Next Generation Sequencing in cardiogenetics

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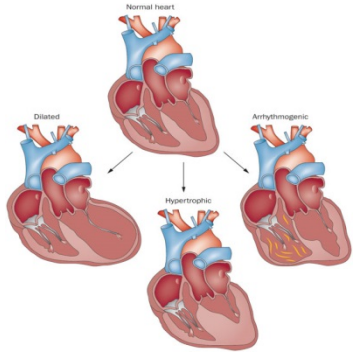
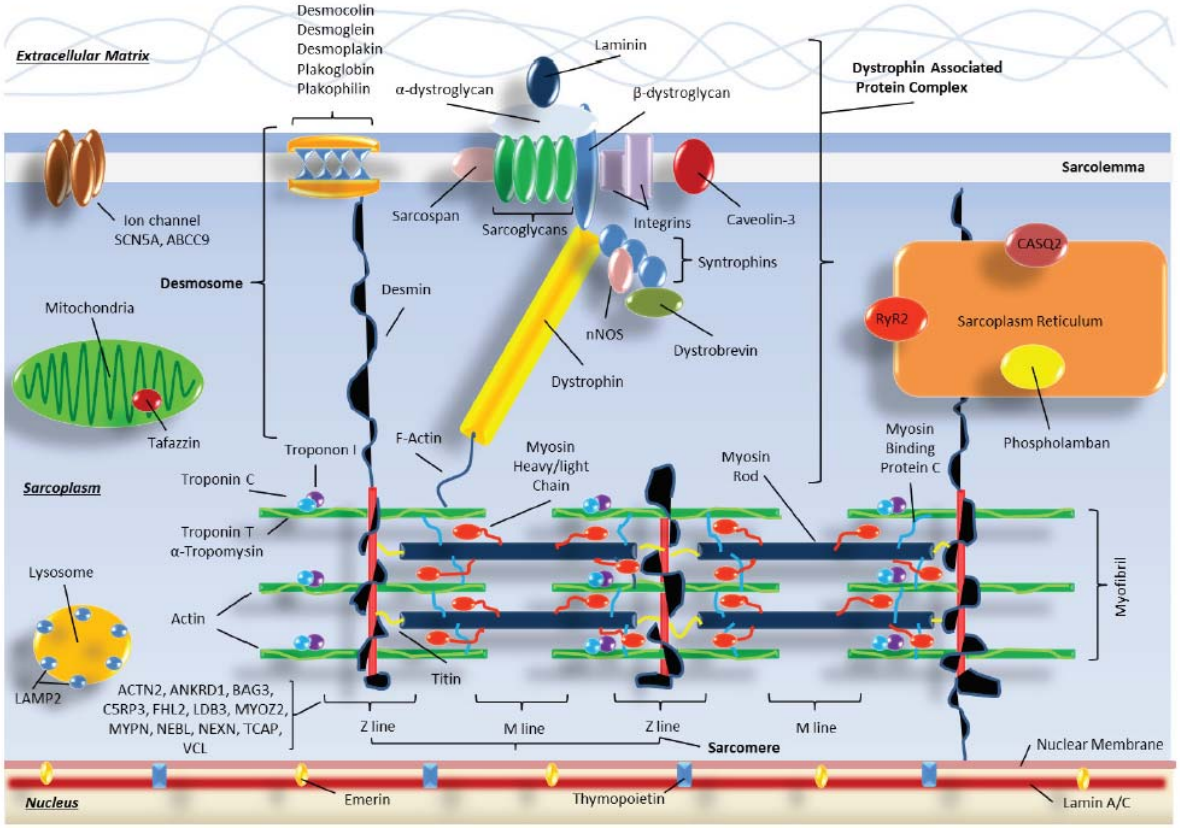
Cardiogenetics

- Search for a genetic cause underlying the cardiac phenotype (cardiomyopathy, arrhythmia)



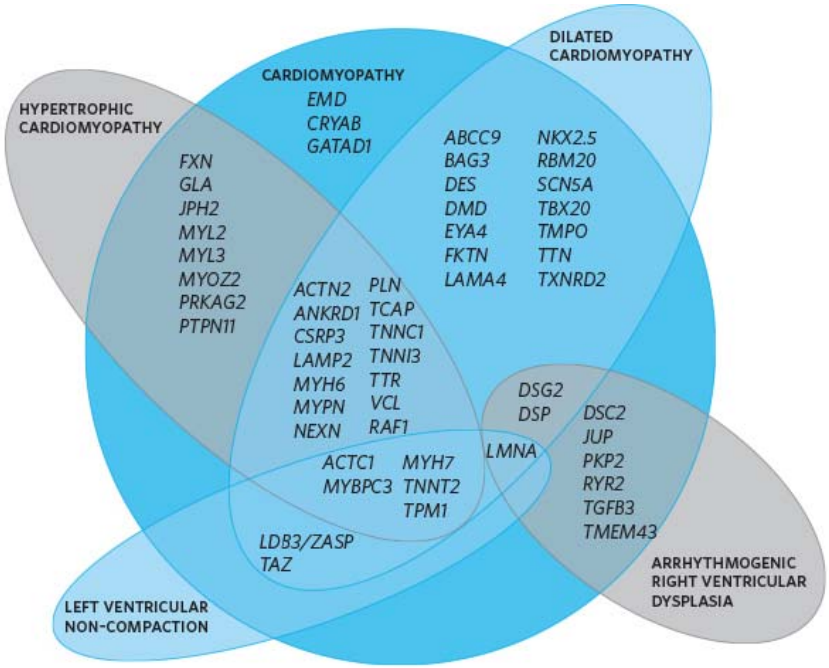
- Identify patients with increased risk of cardiac disease and sudden death
- Indicate periodic cardiologic follow-up
- Give advice regarding treatment, lifestyle, medication (farmacogenetics, personalized medicine)

