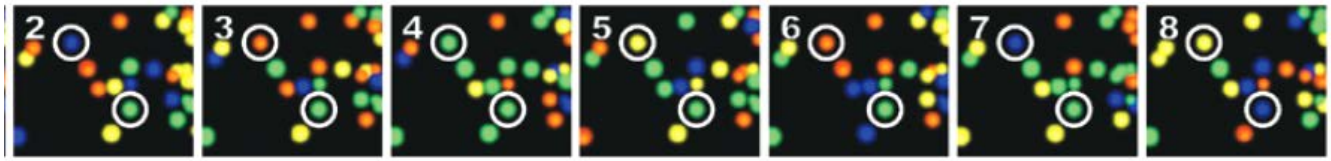
NGS IN THE CLINIC
GENE PANEL TESTING FOR INHERITED CONDITIONS

Genetics Webinar Series – Blue Cross Blue Shields – 01/12/2016

Birgit Funke, PhD, FACMG
Associate Professor of Pathology, Harvard Medical School
Associate Director and Director Clinical R&D; Laboratory for Molecular Medicine



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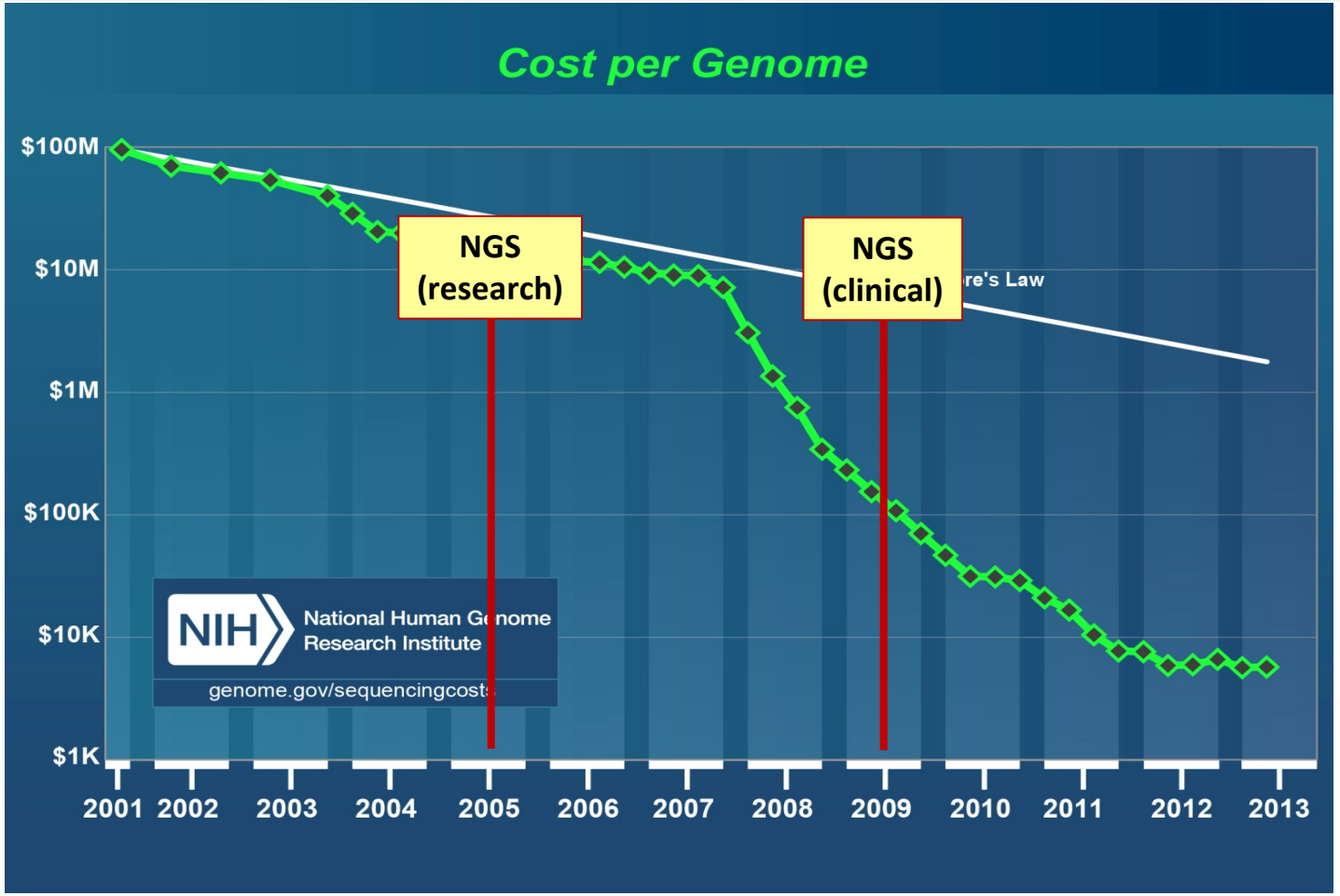
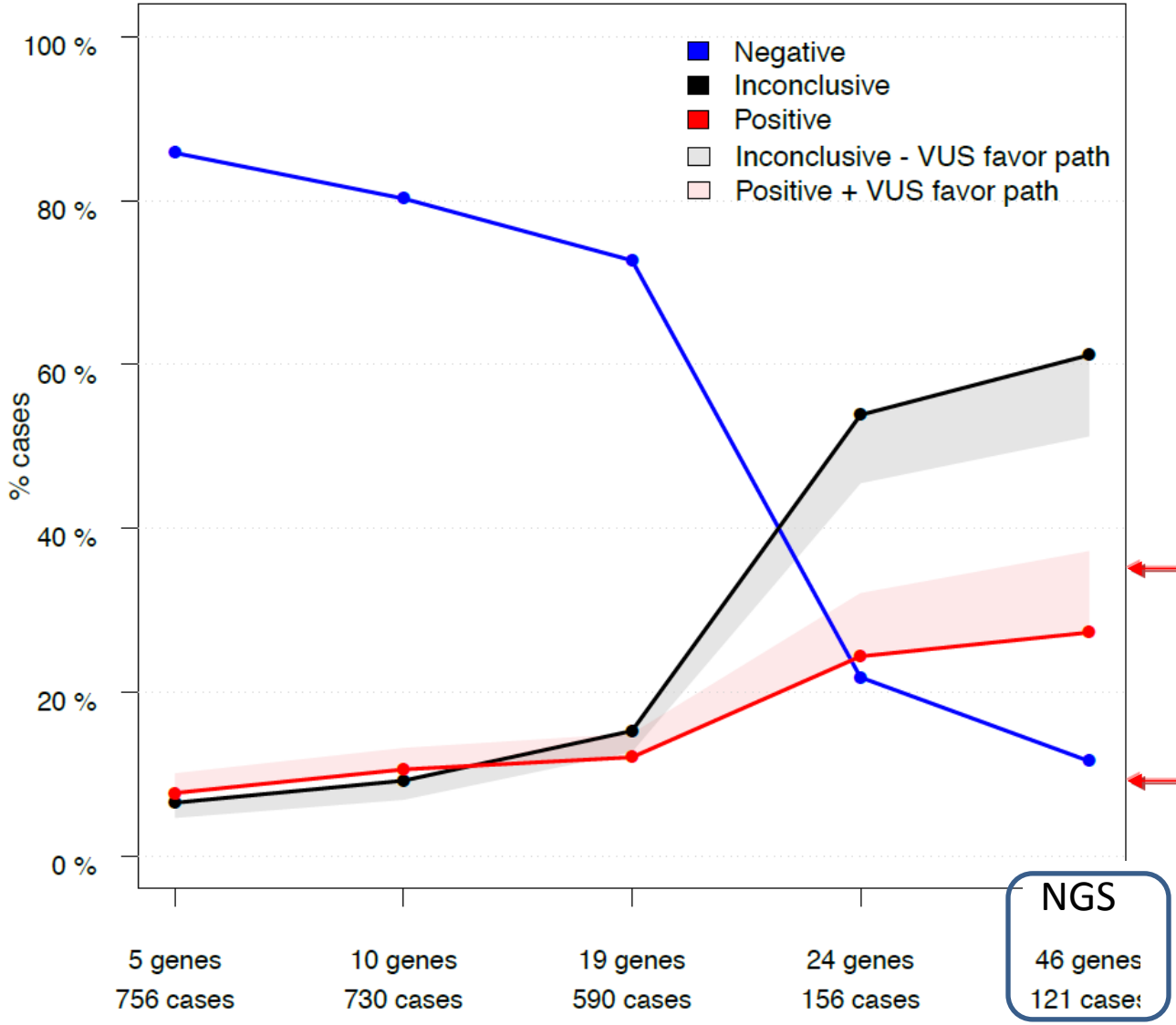
INTRODUCTION

THE DISRUPTIVE NATURE OF NGS



- **Clinical NGS is being implemented in an increasing number of labs**
  - Majority focus on gene panels
  - Implementation of exome/genome sequencing quickly increasing

DETECTION RATE OVER TIME



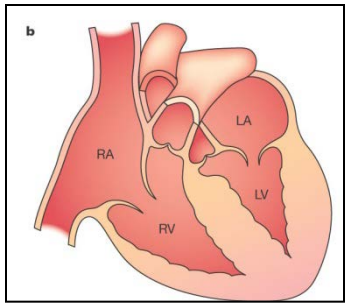
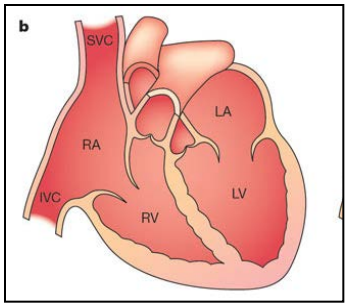
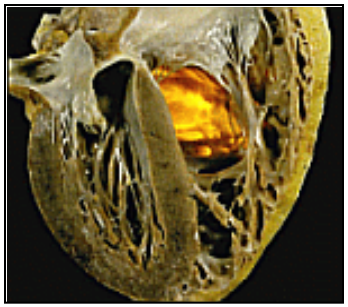
DCM

2011: ~37%

2007: ~10%

WHICH DISORDERS BENEFIT FROM PANEL TESTING?

