Variantyx Genomic Unity® Whole Genome Analysis

Patient Name	Date of Birth	Test	Report Date
John Doe	**Apr 22, 2021**	**MDemo374193291 / 49835**	**Jun 30, 2024**
			Report ID **691bf0e18988**

Test Information

Cohort	Trio
Sample Type	Blood
Sample Collection Date	Jan 22, 2024
Sample Received Date	Jan 24, 2024
Processed Date	Aug 4, 2022
Ordering Clinician	
NPI	N/A

Indication for Testing

Short stature, Tracheal stenosis, Global developmental delay, Peripheral pulmonary artery stenosis, Clinodactyly of the 5th finger, Low-set ears, Enlarged cisterna magna

Included Analyses

- Small Sequence Variants
- Mitochondrial Genome
- Structural Variants
- Short Tandem Repeats

Optional Findings

ACMG Secondary Findings	Opted in
Actionable Findings	Opted in

Results | POSITIVE with Secondary Findings

Primary | ACMG Secondary | Actionable | Carrier | Other Variants | Supplementary

Summary of Findings

A heterozygous, likely pathogenic variant was identified in the *NF1* gene in this individual. Pathogenic variants in this gene have been reported to cause autosomal dominant NF1-related disorders, which include neurofibromatosis type 1 (OMIM 162200), neurofibromatosis-Noonan syndrome (OMIM 601321), Watson syndrome (OMIM 193520), and familial spinal neurofibromatosis (OMIM 162210). The clinical presentation of this patient overlaps with features of neurofibromatosis-Noonan syndrome and Watson syndrome, such as short stature and pulmonary artery stenosis. Other common features of NF1-related disorders were not described in the clinical notes (PMID: 8740913, 2411134, 20301288, 6025371).

In addition, a heterozygous pathogenic potentially clinically-actionable variant was identified in the *BRCA1* gene. Heterozygous pathogenic variants in the *BRCA1* gene have been reported to cause susceptibility to familial breast-ovarian cancer 1, and are considered a secondary finding by the American College of Genetics and Genomics (ACMG).

Findings	Location	Variant	Mode of Inheritance / Disease	Classification
Primary	*NF1* NM_001042492.3	c.-272G>A Heterozygous in proband Not detected in father Not detected in mother	Autosomal dominant neurofibromatosis-Noonan syndrome	Likely Pathogenic PS2, PS4_Moderate, PM2_Supporting
ACMG Secondary	*BRCA1* NM_007294.4	c.1140dup p.Lys381GlufsTer3 rs876659327 Heterozygous Heterozygous in father Not detected in mother	Autosomal dominant susceptibility to familial breast-ovarian cancer 1	Pathogenic PVS1, PS4, PM2_Supporting, PP5

Follow Up Recommendations

Genetic counseling is recommended to review both positive and negative results, as well as secondary and incidental findings, if identified. Test results may benefit from periodic reevaluation for new clinical associations to variants and updated variant classification.

Primary Findings

This section contains variant(s) in genes partially or fully consistent with the clinical phenotype

Primary Findings (Continued)

Findings	Location	Variant	Mode of Inheritance / Disease	Classification
Primary	*NF1* NM_001042492.3	c.-272G>A Heterozygous in proband Not detected in father Not detected in mother	Autosomal dominant neurofibromatosis-Noonan syndrome	Likely Pathogenic PS2, PS4_Moderate, PM2_Supporting

***NF1* NM_001042492.3:c.-272G>A**

This is a variant in the 5'-untranslated region of the *NF1* gene (OMIM: 613113). Pathogenic variants in this gene have been associated with autosomal dominant NF1-related disorders. This variant was identified de novo in this individual (PS2). This variant has been reported in at least 2 unrelated individuals with features of neurofibromatosis type 1 (PMID: 27322474) (PS4_Moderate). It has also been found to segregate with the disease in relatives of the two individuals mentioned above; however, details on the families were not provided (PMID: 27322474). An alternate nucleotide substitution at this position (c.-272G>C) was reported in another affected individual (PMID: 27322474) and an adjacent variant (c.-273A>C) was described in two more patients (one de novo and the other of unknown inheritance). The functional consequence of these variants is uncertain at this time. This variant is absent from control populations (https://gnomad.broadinstitute.org/) (PM2_Supporting). Based on the current evidence, this variant is classified as likely pathogenic for autosomal dominant NF1-related disorders.