Genes involved in cardiomyopathy

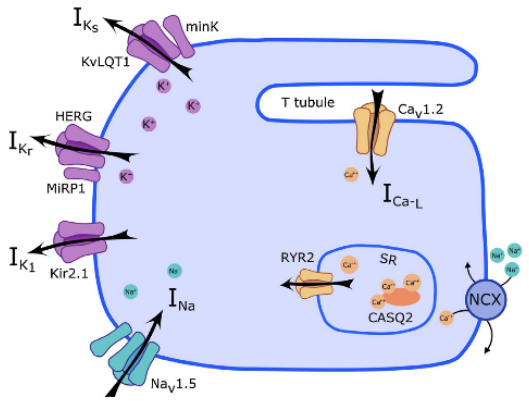


Phelan et al OA Genetics 2013;1(1):9

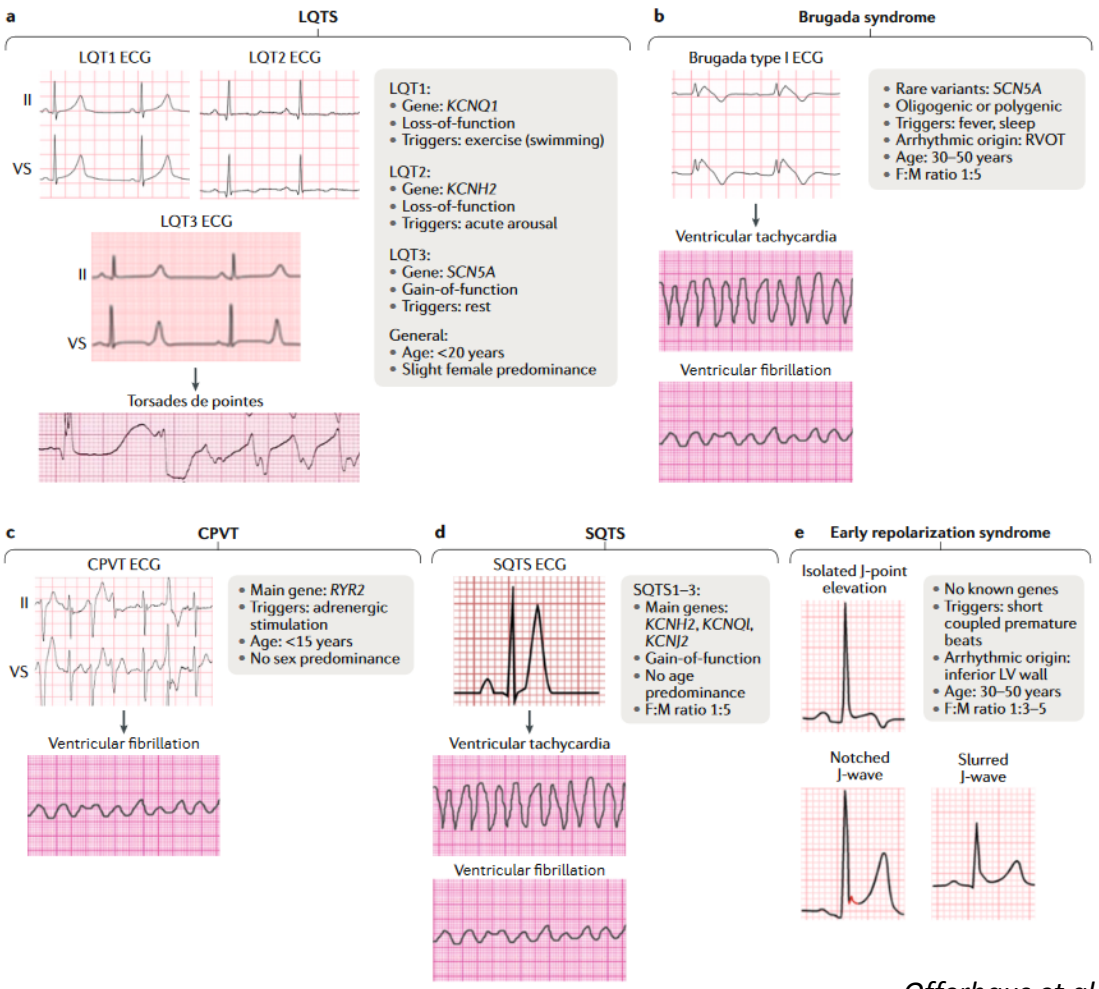
Cardiomyopathy disease genes



Arrhythmia genes (LQT, BrS, CPVT, SQT, SSS)



