Supplementary Tables

Coverage and diagnostic yield of Whole Exome Sequencing for the Evaluation of Cases with Dilated and Hypertrophic Cardiomyopathy

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Supplementary Table S1. Genes covered by three commercial panels for cardiomyopathy

Company	Admera Health[1]	GeneDx[2]	Invitae[3]	Ambry[4]
Product	CardioGxOne (cardiomyopathies general panel)	Cardiomyopathy panel	Cardioyopathies	CMNEXT
Diseases covered	HCM, DCM, RCM, ACM, non-compaction cardiomyopathy, RASopathies, storage diseases, and congenital heart diseases	ARVC, DCM, HCM, LVNC, Noonan syndrome	ARVC, DCM, HCM, Fabry disease, LVNC, transthyretin amyloidosis	ARVD, DCM, HCM, LVNC, RCM
Genes	AARS2, ABCC9, ACAD9, ACADVL, ACTA1, ACTA2, ACTC1, ACTN2, AGK, AGL, AGPAT2, ALMS1, ANK2, ANKRD1, ATPAF2, BAG3, BRAF, BSCL2, CALR3, CASQ2, CAV3, CBL, COQ2, COX15, COX6B1, CRELD1, CRYAB, CSRP3, CTF1, CTNNA3, DES, DLD, DMD, DNAJC19, DOLK, DSC2, DSG2, DSP, DTNA, ELN, EMD, EYA4, FAH, FHL1, FHL2, FHOD3, FKRP, FKTN, FLNA, FLNC, FOXD4, GAA, GATA4, GATA6, GATAD1, GFM1, GJA1, GJA5, GLA, GLB1, GNPTAB, GUSB, HCN4, HFE, HRAS, JAG1, JPH2, JUP, KCNH2, KCNJ2, KCNJ8, KCNQ1, KLF10,	ABCC9 , ACTC1, ACTN2, ALMS1, ALPK3, ANKRD1, BAG3, BRAF, CAV3, CHRM2, CRYAB , CSRP3, DES, DMD, DOLK, DSC2, DSG2, DSP, DTNA, EMD, FHL1, FKRP, FKTN, GATAD1, GLA, HCN4, HRAS, ILK, JPH2, JUP, KRAS, LAMA4, LAMP2, LDB3 , LMNA, MAP2K1, MAP2K2, MIB1, MTND1, MTND5, MTND6, MTTD, MTTG, MTTH, MTTI, MTTK, MTTL1, MTTL2, MTTM, MTTQ, MTTS1, MTTS2, MURC, MYBPC3, MYH6, MYH7, MYL2, MYL3, MYLK2, MYOZ2, MYPN, NEBL , NEXN, NKX2-5, NRAS, PDLIM3, PKP2,	A2ML1, ABCC9, ACADVL, ACTC1, ACTN2, AGL, ALMS1, ANKRD1, BAG3, BRAF, CACNA1C, CALR3, CAV3, CBL, CHRM2, CPT2, CRYAB, CSRP3, CTF1, CTNNA3, DES, DMD, DNAJC19, DOLK, DSC2, DSG2, DSP, DTNA, ELAC2, EMD, EYA4, FHL1, FHL2, FKRP, FKTN, FLNC, GAA, GATA4, GATA6, GATAD1, GLA, HCN4, HRAS, ILK, JPH2, JUP, KRAS, LAMA4, LAMP2, LDB3, LMNA, LRRC10, MAP2K1, MAP2K2, MTO1, MYBPC3, MYH6, MYH7, MYL2, MYL3, MYLK2, MYOM1, MYOZ2, MYPN, NEBL, NEXN,	ABCC9, ACTC1, ACTN2, ANKRD1, BAG3, CRYAB, CSRP3, DES, DMD, DSC2, DSG2, DSP, EMD, EYA4, FKTN, FXN, GATAD1, GLA, JPH2, JUP, LAMA4, LAMP2, LDB3, LMNA, MYBPC3, MYH6, MYH7, MYL2, MYL3, MYOZ2, MYPN, NEXN, NKX2-5, PKP2, PLN, PRKAG2, PTPN11, RAF1, RBM20, RYR2, SCN5A, TAZ, TBX20, TCAP, TGFB3, TMEM43, TMPO, TNNC1, TNNI3, TNNT2, TPM1, TTN, TTR, TXNRD2, VCL, ZASP

