Genesys Diagnostics Inc.
8 Enterprise Lane Oakdale, CT, 06370
Phone: (860) 574-9172 / Fax: (860) 574-9264
http://gdilabs.com

Name: **Doe, Jane**

Accession ID: **2000649**

DOB: **8/26/1960**
Sex: **Female**
Race/Ethnicity:
Family #:

MRN:
Referring facility:
Referring physician: **Physician, Ordering**
Copies to:

Specimen: **Buccal swab**
Date of Collection: **2/17/2020**
Date of Receipt: **2/19/2020**
Date of Report: **9/28/2020**

Test(s) Performed: **Cardiology Full Panel**
Indication for test: **Essential (primary) hypertension**

RESULT: Positive
Two variants of uncertain clinical significance and one risk allele were identified

APPROACH

Sequencing of select genes was done using Next Generation Sequencing and the data was analyzed to identify both previously classified and novel variants in targeted genes. A total of 174 genes with previous implications in various mendelian disorders (see Supplement for a list of genes and coverage information) were covered with minimum read depth of 20X. Note that this test cannot exclude the possibility of variants in genes not analyzed or assayed with incomplete coverage.

VARIANTS RELEVANT TO INDICATION FOR TESTING

DNA sequencing identified two variants of uncertain clinical significance: one in MYLK, and one in PRKAG2. No other variants of clinical significance relevant to the patient's phenotype or family history were identified. It is recommended that this individual and any 1st degree relative receive continued clinical evaluation. Please see below for more detailed variant information. The mean depth coverage is 315x with a quality threshold of 99.3%.

Gene & Transcript	Variant	Allele State	Location	Disorder or Phenotype	Inheritance	Classification
MYLK NM_053025.3	c.3094G>C p.Ala1032Pro	Het.	Exon 18	Familial thoracic aortic aneurysm-7	Autosomal Dominant / Heterozygous	Uncertain Significance
PRKAG2 NM_016203.3	c.704T>G p.Leu235Arg	Het.	Exon 5	Hypertrophic cardiomyopathy-6, Wolff-Parkinson-White syndrome, lethal congenital glycogen storage disease of the heart	Autosomal Dominant / Heterozygous	Uncertain Significance

OTHER VARIANTS OF MEDICAL SIGNIFICANCE (INCIDENTAL FINDINGS)

No incidental findings are possible due to targeted sequencing.

Monogenic Disease Risk

DNA sequencing identified a heterozygous missense variant c.55G>A, p.Asp19His in the ABCG8 gene (NM_022437.2). It replaces aspartic acid with histidine at codon 19 of the ABCG8 protein (p.Asp19His). The aspartic acid residue is moderately conserved and there is a moderate physicochemical difference between aspartic acid and histidine. This variant is present in population databases (6.64% in gnomAD), including multiple homozygous individuals. Population-based case-control studies have shown that this variant is associated with reduced serum phytosterol levels and confers susceptibility to gallstone disease (PMID: 11893785, 17632509, 21039838, 21274884, 22898925). In a large meta analysis with 4,381 cases and 3,765 controls (PMID: 22898925), individuals carrying this variant had an increased overall risk of gallstone disease (2.07, 95% CI: 1.65 2.60). Experimental studies have shown that this missense variant causes a gain of ABCG8 protein function in vitro, contrary to the loss of ABCG8 protein function associated with sitosterolemia (PMID: 22898925). For these reasons, this variant has been classified as an Increased Risk Allele for gallbladder disease. Clinical correlation and genetic counseling are recommended.

Carrier Status

There were NO variants inferring a carrier status of a recessive disorder identified in this individual in genes unrelated to this individual's clinical presentation. Please see limitations for more detail.