Skinner et al Heart Lung Circ. 2019;28(1):22-30



Offerhaus et al Nat Rev Cardiol. 2019 Oct 3

ACMG STANDARDS AND GUIDELINES | Genetics inMedicine

Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology

Sue Richards, PhD[1], Nazneen Aziz, PhD[2,16], Sherri Bale, PhD[3], David Bick, MD[4], Soma Das, PhD[5], Julie Gastier-Foster, PhD[6,7,8], Wayne W. Grody, MD, PhD[9,10,11], Madhuri Hegde, PhD[12], Elaine Lyon, PhD[13], Elaine Spector, PhD[14], Karl Voelkerding, MD[13] and Heidi L. Rehm, PhD[15]; on behalf of the ACMG Laboratory Quality Assurance Committee

Class 5: Pathogenic

Class 4: Likely pathogenic

Class 3: Variant of Unknown Significance

Class 2: Likely benign

Class 1: Benign

Evidence of pathogenicity	Category
Very strong	PVS1 null variant (nonsense, frameshift, canonical ±1 or 2 splice sites, initiation codon, single or multiexon deletion) in a gene where LOF is a known mechanism of disease Caveats: • Beware of genes where LOF is not a known disease mechanism (e.g., *GFAP*, *MYH7*) • Use caution interpreting LOF variants at the extreme 3′ end of a gene • Use caution with splice variants that are predicted to lead to exon skipping but leave the remainder of the protein intact • Use caution in the presence of multiple transcripts
Strong	PS1 Same amino acid change as a previously established pathogenic variant regardless of nucleotide change Example: Val→Leu caused by either G>C or G>T in the same codon Caveat: Beware of changes that impact splicing rather than at the amino acid/protein level PS2 De novo (both maternity and paternity confirmed) in a patient with the disease and no family history Note: Confirmation of paternity only is insufficient. Egg donation, surrogate motherhood, errors in embryo transfer, and so on, can contribute to nonmaternity. PS3 Well-established in vitro or in vivo functional studies supportive of a damaging effect on the gene or gene product Note: Functional studies that have been validated and shown to be reproducible and robust in a clinical diagnostic laboratory setting are considered the most well established. PS4 The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls Note 1: Relative risk or OR, as obtained from case–control studies, is >5.0, and the confidence interval around the estimate of relative risk or OR does not include 1.0. See the article for detailed guidance. Note 2: In instances of very rare variants where case–control studies may not reach statistical significance, the prior observation of the variant in multiple unrelated patients with the same phenotype, and its absence in controls, may be used as moderate level of evidence.
Moderate	PM1 Located in a mutational hot spot and/or critical and well-established functional domain (e.g., active site of an enzyme) without benign variation PM2 Absent from controls (or at extremely low frequency if recessive) (Table 6) in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium Caveat: Population data for insertions/deletions may be poorly called by next-generation sequencing. PM3 For recessive disorders, detected in *trans* with a pathogenic variant Note: This requires testing of parents (or offspring) to determine phase. PM4 Protein length changes as a result of in-frame deletions/insertions in a nonrepeat region or stop-loss variants PM5 Novel missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before Example: Arg156His is pathogenic; now you observe Arg156Cys Caveat: Beware of changes that impact splicing rather than at the amino acid/protein level. PM6 Assumed de novo, but without confirmation of paternity and maternity
Supporting	PP1 Cosegregation with disease in multiple affected family members in a gene definitively known to cause the disease Note: May be used as stronger evidence with increasing segregation data 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 PP3 Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc.) Caveat: Because many in silico algorithms use the same or very similar input for their predictions, each algorithm should not be counted as an independent criterion. PP3 can be used only once in any evaluation of a variant. PP4 Patient's phenotype or family history is highly specific for a disease with a single genetic etiology PP5 Reputable source recently reports variant as pathogenic, but the evidence is not available to the laboratory to perform an independent evaluation

Increased throughput of genetic analysis

<2007: **Sanger sequencing** (single genes, small gene panels)

2007: Affymetrix Custom **resequencing Chip** 300K
Allele Specific Oligonucleotide Hybridizations
34 cardiomyopathy genes
320 patients

2010: **Pyrosequencing** Roche 454 Life Sciences
LR-PCR 34 cardiomyopathy genes
64 patients

2012: Illumina HighSeq2000 **WES**
45 cardiomyopathy, 42 arrhythmia genes
425 patients

