SUPPLEMENTAL MATERIAL

CIRCCVG/2011/959866

Molecular Genetic and Functional Characterization Implicate Muscle-Restricted Coiled-Coil Gene (*MURC*) As a Causal Gene For Familial Dilated Cardiomyopathy

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TABLE 1

A. Sequence of Oligonucleotide Primers Used for Sequencing of *MURC* Exons

(Genomic DNA Sequence Source: NC_000009.11, GI:224589821)

Exon 1 (nucleotides 5- 586)

Forward: 5'CCTGTTGCCTGTTATCAAGCTGAC3'

Reverse: 5'GACACTGGAAACCTCTGATATGAC3'

Exon 2 - Fragment 1 (nucleotides 7637 - 8058)

Forward: 5'CATGGCCAGAAGGTAAAAGAGCTG3'

Reverse: 5'CCTGACTGTCTCAGCCTCTCTC3'

Exon 2 - Fragment 2 (nucleotides 7930 - 8475)

Forward: 5'GACACGGCAGAATCTTGACAAG3'

Reverse: 5'CATGGCACAGAAATGTAGACGAC3'

B. Sequence of Oligonucleotide Primers Used for Mutagenesis

p.N128K

Forward: 5'GTCAAGCAAGAGGAAATAATGAAGAAAAAGAAATTCCGCGTGG3'

Reverse: 5’-CCACGCGGAATTTCTTTTTCTTCATTATTTCCTCTTGCTTGAC3’

p.R140W

Forward: 5’-CAGGAGAAGTTTTGGTGTCCGACATCCCTGTCTG3’

Reverse: 5’-CAGACAGGGATGTCGGACACCAAAACTTCTCCTG3’

p.L153P

Forward: 5’-GTCTGTTGTTAAAGACAGAAACCCAACTGAGAACCAAGAAGAGG3’

Reverse: 5’-CCTCTTCTTGGTTCTCAGTTGGGTTTCTGTCTTTAACAACAGAC3’

p.S307T

Forward: 5’-GTCTCTGGGCCCCATCACTGAGCTCTACTCTG3’

Reverse: 5’-CAGAGTAGAGCTCAGTGATGGGGCCCAGAGAC3’

p.P324L

Forward: 5’-GAACACGAGGCAGCCAGGCTGGTGTATCCTCCCCATGAAG3’

Reverse: 5’-CTTCATGGGGAGGATACACCAGCCTGGCTGCCTCGTGTTC3’

p.S364L

Forward: 5’-GATTTAAAGCACTCATTGGACTACAAAGACGATGACGAC3’

Reverse: 5’-GTCGTCATCGTCTTTGTAGTCCAATGAGTGCTTTAAATC3’

C. Sequence of Oligonucleotide Primers Used in qPCR

Rat atrial natriuretic peptide (ANP or *Nppa*)

Forward: 5'-ATACAGTGCGGTGTCCAACA-3'

Reverse: 5'-CGAGAGCACCTCCATCTCTC-3'

Rat brain natriuretic peptide (BNP or *Nppb*)

Forward: 5'-GGAAATGGCTCAGAGACAGC-3'

Reverse: 5'-CGATCCGGTCTATCTTCTGC-3'

Rat skeletal α-actin (SkA or *Acta1*)

Forward: 5'-CACGGCATTATCACCAACTG-3'

Reverse: 5'-CCGGAGGCATAGAGAGACAG-3'

Rat glyceraldehyde-3-phosphate dehydrogenase (*Gapdh)*

Forward: 5'-ATGGGAAGCTGGTCATCAAC-3'

Reverse: 5'-GTGGTTCACACCCATCACAA-3'

