Ambry Genetics
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REVISED REPORT - 08/27/2018

Ordered By Contact ID:1261158 Org ID:3001	Patient Name: **Sinnott, Andrew**
Physician: Kennelly, Rory, MD	Accession #: **18-097488** Specimen #: BX671155S
Ph:353-1-221-4510 Fx:353-1-221-3198	AP2 Order #: 579881 Specimen: Blood EDTA (Purple top)
Client: St. Vincent's University Hospital (05211) Suite 3 Clinical Research Centre Elm Park County Dublin 4 IE	Birthdate: 12/01/1967 Age: 50y 8m
	Gender: M
	MRN #: N/A Collected: N/A
	Indication: Diagnostic Received: 07/05/2018
	Ethnicity: Caucasian

Additional Authorized Recipient:

Murphy, Donal N/A Ph:353-1-221-4510 Fx:353-1-221-3198
Winter, Des MD Ph:01135312213330 Fx:353-1-221-3198
Frayling, Ian MD, FRCPC Ph:(0)29 2074 4203

APC and MUTYH Analyses with CustomNext-Cancer

REVISION

This report was revised to correct the specimen number from being blank to BX671155S. No changes were made to any test results. This report supersedes all previous reports.

RESULTS

MUTYH	Variant, Unknown Significance:	p.R495H
NTHL1	Variant, Unknown Significance:	p.R21W

SUMMARY

Variants of Unknown Significance Detected

INTERPRETATION

- **No known clinically actionable alterations were detected.**
- Two variants of unknown significance were detected: one in the *MUTYH* gene and one in the *NTHL1* gene.
- **Risk Estimate:** should be based on clinical and family history, as the clinical significance of this result is unknown.
- Genetic testing for variants of unknown significance (VUSs) in family members may be pursued to help clarify VUS significance, but cannot be used to identify at-risk individuals at this time.
- Genetic counseling is a recommended option for all individuals undergoing genetic testing.

This individual is heterozygous for the p.R495H (c.1484G>A) variant of unknown significance in the *MUTYH* gene and heterozygous for the p.R21W (c.61C>T) variant of unknown significance in the *NTHL1* gene, which may or may not contribute to this individual's clinical history. Refer to the supplementary pages for additional information on these variants. No additional pathogenic mutations, variants of unknown significance, or gross deletions or duplications were detected. Genes Analyzed (3 total): ***APC, MUTYH* and *NTHL1* (sequencing and deletion/duplication).**

Order Summary: The following products were included in the test order for this individual. Please note: tests on hold and those that have been cancelled (including reflex testing steps cancelled due to a positive result in a preceding test) are excluded. For additional information, please contact Ambry Genetics.

- APC & MUTYH seq and del/dup (Product Code 8726)
- CustomNext: Cancer (Product Code 9510)

ELECTRONICALLY SIGNED BY

Michael Jarvis, Ph.D., DABMG, CGMB, on 08/27/2018 at 14:41:34 pm

ASSAY INFORMATION

General Information: Cancer is a complex, multifactorial disease in which cells become abnormal and multiply without control to form malignant tumors. It is estimated that approximately 1 out of every 2 men and 1 out of every 3 women will be diagnosed with cancer over the course of a lifetime. Hereditary cancers due to mutations in cancer predisposition genes appear to be responsible for between 5-10% of cancer diagnoses.

