Inherited arrhythmia syndromes V:

Genetics of Sudden Cardiac Death

Targeted screening or whole genome sequencing

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PEDIRHYTHM VII

Thessaloniki, Greece feb 4-7 201

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Why genetic testing in SCD patient?

- Confirm genetic character:
  - Recurrence risk
  - enables genetic cascade screening in **family members** (presymptomatic/ prenatal)
- Confirm diagnosis
- Risk stratification (limited; e.g. LQTS/ LMNA/ PLN/ multiple mutations)
- Scientific point of view (understanding disease; finding new genes/modifiers)

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C

	Pathogenic → Supporting	Moderate	Strong	Very strong
Population data		Absent in 1000G and ESP	Prevalence in affected individuals statistically significantly increased over controls	
Computational and predictive data	Multiple lines of computational evidence support a deleterious effect on the gene/gene product	Novel missense change at an amino acid residue where a different pathogenic missense change has been seen before In-frame INDELs in a nonrepeat region or stop-loss variants	Same amino acid change as an established pathogenic variant	Truncating variant in a gene where LOF is a known mechanism of disease
Functional data	Missense in gene with low rate of benign missense variants and pathogenic missense common		Well-established functional studies show a deleterious effect	
Segregation data	Co-segregation with disease in multiple affected family members	→ Increased segregation data		
***De novo* data**		*De novo* (without paternity and maternity confirmed)	*De novo* (paternity and maternity confirmed)	
Allelic data		For recessive disorders, detected in trans with a pathogenic variant		
Other database	Reputable database = pathogenic			
Other data	Patient's phenotype highly specific for gene			

Not every published "pathogenic" variant is really pathogenic! (10-30%?)

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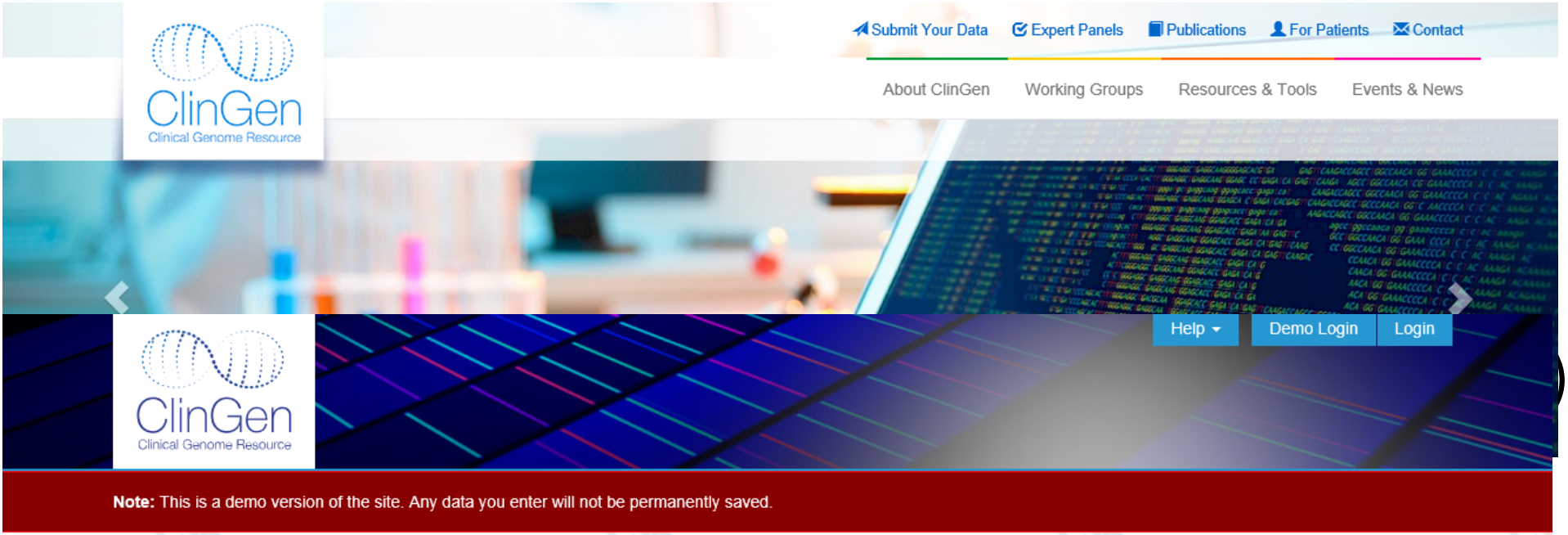
Classification variants

www.clinicalgenome.org



ClinGen Curator Interfaces

- class 1 not pathogenic (<1%)