***NF1* Gene Information**

The *NF1* gene provides instructions for making a protein called neurofibromin. This protein is predominantly active in nervous system tissues, where it functions as a tumor suppressor. Neurofibromin accelerates the conversion of a molecule called Ras from its active form, which promotes cell division, to its inactive form, thus reducing cell growth and division. As a result, neurofibromin plays a crucial role in the regulation of cell proliferation and differentiation.

Mechanism - Pathogenic variants in the *NF1* gene, which encodes for neurofibromin, are recognized as the cause of Neurofibromatosis Type 1 (NF1), neurofibromas, and an increased risk of benign and malignant tumors. Pathogenic variants impair the function of neurofibromin, resulting in uncontrolled cellular proliferation due to the lack of regulation of the RAS pathway, leading to the formation of tumors and other manifestations of the disease. Most cases of NF1 are due to loss-of-function variants, which can be inherited in an autosomal dominant pattern or arise de novo in a significant number of patients. The disease exhibits complete penetrance but with highly variable expressivity, meaning that while NF1 variants affect most individuals carrying them, the severity and specific symptoms can differ widely among those affected.

Epidemiology - NF1 is one of the most common hereditary disorders with an estimated incidence of 1 in every 3,000 live births and it affects all populations. The lifetime risk of cancer in NF1 patients is estimated to be 59.6% with the highest risks for CNS and nerve sheath tumors.

Medline Plus-NF1 (https://medlineplus.gov/genetics/gene/nf1/); NCBI-NF1 (https://www.ncbi.nlm.nih.gov/gene/4763); PMID: 20301288 (https://www.ncbi.nlm.nih.gov/books/NBK1109/); PMID: 26926675

Pathogenic variants in this gene have been associated with the following disorders in OMIM[1]:

Disease	Mode of Inheritance
Neurofibromatosis type 1	AD
Spinal neurofibromatosis	AD
Neurofibromatosis-Noonan syndrome	AD
Juvenile myelomonocytic leukemia	AD
Watson syndrome	AD

[1]For disorders with the [], {}, or ? symbol, refer to https://www.omim.org/help/faq#1_6 for additional information.

ACMG Secondary Findings

This section of the report includes variants identified in a list of genes recommended by the American College of Medical Genetics and Genomics (ACMG) for reviewing and reporting secondary findings.

ACMG Secondary Findings (Continued)

Findings	Location	Variant	Mode of Inheritance / Disease	Classification
ACMG Secondary	*BRCA1* NM_007294.4	c.1140dup p.Lys381GlufsTer3 rs876659327 Heterozygous Heterozygous in father Not detected in mother	Autosomal dominant susceptibility to familial breast-ovarian cancer 1	Pathogenic PVS1, PS4, PM2_Supporting, PP5

***BRCA1* NM_007294.4:c.1140dup (p.Lys381GlufsTer3)**

This is a frameshift variant in the *BRCA1* gene (OMIM: 113705). Pathogenic variants in this gene have been associated with autosomal dominant susceptibility to familial breast-ovarian cancer 1. This variant introduces a premature termination codon in exon 10 out of 23. It is expected to result in loss of function, which is a known disease mechanism for *BRCA1* in this disorder (PMID: 11157798) (PVS1). This is an established founder variant in the Middle Eastern population (PMID: 27082205, 29297111) (PS4). This variant is absent from control populations (https://gnomad.broadinstitute.org/) (PM2_Supporting). Other reputable laboratories have reported this variant as pathogenic or likely pathogenic, and this classification has been validated by an expert panel in ClinVar (PP5). Based on the current evidence, this variant is classified as pathogenic for autosomal dominant susceptibility to familial breast-ovarian cancer 1.

***BRCA1* Gene Information**

The *BRCA1* gene encodes a tumor suppressor protein that plays a crucial role in the maintenance of genomic integrity by facilitating cellular responses to DNA damage. The BRCA1 protein combines with other tumor suppressors, DNA damage sensors, and signal transducers to form a large multi-subunit protein complex. In addition, the BRCA1 protein is a component of the RNA polymerase II holoenzyme and functions as a transcriptional coactivator.

