SUPPLEMENTAL MATERIAL

Supplemental Methods

Quality Control for Mapping and Variant Calling. MAQ was used for calling SNVs and the alignment of paired-end reads, by default, allows for 2 mismatches per read. For each exome sequenced, at least 20000 on-target SNVs are expected. The MAQ filtering criteria for mapped reads was a Phred-like consensus quality greater than 40. Several tolerance parameters are utilized when calling an SNV by MAQ. Listed below are 5 scenarios where an SNV would be discarded based upon the assessment of its location and quality:

- SNVs within 3-bp of a potential INDEL
- SNVs covered by three or fewer reads
- SNVs covered by zero reads with a mapping quality higher than 60
- SNVs with Phred-like consensus quality score less than 40
- If there are 3 or more SNVs within a 10 bp region, all are discarded

BWA alignment was used for calling INDELs and, by default, the alignment of paired-end reads allows for 3 mismatches per read. For each exome sequenced, at least 1000 on-target INDELs are expected. There were no filtering criteria used for these variants. Several tolerance parameters are utilized when calling an INDEL by GATK. Listed below are 3 scenarios where an INDEL would be discarded based upon the assessment of its location and quality:

- Minimum coverage is below 6 (default)
- Fraction of reads supporting the consensus INDEL is below 0.3 (minimum fraction default).
- Out of all of the reads that have an INDEL at a site, any instance where the fraction of those supporting the consensus INDEL is below 0.7 (Minimum consensus fraction default).

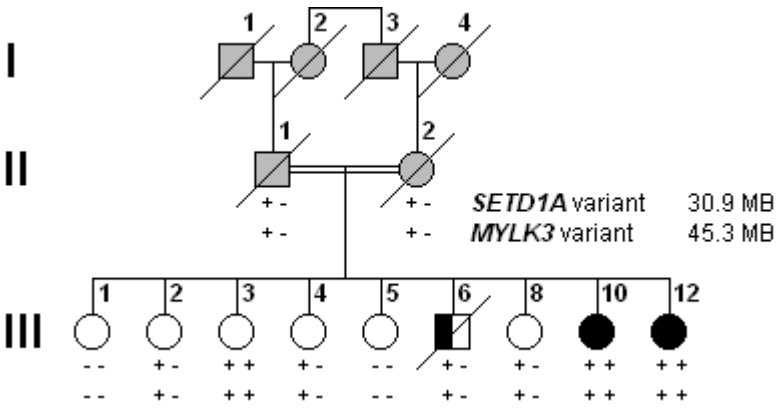
Supplemental Table 1. Summary of Regions Comprised of Contiguous, Homozygous SNVs

Pedigree Number	PHENO	CHR	SNV1	SNV2	POS1	POS2	KB	NSNV	DENSITY	PHOM	PHET
III.12	**2**	7	rs9648899	rs13234589	76539536	93805797	17266.26	2137	8.08	0.999	0.001
III.10	**2**	7	rs2906510	rs13234589	77472916	93805797	16332.88	2033	8.034	0.999	0.001
III.6	**2**	7	rs7789215	rs13234589	77933529	93805797	15872.27	1900	8.354	0.999	0.001
III.4	1	7	rs9648899	rs4728664	76539536	86501745	9962.209	1248	7.983	0.999	0
III.12	**2**	10	rs4935347	rs9414948	54210789	68296132	14085.34	2126	6.625	0.998	0
III.10	**2**	10	rs11592723	rs9414948	47164002	68296132	21132.13	2885	7.325	0.998	0
III.1	1	10	rs11592723	rs10761659	47164002	64115570	16951.57	2489	6.811	0.999	0.001
III.4	1	10	rs11592723	rs9414948	47164002	68296132	21132.13	2885	7.325	0.999	0.001
III.12	**2**	14	rs17278879	rs8016253	43611449	45508248	1896.799	138	13.745	0.986	0.014
III.10	**2**	14	rs17278879	rs8016253	43611449	45508248	1896.799	138	13.745	0.986	0.014
III.2	1	14	rs17278879	rs8016253	43611449	45508248	1896.799	138	13.745	0.986	0.014
III.4	1	14	rs17278879	rs8016253	43611449	45508248	1896.799	138	13.745	0.986	0.014
III.5	1	14	rs17278879	rs8016253	43611449	45508248	1896.799	138	13.745	0.986	0.014
III.12	**2**	16	rs4780514	rs12927233	13254572	32045466	18790.89	2244	8.374	0.999	0.001
III.10	**2**	16	rs4780514	rs12927233	13254572	32045466	18790.89	2244	8.374	0.999	0.001
III.2	1	16	rs4780514	rs739710	13254572	18072199	4817.627	533	9.039	0.998	0.002
III.3	1	16	rs12598321	rs12927233	26066068	32045466	5979.398	904	6.614	0.997	0.001
III.4	1	16	rs4780514	rs16977003	13254572	27745273	14490.7	1683	8.61	0.998	0.001
III.12	**2**	20	rs6080070	rs199541	15907833	19865570	3957.737	869	4.554	0.999	0.001
III.10	**2**	20	rs6131996	rs199541	17683508	19865570	2182.062	436	5.005	1	0
III.5	1	20	rs6080070	rs199541	15907833	19865570	3957.737	869	4.554	0.999	0.001
III.12	**2**	20	rs1409371	rs6076423	24493732	26262901	1769.169	150	11.794	0.993	0.007
III.10	**2**	20	rs1409371	rs6076423	24493732	26262901	1769.169	150	11.794	0.993	0.007
III.5	1	20	rs1409371	rs6076423	24493732	26262901	1769.169	150	11.794	0.993	0.007

