Targeted NGS in DNA diagnostics for cardiomyopathy

Improved diagnostic yield with NGS tests

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Cardiomyopathies NL

Targeted panel/ MIP testing versus WES (different per center)

⬇

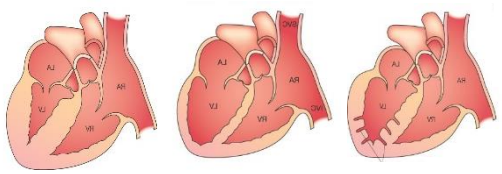
Same test in each center possible?

Goal for now *Screen the same core genes in each center*
Agreement on classification and reporting

Ronald Lekanne Dit Deprez (AMC)
Jan Jongbloed/ Yvonne Vos (UMCG)
Arthur van den Wijngaard/ Debby Hellebrekers (MUMC)
Dennis Dooijes (UMCU)

Diagnostics for Cardiomyopathies

1. 1 panel fits all? Bigger is not always better; prescreen small panel?
   Overlap HCM/DCM/NCCM



2. Variant classification: gnomAD/ splicing effect/ protein pred tools/ ACMG guidelines/
   Gene variant resources: VKGL/ ClinVar/ ClinGen/ HGMD/ Alamut
   Reporting variants; example RYR2/ TTN

3. How we use all these different tools in HCM diagnostics:
   Successes/ Limitations and pitfalls

4. Follow up on VUS in future: where to start?

NGS diagnostics for Cardiomyopathies

Start NGS cardiomyopathies (HCM/DCM/RCM/NCCM) in 2012

↓

46 genes (most of them were core-genes/ Sanger quality)

↓

targeted enrichment

↓

Update panel with ARVC and TTN “complete” in 2013

↓

Change to WES approach in 2017

↓

Evaluation ongoing 5 labs; which genes are core?

