A comprehensive genomic reporting structure for communicating all clinically significant primary and secondary findings

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Supplementary 1: Example of an exome sequencing report: Breast cancer patient with a LP variant in the *BRCA2* gene, VUS in the *MSH6* gene, and SFs associated with a medically actionable condition, carrier status, pharmacogenomics, and common disease risk variants.

ST. MICHAEL'S UNITY HEALTH TORONTO

Sinai Health

Referring Clinic: Cancer Genetics Clinic	**Patient Name:** [insert patient name]	**Specimen Type:** DNA, blood
Physician: Dr. Ganesh Agarwal	**Date of Birth:** 1975-Jun-18	**Accession number:** 12498
Address: 30 Bond St, Toronto ON	**Sex:** Female	**Date Enrolled:** 2020-Sep-09
Counselor: Sofía Fernández	**Ethnicity:** Italian & Guyanese	**Date of Report:** 2021-Mar-23

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Test Ordered: Exome Sequencing (Research Study)

This individual elected to receive analysis of cancer associated findings (154 genes) and secondary findings in the following categories: medically actionable disorders (111 genes), pharmacogenomics (26 variants), common disease risk variants (24 variants), Mendelian disorders (3,773 genes), early-onset neurodegenerative conditions (54 genes), and carrier status (686 genes).

Reason for Referral: Clinical features for this individual include breast cancer diagnosed at age 39. Family history of cancer includes breast cancer (first, second, and third degree relative), colon cancer (third degree relative) and prostate cancer (first and second degree relatives). Family history also includes myocardial infarctions, Alzheimer's disease, and renal impairments.

Results Summary: Exome sequencing identified 40,049 variants in this individual compared to the reference genome.

- **One likely pathogenic variant was identified that is associated with the primary indication**
- **One variant of uncertain significance was identified that is associated with the primary indication**
- **One clinically significant variant was identified that is associated with a medically actionable condition**
- **One clinically significant variant was identified that is associated with carrier status**
- **Three pharmacogenomic variants were identified**
- **Ten common disease risk variants were identified**
- **No clinically significant variants were identified that were associated with Mendelian disorders and early-onset neurodegenerative disorders**

A. PRIMARY CANCER FINDINGS:

Disease (Inheritance)	Gene (Transcript)	Variant; Zygosity	Variant Frequency (gnomAD)	Variant Interpretation
Hereditary Breast and Ovarian cancer (Autosomal Dominant); Fanconi anemia, type D1 (Autosomal Recessive)	*BRCA2* (NM_000059)	c.67+3A>G; heterozygous	-	Likely Pathogenic
Lynch Syndrome (Autosomal Dominant); Mismatch repair cancer syndrome (Autosomal Recessive); Familial endometrial cancer (Autosomal Dominant)	*MSH6* (NM_000179)	c.667A>G (p.N223D); heterozygous	0.000008013	Uncertain Significance

INTERPRETATION SUMMARY: Analysis of 154 genes associated with cancer identified one likely pathogenic variant in the ***BRCA2* (c.67+3A>G)** gene listed above that is associated with autosomal dominant hereditary breast and ovarian cancer as well as autosomal recessive Fanconi anemia. In addition, one variant of uncertain significance was identified that is related to the personal or family history of cancer in the ***MSH6* (p.N223D)** gene; the clinical significance of this variant could not be determined with absolute certainty at this time. Detailed variant information and disease summaries for ***BRCA2* (c.67+3A>G)** and ***MSH6* (p.N223D)** are listed further below on pages 3-4. **Variants in genes not known to be directly related to the personal or family history of cancer are not included on the report. The list of all primary cancer variants identified can be found in Appendix A on page 9**

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B. SECONDARY FINDINGS:

Medically Actionable

Analysis of 121 genes identified one clinically significant variant (***MYBPC3* c.26-2A>G**) associated with the medically actionable conditions: dilated cardiomyopathy, hypertrophic cardiomyopathy, and left ventricular noncompaction. Refer to page 5 for detailed variant and disease information.

Disease (Inheritance)	Gene (Transcript)	Variant; Zygosity	Variant Frequency (gnomAD)	Variant Interpretation
Hypertrophic cardiomyopathy (Autosomal Dominant); Dilated cardiomyopathy (Autosomal Dominant); Left ventricular noncompaction (Autosomal Dominant)	*MYBPC3* (NM_000256)	c.26-2A>G; heterozygous	0.00002741	Pathogenic

Carrier Status

Analysis of 686 genes identified one clinically significant heterozygous variant (***RPGRIP1* p.E539Qfs*2**) consistent with carrier status in this individual. Refer to pages 5-6 for detailed variant and disease information.

Disease (Inheritance)	Gene (Transcript)	Variant; Zygosity	Variant Frequency (gnomAD)	Variant Interpretation
Leber congenital amaurosis-6 (Autosomal Recessive)	*RPGRIP1* (NM_020366)	c.1614_1623del (p.E539Qfs*2); heterozygous	0.00002905	Likely Pathogenic

Note, variants of uncertain significance in genes associated with secondary findings are not reported here. We cannot rule out the possibility that another variant exists (VUS or other) that is not reported or identified using the methodologies described below.

Pharmacogenomic Associations

Three of 26 pharmacogenomic variants alter the metabolizer phenotype in this individual: ***VKORC1* *1/*2 (rs9923231)**, ***CYP2C9* *1/*2 (rs1799853)**, and ***CYP2C19* *1/*17 (rs12248560)**. Refer to Appendix C on page 11 for a full list of medications and dosing recommendations.