Methodology: The **CustomNext-*Cancer*** test is a customizable screen of up to 68 genes associated with hereditary cancer predisposition. Genomic deoxyribonucleic acid (gDNA) is isolated from the patient's specimen using standardized methodology and quantified. Sequence enrichment of the targeted coding exons and adjacent intronic nucleotides is carried out by a bait-capture methodology using long biotinylated oligonucleotide probes followed by polymerase chain reaction (PCR) and Next-Generation sequencing. Additional Sanger sequencing is performed for any regions missing or with insufficient read depth coverage for reliable heterozygous variant detection. Reportable small insertions and deletions, potentially homozygous variants, variants in regions complicated by pseudogene interference, and single nucleotide variant calls not satisfying 100x depth of coverage and 40% het ratio thresholds are verified by Sanger sequencing (Mu W et al. *J Mol Diagn*. 2016 Oct 4. PubMed PMID: 27720647). For *BRCA2* and *MSH2*, the Portuguese founder mutation, c.156_157insAlu (also known as 384insAlu), and the coding exons 1-7 inversion, respectively, are detected by next generation sequencing and confirmed by multiplex ligation-dependent probe amplification (MLPA) or PCR and agarose gel electrophoresis. Gross deletion/duplication analysis for all ordered genes (excluding *MITF* and *PMS2*) is performed using a custom pipeline based on read-depth from NGS data and/or targeted chromosomal microarray with confirmatory MLPA when applicable. For *PMS2*, gross deletion/duplication analysis is performed using MLPA kit P008-B1. If a deletion is detected in exons 13, 14, or 15 of *PMS2*, double stranded sequencing of the appropriate exon(s) of the pseudogene *PMS2CL* will be performed to determine if the deletion is located in the *PMS2* gene or pseudogene. NCBI reference sequences are as follows: *AIP*- NM_003977.2, *ALK*- NM_004304.4, *APC*- NM_000038.5 & NM_001127511.2, *ATM*- NM_000051.3, *BAP1*- NM_004656.2, *BARD1*- NM_000465.2, *BLM*- NM_000057.2, *BMPR1A*- NM_004329.2, *BRCA1*- NM_007294.3, *BRCA2*- NM_000059.3, *BRIP1*- NM_032043.2, *CDH1*- NM_004360.3, *CDK4*- NM_000075.3, *CDKN1B*- NM_004064, *CDKN2A*- NM_000077.4 and NM_058195.3 (p14ARF), *CHEK2*- NM_007194.3, *DICER1*- NM_177438.2, *FANCC*- NM_000136.2, *FH*- NM_000143.3, *FLCN*- NM_144997.5, *GALNT12*- NM_024642.4, *HOXB13*- NM_006361.5, *MAX*- NM_002382.3, *MEN1*- NM_130799.2, *MET*- NM_000245.1, *MITF*- NM_000248.3, *MUTYH*- NM_001128425.1, *MRE11A*- NM_005591.3, *MLH1*- NM_000249.3, *MSH2*- NM_000251.1, *MSH6*- NM_000179.2, *NBN*- NM_002485.4, *NF1*- NM_000267.3, *NF2*- NM_000268.3, *NTHL1*- NM_002528.5, *PALB2*- NM_024675.3, *PHOX2B*- NM_003924.3, *PMS2*- NM_000535.5, *POLD1*- NM_002691.2, *POLE*- NM_006231.2, *POT1*- NM_015450.2, *PRKAR1A*- NM_002734.3, *PTCH1*- NM_000264.3, *PTEN*- NM_000314.4, *RAD50*- NM_005732.3, *RAD51C*- NM_058216.1, *RAD51D*- NM_002878.3, *RB1*- NM_000321.2, *RET*- NM_020975.4, *SDHA*- NM_004168.2, *SDHAF2*- NM_017841.2, *SDHB*- NM_003000.2, *SDHC*- NM_003001.3, *SDHD*- NM_003002.2, *SMAD4*- NM_005359.5, *SMARCA4*- NM_001128849.1, *SMARCB1*- NM_003073.3, *SMARCE1*- NM_003079.4, *STK11*- NM_000455.4, *SUFU*- NM_016169.3, *TMEM127*- NM_017849.3, *TP53*- NM_000546.4, *TSC1*- NM_000368.4, *TSC2*- NM_000548.3, *VHL*- NM_000551.3, *XRCC2*- NM_005431.1.

Analytical Range: The **CustomNext-*Cancer*** test targets detection of DNA sequence mutations in up to 68 genes by either Next-Generation or Sanger sequencing of all coding domains and well into the flanking 5' and 3' ends of all the introns and untranslated regions. In addition, sequencing of the promoter region is performed for the following genes if ordered: *PTEN* (c.-1300 to c.-745), *MLH1* (c.-337 to c.-194), and *MSH2* (c.-318 to c.-65). For *MITF*, only the status of the c.952G>A (p.E318K) alteration is analyzed and reported. For *POLD1* and *POLE*, missense variants located outside of the exonuclease domains (codons 311-541 and 269-485, respectively) are not routinely reported. For *ALK*, only variants located within the kinase domain (c.3286-c.4149) are reported. For *PHOX2B*, the polyalanine repeat region is excluded from analysis. Gross deletion/duplication analysis determines gene copy number for the covered exons and untranslated regions of all ordered genes, excluding *MITF*. For *GREM1*, only the status of the 40kb 5'UTR gross duplication is analyzed and reported. For *EPCAM*, only gross deletions encompassing the 3' end of the gene are reported. For *NTHL1*, only full-gene gross deletions and duplications are detected. For *APC*, all promoter 1B gross deletions as well as single nucleotide substitutions within the promoter 1B YY1 binding motif (NM_001127511 c.-196_-186) are analyzed and reported.