2015: Single-Molecule Molecular Inversion Probes (**smMIPs**)
cardio45, arrhythmia27, TTN, (2018: FLNC)
Illumina NextSeq500

Maastricht UMC+

Laboratorium Klinische Genetica

Cardiogenetica

NGS targeting-panel*, kwaliteit A

(uitslagtermijn 1-2 maanden)

☐ **Cardiomyopathie core targeting-panel (45 genen)***
(HCM, DCM, ARVD/C, LNVC, RCM)

ACTC1, ACTN2, ANKRD1, BAG3, CAV3, CRYAB, CSRP3, CTNNA3, DES, DSC2, DSG2, DSP, EMD, FLH1, GLA, JPH2, JUP, LAMA4, LAMP2, LDB3, LMNA, MIB1, MYBPC3, MYH6, MYH7, MYL2, MYL3, MYOZ2, MYPN, NEXN, PKP2, PLN, PRDM16, PRKAG2, RBM20, SCN5A, TAZ, TCAP, TMEM43, TNNC1, TNNI3, TNNT2, TPM1, TTR, VCL.

☐ **Aritmie targeting-panel (27 genen)***
(LQT, SQT, BrS, CPVT, SSS)

ABCC9, AKAP9, ANK2, CACNA1C, CACNA2D1, CACNB2, CALM1, CASQ2, CAV3, DPP6 c.-340C>T (founder), GPD1L, HCN4, KCNE1, KCNE2, KCNE3,KCNH2, KCNJ2,KCNJ8, KCNQ1, LMNA, RYR2, SCN1B, SCN3B, SCN5A, SNTA1,TNNT2, TRDN

☐ **FLNC***

☐ **TTN***

Whole exome sequencing (WES), kwaliteit C**

(uitslagtermijn 120 dagen)

☐ **Hartpanel** (298 genen)** alleen na NGS targeting-pakket, of anders na overleg.

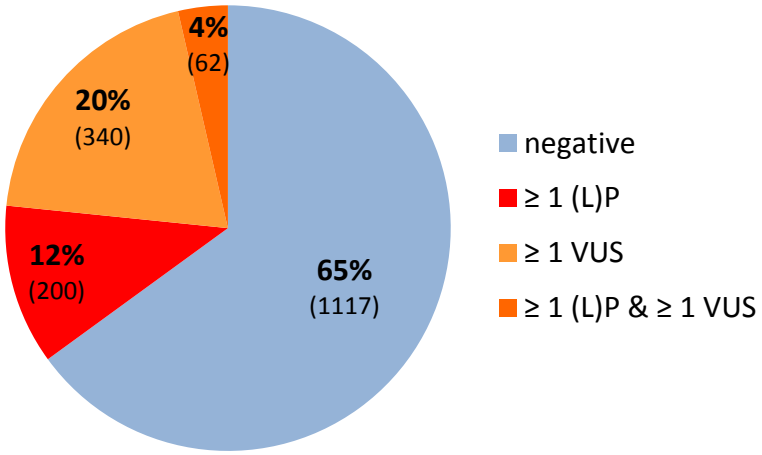
☐ **Congenitale hartziektepanel** (58 genen)**

WES hartziekte en/of congenitale hartziekte inclusief CNV-analyse kunt u ook via het WES-aanvraagformulier aanvragen, zie https://klinischegenetica.mumc.nl/aanvraagformulieren-laboratoriumdiagnostiek

Voor de samenstelling van de WES-panels zie:
https://www.radboudumc.nl/patientenzorg/onderzoeken/erfelijkheidsonderzoek-exoomsequencing-wes/exoompanelsvoorgaandeversies

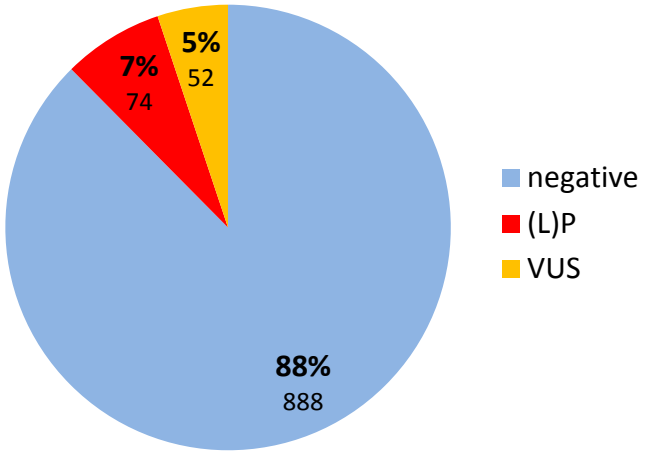
Yield CM panel (45 genes) & TTN
2015-present

1719 patients (45 genes)