Gene & Genotype (SNP ID)	Metabolizer Phenotype	Associated Medication(s)	Dosing Recommendation(s)
VKORC1 *1/*2 (rs9923231)	Intermediate Metabolizer	*Warfarin* (blood thinner)	Warfarin: Based on this individual's *VKORC1* and *CYP2C9* genotypes, a lower warfarin starting dose is recommended. Standard dosing is recommended for phenytoin, fosphenytoin, celecoxib, flurbiprofen, ibuprofen, lornoxicam, meloxicam, piroxicam and tenoxicam.
CYP2C9 *1/*2 (rs1799853)	Intermediate Metabolizer	*Warfarin* (blood thinner) *Phenytoin & Fosphenytoin* (anti-seizure medications) *Celecoxib, Flurbiprofen, Ibuprofen, Lornoxicam, Meloxicam, Piroxicam & Tenoxicam* (nonsteroidal anti-inflammatory drugs; NSAIDs)	
CYP2C19 *1/*17 (rs12248560)	Rapid Metabolizer	*Amitriptyline* (anti-depressant & anti-pain medication) *Citalopram & Escitalopram* (anti-depressants) *Voriconazole* (anti-fungal medication) *Lansoprazole, Omeprazole & Pantoprazole* (proton pump inhibitors; stomach and esophageal medications) *Clopidogrel* (blood thinner)	Alternative therapy may be recommended for amitriptyline, citalopram, escitalopram and voriconazole due to the potential for therapeutic failure. Increased dosing is recommended for lansoprazole, omeprazole and pantoprazole due to the potential for therapeutic failure. Standard dosing is recommended for clopidogrel.

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Common Disease Variants

Ten of 24 common disease risk variants were identified in this individual and are associated with increased risk for age-related macular degeneration (***CFH* rs1061170; *SKIV2L* rs429608**; ***CFB* rs641153; *C2* rs9332739; *NELFE* rs522162**), Crohn's disease (***IL23R* rs11209026, rs11465804**),Type 1 diabetes (***INS* rs689; *HLA-DQA1* rs9272346**), and Celiac disease (***HLA-DQA1* rs2187668**). *Developing these conditions is also dependent on other genetic and environmental risk factors, therefore the presence of these variants accounts for a small contribution of the overall disease risk.*

Disease (% Prevalence)	Gene; rs #; Zygosity for risk allele	Reported Odds Ratio; Publication ID	Risk Allele Frequency (gnomAD)
Age-related macular degeneration (8.69%[1])	*CFH*; rs1061170; heterozygous	2.41; PMID: 21665990	0.32133
	SKIV2L; rs429608; heterozygous	2.16; PMID: 20385819	0.85711
	CFB; rs641153; heterozygous	2.22; PMID: 22705344	0.90424
	C2; rs9332739; homozygous	2.17; PMID: 21665990	0.9647
	NELFE; rs522162; homozygous	2.33; PMID: 23577725	0.8884
Crohn's disease (0.37%[2])	*IL23R*; rs11209026; homozygous	2.66; PMID: 21102463	0.95768
	IL23R; rs11465804; heterozygous	2.5; PMID: 18587394	0.95568
Type I diabetes (0.90%[3])	*INS*; rs689; heterozygous	2.38; PMID: 25751624	0.73433
	HLA-DQA1; rs9272346; heterozygous	5.49; PMID: 17554300	0.525
Celiac disease (0.5-1%[4])	*HLA-DQA1*; rs2187668; heterozygous	6.23; PMID: 20190752	0.0777

[1]Wong_2014_PMID: 25104651, [2]Fedorak_2010_PMID: 21157579, [3]Diabetes in Canada: Highlights from the Canadian Chronic Disease Surveillance System, Public Health Agency of Canada, [4]Gujral_2012_PMID: 23155333

Recommendation:

Genetic counselling and clinical correlation are recommended for this individual and their family members. Clinical confirmation of clinically significant variants may be recommended. Familial or known variant testing may be recommended for other family members. Increased surveillance or management strategies may be recommended according to established guidelines for clinically significant findings. Genetic testing of additional family members, particularly those who are affected, may help to understand the clinical significance of the variants of uncertain significance. Follow up for variants of uncertain significance identified in this individual should be made at the discretion of the referring clinician. Copy number variant (CNV) or MLPA analysis may be recommended to rule out the presence of large deletions/duplications that are not detectable using the below methodology. To check on the status of a variant or for assistance in locating nearby genetic counselling services please contact Advanced Molecular Diagnostics by email at jordan.lerner-ellis@sinaihealth.ca or our study coordinator at emma.reble@unityhealth.to.

VARIANT INFORMATION:

***BRCA2*, c.67+3A>G, Heterozygous, Likely Pathogenic**

Variant Interpretation: The *BRCA2* c.67+3A>G variant was identified in the literature in an Australian patient with bilateral breast cancer who had a strong family history of cancer (sister with endometrial cancer, mother with breast cancer, maternal grandmother with an unknown cancer type) (Parsons_2015_PMID:24302565). This variant was also reported in a compound heterozygous proband with Fanconi anaemia and leukemia who died at 17 months; the *BRCA2* c.67+3A>G variant was inherited from the unaffected mother and the proband's second *BRCA2* variant (p.I924Rfs*38) was inherited from the unaffected father (Feben_2017_PMID:28185119). The variant was identified in dbSNP (ID: rs1593880835) and ClinVar (classified as likely pathogenic by Ambry Genetics for hereditary cancer-predisposing syndrome). The variant was not identified in the COSMIC database or in the following control databases: the 1000 Genomes Project, the NHLBI GO Exome Sequencing Project, or the Genome Aggregation Database (March 6, 2019, v2.1.1). The c.67+3A>G variant is located in the 5' splice region but does not affect the invariant +1 and +2 positions. However, positions +3 to +6 are part of the splicing consensus sequence and variants involving these positions sometimes affect splicing. In addition, in silico or computational prediction software programs (Splice AI exome, Splice AI genome, dbscSNV Ada, RF) predict deleterious effect on splicing. Further, through in vitro mRNA analysis the c.67+3A>G variant was found to result in exon 2 skipping, resulting in the loss of the consensus ATG start site (Parsons_2015_PMID:24302565). In summary, based on the above information the clinical significance of this variant cannot be determined with certainty at this time although we would lean towards a more pathogenic role for this variant. This variant is classified as likely pathogenic.