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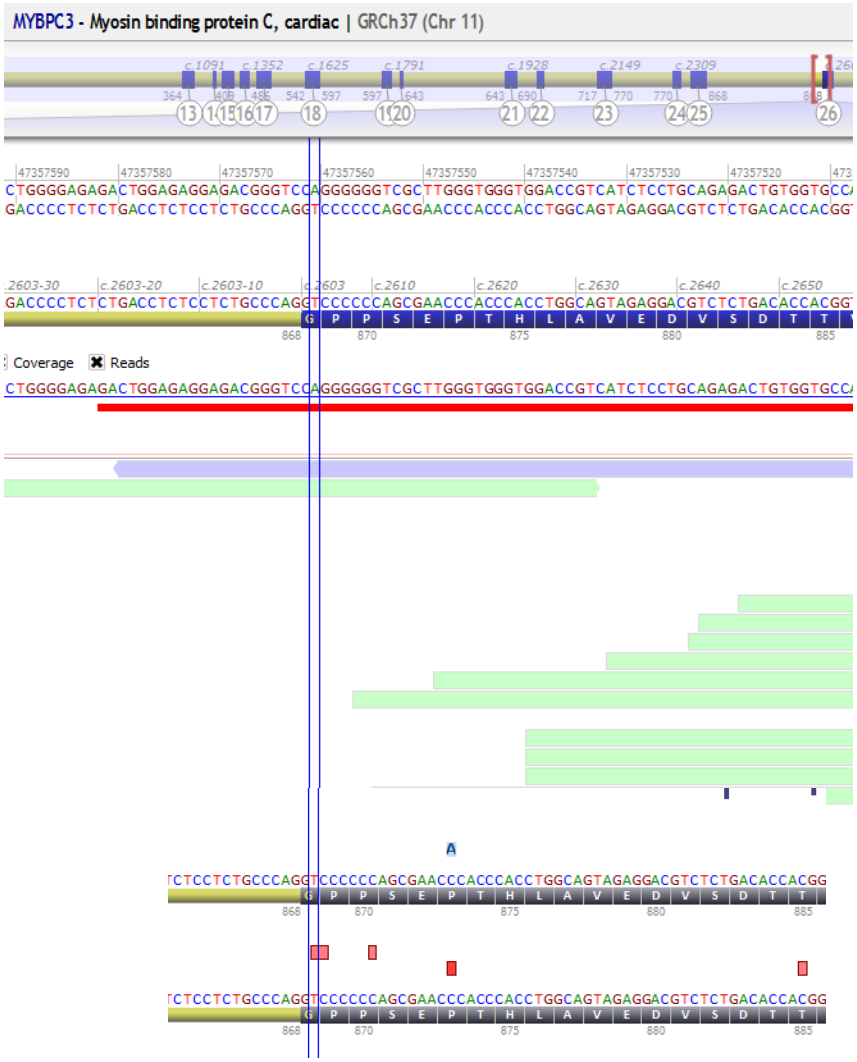
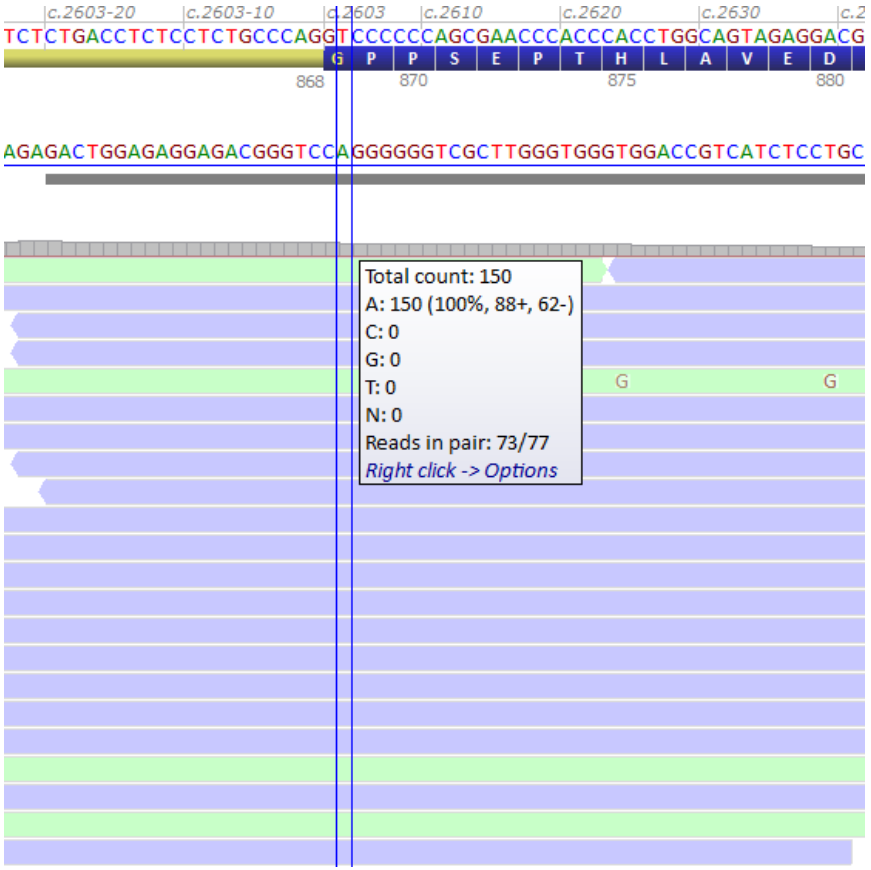
Improved WES coverage