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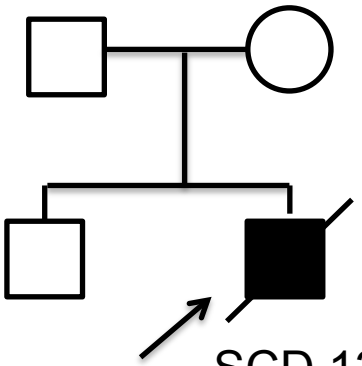


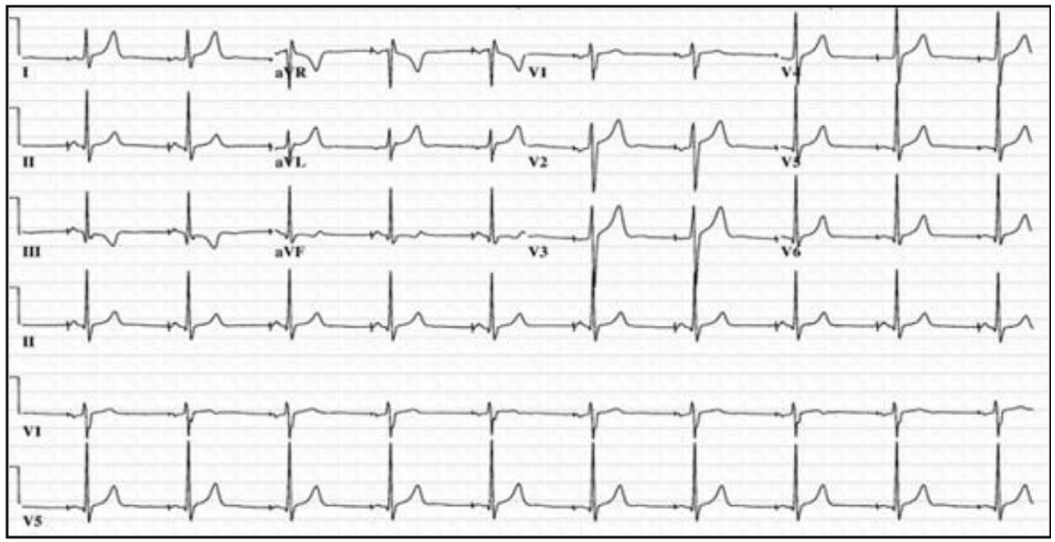
SCD 13 yrs

(brief) SV parox.
VT: judged as NSVT

Dx LQTS

KCNQ1-V133I
“probable
deleterious mutation.”

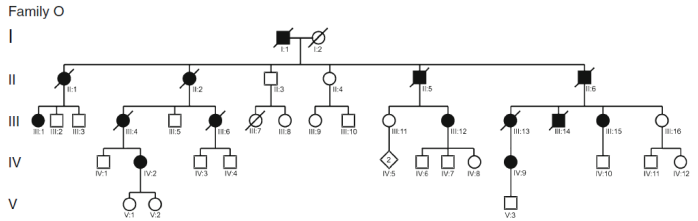
ICD



>24 family members identified!

Yet: “mutation” not present in 13 yo boy!

WES: de novo DES N342D mutation (class 5!)



Neth Heart J. 2012 ;20(5): 219-28

Mayo Clin Proc 2016;91:1606-16

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Paramount in genetic testing

- Test the right person (Dx)
- Do your own homework

  (confirm diagnoses/ ECGs/ genetics/pathology)
- Let phenotype guide your actions!
- Related test result to clinical diagnosis

  (pretest probability)
- Be critical: 10-30% published mutations wrongly classified!

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WHICH DIAGNOSTIC DNA TEST?

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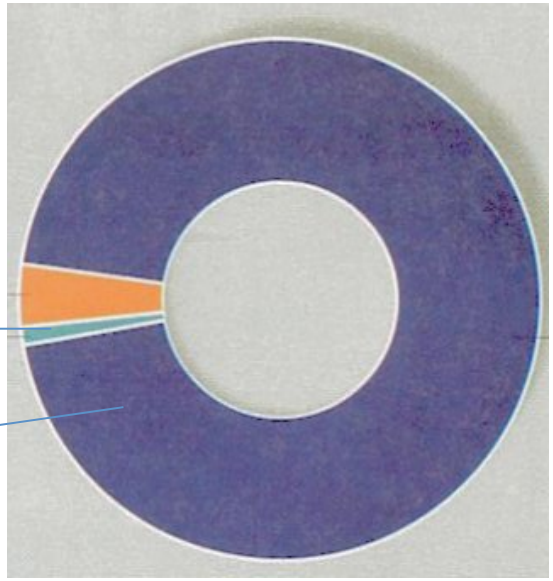
Next generation sequencing: DNA

- single *gene(s)*
- *panels* 23-100+ genes

  (cm/ arrh/ etc combi)
- *Exome sequencing* 20,000 genes
  (=1%-2% of DNA)
- *Genome sequencing*?