Result Reports: In result reports, alterations in the following classifications are always reported:

- **Pathogenic Mutation**: alterations with sufficient evidence to classify as pathogenic (capable of causing disease). Targeted testing of at-risk relatives and appropriate changes in medical management for pathogenic mutation carriers recommended. Previously described pathogenic mutations, including intronic mutations at any position, are always reported when detected.
- **Variant, Likely Pathogenic (VLP)**: alterations with strong evidence in favor of pathogenicity. Targeted testing of at-risk relatives and appropriate changes in medical management for VLP carriers typically recommended. Previously described likely pathogenic variants, including intronic VLPs at any position, are always reported when detected.
- **Variant, Unknown Significance (VUS)**: alterations with limited and/or conflicting evidence regarding pathogenicity. Familial testing via the Family Studies Program recommended. Medical management to be based personal/family clinical histories, not VUS carrier status. Note, intronic VUSs are always reported out to 5 basepairs from the splice junction when detected.

Alterations of unlikely clinical significance (those classified as "likely benign" and "benign") are not routinely included on results reports.

Assay Information Continued on Next Page

ASSAY INFORMATION *(Supplement to Test Results - Continued)*

Resources: The following references are used in variant analysis and classification when applicable for observed genetic alterations.

1. The 1000 Genomes Project Consortium. An integrated map of genetic variation from 1092 human genomes. *Nature*. 2012;491:56-65.
2. ACMG Standards and guidelines for the interpretation of sequence variants. *Genet Med.* 2015 May;17(5):405-23.
3. Ambry Genetics Variant Classification Scheme. http://www.ambrygen.com/variant-classification.
4. Berkeley Drosophila Genome Project [Internet]. Reese MG et al. *J Comp Biol.* 1997;4:311-23. http://www.fruitfly.org/seq_tools/splice.html.
5. Database of Single Nucleotide Polymorphisms (dbSNP) [Internet]. Bethesda (MD): National Center for Biotechnology Information, National Library of Medicine (dbSNP Build ID:135) Available from: www.ncbi.nlm.nih.gov/SNP. Accessed Jan 2012).
6. ESEfinder [Internet]. Smith PJ, et al. (2006) *Hum Mol Genet.* 15(16):2490-2508 and Cartegni L, et al. *Nucleic Acid Research.* 2003;31(13):3568-3571. http://rulai.cshl.edu/cgi-bin/tools/ESE3/esefinder.cgi?process=home.
7. Exome Variant Server, NHLBI Exome Sequencing Project (ESP) [Internet], Seattle WA. Available from: evs.gs.washington.edu/EVS.
8. Grantham R. Amino acid difference formula to help explain protein evolution. *Science.* 1974;185(4151):862-864.
9. HGMD® [Internet]: Stenson PD et al. *Genome Med.* 2009;1(1):13. www.hgmd.cf.ac.uk.
10. Landrum MJ et al. ClinVar: public archive of relationships among sequence variation and human phenotype. *Nucleic Acids Res*. 2014 Jan 1;42(1):D980-5. doi: 10.1093/nar/gkt1113. PubMed PMID: 24234437.
11. Online Mendelian Inheritance in Man, OMIM®. McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University (Baltimore, MD), Copyright® 1966-2012. World Wide Web URL: http://omim.org.
12. PolyPhen [Internet]: Adzhubei IA, et al. *Nat Methods.* 2010;7(4):248-249. genetics.bwh.harvard.edu/pph2.
13. SIFT [Internet]: Ng PC & Henikoff S. *Hum Genet.* 2006;7:61-80. http://sift.jcvi.org.
14. Exome Aggregation Consortium (ExAC) [Internet], Cambridge, MA. Available from: http://exac.broadinstitute.org.
15. Genome Aggregation Database (gnomAD) [Internet], Cambridge, MA. Available from: http://gnomad.broadinstitute.org.
16. Lek M et al. Analysis of protein-coding genetic variation in 60,706 humans. *Nature*. 2016 Aug 17;536(7616):285-91. PMID: 27535533