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TABLE 2

Non-Synonymous Variants Identified in the Probands and Controls

Variants		Controls		DCM		HCM	
		N=509		N=383		N=307	
		AA	C	AA	C	AA	C
		316	193	179	204	49	258
AA	Nucleotide						
p.S20T	g. 149G>C	2	0	1	0	0	0
p.S78L	g.323C>T	13	0	6	1	2	0
p.N81K	g.333T>G	1	0	1	0	1	0
p.R131H	g.482G>A	1	2	2	1	0	0
p.D163G	g.7791A>G	9	0	4	0	1	0
p.R231K	g.7795G>A	2	0	0	0	1	0
Total number of non-synonymous variants per group and ethnic background		28	2	14	2	5	0
Total number of non-synonymous variants per group		30 (5.9%)		16 (4.2%)		5 (1.6%)	

The prevalence of these non-synonymous variants was not significantly different between the DCM probands and controls. Likewise, the mean and median ages of individuals with these rare non-synonymous variants in the control and the DCM groups were similar (mean: 48.7 ± 8.6 vs. 47.9 ± 3.9, respectively, p=0.537; median: 45 vs. 49 years; range: 22 to 81 vs. 23 to 72 years).

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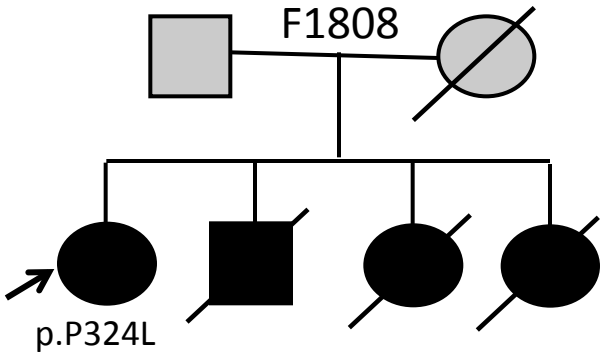
Table 3

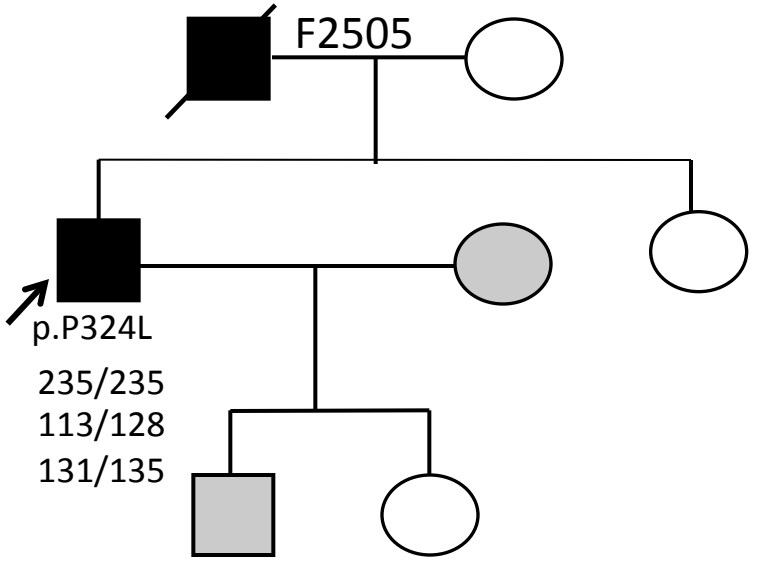
Synonymous, non-coding and deletion variants identified in the study population

