The HudsonAlpha CLINICAL SERVICES LAB

601 Genome Way, Room 3001, Huntsville, AL 35806

Phone: (256) 327-9670 Fax: (256) 327-9760

www.clinicallab.org

Patient Name	John Doe	**Ordering Physician**	Dr. Gene	**Specimen Type**	Blood
Date of Birth	01/01/1900	**Date of Report**	06/25/2020	**Test Type**	Screening Array
Race/Ethnicity	Caucasian	**Sample Collection Date**	05/18/2020	**Accession #**	ABC123
Gender	Male	**Sample Received Date**	05/20/2020	**Provider Accession ID**	MRN987654
		Test Request Date	05/20/2020		

Clinical Indications

We were requested to perform a screening array on a sample from this individual. Result categories are reported as requested on the Test Requisition Form. Orthogonal confirmation is performed for actionable findings only.

Results Summary of Actionable Findings

The following variant(s) were identified:

Gene & Transcript	Location	Variant	Zygosity	Disease or Phenotype	Inheritance Pattern of Disease	Variant Classification[1]
KCNH2, NM_172056.2	Interior Intron, Canonical 5' Splice Site	chr7:150977836A>C (GRCh38) c.76+2T>G	Heterozygous	Long QT syndrome 2 (OMIM 613688)	Autosomal Dominant	Pathogenic

[1] Based on ACMG guidelines (Richards, 2015)

Interpretation and Recommendation Summary for Actionable Findings

This individual is heterozygous for a potentially clinically significant variant in KCNH2. Pathogenic variants in this gene are associated with long QT syndrome 2. Evaluation for this condition may be appropriate.

These results are for screening purposes and should be interpreted in the context of the individual's medical evaluation, family history, and racial/ethnic background. Genetic counseling is recommended to discuss the implications of this report. Please note that variant classification and/or interpretation, as well as our knowledge of the genome, will change over time as more information becomes available.

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Results Summary for Carrier Findings

The following variant(s) were identified:

Gene & Transcript	Location	Variant	Zygosity	Disease or Phenotype	Inheritance Pattern of Disease	Variant Classification[1]
MUTYH, NM_001048174.1	Interior Intron, Canonical 3' Splice Site	chr1:45331558T>C (GRCh38) c.1103-2A>G	Heterozygous	Adenomas, multiple colorectal (OMIM 608456)	Autosomal Recessive	Pathogenic

[1] Based on ACMG guidelines (Richards, 2015)

Interpretation and Recommendation Summary for Carrier Findings

This individual is heterozygous for a potentially clinically significant variant in MUTYH. Pathogenic variants in this gene are associated with multiple colorectal adenomas. This result suggests that this individual is a carrier for this disease.

These results are for screening purposes and should be interpreted in the context of the individual's medical evaluation, family history, and racial/ethnic background. Genetic counseling is recommended to discuss the implications of this report. Please note that variant classification and/or interpretation, as well as our knowledge of the genome, will change over time as more information becomes available.

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Detailed Description of Actionable Findings

Gene & Transcript	Location	Variant	Zygosity	Disease or Phenotype	Inheritance Pattern of Disease	Variant Classification
KCNH2, NM_172056.2	Interior Intron, Canonical 5' Splice Site	chr7:150977836A>C (GRCh38) c.76+2T>G	Heterozygous	Long QT syndrome 2 (OMIM 613688)	Autosomal Dominant	Pathogenic

Genomic Position	Variant Frequency
chr7:150977836A>C (GRCh38)	5/38438 of chromosomes in gnomAD Genome 0/0 of chromosomes in gnomAD Exome

Variant Interpretation and Disease Information

This individual is heterozygous for a potentially clinically significant variant in KCNH2. Pathogenic variants in this gene are associated with long QT syndrome 2. Evaluation for this condition may be appropriate.

The c.76+2T>G variant in the KCNH2 gene has been previously reported (ClinVar Variation ID 200596). The c.76+2T>G is a canonical splice site variant, which is predicted to result in loss of function in the KCNH2 gene where loss of function is a known mechanism of long QT syndrome 2. The Genome Aggregation Database (gnomAD) reports that the c.76+2T>G variant was observed in 5/38438 alleles and was not observed for genome and exome data, respectively; consistent with the expected frequency of a pathogenic variant in long QT syndrome 2. The c.76+2T>G variant has been reported as pathogenic by a reputable source (ClinVar Variation ID 200596). In summary, this collective evidence supports c.76+2T>G as a pathogenic variant for long QT syndrome 2.

Long QT syndrome 2 arises from the presence of a single pathogenic variant in KCNH2.

Detailed Description of Carrier Findings

Gene & Transcript	Location	Variant	Zygosity	Disease or Phenotype	Inheritance Pattern of Disease	Variant Classification
MUTYH, NM_001048174.1	Interior Intron, Canonical 3' Splice Site	chr1:45331558T>C (GRCh38) c.1103-2A>G	Heterozygous	Adenomas, multiple colorectal (OMIM 608456)	Autosomal Recessive	Pathogenic

Genomic Position	Variant Frequency
chr1:45331558T>C (GRCh38)	1/143302 of chromosomes in gnomAD Genome 6/249628 of chromosomes in gnomAD Exome

Variant Interpretation and Disease Information

This individual is heterozygous for a potentially clinically significant variant in MUTYH. Pathogenic variants in this gene are associated with multiple colorectal adenomas. This result suggests that this individual is a carrier for this disease.