In 2012 WES coverage



In 2017 WES coverage



Improved WES coverage

In 2012 WES coverage

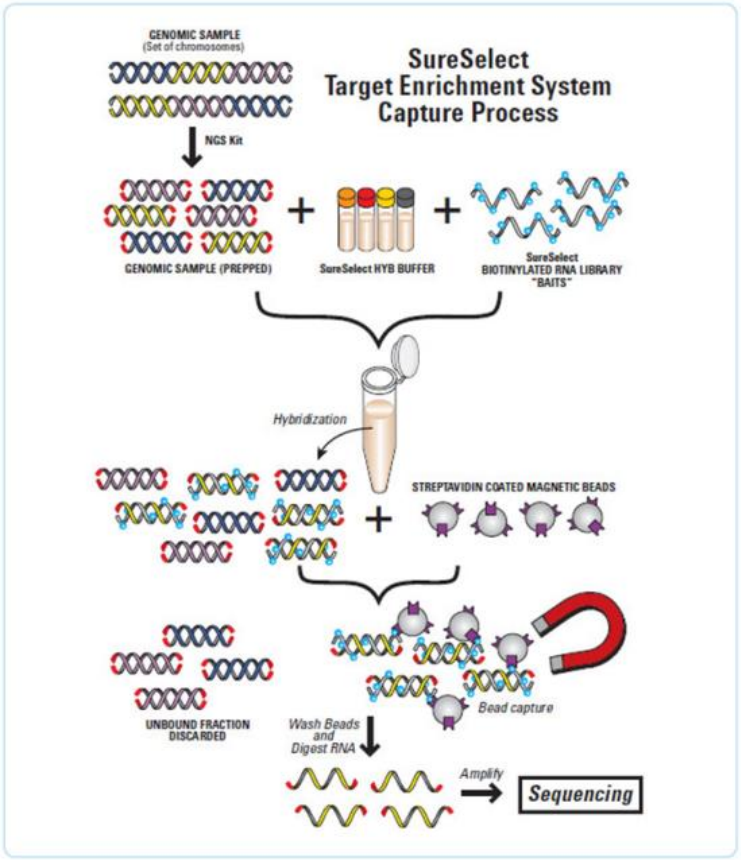
In 2017 WES coverage

WES coverage HCM genes

HGNC approved gene symbol	Phenotype description including OMIM phenotype ID(s)	OMIM gene ID	median depth	% covered >10x	% covered >20x	% covered >30x
MYBPC3	Cardiomyopathy, dilated, 1MM, 615396 Cardiomyopathy, hypertrophic, 4, 115197 Left ventricular noncompaction 10, 615396	600958	169	100	100	98
MYH6	Atrial septal defect 3, 614089 Cardiomyopathy, dilated, 1EE, 613252 Cardiomyopathy, hypertrophic, 14, 613251 {Sick sinus syndrome 3}, 614090	160710	145	100	99	98
MYH7	Cardiomyopathy, dilated, 1S, 613426 Cardiomyopathy, hypertrophic, 1, 192600 Laing distal myopathy, 160500 Left ventricular noncompaction 5, 613426 Myopathy, myosin storage, 608358 Myopathy, myosin storage, 255160 Scapuloperoneal syndrome, myopathic type, 181430	160760	176	100	100	100
MYL2	Cardiomyopathy, hypertrophic, 10, 608758	160781	170	100	100	100
MYL3	Cardiomyopathy, hypertrophic, 8, 608751	160790	118	100	100	100

100%

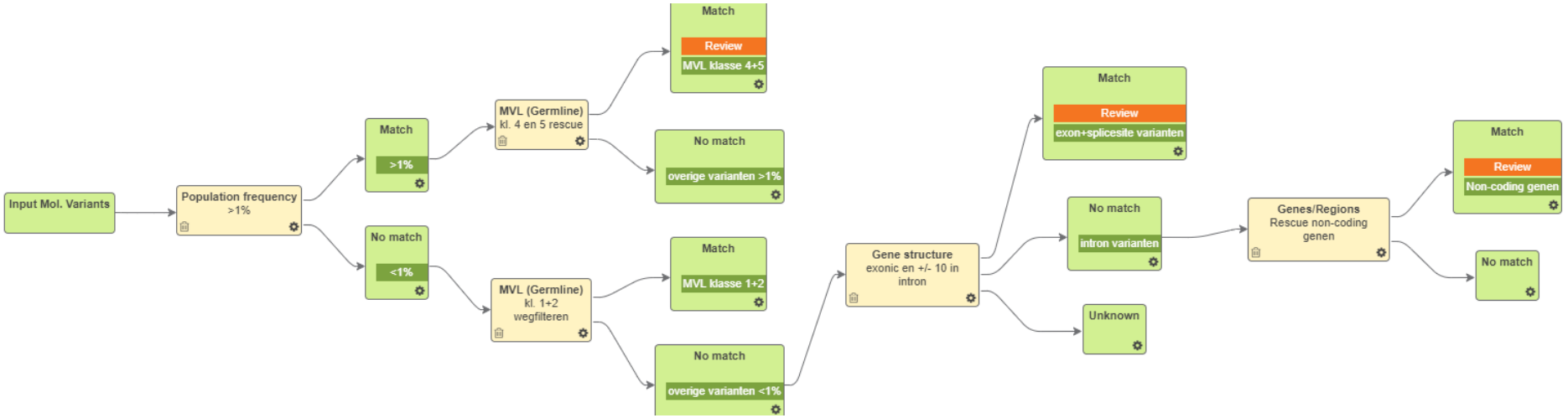
NGS flow



Mapping reads and calling variants with bioinformatic tools
Filtering and annotation with Cartagenia software
Classification with Alamut and available resources