EXAMPLE: INHERITED CARDIOMYOPATHIES

HCM	DCM	ARVC	OTHER (RARE)
			LVNC RCM
1:500	> 1:2,500	1:1,000 - 1:5,000	

- Collective incidence: > 1/500
- Can lead to SCD
- Substantial genetic component
- Incentive for predictive testing

WHY SCREEN FOR MUTATIONS? (HCM)

CLINICAL MANAGEMENT

- Cardiac variant of Fabry disease can masquerade as isolated HCM: therapeutic intervention (enzyme replacement therapy)

COST

Current guidelines recommend clinical screening of 1st degree relatives

Child of an HCM patient

- $6,000 through puberty
- $20,000 over lifetime

Compare to genetic testing

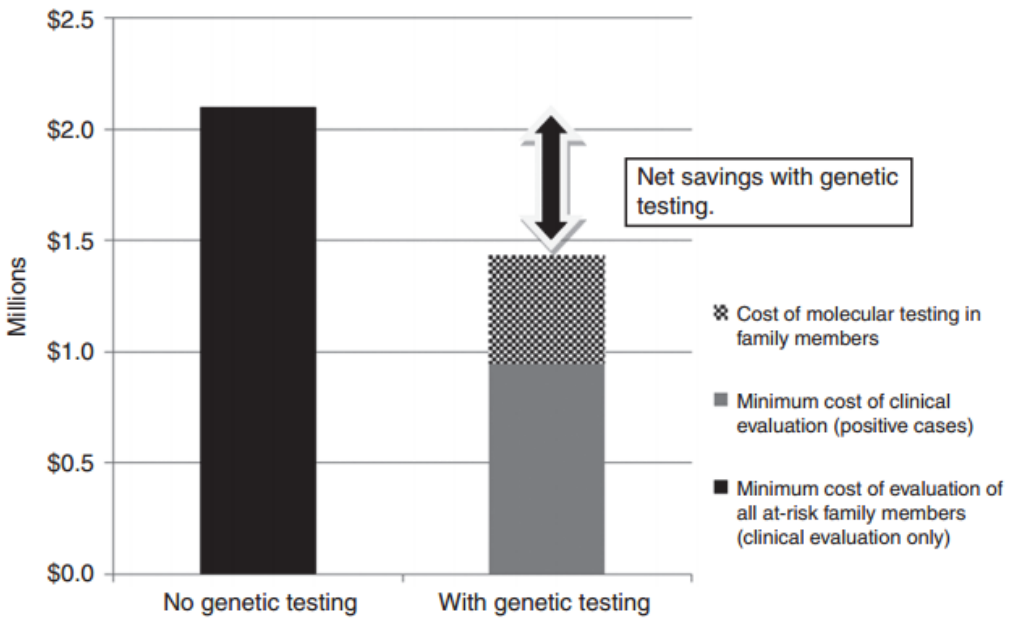
- $3,000 for proband
- $500 per mutation per family member
- Clinical F/U reduced to mutation-positive family members

Ho 2010, Circulation 122:2430

Results of clinical genetic testing of 2,912 probands with hypertrophic cardiomyopathy: expanded panels offer limited additional sensitivity

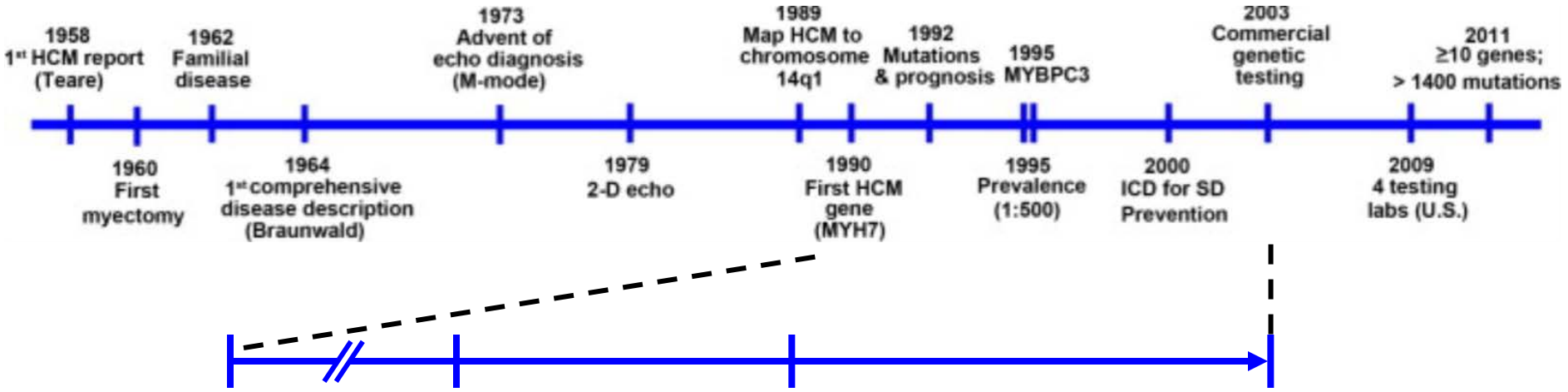
Ahmed A. Alfares, MD[1,2], Melissa A. Kelly, MS[1], Gregory McDermott, BA[1], Birgit H. Funke, PhD[1,3,4], Matthew S. Lebo, PhD[1,3,4], Samantha B. Baxter, MS[1], Jun Shen, PhD[1,3,4], Heather M. McLaughlin, PhD[1,3,4], Eugene H. Clark, BM[1], Larry J. Babb, BS[1], Stephanie W. Cox, BS[1], Steven R. DePalma, PhD[5,6], Carolyn Y. Ho, MD[7], J.G. Seidman, PhD[6], Christine E. Seidman, MD[5,6,7] and Heidi L. Rehm, PhD[1,3,4]

- 691 of 1,209 asymptomatic family members of a positive proband tested negative
- no longer required cardiac evaluations recommended for high-risk family members



Alfares 2015 *(Genetics in Medicine)*

GENETIC TESTING FOR CARDIOMYOPATHY



1990

1st HCM gene (MYH7)

2003

Clinical Genetic Testing
HCM

2007

Clinical Genetic Testing
DCM + ARVC

2011

Testing covers all inherited cardiomyopathies
HCM, DCM, ARVC, RCM, LVNC

5 genes/patient → **51 genes/patient**

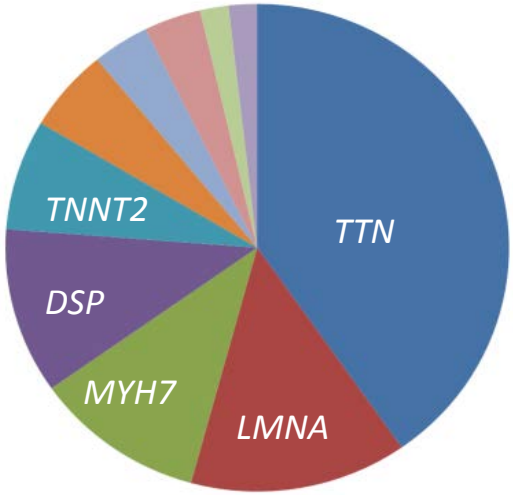
Adapted from: Maron 2012

CHALLENGES

- **Locus heterogeneity** - 1 disease / many genes
- **Allelic heterogeneity** - many disease causing variants/gene
- **Spectrum of pathogenic variation** - not yet well understood
- **Phenotypic overlap** - can complicate testing process