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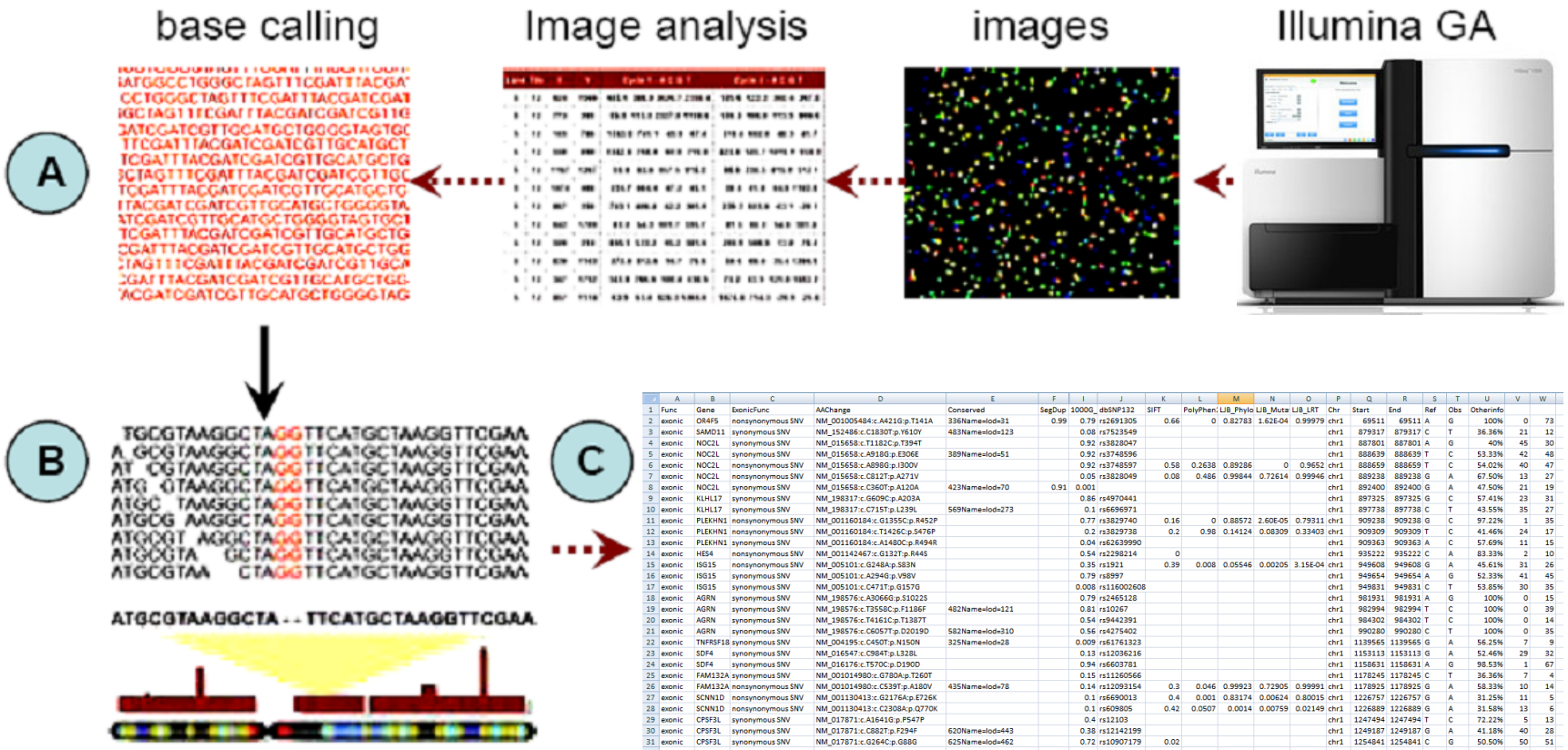
Next generation sequencing: DNA