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Disease Information: *BRCA1*- and *BRCA2*-associated hereditary breast and ovarian cancer syndrome (HBOC) is characterized by an increased risk for male and female breast cancer, ovarian cancer (includes fallopian tube and primary peritoneal cancers), and to a lesser extent other cancers such as prostate cancer, pancreatic cancer, and melanoma primarily in individuals with a BRCA2 pathogenic variant. Breast cancer is the most common malignancy in individuals with a germline *BRCA1* or *BRCA2* pathogenic variant with a lifetime risk ranging from 46% to 87% (GeneReviews).

Homozygous or compound heterozygous pathogenic variants in the *BRCA2* gene can also cause Fanconi anemia, complementation group D1. Fanconi anemia (FA) is a clinically and genetically heterogeneous disorder that causes genomic instability. Characteristic clinical features include developmental abnormalities in major organ systems, early-onset bone marrow failure, and a high predisposition to cancer (MIM: 605724).

Familial Risk: Hereditary breast and ovarian cancer syndrome is an autosomal dominant condition. Each offspring of an individual with a pathogenic variant has a 50% chance of inheriting the variant. Fanconi anemia, complementation group D1 is an autosomal recessive condition. At conception, the siblings of an affected individual have a 25% chance of being affected, a 50% chance of being asymptomatic carriers, and a 25% chance of being unaffected and not carriers. Carrier testing for at-risk relatives is possible if the pathogenic variants in the family are known.

***MSH6,* c.667A>G, p.N223D, Heterozygous, Uncertain Significance**

Variant Interpretation: The *MSH6* p.N223D variant was assessed to have no impact on the MSH6 protein using a bioinformatics tool CoDP (Combination of the Different Properties) that integrates the prediction results of MAPP, PolyPhen-2 and SIFT, in addition to 2 other structural properties (solvent accessibility and change in the number of heavy atoms of amino acids) (Terui_2013_23621914). The variant was also identified in dbSNP (ID: rs374041375), ClinVar (classified uncertain significance by Ambry Genetics, Invitae, Color and Integrated Genetics), Clinvitae (2x), and in control databases in 2 of 249596 chromosomes at a frequency of 0.000008013 (Genome Aggregation Database March 6, 2019, v2.1.1). Breakdown of the observations by population include European (non-Finnish) in 2 of 113178 chromosomes (freq: 0.00001767), while not observed in the African, Other, Latino, Ashkenazi Jewish, East Asian, European Finnish, and South Asian populations. The variant was not identified in GeneInSight-COGR, Cosmic, MutDB, UMD-LSDB, Insight Colon Cancer Gene Variant Database, Zhejiang Colon Cancer Database, Mismatch Repair Genes Variant Database, or Insight Hereditary Tumors Database. The p.N223 residue is conserved in in mammals but not in more distantly related organisms however computational analyses (PolyPhen-2, SIFT, AlignGVGD, BLOSUM, MutationTaster) do not suggest a high likelihood the variant Asp impacts the protein; this information is not predictive enough to rule out pathogenicity. The variant occurs outside of the splicing consensus sequence and in silico or computational prediction software programs (SpliceSiteFinder, MaxEntScan, NNSPLICE, GeneSplicer, HumanSpliceFinder) do not predict a difference in splicing. In summary, based on the above information the clinical significance of this variant cannot be determined with certainty at this time. This variant is classified as a variant of uncertain significance.

Disease Information: Heterozygous pathogenic variants in the *MSH6* gene are associated with hereditary nonpolyposis colorectal cancer (HNPCC) or Lynch syndrome. Lynch syndrome is characterized by an increased risk for colorectal cancer (CRC) and cancers of the endometrium, stomach, ovary, small bowel, hepatobiliary tract, urinary tract, brain, and skin. The following cancer risks are associated with Lynch syndrome: colon cancer risk of up to 82% (mean age of diagnosis 44-61 years), endometrial cancer risk of up to 14-71% (mean age of diagnosis 48-62 years), gastric cancer risk of up to 13% (mean age of diagnosis 56 years), ovarian cancer risk of up to 24% (mean age of diagnosis 42.5 years). Risks for cancers of the prostate, small intestine, hepatobiliary tract, urinary tract, pancreas, and brain are also increased (Kohlmann_2004_PMID:20301390; Giardiello_2014_PMID:25070057; Raymond_2013_PMID:23530095). The risk for other Lynch syndrome-related cancers is lower, though substantially increased over general population rates (GeneReviews; Invitae Clinical Notes).

Biallelic pathogenic variants in the *MSH6* gene are associated with mismatch repair cancer syndrome, a rare childhood cancer predisposition syndrome with 4 main tumor types: hematologic malignancies, brain/central nervous system tumors, colorectal tumors and multiple intestinal polyps, and other malignancies including embryonic tumors and rhabdomyosarcoma (MIM: 276300).

Familial Risk: Lynch syndrome is an autosomal dominant condition. Each offspring of an individual with a pathogenic variant has a 50% chance of inheriting the variant. Mismatch repair cancer syndrome is an autosomal recessive condition. At conception, the siblings of an affected individual have a 25% chance of being affected, a 50% chance of being asymptomatic carriers, and a 25% chance of being unaffected and not carriers. Carrier testing for at-risk relatives is possible if the pathogenic variants in the family are known.

