseudogene *CYP21A1P*, next generation sequencing technologies with short sequencing reads have significant limitations to detect disease-causing variants from this region. The identified variant was therefore confirmed with a custom confirmation assay utilizing long-range PCR with modified primers for increased specificity (see below for details). Based on NGS data this variant was detected in 109/166 reads (allele ratio 66%), whereas based on Sanger sequencing the patient is homozygous for the variant. In addition to the *CYP21A2* c.844G>T variant, two benign upstream homozygous variants, c.738+12_738+13delinsGT and c.747C>G, p.(Leu249=), were detected by both methods. All three sequence changes are in positions that are known to be different between *CYP21A2* and *CYP21A1P*, therefore this finding may indicate a micro-conversion event between *CYP21A2* and *CYP21A1P* (small sequences transferred from *CYP21A1P* to *CYP21A2*). Micro-conversion is a common disease mechanism leading to 21-hydroxylase deficiency (PMID: 26804566).

CYP21A2 c.844G>T, p.(Val282Leu)

There are 1504 individuals heterozygous and 2 individuals homozygous for this variant in the Genome Aggregation Database (gnomAD, n>120,000 exomes and >15,000 genomes). The variant is relatively common with allele frequency 0.005515 based on the whole gnomAD dataset and 0.02446 in the Ashkenazi Jewish population. Database curators have made every effort to exclude individuals with severe pediatric diseases from these cohorts. Most *in silico* tools utilized predict this variant to be damaging to protein structure and function. The variant p.Val282Leu (also known as p.Val281Leu) is a frequently identified *CYP21A2* variant associated with the non-classical form of congenital adrenal hyperplasia (CAH) (PMID: 21609351, 20661889, 23359698, 12915679, 25481255, 18392095, 28541281, GeneReviews NBK1171). It has been classified as pathogenic in ClinVar during clinical testing of at least five patients with 21-hydroxylase deficiency (Variation ID: 12151). New et al. 2013 reported that 98% (n = 497) of individuals with non-classical CAH harbored this variant and its frequency was very high in affected individuals from the Ashkenazi Jewish population (PMID: 23359698). Additionally, we have identified this variant in multiple patients affected with 21-hydroxylase deficiency (BpG, unpublished observations). Although this variant is associated with non-classical CAH, individuals with classical CAH were also found to have this variant (PMID: 23359698). Functional studies found that co-expression of this mutant protein and wild-type (WT) protein results in a dominant negative effect on the enzymatic activity of WT protein (PMID: 14513879). Other studies indicate variant p.(Val281Leu) to cause reduced enzyme activity (20-50%) (PMID: 2249999, 1864962, 23359706).

CYP21A2

CYP21A2 gene (MIM* *613815)* encodes the 21-hydroxylase enzyme, which is essential for adrenal steroidogenesis. 21-hydroxylase deficiency (21-OHD) (MIM# *201910*) is the most common cause of congenital adrenal hyperplasia (CAH), a family of autosomal recessive disorders involving impaired synthesis of cortisol from cholesterol by the adrenal cortex. The estimated prevalence of classic CAH is 1:10,000 and annual incidence ranges from 1:5,000 to 1:15,000. The highest ethnic-specific non-classic disease prevalence (1:27) is found among Ashkenazi Jewish (*GeneReviews NBK1171*). There are four recognized clinical forms of congenital adrenal hyperplasia, the majority of cases being associated with 21-hydroxylase deficiency: salt-wasting (SW), simple virilizing (SV), nonclassic (NC) late-onset (also called attenuated and acquired), and cryptic. In 21-OHD CAH, excessive adrenal androgen biosynthesis results in virilization in all individuals and salt wasting in some individuals. A classic form with severe enzyme deficiency and prenatal onset of virilization is distinguished from a non-classic form with mild enzyme

deficiency and postnatal onset. The classic form is further divided into the simple virilizing form (~25% of affected individuals) and the salt-wasting form, in which aldosterone production is inadequate (≥75% of individuals). Newborns with salt-wasting 21-OHD CAH are at risk for life-threatening salt-wasting. In female newborns, the external genitalia are masculinized; gonads and internal genitalia are normal. Postnatally, untreated males as well as females show clinical signs of peripheral precocious puberty, including rapid growth, penile or clitoral enlargement, and ultimately early epiphyseal closure and short stature. The laboratory findings of 21-OHD CAH include elevated 17-OHP (typically >300 nmol/l in the classic form), androstendione and testosterone levels, and in severe classic form with salt wasting, serum sodium is low, potassium may be elevated and plasma renin activity is increased. Early detection and treatment of 21-OHD CAH may be life-saving; salt-wasting crisis often occurs during the second week of life. The treatment includes hydrocortisone and fludrocortisone, and during infancy sodium supplementation is also typically needed. During follow-up, the treatment is titrated based on adrenal androgen, sodium and plasma renin activity levels. A mild form of late-onset adrenal hyperplasia due to 21-hydroxylase deficiency can occur in adults and has hirsutism as the only manifestation in the most attenuated form. (MIM# *201910*, *GeneReviews NBK1171*).

