PARTNERS HEALTHCARE | PERSONALIZED MEDICINE
MASSACHUSETTS GENERAL HOSPITAL
BRIGHAM AND WOMEN'S HOSPITAL

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Lab Accession: **PM-13-X00000**
Patient Name: **DOE, GENE**
Birth Date: **01/01/1950**
Age Sex: **61 year old Female**


# MOLECULAR DIAGNOSTICS REPORT

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| **Specimen Type:** | Blood, Peripheral | **Received Date:** | 08/07/2008 |
| **Related Accession(s):** | | **Referring Facility:** | UNIV OF AMERICA |
| **Referring Physician:** | DR. SMITH | **Referring Fac. MRN:** | 12345678 |
| **Copies To:** | OTHER CONTACTS, MS, CGC<br>SENDOUT UNIVERSITY OF AMERICA | **Lab Control Number:**<br>**Family Number:** | 00-222-55555<br>F000000 |

**TEST DESCRIPTION –** Pan Cardiomyopathy Panel (51 Genes)
Sequence Confirmation Test
Copy Number Variation Analysis

**TEST PERFORMED –** PCM-pnlAv2; SeqConfirm; CNV-a

**INDICATION FOR TEST –** Clinical diagnosis and family history of DCM

**RESULTS**

**DNA VARIANTS:**

**Heterozygous c.244G>A (p.Glu82Lys), Exon 1, LMNA, Pathogenic**
Heterozygous c.2267G>A (p.Ser756Asn), Exon 21, RYR2, Unknown Significance
Heterozygous c.3878T>C (p.Phe1293Ser), Exon 22, SCN5A, Unknown Significance
Heterozygous c.98876A>T (p.Glu32959Val), Exon 309, TTN, Unknown Significance
Heterozygous c.2238-1G>A, Intron 17, ABCC9, Unknown Significance

**INTERPRETATION:**

**Positive.** DNA sequencing and copy number assessment of the coding regions and splice sites of 51 cardiomyopathy genes (see methodology section below) identified the variants listed above.

**SUMMARY (see below for variant interpretations):** This individual carries a pathogenic variant in LMNA. The LMNA gene is strongly associated with DCM, which is consistent with the reported clinical diagnosis.

In addition, 4 variants of unknown significance were identified (1 in RYR2, 1 in SCN5A, 1 in TTN, and 1 in ABCC9). Their clinical significance cannot be determined due to a lack of available data, though the frequency of the ABCC9 suggests that it is more likely benign. Additional information is needed to determine if these variants also contribute to disease.

Genetic testing of this individual's biological relatives, particularly those who are affected, may help to clarify the significance of these unknown significance variants.

Cardiomyopathy due to pathogenic variants in the LMNA gene is typically inherited in an autosomal dominant pattern. Each first-degree relative has a 50% (or 1 in 2) chance of inheriting a variant and its risk for cardiomyopathy. Disease penetrance and severity can vary due to modifier genes and/or environmental factors. The significance of a variant should therefore be interpreted in the context of the individual's clinical manifestations.

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For a list of disease-gene associations, please visit our website at www.partners.org/personalizedmedicine/lmm.

**VARIANT INTERPRETATIONS:**

The **Glu82Lys** variant in LMNA has been reported in at least 2 families with DCM and conduction system abnormalities, and segregated with disease in 8 affected relatives (Wang 2006, Wu 2010). It has not been identified in large population studies. Functional studies (in vitro) suggest an effect on protein function and mice carrying the variant exhibited clinical features of DCM (Wang 2006, Lu 2010, Sun 2010). In summary, this variant meets our criteria to be classified as pathogenic (www.partners.org/personalizedmedicine/lmm)based upon segregation studies, absence from controls, and functional evidence.

The **Ser756Asn** variant in RYR2 has now been identified by our laboratory in 2 Caucasian individuals with DCM, but has also been identified in 2/8720 European American chromosomes by the NHLBI Exome Sequencing Project (http://evs.gs.washington.edu/EVS/; dbSNP rs193922623). Computational analyses (biochemical amino acid properties, conservation, AlignGVGD, PolyPhen2, and SIFT) suggest that this variant may impact the protein, though this information is not predictive enough to determine pathogenicity. At this time, additional information is needed to fully assess the clinical significance of this variant.

The **Phe1293Ser** variant in SCN5A has been reported in 2 individuals with Brugada syndrome (Priori 2002, Sommariva 2012), one of whom carried a frameshift variant in the other copy of SCN5A, but has also been identified in 1/590 Caucasian control chromosomes (Ackerman 2004) and in 4/8412 European American chromosomes by the NHLBI Exome Sequencing Project (http://evs.gs.washington.edu/; dbSNP rs41311127). Computational analyses (biochemical amino acid properties, conservation, AlignGVGD, PolyPhen2, and SIFT) do not provide strong support for or against an impact to the protein. At this time, additional information is needed to fully assess the clinical significance of this variant.

The **Glu32959Val** variant in TTN has now been identified by our laboratory in 1 Ashkenazi Jewish individual with HCM and 1 Caucasian individual with DCM. This variant has also been identified in 3/569 European chromosomes from the ClinSeq project (dbSNP rs55725279) and in 3/8124 European American chromosomes by the NHLBI Exome Sequencing Project (http://evs.gs.washington.edu/EVS). Computational analyses (biochemical amino acid properties, conservation, AlignGVGD, PolyPhen2, and SIFT) do not provide strong support for or against an impact to the protein. Additional information is needed to fully assess its clinical significance.

