Supplemental Data

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Genome-wide Studies of Copy-Number Variation and Exome Sequencing Identify Rare Variants in *BAG3* as Cause for Dilated Cardiomyopathy

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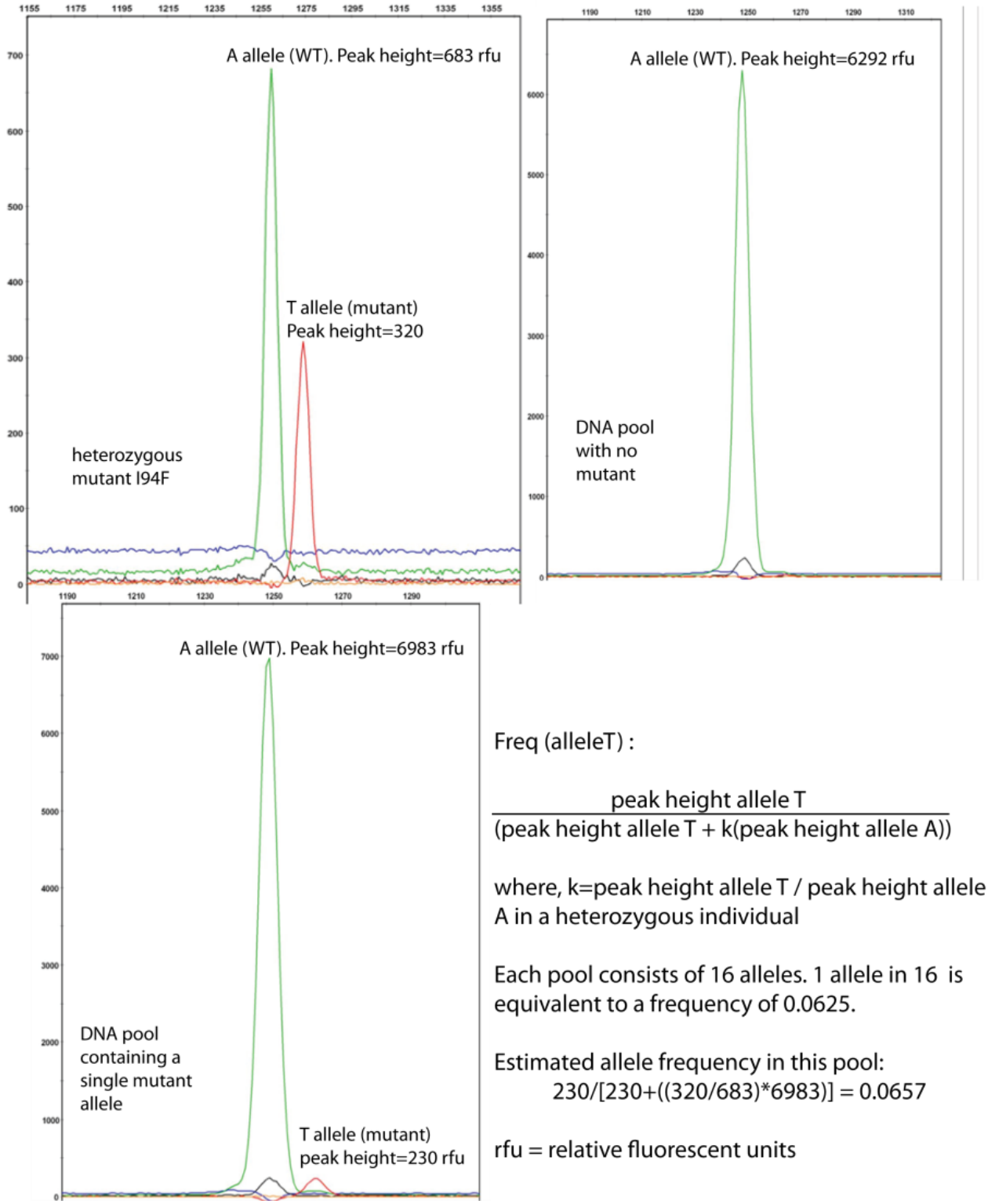
Figure S1. Primer Extension Proof of Principle Data in Control Pools (n=8)



Freq (alleleT) :

$$\frac{\text{peak height allele T}}{\text{(peak height allele T + k(peak height allele A))}}$$

where, k=peak height allele T / peak height allele A in a heterozygous individual

Each pool consists of 16 alleles. 1 allele in 16 is equivalent to a frequency of 0.0625.