Mechanism - Pathogenic variants in *BRCA1* may lead to the production of a truncated or otherwise nonfunctional protein, which can compromise the cellular ability to repair damaged DNA. This impairment can result in accumulation of genetic alterations that contribute to the initiation and progression of cancer. When BRCA1 function is compromised, cells are at an increased risk for malignancy, particularly in tissues with rapid turnover rates such as the breast and ovarian epithelium.

Epidemiology - The estimated prevalence of *BRCA1/2* pathogenic variants in the population is between 1 in 400 to 1 in 500, with higher prevalence in specific subpopulations such as Ashkenazi Jewish individuals, where prevalence is estimated to be as high as 1 in every 40 individuals. Approximately 45-85% of women who carry a pathogenic variant will develop breast cancer, and approximately 10-46% will develop ovarian cancer, depending on age, the nature of the specific variant, and other factors.

MedlinePlus-BRCA1 (https://medlineplus.gov/genetics/gene/brca1/);OMIM:113705 (https://omim.org/entry/113705); PMID: 20301425 (https://www.ncbi.nlm.nih.gov/books/NBK1247/); https://www.cancer.gov/about-cancer/causes-prevention/genetics/brca-fact-sheet; PMID:9159119; PMID:9662397

Pathogenic variants in this gene have been associated with the following disorders in OMIM[1]:

Disease	Mode of Inheritance
Susceptibility to familial breast-ovarian cancer 1	AD
Fanconi anemia, complementation group S	AR
Susceptibility to pancreatic cancer 4	AD

[1]For disorders with the [], {}, or ? symbol, refer to https://www.omim.org/help/faq#1_6 for additional information.

Actionable Findings

No other actionable findings were identified at the time of reporting.

Carrier Findings

No carrier findings were identified at the time of reporting.

Carrier Findings (Continued)

Other Variants Findings

No other findings were identified at the time of reporting.

Supplementary Findings

No supplementary findings were identified at the time of reporting.

Sample Information

Patient	Sex	Date of Birth	Specimen Type	Date Collected	Date Received
Proband John Doe MDemo374193291	M	Apr 22, 2021	Blood	Jan 22, 2024	Jan 24, 2024
Mother MDemo182301283	F	-	Saliva	Sep 7, 2022	Sep 9, 2022
Father MDemo290130237	M	-	Saliva	Sep 7, 2022	Sep 9, 2022

General Information

The Genomic Unity® Whole Genome Analysis is a whole genome sequence based test designed to identify genetic variants that correlate with the patient's clinical symptoms. This test includes sequence analysis (single nucleotide variants, deletions/insertions, intronic, regulatory and intergenic variants); analysis of copy number variants, duplications, deletions, regions of homozygosity, uniparental disomy, mobile element insertions, inversions, and aneuploidy; mitochondrial genome sequence analysis with heteroplasmy and large deletions; and short tandem repeat expansion analysis in select genes.

Methods

Whole genome short read sequencing was performed by next generation sequencing (NGS). Analyses were performed to detect, analyze and report clinically relevant variants using the Variantyx Genomic Intelligence® platform version 3.6.2.0. Orthogonal confirmation is performed as needed by Oxford Nanopore Technologies (ONT) PromethION 24.

Statistics
The sensitivity, specificity and positive predictive value of the assay is greater than 0.99 for single nucleotide variants. The sensitivity and positive predicted value of small insertions and deletions of fewer than 50 base pairs is greater than 0.95 and 0.92, respectively. The analytical sensitivity for copy number variants reported in this assay is greater than 0.80 for variants greater than 300 base pairs, while the clinical sensitivity for copy number variants of any size is greater than 0.96. The clinical sensitivity of this test is greater than 0.99 for pathogenic short tandem repeats.