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***MYBPC3*, c.26-2A>G, Heterozygous, Pathogenic**

Variant Interpretation: The *MYBPC3* c.26-2A>G variant was identified in at least 14 individuals with hypertrophic cardiomyopathy and in one unaffected individual (Ehlermann_2008_PMID: 18957093; Waldmüller_2011_PMID: 21750094; Natarajan_2016_PMID: 27831900; Witjas-Paalberends_2013_PMID: 23674513; Kapplinger_2014_PMID: 24510615; Walsh_2017_PMID: 27532257). In addition, this variant was found to segregate with disease in at least two members from one family (Ehlermann_2008_PMID: 18957093). The variant was identified in dbSNP (ID: rs376395543) and ClinVar (classified as pathogenic by Laboratory for Molecular Medicine, Invitae, GeneDx and six other submitters, and as likely pathogenic by Color and one other submitter). The variant was identified in control databases in 7 of 255378 chromosomes (0 homozygous) at a frequency of 0.00002741, and was observed at the highest frequency in the European (non-Finnish) population in 6 of 115748 chromosomes (freq: 0.00005184) (Genome Aggregation Database March 6, 2019, v2.1.1). The c.26-2A>G variant is predicted to cause abnormal splicing because the nucleotide substitution occurs in the invariant region of the splice consensus sequence and may lead to a truncated or absent protein and loss of function. In addition, in silico or computational prediction software programs (Splice AI, RF, dbscSNV Ada) predict a deleterious effect on splicing. Loss of function variants of the *MYBPC3* gene are an established mechanism of disease in autosomal dominant hypertrophic cardiomyopathy and is the type of variant expected to cause the disorder. In summary, based on the above information this variant meets our laboratory's criteria to be classified as pathogenic.

Disease Information: Pathogenic variants in the *MYBPC3* are most commonly associated with autosomal dominant hypertrophic cardiomyopathy (MIM: 115197), but have also been reported in individuals with dilated cardiomyopathy (MIM: 615396) and left ventricular noncompaction (MIM: 615396).

Familial hypertrophic cardiomyopathy (HCM) is a heart condition characterized by thickening (hypertrophy) of the cardiac muscle. Cardiac hypertrophy often begins in adolescence or young adulthood, although it can develop at any time throughout an individual's lifetime. The symptoms of familial hypertrophic cardiomyopathy are variable, even within the same family. Many affected individuals have no symptoms. Other people with familial hypertrophic cardiomyopathy may experience chest pain; shortness of breath, especially with physical exertion; a sensation of fluttering or pounding in the chest (palpitations); lightheadedness; dizziness; and fainting. While most people with familial hypertrophic cardiomyopathy are symptom-free or have only mild symptoms, this condition can have serious consequences such as arrhythmias, increased risk of sudden death and a small number of affected individuals develop potentially fatal heart failure, which may require heart transplantation. Heterozygous, homozygous and compound heterozygous variants in the *MYBPC3* gene have been identified in multiple individuals and families with HCM (Rottbauer_1997_PMID: 9218526; Carrier_1997_PMID: 9048664; Niimura_1998_PMID: 9562578; Daehmlow_2002_PMID: 12379228; Richards_1999_PMID: 10424815).

Familial dilated cardiomyopathy (DCM) occurs when cardiac muscle becomes thin and weakened in at least one chamber of the heart, causing the open area of the chamber to become enlarged (dilated). It usually takes many years for symptoms of familial dilated cardiomyopathy to cause health problems. They typically begin in mid-adulthood, but can occur at any time from infancy to late adulthood. Signs and symptoms of familial dilated cardiomyopathy can include arrhythmias, dyspnea, fatigue, fainting episodes (syncope), and swelling of the legs and feet. Left ventricular noncompaction (LVNC) is another cardiac muscle disorder that occurs when the lower left chamber of the heart (left ventricle) does not develop correctly. Some individuals with LVNC experience no symptoms at all; others have heart problems overlapping with DCM and can include sudden cardiac death. Although not as common as HCM, heterozygous variants in the *MYBPC3* gene have been identified in individuals with DCM or LVNC (Daehmlow_2002_PMID: 12379228; Probst_2011_PMID: 21551322).

Familial Risk: *MYBPC3*-related cardiomyopathies are inherited in an autosomal dominant manner. Each offspring of an individual with a pathogenic variant has a 50% chance of inheriting the variant.

***RPGRIP1*, c.1614_1623del, p.E539Qfs*2, heterozygous, Likely Pathogenic**

Variant Interpretation: The *RPGRIP1* p.E539Qfs*2 variant was identified in two compound heterozygous siblings with retinal degeneration, as well as another compound heterozygous individual with retinitis pigmentosa; however, the unaffected father shared the same genotype (Jamshidi_2019_PMID: 30072743; Booij_2011_PMID: 16272259). The variant was identified in dbSNP (ID: rs1420750126) and ClinVar (classified as pathogenic by Sharon Lab and as uncertain significance by Broad Institute Rare Disease Group). The variant was identified in control databases in 8 of 275384 chromosomes at a frequency of 0.00002905 (Genome Aggregation Database March 6, 2019, v2.1.1). The c.1615_1624del variant is predicted to cause a frameshift, which alters the protein's amino acid sequence beginning at codon 539 and leads to a premature stop codon 2 codons downstream. This alteration is then predicted to result in a truncated or absent protein and loss of function. Loss of function variants of the *RPGRIP1* gene are an established mechanism of disease in Leber congenital amaurosis-6 and is the type of variant expected to

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cause the disorder. In summary, based on the above information the clinical significance of this variant cannot be determined with certainty at this time although we would lean towards a more pathogenic role for this variant. This variant is classified as likely pathogenic.

Disease Information: Pathogenic variants in the *RPGRIP1* gene are associated with Leber congenital amaurosis-6 (MIM: 613826). Leber congenital amaurosis comprises a group of early-onset childhood retinal dystrophies characterized by vision loss, nystagmus, and severe retinal dysfunction. Patients usually present at birth with profound vision loss and pendular nystagmus. Electroretinogram (ERG) responses are usually nonrecordable. Other clinical findings may include high hypermetropia, photodysphoria, oculodigital sign, keratoconus, cataracts, and a variable appearance to the fundus (Chung&Traboulsi_2009_PMID: 20006823). The *RPGRIP1* gene encodes a photoreceptor protein and is a key component of cone and rod photoreceptor cells. Missense and truncating variants in this gene have been reported in several LCA patients (Dryja_2001_PMID: 11283794; Gerber_2001_PMID: 11528500).