RECOMMENDATIONS

Genetic counseling is recommended for this individual and their family. Genesys Diagnostics provides assistance in genetic counseling services, please contact the laboratory at (860) 5749172 for further information. A medical provider can request reanalysis of the variants, and this is recommended on an annual basis. Data from this next generation sequencing analysis can be reassesed for the presence of any new variants that may be newly linked to established genes or to newly characterized genes and/or disorders identified since the data used in this report was generated. A charge may be applied for reanalysis. Please contact the laboratory for more information at the time reanalysis is requested.

DETAILED VARIANT INFORMATION (VARIANTS RELEVANT TO INDICATION FOR TESTING)

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Gene & Transcript	Variant	Allele State	Location	Disorder or Phenotype	Inheritance	Classification
MYLK NM_053025.3	c.3094G>C p.Ala1032Pro	Het.	Exon 18	Familial thoracic aortic aneurysm-7	Autosomal Dominant / Heterozygous	Uncertain Significance
Genomic Position			**Variant Frequency**			
Chr3:NC_000003.11:g.123419221C>G			Not identified in large population studies			

VARIANT INTERPRETATION: The missense variant p.Ala1032Pro in MYLK (NM_053025.3) is novel (has not been identified in any individuals) in gnomAD and ClinVar. To the best of our knowledge, this variant has not been reported in the literature. There is a small physicochemical difference between alanine and proline, which is not likely to impact secondary protein structure. In-silico analyses, including protein predictors and evolutionary conservation, supports a deleterious effect as the missense change is predicted to be damaging by both SIFT and PolyPhen2 and the nucleotide is conserved in mammalian species. For these reasons, this variant has been classified as Uncertain Significance. Pathogenic variants in MYLK are associated with autosomal dominant familial thoracic aortic aneurysm-7 (OMIM#: 613780) and autosomal recessive megacystismicrocolon-intestinal hypoperistalsis syndrome (OMIM#: 249210). Clinical correlation and genetic counseling are recommended.

Gene & Transcript	Variant	Allele State	Location	Disorder or Phenotype	Inheritance	Classification
PRKAG2 NM_016203.3	c.704T>G p.Leu235Arg	Het.	Exon 5	Hypertrophic cardiomyopathy-6, Wolff-Parkinson-White syndrome, lethal congenital glycogen storage disease of the heart	Autosomal Dominant / Heterozygous	Uncertain Significance
Genomic Position			**Variant Frequency**			
Chr7:NC_000007.13:g.151329205A>C			Not identified in large population studies			

VARIANT INTERPRETATION: The missense variant p.Leu235Arg in PRKAG2 (NM_016203.3) is observed in 8/191708 alleles in gnomAD and has been reported to ClinVar as a variant of uncertain significance (Variation ID 410720). To the best of our knowledge, this variant has not been reported in the literature in individuals with a PRKAG2-related disease. The leucine residue is weakly conserved and there is a moderate physicochemical difference between leucine and arginine. Algorithms developed to predict the effect of missense changes on protein structure and function suggest that this variant is likely to be tolerated, but disagree on the conservation of the nucleotide. In summary, the available evidence is currently insufficient to determine the role of this variant in disease. For these reasons, this variant has been classified as Uncertain Significance. Pathogenic variants in PRKAG2 are associated with autosomal dominant hypertrophic cardiomyopathy-6 (OMIM#: 600858), autosomal dominant lethal congenital glycogen storage disease of the heart (OMIM#: 261740) and autosomal dominant Wolff-Parkinson-White syndrome (OMIM#: 194200). Clinical correlation and genetic counseling are recommended.