Report Standards
Variants are reported using Human Genome Variation Society (HGVS) recommendations, when available. Variants are classified using one of five interpretation categories recommended by the American College of Medical Genetics and Genomics (ACMG): pathogenic, likely pathogenic, uncertain, likely benign, and benign (PMID: 25741868). Benign and likely benign variants are typically not reported. Variants of uncertain clinical significance are reported in select cases where there is a strong clinical correlation to the provided clinical symptoms of the patient and/or the family history. The genetic results are interpreted in the context of the provided personal medical and family history. Accurate interpretation of results is dependent on complete and accurate clinical information. Variants of uncertain clinical significance will only be reported if found to be associated with patient phenotype. Variants of uncertain clinical significance will not be reported in targeted analysis (phenotypic based analyses) unless sufficient clinical information was provided. Regions of homozygosity (ROH) and uniparental disomy (UPD) are detectable with this analysis. ROH for non-imprinted autosomal chromosomes and the X chromosome is reported for regions greater than or equal to 10 Mb. ROH is reported for regions greater than or equal to 5 Mb for imprinted chromosomes (6, 7, 11, 14, 15 and 20). Multiple regions of ROH can be indicative of shared common ancestry or consanguinity. Although the results of ROH are not interpreted, variants in genes associated with autosomal or X-linked recessive conditions related to the patient phenotype or severe early onset disorders will be reported if detected. UPD will only be determined when testing is run as a trio analysis (i.e. both parental samples are available). UPD will be reported for clinically relevant regions on the imprinted chromosomes. If relevant, additional testing may aid in diagnosis.

Annotations
To maintain the most up-to-date annotations, the Variantyx database is updated quarterly and, as a result, variant classification and/or interpretation may change over time as more information becomes available. Sequence variation is compared to reference data using genome build GRCh38 and the lab cannot guarantee the accuracy of this data nor the accuracy of databases listed below. The following databases and tools are included in Variantyx Genomic Intelligence® platform:

1. Disease association: HGMD Professional (http://www.hgmd.cf.ac.uk/), ClinVar (http://www.ncbi.nlm.nih.gov/clinvar/), OMIM (http://www.omim.org/), Orphanet (www.orpha.net/), GeneTests (https://www.genetests.org/).
2. Population frequencies: gnomAD (http://gnomad.broadinstitute.org/), dbSNP (http://www.ncbi.nlm.nih.gov/SNP/), ensembl (www.ensembl.org/), 1000 Genomes Project (www.1000genomes.org/), DGV (http://dgv.tcag.ca/) and the Variantyx allele frequency database (http://variantyx.com/).
3. In silico pathogenicity prediction: REVEL (PMID: 36413997, 27666373)
4. Gene Essentiality: According to published work 10.1371/journal.pgen.1003484
5. Gene tolerance: RVIS score, according to published work 10.1371/journal.pgen.1003709
6. Haploinsufficiency and Triplosensitivity using ClinGen Dosage Sensitivity Map(https://dosage.clinicalgenome.org)
7. Pathogenicity scoring - ACMG classification for SV based on ClinGen CNV Pathogenicity Calculator (https://cnvcalc.clinicalgenome.org/cnvcalc/)
8. Human Genome Variation Society (http://varnomen.hgvs.org/)
9. International System for Human Cytogenomic Nomenclature 2020 (ISCN 2020)

Methods (Continued)

10. MITOMAP - A human mitochondrial genome database (https://mitomap.org/MITOMAP)
11. SFARI - Simons Foundation Autism Research Initiative (https://www.sfari.org/)

A glossary of terms can be found at https://www.variantyx.com/glossary/

Single Nucleotide Variants
Single nucleotide variants and small deletion/insertions (<50 bp) are reported if there is clinical correlation to the patient's clinical symptoms.

Structural Variants
Structural variants classified as pathogenic, likely pathogenic and uncertain are reported if there is clinical correlation with the genes and or region. Parental inheritance will be reported for structural variants when both parents are available for testing.

