Blueprint Genetics

*Sample report as of July 7, 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***

# Premature Ovarian Failure Panel Plus

THE ORIGINAL PANEL CONTENT HAS BEEN MODIFIED BY THE CUSTOMER.

## REFERRING HEALTHCARE PROFESSIONAL

**NAME** **HOSPITAL**

## PATIENT

**NAME** **DOB** **AGE** **GENDER** **ORDER ID**

**PRIMARY SAMPLE TYPE**
DNA

**SAMPLE COLLECTION DATE**

**CUSTOMER SAMPLE ID**
DNA# , HC#, Medicare#

## SUMMARY OF RESULTS

### PRIMARY FINDINGS

The patient is heterozygous for *GNAS* c.677G>A, p.(Gly226Asp), which is a variant of uncertain significance (VUS).

#### PRIMARY FINDINGS: SEQUENCE ALTERATIONS

| GENE | TRANSCRIPT | NOMENCLATURE | GENOTYPE | CONSEQUENCE | INHERITANCE | CLASSIFICATION |
|---|---|---|---|---|---|---|
| GNAS | NM_000516.6 | c.677G>A, p.(Gly226Asp) | HET | missense_variant | AD | **Variant of uncertain significance** |
| | **ID** | **ASSEMBLY** | **POS** | **REF/ALT** | | |
| | | GRCh37/hg19 | 20:57484593 | G/A | | |
| | **gnomAD AC/AN** | **POLYPHEN** | **SIFT** | **MUTTASTER** | **PHENOTYPE** | |
| | 0/0 | probably damaging | deleterious | disease causing | Albright hereditary osteodystrophy, McCune-Albright syndrome, Progressive osseous heteroplasia, Pseudohypoparathyroidism | |

#### SEQUENCING PERFORMANCE METRICS

| PANEL | GENES | EXONS / REGIONS | BASES | BASES > 20X | MEDIAN COVERAGE | PERCENT > 20X |
|---|---|---|---|---|---|---|
| Premature Ovarian Failure Panel | 42 | 582 | 122370 | 122234 | 230 | 99.89 |

#### TARGET REGION AND GENE LIST

The Blueprint Genetics Premature Ovarian Failure Panel includes sequence analysis and copy number variation analysis of the following genes: *BMP15*, *CYP17A1*, *CYP19A1*, *FOXL2*, *FSHR*, *GALT*, *GNAS*, *LHCGR*, *LMNA*, *NOBOX*, *NR5A1*, *POLG*, *POR*, *STAG3**, *STAR* and *WT1*.

This panel targets protein coding exons, exon-intron boundaries (± 20 bps) and selected non-coding, deep intronic variants (listed in the SUMMARY OF THE TEST section). This panel should be used to detect single nucleotide variants and small insertions 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 detect balanced translocations or complex rearrangements, 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 20-year-old female with IUGR, short stature, delayed puberty, premature ovarian insufficiency. Slightly delayed early milestones and question of learning difficulties (not formally assessed to date). Dental crowding. Longstanding history of previous labial agglutination and recurrent lichen sclerosis. Previous genetic tests performed in other laboratories: Karyotype with 46,XXinv(13)(q21.2q32.3) found to be inherited from apparently unaffected mother, follow-up microarray NORMAL. Methylation study GNAS for Albright hereditary osteodystrophy normal. Fragile X and RAD normal. CBC, Calcium, phosphorous, ALK phos, TSH all normal. PTH high. Family history non-contributory overall, though sister with irregular menses/later puberty but still within normal; father with reduced facial/body hair but normal growth.

**Clinical reason for the custom panel:** Reviewed with other local Geneticists and although not highly suspicious Noonan and Coffin-Siris genes proposed as differential

### CLINICAL REPORT

Sequence analysis using the Blueprint Genetics (BpG) FLEX Premature Ovarian Failure Panel identified a heterozygous missense variant *GNAS* c.677G>A, p.(Gly226Asp).