	A	B	C	D	E	F	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W
1	Func	Gene	ExonicFunc	AAChange	Conserved	SegDup	1000G_	db5NP132	SIFT	PolyPhen2	LIB_Phylo	LIB_Mutat	LIB_LRT	Chr	Start	End	Ref	Obs	Otherinfo		
2	exonic	OR4F5	nonsynonymous SNV	NM_001005484:c.A421G:p.T141A	336Name=lod=31	0.99	0.79	rs2691305	0.66	0	0.82783	1.62E-04	0.99979	chr1	69511	69511	A	G	100%	0	73
3	exonic	SAMD11	synonymous SNV	NM_152486:c.C1830T:p.Y610Y	483Name=lod=123		0.08	rs7523549						chr1	879317	879317	C	T	36.36%	21	12
4	exonic	NOC2L	synonymous SNV	NM_015658:c.T1182C:p.T394T			0.92	rs3828047						chr1	887801	887801	A	G	40%	45	30
5	exonic	NOC2L	synonymous SNV	NM_015658:c.A918G:p.E306E	389Name=lod=51		0.92	rs3748596						chr1	888639	888639	T	C	53.33%	42	48
6	exonic	NOC2L	nonsynonymous SNV	NM_015658:c.A898G:p.I300V			0.92	rs3748597	0.58	0.2638	0.89286	0	0.9652	chr1	888659	888659	T	C	54.02%	40	47
7	exonic	NOC2L	nonsynonymous SNV	NM_015658:c.C812T:p.A271V			0.05	rs3828049	0.08	0.486	0.99844	0.72614	0.99946	chr1	889238	889238	G	A	67.50%	13	27
8	exonic	NOC2L	synonymous SNV	NM_015658:c.C360T:p.A120A	423Name=lod=70	0.91	0.001							chr1	892400	892400	G	A	47.50%	21	19
9	exonic	KLHL17	synonymous SNV	NM_198317:c.C609T:p.A203A			0.86	rs4970441						chr1	897325	897325	G	C	57.41%	23	31
10	exonic	KLHL17	synonymous SNV	NM_198317:c.C715T:p.L239L	569Name=lod=273		0.1	rs6696971						chr1	897738	897738	C	T	43.55%	35	27
11	exonic	PLEKHN1	nonsynonymous SNV	NM_001160184:c.G1355C:p.R452P			0.77	rs3829740	0.16	0	0.88572	2.60E-05	0.79311	chr1	909238	909238	G	C	97.22%	1	35
12	exonic	PLEKHN1	nonsynonymous SNV	NM_001160184:c.T1426C:p.S476P			0.2	rs3829738	0.2	0.98	0.14124	0.08309	0.33403	chr1	909309	909309	T	C	41.46%	24	17
13	exonic	PLEKHN1	synonymous SNV	NM_001160184:c.A1480C:p.R494R			0.04	rs62639990						chr1	909363	909363	A	C	57.69%	11	15
14	exonic	HES4	nonsynonymous SNV	NM_001142467:c.G132T:p.R44S			0.54	rs2298214	0					chr1	935222	935222	C	A	83.33%	2	10
15	exonic	ISG15	nonsynonymous SNV	NM_005101:c.G248A:p.S83N			0.35	rs1921	0.39	0.008	0.05546	0.00205	3.15E-04	chr1	949608	949608	G	A	45.61%	31	26
16	exonic	ISG15	synonymous SNV	NM_005101:c.A294G:p.V98V			0.79	rs8997						chr1	949654	949654	A	G	52.33%	41	45
17	exonic	ISG15	synonymous SNV	NM_005101:c.C471T:p.G157G			0.008	rs116002608						chr1	949831	949831	C	T	53.85%	30	35
18	exonic	AGRN	synonymous SNV	NM_198576:c.A3066G:p.S1022S			0.79	rs2465128						chr1	981931	981931	A	G	100%	0	15
19	exonic	AGRN	synonymous SNV	NM_198576:c.T3558C:p.F1186F	482Name=lod=121		0.81	rs10267						chr1	982994	982994	T	C	100%	0	39
20	exonic	AGRN	synonymous SNV	NM_198576:c.T4161C:p.T1387T			0.54	rs9442391						chr1	984302	984302	T	C	100%	0	14
21	exonic	AGRN	synonymous SNV	NM_198576:c.C6057T:p.D2019D	582Name=lod=310		0.56	rs4275402						chr1	990280	990280	C	T	100%	0	35