Short Tandem Repeats
Short tandem repeats (e.g. trinucleotide repeat expansions) in pathogenic ranges, when identified and reported for the following genes: *AFF2, AR, ARX, ATN1, ATXN1, ATXN2, ATXN3, ATXN7, ATXN8OS, ATXN10, C9ORF72, CACNA1A, CNBP, CSTB, DMPK, DIP2B, FGF14, FMR1, FOXL2, FXN, GIPC1, GLS, HTT, JPH3, LRP12, NOP56, NOTCH2NLC, PABPN1, PPP2R2B, RFC1, SOX3, TBP, PHOX2B, TCF4, VWA1, ZIC2.* Carrier status is typically not reported for *RFC1.*

Regions of Homozygosity
Regions or runs of homozygosity (ROH), also known as loss of heterozygosity (LOH) or absence of heterozygosity (AOH), are genomic segments showing a continuous stretch of homozygous variants with no statistically significant intervening heterozygous variants. ROH may be representative of uniparental disomy (UPD), ancestral homozygosity or regions inherited from a more recent common ancestor that are identical by descent (IBD). If the analysis includes sequence analysis, these regions will be interrogated for homozygous pathogenic or likely pathogenic variants and reported as indicated. Reporting ROH follows the ACMG guidelines for ROH and UPD (PMID: 23328890, 32296163).

Uniparental Disomy
Uniparental disomy (UPD) occurs when both homologues of a chromosome pair are inherited from one parent, and the other parent's chromosome for that pair is missing. Uniparental disomy for certain chromosomes may not have clinical consequences, but for several chromosomes can result in abnormalities due to parent-of-origin differences in gene expression. Chromosomes that have been reported to have a phenotypic effect due to UPD are 6, 7, 11, 14, 15, and 20. UPD will be reported as either isodisomy, inheritance of both sister chromatids from the same parent, or heterodisomy, inheritance of both homologous chromosomes from the same parent.

Mitochondrial Variants
Mitochondrial variants are reported in the mitochondrial genome if they are pathogenic/likely pathogenic, or a variant of uncertain clinical significance if there is correlation to the patient's clinical symptoms. Heteroplasmy is reported for single nucleotide variants, however, heteroplasmy may not be reported for large deletions. Duplications are not detected. The false negative rate for large mitochondrial deletions has not been determined.

Previously reported variants
This section of the report includes variants reported by previous genetic testing, either in this individual or in a family member, that do not otherwise meet our clinical diagnostic reporting criteria. Disease associations for the gene(s) are not provided. Variantyx reviews clinical notes and copies of previous test results provided with the test submission. Variants are only included in the report if sufficient variant information was provided at the time of testing. The detection and reporting of previously reported results depends on the provided detailed variant information accompanying the test requisition. Of note, discordant results may be due to differences in technical methods or due to reference sequence errors. Any previously reported variants that meet our clinical diagnostic reporting criteria will appear in other sections of this report. Any differences in variant nomenclature from previous results may be due to differences in reference transcript, genome build, testing methodology, and/or bioinformatics platforms used.

Limitations

Technical Limitations
A negative result from this analysis does not rule out the possibility that the tested individual carries a rare, unexamined pathogenic variant or a pathogenic variant in an undetectable region. All next generation sequencing (NGS) technologies, including whole genome sequencing analysis, may generate false positive and false negative results. Results are applicable to the tissue type used for this sequence test and may not reflect the variation in other tissue types. Each individual may have slightly different coverage yield distributions within the genome. While most structural variants are detectable, some genetic aberrations, such as gross genomic rearrangements or variants in portions of genes with highly homologous pseudogenes (including *HBA1/HBA2*), mosaicism (with the exception of full chromosomal mosaic aneuploidy), are identified with a lower efficiency. Deletions and duplications in the range of 50-300 base pairs are detected with a reduced sensitivity (0.19). For short tandem repeat expansions, due to possible somatic expansion in the tissue being tested and/or sampling bias, the median size of the expanded allele may not be representative of the actual event in the biologically relevant tissue. In addition, this test detects direct DNA sequence changes, and not indirect changes and aberrations, such as gene expression, epigenetic modifications, fusion, chromosome conformational changes, and other unknown abnormalities. Variants are not reported if they are not uniquely mappable, are of low coverage or are otherwise determined to be of low quality. Variantyx is not responsible for specimen errors (e.g. labeling, extraction) of samples received that may have occurred prior to our receipt. This test will not typically report variants related to infertility or variants that increase a statistical risk for a disease. *ARX* repeat expansions will be reported only in cases where the clinical symptoms of the patient include early onset seizures. *TCF4* repeat expansions will be reported only in cases where the clinical symptoms of the patient include corneal dystrophy. Variants are not confirmed unless stated and confirmations are not included in published turnaround times.