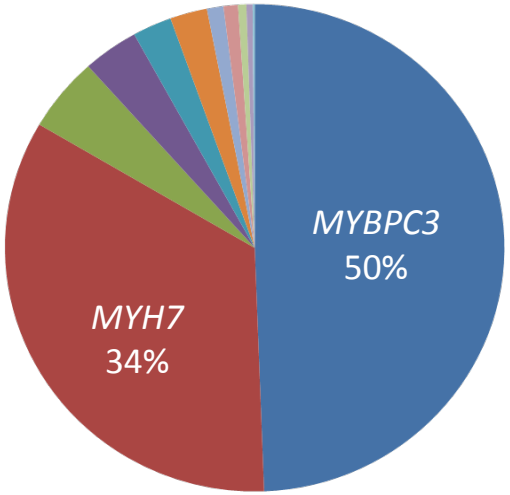
LOCUS HETEROGENEITY

DCM



Melissa Kelly

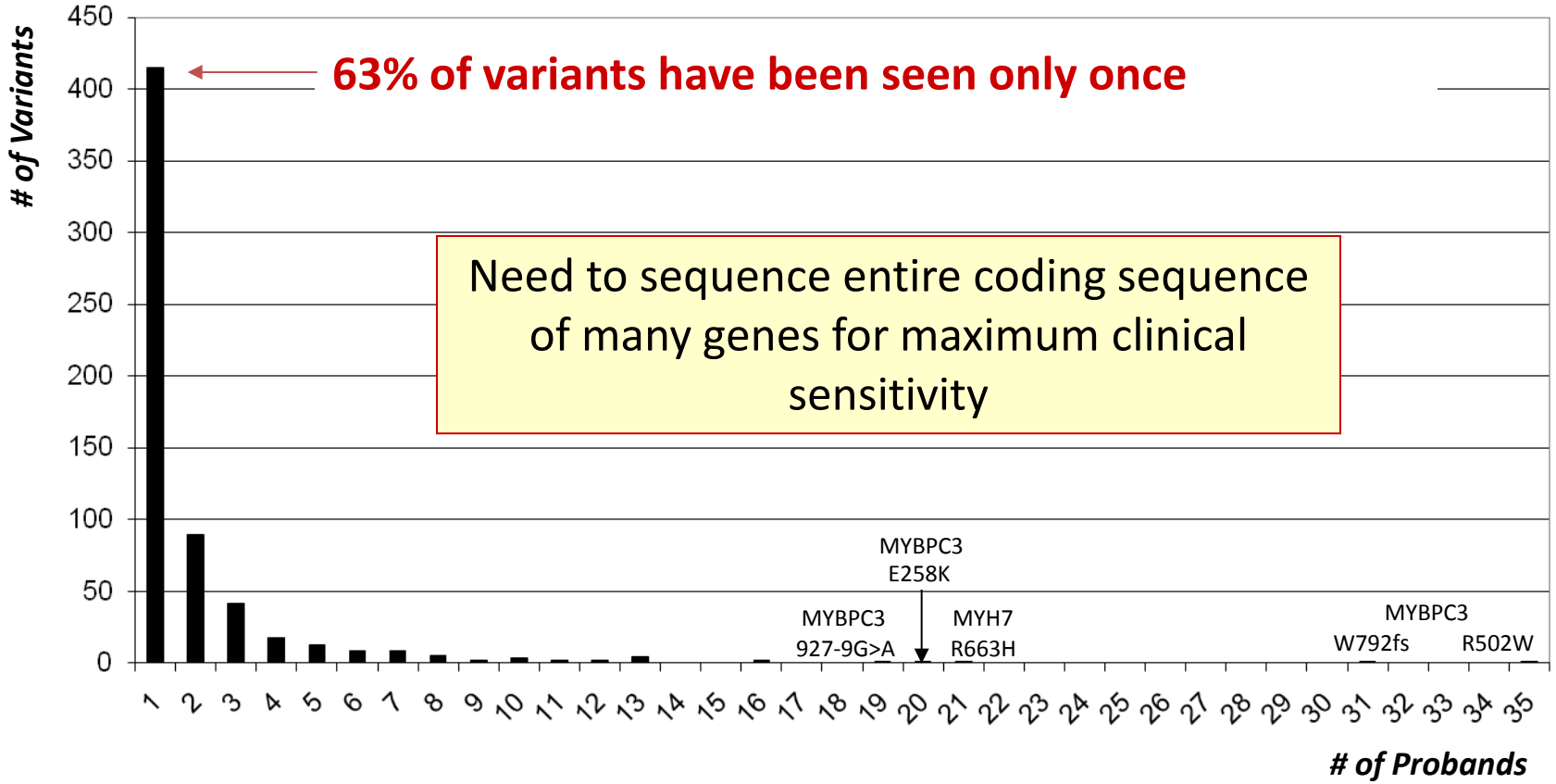
HCM



Ahmed Alfares

ALLELIC HETEROGENEITY

Hypertrophic Cardiomyopathy: 10 years – 11 genes tested

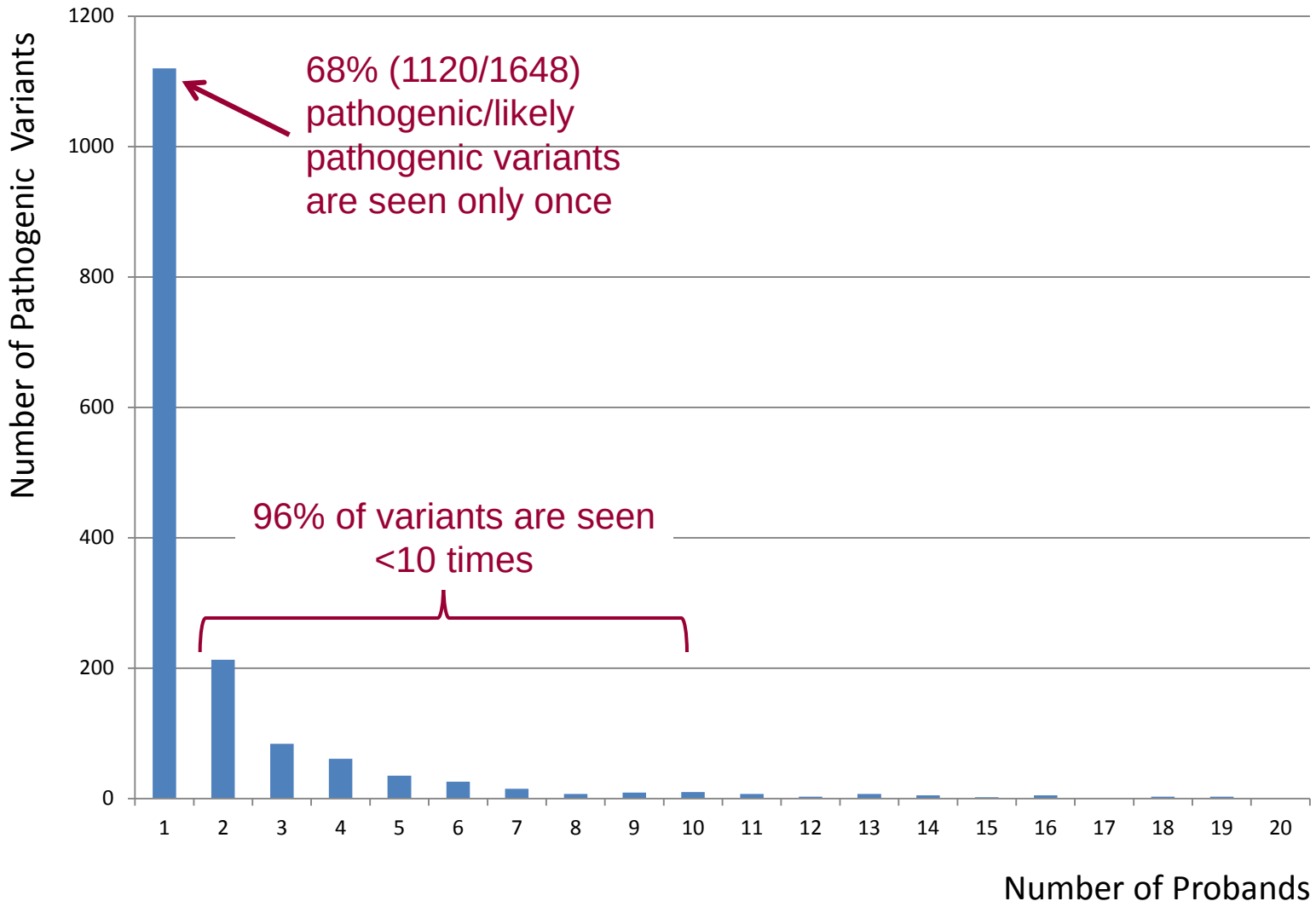


Courtesy of Ahmed Alfares and Heidi Rehm

NOT JUST CARDIOMYOPATHY….

DIAGNOSTIC TESTING OF 15,000 LMM PROBANDS

(cardiomyopathy, hearing loss, rasopathies, aortopathies, somatic and hereditary cancer, pulmonary disorders, skin disorders, other genetic syndromes)

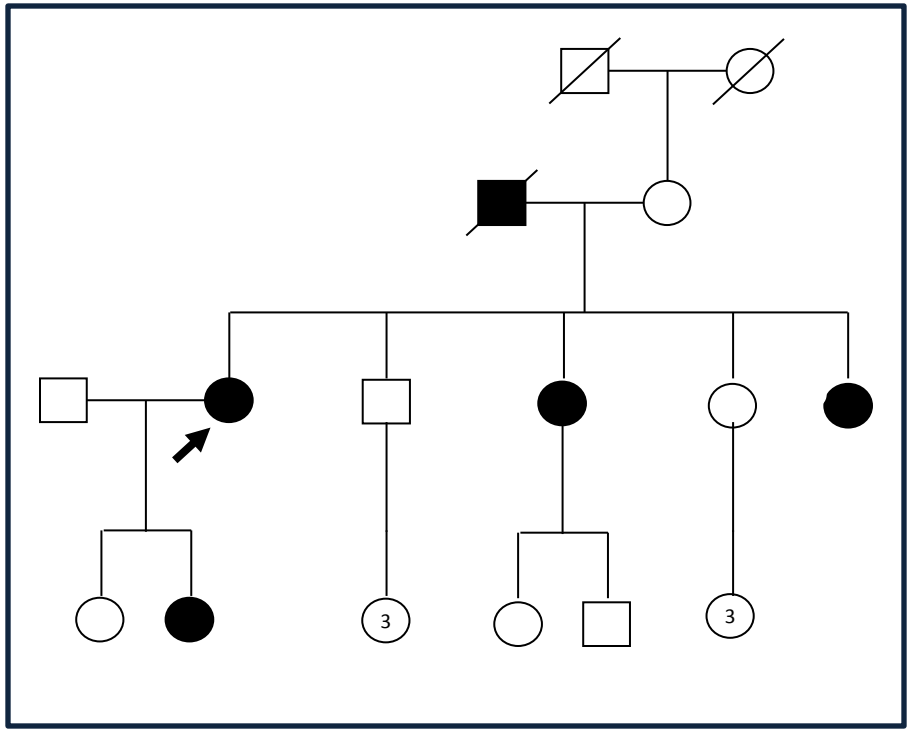


Courtesy of Heidi Rehm

CLINICAL HETEROGENEITY

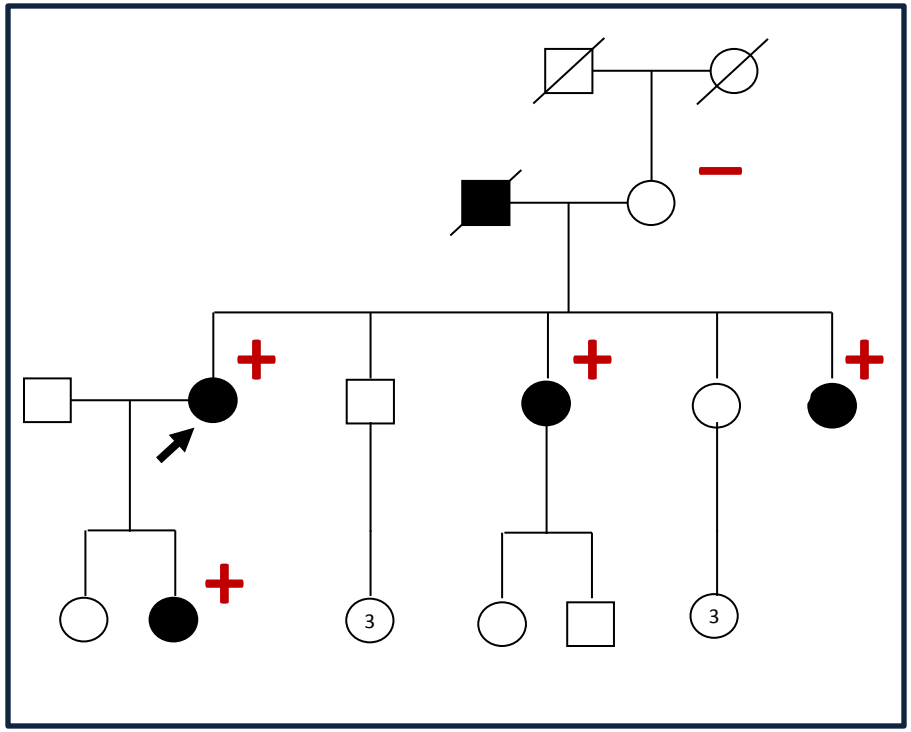
Traditional genetic testing : 1 gene panel for each diagnosis

- **Proband with clinical Dx + family history of DCM**
- **DCM gene panel detects a variant of uncertain significance**
- **Variant did not segregate**