Estimated allele frequency in this pool:
230/[230+((320/683)*6983)] = 0.0657

rfu = relative fluorescent units

Figure S1. Primer Extension Proof of Principle Data in Control Pools (n=8)

Supplementary figure 1 shows the primer extension data for the I94F variant which was detected in 2 pools. Primer extension assays have been shown to sometimes yield unequal peak heights in a single heterozygous individual, either due to differential amplification within the PCR reaction, or unequal incorporation of ddNTPs in the primer extension reaction.[1] In the worst case scenario, failure to detect a mutant allele within a pool could result from either of these possibilities. As proof of principle that the DNA pools are reliable under the above experimental conditions, we show data for I94F, where the mutant allele was clearly detected despite a bias towards the wild type allele, which in a single heterozygous individual showed a peak height double that of the mutant peak height. Calculation of allele frequency in the pool showed an estimated absolute allele frequency of 0.0657, compared to an actual allele frequency 0.0625 (1 allele in 16), an accuracy of 99.7%.

Figure 2. Exon 2 Morpholino PCR Products

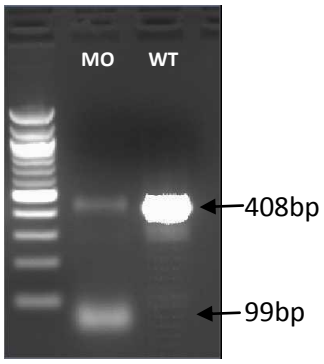


Figure 2. Exon 2 Morpholino PCR Products
For the exon 2 morpholino, PCR products were identified for both the wild type message and the spliced message, demonstrating that to some extent, this model mimics the human DCM condition, whereby heterozygous disease individuals have one copy of the ‘mutant’ message and one copy of the wildtype message.
MO, morpholino; WT, wild type.