	KRAS, LAMA2, LAMA4, LAMP2, LDB3, LIAS, LMNA, MAP2K1, MAP2K2, MIB1, MLYCD, MRPL3, MRPS22, MTO1, MURC, MYBPC3, MYH11, MYH6, MYH7, MYL2, MYL3, MYLK2, MYOT, MYOZ2, MYPN, NEBL, NEXN, NKX2-5, NOTCH1, NRAS, OBSL1, PDHA1, PDLIM3, PHKA1, PITX2, PKP2, PLN, PMM2, PRDM16, PRKAG2, PSEN1, PSEN2, PTPN11, RAF1, RBM20, RYR2, SCN5A, SGCA, SGCB, SGCD, SHOC2, SLC22A5, SLC25A4, SMAD3, SOS1, SPRED1, SURF1, TAZ, TBX1, TBX20, TBX5, TCAP, TGFB3, TMEM43, TMEM70, TMPO, TNNC1, TNNI3, TNNT2, TPM1, TRIM63, TSFM, TTN, TTR, TXNRD2, VCL (n=149)	PLN, PRDM16, PRKAG2, PTPN11, RAF1, RBM20, RIT1, RYR2, SCN5A, SGCD, SOS1, TAZ, TCAP, TGFB3, TMEM43, TMPO, TNNC1, TNNI3, TNNT2, TPM1, TTN, TTR, TXNRD2, VCL (n=91)	NF1, NKX2-5, NPPA, NRAS, PDLIM3, PKP2, PLEKHM2, PLN, PRDM16, PRKAG2, PTPN11, RAF1, RASA1, RBM20, RIT1, RRAS, RYR2, SCN5A, SDHA, SGCD, SHOC2, SLC22A5, SOS1, SOS2, SPRED1, TAZ, TCAP, TGFB3, TMEM43, TMEM70, TMPO, TNNC1, TNNI3, TNNT2, TPM1, TTN, TTR, TXNRD2, VCL (n=105)	(n=56)

1. http://www.admerahealth.com/cardiogxone/cardiomyopathies-general-panel/
2. https://www.genedx.com/test-catalog/available-tests/cardiomyopathy-panel/
3. https://www.invitae.com/en/physician/tests/02251/#test-order
4. http://www.ambrygen.com/tests/cmne

Supplementary Table S2. Methods for applying the ACMG guidelines

Evidence of pathogenicity	ACMG definition	Our working definition
Very strong	PVS1: Null variant (nonsense, frameshift, canonical +/1 1 or 2 splice sites, initiation codon, single or multiexon deletion) in a gene where Truncating is a known mechanism of disease	Truncating variants in genes whose truncating variants are significantly associated with HCM/DCM according to Walsh et al[6] (after correction for multiple testing).
Strong	PS1: Same amino acid change as a previously established pathogenic variant regardless of nucleotide change	A previously established pathogenic variant was defined as one which had a Pathogenic call from the ACMG guideline.
	PS2: De novo in a patient with the disease and no family history	based on literature review
	PS3: Well-established in vitro or in vivo functional studies supportive of a damaging effect on the gene or gene product	based on literature review
	PS4: The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls	Not applicable.
Moderate	PM1: Located in a mutational hot spot and/or critical and well-established functional domain without benign variation	Mutational hotspots for 190 genes were given in the Supplementary material of Maxwell et al[7]
	PM2: Absent from controls in Exome sequencing project, 1000 Genomes Project, or Exome Aggregation Consortium	Absent in the Exac database, the 1000 Genome database, and a cohort of 712 local DDD patients
	PM3: For recessive disorders, detected in trans with a pathogenic variant	Not applicable
	PM4: Protein length changes as a result of in-frame deletions/insertions in a nonrepeat region or stop-loss variants	nonframeshift variants
	PM5: Novel missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before	Like PS1, a previously established pathogenic variant was defined as one which had a Pathogenic call from the ACMG guideline.
	PM6: Assumed de novo, but without confirmation of paternity and maternity	based on literature review
Supporting	PP1: Cosegregation with disease in multiple affected family members in a gene definitively known to cause the disease	If evidence found in Clinvar.