22	exonic	TNFRSF18	synonymous SNV	NM_004195:c.C450T:p.N150N	325Name=lod=28		0.009	rs61761323						chr1	1139565	1139565	G	A	56.25%	7	9
23	exonic	SDF4	synonymous SNV	NM_016547:c.C984T:p.L328L			0.13	rs12036216						chr1	1153113	1153113	G	A	52.46%	29	32
24	exonic	SDF4	synonymous SNV	NM_016176:c.T570C:p.D190D			0.94	rs6603781						chr1	1158631	1158631	A	G	98.53%	1	67
25	exonic	FAM132A	synonymous SNV	NM_001014980:c.G780A:p.T260T			0.15	rs11260566						chr1	1178245	1178245	C	T	36.36%	7	4
26	exonic	FAM132A	nonsynonymous SNV	NM_001014980:c.C539T:p.A180V	435Name=lod=78		0.14	rs12093154	0.3	0.046	0.99923	0.72905	0.99991	chr1	1178925	1178925	G	A	58.33%	10	14
27	exonic	SCNN1D	nonsynonymous SNV	NM_001130413:c.G2176A:p.E726K			0.1	rs6690013	0.4	0.001	0.83174	0.00624	0.80015	chr1	1226757	1226757	G	A	31.25%	11	5
28	exonic	SCNN1D	nonsynonymous SNV	NM_001130413:c.C2308A:p.Q770K			0.1	rs609805	0.42	0.0507	0.0014	0.00759	0.02149	chr1	1226889	1226889	G	A	31.58%	13	6
29	exonic	CPSF3L	synonymous SNV	NM_017871:c.A1641G:p.P547P			0.4	rs12103						chr1	1247494	1247494	T	C	72.22%	5	13
30	exonic	CPSF3L	synonymous SNV	NM_017871:c.C882T:p.F294F	620Name=lod=443		0.38	rs12142199						chr1	1249187	1249187	G	A	41.18%	40	28
31	exonic	CPSF3L	synonymous SNV	NM_017871:c.G264C:p.G88G	625Name=lod=462		0.72	rs10907179	0.02					chr1	1254841	1254841	C	G	50.50%	50	51
32	exonic	GLTPD1	synonymous SNV	NM_001029885:c.C468T:p.R156R	496Name=lod=138		0.78	rs307349						chr1	1262966	1262966	C	T	80%	3	12
33	exonic	TAS1R3	synonymous SNV	NM_152228:c.C1248T:p.P416P			0.11	rs3813210						chr1	1268159	1268159	C	T	54.35%	21	25
34	exonic	TAS1R3	nonsynonymous SNV	NM_152228:c.T2269C:p.C757R			0.95	rs307377	1	0	0.0966	1.26E-04	0.97622	chr1	1269554	1269554	T	C	100%	0	93
35	exonic	DVL1	synonymous SNV	NM_004421:c.A366G:p.P122P	513Name=lod=162		1	rs307362						chr1	1277533	1277533	T	C	100%	0	33
36	exonic	ATAD3C	synonymous SNV	NM_001039211:c.G921T:p.A307A	573Name=lod=283		0.21	rs72896283						chr1	1396238	1396238	G	T	46.03%	34	29
37	exonic	ATAD3B	synonymous SNV	NM_031921:c.C1005A:p.T335T	522Name=lod=176	0.91	0.37	rs56587801						chr1	1421531	1421531	C	A	42.03%	40	29
38	exonic	ATAD3B	nonsynonymous SNV	NM_031921:c.G1157A:p.R386Q	640Name=lod=530	0.91	0.19	rs77225021	0.01	0.996	0.98032	0.98861	1	chr1	1421991	1421991	G	A	40.45%	53	36
39	exonic	ATAD3B	synonymous SNV	NM_031921:c.A1239G:p.A413A		0.91	0.42	rs819977						chr1	1423267	1423267	A	G	45.83%	39	33
40	exonic	ATAD3A	nonsynonymous SNV	NM_001170535:c.G646T:p.A216S	284Name=lod=19	0.91		rs2767471	0.76	0	0.99753	0.81851	1	chr1	1455652	1455652	G	T	39.02%	50	32
41	exonic	ATAD3A	synonymous SNV	NM_001170535:c.T882G:p.L294L	374Name=lod=44	0.91	0.04	rs11546154						chr1	1458255	1458255	T	G	36%	48	27