The **2238-1G>A** variant in ABCC9 has not been reported in individuals with cardiomyopathy, but has been identified in 0.1% (10/8600) of European American chromosomes by the NHLBI Exome Sequencing Project (http://evs.gs.washington.edu/EVS/; dbSNP rs141281214). This variant is expected to abolish the 3’ splice site of exon 18. Computational tools predict the creation of a novel spice site 3 bases further downstream, which would result in an in-frame deletion of one amino acid. While the frequency of this variant suggests that it is more likely benign, it is too low to confidently rule out a disease-causing role. Additional information is needed to determine the splice impact of this variant, as well as fully assess its clinical significance.

**RECOMMENDATION:**

Genetic counseling is recommended for this individual and their relatives. Familial variant testing is available for other relatives if desired. For

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assistance in locating genetic counseling services or disease specialists, please call the laboratory at 617-768-8500 or email at LMM@partners.org.

Please note that variant classification, particularly of unknown significance, may change over time if more information becomes available. Please contact us at 617-768-8500 or LMM@partners.org.

**COMMENTS:**
Common sequence variants of unlikely clinical significance that are classified as benign are not included in this report but are available upon request.

In addition, the following less common sequence variants have been identified. These variants are believed to be likely benign because they do not change an amino acid and/or are within a non-conserved region of the intron and/or have a frequency in the general population that strongly argues against a pathogenic effect (please see www.partners.org/personalizedmedicine/lmm for our classification rules). Although they are likely benign we cannot rule out that they may be pathogenic or contribute to disease.

Silent variants that are not located near a splice junction:
His3539His in exon 45A of TTN (allele frequency = 0.1%, 6/7020) **
Ser32958Ser in exon 309 of TTN (rs55838839; allele frequency = 3/569)

Missense variants with frequencies that make a disease causing role unlikely:
Gly5814Asp in exon 70 of TTN (rs72648964; allele frequency = 0.6%, 20/3112) **
Leu9540Val in exon 125 of TTN (rs72650029; allele frequency = 0.9%, 28/3110) **

** NHLBI Exome Sequencing Project (http://evs.gs.washington.edu/EVS)

**INCIDENTAL VARIANTS:**
Heterozygous c.10617T>C (p.His3539His), Exon 45A, TTN, Likely Benign
Heterozygous c.17441G>A (p.Gly5814Asp), Exon 70, TTN, Likely Benign
Heterozygous c.28618C>G (p.Leu9540Val), Exon 125, TTN, Likely Benign
Heterozygous c.98874T>A (p.Ser32958Ser), Exon 309, TTN, Likely Benign

## TEST INFORMATION

**BACKGROUND:**
Dilated cardiomyopathy (DCM) is characterized by ventricular chamber enlargement and systolic dysfunction with normal left ventricular wall thickness. The estimated prevalence of DCM is 1/2,500 and about 20-35% of cases have a family history showing a predominantly autosomal mode of inheritance. To date, over 40 genes have been demonstrated to cause DCM, encoding proteins involved in the sarcomere, Z-disk, nuclear lamina, intermediate filaments and the dystrophin-associated glycoprotein complex. Variants in some genes can cause additional abnormalities in conjunction with DCM (BAG3, CSRP3, DES, EMD, LAMP2, LDB3, LMNA, SCN5A, SGCD, TCAP, and TTN), including conduction system disease and skeletal myopathy. Variants in the TAZ gene cause Barth syndrome, an X-linked cardioskeletal myopathy in infants.

**METHODOLOGY:**
The Pan Cardiomyopathy Panel includes the following 51 genes: ABCC9, ACTC1, ACTN2, ANKRD1, BAG3, CASQ2, CAV3, CRYAB, CSRP3, CTF1, DES, DSC2, DSG2, DSP, DTNA, EMD, FHL2, GATAD1, GLA, JUP, LAMA4, LAMP2, LDB3, LMNA, MYBPC3, MYH6, MYH7, MYL2, MYL3, MYLK2, MYOZ2, NEBL, NEXN, PKP2, PLN, PRKAG2, RBM20, RYR2, SCN5A, SGCD, TAZ, TCAP, TMEM43, TMPO, TNNC1, TNNI3, TNNT2, TPM1, TTN, TTR, and VCL. For reference

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sequences and exons covered, please visit our website (www.partners.org/personalizedmedicine/lmm).

This test is performed by next generation sequencing using Agilent SureSelect capture followed by sequencing of the coding regions and splice sites using Illumina sequencing technologies. Variant calls are generated using the Burrows-Wheeler Aligner followed by GATK analysis. Detection of copy number variants (CNVs) encompassing 1 or more exons is performed using VisCap™ analysis. Sanger sequencing is used to fill in regions with insufficient coverage. All clinically significant variants are confirmed by Sanger sequencing or droplet digital PCR. Variants classified as likely benign or benign are not confirmed. This test does not detect variants in non-coding regions, aside from the splice junctions, that could affect gene expression and a few exons have been excluded due to technical difficulties. CNV analysis is only performed when data meets necessary quality standards and may not be available for all cases.

Variants are reported according to HGVS nomenclature (www.hgvs.org/mutnomen).

This test was developed and its performance characteristics determined by the Laboratory for Molecular Medicine at the Partners HealthCare Personalized Medicine (LMM, 65 Landsdowne St, Cambridge, MA 02139; 617-768-8500; CLIA#22D1005307). It has not been cleared or approved by the U.S Food and Drug Administration (FDA). The FDA has determined that such clearance or approval is not necessary.

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