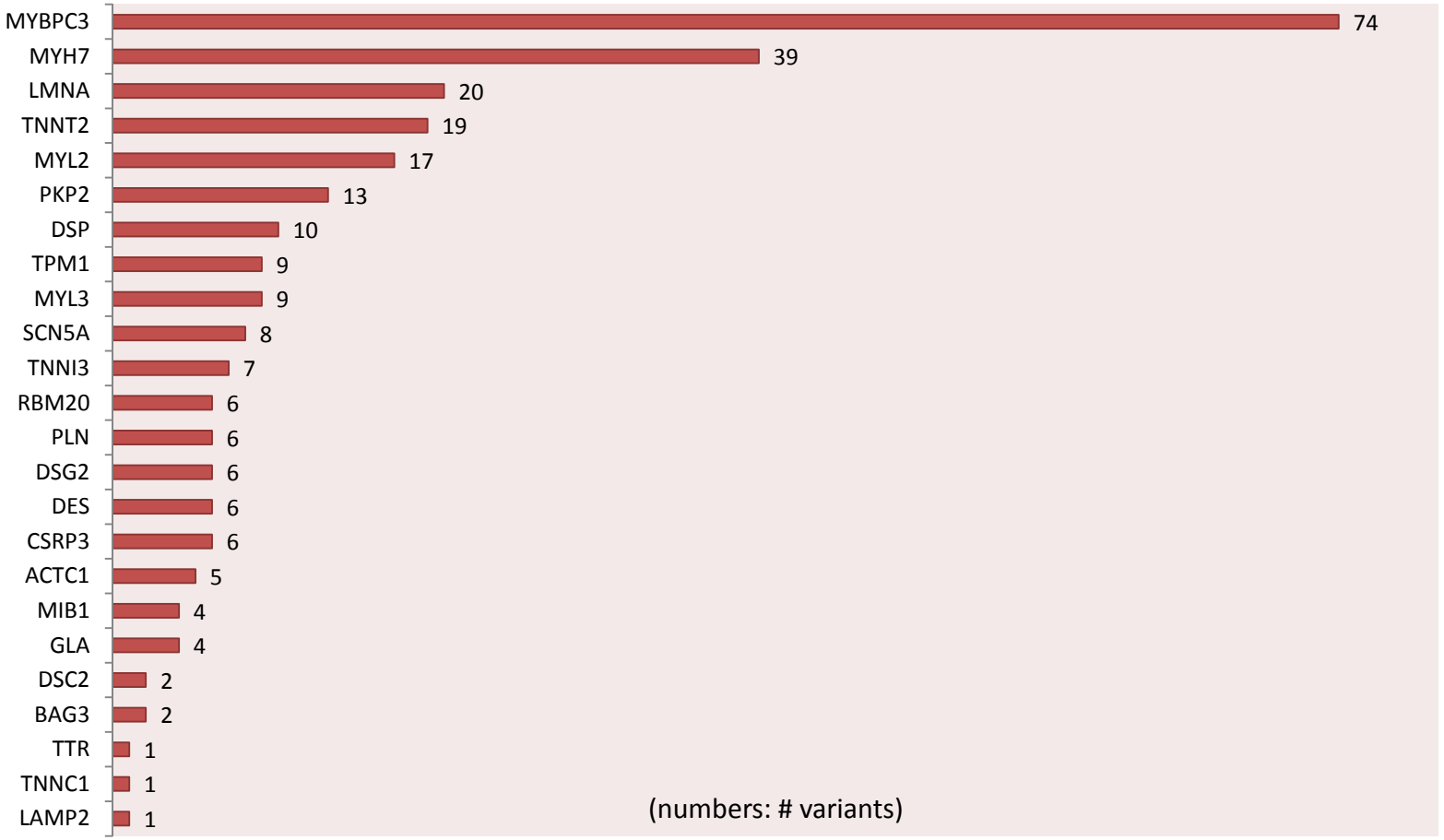
% of patients
(# of patients)

1014 patients (TTN)

