PREVENTION GENETICS part of EXACT SCIENCES

NAME	PATIENT ID
LAST, First	####-###-###

PATIENT INFORMATION	SPECIMEN INFORMATION	PROVIDER INFORMATION
LAST, First ID#: DOB: Month, Day, Year Sex:	Type: Collected: Month, Day, Year Received: Month, Day, Year PG ID: ####-###-### Test Method: PGxome	Physician Genetic Counselor Institution

PGxome Health Screen – Whole Exome Sequencing

Singleton Analysis

SECONDARY FINDINGS: Heterozygous for a Pathogenic Variant in *BRCA1*; Heterozygous for a Likely Pathogenic Variant in *MNX1*

CARRIER STATUS: Heterozygous for a Pathogenic Variants in *HOGA1*; Heterozygous for a Likely Pathogenic Variant in *MESP2*

Medically actionable variants in guideline recommended genes*:

*Recommended list of genes (Miller et al. 2023. PubMed ID: 37347242)

Sequence Variant(s):

Gene, Transcript	Mode of Inheritance, Gene OMIM	DNA Variations, Predicted Effects, Zygosity	ClinVar ID	Highest Allele Frequency in a gnomAD Population	In Silico Missense Predictions	Interpretation
BRCA1, NM_007294.3	AR, AD, 113705	c.5123C>A, p.Ala1708Glu, Heterozygous	113705	0.0058%, Latino	Damaging	PATHOGENIC

Mode of Inheritance: Autosomal Dominant=AD, Autosomal Recessive=AR, X-Linked=XL
ClinVar ID: Variant accession (www.ncbi.nlm.nih.gov/clinvar)
GnomAD: Allele Frequency registered in a large population database (gnomad.broadinstitute.org). Value listed is the highest allele frequency reported within one of seven population categories recognized in gnomAD v.2.1.1 (The "Other" population is excluded).
Missense Predictions: Summarized output (Damaging, Conflicting, or Tolerated) via PolyPhen-2, SIFT, MutationTaster, and FATHMM (PMID: 26555599).

BRCA1 VARIANT INFORMATION:

This patient is heterozygous in the *BRCA1* gene for a sequence variant designated c.5123C>A, which is predicted to result in the amino acid substitution p.Ala1708Glu. This variant (also known as c.5242C>A) has been reported in numerous families of Spanish origin with a history of breast and ovarian cancer (de Juan Jiménez et al. 2013. PubMed ID: 23479189; Janavicius 2010. PubMed ID: 23199084; Table 2, Díez et al. 2003.

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PubMed ID: 12955716). Functional studies have also reported that this variant impacts protein stability (Rowling et al. 2010. PubMed ID: 20378548) and results in skipping of exon 18 (Millevoi et al. 2010. PubMed ID: 19404736). In ClinVar, it is reported as pathogenic by several laboratories (https://www.ncbi.nlm.nih.gov/clinvar/variation/55407/). We classify this variant as pathogenic.

Pathogenic variants in *BRCA1* have been associated with autosomal dominant familial breast-ovarian cancer 1 (OMIM #604370), autosomal dominant susceptibility to pancreatic cancer 4 (OMIM #614320), and autosomal recessive Fanconi anemia, complementation group S (OMIM #617883). Only one variant was detected in this gene. It is possible that a second causative variant is not detectable by our sequencing test. Targeted testing for this variant in biological relatives is available.

Variants in genes not associated with phenotype but may result in a Mendelian disorder:

Sequence Variant(s):

Gene, Transcript	Mode of Inheritance, Gene OMIM	DNA Variations, Predicted Effects, Zygosity	ClinVar ID	Highest Allele Frequency in a gnomAD Population	In Silico Missense Predictions	Interpretation
MNX1, NM_005515.3	AD, 142994	c.817G>T, p.Glu273*, Heterozygous	Not listed in ClinVar	Not Present	Damaging	LIKELY PATHOGENIC

*For footnotes, please see previous table.