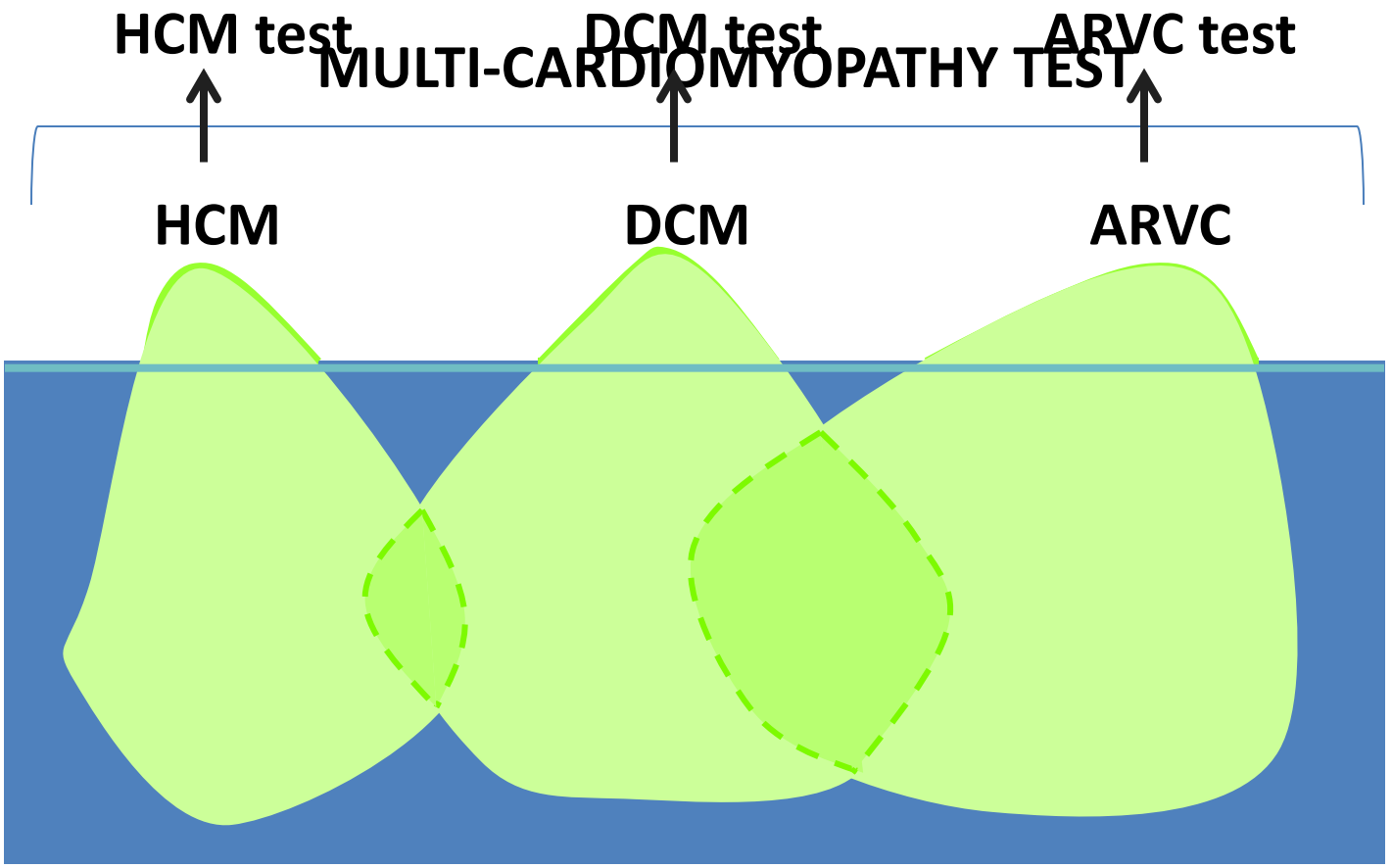
PREVENTING DIAGNOSTIC ODYSSEYS

- Patient was seen again , diagnosis was revised to ARVC
- ARVC panel identified a likely pathogenic variant



Traditional testing (disease centric) does not make sense for disorders with clinical and genetic overlap

MULTI-DISEASE GENE PANELS

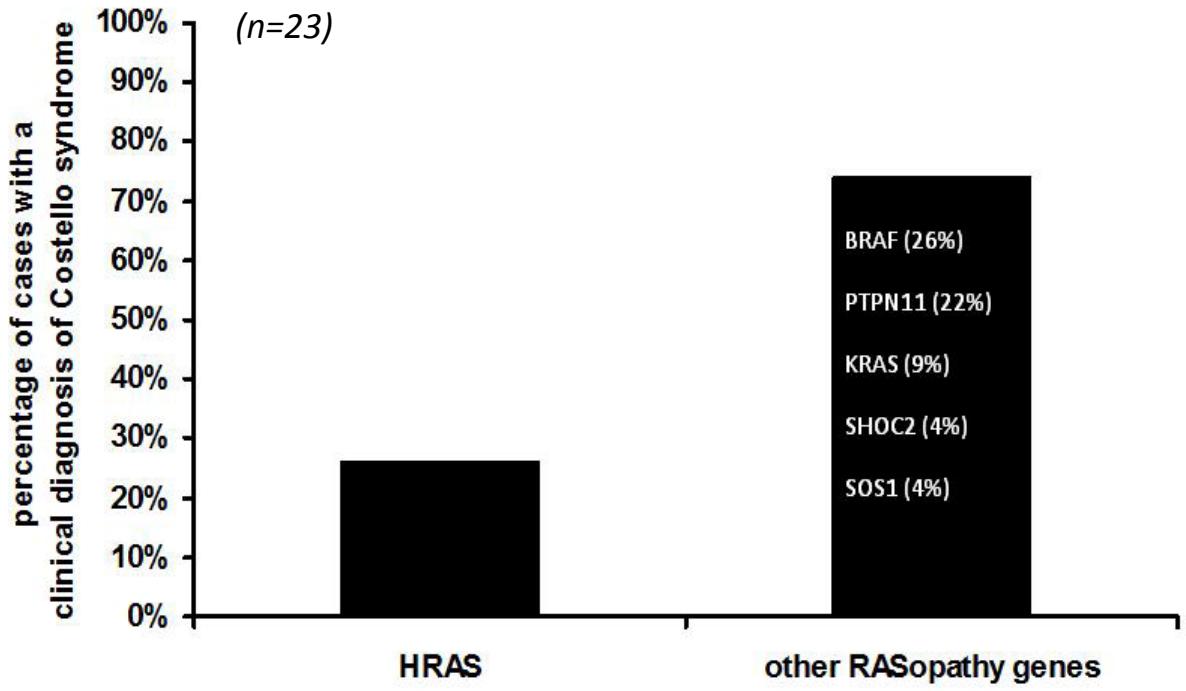


- ~3% of **DCM** patients carry a pathogenic variant in an **ARVC** gene

MULTI DISEASE GENE PANELS IMPROVE CLINCAL DIAGNOSIS

GeneReviews (2012): *"80-90% of patients with Costello syndrome carry a mutation in HRAS."*

LMM broad referral population data:



Most patients would have received a **NEGATIVE** report if only HRAS had been tested

MULTI DISEASE GENE PANELS IMPROVE CLINCAL DIAGNOSIS

Phenotypic expansion

- Original clinical definition based on most severe cases
- Often too narrow, full range of clinical variability emerges over time

Phenotypic overlap

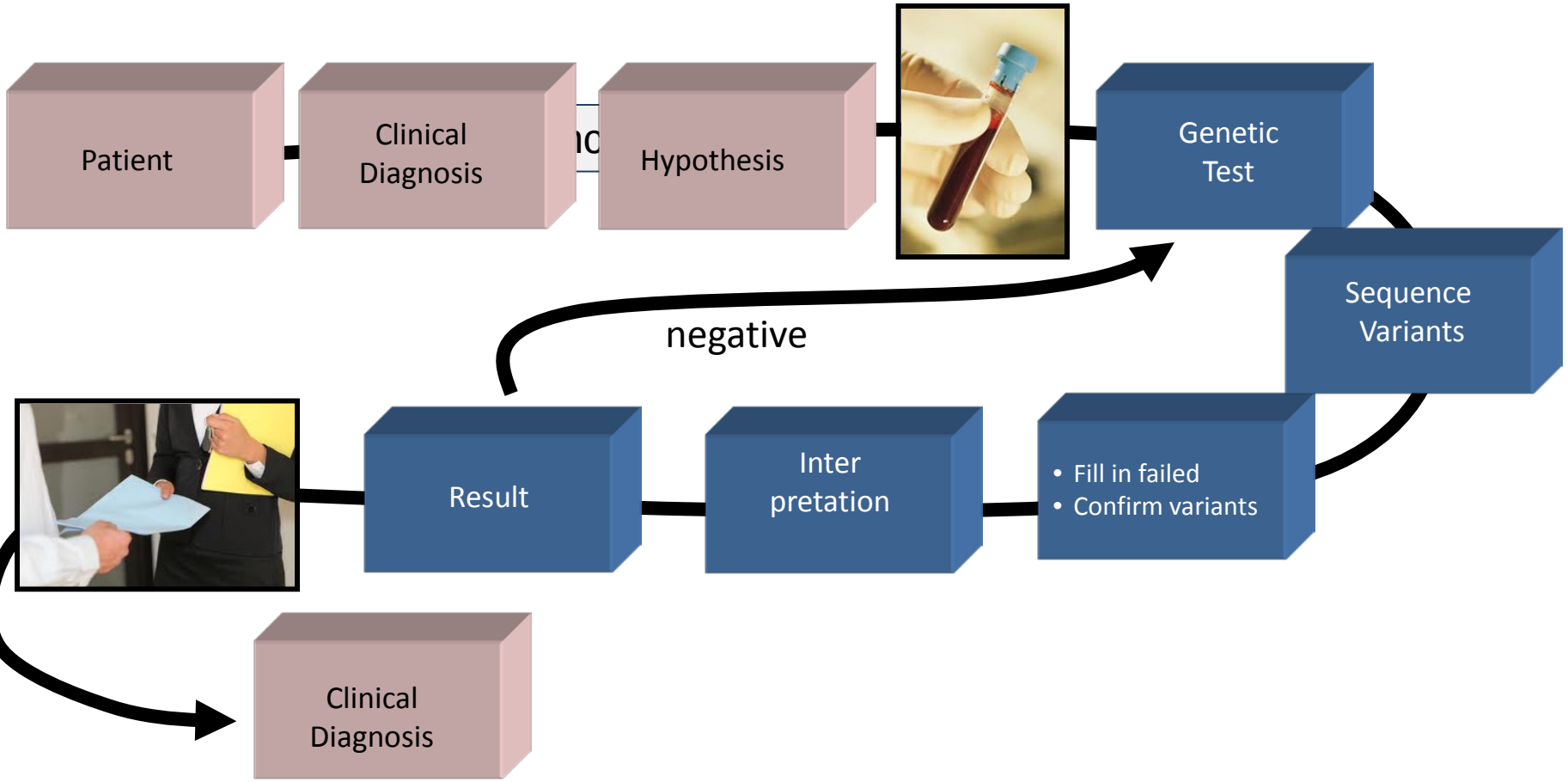
- Disorders present the same -> diagnostic “error”
- Happens more often as genetic testing is moving out of specialty clinics to more general (genetics) care