Cartagenia/ Alissa Interpret Filtering

Variants loaded with genepanel filter
Filtering: population frequency, in-house and VKGL database, coding +/- 10, + regions of interest added



Important:
-quality of reads
-quality of base
-coverage of variant

➡ BAM file

Variant classification

Gene	Position	Ref	Patient	Read Depth	Type	Transcript	cDNA	Location	Exon	Effect	Protein
TTN	2:179,454,135	G	G A	40	snp	NM_001267550.1	c.62317C>T	exonic	304	synonymous	p.Leu20773=
EYA4	6:133,783,573	G	G A	79	snp	NM_004100.4	c.538G>A	exonic	8	nonsynonymous	p.Ala180Thr
MYBPC3	11:47,359,280	.	. C	160	insertion	NM_000256.3	c.2373dupG	exonic	24	frameshift	p.Trp792Valfs*41

HGMD Professional

Gene	Position	Classification	External Databases	MVL Eras	MVL Gene	MVL niet	MVL verk	MVL Other	MVL VKGL	MVL Simil.
TTN	2:179,454,135	Likely benign								
EYA4	6:133,783,573	VOUS								
MYBPC3	11:47,359,280	Pathogenic								

HGMD accession	Reported disease/phenotype	Variant class	Gene symbol	Insertion
CI983160	Cardiomyopathy, hypertrophic	DM	MYBPC3	CACAGTA^CAGgTGGGAGCCGC

Literature citation	Citation type	Support	
1. Niimura (1998) *N Engl J Med* **338:** 1248 PubMed: 9562578 Mutations in the gene for cardiac myosin-binding protein C and late-onset familial hypertrophic cardiomyopathy.	Primary literature report	✓	aka c.2373_2374insG/c.2373dupG.
2. Marston (2009) *Circ Res* **105:** 219 PubMed: 19574547 Evidence from human myectomy samples that MYBPC3 mutations cause hypertrophic cardiomyopathy through haploinsufficiency.	Additional literature report		None
3. van Dijk (2009) *Circulation* **119:** 1473 PubMed: 19273718 Cardiac Myosin-Binding Protein C Mutations and Hypertrophic Cardiomyopathy. Haploinsufficiency, Deranged Phosphorylation, and Cardiomyocyte Dysfunction.	Functional characterisation		None
4. Christiaans (2010) *Neth Heart J* **18:** 248 PubMed: 20505798 Founder mutations in hypertrophic cardiomyopathy patients in the Netherlands.	Additional literature report	✓	Founder mutation in Dutch.
5. Yiu (2012) *PLoS One* **7:** e36115 PubMed: 22574137 Myocardial structural alteration and systolic dysfunction in preclinical hypertrophic cardiomyopathy mutation carriers.	Additional literature report		None
6. Birket (2015) *Cell Rep* **13:** 733 PubMed: 26489474 Contractile Defect Caused by Mutation in MYBPC3 Revealed under Conditions Optimized for Human PSC-Cardiomyocyte Function.	Functional characterisation	✓	None
7. Murphy (2016) *J Cardiovasc Transl Res* **9:** 153 PubMed: 26914223 Evaluation of the Mayo Clinic Phenotype-Based Genotype Predictor Score in Patients with Clinically Diagnosed Hypertrophic Cardiomyopathy.	Additional literature report	✓	Descr. in Supplemental Table 2 (online).
8. Wijnker (2016) *J Mol Cell Cardiol* **97:** 82 PubMed: 27108529 Comparison of the effects of a truncating and a missense MYBPC3 mutation on contractile parameters of engineered heart tissue.	Functional characterisation	✓	None
9. Baudhuin (2017) *Circ Cardiovasc Genet* **10:** e001844 PubMed: 29237689 Technical Advances for the Clinical Genomic Evaluation of Sudden Cardiac Death: Verification of Next-Generation Sequencing Panels for Hereditary Cardiovascular Conditions Using Formalin-Fixed Paraffin-Embedded Tissues and Dried Blood Spots.	Additional case report	✓	None
10. Burns (2017) *Circ Cardiovasc Genet* **10:** e001666 PubMed: 28790153 Multiple Gene Variants in Hypertrophic Cardiomyopathy in the Era of Next-Generation Sequencing.	Additional literature report	✓	None
11. Miller (2017) *Circ Cardiovasc Genet* **10:** e001735 PubMed: 29212898 Genetic Testing in Pediatric Left Ventricular Noncompaction.	Additional phenotype	✓	Noncompaction, left ventricular
12. van Velzen (2017) *Circ Cardiovasc Genet* **10:** e001660 PubMed: 28794111 Clinical Characteristics and Long-Term Outcome of Hypertrophic Cardiomyopathy in Individuals With a MYBPC3 (Myosin-Binding Protein C) Founder Mutation.	Additional literature report	✓	None
13. Viswanathan (2017) *PLoS One* **12:** e0187948 PubMed: 29121657 Hypertrophic cardiomyopathy clinical phenotype is independent of gene mutation and mutation dosage.	Additional literature report	✓	Potentially recessive. See Table S4 and S5.