Figure S3a. Coverage Distribution for Individual Exomes across Chromosome 1

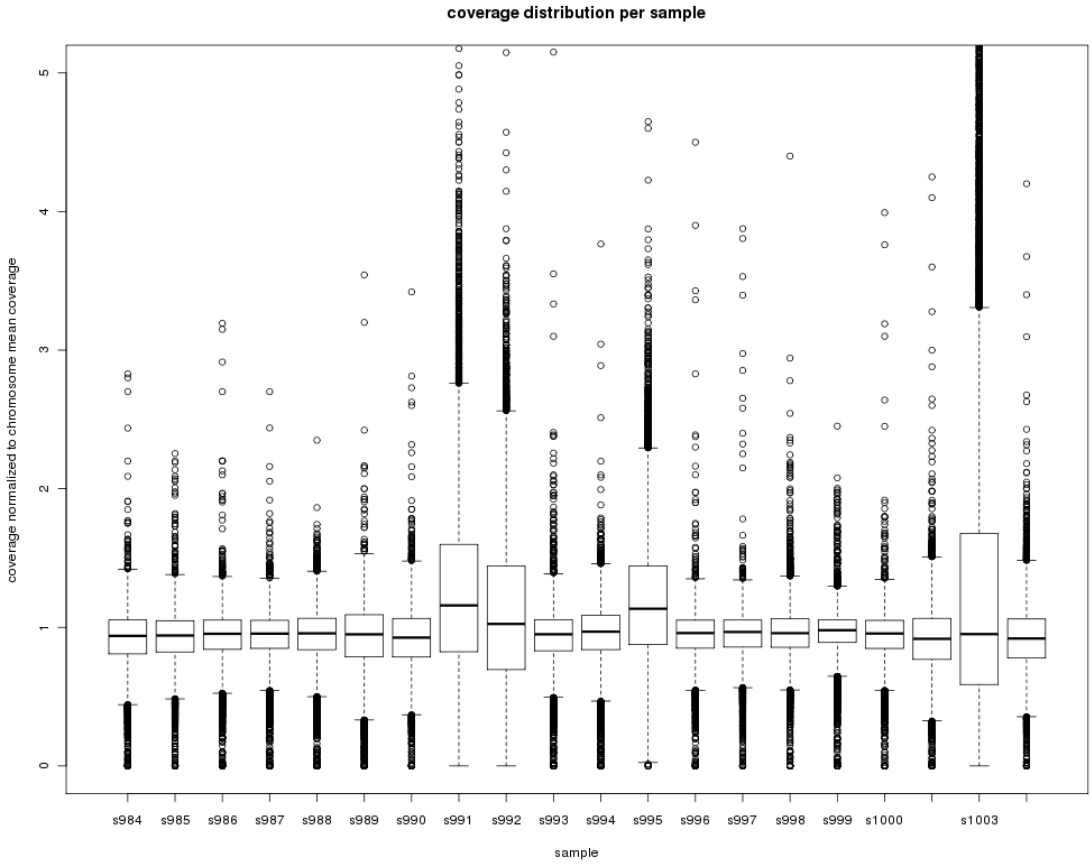


Figure S3a. Coverage Distribution for Individual Exomes across Chromosome 1

We assessed the exome sequence data post-hoc to identify to identify the deletion in *BAG3* in pedigree A using exome data read depth. Analysis of coverage distribution per individual for each of the 20 exomes revealed four samples as outliers, indicating overall coverage in these samples was slightly lower. Data for chromosome 1 is shown. These individuals were removed from further analysis.

Figure S3b. Normalized Sequence Coverage across *BAG3,* Exon 4 from 16 Exome Sequences