- Data per sample (patiënt)
- Map reads against "reference genome"
- Detect variants
- Annotate variants

- **Targeted Panels:**

+: high quality , cheap (less time molecular geneticist)

+: no incidental findings, CNV

-: inflexible (new genes), enrichment

- **Whole Exome sequencing**:

+: fast, flexible, 1 test,

+/-: costs

-: quality/coverage of some "core" genes, enrichment, CNV (>3 exons)

-: incidental findings

- **(Panel based upon WES)**

- **Whole Genome Sequencing**:

+: no enrichment, fast, highly flexible, coverage?, detects CNV, one-test

-: quality (increasing); costs, incidental findings

panel-exome-genome

	panel	WES	WGS
Quality/coverage	+++	++	++(+)
CNVs (large ins/del)	++	+	++
enrichment	yes	yes	no
flexible	-	+	+
Incidental findings	no	yes	yes
costs	$	$$	$$$$

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Panel-exome-genome:

The more you evaluate, the more you will find

- Proportionality WGS if you only need 12 genes ?
- Genes of interest
- Unsolicted findings!

N=600 WES

2% unsolicited/ incidental findings

7% heterozygote carrier (for recessive disease like Cystic Fibrosis, Tay Sachs etc)

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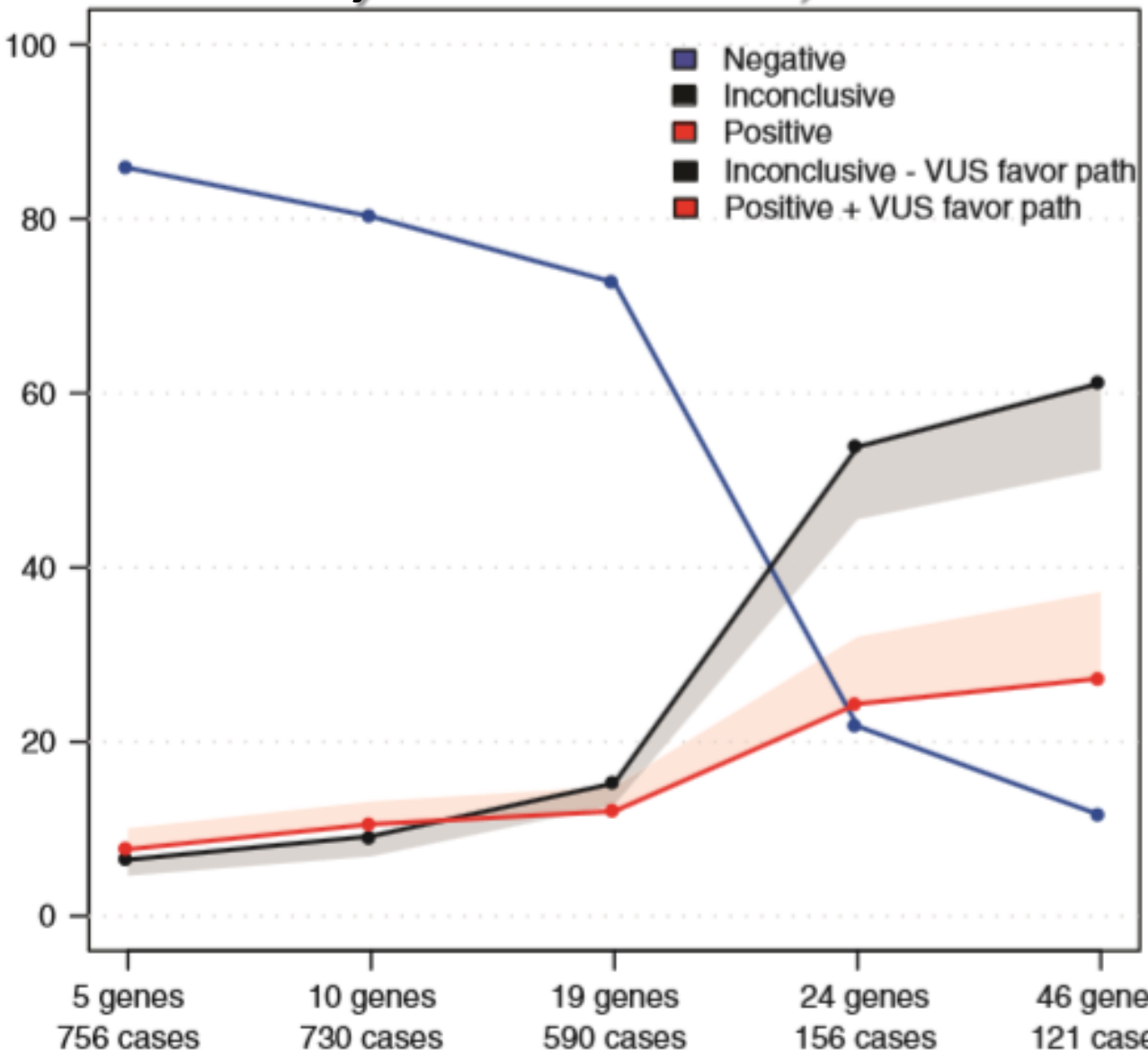
Panel-exome-genome:

The more you evaluate, the more you will find



DCM patients

Pugh et al Genet Med 2014

Class 3

Genetic purgatory

Class 4

Class 5

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PANEL-WES-WGS?

WHICH TEST TO USE?

Dependent of your goal/ infrastructure

- Clinical vs research?
- Skills and infrastructure “cardiogenetics team” *(counseling, interpretation)*
- Interpretation is teamwork

  (-pediatric- cardiologists, pathologists, clinical geneticists, gen counselors, psychologist, molecular geneticists)
- Families available: segregational studies?
- Network: functional follow up

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Targeted sequencing (-exome based-targeted panel) is preferred in diagnostics

- Given the goal of genetic screening (Dx, facilitate cascade screening & some risk stratification): solid genes & mutations)

- limited set of solid genes cover most of the yield

- Whole exome/genome sequencing: great for research and specific other indications

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Thank you!