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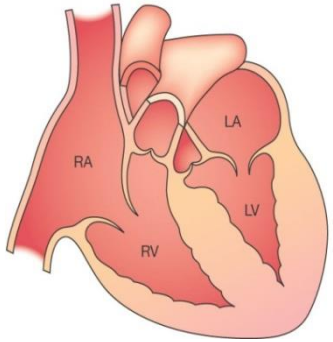
What to confirm/ report in this case?



Single HCM patient with current CMP panel;

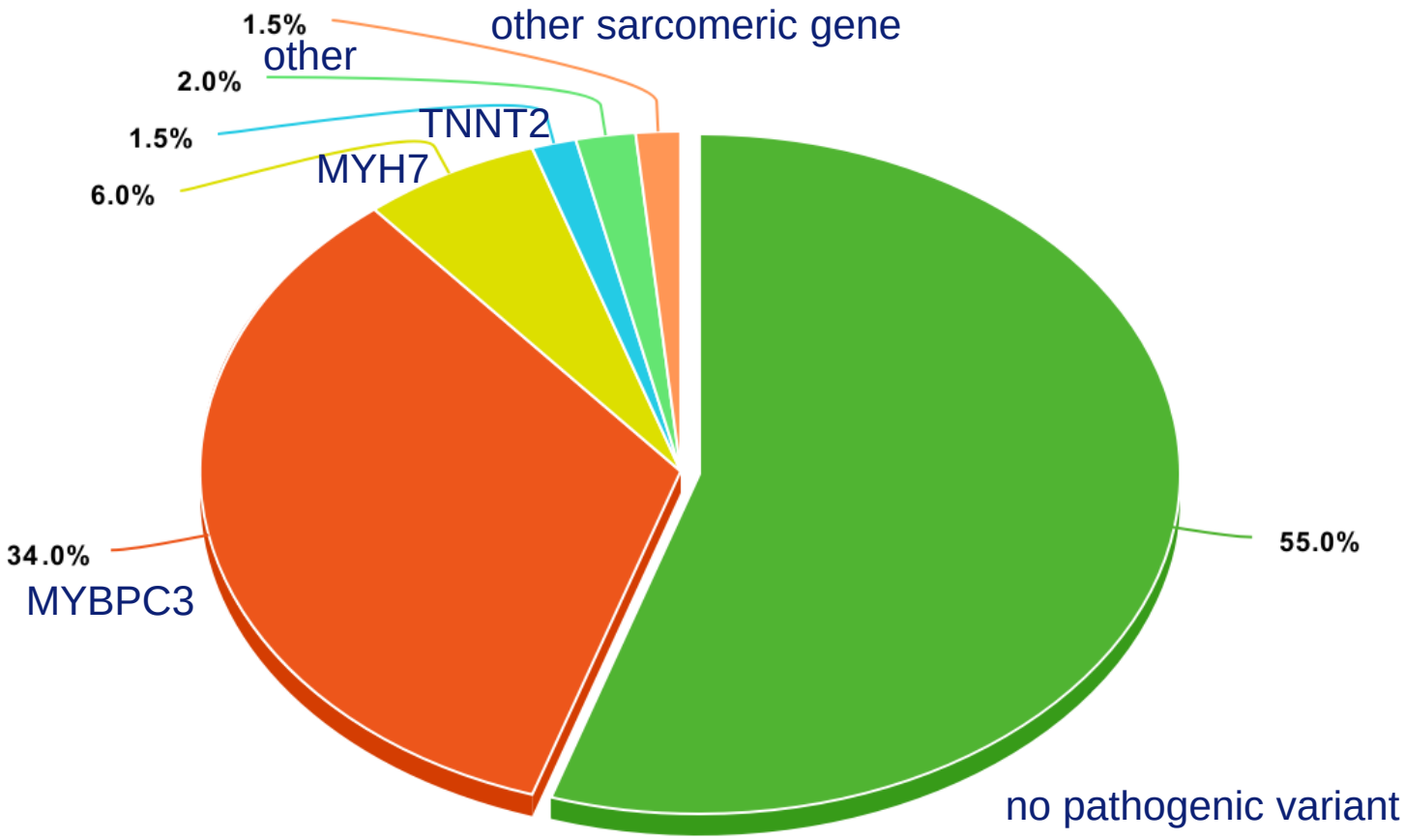
Class 4 or 5 that confirms the diagnosis; MYBPC3 pathogenic variant