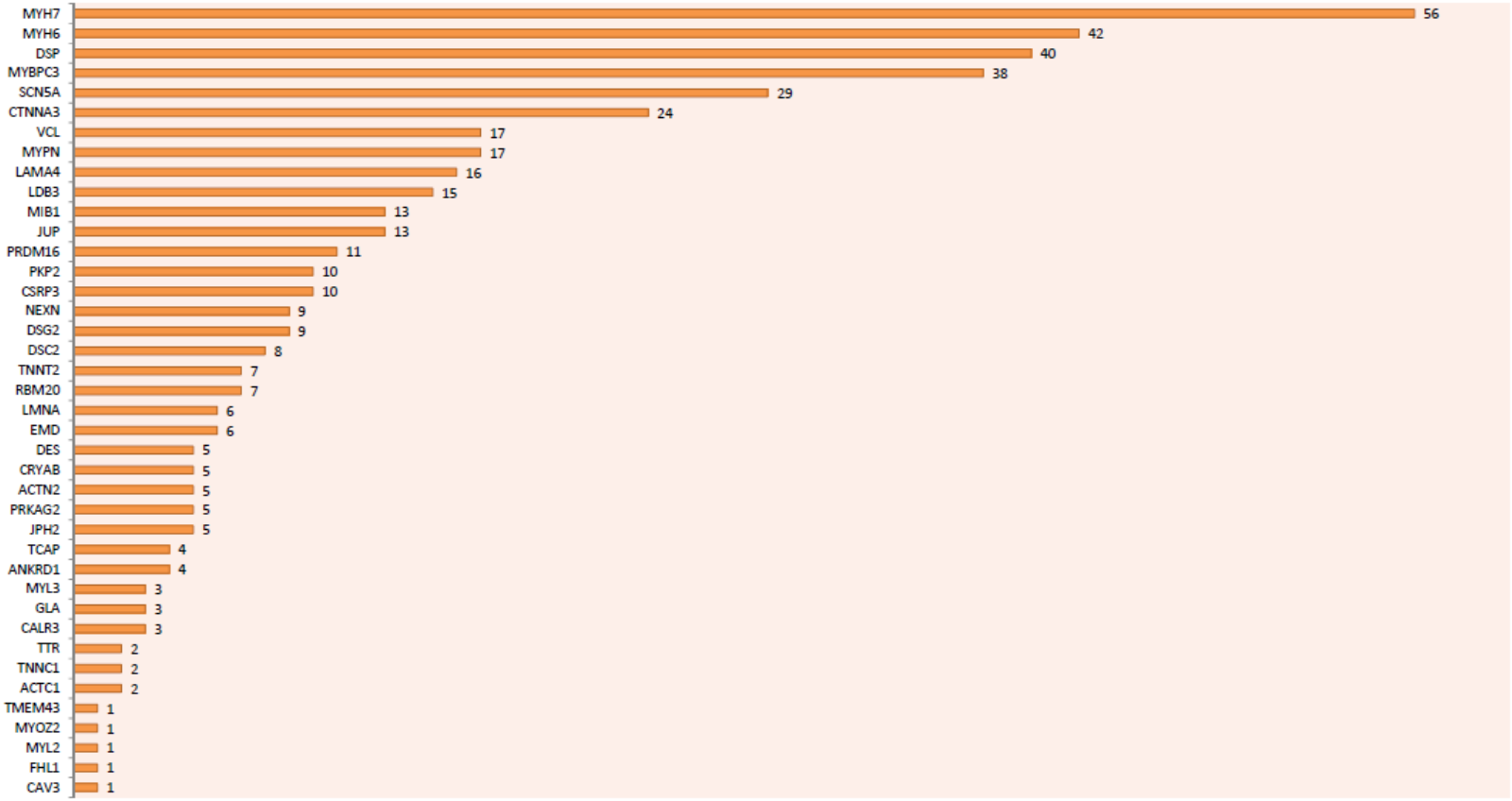


% of patients
(# of patients)

274 (L)P variants in CM panel (45 genes)