Annotation Limitations
Sequence variation is compared to reference data using genome build GRCh38 and the lab cannot guarantee the accuracy of this data nor the accuracy of databases listed in the 'Annotations' section of this report.

Parental Analysis
Comparator samples are parental samples or other familial samples used solely for the purpose of comparison of variants with the tested individual (or proband). As such, no specific parental results are issued under the family member's name, however, parental inheritance is reported for the proband and therefore will reveal parental results for select genes. Additionally, method platform and/or quality thresholds may vary for comparator samples used for the purposes of identifying inheritance. Therefore, if a familial comparator sample is used later as a proband sample, a new collection may be necessary to support validated test performance.

ACMG Secondary Findings
The American College of Medical Genetics and Genomics (ACMG) recommends reporting pathogenic and likely pathogenic variants in a list of genes in both a gene-specific and variant-specific manner. Variantyx evaluates the secondary findings list of genes V3.2, which can be found on the Variantyx website (ACMG Secondary Findings). These variants are not typically reviewed during routine processing of patient samples, but are actively sought and reported to the patient. The ACMG recommends reviewing variants in the genes in their recommended list because the genes are related to conditions that are considered 'actionable', meaning that there are steps that can be taken to mitigate the onset or severity of the clinical outcome. It is important to understand that it is possible to have a pathogenic variant, but to have it not detected by the assay. In addition, variants of uncertain significance are not reported in these genes. If a variant is of uncertain significance, and later is considered pathogenic, it cannot be determined without a reanalysis of the data. ACMG secondary findings are reported if opted-in.

Actionable Findings
Other actionable findings are likely pathogenic/pathogenic variants detected unexpectedly during routine processing of patient samples. These variants are in genes apparently unrelated to the patient's reported phenotype, but with some degree of clinical actionability. These genes are not restricted to a specific list (such as the ACMG Secondary Findings list), but are similar in that they could impact medical management and decision making. Other actionable findings are not actively sought and therefore all actionable variants may not be

Limitations (Continued)

identified during processing. Variants of uncertain clinical significance are not reported in these genes. Some examples of these findings are pathogenic/likely pathogenic variants in high penetrance oncogenic related genes, polycystic kidney related genes with increased surveillance recommendations and/or genes associated with conditions for which possible treatment is available. Variants for late-onset conditions unrelated to the patient's phenotype, such as adult onset neurodegenerative diseases, will typically not be reported.

Reanalysis and Reclassification of Variants
Variant classification and/or interpretation may change over time as more information becomes available on the clinical symptoms associated with the genes/variants. Variants of uncertain significance identified by sequencing are typically not reported in this test, however may be reported when they are rare and in genes associated with diseases with symptoms that partially or completely correlate with the patient's disease spectrum and severity. Variants of uncertain significance are neither pathogenic nor benign, but are likely to be reclassified as such over time as more evidence becomes available. Variants in 5' or 3' untranslated regions are typically not reported. New associations of symptoms to diseases and genes are likely to occur with time. In addition, if the clinical symptoms reported to Variantyx were incomplete or if there has been a change in symptoms, new correlations may be revealed upon reanalysis. Therefore, it is recommended that results be reinterpreted periodically to determine if they may be related to disease. Reanalysis is an analysis of the original data only; Any variant(s) identified during reanalysis that require additional sequencing for variant confirmation (e.g. potentially expanded STR gene) will require an additional payment and may require additional sample(s).

Use of Test Results by Clinician
Results should be interpreted by the ordering clinician in the context of the patient's personal medical and family history. Genetic counseling is recommended to assist in the interpretation of genetic results. Genetic counselors in your area may be found by visiting the National Society of Genetic Counselors (NSGC) website at https://www.nsgc.org/ or at https://www.findageneticcounselor.com/.

FDA Notes
This laboratory is certified under the Clinical Laboratory Improvement Amendments (CLIA) of 1988 as qualified to perform high complexity laboratory testing. The US Food and Drug Administration (FDA) does not require this test to go through premarket clearance. This lab developed test (LDT) was developed and its performance characteristics determined by Variantyx, Inc. to be used for clinical purposes and not as investigational or as research. These results should be used in the context of the patient's clinical findings and family history and not as the sole basis for diagnosis and/or treatment.