Disclaimer: This test was developed and its performance characteristics were determined by Ambry Genetics Corporation. It has not been cleared or approved by the US Food and Drug Administration. The FDA does not require this test to go through premarket FDA review. It should not be regarded as investigational or for research. This test should be interpreted in context with other clinical findings. This report does not represent medical advice. Any questions, suggestions, or concerns regarding interpretation of results should be forwarded to a genetic counselor, medical geneticist, or physician skilled in interpretation of the relevant medical literature. This laboratory is certified under the Clinical Laboratory Improvement Amendments (CLIA) as qualified to perform high complexity clinical laboratory testing. The **CustomNext-*Cancer*** test analyzes the following types of mutations: nucleotide substitutions, small deletions (up to 25 bp), small insertions (up to 10 bp), small indels and gross deletions/duplications. Unless otherwise noted in the methodology section above, it is not intended to analyze the following types of alterations: gross rearrangements, deep intronic variations, Alu element insertions, and other unknown abnormalities. The pattern of mutation types varies with the gene tested and this test detects a high but variable percentage of known and unknown mutants of the classes stated. A negative result from the analysis cannot rule out the possibility that the tested individual carries a rare unexamined mutation or mutation in the undetectable group. The **CustomNext-*Cancer*** test is designed and validated to be capable of detecting ~99% of described mutations in the 68 orderable genes on the test (analytical sensitivity). The clinical sensitivity of the **CustomNext-*Cancer*** test may vary widely according to the specific clinical and family history. Cancer is a complex clinical disorder. Mutations in other genes or the regions not analyzed by the **CustomNext-*Cancer*** test can also give rise to similar clinical conditions. Although molecular tests are highly accurate, rare diagnostic errors may occur. Possible diagnostic errors include sample mix-up, erroneous paternity identification, technical errors, clerical errors, and genotyping errors. Genotyping errors can result from trace contamination of PCR reactions, from maternal cell contamination in fetal samples, from rare genetic variants that interfere with analysis, low-level mosaicism, presence of pseudogenes, technical difficulties in regions with high GC content or homopolymer tracts, presence of pre-malignant or malignant cells in the sample, or from other sources. Rare variants present in the human genome reference sequence (GRCh37.p5/hg19) or rare misalignment due to presence of pseudogenes can lead to misinterpretation of patient sequence data.

MUTYH NM_001128425 c.1484G>A p.R495H

VARIANT DETAILS:

The **p.R495H** variant (also known as c.1484G>A), located in coding exon 15 of the *MUTYH* gene, results from a G to A substitution at nucleotide position 1484. The arginine at codon 495 is replaced by histidine, an amino acid with highly similar properties. In one functional study, this variant demonstrated DNA glycosylase activity similar to that of wild type MUTYH in a DNA cleavage activity assay and a supF forward mutation assay (Shinmura K et al. *Hum. Mutat.* 2016 Apr;37:350-3). This amino acid position is highly conserved in available vertebrate species. In addition, this alteration is predicted to be benign and deleterious by PolyPhen and SIFT *in silico* analyses, respectively. Since supporting evidence is limited at this time, the clinical significance of this alteration remains unclear.

GENE INFORMATION:

The *MUTYH* gene (NM_001128425.1), which is classically associated with an autosomal recessive form of hereditary polyposis, is involved in the base excision repair pathway and cellular response to oxidative DNA damage. Two common mutations, p.Y179C and p.G396D (originally designated as p.Y165C and p.G382D), account for the majority of pathogenic *MUTYH* alterations reported to date. Current data regarding cancer risks for monoallelic mutation carriers is limited and conflicting; however, recent studies have estimated that carriers of some *MUTYH* mutations have as high as a 1.5 fold increased risk of female breast cancer (specific to the North African Jewish population) and a 2 fold increase in colorectal cancer risk compared to the general population (Jones N et al. *Gastroenterology.* 2009 Aug;137(2):489-94; Win AK et al. *Int J Cancer.* 2011 Nov 1;129(9):2256-62; Rennert G et al. *Cancer.* 2012 Apr 15;118(8):1989-93). Breast cancer risk estimates for male *MUTYH* mutation carriers are not currently available. Biallelic mutations in the *MUTYH* gene are known to cause *MUTYH*-associated polyposis (MAP), an autosomal recessive condition predisposing to gastrointestinal polyposis and colorectal cancer. Parents who each carry a *MUTYH* mutation have a 25% chance for a child with MAP in every pregnancy. These risks should be discussed with *MUTYH* mutation carriers of reproductive age.