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METHODOLOGY

Genomic DNA was extracted from the patient's sample submitted to our laboratory using QIAamp mini kit. The Nextera Flex for Enrichment (Illumina) and Illumina's TruSight Cardio Enrichment Oligos were used to target the requested genes of tagmented genomic DNA. These targeted regions were then sequenced using Illumina MiSeq Dx with pair end reads. The DNA sequence was mapped to, and analyzed in comparison with, the published human genome build (UCSC hg19 reference sequence). The targeted coding genes and splice junctions of the known protein coding RefSeq genes were assessed for the average depth of coverage and the data quality threshold values. All reportable pathogenic/likely pathogenic sequence variants are confirmed by Sanger sequencinganalysis using a separate DNA preparation. All genes in the panel were evaluated for large deletions and/or duplications. However, small deletions or duplications only involving several exons may not be detected by this assay, nor will copy number alterations in regions of genes with poor coverage or pseudogenes. Putative deletions or duplications identified are confirmed by microarray or qPCR analysis using a separate DNA preparation.
The values below represent metrics from this individual's exome sequencing:
Mean depth of coverage refers to the sequence mean read depth across the targeted region. The quality threshold refers to the percentage of the defined target region where read depth was at least 20x coverage to permit high quality exome variant base calling, annotation and evaluation. Average quality thresholds may range from>85 of the targeted region, indicating a small portion of the target region may not be covered with sufficient depth or quality to confidently call variant positions.
The genes covered in this panel and the accession numbers of the reference are: *ABCC9* (NM_020297.3), *ABCG5* (NM_022436.3), *ABCG8* (NM_022437.3), *ACTA1* (NM_001100.4), *ACTA2* (NM_001141945.2), *ACTC1* (NM_005159.5), *ACTN2* (NM_001278344.1), *AKAP9* (NM_005751.4), *ALMS1* (NM_015120.4), *ANK2* (NM_001148.6), *ANKRD1* (NM_014391.2), *APOA4* (NM_000482.4), *APOA5* (NM_052968.5), *APOB* (NM_000384.3), *APOC2* (NM_000483.5), *APOE* (NM_001302690.1), *BAG3* (NM_004281.3), *BRAF* (NM_001374258.1), *CACNA1C* (NM_001129827.2), *CACNA2D1* (NM_001366867.1), *CACNB2* (NM_201571.4), *CALM1* (NM_001363670.1), *CALR3* (NM_145046.5), *CASQ2* (NM_001232.3), *CAV3* (NM_033337.3), *CBL* (NM_005188.4), *CBS* (NM_001321072.1), *CETP* (NM_000078.3), *COL3A1* (NM_000090.3), *COL5A1* (NM_001278074.1), *COL5A2* (NM_000393.5), *COX15* (NM_001372025.1), *CREB3L3* (NM_032607.3), *CRELD1* (NM_001374318.1), *CRYAB* (NM_001885.3), *CSRP3* (NM_003476.5), *CTF1* (NM_001330.3), *DES* (NM_001927.4), *DMD* (NM_004010.3), *DNAJC19* (NM_001190233.1), *DOLK* (NM_014908.4), *DPP6* (NM_130797.4), *DSC2* (NM_004949.5), *DSG2* (NM_001943.5), *DSP* (NM_004415.4), *DTNA* (NM_032975.3), *