Now widely recognized

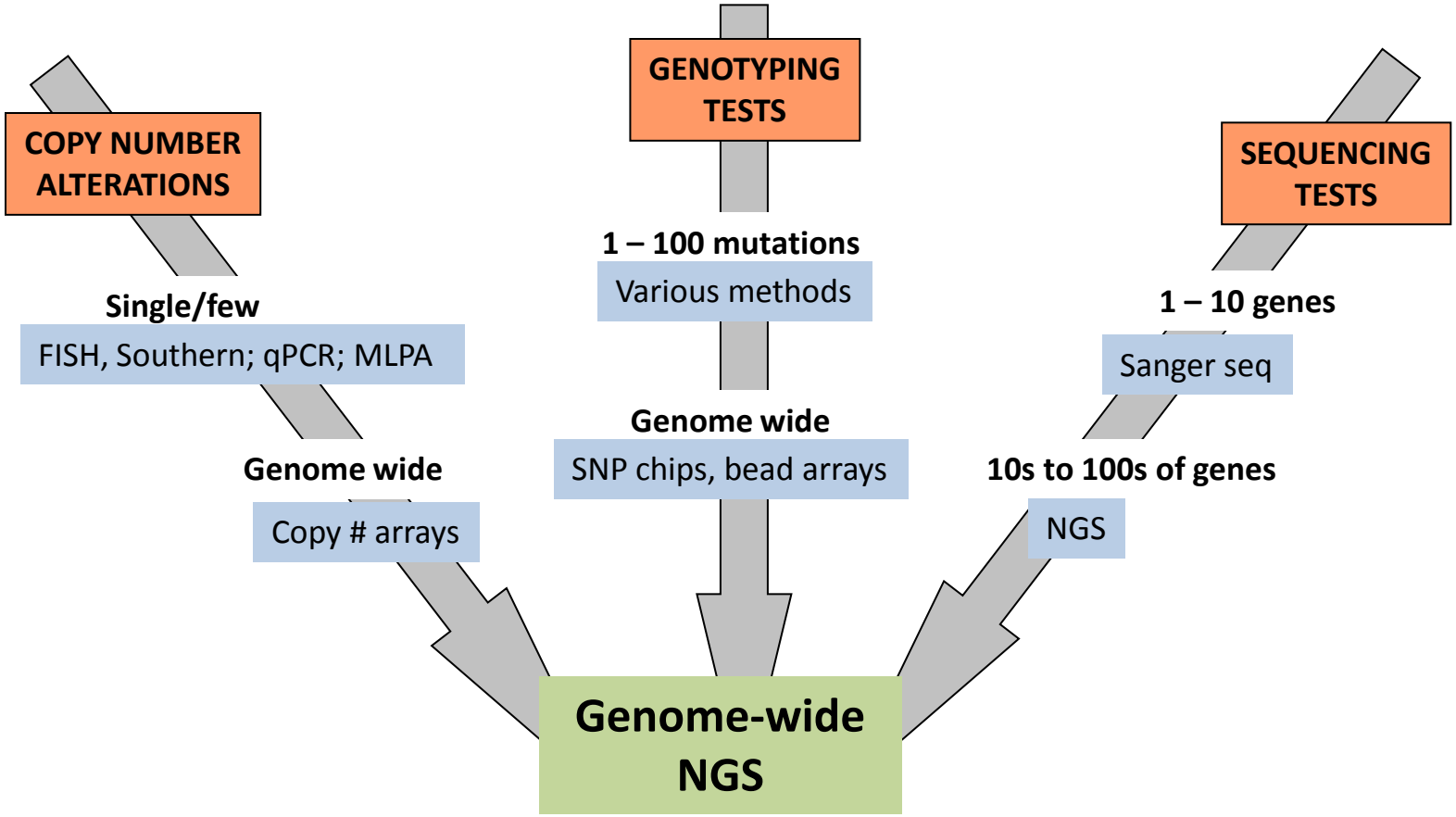
- Many physicians are beginning to change workflow

THE CHANGING GENOMIC TESTING WORKFLOW

"SEQUENCE FIRST"



TREND TOWARDS GENOME WIDE TESTING



Expected to eventually consolidate most genetic testing

WHICH GENES SHOULD BE ON A PANEL?

ASSESSING CLINICAL VALIDITY OF VARIANTS AND GENES

DEVELOPING STANDARDS FOR ASSESSING CLINICAL VALIDITY

ACMG + AMP (2015)

New guideline for clinical grade variant classification (Mendelian disorders)

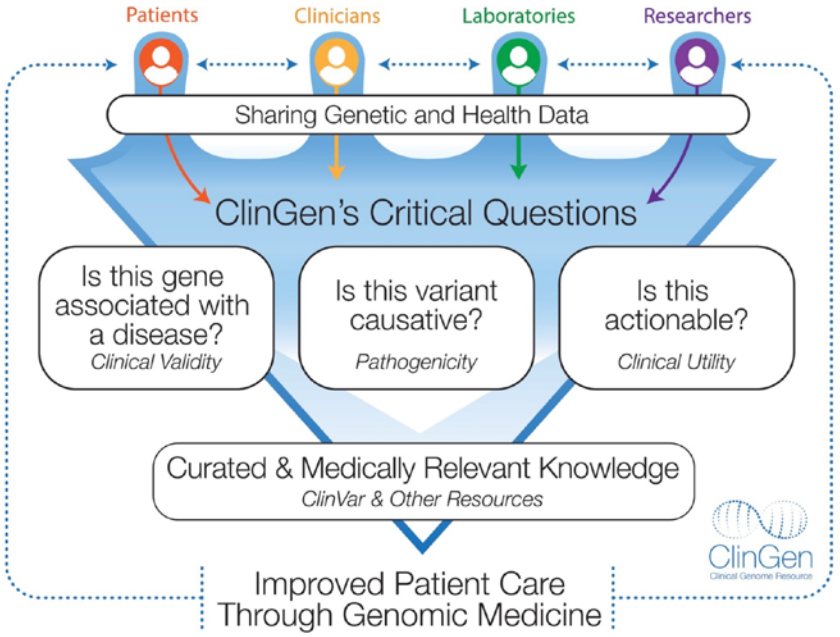
ClinGen

Unite medical genetics community by developing approaches to curate, centralize and share genetic data

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



CLINICAL VALIDITY – VARIANTS

Questions

- Does the variant affect protein/gene function?
- Does this cause disease?

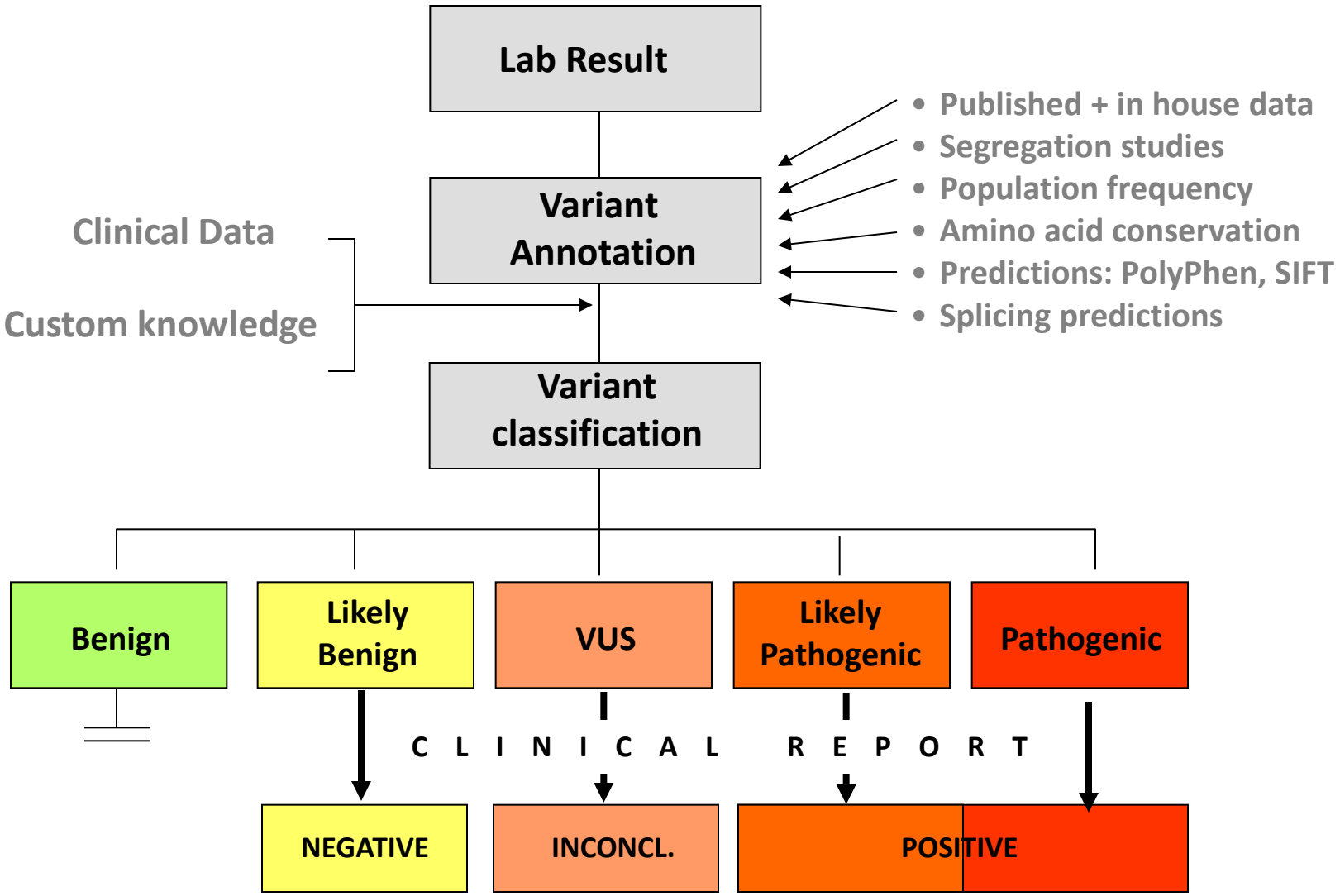
Classify variants based on available evidence

- Pathogenic
- Likely pathogenic
- Variant of uncertain significance (VUS)
- Likely benign
- Benign