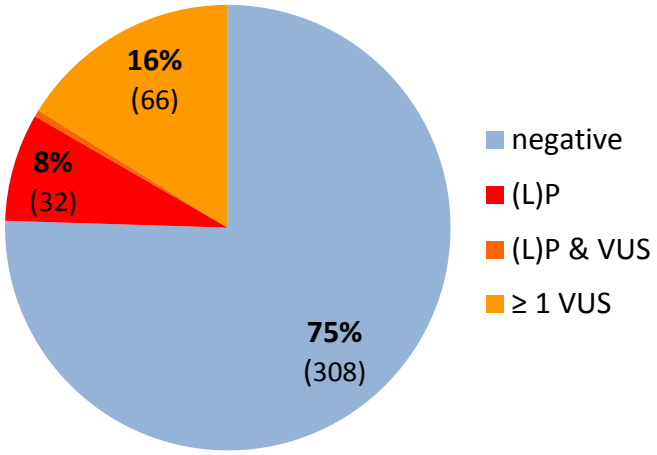


457 VUS in CM panel (45 genes)



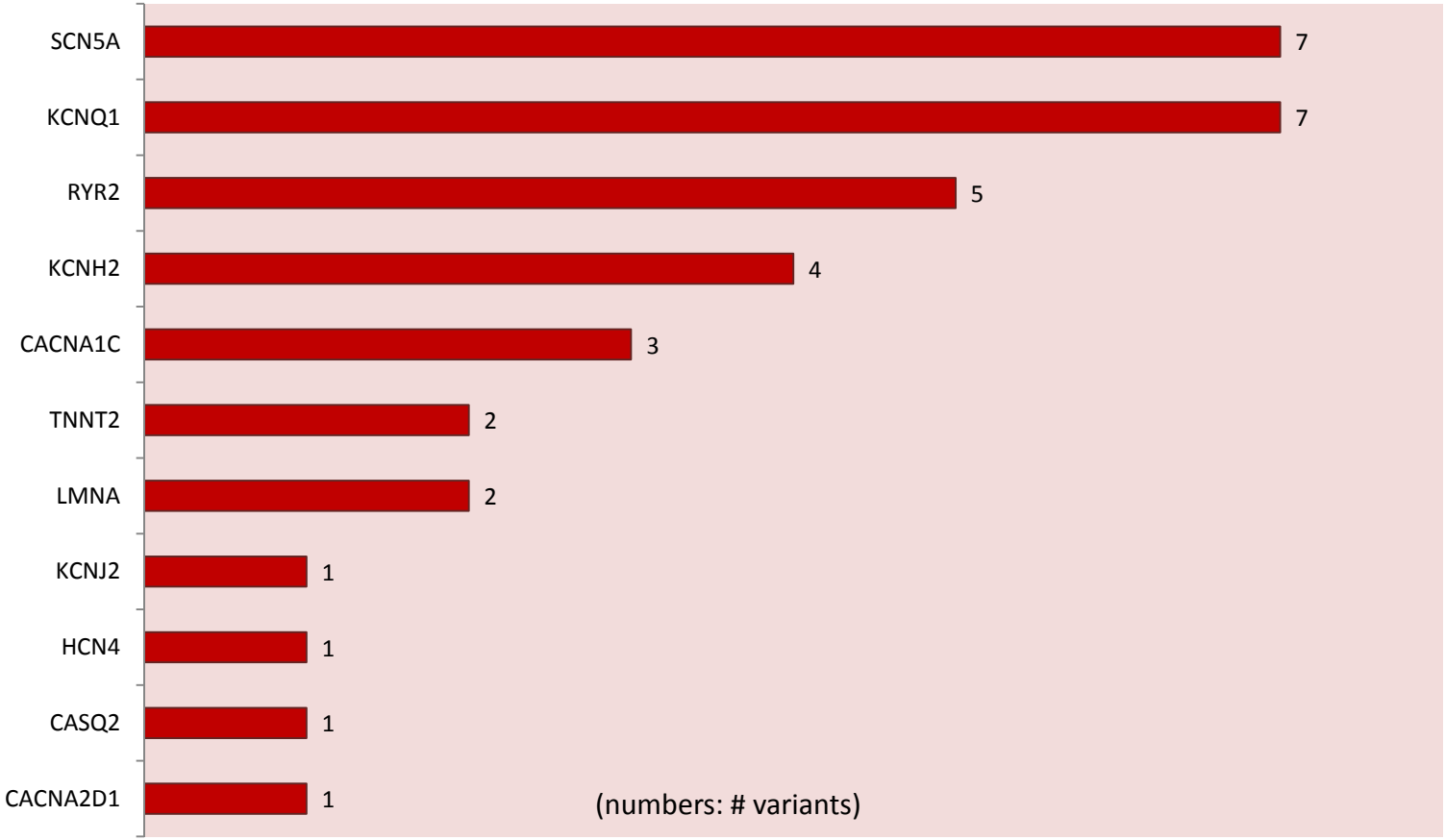
(numbers: # variants)