EFEMP2* (NM_016938.5), *ELN* (NM_001278939.1), *EMD* (NM_000117.3), *EYA4* (NM_004100.5), *FBN1* (NM_000138.5), *FBN2* (NM_001999.4), *FHL1* (NM_001159704.1), *FHL2* (NM_201555.2), *FKRP* (NM_001039885.3), *FKTN* (NM_001351502.2), *FXN* (NM_181425.3), *GAA* (NM_000152.5), *GATAD1* (NM_021167.5), *GCKR* (NM_001486.4), *GJA5* (NM_005266.6), *GLA* (NM_000169.3), *GPD1L* (NM_015141.3), *GPIHBP1* (NM_178172.6), *HADHA* (NM_000182.5), *HCN4* (NM_005477.3), *HFE* (NM_139006.3), *HRAS* (NM_176795.4), *HSPB8* (NM_014365.2), *ILK* (NM_001014795.3), *JAG1* (NM_000214.3), *JPH2* (NM_020433.5), *JUP* (NM_001352773.1), *KCNA5* (NM_002234.4), *KCND3* (NM_004980.4), *KCNE1* (NM_000219.6), *KCNE2* (NM_172201.1), *KCNE3* (NM_005472.4), *KCNH2* (NM_000238.4), *KCNJ2* (NM_000891.3), *KCNJ5* (NM_001354169.2), *KCNJ8* (NM_004982.4), *KCNQ1* (NM_000218.3), *KLF10* (NM_001032282.3), *KRAS* (NM_033360.4), *LAMA2* (NM_000426.3), *LAMA4* (NM_001105206.3), *LAMP2* (NM_002294.3), *LDB3* (NM_001171610.2), *LDLR* (NM_000527.5), *LDLRAP1* (NM_015627.3), *LMF1* (NM_001352018.2), *LMNA* (NM_170707.4), *LPL* (NM_000237.3), *LTBP2* (NM_000428.3), *MAP2K1* (NM_002755.3), *MAP2K2* (NM_030662.3), *MIB1* (NM_020774.3), *MURC* (NM_001018116.2), *MYBPC3* (NM_000256.3), *MYH11* (NM_001040113.2), *MYH6* (NM_002471.3), *MYH7* (NM_000257.4), *MYL2* (NM_000432.3), *MYL3* (NM_000258.3), *MYLK* (NM_053025.4), *MYLK2* (NM_033118.4), *MYO6* (NM_001368865.1), *MYOZ2* (NM_016599.5), *MYPN* (NM_001256268.1), *NEXN* (NM_144573.3), *NKX2-5* (NM_001166175.2), *NODAL* (NM_018055.5), *NOTCH1* (NM_017617.5), *NPPA* (NM_006172.4), *NRAS* (NM_002524.5), *PCSK9* (NM_174936.4), *PDLIM3* (NM_014476.6), *PKP2* (NM_004572.3), *PLN* (NM_002667.5), *PRDM16* (NM_022114.4), *PRKAG2* (NM_016203.4), *PRKAR1A* (NM_212471.2), *PTPN11* (NM_001330437.2), *RAF1* (NM_002880.3), *RANGRF* (NM_001177802.2), *RBM20* (NM_001134363.3), *RYR1* (NM_000540.3), *RYR2* (NM_001035.3), *SALL4* (NM_020436.5), *SCN1B,* (NM_199037.5), *SCN2B* (NM_004588.5), *SCN3B* (NM_018400.3), *SCN4B* (NM_174934.3), *SCN5A* (NM_000335.5), *SCO2* (NM_001169109.1), *SDHA* (NM_004168.4), *SEPN1* (NM_020451.3), *SGCB* (NM_000232.4), *SGCD* (NM_000337.5), *SGCG* (NM_000231.2), *SHOC2* (NM_001324337.1), *SLC25A4* (NM_001151.4), *SLC2A10* (NM_030777.4), *SMAD3* (NM_005902.4), *SMAD4* (NM_005359.6), *SNTA1* (NM_003098.3), *SOS1* (NM_005633.3), *SREBF2* (NM_004599.4), *TAZ* (NM_001303465.2), *TBX20* (NM_001077653.2), *TBX3* (NM_016569.4), *TBX5* (NM_000192.3), *TCAP* (NM_003673.4), *TGFB2* (NM_001135599.3), *TGFB3* (NM_003239.4), *TGFBR1* (NM_001306210.2), *TGFBR2* (NM_001024847.2), *TMEM43* (NM_024334.2), *TMPO* (NM_001032283.2), *TNNC1* (NM_003280.3), *TNNI3* (NM_000363.5), *TNNT2* (NM_001276347.2), *TPM1* (NM_001365776.1), *TRDN* (NM_006073.4), *TRIM63* (NM_032588.3), *TRPM4* (NM_017636.4), *TTN* (NM_001267550.2), *TTR* (NM_000371.3), *TXNRD2* (NM_001352300.2), *VCL* (NM_003373.4), *ZBTB17* (NM_001287603.1), *ZHX3* (NM_015035.4), *ZIC3* (NM_003413.4)