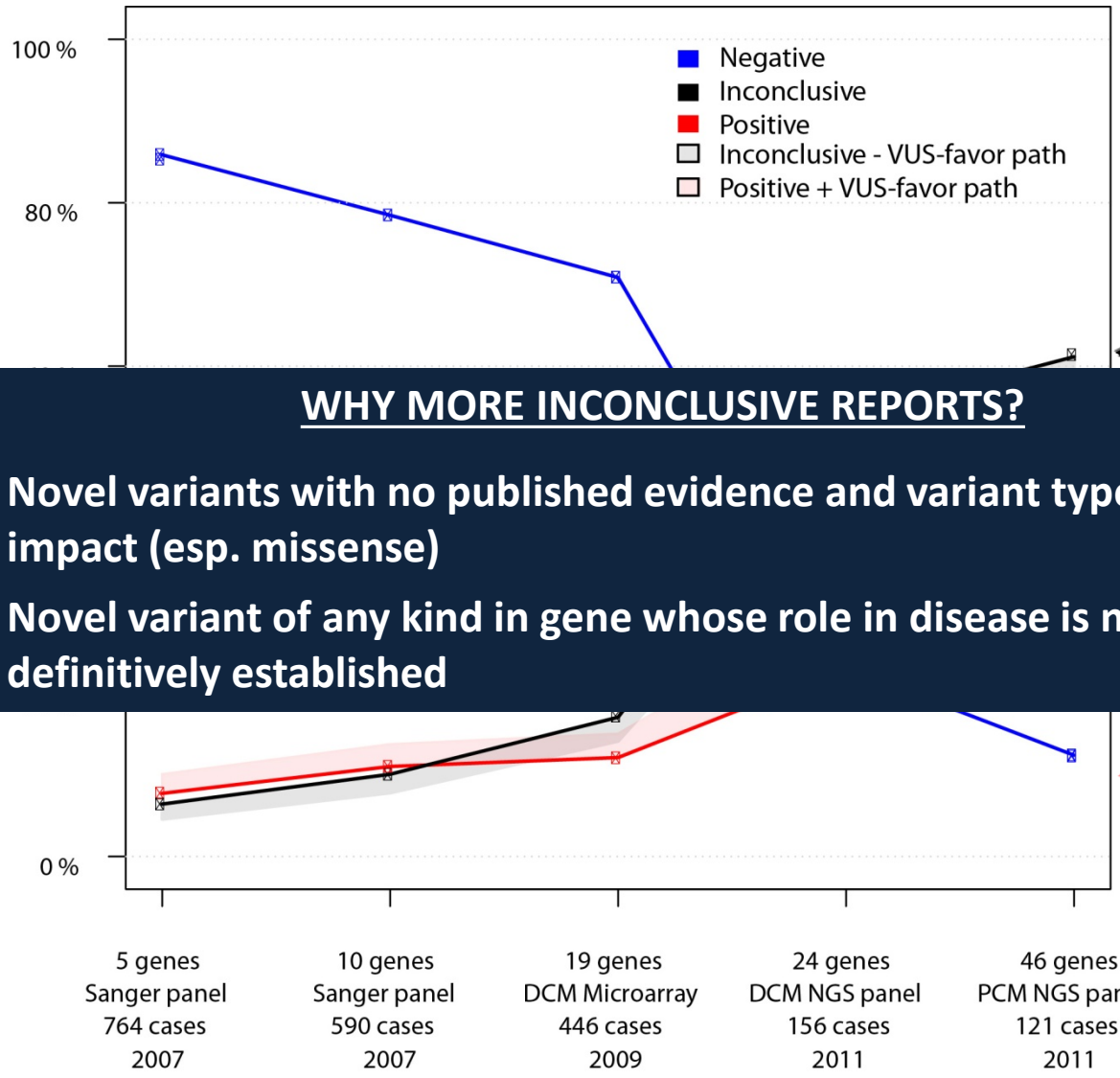
Integrate result with patient's clinical presentation:

- Does the variant cause **THIS** patient's disease?

CLINICAL RESULT REPORTING



THE GOOD, THE BAD AND THE UGLY



DCM

INCONCLUSIVE

7%

2007: ~10%

WHY MORE INCONCLUSIVE REPORTS?

- **Novel variants with no published evidence and variant type of unclear impact (esp. missense)**
- **Novel variant of any kind in gene whose role in disease is not definitively established**

THE DEBATED VALUE OF VUSs

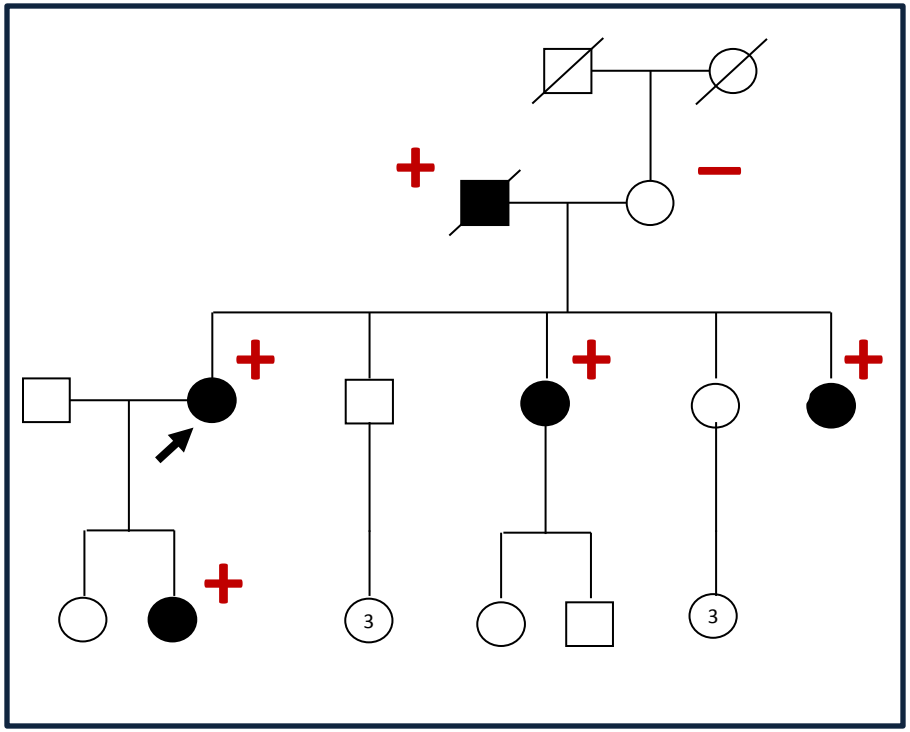
- **Novel variants with no published evidence and variant type of unclear impact (esp. missense)**

- **With the good (improved diagnostic sensitivity) comes some bad - how bad is the bad?**
- **Depends on many factors**
  - Patient ability to deal with uncertainty
  - Presence of a family history – can turn VUSs into Pathogenic!
  - Also – now the world is moving closer together, ability to solve cases by connecting patients around the globe

For disorders with a high degree of allelic heterogeneity there would **NEVER** be progress if one tested only what is already known..

FROM VUS TO DISEASE CAUSING VARIANT

Novel variants can start out as a VUS but can have clinical utility



NEW!
ASSESSMENT OF GENE-DISEASE RELATIONSHIPS

- **Novel variant of any kind in gene whose role in disease is not definitively established**

- Most novel variants in genes that are not strongly linked with disease cannot be interpreted
- Traditionally not a problem because old tests could not accommodate more than a few genes
- That barrier is gone…. How to select valid disease genes?

Many published claims for a gene-disease relationship do not withstand the rigor of **CLINICAL GRADE** curation

Evidence Level		Evidence Description
Supportive Evidence	DEFINITIVE	The role of this gene in this particular disease has been repeatedly demonstrated in both the research and clinical diagnostic settings, and has been upheld over time (in general, at least 3 years). No convincing evidence has emerged that contradicts the role of the gene in the specified disease.
	STRONG	The role of this gene in disease has been independently demonstrated in at least two separate studies providing **strong** supporting evidence for this gene's role in disease, such as the following types of evidence: • Strong variant-level evidence demonstrating numerous unrelated probands with variants that provide convincing evidence for disease causality[1] • Compelling gene-level evidence from different types of supporting experimental data[2]. In addition, no convincing evidence has emerged that contradicts the role of the gene in the noted disease.
		There is **moderate** evidence to support a causal role for this gene in this disease, such as: • At least 3 unrelated probands with variants that provide convincing evidence for convincing evidence has emerged that contradicts the role of the gene in the noted disease.
	NO REPORTED EVIDENCE	Evidence for a causal role in disease has not been reported. These genes might be "candidate" genes based on linkage intervals, animal models, implication in pathways known to be involved in human diseases, etc., but no reports have directly implicated the gene in human disease cases.
Contradictory Evidence	CONFLICTING EVIDENCE REPORTED	Although there has been an assertion of a gene-disease association, conflicting evidence for the role of this gene in disease has arisen since the time of the initial report indicating a disease association. Depending on the quantity and quality of evidence disputing the association, the association may be further defined by the following two sub-categories: 1. **Disputed** a. Convincing evidence *disputing* a role for this gene in this disease has arisen since the initial report identifying an association between the gene and disease. b. Refuting evidence need not outweigh existing evidence supporting the gene:disease association. 2. **Refuted** a. Evidence refuting the role of the gene in the specified disease has been reported and significantly outweighs any evidence supporting the role. b. This designation is to be applied at the discretion of clinical domain experts after thorough review of available evidence

Pillars of evidence

- # of clearly pathogenic variants reported
- # of independent studies / # of probands with pathogenic variants
- Statistical evidence from case/control cohorts
- Strength of supporting experimental data (animal models, *in vitro* data)

Clinicalgenome.org

NEXILIN REVISITED

ARTICLES

nature medicine

-Candidate gene analysis (2009)
-Zebrafish morpholino knockdown results in DCM
-Sequenced patient cohort, 6/910 (**0.3%)** patients have same 3 base del (Gly650del)
-Absent from>2,500 ctrl chromosomes - **but present in 0.7% (58/7842 ESP)**
-mRNA injection of mutant RNA shows effect of this variant on Z-disk