Study Population		Total	Control		DCM		HCM	
			AA	C	AA	C	AA	C
		1,199	316	193	179	204	49	258
Nucleotide	Amino acid	Number of Individuals with DNA Sequence variants						
		1,183	264	233	178	211	47	250
g.34G>A	Non-coding	10	1	3	3	2	1	0
g.34G>R	Non-coding	6	1	0	0	5	0	0
g.79G>R	Non-coding	1	0	0	1	0	0	0
g.147G>R	p.S19S	5	2	0	2	0	1	0
g.267G>R	p.R59R	1	0	1	0	0	0	0
g.339G>K	p.G83G	1	0	0	0	1	0	0
g.504C>M	Non-coding	11	4	0	7	0	0	0
g.507G>R	Non-coding	2	0	0	1	0	1	0
g.526T>Y	Non-coding	3	1	0	2	0	0	0
g.540T>C	Non-coding	75	10	21	7	13	2	22
g.540T>Y	Non-coding	361	96	75	43	63	10	74
g.7729G>R	p.P142P	1	0	0	0	0	1	0
g.7822G>S	p.S173S	1	0	0	0	0	0	1
g.7873A>T	p.S190S	1	0	0	1	0	0	0
g.7873A>W	p.S190S	81	26	8	25	5	6	11
g.7918A>R	p.E205E	3	2	0	1	0	0	0
g.7981G>K	p.V226V	1	0	0	1	0	0	0
g.7987G>R	p.P228P	1	0	0	1	0	0	0
g. 8005A>R	p.L234L	5	0	1	0	1	0	3
g. 8008G>R	p.R235R	6	0	2	0	1	0	3
g.8017A>R	p.G238G	1	0	1	0	0	0	0
g.8203C>Y	p.S300S	1	1	0	0	0	0	0
g.8299G>A	p.R332R	150	17	33	15	29	6	50
g.8299G>R	p.R332R	434	94	87	60	89	18	86
g.8427A>R	Non-coding	3	0	1	0	2	0	0
g.8444T>Y	Non-coding	18	9	0	8	0	1	0