All other class 3 variants! EYA4 variant (involvement in DCM?);
this might change in future

Conclusion in letter: Diagnosis HCM has been confirmed.
Further testing for the pathogenic variant in the familiy of tested person is possible

With smaller HCM panel only MYBPC3 found and reported

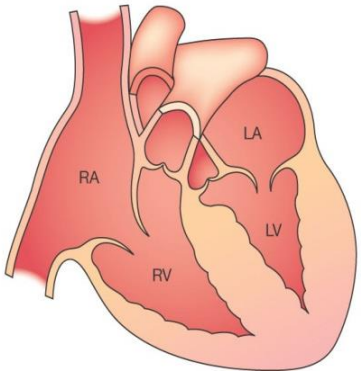
Diagnostic yield NGS for HCM



meta-chart.com

Pathogenic variants HCM

Gene	#
MYBPC3	430
MYH7	76
TNNT2	18
TNNI3	5
TPM1	7
MYL2	5
CSRP3	1
TCAP	X
ACTC1	1
MYL3	3
GLA	2
LAMP2	1
PRKAG2	1
TTR	4
FHL1	1
FLNC	X
DES	X



Gene	#
PTPN11	1
ACTN2	X
ALPK3	AD/ AR
DSC2	1XSS
TAZ	1
PLN	3
CRYAB	1XSS
PKP2	3
RBM20	X
TNNC1	1
TTN	X
JPH2	X

Panel evaluation

UMCU	AMC	EURMC	MUMC	UMCG
ABCC9		ABCC9		ABCC9
ACTC1	ACTC1	ACTC1	ACTC1	ACTC1
ACTN2	ACTN2	ACTN2	ACTN2	ACTN2
ANKRD1		ANKRD1	ANKRD1	ANKRD1
	ALPK3	ALPK3		ALPK3
BAG3	BAG3	BAG3	BAG3	BAG3
CALR3		CALR3	CALR3	CALR3
CAV3		CAV3	CAV3	CAV3
CRYAB		CRYAB	CRYAB	CRYAB
CSRP3	CSRP3	CSRP3	CSRP3	CSRP3
CTNNA3	CTNNA3	CTNNA3	CTNNA3	CTNNA3
DES	DES	DES	DES	DES
DSC2	DSC2	DSC2	DSC2	DSC2
DSG2	DSG2	DSG2	DSG2	DSG2
DSP	DSP	DSP	DSP	DSP
FHL1	FHL1	FHL1	FHL1	FHL1
FLNC	FLNC	FLNC	FLNC	FLNC
GLA	GLA	GLA	GLA	GLA
JPH2	JPH2	JPH2	JPH2	JPH2
JUP	JUP	JUP	JUP	JUP
LAMA4	LAMA4	LAMA4	LAMA4	LAMA4
LAMP2	LAMP2	LAMP2	LAMP2	LAMP2
LDB3	LDB3	LDB3	LDB3	LDB3
LMNA	LMNA	LMNA	LMNA	LMNA
MIB1	MIB1	MIB1	MIB1	MIB1
MYBPC3	MYBPC3	MYBPC3	MYBPC3	MYBPC3
MYH6	MYH6	MYH6	MYH6	MYH6
MYH7	MYH7	MYH7	MYH7	MYH7