HGMD Professional (2020.2) lists over 310 disease-causing variants in *CYP21A2,* including missense (35%), nonsense (18.5%), start-lost (1.3%), and splicing (7%) variants, as well as small insertions, deletions, and indels (15%). In addition, complex rearrangements (17%), gross deletions and insertions (5.4%), and 4 regulatory variants are reported. The majority of pathogenic variants (>90%) that cause 21-hydroxylase deficiency are due to recombination resulting in gene conversions between *CYP21A2* and a highly homologous, closely linked pseudogene (*CYP21A1P*) (PMID: *10857554*, *2845408*); 75% of the intergenic recombinations are represented by microconversion events which result in the transfer of mutations normally present in the pseudogene to the functional gene. The remaining 25% of the intergenic recombinations result in *CYP21A2* gene deletions and deletions involving the 3′end of *CYP21A1P* and the 5′end of *CYP21A2 (*PMID: 10794740, 7479886). The region between intron 2 and the 3’ end of exon 3 in *CYP21A2* is considered a hotspot for recombinations and microconversions (PMID: 7479886).

Mutation nomenclature is based on GenBank accession NM_000500.7 (*CYP21A2*) with nucleotide one being the first nucleotide of the translation initiation codon ATG.

CONCLUSION

CYP21A2 c.844G>T, p.(Val282Leu) is classified as pathogenic, based on currently available evidence supporting its disease-causing role. Disease caused by *CYP21A2* variants is inherited in an autosomal recessive manner. The patient is homozygous for the variant, which is consistent with autosomal recessive inheritance. If the patient’s parents are each confirmed to be carriers of this variant, any siblings of the patient will have a 25% chance of being homozygous for the variant and thus affected, a 50% chance of being an asymptomatic carrier, and a 25% chance of being an unaffected non-carrier. Genetic counselling and family member testing are recommended.

CONFIRMATION

CYP21A2 c.844G>T, p.(Val282Leu) has been confirmed with a custom confirmation assay.

Details on the custom confirmation assay: long range PCR with modified primers for increased specificity were used to separately amplify *CYP21A2* coding region and its pseudogene *CYP21A1P*. In addition, two fragments of known common rearrangements were amplified for the detection of large deletions between *CYP21A1P* and *CYP21A2* resulting in a *CYP21A1P-CYP21A2* fusion, as well as for the detection of rearrangements resulting in *CYP21A2-CYP21A1P* fusion. The obtained PCR amplicons were subsequently Sanger sequenced.

STEP	DATE
Order date	
Sample received	
Sample in analysis	
Reported	

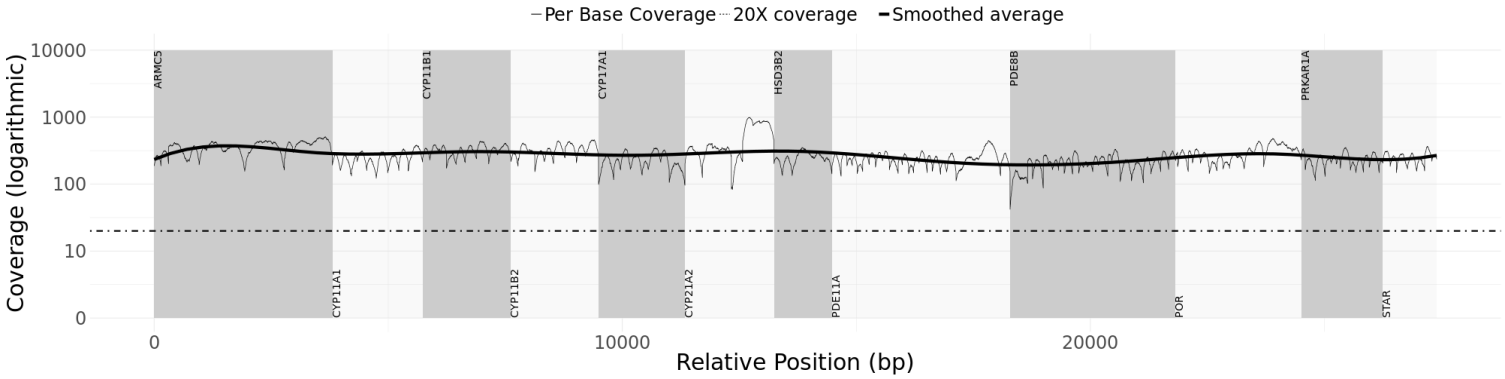
(This statement has been prepared by our geneticists and physicians, who have together evaluated the sequencing results.)

Signature

Name

Title

Readability of the coverage plot may be hindered by faxing. A high quality coverage plot can be found with the full report on nucleus.blueprintgenetics.com.