#### *GNAS* c.677G>A, p.(Gly226Asp)

This variant is absent in gnomAD, a large reference population database (n>120,000 exomes and >15,000 genomes) which aims to exclude individuals with severe pediatric disease. The affected amino acid is highly conserved in mammals as well as in evolutionarily more distant species, which suggests that this position does not tolerate variation. Additionally, there is a moderate physicochemical difference between Gly and Asp (Grantham distance: 94 [0-215]), and all *in silico* tools utilized predict that this variant will be damaging to protein structure and function. To the best of our knowledge, this variant has not been described in the medical literature or reported in disease-related variation databases such as ClinVar or HGMD. However, missense variants affecting nearby codons in *GNAS*, such as p.(Met221Thr), p.(Arg228Cys), and p.(Arg228Ser), have been reported in HGMD (Professional 2021.3) in association with Pseudohypoparathyroidism 1a.

#### *GNAS*

*GNAS* (MIM*139320) is a complex imprinted locus that produces multiple transcripts through the use of alternative promoters and alternative splicing. The *GNAS* locus encodes four main transcripts, Gs-alpha, XLAS, NESP55, and the A/B transcript, as well as an antisense *GNAS* transcript (*GNAS*-AS1; MIM* 610540). The four main transcripts are produced through the use of

alternative promoters and splicing of four unique first exons onto the shared exons 2 through 13. Both activating and inactivating mutations have been characterized in *GNAS*. The most well-characterized transcript derived from *GNAS*, Gs-alpha (Gsα), encodes the alpha subunit of the stimulatory guanine nucleotide-binding protein (G protein). Gsα plays essential roles in a multitude of physiologic processes. Other transcripts produced by *GNAS* are expressed exclusively from either the paternal or the maternal allele but Gsα is biallelically expressed in most tissues, except in a small number of tissues where it is predominantly expressed from the maternal allele. These tissues include renal proximal tubules, thyroid, gonads, and pituitary. Disorders caused by Gsα inactivation are inherited in an autosomal dominant manner with the specific phenotype determined by the parental origin of the defective allele.

Pseudohypoparathyroidism is a term applied to a heterogeneous group of disorders that share as a common feature end-organ resistance to parathyroid hormone (PTH; 168450). Pseudohypoparathyroidism 1a (PHP1A; MIM #103580), also referred to as Albright Hereditary Osteodystrophy (AHO), is caused by heterozygous inactivating variants of Gsα. Generally, pathogenic variants in the maternal allele lead to the PHP1A phenotype, while variants of paternal origin result in pseudopseudohypoparathyroidism (PPHP; MIM #612463), characterized by the physical findings of PHP1A but without hormone resistance or obesity. Pseudohypoparathyroidism 1b (PHP1B; MIM #603233) on the other hand is caused by deletions in the differentially methylated region (DMR) of the *GNAS* locus, affecting expression. Furthermore, a subset of patients with a heterozygous variant on the maternal allele has a diagnosis of pseudohypoparathyroidism 1c (PHP1C; MIM #612462). Because the phenotype of PHP1C is essentially identical to that of PHP1A, PHP1C may represent a subgroup of PHP1A, and the differences may result from the location of the variant.

PHP1A and PHP1C are characterized by end-organ resistance to endocrine hormones including parathyroid hormone (PTH), thyroid-stimulating hormone (TSH), gonadotropins (LH and FSH), growth hormone-releasing hormone (GHRH), and CNS neurotransmitters (leading to obesity and variable degrees of intellectual disability and developmental delay) as well as by Albright hereditary osteodystrophy (AHO) phenotype (short stature, round face with a low, flat nasal bridge, obesity, subcutaneous ossification, intracranial calcification, and variable degrees of intellectual disability) and brachydactyly type E (shortening mainly of the 4th and/or 5th metacarpals and metatarsals and distal phalanx of the thumb). Although PHP1B is characterized principally by PTH resistance, some individuals also have partial TSH resistance and mild features of AHO (e.g., brachydactyly), as well as patterns of excessive growth or weight gain during the perinatal period, early infancy, and childhood (GeneReviews). It has been estimated that at least 65% of PHP1A/PHP1C patients younger than age 18 years are obese and obesity can begin in infancy (PMID: 19364695, 19364695). Obesity tends to be the most difficult manifestation to treat, as individuals with PHP1A and PHP1C have decreased resting energy expenditure and hyperphagia; thus, the usual recommendation of reduced caloric intake and increased physical activity may be less successful than in persons with obesity from other causes.