Yield arrhythmia panel (27 genes)
408 patients, 2015-present

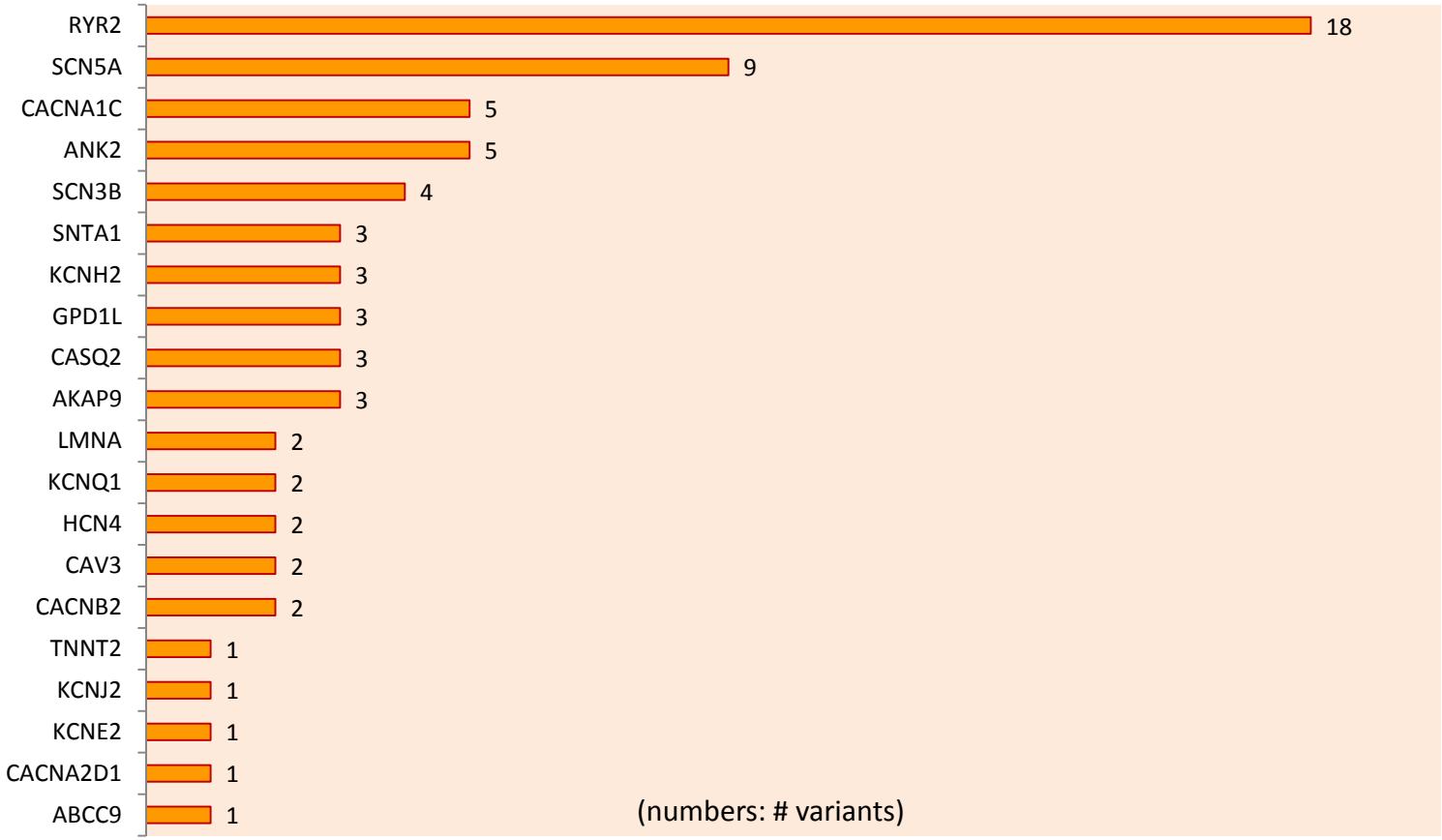


% of patients
(# of patients)

34 (L)P variants in arrhythmia panel (27 genes)



71 VUS in arrhythmia panel (27 genes)



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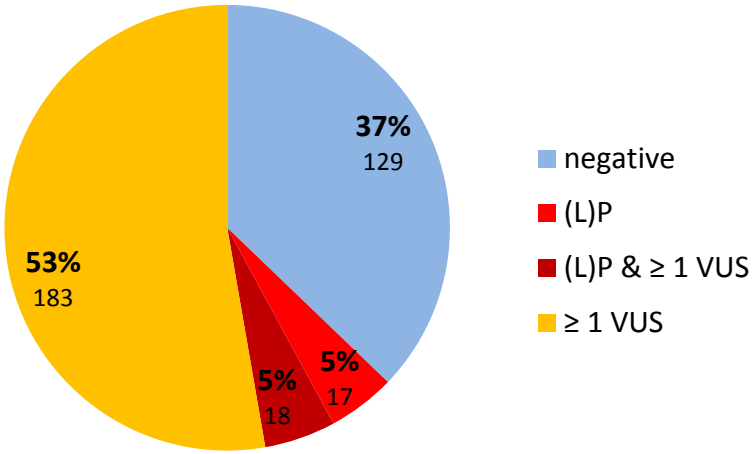
Heart WES panel

- Cardiomyopathy (45) and arrhythmia (27) genes, TTN, FLNC
- Congenital Heart Disease genes (52)
- Thoracic Aortic Aneurysm and Dissection (30)
- Noonan genes with HCM
- LGMD genes with heart phenotype
- Metabolic genes with heart phenotype
- Other genes with cardiac phenotype in HGMD

Yield WES heart panel (350 genes)
347 patients, 2016 - March 2019

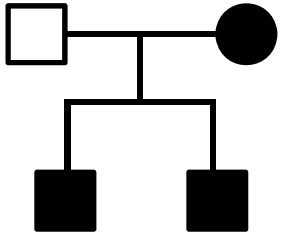


(numbers: # patients)

Yield WES heart panel (350 genes)
347 patients, 2016 - March 2019

- 35 (L)P variants: 27/35 already found in CM/arrhythmia panel
  3/35 class 5: explaining the clinical phenotype
  5/35 class 4: not explaining the clinical phenotype
- Many VUS: some in genes lacking convincing evidence of being a cardiac disease gene
- Revise heart gene panel (350 → 300 genes) by removing:
  - genes of which association to heart disease is too weak
  - genes associated with clear extracardiac phenotype (syndromic)
- WES heart panel in case of:
  - age < 50
  - extra-cardiac phenotype
  - pedigree

Identification of novel disease genes: ‘shared’ WES



- Common missense variant in *FLNC* gene

Conclusions

- Parallel sequencing of ‘core genes’ for genetic testing of patients suspected of genetic cardiomyopathy / arrhythmia gives the highest diagnostic yield
- Critical re-evaluation NL ‘core panel’
- Additional genes (Heart WES gene panel): minimal additional yield
- Heart panel only for limited cases (age < 50, extra-cardiac phenotype, pedigree)

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