APPENDIX 5: SUMMARY OF THE TEST

PLUS ANALYSIS

Laboratory process: When required, the total genomic DNA was extracted from the biological sample using bead-based 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. Sequencing library was prepared by ligating sequencing adapters to both ends of DNA fragments. Sequencing libraries were size-selected with bead-based method to ensure optimal template size and amplified by polymerase chain reaction (PCR). Regions of interest (exons and intronic targets) were targeted using hybridization-based target capture method. The quality of the completed sequencing library was controlled by ensuring the correct template size and quantity and to eliminate the presence of leftover primers and adapter-adapter dimers. Ready sequencing libraries that passed the quality control were sequenced using the Illumina's sequencing-by-synthesis method using paired-end sequencing (150 by 150 bases). Primary data analysis converting images into base calls and associated quality scores was carried out by the sequencing instrument using Illumina's proprietary software, generating CBCL files as the final output.
Bioinformatics and quality control: Base called raw sequencing data was transformed into FASTQ format using Illumina's software (bcl2fastq). 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) for nDNA. Variant data for was annotated using a collection of tools (VcfAnno and VEP) with a variety of public variant databases including but not limited to gnomAD, ClinVar and HGMD. The median sequencing depth and coverage across the target regions for the tested sample were calculated based on MQ0 aligned reads. 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 including assessments for contamination and sample mix-up. 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. 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: The clinical interpretation team assessed the pathogenicity of the identified variants by evaluating the information in the patient requisition, reviewing the relevant scientific literature and manually inspecting the sequencing data if needed. All available evidence of the identified variants was compared to classification criteria. Reporting was carried out using HGNC-approved gene nomenclature and mutation nomenclature following the HGVS guidelines. Likely benign and benign variants were not reported.
Variant classification: Our variant classification follows the Blueprint Genetics 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.
Databases: The pathogenicity potential of the identified variants were assessed by considering the predicted consequence of the 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 gnomAD, ClinVar, HGMD Professional and Alamut Visual. In addition, the clinical relevance of any identified CNVs was evaluated by reviewing the relevant literature and databases such as Database of Genomic Variants and DECIPHER. For interpretation of mtDNA variants specific databases including e.g. Mitomap, HmtVar and 1000G were used.
Confirmation of sequence alterations: Sequence variants classified as pathogenic, likely pathogenic and variants of uncertain significance (VUS) were confirmed using bi-directional Sanger sequencing when they did not meet our stringent NGS quality metrics for a true positive call. In addition, prenatal case with diagnostic findings were confirmed.
Confirmation of copy number variants: CNVs (Deletions/Duplications) were confirmed using a digital PCR assay if they covered less than 10 exons (heterozygous), less than 3 exons (homo/hemizygous) or were not confirmed at least three times previously at our laboratory. Furthermore, CNVs of any size were not confirmed when the breakpoints of the call could be

determined.
Analytic validation: The detection performance of this panel is expected to be in the same range as our high-quality, clinical grade NGS sequencing assay used to generate the panel data (nuclear DNA: sensitivity for SNVs 99.89%, indels 1-50 bps 99.2%, one-exon deletion 100% and five exons CNV 98.7%, and specificity >99.9% for most variant types). It does not detect very low level mosaicism as a variant with minor allele fraction of 14.6% can be detected in 90% of the cases. Detection performance for mtDNA variants (analytic and clinical validation): sensitivity for SNVs and INDELs 100.0% (10-100% heteroplasmy level), 94.7% (5-10% heteroplasmy level), 87.3% (<5% heteroplasmy level) and for gross deletions 100.0%. Specificity is >99.9% for all.
Test restrictions: 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.
Technical limitations: This test does not detect the following: complex inversions, gene conversions, balanced translocations, repeat expansion disorders unless specifically mentioned, non-coding variants deeper than ±20 base pairs from exon-intron boundary unless otherwise indicated (please see the list of non-coding variants covered by the test). Additionally, this test may not reliably detect the following: low level mosaicism, stretches of mononucleotide repeats, indels larger than 50bp, single exon deletions or duplications, and variants within pseudogene regions/duplicated segments. The sensitivity of this test may be reduced if DNA is extracted by a laboratory other than Blueprint Genetics. Laboratory error is also possible. Please see the Analytic validation above.
Regulation and accreditations: This test was developed and its performance characteristics determined by Blueprint Genetics (see Analytic validation). It has not been cleared or approved by the US Food and Drug Administration. 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 (excluding mtDNA testing and digital PCR confirmation).

NON-CODING VARIANTS COVERED BY THE PANEL:

NM_000497.3(CYP11B1):c.595+16G>T
NM_000500.7(CYP21A2):c.293-13C>G
NM_000941.2(POR):c.-5+4A>G
NM_002734.4(PRKAR1A):c.-97G>A
NM_002734.4(PRKAR1A):c.-7G>A
NM_002734.4(PRKAR1A):c.-7+1G>A
NM_002734.4(PRKAR1A):c.550-17T>A
NM_002734.4(PRKAR1A):c.709-7_709-2delTTTTTA
NM_000349.2(STAR):c.466-11T>A

GLOSSARY OF USED ABBREVIATIONS:

AD = autosomal dominant
AF = allele fraction (proportion of reads with mutated DNA / all reads)
AR = autosomal recessive
CNV = Copy Number Variation eg, 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
MT = Mitochondria
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.