PHENO: Phenotype, 1=Unaffected, 2=Affected; CHR, Chromosome; SNV1, SNV (single nucleotide variant) at start of region; SNV2, SNV at end of region; POS1, physical position in base-pairs at start of region; POS2, physical position in base-pairs at end of region; KB, length of region in kilobases; NSNV, number of SNVs in the region; DENSITY, number of SNVs/1 kb in region; PHOM, proportion of calls homozygous for subject in region; PHET, proportion of calls heterozygous for subject in region.

Supplemental Figure 1

A



B

588	D	D	R	G	G	S	P	P	**P**	A	P	T	P	P	Q	Q	P	604 **Human**
588	•	•	•	•	•	•	•	•	**L**	•	•	•	•	•	•	•	•	604 **P596L Substitution**
588	•	•	•	•	•	•	•	A	•	•	•	•	•	•	•	•	•	604 **Chimpanzee**
594	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	610 **Dog**
587	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	603 **Cow**
601	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	617 **Mouse**

C

631	Y	I	L	H	L	D	L	K	**P**	E	N	I	L	C	V	N	Q	647 **Human**
631	•	•	•	•	•	•	•	•	**L**	•	•	•	•	•	•	•	•	647 **P639L Substitition**
615	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	631 **Chimpanzee**
828	•	V	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	844 **Dog**
594	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	610 **Cow**
607	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	S	•	623 **Mouse**
598	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	S	•	614 **Rat**
729	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	H	745 **Chicken**
382	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	398 **Platypus**
584	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	R	600 **Frog**
520	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	S	536 **Zebrafish**

Supplemental Figure 1. (A) In addition to *GATAD1*, exome sequencing revealed homozygosity in two other genes for unreported, nonsynonymous SNVs shared by the affected sisters (III.10 and III.12). On further investigation, all 3 variants were also absent in 23 additional Mayo Clinic exomes and 200 Danish exomes[1] The other variants occurred in *SETD1A* (P596L) and *MYLK3* (P639L), both of which map to the same region of chromosome 16. The individual with idiopathic LVE (III.6) was heterozygous for both variants, raising the possibility of a disease-modifying effect related to mutation dose. However, homozygosity for both variants in an elderly unaffected individual (III.3) ruled these genes out as primary pathogenetic bases of DCM in this family. This individual had a normal echocardiogram and Sestamibi scan at ages 64 and 79, respectively, and had no symptoms of heart failure at age 86. Additionally, 3 siblings (III.2, III.4, and III.8) were heterozygous carriers of both mutations had normal echocardiograms at ages 81, 79, and 55. Conservation of the substituted amino acids in (B) SETD1A and (C) MYLK3 as well as 8 flanking residues is illustrated with the ● symbol indicating a conserved residue. Remarkably, both genes would be plausible candidates for DCM. MYLK3 is a cardiac specific kinase that plays an integral role in the regulation of sarcomere assembly[2] whereas SETD1A is a member of a methyltransferase complex which is known to be involved with the precise histone modification (H3K4Me3) with which GATAD1 was shown to interact.[3]

SUPPLEMENTAL REFERENCES

1. Li Y, Vinckenbosch N, Tian G, Huerta-Sanchez E, Jiang T, Jiang H, Albrechtsen A, Andersen G, Cao H, Korneliussen T, Grarup N, Guo Y, Hellman I, Jin X, Li Q, Liu J, Liu X, Sparsø T, Tang M, Wu H, Wu R, Yu C, Zheng H, Astrup A, Bolund L, Holmkvist J, Jørgensen T, Kristiansen K, Schmitz O, Schwartz TW, Zhang X, Li R, Yang H, Wang J, Hansen T, Pedersen O, Nielsen R, Wang J. Resequencing of 200 human exomes identifies an excess of low-frequency non-synonymous coding variants. Nat Genet. 2010; 42:969-972.

2. Seguchi O, Takashima S, Yamazaki S, Asakura M, Asano Y, Shintani Y, Wakeno M, Minamino T, Kondo H, Furukawa H, Nakamaru K, Naito A, Takahashi T, Ohtsuka T, Kawakami K, Isomura T, Kitamura S, Tomoike H, Mochizuki N, Kitakaze M. A cardiac myosin light chain kinase regulates sarcomere assembly in the vertebrate heart. J Clin Invest. 2007; 117:2812-2824.

3. Tate CM, Lee JH, Skalnik DG. CXXC finger protein 1 restricts the Setd1A histone H3K4 methyltransferase complex to euchromatin. FEBS J. 2010; 277:210-223.