UMCU	AMC	EURMC	MUMC	UMCG
MYL2	MYL2	MYL2	MYL2	MYL2
MYL3	MYL3	MYL3	MYL3	MYL3
MYPN	MYPN	MYPN	MYPN	MYPN
NEXN	NEXN	NEXN	NEXN	NEXN
PKP2	PKP2	PKP2	PKP2	PKP2
PLN	PLN	PLN	PLN	PLN
	PPA2	PPA2		
PRKAG2	PRKAG2	PRKAG2	PRKAG2	PRKAG2
RBM20	RBM20	RBM20	RBM20	RBM20
RYR2		RYR2		RYR2
SCN5A	SCN5A	SCN5A	SCN5A	SCN5A
TAZ	TAZ	TAZ	TAZ	TAZ
TCAP	TCAP	TCAP	TCAP	TCAP
TMEM43	TMEM43	TMEM43	TMEM43	TMEM43
TNNC1	TNNC1	TNNC1	TNNC1	TNNC1
TNNI3	TNNI3	TNNI3	TNNI3	TNNI3
TNNT2	TNNT2	TNNT2	TNNT2	TNNT2
TPM1	TPM1	TPM1	TPM1	TPM1
TTN	TTN	TTN	TTN	TTN
TTR	TTR	TTR	TTR	TTR
VCL		VCL	VCL	VCL

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Genes for HCM

DEFINITIVE-MODERATE-LIMITED-NO EVIDENCE

Circ Genom Precis Med. 2019 Feb;12(2):e002460. doi: 10.1161/CIRCGEN.119.002460.

Evaluating the Clinical Validity of Hypertrophic Cardiomyopathy Genes.

Ingles J[1,2], Goldstein J[3], Thaxton C[3], Caleshu C[4], Corty EW[3], Crowley SB[3], Dougherty K[5], Harrison SM[6], McGlaughon J[3], Milko LV[3], Morales A[7], Seifert BA[3], Strande N[3], Thomson K[8], Peter van Tintelen J[9], Wallace K[3], Walsh R[10,11], Wells Q[12], Whiffin N[10,11], Witkowski L[13], Semsarian C[1,2], Ware JS[10,11], Hershberger RE[7,14], Funke B[13].

Conclusions

- 2500 patients screened/ diagnostic yield;
  - HCM; 45%
  - DCM; 30%
  - NCCM; 26%
- Diagnostic yield in HCM is slightly increased
  - many patients that were negative for Sanger were tested again
  - less founder variants identified
- Diagnostic yield is doubled for DCM and NCCM (TTN)
- Turn-around-time improved
- Pathogenis variants found in HCM in ARVC genes
- Datasharing has improved diagnostic outcome

Advantage WES in DNA diagnostics

- Cardial screening because SCD brother 14-years
- MRI: Dilated LV
- Dec 2017: hospitalized because chestpain and dyspnoea after alcohol consumption. Elevated CK and myoglobin; rhabdomyolyse
- ICD implantation
- NGS cardio and mitochip (MUMC) negative.
- Literature screen: alcohol induced phenotype: PPA2 gene.
- In WES data 2 known pathogenic missense variants in PPA2

REPORT

Biallelic *PPA2* Mutations Cause
Sudden Unexpected Cardiac Arrest in Infancy

Anne Guimier,[1] Christopher T. Gordon,[1] François Godard,[2] Gianina Ravenscroft,[3] Myriam Oufadem,[1]

Questions?

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