REPORT

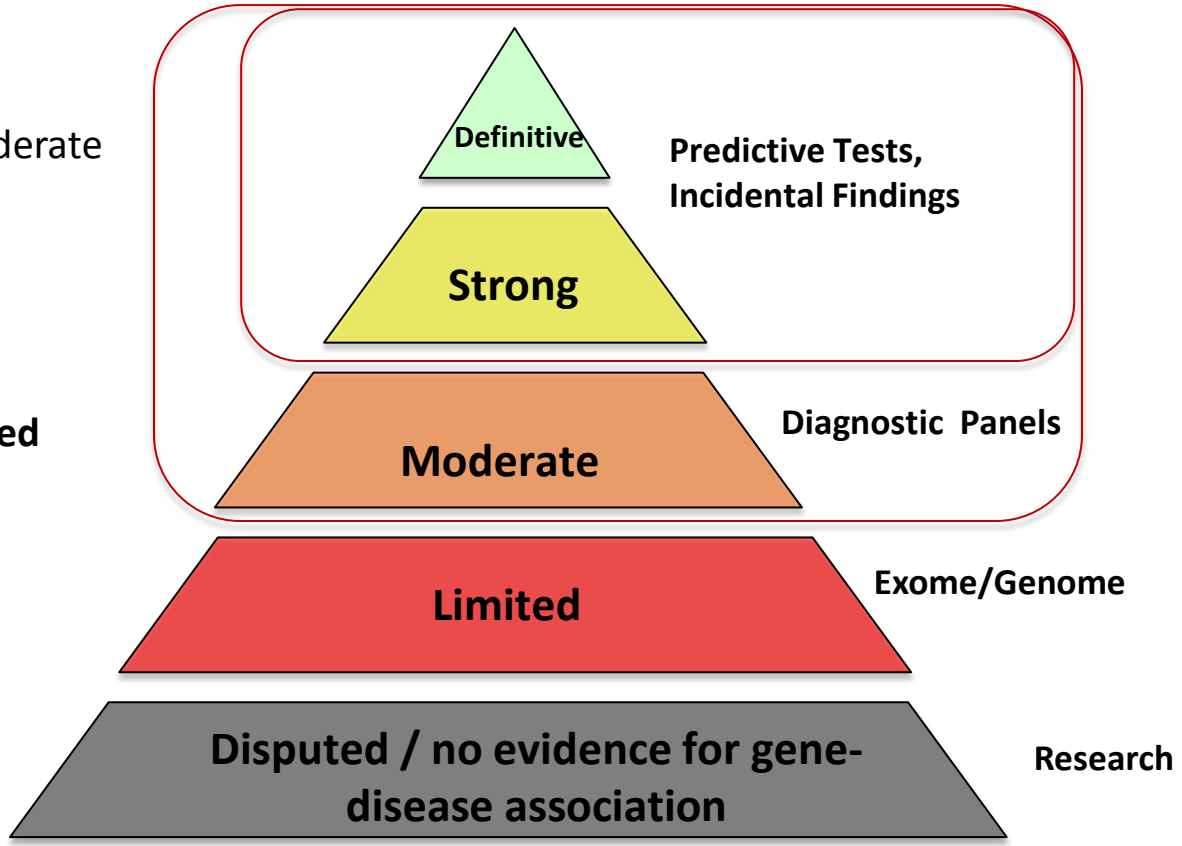
- Candidate gene analysis
- 2 missense variants, each present in 3 affected individuals/family; **1 classified as likely benign by LMM based on frequency in Chinese (5/394 chrom)**
- in vitro studies show local accumulation of protein

CLINICAL VALIDITY

Diagnostic testing

- Usually includes genes with moderate to definitive disease association
- Genes with credible variants +/- additional data

Expert consensus guidelines needed



Courtesy of ClinGen (Heidi Rehm)

CLINGEN'S CARDIOVASCULAR DOMAIN WORKING GROUP

Co-chairs: Birgit Funke, Ray Hershberger

PROJECT AREA 1: Outreach

- Identify experts and resources around the globe
- Facilitate submission of existing variants into ClinVar

PROJECT AREA 2: Variant curation

- Use high impact genes to develop framework

PROJECT AREA 3: Gene curation

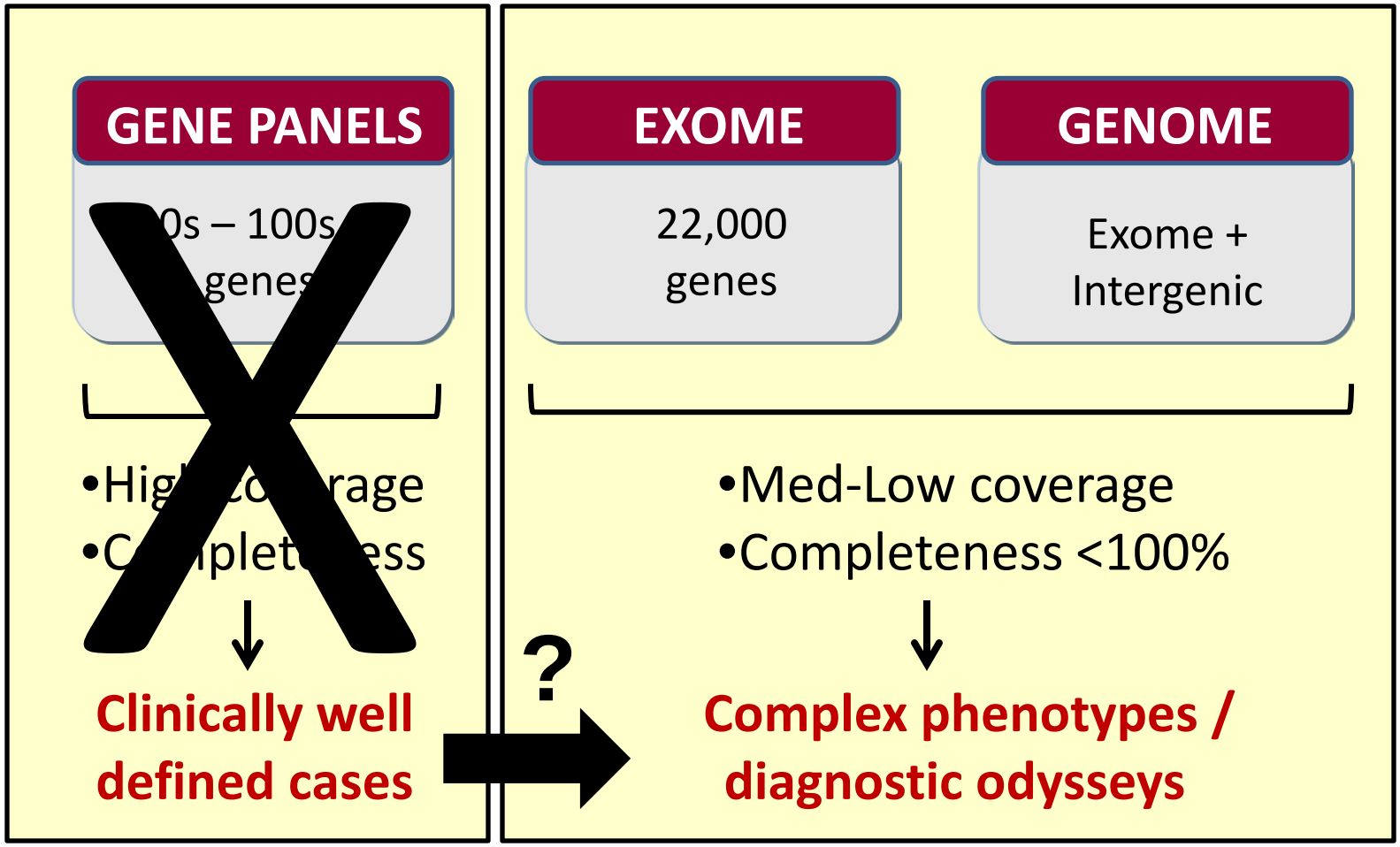
- Curate evidence for gene-disease relationships
- Use ClinGen's clinical validity scheme

KEEPING UP WITH ACCELERATING KNOWLEDGE

- Utility of multi-gene and multi-disease panels is no longer debated
- Higher risk of detecting VUSs is the only negative but can be minimized with rigorous gene selection

- Increasing rate of disease gene discovery – how to keep up??
- Cost of developing and updating gene panels is not sustainable

THE CURRENT DEBATE: PANELS OR EXOME?



INCENTIVES

- A large fraction of panels are **NEGATIVE** (often >50%)
- Growing appreciation of “phenotypic expansion” – argument for hypothesis fee testing
- Additional tests often end up being more expensive than WES
- Always up to date (accelerating pace of gene discovery)
- Easier to maintain for labs than growing # of gene panels

BARRIERS

BARRIERS

- Cost (though gap is closing)
- Incomplete coverage (suboptimal design)

RISK

- Loss of intimate /a priori knowledge on tested genes

BARRIERS ARE FAST DISAPPEARING

COST

Small tests can end up being more expensive than WES

EXAMPLE

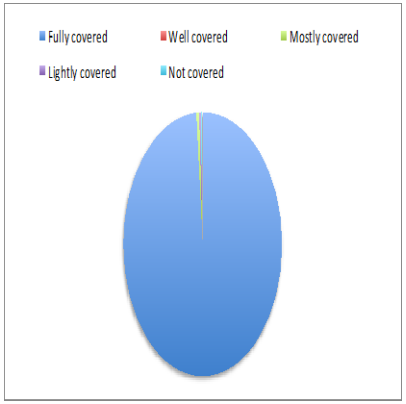
- **Ordered test: CMT Sequencing Test, Lab XXX**
  - 23 genes including CNV analysis
  - Clin. Sens. = 65%
- **Enhanced Exome (would need additional PMP22 del/dup)**
  - 34 genes (99.5% bases >20x)
  - Clin. Sens. = 75-80%
- **Exome turned out to be cheaper (enough to add PMP22del/dup)**

BARRIERS ARE FAST DISAPPEARING

LACK OF COMPLETENESS

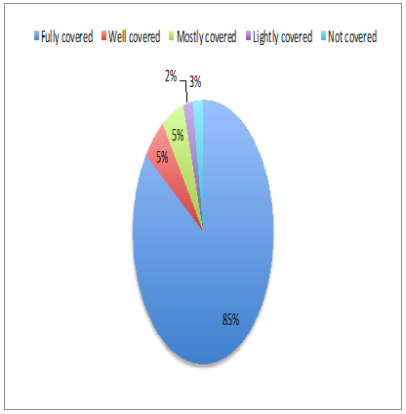
Pan Cardiomyopathy Panel – 51 genes

Targeted capture (Agilent)



- 0.7% bp < 20x
- **<1% exons not fully covered**

Exome data (Agilent v5)



- 3.7% bp < 20x
- **15% exons not fully covered**

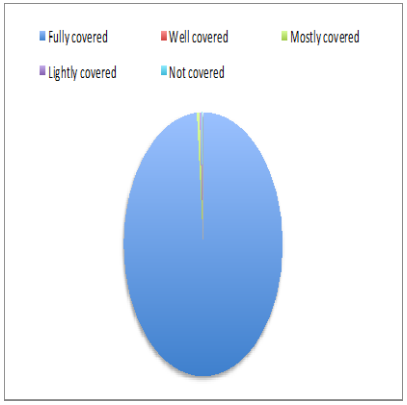
BARRIERS ARE FAST DISAPPEARING

LACK OF COMPLETENESS

Medical Exome Team (A. Santani, M. Hegde, B. Funke and teams)