***MNX1* VARIANT INFORMATION:**
This patient is heterozygous in the *MNX1* gene for a variant designated c.817G>T, which is predicted to result in a premature protein termination (p.Glu273*). To our knowledge, this variant has not been reported in the literature or in a large population database (http://gnomad.broadinstitute.org), indicating this variant is rare. Nonsense variants in *MNX1* are expected to be pathogenic. This variant is interpreted as likely pathogenic.

Pathogenic variants in *MNX1* have been associated with autosomal dominant Currarino syndrome (OMIM #176450).

Carrier status for autosomal and X-linked recessive disorders:

Sequence Variant(s):

Gene, Transcript	Mode of Inheritance, Gene OMIM	DNA Variations, Predicted Effects, Zygosity	ClinVar ID	Highest Allele Frequency in a gnomAD Population	In Silico Missense Predictions	Interpretation
HOGA1, NM_138413.3	AR, 613597	c.700+5G>T, Intronic, Heterozygous	204285	0.20% European (Non-Finnish)	Not Applicable	PATHOGENIC
MESP2, NM_001039958.1	AR, 605195	c.258_261del, p.Glu88Glyfs*31, Heterozygous	555370	0.0044%, Latino	Not Applicable	LIKELY PATHOGENIC

*For footnotes, please see previous table.

***HOGA1* VARIANT INFORMATION:**
This patient is heterozygous in the *HOGA1* gene for a sequence variant designated c.700+5G>T, which is predicted to interfere with splicing. This variant has been reported to be pathogenic for primary hyperoxaluria

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(Hopp et al. 2015. PubMed ID: 25644115; Beck et al. 2013. PubMed ID: 22781098; reported as c.701+4G>T in Belostotsky et al. 2010. PubMed ID: 20797690). This variant is reported in 0.20% of alleles in individuals of European (Non-Finnish) descent in gnomAD (http://gnomad.broadinstitute.org/variant/10-99359925-G-T). In summary, this variant is interpreted as pathogenic.

Pathogenic variants in *HOGA1* have been associated with autosomal recessive primary hyperoxaluria type III (OMIM #613616).

***MESP2* VARIANT INFORMATION:**
This patient is heterozygous in the *MESP2* gene for a variant designated c.258_261del, which is predicted to result in a frameshift and premature protein termination (p.Glu88Glyfs*31). This variant is reported in 0.0044% of alleles in individuals of Latino descent in gnomAD (http://gnomad.broadinstitute.org/variant/15-90319843-CGCCA-C). Frameshift variants in *MESP2* are expected to be pathogenic. This variant is interpreted as likely pathogenic.

Pathogenic variants in *MESP2* have been associated with autosomal recessive Spondylocostal dysostosis 2 (OMIM #608681).

This patient is apparently negative for clinically relevant copy number variants (CNVs). This patient is also negative for known microdeletion and microduplication syndromes, autosomal and sex chromosome aneuploidy, and triploidy.

These results should be interpreted in the context of clinical findings, family history and other laboratory data. All genetic tests have limitations. Please see limitations and other information for this test on the following pages.

NOTES:

1) Based on these findings, the sequence variants considered in this patient are not eligible for free family follow up testing. Copy number variants are not eligible for free family follow up testing. For more information, please visit https://www.preventiongenetics.com/About/Resources/familytesting.

2) Genetic counseling is recommended.

SUMMARY STATISTICS:

Pipeline	Version	Average NGS Coverage	Fraction Bases Covered with NGS
Infinity_Pipeline	4.0.0	172x	97.6%

Minimum NGS coverage is ≥20x for all coding exons and +/-10bp of flanking DNA.

Electronically signed on by:	Electronically signed and reported on by:

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SUPPLEMENTAL INFORMATION V.23.08

Background: PGxome is PreventionGenetics' whole exome sequencing (WES) assay. The PGxome assesses almost all genes from the human genome including coding regions and adjacent introns. The PGxome Health Screen is intended for patients who are basically healthy, but who want to learn their carrier status for recessive disease and/or their susceptibility to adult onset disorders.