The PHP1A and PPHP phenotypes have been observed to segregate within the same family and thus represent variable expressivity of the same variant. All such patients show a 50% reduction in Gsα activity in all tissues studied when compared to normal controls. There is an apparent parent-of-origin effect, maternal transmissions usually leading to the PHP1A phenotype, while variants of paternal origin result in PPHP. Mutations observed in PHP1A patients are distributed throughout the gene. Small insertions/deletions and amino acid substitutions predominate, but nonsense mutations and point mutations that lead to altered translation initiation or aberrant mRNA splicing have also been documented. In several cases the variant has been demonstrated to have arisen *de novo* (PMID: 10980525).

Disorders of *GNAS*/Gsα inactivation also include progressive osseous heteroplasia (POH, MIM #166350), which results from heterozygous pathogenic variants on the paternal allele. POH has a variable disease severity and progression and is characterized by dermal ossification that has an onset in infancy, which is followed by increasing and extensive bone formation in deep muscle and fascia. It has been suggested that POH could represent an extreme end of the spectrum of AHO features. Some of the variants identified in POH are identical to those found in patients with either PHP1A or PPHP (MIM #166350, 15711092).

In addition to inactivating variants, specific amino acid substitutions occurring at codons Arg201 (>95%) and Gln227 (<5%) confer a gain-of-function on Gsα. Replacement of either amino acid results in constitutive activation of Gsα and hence of adenylyl cyclase. Such variants were first characterized in pituitary tumors that secreted human growth hormone and showed increased adenylyl cyclase activity. Activating variants at codon Arg201 can also lead to a condition known as McCune-Albright syndrome (MAS, also known as fibrous dysplasia (FD); MIM #174800). Individuals with MAS are somatic mosaics, presumably as a result of mutation during embryonic development, and the variant is most readily detectable in the affected tissues. Germline

transmission of these activating variants has not been observed, suggesting that they are likely to be embryonic lethal if present in non-mosaic form (ORPHA562).

There are currently over 300 variants in *GNAS* annotated as disease-causing (DM) in the HGMD Professional variant database (version 2021.3), including both missense and truncating variants.

Mutation nomenclature is based on GenBank accession NM_000516.6 (*GNAS*) with nucleotide one being the first nucleotide of the translation initiation codon ATG.

**CONCLUSION**

*GNAS* c.677G>A, p.(Gly226Asp) is classified as a variant of uncertain significance (VUS), as there is currently insufficient evidence to support its disease-causing role. However, it should be noted that there is a strong association between the gene and the patient's phenotype, the variant is absent in control populations, and *in silico* tools predict the substitution deleterious. At present, screening for this variant should not be used for risk evaluation in family members. However, family member testing could assist in the further classification of the variant. Management of the patient and family should be based on clinical evaluation and judgment. Genetic counseling is recommended.

The identified *GNAS* c.677G>A, p.(Gly226Asp) variant is eligible for the BpG VUS Clarification Service if: 1) the index patient is the only affected in the family, 2) both biological parents are unaffected and 3) both biological parents are willing to provide a sample.
If indicated, complete a requisition form for each parental sample and use the Promotion code "VUS1_ID170265" when submitting the parental samples. Include phenotypic information and the relationship to the index patient. Testing will only begin after both parental samples have arrived in our laboratory. All required samples must be received within 6 months from the date when the index patient's result was reported, or the promotion code is no longer valid. The BpG VUS Clarification Service is offered when testing additional family members is likely to result in reclassification of the variant to likely pathogenic. For more information, please visit https://blueprintgenetics.com/vus-clarification/

| STEP | DATE |
|---|---|
| Order date | |
| Sample received | |
| Sample in analysis | |
| Reported | |

