Example report - Do not use for patient care decisions

Genome-wide Sequencing Ontario Laboratory Name
Address, Ontario
Phone: ###-###-#### Fax: ###-###-####

Ordering provider: Dr. Ordering Provider
Submitter clinic/institution: Medical Clinic, Hospital

Patient: **Last name, First name**

GSO Order ID: ########	Family number: ######	Report date:	00-00-0000 00:00
MRN#: ########	DOB: 00-00-0000	Received date:	00-00-0000
Sex: M/F	Specimen type: specimen	Collection date:	00-00-0000

TEST PERFORMED: Whole Genome Sequencing – Trio

REASON FOR REFERRAL:
Intellectual disability, autism, epilepsy, dysmorphic features

RESULTS:

- Causative variant(s) in disease genes related to the reported phenotype: **Positive – see below**
- Variant(s) in genes possibly related to the reported phenotype: **None identified**
- Variant(s) in candidate genes with potential relationship to reported phenotype: **None identified**
- ACMG Secondary findings analysis: **Positive – see below**

Sequence variants:

Category	Gene	Associated disorder	Mode of inheritance	Sequence variant	Zygosity	Inherited from	Interpretation
Related to the phenotype	*ANKRD11*	KBG syndrome	AD	c.1903_1907del p.Lvs635Glnfs*26	Heterozygous	Unknown; not maternal	Pathogenic
Secondary finding	*BRCA1*	Familial breast and ovarian cancer 1	AD	c.3756_3759del p.(Ser1253Argfs*10)	Heterozygous	Unknown; not maternal	Pathogenic

Acronyms: AD: Autosomal dominant, AR: Autosomal recessive, XL=X-linked, UPD: Uniparental disomy, AOH: Absence of heterozygosity

INTERPRETATION
SUMMARY :

- Duo analysis was performed using a maternal sample (MO26-00001).
- This individual is heterozygous for a pathogenic variant in the *ANKRD11* gene. This finding supports a clinical diagnosis of KBG syndrome.
- The secondary findings analysis detected a known pathogenic variant in the *BRCA1* gene in this individual.

VARIANT DETAILS
Findings related to the reported phenotype:

ANKRD11
(NM_013275.6, GRCh38/hg38)
Pathogenic variants in the *ANKRD11* gene are associated with autosomal dominant KBG syndrome (OMIM #148050). KBG syndrome is characterized by neurological features including developmental delay, intellectual disability, seizures, as well as macrodontia of the upper central incisors, distinctive facial features, and short stature. Other features include feeding difficulties, skeletal anomalies, and hearing loss (GeneReviews: PMID 29565525).

Variant: c.1903_1907del, p.(Lys635Glnfs*26), heterozygous, unknown inheritance, pathogenic
This variant is predicted to cause a premature stop codon, leading to nonsense mediated mRNA decay or a truncated protein, in a gene where loss of function is a known disease mechanism of disease. It has been previously reported in numerous affected individuals (PMID 33955014, 25413698, 32581362, 27667800, 28449295, 27605097, 28250421, 25424714). ClinVar has an entry for this variant (VCV000279678). It has not been observed in population controls of the Genome Aggregation Database (gnomAD). This variant was not detected in this individual's mother (in blood). As this is a duo analysis, it is not known if this variant was inherited from the father or if it is de novo. Based on the evidence above, this variant is classified as pathogenic.

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Secondary and incidental findings:

BRCA1

(NM_007294.4, GRCh38/hg38)

Pathogenic variants in the *BRCA1* gene are associated with autosomal dominant familial breast and ovarian cancer 1 (BROVCA1, OMIM #604370). In addition to an increased risk of developing breast and ovarian cancer, this condition is also associated with an increased risk for other cancers such as prostate cancer, pancreatic cancer, and melanoma (GeneReviews: PMID 20301425).

Variant: c.3756_3759del, p.(Ser1253Argfs*10),heterozygous, unknown inheritance, pathogenic

This variant is predicted to cause a premature stop codon, leading to nonsense mediated mRNA decay or a truncated protein, in a gene where loss of function is a known disease mechanism of disease. This is a well-established pathogenic variant that has been reported previously in many individuals with breast and/or ovarian cancer (PMID 29492181, 29614442, 23633455, 24728189, 25682074, 25556971, 27836010, 36367610, 32341426, 33478551, and others). ClinVar has an entry for this variant (VCV000017673), and it has been classified as pathogenic by the ENIGMA BRCA1/2 expert panel (ClinVar, June 24, 2016). This variant is observed at an allele frequency of 0.002% in population controls of the Genome Aggregation Database (gnomAD). This variant was not detected in this individual's mother (in blood). As this is a duo analysis, it is not known if this variant was inherited from the father or if it is de novo. Based on the evidence above, this variant is classified as pathogenic.

RECOMENDATIONS

- Clinical correlation is recommended: The results should be interpreted in the context of clinical findings, family history, and other experimental data.
- If clinically indicated, targeted testing for the *ANKRD11* variant in this individual's biological father (if available) is available through GSO at the Lab Name Diagnostic Laboratory, and can be initiated by using the GSO Family Member Requisition.
- Targeted testing of the *BRCA1* variant in other family members can be pursued at a clinical lab that offers this test. See https://www.ontariohealth.ca/clinical/genetics/test-directory for a list of tests available in Ontario.
- Genetic counselling is recommended.

METHODS

Genome sequencing and variant analysis

Whole genome sequencing was performed using TruSeq PCR-free library preparation followed by 2x150bp paired end sequencing on the Illumina Novaseq6000 platform to an average depth of coverage of at least 30X. Genome alignment (Build GRCh38/hg38) and small sequence variant (substitutions, insertions, duplications and deletions), copy number variant (deletions and duplications >10kb), and structural variant (deletions, duplications, and insertions of 50bps to <=20kb) analysis was performed with the Illumina Dragen Germline analysis platform.