Although we sequence nearly all human genes, we analyze and report sequence variants only in genes that have been proven with high confidence to be involved in Mendelian (also called single gene) disorders (MacArthur et al. 2014. PubMed ID: 24759409). Our list of "clinically-relevant" genes currently includes about 4500 genes and is updated quarterly. We do not report variants in genes that for technical reasons cannot be accurately sequenced (primarily due to the presence of pseudogenes).

In addition, although we identify and interpret all sequence variants (differences between the patient's sequence and the reference sequence (build hg19), we report only Pathogenic and Likely Pathogenic variants (Richards et al. 2015. PubMed ID: 25741868).

We have found through our exome sequencing at PreventionGenetics that the average person is a recessive disease carrier for less than five Pathogenic or Likely Pathogenic variants. Note, however, that the average person also carries approximately 75 variants of Uncertain significance and thousands of Benign variants.

For this test, patients also have the option of receiving results of Pathogenic and Likely Pathogenic variants in genes that predispose to or confirm a diagnosis of adult onset disorders such as cancer and heart disease (v3.2; Miller et al. 2023. PubMed ID: 37347242).

Because new Mendelian disease genes are being continuously identified and because our ability to interpret sequence variants is steadily improving, it is important to consider reanalysis and reinterpretation of exome data in future. For this purpose, PreventionGenetics retains raw sequence data indefinitely from each exome test.

Test Methods: For the PGxome we use Next Generation Sequencing (NGS) technologies to cover the coding regions of targeted genes plus ~10 bases of non-coding DNA flanking each exon. As required, genomic DNA is extracted from patient specimens. Patient DNA corresponding to these regions is captured using hybridization probes. Captured DNA is sequenced on the NovaSeq 6000 using 2x150 bp paired-end reads (Illumina, San Diego, CA, USA). The following quality control metrics are generally achieved: >97% of target bases are covered at >20x and mean coverage of target bases >100x. Data analysis and interpretation is performed by the internally developed Infinity pipeline. Variant calls are made by the GATK Haplotype caller and annotated using in house software and Jannovar. Common benign, likely benign, and low quality variants are filtered from analysis.

Copy number variants (CNVs) are also detected from NGS data. We utilize a CNV calling algorithm that compares mean read depth and distribution for each target in the test sample against multiple matched controls. Neighboring target read depth and distribution and zygosity of any variants within each target region are used to reinforce CNV calls. In rare instances, confirmation of copy number variant results may be provided by the Wisconsin State Laboratory of Hygiene (465 Henry Mall, Madison, WI 53706; CLIA #52D0669558, CAP#1783801).

Reporting: Reports will consist of up to three different sections:

- **Guideline Recommended Genes (if opted in on the requisition form)**: Recent recommendations are for labs performing WES or WGS to report pathogenic variants in selected medically actionable genes that cause

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(mostly) dominantly inherited disorders (v3.2; Miller et al. 2023. PubMed ID: 37347242). Please note that if secondary findings were selected with concurrent PGxome diagnostic testing, Guideline Recommended Genes will be reported with PGxome diagnostic testing.

- **Other Predispositions/Diagnoses (if opted in on the requisition form)**: This secondary finding option refers to a very broad range of disorders beyond the Recommended Genes above. Variants that are likely to result in a Mendelian (single gene) disorder will be reported (i.e., one variant in a dominant gene or X-linked gene or two variants in a recessive gene).
- **Carrier Status**: Variants in any gene that relate to an autosomal recessive or X-linked recessive (in females) disorder will be reported if this option is selected.

All differences from the reference sequences (sequence variants) are assigned to one of seven interpretation categories (Pathogenic, Likely Pathogenic, Variant of Uncertain Significance, Likely Benign, Benign, Risk, and Pseudodeficiency) per ACMG Guidelines (Richards et al. 2015. PubMed ID: 25741868). Only pathogenic and likely pathogenic variants will be reported. Rare and undocumented synonymous variants are nearly always classified as likely benign if there is no indication that they alter protein sequence or disrupt splicing. Variants of Uncertain Significance, Benign, Likely Benign, Risk, and pseudodeficiency variants are not reported. Sequencing data is available to the ordering physician upon request.