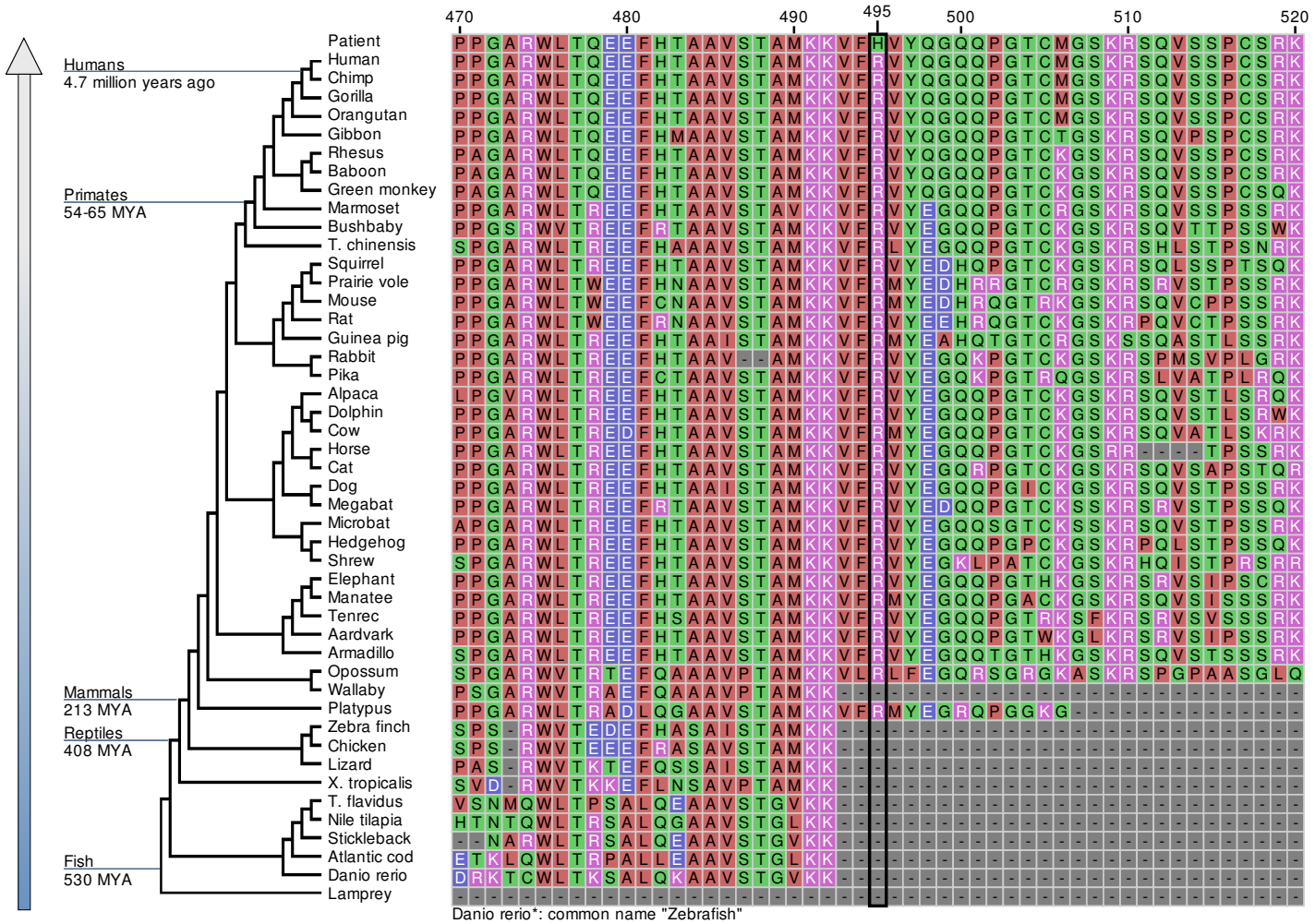
ADDITIONAL SUPPORTING INFORMATION:

Co-Segregation	Co-segregation data for this variant is currently unavailable.
Co-occurrence	No significant co-occurrence data is currently available at our laboratory.
Frequency	Internal Frequency: <0.01% (32/382000) total alleles studied.
	ESP: 0.02% (2/13006) total alleles studied., 0.02% (2/8600) European American alleles
	ExAC: <0.01% (4/72768) total alleles studied (TCGA excluded).
Grantham Score	29 (highly similar amino acid substitution)
Polyphen	Benign (0.018)
SIFT	Deleterious (0.050)

MUTYH NM_001128425 c.1484G>A p.R495H

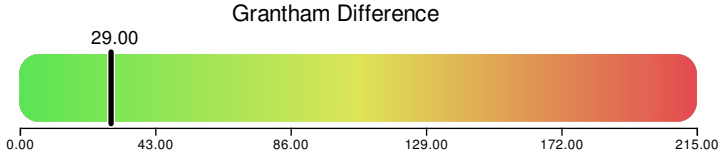
Evolutionary conservation diagram: Amino Acid Alignment

This amino acid position is highly conserved in available vertebrate species.



Amino Acid Change:

Trait	Arg(R)	His(H)
Amino Acid Name	Arginine	Histidine
Polarity/Charge	positively charged	positively charged
pH	basic	basic
Residue Weight	156	137
Hydrophobicity Score	-4.5	-3.2
Hydrophilicity Score	3	-0.5
Secondary Structure Propensity	α indifferent / β indifferent	weak α former / β former



NTHL1 NM_002528 c.61C>T p.R21W

VARIANT DETAILS:

The **p.R21W** variant (also known as c.61C>T), located in coding exon 1 of the *NTHL1* gene, results from a C to T substitution at nucleotide position 61. The arginine at codon 21 is replaced by tryptophan, an amino acid with dissimilar properties. This amino acid position is not well conserved in available vertebrate species. In addition, this alteration is predicted to be benign and deleterious by PolyPhen and SIFT *in silico* analyses, respectively. Since supporting evidence is limited at this time, the clinical significance of this alteration remains unclear.