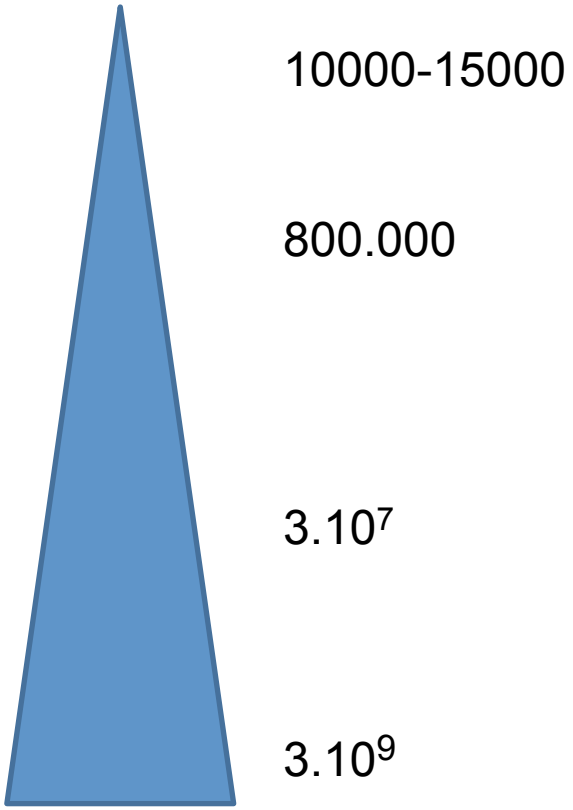
VUmc amc Durrer center for cardiogenetic research CVON Cardio Vasculair Onderzoek Nederland

Next generation sequencing: DNA

- <1995: niets
- 1995-2012: meestal 1-2 *genen* per keer; sequentieel. Max ca. 6
- Vanaf 2012: *panels* 23-100+ genen

(cardiomyopathie/ aritmie/ combi/ aorta-pakket)

- Na 2014 *Exoom sequencing* 20.000 genen (=1%-2% van het DNA)
- Na 2017?: *Genoom sequencing*?



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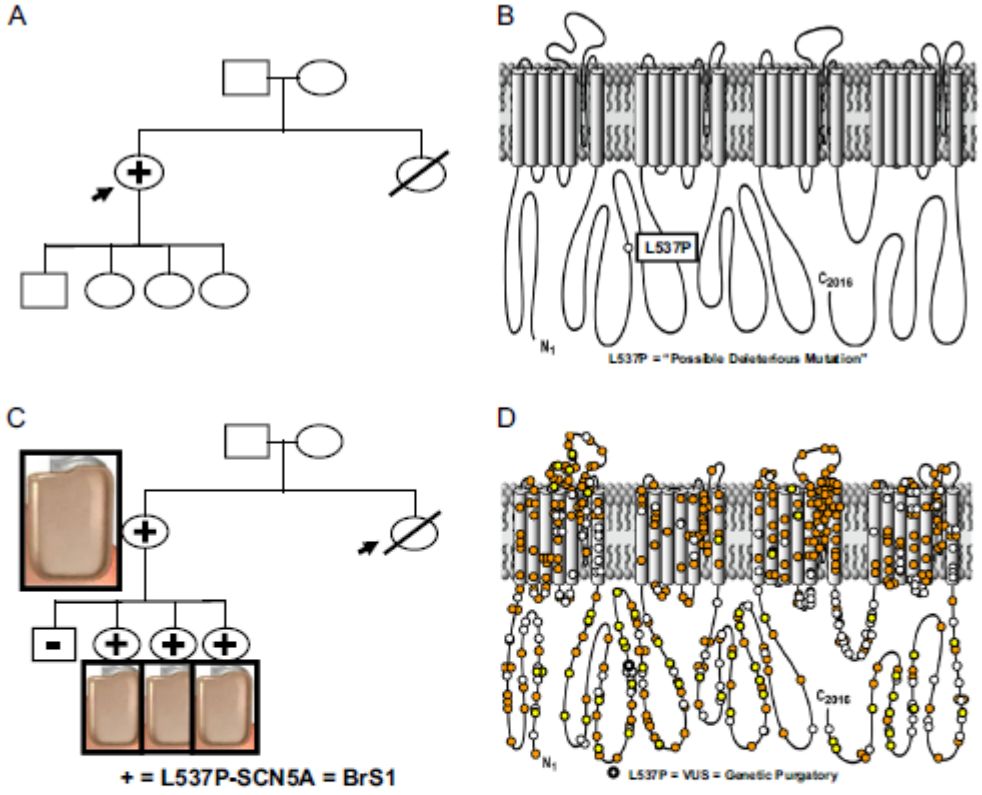
Keuze genoom, WES of pakket

	geen verrijking	wel verrijking	
praktisch	genoom	WES	genpakket
analyse	NIPT	WES	bindweefsel
	arrays?	ID pakket	BRCA1/2
	research	FADS pakket	
		wittestofpakket	
		onco	
voordeel	snel	flexibel	hoge kwaliteit
		1 test voor alles	
flexibiliteit	hoog	hoog	laag
kwaliteit	te laag voor diagnostiek (nog)	voldoende, niet voor core genes	hoog
kosten	hoog	gemiddeld	laag