Analytical sensitivity

The sensitivity of this test is 99.5% (95% confidence interval: 99.5 to 99.5%) for detecting substitution variants and 99.8% (95% confidence interval: 99.8 to 99.8%) for detecting small insertions, duplications and deletions. Sensitivity for variants was assessed using a truth set constructed from the high confidence regions of reference standards according to best practices (Kruche et al. (2019) PMID: 30858580). The sensitivity of this test is 99.9% (95% confidence interval: 95.7 to 99.9%) for detecting copy number variants greater than or equal to 10kb in size. Sensitivity was assessed for copy number variants using the comparator method against a truth set constructed from positive clinical chromosomal microarray analysis (CMA) results. The sensitivity of this test is 94.0% (95% confidence interval: 85.6% to 97.7%) for detecting deletions and tandem duplications greater than or equal to 50bps and less then or equal to 20kb in size. Sensitivity was assessed for structural variants using the comparator method against a truth set constructed from chromosomal microarray analysis (CMA) results, multiplex ligation-dependent probe amplification (MLPA), quantitative PCR (qPCR), and next generation sequencing derived copy number variant results. The sensitivity of this test for detecting insertions is not known . Reportable insertions greater than or equal to 50bps in size will be confirmed by an orthogonal method prior to reporting Individual quality metrics are available upon request.

Clinical sensitivity

The clinical sensitivity of genome-wide sequencing is dependent on the proband's reported phenotype, the type of testing performed, and the analysis strategy. A review of several large studies reported the diagnostic yield of genome-wide sequencing to range between 26-58%, with molecular diagnoses more likely to be identified when parental sequence data is available for concurrent analysis (Clark et al. (2018) PMID: 30002876).

LIMITATIONS AND OTHER TEST NOTES

Whole genome sequencing may not detect all sequence variants associated with the reported clinical indication. Approximately 5.9% of the genome is outside of regions for which we can confidently detect variation; this is a technical limitation, and we are unable to assess the accuracy in these low confidence regions. Sequencing technologies , library preparation, and associated bioinformatics analysis have limitations and may result in failure to detect some sequence variants. Highly-homologous and pseudogene sequences as well as GC rich and repetitive regions may interfere with accurate detection of variants in sequencing analyses. Certain types of genomic alterations known to cause disease may not be detected by this genome assay, including but not limited to, translocations or inversions, mitochondrial variants and repeat expansions. This genome assay is not designed or validated to detect somatic mosaicism.

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Interpretation
Absence of a plausible explanation for the reported phenotype by genome-wide sequencing does not exclude a genetic basis of the individual's condition. The analysis for the proband includes evaluation of variants under the various inheritance models (autosomal dominant, recessive and X-linked) and includes the analysis of de nova variants when both parents are submitted for concurrent analysis. The mode of inheritance for each gene is obtained from OMIM, when available. If not available in OMIM, the mode of inheritance is obtained from the scientific literature, and references are included in the report. In silica prediction algorithms used to predict the impact of variants on protein structure and/or function or splicing include MutationTaster, SIFT, PolyPhen-2, REVEL, SpliceSiteFinder-like, MaxEntScan, NNSplice and GeneSplicer. A large number of variants are detected by genome-wide sequencing. The prioritization of variants of interest relies on the accurate description of family structure, and a complete description of the reported phenotype and clinical and/or differential diagnosis at the time of testing. This report provides a focused list of variants of interest in genes related to the phenotype of the proband. Variants of uncertain significance in disease genes related to the proband's phenotype may be reported at the discretion of the laboratory. Common variants that may be associated with multifactorial and polygenic disorders are not reported.

Sequence variants are classified using the criteria outlined by the ACMG (Richards et al. (2015) PMID: 25741868) and are interpreted using the current knowledge of disease genes. Variant classification, particularly for variants of uncertain significance, may change as more information becomes available or as inheritance and allelic information is obtained from familial segregation studies. When genome-wide sequencing and analysis on families (e.g. trio, duo, quad) is performed, accurate assignment of family relationships is critical to the interpretation of test results, therefore a separate test to confirm the family relationships is performed. Independent analysis and reporting of variants on family members is not performed. Genes that do not currently have an established association with a disease but where evidence is emerging (candidate genes) may be reported at the discretion of the laboratory. Classification of candidate genes may also change as additional genetic or experimental evidence becomes available (Strande et al. (2017) PMID: 28552198). Test results should only be used in conjunction with the patient's clinical history and any previous analysis of appropriate family members. The chance of a false positive or false negative result due to laboratory error during any phase of testing cannot be completely excluded .

Secondary and incidental findings analysis
Analysis of secondary findings is performed on the proband only. Variants in the genes recommended by the ACMG SF v3.3 or the medically actionable in childhood subset defined by Genome-Wide Sequencing Ontario are reported when they are known or expected to be pathogenic (Lee et al. (2025), PMID: 40568962). Variants in other disease genes that meet the actionability criteria outlined in the ACMG policy statement may also be reported at the discretion of the Laboratory when identified through genome-wide sequencing analysis (i.e. incidental findings). Information about segregation of secondary and incidental findings in relatives submitted for familial analysis is reported unless the family has opted out of receiving this information. The absence of reportable secondary and incidental findings does not exclude the possibility that the proband or family members submitted for analysis carry variants that may confer susceptibility to the disorders included in the ACMG policy statement.

Regulatory notes
This test was developed and its performance characteristics determined by the SickKids Genome Diagnostics and CHEO Genetic Diagnostics Laboratories. This test is used for clinical purposes and therefore validation was completed in accordance with Accreditation Canada requirements .

Report electronically signed by:

Analyst Director