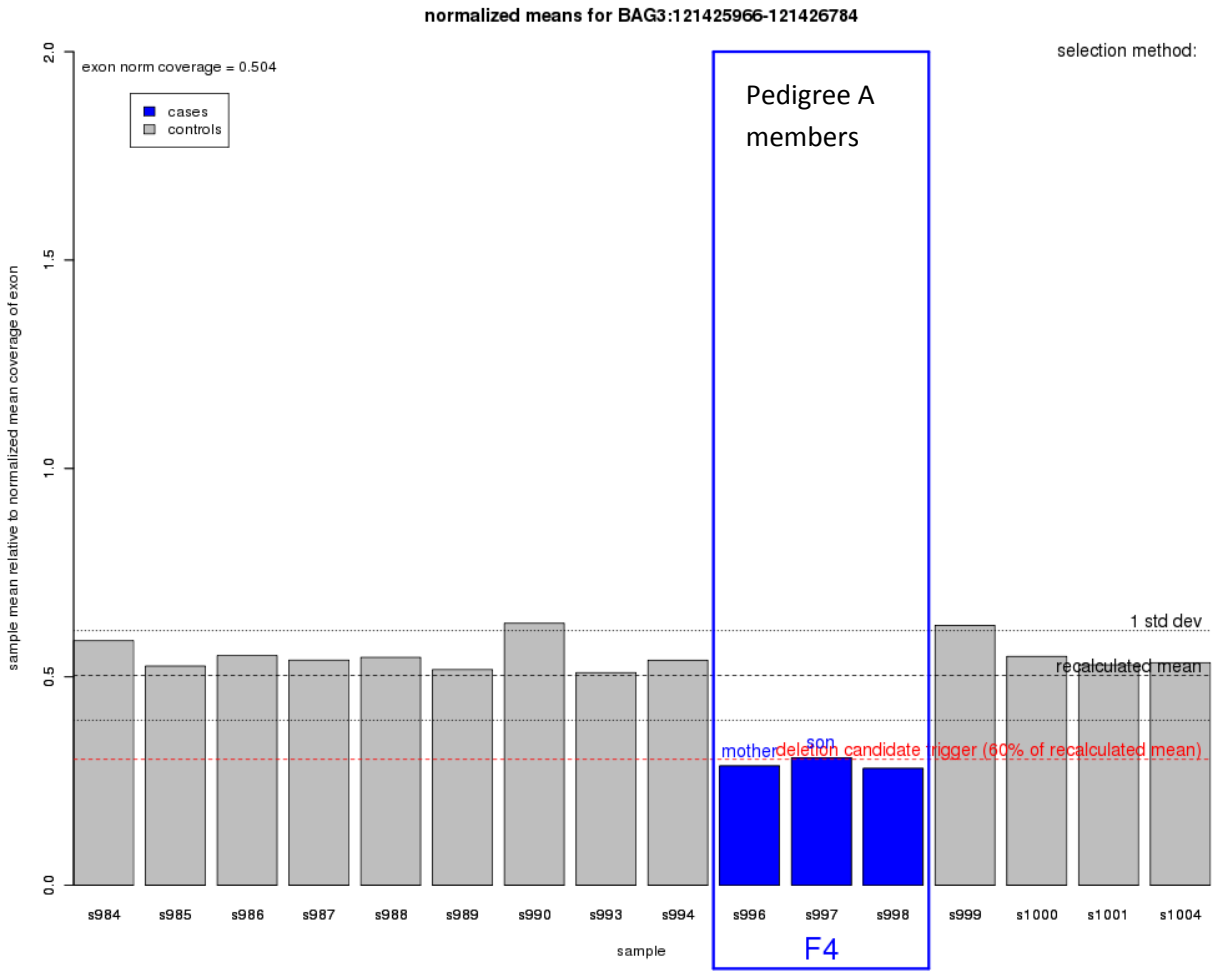


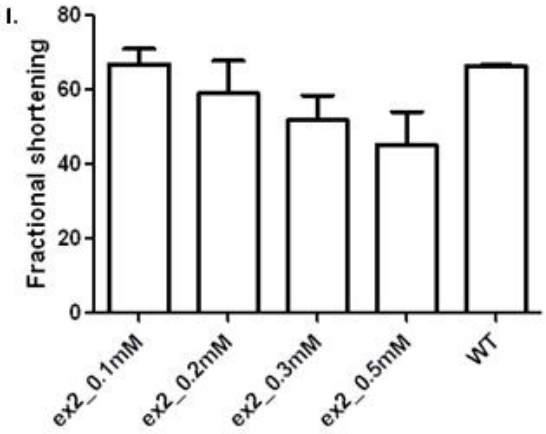
Figure S3b. Normalized Sequence Coverage across *BAG3,* Exon 4 from 16 Exome Sequences

We normalized read depth per exon against the average read depth per chromosome at the level of the individual and looked across individuals for a difference in this ratio that was shared across the affected members of pedigree A. The ratio for *BAG3* exon 4 in the affected members of pedigree A was approximately 50% compared to the other individuals.

Supplementary figure 4

H.

Dosage	straight	axis curvature (%)
0.1mM	22	13 (37.1)
0.2mM	18	33 (64.7)
0.3mM	22	58 (72.5)
0.5mM	16	43 (72.9)



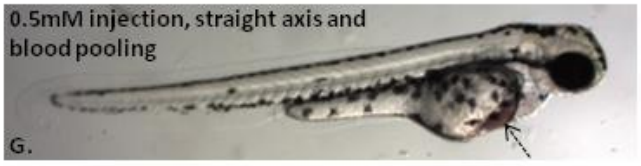


Figure S4. Additional Exon 2 Morpholino Data

Significant phenotypic variability was observed with the exon 2 morpholino, with some fish showing only heart failure (A, E), only axis curvature (B, C) or both axis curvature and heart failure (D, F). The number of fish with axis curvature and heart failure measured by fractional shortening increased in a dosage dependent manner (H, I). Pericardial effusion is marked by a solid arrow. Blood pooling was also observed in some fish, marked with a dotted arrow. In (I) mean values are shown. Error bars represent SEM.

Table S1. Primer Sequences

Region	PCR primer F	PCR primer R	Extension primer
BAG3 ex2	GCCAGGAGGGTTCACTTCC	CCCTGCATGTGAACAGGTG	CCTCTGCCAATGGCCCTTCC (R71W)
			CAGCTCCGACCAGGCTAC (I94F)
			ACCCTGTGTACCCCCAGCTC (R90stop)
			CGCTGAGAACCGGCAGGTGC (H109R)
			AGCCTGGGATGCAGCGATTC (R123stop)
			CTCTGCGGGGCATGCCAGAA (T144A)
BAG3 ex3	CAAGCCAGGGGAGTCATTT	CTGCACCCCTGGAGACATAC	ACACGAGCAGAACGTTACCC (R218Q)
			TACACGAGCAGAACGTTACC (R218Gfs89)
			GAGCCCCGGCCCCTGCGG (A262T)
BAG3 ex4 frag1	AGCTACAAACAATTTCTGTGACTTT	GCCAGCAGCTCTTTGGTC	N/A
BAG3 ex4 frag2	CTGGAGCAGGCTGTAGACAA	CCTAAAGCACACATCGGTTC	AGGGACGAGCCGATGTGC (R477H)
BAG3 deletion	GACGAGCCAGTCTTGTCTCC (Del)	GCTTGGCTCTGTCAATCCTC	N/A
	GGTTCTCCAGCTTCCTCCTC (WT)		
ATE1 deletion	AGCTGCTAAGCGGTGATTGT	TGCCTGTACCCCCATTGTAT (Del)	N/A
		ATAAGCAGGGTAGGGCTGGT (WT)	

Supplemental Refereces

1. Hoogendoorn, B., Norton, N., Kirov, G., Williams, N., Hamshere, M.L., Spurlock, G., Austin, J., Stephens, M.K., Buckland, P.R., Owen, M.J., and O'Donovan, M.C. (2000). Cheap, accurate and rapid allele frequency estimation of single nucleotide polymorphisms by primer extension and DHPLC in DNA pools. Hum. Genet. 107, 488–493.