Pan Cardiomyopathy Panel – 51 genes

Targeted capture data

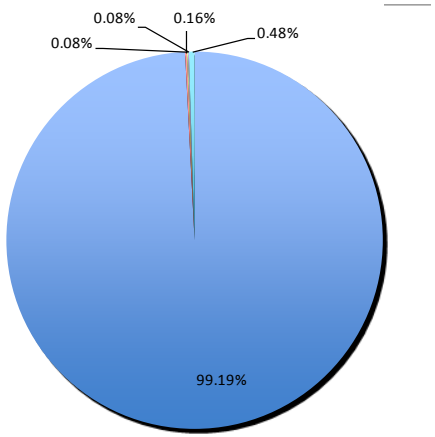


>99% of exons fully covered
(every base ≥ 20x)

HiSeq25000
10 samples/lane
~400x avg coverage

=

Exome data (v5-**plus**)

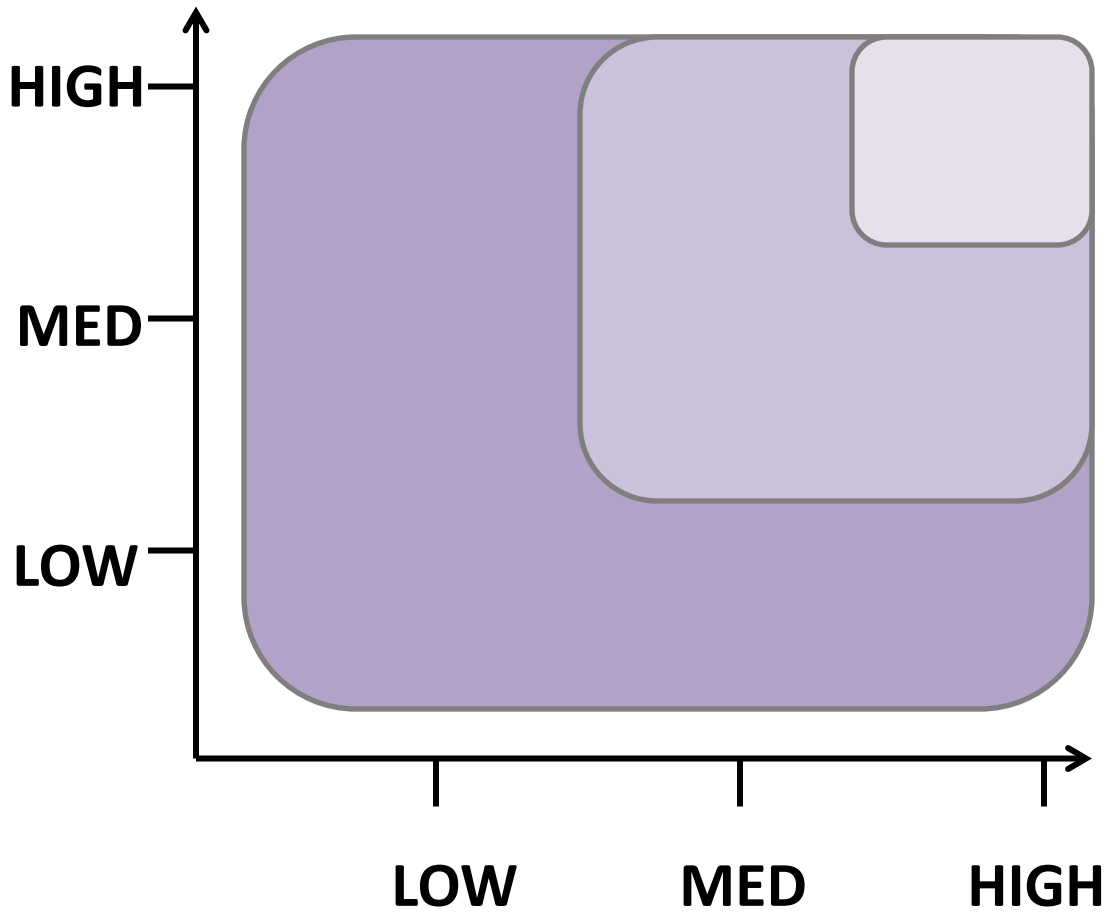


99% of exons fully covered
(every base ≥ 20x)

HiSeq2500
4 samples/lane
~200x avg coverage

THIS BARRIER IS NOT SO EASY TO ELIMINATE

NEED TO EDUCATE



TODAY
Exome sequencing ordered by experts and analyzed by experts

FUTURE
Need pre-curated information to educate + ensure adequate testing

RE-DEFINING
THE QUESTION

WE ARE ASKING THE WRONG QUESTION!

Assuming adequate ***coverage and assay cost***, WES and WGS sequencing can be used in various ways!!

It is expected that this will be reality in the near term future.

- Genotyping (interrogate only known pathogenic variants)
- Sequencing - Panel testing (well established genes)
- Sequencing - All genes – when clinical dx not clear but family Hx suggests genetic etiology

The critical question

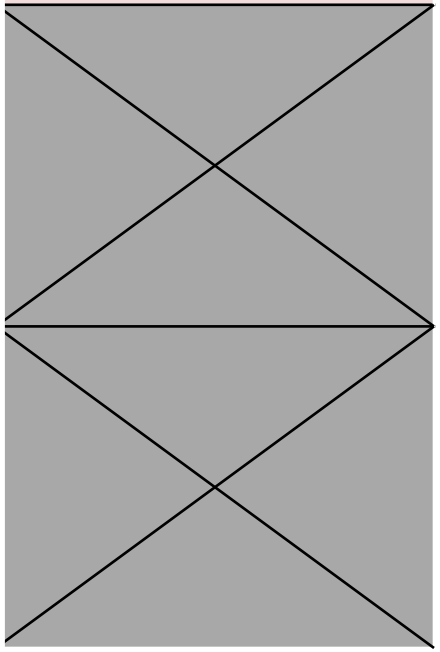
How **specific** is the patient's **phenotype**? → will dictate which set of genes we look at first and how deep the analysis needs to be

RETHINKING DISEASE-TARGETED PANELS

Use exome but guarantee full coverage of critical genes

TRADITIONAL DISEASE FOCUSED PANEL

- Coverage: 100% with fill-in
- Report: P, LP, VUS, LB



IMPORTANCE OF A STANDARDIZED AND STRUCTURED EVALUATION OF GENES

Goal: define indication driven gene panel (inherited renal disorders)

- Used ontology driven databases/tools to create a draft gene list (n=279)
  - Clinical expert opinion
  - ClinGen matrix –based clinical validity assessment

Category	n	Definitive Evidence	Strong Evidence	Moderate Evidence	Limited Evidence	No Evidence
Mission Critical	126	**55**	**32**	20	17	2
Nice to Have	22	8	3	4	6	1
Rest	131	**33**	**28**	27	27	17
TOTAL	279	**96**	**63**	51	50	20

SUMMARY

- Multi-gene and multi-disease testing is useful for disorders with clinical and genetic heterogeneity
- A genome will soon be cheap enough to be the first line test for all genetic disorders
- Understanding the clinical scenario is key – the test becomes an informatics exercise
  - Analyze just a few sites of known pathogenic variation (→ achondroplasia)
  - Analyze a single gene (→ Birt Hogg Dube: >90% of variants in FLNC)
  - Analyze a set of genes (→ Patient with classic HCM + family history of HCM)
  - Analyze exome (→patient with complex phenotype, no clear Dx but family Hx suggestive of genetic etiology)
- Curating the validity of gene-disease relationships is essential

ACKNOWLEDGMENTS

LMM

- **Heidi Rehm**
- Matt Lebo
- Sami Amr
- Heather McLaughlin
- Heather Mason-Suares
- Jun Shen

Operations

- ***Lisa Mahanta***
- Robin Hill Cook
- Laura Hutchinson
- Shangtao Liu
- Ashley Frisella
- Timothy Fiore
- Jiyeon Kim
- Maya Miatkowski
- William Camara
- Eli Pasackow

Development

- ***Birgit Funke***
- Beth Duffy-Hynes
- Mark Bowser
- Diana Mandelker

Fellows

- Ozge Birsoy
- Yan Meng
- Ahmad A. Tayoun
- Jillian Buchan
- Fahad Hakami
- Hana Zouk

Genetic Counselors

- ***Amy Lovelette***
- ***Melissa Kelly***
- Mitch Dillon
- Shana White
- Andrea Muirhead
- Danielle Metterville

Office

- Peta-Gaye Brooks
- Kim O'Brien
- Lauren Salviati
- Sarah Yuhas
- Summer Spillane

Transl. Genomics Core

Sami Amr

- All team members

IT Team

- **Sandy Aronson**
- Natalie Boutin
- All team members

Bioinformatics

- **Matt Lebo**
- Rimma Shakhbatyan
- Jason Evans
- Himanshu Sharma
- Peter Rossetti
- Ellen Tsai
- Chet Graham

Leadership and Faculty

- **Scott Weiss**
- Meini Sumbada-Shin
- Robert Green
- Immaculata deVivo
- Mason Freeman
- John Iafrate
- Mira Irons
- Isaac Kohane
- Raju Kucherlapati
- Cynthia Morton
- Soumya Raychaudhuri
- Patrick Sluss

ClinGen leadership and cardiovascular domain working group


ACATAATCTACGAACTATCAATGTTTATGATGC
ATTTGAATCTGATAATGCGAAGAGTTGCTAATA
ATCTTGGATTCTATCGATAACAGCCGAGGTGCC
TACTTTGGATACTTTGACAGGTGGACACTCAAA
ATGGCAAACGTATTCCTGAGACTGCCAGAGCTC
GGCTTTATTGAAGTACCATCTTACATTTTAAAC
TCACGTTACGAAAGATAACATTHANKS!AGTCT
ATATCAAAAGTGATCATAATTCTGAAAATCCTT
AATGGATATTTAACCTTGGCTCCTAATTTCGGT
AGGTGGTTTTGTAACTATTTGCAGACATCCATC
GTATAATAAAAAAGATCAGAAGGGTTTACTATTA
TTTAATGCTGATTTTGATGGAGATGAGATGACA
CAATCTCGAACAAGCTTTGATTATGAACTCACG
GCAATCCAATGTTCGGCTTGGTCCAAGATCAAA