VARIANT ASSESSMENT PROCESS

The following databases and insilico algorithms are used to annotate and evaluate the impact of the variant in the context of human disease: 1000 genomes, gnomAD, ClinVar, OMIM, dbSNP, NCIB RefSeq Genes, ExAC Gene Constraints, VSSIFT, VSPolyPhen2, PhyloP, GERP++, GeneSplicer, MaxEntScan, NNSplice, PWM Splice Predictor. Analysis was reported using the to HGVS nomenclature (www.hgvs.org/mutnomen) as implemented by the VarSeq transcript annotation algorithm. The reported transcript matches that used most frequently by the clinical labs submitting to ClinVar. Following variants are not reported but are available upon request: (1) variants classified as likely benign or benign; (2) synonymous or noncoding variants which are predicted not to affect splicing by insilico algorithms; (3) variants of uncertain clinical significance in a gene associated with autosomal recessive diseases without a second hit; (4) variants in a gene of uncertain clinical significance; (5) rare missense variants or inframe insertions/deletions in the TTN gene as several recent studies have demonstrated that these variants are unlikely to be causative for heart diseases (PMID: 26567375, 27886618, 29750433, 30858397).

LIMITATIONS

Certain regions in various genes have poor coverage and are not included in the panel (if you would like more coverage information regarding any specific genes of interest, please contact Genesys Diagnostics Inc.). All genes that have pseudogenes will have poorer performance on the MiSeq instrument. Variants in genes with their pseudogenes may not be reliably detected. DNA alterations in regions not covered by this test such as deep intronic or regulatory regions, or in poorly covered regions will not be detected using Next Generation Sequencing analysis. There are technical limitations on the ability of Next Generation Sequencing to detect small insertions and deletions and these types of alterations are not detected as reliably as single nucleotide variants. This assay is not designed or validated for the detection of low-level mosaicism or somatic mutations. This assay will not detect certain types of genomic aberrations such as translocations, inversions, or repeat expansions. Large deletions or duplications may be identified, but not guaranteed to be detected by this test. Rare diagnostic errors can result from incorrectly assigned specimens (e.g. sample mixup), family relationships (e.g. nonpaternity), or DNA alterations (e.g. DNA variant under a primer or probe binding site and the presence of pseudogene artifacts).Additionally, the classification and interpretation of variants reported reflects the current state of scientific understanding at the time this report is issued. It is possible that a particular genetic abnormality may not be recognized as the underlying cause of the genetic disorder due to incomplete scientific knowledge about the function of all genes in the human genome and the impact of variants in those genes.
Disclaimer:
This test was developed and its performance characteristics determined by Genesys Diagnostics Inc (CLIA# 07D2046796). The U.S. Food and Drug Administration has not approved or cleared this test; however, FDA clearance or Approval is not currently required for clinical use. The results are not intended to be used as the sole means for clinical diagnosis or patient management decisions.

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REFERENCES

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Kirchhof P, B. S. (2016). 2016 ESC Guidelines for the management of atrial fibrillation developed in collaboration with EACTS: The Task Force for the management of atrial fibrillation of the European Society of Cardiology (ESC). European Heart Journal, 28932962.

Michael J. Ackerman, M. P. (2011). HRS/EHRA Expert Consensus Statement on the State of Genetic Testing for the Channelopathies and Cardiomyopathies: This document was developed as a partnership between the Heart Rhythm Society (HRS) and the European Heart Rhythm Association (EHRA) . EP Europace, 10771109.

Santani A, M. J.G. (2017). Development and Validation of Targeted NextGeneration Sequencing Panels for Detection of Germline Variants in Inherited Diseases. Arch Pathol Lab Med., 141(6):787797.

Approved by:
Jun Liao (1/22/2021)