FAMILY STUDIES PROGRAM:

Ambry Genetics offers complimentary genetic studies for variants of unknown significance (VUSs) meeting specific criteria in appropriate family members. Review of clinical information is required. Additional information, application instructions and required forms, and patient education materials are available at http://ambrygen.com/family-studies-program. For additional information, please email us at GeneticCounselor@ambrygen.com or call 949-900-5500 and ask to speak with a genetic counselor.

Please note that the classification of variants may change over time as additional information becomes available. Alerts are disseminated via fax and/or AmbryPort email to clinicians upon clinically relevant variant reclassifications. If no updates are received, clinicians are encouraged to contact the laboratory at 949-900-5500 once a year to review the status of previously reported variants.

GENE INFORMATION:

The nth-like DNA glycosylase 1 (*NTHL1*, OMIM: *602656, NM_002528.5) gene is located on chromosome 16p13.3 and encodes a 311 amino acid DNA N-glycosylase protein of the endonuclease III family that is involved in the process of DNA base-excision repair (BER) pathway. BER is the primary repair pathway in response to oxidative damage and is crucial for maintaining genomic integrity (Ocampo MT et al. *Mol Cell Biol.* 2002;(17):6111-21; Weren RD et al. *Nat Genet.* 2015; 47(6): 668-71). Biallelic pathogenic mutations in *NTHL1* are associated with increased risk for adenomatous polyposis and colorectal cancer; however, exact risk estimates have not been determined (Belhadj S et al. *Clin Gastroenterol Hepatol.* 2017; 15(3):461:462; Rivera B et al. *N Engl J Med.* 2015; 373(20):1985-6; Weren RD et al. *Nat Genet.* 2015; 47(6): 668-71). Association between *NTHL1* biallelic mutations and elevated incidence of extra-intestinal tumors including skin, breast and endometrial cancer has been suggested, although data is currently limited. Risk estimates for monoallelic *NTHL1* carriers are not currently available.

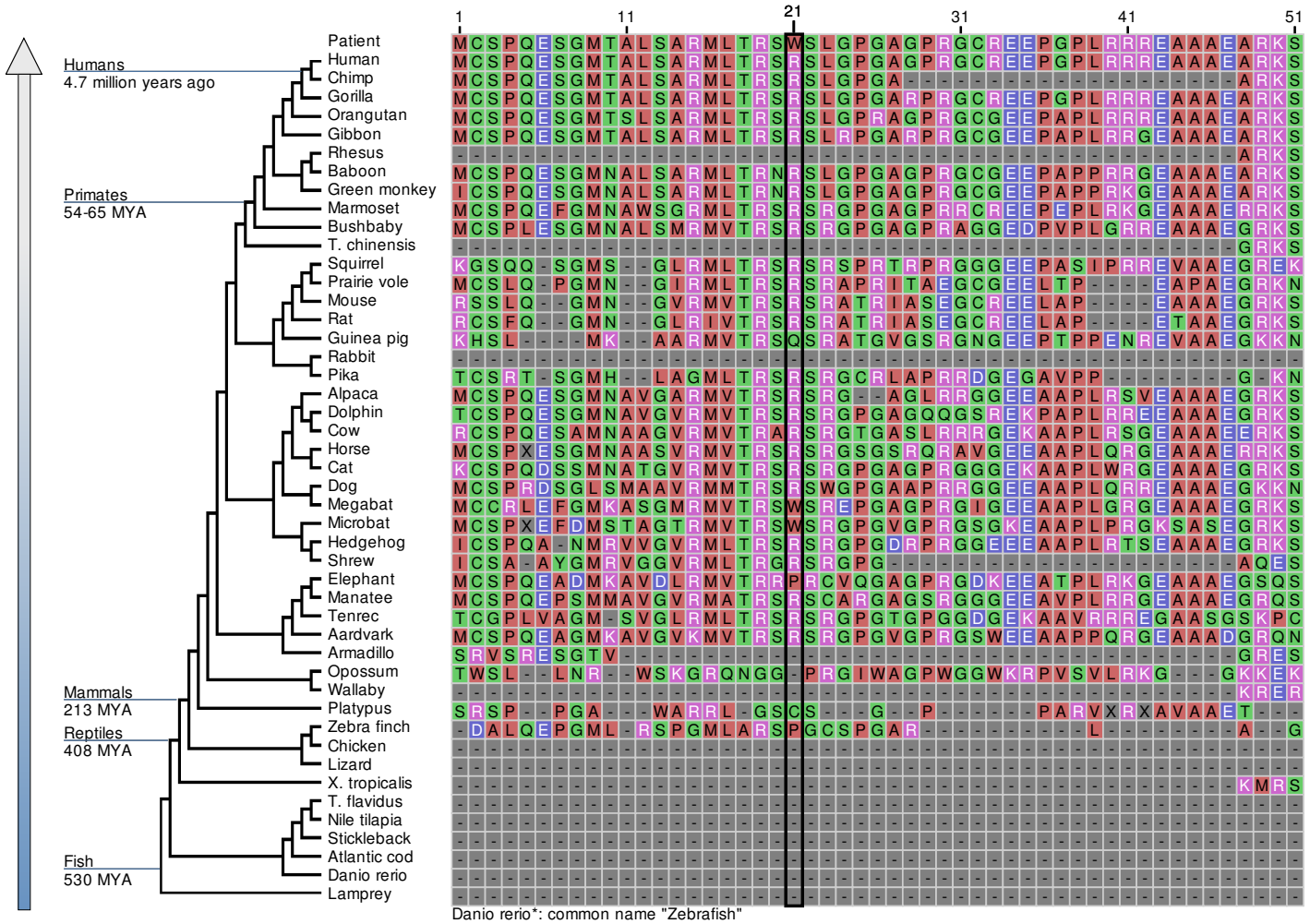
ADDITIONAL SUPPORTING INFORMATION:

