NGS IN THE CLINIC
GENE PANEL TESTING FOR INHERITED CONDITIONS

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http://www.nature.com/nrg/journal/v11/n1/images/nrg2626-f2.jpg
INTRODUCTION
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

- 2007: ~10%
- 2011: ~37%

NGS
WHICH DISORDERS BENEFIT FROM PANEL TESTING?
EXAMPLE: INHERITED CARDIOMYOPATHIES

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

<table>
<thead>
<tr>
<th>Condition</th>
<th>Incidence</th>
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<tbody>
<tr>
<td>HCM</td>
<td>1:500</td>
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<tr>
<td>DCM</td>
<td>&gt; 1:2,500</td>
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<tr>
<td>ARVC</td>
<td>1:1,000 - 1:5,000</td>
</tr>
<tr>
<td>OTHER (RARE)</td>
<td></td>
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<td></td>
<td>LVNC</td>
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<td>RCM</td>
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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, MD¹,², Melissa A. Kelly, MS¹, Gregory McDermott, BA¹, Birgit H. Funke, PhD¹,³,⁴, Matthew S. Lebo, PhD¹,³,⁴, Samantha B. Baxter, MS¹, Jun Shen, PhD¹,³,⁴, Heather M. McLaughlin, PhD¹,³,⁴, Eugene H. Clark, BM¹, Larry J. Babb, BS¹, Stephanie W. Cox, BS¹, Steven R. DePalma, PhD⁵,⁶, Carolyn Y. Ho, MD⁷, J.G. Seidman, PhD⁶, Christine E. Seidman, MD⁵,⁶,⁷ and Heidi L. Rehm, PhD¹,³,⁴

- 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

1958 1st HCM report (Teare)
1962 Familial disease
1964 1st comprehensive disease description (Braunwald)
1973 Advent of echo diagnosis (M-mode)
1979 2-D echo
1989 Map HCM to chromosome 14q1
1990 1st HCM gene (MYH7)
1992 Mutations & prognosis
1995 MYBPC3
1995 Prevalence (1:500)
2000 ICD for SD Prevention
2003 Commercial genetic testing
2011 ≥10 genes; > 1400 mutations
2009 4 testing labs (U.S.)


1st HCM gene (MYH7)
Clinical Genetic Testing
Clinical Genetic Testing
Testing covers all inherited cardiomyopathies

51 genes/patient

5 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

- TTN
- TNNT2
- DSP
- MYH7
- LMNA

Melissa Kelly

HCM

- MYBPC3 50%
- MYH7 34%

Ahmed Alfares
Hypertrophic Cardiomyopathy: 10 years – 11 genes tested

63% of variants have been seen only once

Need to sequence entire coding sequence of many genes for maximum clinical sensitivity

Courtesy of Ahmed Alfares and Heidi Rehm
(cardiomyopathy, hearing loss, rasopathies, aortopathies, somatic and hereditary cancer, pulmonary disorders, skin disorders, other genetic syndromes)

NOT JUST CARDIOMYOPATHY....

DIAGNOSTIC TESTING OF 15,000 LMM PROBANDS

68% (1120/1648) pathogenic/likely pathogenic variants are seen only once

96% of variants are seen <10 times

Number of Probands

Number of Pathogenic Variants

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
• 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
• ~3% of **DCM** patients carry a pathogenic variant in an **ARVC** gene
**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.
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"

1. **Patient** → **Clinical Diagnosis** → **Hypothesis** → **Genetic Test** → **Sequence Variants**

   - **Result**: negative

   - **Interpretation**:
     - Fill in failed
     - Confirm variants

   - **Clinical Diagnosis**
TREND TOWARDS GENOME WIDE TESTING

COPY NUMBER ALTERATIONS
- Single/few
  - FISH, Southern; qPCR; MLPA
- Genome wide
  - Copy # arrays

GENOTYPING TESTS
- 1 – 100 mutations
  - Various methods
- Genome wide
  - SNP chips, bead arrays