Familial Risk: Leber congenital amaurosis is an autosomal recessive condition. At conception, the siblings of an affected individual have a 25% chance of being affected, a 50% chance of being asymptomatic carriers, and a 25% chance of being unaffected and not carriers. Carrier testing for at-risk relatives is possible if the pathogenic variants in the family are known.

Testing Methodologies:

Exome sequencing was performed using next generation sequencing (NGS) on the Illumina HiSeq 2500 platform. Approximately 98% of the exome was sequenced at greater than or equal to 10X depth coverage. The coding regions and splice sites with a minimum coverage equal to or greater than 10x and an allelic fraction equal to or greater than 25 percent (+/- 15 base pairs from the exon boundaries) were included in the analysis. Library preparation was performed using Agilent SureSelectXT Clinical Research Exome (Cat# 5190-7338) (Agilent Technologies, Santa Clara, CA, USA) according to the manufacturer's instructions. Two-to-three micrograms of DNA was fragmented to 150-200 bp using Covaris LE220 instrument (Covaris Inc., Woburn, MA) acoustic shearing. Exonic libraries were evaluated on a Bioanalyzer 2100 DNA High Sensitivity chip (Agilent Technologies) for size and quantity by qPCR using the Kapa Library Quantification Illumina/ABI Prism Kit protocol (KAPA Biosystems). An equimolar amount of eight libraries were pooled together. Pooled library underwent qPCR for quantification of each index-tagged library, followed by cluster generation, followed by paired-end sequencing on Illumina HiSeq 2500 instruments (using V4 chemistry, and 2x126 reads). The sequencing of the DNA was performed on a research basis at The Center for Applied Genomics (TCAG; Hospital for Sick Children, Toronto, ON).

The raw sequence reads produced from the sequencing technology were analyzed using the TCAG DNA-Seq pipeline v1.0.5. Base calling is performed using bcl2fastq 2.20 (for HiSeq 2500). The TCAG DNA-Seq pipeline uses GATK 3.7 and follows the GATK best practices. Reads are mapped to the b37 reference sequence using the bwa-mem algorithm and duplicate reads are marked using Picard Tools. Local realignment and base quality score recalibration using GATK follows. Variants are called using GATK HaplotypeCaller. For whole exome sequencing (WES) or targeted sequencing, variants are hard filtered (as recommended by GATK when VQSR cannot be used). Hard filters for SNPs are (QD < 2.0 || FS > 60.0 || MQ < 40.0 || MQRankSum < -12.5 || ReadPosRankSum < -8.0 || SOR > 3.0). Hard filters for indels are (QD < 2.0 || FS > 200.0 || ReadPosRankSum < -20.0 || SOR > 10.0). Variants are annotated using a custom TCAG annotation pipeline based on Annovar. Processed data was then run on the Lerner-Ellis Lab exome pipeline described in the analysis methodologies below.

Analysis methodologies:

The list of 154 cancer genes (Appendix B) for the primary indication was generated using genes from hereditary cancer panels issued by Invitae, GeneDx, and Ambry Genetics reputable labs as well as the Catalogue of Somatic Mutations in Cancer (COSMIC) v89 database, June 2019. In order to conduct a primary analysis in accordance to the reason for referral, annotated variants were included if they met the following filtration parameters: GnomAD and ExAC frequency <0.05; ClinVar Pathogenic/Likely Pathogenic variant calls of any frequency were included unless called only by OMIM or GeneReviews; synonymous and splicing variants ± 15bp from the exon boundaries were included unless no splicing effect was predicted by SpliceSiteFinder-like, MaxEntScan, NNSPLICE, and GeneSplicer (variants outside the region of analysis were included only if they were called Pathogenic/Likely Pathogenic in ClinVar); ClinVar Benign/Likely Benign calls with ≥ 2 observations from reputable labs (Appendix A[4]) were excluded and ClinVar Benign/Likely Benign calls with ≥ 4 observations from any lab were also excluded.

In addition to the reason for referral, study participants provided consent to receive secondary findings associated with certain disease types of their choice which may have included: medically actionable diseases, pharmacogenomics, common disease risk variants, rare Mendelian diseases (non-medically actionable), early-onset neurodegenerative disorders, and carrier status for autosomal recessive conditions. Annotated variants were filtered using various parameters described below according to the

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selected category. Variants may be reported in another category if the scope of the associated disease or phenotype fits better than in the category in which it was identified.

The list of medically actionable genes was developed according to specifications of medically actionable genes as reported by both the American College of Medical Genetics and Genomics (ACMG SF v2.0, Kalia_2017_PMID: 27854360) and The Clinical Genome Resource (ClinGen, https://search.clinicalgenome.org/kb/actionability, February 2020). The ClinGen list consists of 177 genes including the ACMG 59 gene list and an additional 118 genes. Sixty-six of these genes were found to be associated with cancer and therefore, were removed from the medically actionable gene list as they would instead be reported in relation to the primary cancer indication, leaving a total of 111 medically actionable genes. Medically actionable genes were filtered using the following parameters: variants with GnomAD and ExAC frequency of <0.01 across all populations were included; ClinVar Pathogenic/Likely Pathogenic variant calls of any frequency were included unless called only by OMIM or GeneReviews; synonymous and splicing variants ± 15bp from the exon boundaries were included unless no splicing effect was predicted by SpliceSiteFinder-like, MaxEntScan, NNSPLICE, and GeneSplicer (variants outside the region of analysis were included only if they were called Pathogenic/Likely Pathogenic in ClinVar); ClinVar Benign/Likely Benign variant calls with ≥ 2 observations from reputable labs (Appendix A[4]) were excluded and ClinVar Benign/Likely Benign calls with ≥ 4 observations from any lab were excluded; heterozygous autosomal recessive variants (mode of inheritance as reported by OMIM) were excluded unless found in the compound heterozygous state and variants with frequencies between > 0.001-0.0001 in at least three control populations were removed.