Co-Segregation	Co-segregation data for this variant is currently unavailable.
Co-occurrence	No significant co-occurrence data is currently available at our laboratory.
Frequency	ExAC:<0.01% (3/43106) total alleles studied (TCGA excluded).
Grantham Score	101 (dissimilar amino acid substitution)
Polyphen	Benign (0.001)
SIFT	Deleterious (0.010)

NTHL1 NM_002528 c.61C>T p.R21W

Evolutionary conservation diagram: Amino Acid Alignment

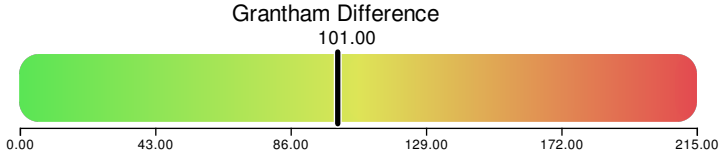
This amino acid position is not well conserved in available vertebrate species.



Danio rerio*: common name "Zebrafish"

Amino Acid Change:

Trait	Arg(R)	Trp(W)
Amino Acid Name	Arginine	Tryptophan
Polarity/Charge	positively charged	non-polar
pH	basic	neutral
Residue Weight	156	186
Hydrophobicity Score	-4.5	-0.9
Hydrophilicity Score	3	-3.4
Secondary Structure Propensity	α indifferent / β indifferent	α former / β former



Ambry Genetics®

Understanding Your VUS Hereditary Cancer Genetic Test Result

INFORMATION FOR PATIENTS WITH A **VARIANT OF UNKNOWN SIGNIFICANCE**

Result	**VUS**	Your testing found at least one variant of unknown significance (VUS) in a gene tested. A VUS is a change in a gene from what we expect to see, but we do not know if it causes an increased risk for cancer or not.
Reclassification	**POSSIBLE**	Collecting information about a VUS is an ongoing process, so it is possible that your result may be better understood in the future. The healthcare provider that ordered your test will be notified if new information becomes available about your VUS.
Cancer Risk	**VARIES**	Even though your genetic test result was a VUS, you and your relatives may still have an increased risk of developing cancer based on other factors, including your medical and/or family history. Your healthcare provider can help you learn more about this.
Risk Management	**VARIES**	Risk management decisions are very personal and depend on many factors. Talk to your doctor about which, if any, options may be right for you.
Family Members	**POSSIBLE FURTHER TESTING**	Certain family members may be eligible for genetic testing through our Family Studies Program. In some cases, this may help add to the understanding of your result. If you and your relatives are interested in this, please speak to your healthcare provider about it.
Next Steps	**DISCUSS**	It is recommended that you stay in contact with your healthcare provider on a regular basis for possible new information about your result.
Reach Out	**RESOURCES**	• Ambry's Hereditary Cancer Site for Families patients.ambrygen.com/cancer • American Cancer Society cancer.org • Genetic Information Nondiscrimination Act (GINA) ginahelp.org • National Society of Genetic Counselors nsgc.org • Canadian Association of Genetic Counsellors cagc-accg.ca

Please discuss this information with your healthcare provider. The cancer genetics field is continuously evolving, so updates related to your genetic test result, medical recommendations, genetic testing options, and/or potential treatments may be available over time. This information is not meant to replace a discussion with a healthcare provider, and should not be considered or interpreted as medical advice.