SEQUENCING TESTS
- 1 – 10 genes
  - Sanger seq
- 10s to 100s of genes
  - NGS
- Genome-wide NGS
  - 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

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, PhD1, Nazneen Aziz, PhD1,2, Sherri Bale, PhD1, David Bick, MD1, Soma Das, PhD1, Julie Gastier-Foster, PhD1,2,8, Wayne W. Grody, MD, PhD3,10,11, Madhuri Hegde, PhD1,2, Elaine Lyon, PhD1, Elaine Spector, PhD1,4, Karl Voelkerding, MD1 and Heidi L. Rehm, PhD1; on behalf of the ACMG Laboratory Quality Assurance Committee

Disclaimer: These ACMG Standards and Guidelines were developed primarily as an educational resource for clinical laboratory geneticists to help them provide quality clinical laboratory services. Adherence to these standards and guidelines is voluntary and does not necessarily assure a successful medical outcome. These Standards and Guidelines should not be considered inclusive of all proper procedures and tests or exclusive of other procedures and tests that are reasonably directed to obtaining the same results. In determining the propriety of any specific procedure or test, the clinical laboratory geneticist should apply his or her own professional judgment to the specific circumstances presented by the individual patient or specimen. Clinical laboratory geneticists are encouraged to document in the patient’s record the rationale for the use of a particular procedure or test, whether or not it is in conformance with these Standards and Guidelines. They also are advised to take notice of the date any particular guideline was adopted and to consider other relevant and scientific information that becomes available after that date. It also would be prudent to consider whether intellectual property interests may restrict the performance of certain tests and other procedures.
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

Lab Result

Variant Annotation

- Published + in house data
- Segregation studies
- Population frequency
- Amino acid conservation
- Predictions: PolyPhen, SIFT
- Splicing predictions

Clinical Data

Custom knowledge

Variant classification

Benign

Likely Benign

VUS

Likely Pathogenic

Pathogenic

Clinical Data

CLINICAL REPORTING

NEGATIVE

INCONCL.

POSITIVE
THE GOOD, THE BAD AND THE UGLY

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

DCM

2007: ~10%

2007: ~10%

7%
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..
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
### 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)
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

- 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
Diagnostic testing

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

Expert consensus guidelines needed
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
• 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?

**GENE PANELS**
- 10s – 100s of genes
- High coverage
- Completeness

Clinically well defined cases

**EXOME**
- 22,000 genes
- Med-Low coverage
- Completeness <100%

Complex phenotypes / diagnostic odysseys

**GENOME**
- Exome + Intergenic
• 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

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

RISK

• Loss of intimate /a priori knowledge on tested genes
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)
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
Pan Cardiomyopathy Panel – 51 genes

**Targeted capture data**

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

**Exome data (v5-plus)**

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

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**Medical Exome Team (A. Santani, M. Hegde, B. Funke and teams)**
Need pre-curated information to educate + ensure adequate testing

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

<table>
<thead>
<tr>
<th>TRADITIONAL DISEASE FOCUSED PANEL</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Coverage: 100% with fill-in</td>
</tr>
<tr>
<td>• Report: P, LP, VUS, LB</td>
</tr>
</tbody>
</table>
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

### IMPORTANCE OF A STANDARDIZED AND STRUCTURED EVALUATION OF GENES

<table>
<thead>
<tr>
<th>Category</th>
<th>n</th>
<th>Definitive Evidence</th>
<th>Strong Evidence</th>
<th>Moderate Evidence</th>
<th>Limited Evidence</th>
<th>No Evidence</th>
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<tbody>
<tr>
<td>Mission Critical</td>
<td>126</td>
<td>55</td>
<td>32</td>
<td>20</td>
<td>17</td>
<td>2</td>
</tr>
<tr>
<td>Nice to Have</td>
<td>22</td>
<td>8</td>
<td>3</td>
<td>4</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>Rest</td>
<td>131</td>
<td>33</td>
<td>28</td>
<td>27</td>
<td>27</td>
<td>17</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>279</td>
<td>96</td>
<td>63</td>
<td>51</td>
<td>50</td>
<td>20</td>
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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
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