Aantal genen (~50)
Aantal core genes
Aantal patiënten
Aantal nieuwe genen
Kosten

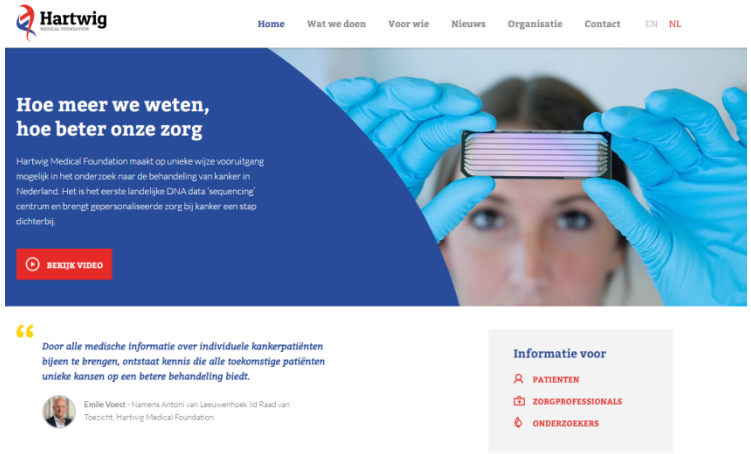
pr: ookbs



Ackerman MJ et al
2015;12:2325-31

Whole Genome Sequencing, waarom?

- 1 test ipv 2 (of meer) want ook CNV detectie mogelijk
- Betere gemiddelde coverage
- Genetische informatie buiten exoom: verbetering diagnostiek in de toekomst
  - Intronische varianten
  - Promoter
  - Enhancers
  - …
- Detectie structurele varianten mogelijk



Huidige stand van zaken:

Outsourcing: Hartwig Medical Foundation (samenwerking)

Implementatie traject gestart, de eerste 2 series zijn in progress

Exome sequencing: all genes; 2% of DNA

Myocardial and Pericardial Diseases
ESC Working Group

The current role of Next generation DNA sequencing in routine care of patients with hereditary cardiovascular conditions

A viewpoint paper of the European Society of Cardiology working group on myocardial and pericardial diseases and members of the European Society of Human Genetics

Authors: Jens Mogensen[1*], J. Peter van Tintelen[2], Siv Fokstuen[3], Perry Elliott[4], Irene M van Langen[5], Benjamin Meder[6], Pascale Richard[7,8], Petros Syrris[9], Alida LP Caforio[10], Yehuda Adler[11], Aris Anastasakis[12], Juan R. Gimeno[13], Karin Klingel[14], Ales Linhart[15], Massimo Imazio[16], Yigal Pinto[17], Ruth Newbery-Ecob[18], Joerg Schmidtke[19], and Philippe Charron[8,20].

Whole exome/genome sequencing is considered to be a diagnostic method in development and should be used for genetic diagnosis only if filtered against recognised disease genes. The coverage should allow identification of all exomic variants in these genes

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Exome sequencing: all genes; 2% of DNA

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Unsolicited findings:

- Before genetic testing it is important to inform the patient about the challenges in interpretation of sequencing results of multiple genes and discuss the implications of unsolicited findings
- In a clinical diagnostic setting only recognised disease genes should be investigated in patients fulfilling diagnostic criteria of a specific cardiovascular condition

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Experimental Evidence Scoring

Evidence Category	Evidence Type	Score Range	Recommended points/ evidence	Points Given	Max Score
Function	Biochemical Function	½ - 2	½ point for each piece of evidence in any category	1.5	2
	Protein Interaction	½ - 2			
	Expression	½ - 2			
Functional Alteration	Patient cells	1 - 2	1 point	1	2
	Non-patient cells	½ - 1	½ point	NA	
Models & Rescue	Animal model	1 - 4	2 points	NA	4
	Cell culture model system	½ - 2	1 point	NA	
	Rescue in animal model	1 - 4	2 points	NA	
	Rescue in engineered equivalent	½ - 2	1 point	NA	
			Total Final Score	2.5	**0 - 8**

Assertion criteria	Genetic Evidence (0-12 points)	Experimental Evidence (0-6 points)	Total Points (0-18)	Replication Over Time (Y/N)
Description	Case-level, family segregation, or case-control data that support the gene-disease association	Gene-level experimental evidence that support the gene-disease association	Sum of Genetic & Experimental Evidence	> 2 pubs w/ convincing evidence over time (>3 yrs)
Assigned Points	**9.75**	**2.5**	**12.25**	**Y**

CALCULATED CLASSIFICATION	**LIMITED**	**1-6**
	MODERATE	**7-11**
	STRONG	**12-18**
	DEFINITIVE	**12-18 AND replication over time**

Valid contradictory evidence? (Y/N)	List PMIDs and describe evidence:

CURATOR CLASSIFICATION	**STRONG???**
FINAL CLASSIFICATION	