Ambry Genetics®

Understanding Your VUS Hereditary Cancer Genetic Test Result

INFORMATION FOR PATIENTS WITH A **VARIANT OF UNKNOWN SIGNIFICANCE**

PATHOGENIC MUTATION (POSITIVE TEST RESULT)	Contains enough evidence showing it can cause a disease
VARIANT, LIKELY PATHOGENIC (VLP, POSITIVE TEST RESULT)	Strong evidence to suggest it causes a disease
VARIANT UNKNOWN SIGNIFICANCE (VUS)	Limited and/or conflicting evidence to suggest it may cause a disease
VARIANT, LIKELY BENIGN (VLB, NEGATIVE TEST RESULT)	Strong evidence to suggest it does not cause a disease
BENIGN (NEGATIVE TEST RESULT)	Contains enough evidence to show it does not cause a disease

1. **Does finding a VUS on genetic testing change medical management recommendations?**

   VUS by definition have not been proven to increase an individual's risk for disease or to be the cause of the disease within a family. Medical recommendations should be based on personal and/or family history of a specific disease.

2. **What percentage of VUS are reclassified?**

   Of the VUS that are reclassified, the vast majority will be reclassified to VLB or benign, although many VUS will not be reclassified at all due to lack of additional information. Only a small percentage of VUS will ultimately be reclassified to VLP or pathogenic.

3. **How long does it take to reclassify a VUS?**

   This depends upon several factors:
   - How often the VUS is found in individuals (rare variants may take longer to reclassify)
   - How common the disease is in the general population and how strongly the gene has been linked to the disease
   - Participation of certain families with the VUS in our Family Studies Program
   - Eligibility for additional specialized testing performed by Ambry's Translational Genomics (ATG) laboratory
   - Amount of active research taking place on a particular gene or VUS

4. **Who is notified if a VUS gets reclassified?**

   When enough evidence becomes available to cause a significant change, Ambry will make every attempt to send reclassification alerts for a VUS that gets reclassified to the healthcare provider.

5. **What is Ambry's Family Studies Program, and is it worth participating in it?**

   Our Family Studies Program and ATG lab include follow-up testing for you or certain family members after a VUS has been found. These studies can be worthwhile if many family members (especially those with the disease) are willing to participate. For more information, please visit our website for the Family Studies Program or ATG lab.

6. **Does Ambry perform family studies for VUS in all genes?**

   Not all genes are well suited for family studies. To find out if the VUS found is eligible for family studies contact FamilyStudies@ambrygen.com

7. **How often does Ambry check to see if there is new information about a VUS?**

   Ambry regularly assesses the data and emerging evidence related to a specific variant. Healthcare providers are welcome to contact Ambry Genetics at +1.866.262.7943 on a yearly basis to request the most current assessment of a particular variant.

7 Argonaut, Aliso Viejo, CA 92656 USA Toll Free +1 866.262.7943 Fax +1 949.900.5501 ambrygen.com

Opportunity to Enroll in Hereditary Cancer Research

Genetic testing can help individuals and families by giving them a clearer idea of their cancer risks. Genetic tests (called multi-gene or multiplex panels) look for changes in several different genes, all in a single test. While all of the genes on these panels have been tied to an increased risk of cancer, we understand the risks associated with some of the genes better than we understand others. One way to help improve our understanding is to enroll people with pathogenic mutations or variants of unknown significance in registries. Registries typically follow people over many years to learn more about these alterations and how they impact their health.

How can I find a research registry?

There are several hereditary cancer research registries that are studying individuals who have had multi-gene panel testing. One registry that is open to individuals nationwide is PROMPT (or Prospective Registry Of MultiPlex Testing). PROMPT is an online registry for patients and families who have had multi-gene testing and have been found to have a genetic variation which may be linked to an increased risk of cancer. PROMPT is a joint effort involving several academic medical centers and commercial laboratories, working together to learn more about the genes that are studied on multi-gene panels. PROMPT will allow researchers to better understand the cancer risks associated with changes in these genes and thus provide a better understanding of the best way to take care of individuals who have such changes.

What is involved in participation?

Participation in the study simply involves completing online surveys. You can take part without providing any personal information to the PROMPT study. But, if you are interested, the PROMPT team will reach out to you to talk about ways that you can get more involved with the research effort. Either way, your participation will help researchers learn more and improve the ability of this genetic testing to help people.

How do I enroll?

You can learn more about or register for PROMPT by going to www.promptstudy.org or by scanning the QR code to the right.

Thank you again for considering taking part in PROMPT!

If you would like to read more about multi-gene panels, including details about specific genes, please visit our informational website at www.promptstudy.info.

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