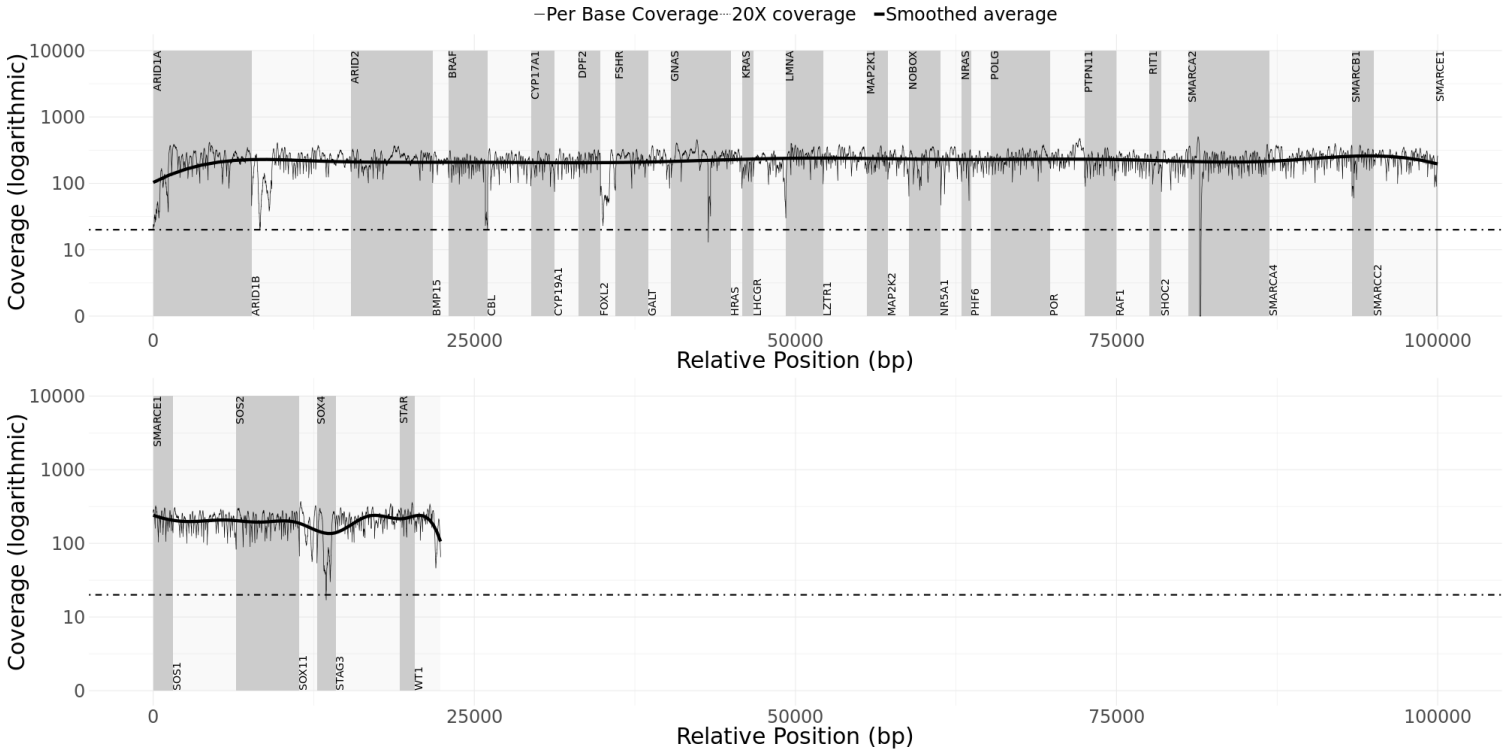
(This statement has been prepared by our geneticists and physicians, who have together evaluated the sequencing results.)

## 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 at Blueprint Genetics. 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.These steps were performed at Blueprint Genetics.
**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. Bioinformatics and quality control processes were performed by Blueprint Genetics.
**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. The interpretation was performed at Blueprint Genetics.
**Variant classification:** 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. The classification and interpretation of the variant(s) identified reflect the current state of Blueprint Genetics' understanding at the time of this report. Variant classification and interpretation are subject to professional judgment, and may change for a variety of reasons, including but not limited to, updates in classification guidelines and availability of additional scientific and clinical information. This test result should be used in conjunction with the health care provider's clinical evaluation. Inquiry regarding potential changes to the classification of the variant is strongly recommended prior to making any future clinical decision. For questions regarding variant classification updates, please contact us at support@blueprintgenetics.com
**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.The confirmation of sequence alterations was performed at Blueprint Genetics.

**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.The confirmation of copy number variants was performed at Blueprint Genetics.

**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).

**PERFORMING SITE:**

BLUEPRINT GENETICS OY, KEILARANTA 16 A-B, 02150 ESPOO, FINLAND Laboratory Director: MD, PhD, CLIA: 99D2092375

**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 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
**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.