Online Figure 1

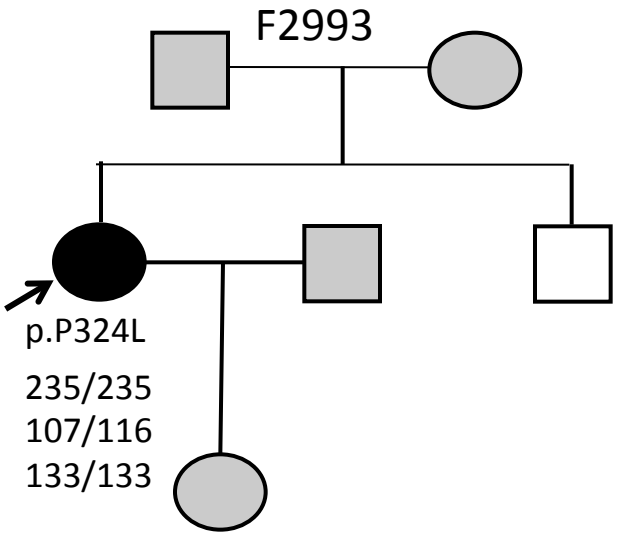


D9S180	231/233
D9S910	110/125
D9S176	151/153



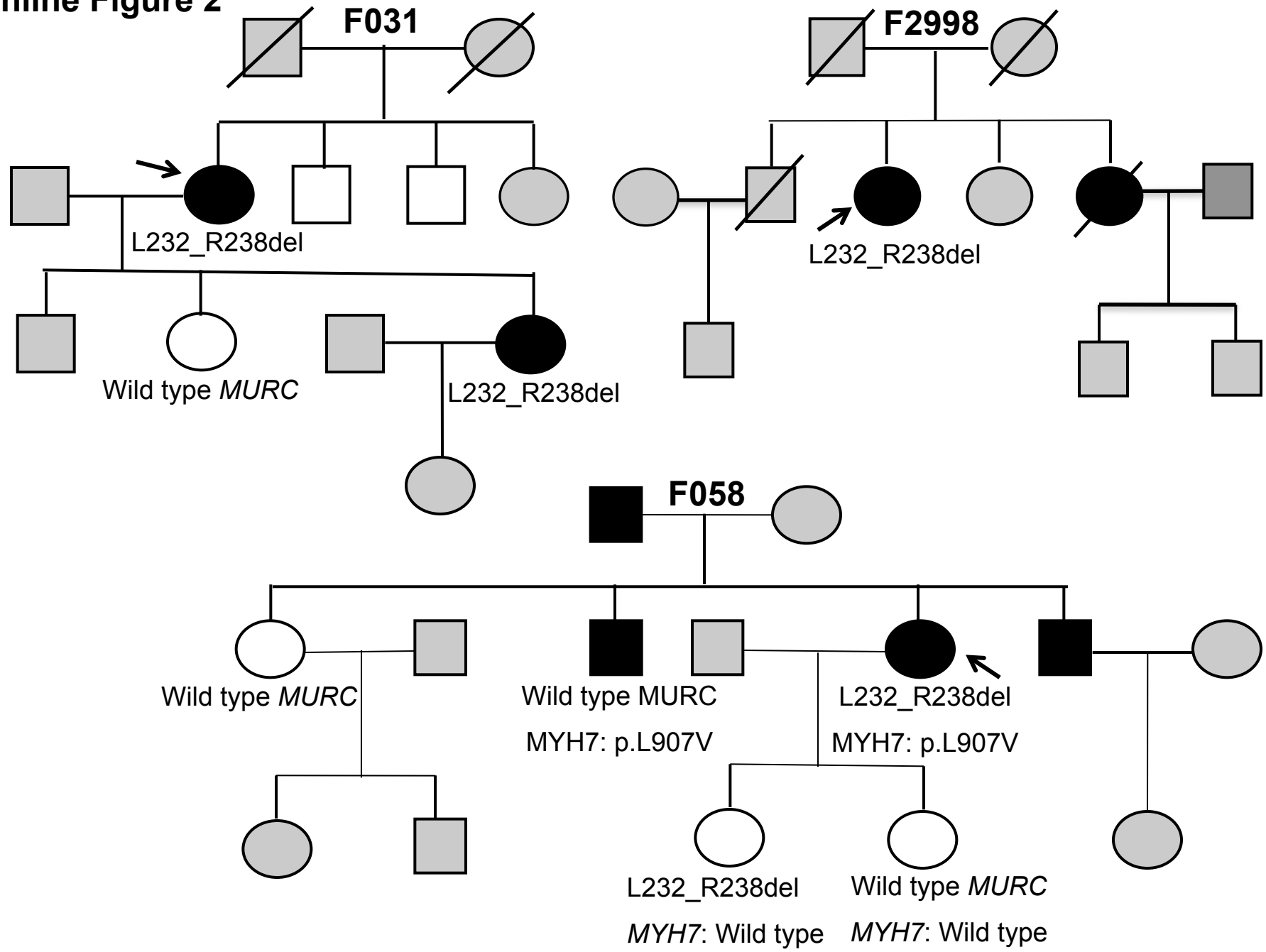
D9S180	235/235
D9S910	113/128
D9S176	131/135

D9S180	235/235
D9S910	110/113
D9S176	131/143



D9S180	235/235
D9S910	107/116
D9S176	133/133

Online Figure 2



Supplemental figure legend

Supplemental Figure 1. Genotypes of three probands with familial dilated cardiomyopathy and the p.P324L variant in *MURC* gene, who were typed for three short tandem repeat markers at the *MURC* locus. As shown the probands do not share a common locus haplotypes, indicating an independent origin of the p.P324L variants in three probands. Pedigree symbols are per convention (full circles and squares indicate affected females and males respectively. Empty circles and squares indicate normal females and males respectively. Grey symbols indicate individuals that were not phenotyped.

Supplemental Figure 2. Detection of the deletion variant (L232-R238del) in three families with hypertrophic cardiomyopathy. The deletion variant did not co-segregate with the phenotype in Family F058 as one clinically affected family member did not have the deletion mutation. A second mutation p.L907V in MYH7 was detected in two affected members in this family. Symbols are as in Supplemental Figure 1.