Pharmacogenomic information was extracted from the Pharmacogenomics Knowledgebase (PharmGKB, February 2020), which is a publically available interactive database of pharmacogenomics variants. The PharmGKB annotates pharmacogenomics based drug dosing guidelines published by the Clinical Pharmacogenetics Implementation Consortium (CPIC), the Royal Dutch Association for the Advancement of Pharmacy - Pharmacogenetics Working Group (DPWG), the Canadian Pharmacogenomics Network for Drug Safety (CPNDS) and other professional societies [https://www.pharmgkb.org]. CPIC class A pharmacogenomic variants with peer-reviewed dosing guidelines were selected and were then further chosen based on consultations with a clinical pharmacogenetics advisor. Additionally, only haplotypes that were covered by our sequencing kit were included in the analysis; these are listed in the Pharmacogenomic Information variant table in Appendix C. Metabolizer phenotypes are determined based only on SNPs that are covered by our sequencing kit.

Common disease risk variants were selected from the NHGRI-EBI Catalog (v1.0.2) of published genome-wide association studies. All variants with pharmacogenetics, cancer or pediatric associations were excluded. Variants that reached genome-wide significance (p value < 5 x 10-8) and had a reported odds ratio ≥ 2.0 were included. Only variants with ethnically relevant associations are reported here. These variants are associated with risk of developing the following disorders: Crohn's disease, Diabetes Mellitus Types 1 and 2, Macular Degeneration, Celiac disease, Ulcerative colitis, Chronic obstructive pulmonary disease and stroke. A total of 24 variants were investigated and referring literature is available upon request.

Online Mendelian Inheritance in Man (OMIM®; February 2020) was used to generate a list of genes associated with rare Mendelian disorders that were not included in the primary cancer indication, medically actionable, pharmacogenomics and early-onset neurodegenerative disorders categories. This list consisted of 3,773 genes. OMIM genes were included if molecular basis is known and a pathogenic variant has been previously identified in the gene. Annotated variants were filtered using the following parameters: variants with a GnomAD and ExAC frequency of <0.01 were included; ClinVar Pathogenic/Likely Pathogenic variant calls of any frequency were included unless called only by OMIM or GeneReviews; synonymous and splicing variants ± 15bp from the exon boundaries were included unless no splicing effect was predicted by SpliceSiteFinder-like, MaxEntScan, NNSPLICE, and GeneSplicer (variants outside the region of analysis were included only if they were called Pathogenic/Likely Pathogenic in ClinVar); ClinVar Benign/Likely Benign variant calls with ≥ 2 observations from reputable labs (Appendix A[4]) were excluded; ClinVar Benign/Likely Benign variant calls with ≥ 4 observations from any lab were excluded; heterozygous autosomal recessive variants were excluded unless found in compound heterozygous state and variants with frequencies between > 0.001-0.0001 in at least three control populations were removed.

Early onset neurodegenerative disorders were curated from OMIM® phenotypes (February 2020). This list consisted of 54 genes. Genes involving repeat expansion disorders were not analyzed nor reported. Annotated variants were filtered using the following parameters: variants with a GnomAD and ExAC frequency of <0.01 were included; ClinVar Pathogenic/Likely Pathogenic variant calls of any frequency were included unless called only by OMIM or GeneReviews; synonymous and splicing variants ± 15bp from the exon boundaries were included unless no splicing effect was predicted by SpliceSiteFinder-like, MaxEntScan, NNSPLICE, and GeneSplicer (variants outside the region of analysis were included only if they were called Pathogenic/Likely Pathogenic in

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ClinVar); ClinVar Benign/Likely Benign variant calls with ≥ 2 observations from reputable labs (Appendix A[4]) were excluded and ClinVar Benign/Likely Benign variant calls with ≥ 4 observations from any lab were excluded.

Clinical Sequencing Exploratory Research (CSER) consortium was used to generate the carrier status gene list (Himes_2017_PMID: 28079899). This list consisted of 710 genes. Twenty-four of these genes were found to be associated with cancer and therefore, were removed from the carrier status gene list as they would instead be reported in relation to the primary cancer indication, leaving a total of 686 genes. Carrier status variants identified in other genes associated with medically actionable or rare Mendelian disorders were reported if analyzed. Annotated variants were filtered using the following parameters: variants with a GnomAD and ExAC frequency of <0.01 were included; ClinVar Pathogenic/Likely Pathogenic variant calls of any frequency were included unless called only by OMIM or GeneReviews; synonymous and splicing variants ± 15bp from the exon boundaries were included unless no splicing effect was predicted by SpliceSiteFinder-like, MaxEntScan, NNSPLICE, and GeneSplicer (variants outside the region of analysis were included only if they were called Pathogenic/Likely Pathogenic in ClinVar); ClinVar Benign/Likely Benign variant calls with ≥ 2 observations from reputable labs (Appendix A[4]) were excluded; ClinVar Benign/Likely Benign variant calls with ≥ 4 observations from any lab were excluded; homozygous variants were excluded and variants with frequency > 0.001-0.0001 in at least three control populations removed.

Additional data (e.g. quality and coverage parameters; a complete list of all variants identified) is available upon request from the referring physician. DNA variant positions are provided using HGVS nomenclature. Computational programs used to predict the potential impact to the protein were PolyPhen-2, SIFT, AlignGVGD, BLOSUM and MutationTaster. Benign or common sequence variants of unlikely clinical significance are not included on this report but are available upon request. Likely pathogenic and pathogenic variants are included on the report. Variants of uncertain significance are not included on the report unless they occur in genes that match or are related to the clinical phenotype. Note: Common pathological variants at a population frequency of greater than 0.01 percent may not be detected using the filtration strategies listed above.