The c.1103-2A>G variant in the MUTYH gene has been previously reported (ClinVar Variation ID 141282; Farrington, 2005; Wang, 2004). The c.1103-2A>G is a canonical splice site variant, which is predicted to result in loss of function in the MUTYH gene where loss of function is a known mechanism of multiple colorectal adenomas. The c.1103-2A>G variant has been shown to damage the gene or gene-product based on a well-established functional assay, cDNA analysis (Farrington, 2005). The Genome Aggregation Database (gnomAD) reports that the c.1103-2A>G variant was observed in 1/143302 and 6/249628 alleles for genome and exome data, respectively; consistent with the expected frequency of a pathogenic variant in multiple colorectal adenomas. The c.1103-2A>G variant has been observed to be in trans with a pathogenic variant based on segregation analysis in individuals with multiple colorectal adenomas (Farrington, 2005; Wang, 2004). The c.1103-2A>G variant has been reported as pathogenic by a reputable source (ClinVar Variation ID 141282). In summary, this collective evidence supports c.1103-2A>G as a pathogenic variant for multiple colorectal adenomas.

Multiple colorectal adenomas arises from the presence of a pathogenic variant in each copy of MUTYH. This analysis detected only a single variant of potential clinical relevance.

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References

Farrington SM, Tenesa A, Barnetson R, et al. Germline susceptibility to colorectal cancer due to base-excision repair gene defects. Am J Hum Genet. 2005;77(1):112-9.

Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17(5):405-24.

Wang L, Baudhuin LM, Boardman LA, et al. MYH mutations in patients with attenuated and classic polyposis and with young-onset colorectal cancer without polyps. Gastroenterology. 2004;127(1):9-16.

https://www.ncbi.nlm.nih.gov/clinvar/variation/141282

https://www.ncbi.nlm.nih.gov/clinvar/variation/200596

Test Methodology and Limitations

This screening test was performed by the HudsonAlpha Clinical Services Lab, LLC (“CSL”), an Alabama limited liability company (CLIA: #01D2086581; CAP: #8051488; Lab Director: Ghunwa Nakouzi, PhD), on the provided sample. DNA was checked for concentration and quality and tested using the Illumina Global Diversity Array with Enhanced PGx. Genotypes were called using Illumina’s AutoCall software, and Variant Call Format (VCF) files were generated with the Array Analysis CLI tool using reference genome GRCh38. Quality metrics were evaluated at each stage of sample preparation and analysis to ensure quality data

Sequence variants were loaded into a custom software analysis application called Codicem for interpretation. Within Codicem all sequence variants were annotated with relevant reference information from established data sources to provide support for the interpretations set forth in this test report. Population allele frequencies were derived from data collected by the gnomAD consortium (Karczewski, 2020). A listing of data sources is available upon request. All actionable variants were confirmed by an orthogonal technology (Sanger (dideoxy) sequencing).

The ability of this test to identify abnormal variants (the analytical sensitivity) is dependent on the presence of the variants in the sequencing data provided to Codicem for evaluation. Analytical sensitivity and specificity for the Illumina Global Screening Array is >95%. Only targeted variants were assessed by the array; other variants and/or genes may exist that are not detected by this test which may or may not be clinically relevant. This assay is not intended to detect mosaicism and is for screening purposes only.

GDA Pipeline Version: VCFv4.1/array-analysis-cli1.0.1

Data Store Version: 0.51.1

Codicem Version (Annotation): 5.3.3

Codicem Version (Report): 5.3.2

Interpretation Limitations

The interpretations set forth in this report assume that all information provided to the CSL, including any family relationships and all information stated on the sample submission or test requisition form, are accurate and fully answered. Because of the limitation of today’s technology and scientific knowledge, a genetic abnormality may still exist even if a variant is not included in this report. If specific clinical disorders are suspected, specific evaluation of

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known genes by alternate test methods should be considered. False positive, negative or misleading results may occur for reasons that include genetic variants, blood transfusions, bone marrow transplantation, erroneous representation of family relationship, or contamination of a specimen. However, the CSL has standard and effective procedures in place to protect against such technical and operational problems.

The interpretations set forth in this report are based only upon current scientific knowledge and technology. Each variant is interpreted independently of all other variants. This test attempts to use current scientific knowledge to identify possible genetic variants; however, current scientific knowledge about the function of variants, genes, and other portions of the genome, and the ways in which genetic disorders are inherited, is incomplete. For example, variants in different genes may sometimes interact to cause disease, and variants may modify the phenotype of a monogenic disease. It cannot be excluded that pathogenic variants were missed due to limitations inherent in the analysis method used in this test.

These results should be interpreted in the context of an individual's medical evaluation, family history, and racial/ethnic background. The data, interpretations and results of this test are not intended to recommend or discourage any specific treatment plan, product or course of action in an individual's medical care.

The identification of some variants, including those associated with disease, is limited by the current state of knowledge in the genomics field and the annotations of variants in currently available public and private databases. Variants found in the individual that are benign, likely benign, or of uncertain significance, as identified in the medical literature based on ACMG criteria, are not reported. Please note that variant classification and/or interpretation may change over time if and when more information becomes available. Therefore, it is possible that re-interpretation of these test results after any scientific advancement could lead to new information about a medical condition or clinical disorder an individual may be experiencing. Such re-interpretation must be requested by an individual's health care provider, and will involve additional costs.

This test was developed, and the associated orthologous methods and performance characteristics were determined by, the CSL. It has not been cleared or approved by the U.S. Food and Drug Administration. To date, the U.S. Food and Drug Administration has determined that such clearance or approval is not necessary. These tests are used for clinical purposes, and therefore validation was done as required under the requirements of the Clinical Laboratory Improvement Act of 1988. These test results should not be regarded as investigational or for research. The chance of a false negative or a false positive result due to laboratory error cannot be completely excluded.

CLIA #01D2086581

CAP #8051488

Signatures

Elaine Lyon, PhD, FACMG, Director, Clinical Services Laboratory — Electronically signed by Elaine Lyon on 06/25/2020