Evidence of pathogenicity	ACMG definition	Our working definition
	PP2: Missense variant in a gene that has a low rate of benign missense variation and in which missense variants are a common mechanism of disease	non-truncating variant found in gene whose RVIS (Residual Variation Intolerance Score) [1] is in the most intolerant 10%, or Truncating variant found in gene whose pLI (probability of Loss-of-function intolerant) [2] score is in the most intolerant 10%.
	PP3: Multiple lines of computational evidence support a deleterious effect on the gene or gene product	Predicted to be pathogenic by Polyphen (HDIV), Polyphen (HVAR)[3], deleterious by SIFT (Sorting Intolerant From Tolerant) [4],and Mutation Taster[5].
	PP4: Patient's phenotype or family history is highly specific for a disease with a single genetic etiology	Not applicable
	PP5: Reputable source recently reports variant as pathogenic, but the evidence is not available to the laboratory to perform an independent evaluation	Not applicable
Benign (stand-alone)	BA1: Allele frequency is >5% in Exome sequencing project, 1000 Genome project, or Exome Aggregation consortium	Not applicable
Benign (Strong)	BS1: Allele frequency is greater than expected for disorder	MAF > 0.1%
	BS2: Observed in a healthy adult individual with full penetrance expected at an early age	Not applicable. Cardiomyopathy not fully penetrant at early age
	BS3: Well-established in vitro or in vivo function studies show no damaging effect on protein function or splicing	Literature review
	BS4: Lack of segregation in affected members of a family	Literature review
Benign (Supporting)	BP1: Missense variant in a gene for which primarily truncating variants are known to cause disease	Missense variants in TTN or SCN5A (according to Walsh et al)
	BP2: Observed in trans with a pathogenic variant for a fully penetrant dominant gene/disorder or observed in cis with a pathogenic variant in any inheritance pattern	Not applicable. Information not available.
	BP3: In-frame deletions/insertions in a repetitive region without a known function	Not applicable. Information not available.
	BP4: Multiple lines of computational evidence suggest no impact on gene or gene product	Predicted to be benign by Polyphen (HDIV), Polyphen (HVAR)[4], deleterious by SIFT[5],and Mutation Taster[3].

Evidence of pathogenicity	ACMG definition	Our working definition
	BP5: Variant found in a case with a an alternate molecular basis for disease	awarded to variants found in patients with variants classified as “Pathogenic”
	BP6: Reputable source recently reports variant as benign	One “Benign” or “Likely benign” classification in Clinvar
	BP7: A synonymous variant for which splicing prediction algorithms predict no impact to the splice consensus sequence nor the creation of a new splice site and the nucleotide is not highly conserved	Not applicable. Synonymous variants are not considered

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2. Lek, M. *et al.* Analysis of protein-coding genetic variation in 60,706 humans. *Nature* **536,** 285–291 (2016).
3. Adzhubei, I. A. *et al.* A method and server for predicting damaging missense mutations. *Nat. Methods* **7,** 248–249 (2010).
4. Kumar, P., Henikoff, S. & Ng, P. C. Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. *Nat. Protoc.* **4,** 1073–1081 (2009).
5. Schwarz, J. M., Cooper, D. N., Schuelke, M. & Seelow, D. MutationTaster2: mutation prediction for the deep-sequencing age. *Nat. Methods* **11,** 361–2 (2014).
6. Walsh, R. *et al.* Reassessment of Mendelian gene pathogenicity using 7,855 cardiomyopathy cases and 60,706 reference samples. *Genet. Med.* 41111 (2016). doi:10.1038/gim.2016.90
7. Maxwell, K. N. *et al.* Evaluation of ACMG-Guideline-Based Variant Classification of Cancer Susceptibility and Non-Cancer-Associated Genes in Families Affected by Breast Cancer. *Am. J. Hum. Genet.* **98,** 801–817 (2016).