Limitations:

This test does not sequence all bases in the human exome and not all variants have been identified or interpreted. DNA alteration may be missed due to low coverage in certain regions or due to limitations of the computational pipeline to identify certain types of DNA variations. Repeat expansions, translocations, rearrangements and large copy number events (CNVs) including deletions, insertions or duplications and variants within pseudogene regions are not reliably detected using these methodologies. Sequencing does not detect variants in non-coding regions that could affect gene expression or splicing. This test was not designed to detect somatic variants or somatic mosaicism. The methodology we use to filter variants may not identify variants in genes that have not been associated with a disease. Not all genes have been associated with a disease, some gene variants may be associated with more than one disease and the clinical significance of many gene variants is not well understood. Furthermore, the presence of more than one DNA variant may modify the expression of a disease or increase the severity of a disease such as resulting in an earlier age of onset. Periodic review of the exome data may be recommended at the request of the referring physician, especially when new symptoms arise or with the emergence of new scientific evidence, phenotypes or modes of inheritance. Sequencing data or other annotations performed by the lab are available at the request of the referring physician. The classification of DNA variants may change over time as new information becomes available. The significance of a DNA alteration should always be interpreted in the context of the individual's clinical manifestations. The analytical performance parameters of this test were not validated; thus this test was performed on a research basis and is not meant to be used as a clinical test. DNA variants or alterations outside the region of analysis will not be detected. These test results presuppose that the sample received by the Laboratory for testing, was in fact from this patient, and was not contaminated nor subject to sample mix-up. Results do not include the possibility of sample mix-up or laboratory error. Rare diagnostic errors can result from incorrectly assigned family relationships (e.g. non-paternity) or DNA alterations (e.g. DNA variant under a primer or probe binding site; the presence of pseudogene artifacts).

This test was performed on a research basis and should not be used for clinical decision making. Results should be confirmed clinically by the referring clinic. The study participant has agreed to have this report included in their medical records, however these results are confidential and are not to be shared without the patient's consent.

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FOR RESEARCH PURPOSES ONLY

Referring Clinic: Cancer Genetics Clinic
Physician: Dr. Ganesh Agarwal
Address: 30 Bond St, Toronto ON
Counselor: Sofía Fernández

Patient Name: [insert patient name]
Date of Birth: 1975-Jun-18
Sex: Female
Ethnicity: Italian & Guyanese

Specimen Type: DNA, blood
Accession number: 12498
Date Enrolled: 2020-Sep-09
Date of Report: 2021-Mar-23

Appendix A: List of filtered* variants identified with associated clinical phenotypes of cancer or tumour predisposition
EXOME SEQUENCING RESEARCH STUDY

Gene	Chr	Transcript	Zygosity[1]	Coding Variant	Protein Change	GnomAD Frequency	Disease (Inheritance[2])	Classification (ClinVar)[3]
BRCA2	17	NM_000059	het	c.67+3A>G	.	-	Hereditary Breast and Ovarian cancer (AD); Fanconi anemia, type D1 (AR)	LP (LP)
FAT1	4	NM_005245	het	c.5646A>G	p.P1882P	0.00140641	No disease information; limited evidence for susceptibility to pancreatic cancer	-- (--)
MC1R	16	NM_002386	het	c.252C>A	p.D84E	0.004929	Cutaneous malignant melanoma 5 (AD); UV-induced skin damage (AR)	US (CI)
MSH6	2	NM_000179	het	c.667A>G	p.N223D	0.000008013	Lynch Syndrome (AD); Mismatch repair cancer syndrome (AR); Familial endometrial cancer (AD)	US (US)
POLQ	3	NM_199420	het	c.345T>C	p.A115A	0.00253818	No disease information; limited evidence for susceptibility to breast cancer	-- (--)
PTCH2	1	NM_003738	het	c.2127C>T	p.D709D	0.0133549	Basal cell nevus syndrome (AD)	-- (B)
SAMD9	7	NM_001193307	het	c.257C>T	p.S86F	0.00423665	MIRAGE syndrome (AD); Familial normophosphatemic tumoral calcinosis (AR)	LB (B)
SAMD9L	7	NM_001350082	het	c.1977C>G	p.L659L	-	Ataxia-pancytopenia syndrome (AD)	-- (--)
SMARCA4	19	NM_001128849	het	c.2123+12C>T	.	0.0248741	Rhabdoid tumor predisposition syndrome (AD); Coffin-Siris syndrome (AD)	-- (B)

[2]AD = autosomal dominant; AR = autosomal recessive; XLR = X-linked recessive; XLD = X-linked dominant; MT = Mitochondrial; Di = Digenic; -- = unknown

[3]Variant classification as analyzed by the study team; ClinVar classification is noted in brackets; P = Pathogenic; LP = Likely Pathogenic; US = Uncertain significance; LB = Likely Benign; B = Benign; O = Other; CI = Conflicting Interpretations; -- = not assessed

[4]Reputable Labs: Ambry Genetics, Color Genomics Inc., GeneDx, Invitae, Partners Laboratory for Molecular Medicine, University of Chicago, Mayo Clinic, Prevention Genetics, Department of Pathology and Laboratory Medicine Sinai Health Systems, EGL Genetics, Fulgent Genetics

***Final filters applied:** GnomAD and ExAC frequency <0.05 for cancer genes; Cancer genes acquired from Invitae, GeneDx and Ambry Genetics hereditary cancer panels as well as the Catalogue Of Somatic Mutations In Cancer (COSMIC) v89 database ; ClinVar Pathogenic/Likely Pathogenic calls were included unless called only by OMIM or GeneReviews; only exonic and splicing variants ± 15bp from the exon boundaries were included; ClinVar Benign/Likely Benign calls > 2 from reputable labs[4] were excluded; ClinVar Benign/Likely Benign calls > 4 from any lab were excluded; variants with coverage of less than 10x or with an allelic fraction <25% were excluded.*

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Referring Clinic: Cancer Genetics Clinic
Physician: Dr. Ganesh Agarwal
Address: 30 Bond St, Toronto ON
Counselor: Sofía Fernández

Patient Name: [insert patient name]
Date of Birth: 1975-Jun-18
Sex: Female
Ethnicity: Italian & Guyanese

Specimen Type: DNA, blood
Accession number: 12498
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Appendix B: 154 Cancer Gene List