Human Genome Variation Society (HGVS) recommendations are used to describe sequence variants (http://www.hgvs.org).

Limitations and Other Test Notes: Interpretation of the test results is limited by the information that is currently available. Better interpretation should be possible in the future as more data and knowledge about human genetics and genetic disorders improves.

Sequencing: When sequencing does not reveal any heterozygous differences from the reference sequence, we cannot be certain that we were able to detect both patient alleles. Occasionally, a patient may carry an allele which does not capture or amplify, due for example to a large deletion or insertion. In these cases, the report may contain no information about the second allele.

For technical reasons, the PGxome test is not 100% sensitive. Some exons cannot be efficiently captured, and some genes cannot be accurately sequenced because of the presence of multiple copies in the genome. Therefore, a small fraction of sequence variants relevant to the patient's health will not be detected.

We sequence coding exons for most given transcripts, plus ~10 bp of flanking non-coding DNA for each exon. Unless specifically indicated, test reports contain no information about other portions of the gene, such as regulatory domains, deep intronic regions, uncharacterized alternative exons, chromosomal rearrangements, repeat expansions, and mitochondrial genome variants.

In most cases, we are unable to determine the phase of sequence variants. In particular, when we find two likely causative variants for recessive disorders, we cannot be certain that the variants are on different alleles.

The ability to detect low-level mosaicism of variants is limited.

Runs of mononucleotide repeats (eg (A)n or (T)n) with n >8 in the reference sequence are generally not analyzed because of strand slippage during amplification.

Unless otherwise indicated, DNA sequence data is obtained from a specific cell-type (usually leukocytes if taken from whole blood). Test reports contain no information about the DNA sequence in other cell-types.

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We cannot be certain that the reference sequences are correct. Genome build hg19, GRCh37 (Feb2009) is used as our reference in nearly all cases.

Copy Number Variant Analysis: The PGxome test detects most deletions and duplications including intragenic CNVs and large cytogenetic events; however aberrations in a small percentage of regions may not be accurately detected due to sequence paralogy (e.g., pseudogenes, segmental duplications), sequence properties, deletion/duplication size (e.g., 1-3 exons vs. 4 or more exons), and inadequate coverage. In general, sensitivity for single, double, or triple exon CNVs is ~70% and for CNVs of four exon size or larger is >95%, but may vary from gene-to-gene based on exon size, depth of coverage, and characteristics of the region.

Balanced translocations or inversions are only rarely detected.

Certain types of sex chromosome aneuploidy may not be detected.

In nearly all cases, our ability to determine the exact copy number change within a targeted region is limited.

Our ability to detect CNVs due to somatic mosaicism is limited.

The sensitivity of this test is dependent on DNA quality.

General: We have confidence in our ability to track a specimen once it has been received by PreventionGenetics. However, we take no responsibility for any specimen labeling errors that occur before the specimen arrives at PreventionGenetics.

A negative finding does not rule out a genetic diagnosis.

Genetic counseling to help to explain test results to the patients and to discuss reproductive options is recommended. Results of PGxome testing can be used for both diagnostic and scientific research purposes.

References: Please see the test description for this test on our website (www.preventiongenetics.com) for a full list of complete citations.

Regulatory Information: These results should be used in the context of available clinical findings, and should not be used as the sole basis for treatment. This test was developed and its performance characteristics determined by PreventionGenetics. US Food and Drug Administration (FDA) does not require this test to go through premarket FDA review. This laboratory is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) as qualified to perform high complexity clinical laboratory testing.

If the results of this test will be utilized as part of a clinical study in the European Union, please note this test is intended for performance evaluation only. For all other testing within the European Union, this test is intended for research use only.

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