EXOME SEQUENCING RESEARCH STUDY

ABRAXAS1	*CDC73*	*ENG*	*FH*	*MITF*	*PMS2*	*RFWD3*	*SPOP*	*XPC*
AIP	*CDH1*	*EPCAM*	*FLCN*	*MLH1*	*POLD1*	*RINT1*	*SRP72*	*XRCC2*
AKT1	*CDH10*	*ERCC2*	*GALNT12*	*MLH3*	*POLE*	*RNF43*	*STAT3*	
ALK	*CDK4*	*ERCC3*	*GATA2*	*MPL*	*POLQ*	*RPS20*	*STK11*	
ANKRD26	*CDKN1B*	*ERCC4*	*GEN1*	*MRE11*	*POT1*	*RUNX1*	*SUFU*	
APC	*CDKN1C*	*ERCC5*	*GPC3*	*MSH2*	*PRF1*	*SAMD9*	*TERC*	
APOBEC3B	*CDKN2A*	*ETV6*	*GREM1*	*MSH3*	*PRKAR1A*	*SAMD9L*	*TERT*	
ATM	*CEBPA*	*EXT1*	*HNF1A*	*MSH6*	*PTCH1*	*SBDS*	*TGFBR2*	
ATR	*CEP57*	*EXT2*	*HOXB13*	*MUTYH*	*PTCH2*	*SCG5*	*TMEM127*	
AXIN2	*CHEK2*	*EZH2*	*HRAS*	*NBN*	*PTEN*	*SDHA*	*TP53*	
BAP1	*CTNNA1*	*FANCA*	*KDR*	*NF1*	*PTPN13*	*SDHAF2*	*TP63*	
BARD1	*CTR9*	*FANCC*	*KIF1B*	*NF2*	*RAD50*	*SDHB*	*TSC1*	
BLM	*CYLD*	*FANCD2*	*KIT*	*NTHL1*	*RAD51C*	*SDHC*	*TSC2*	
BMPR1A	*DDB2*	*FANCE*	*LMO1*	*PALB2*	*RAD51D*	*SDHD*	*TSHR*	
BRCA1	*DDX41*	*FANCF*	*LZTR1*	*PALLD*	*RB1*	*SETBP1*	*VHL*	
BRCA2	*DICER1*	*FANCG*	*MAX*	*PDGFRA*	*RECQL*	*SMAD4*	*WAS*	
BRIP1	*DIS3L2*	*FANCM*	*MC1R*	*PHOX2B*	*RECQL4*	*SMARCA4*	*WRN*	
BUB1B	*EGFR*	*FAT1*	*MEN1*	*PIK3CA*	*REST*	*SMARCB1*	*WT1*	
CASR	*EGLN1*	*FEN1*	*MET*	*PMS1*	*RET*	*SMARCE1*	*XPA*	

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Appendix C: Pharmacogenomic Information

Gene	Genotype SNP ID[1]	Metabolizer Phenotype	Associated Medications	Dosing Recommendations
VKOCR1	***1/*2** **rs9923231**	**Intermediate Metabolizer**	***Warfarin* (blood thinner)**	**Warfarin: Based on this individual's *VKORC1* and *CYP2C9* genotypes, a lower warfarin starting dose is recommended.** **Standard dosing is recommended for phenytoin, fosphenytoin, celecoxib, flurbiprofen, ibuprofen, lornoxicam, meloxicam, piroxicam and tenoxicam**
CYP2C9	***1/*2** **rs1799853** rs1057910	**Intermediate Metabolizer**	***Warfarin* (blood thinner)** ***Phenytoin & Fosphenytoin* (anti-seizure medications)** ***Celecoxib, Flurbiprofen, Ibuprofen, Lornoxicam, Meloxicam, Piroxicam & Tenoxicam* (nonsteroidal anti-inflammatory drugs; NSAIDs)**	
CYP2C19	***1/*17** rs4244285 **rs12248560** rs4986893	**Rapid Metabolizer**	***Amitriptyline* (anti-depressant & anti-pain medication)** ***Citalopram & Escitalopram* (anti-depressants)** ***Voriconazole* (anti-fungal medication)** ***Lansoprazole, Omeprazole & Pantoprazole* (proton pump inhibitors; stomach and esophageal medications)** ***Clopidogrel* (blood thinner)**	**Alternative therapy may be recommended for amitriptyline, citalopram, escitalopram and voriconazole due to the potential for therapeutic failure.** **Increased dosing is recommended for lansoprazole, omeprazole and pantoprazole due to the potential for therapeutic failure.** **Standard dosing is recommended for clopidogrel.**
DPYD	*1/*1 rs67376798 rs3918290 rs55886062	Normal Metabolizer	*Capecitabine & Fluorouracil* (chemotherapy medications)	Standard dosing is recommended.
G6PD	B/B rs1050828 rs1050829 rs5030868	Normal Function	*Rasburicase* (chemotherapy adjunct to prevent tumour lysis syndrome)	Standard dosing is recommended.
CYP3A5	*1/*1 rs776746	Normal Metabolizer	*Tacrolimus* (immunosuppressant)	Standard dosing is recommended.
NUDT15	CC rs116855232	Normal Function	*Azathioprine* (immunosuppressant) *Mercaptopurine* (immunosuppressant & cancer medication) *Thioguanine* (cancer medication)	Standard dosing is recommended.
TPMT	*1/*1 rs1142345 rs1800584 rs1800460 rs1800462	Normal Metabolizer		
UGT1A1	*1/*1 rs3064744	Normal Function	*Atazanavir* (antiretroviral medication for HIV/AIDS)	Standard dosing is recommended.
CYP2B6	*1/*1 rs2279343 rs34223104 rs3745274 rs28399499	Normal Metabolizer	*Efavirenz* (antiretroviral medication for HIV/AIDS)	Standard dosing is recommended.
SLCO1B1	TT rs4149056	Normal Function	*Simvastatin* (lipid-lowering medication)	Standard dosing is recommended.

[1]Metabolizer phenotype is only determined based on indicated SNPs. Other rare SNPs were not captured.

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