Agenda

- What’s new at Invitae?
  Oncology Marketing Manager, Invitae
  Mona Varia

- Clinical Validity and Actionability of Multigene Tests for Hereditary Breast and Ovarian Cancer
  Head of Scientific Affairs, Invitae
  Stephen E. Lincoln

- A systematic and accurate approach to variant classification
  Head of Clinical Genomics, Invitae
  Scott Topper, PhD, FACMG
The new Invitae test catalog: Building on our promise

Hundreds of new genes.
Dramatically expanded test catalog.
Same mission.
Invitae’s new test catalog

**BROADER, DEEPER MENU WITH FLEXIBILITY TO FIT YOUR NEEDS**

**NEW:** Common Hereditary Cancers Panel and Gastric Cancer Panel

**EXPANDED:** Panels for breast, gynecologic, colon, and pancreatic cancer

**INCLUDES:** Promoter regions for relevant genes, MSH2 inversion, and full PMS2 and CHEK2

**MORE THAN 30 TESTS AND MANY NEW CONDITIONS**

**NEW:** Invitae Arrhythmia and Cardiomyopathy Comprehensive Panel

**NEW TESTING CATEGORIES:** Familial hypercholesterolemia, aortopathies, pulmonary hypertension, and congenital heart disease

**FLEXIBLE ORDERING:** Combine genes and panels to build the right test
Invitae’s new test catalog

MORE THAN 100 NEW CAREFULLY CURATED GENES

NEW: Tests for various congenital structural heart defects, a strong option for patients with negative or uncertain chromosomal microarray results

EXPANDED: Offerings for RASopathies and ciliopathies, including a comprehensive targeted panel for primary ciliary dyskinesia

BROAD, AFFORDABLE MENU WITH PRE-CURATED PANELS AND FLEXIBLE ORDERING

NEW: Panels for Duchenne/Becker muscular dystrophy and dystonia

EXPANDED: Offerings for Charcot-Marie-Tooth and hereditary spastic paraplegia
Invitae advantage remains the same

**FLEXIBLE TEST OPTIONS.**
Select a pre-curated gene panel, customize with one click, or design your own.

**AFFORDABLE, TRANSPARENT PRICING.**
Our pricing remains the same even with our newly expanded test catalog.

**SUPPORT EVERY STEP OF THE WAY.**
From clinical consultations to help with ordering, billing, and understanding results.

**CONFIDENCE AND QUALITY.**
Proven results based on published data and backed by our team of genetic and medical experts.
Clinical Validity and Actionability of Multigene Tests for Hereditary Breast and Ovarian Cancer

STEPHEN E. LINCOLN
Scientific Affairs, Invitae

September 2015
• Leif Ellisen
• Andrea Desmond
• Michelle Gabree
• Kristen Shannon

• Jim Ford
• Allison Kurian
• Meredith Mills

• Nadine Tung

• Steve Lincoln
• Shan Yang
• Yuya Kobayashi
• Michael Anderson
• Invitae Clinical Genomics Group

• Kevin Jacobs
• Geoff Nilsen
• Josh Paul
• Mike Kennemer
• Dan Kvitek

• Scott Topper
• Martin Powers
• Federico Monzon
Genetic Heterogeneity

- BRCA1/2
- TP53
- CDH1
- PTEN
- NF1
- CHEK2
- MRE11A
- RAD51C
- MSH2
- MLH1
- RAD50
- MSH6
- PMS2
- STK11
- PALB2
- ATM
- NBN
- BRIP1
- Others
gene list from Easton et al. NEJM 2015
Figure adapted from Tuya Pal, Moffitt Cancer Center
**Clinical Heterogeneity**

<table>
<thead>
<tr>
<th>Genes</th>
<th>Elevated Cancer Risks</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRCA1, BRCA2</td>
<td>Breast, ovary, fallopian tube, peritoneal, prostate, pancreas, male breast</td>
</tr>
<tr>
<td>MLH1, MSH2, MSH6</td>
<td>Colon, endometrium, ovary, stomach, urinary tract, small bowel</td>
</tr>
<tr>
<td>PMS2, EPCAM</td>
<td></td>
</tr>
<tr>
<td>STK11</td>
<td>Breast, ovary, colon, stomach, pancreas, colon, lung</td>
</tr>
<tr>
<td>CHEK2</td>
<td>Breast, ovary, prostate, colon, thyroid, kidney</td>
</tr>
<tr>
<td>TP53</td>
<td>Bone, breast, brain, andrenocortical, colon, leukemia</td>
</tr>
<tr>
<td>PTEN</td>
<td>Breast, thyroid, endometrium, colon, kidney</td>
</tr>
<tr>
<td>CDH1</td>
<td>Stomach, breast, colorectal</td>
</tr>
<tr>
<td>Others…</td>
<td>Multiple…</td>
</tr>
</tbody>
</table>

Cragun et al. Clinical Genetics 2014
NCI BRCA1/2 Fact Sheet, 2014
360 patients with ovarian, fallopian, or peritoneal cancer
Unselected for age of onset or family history
21-gene germline test

17.5% BRCA1/2 positive
6.1% non-BRCA1/2 positive
  - BARD1, BRIP1, CHEK2, MRE11A, MSH6,
  - NBN, PALB2, RAD50, RAD51C, TP53

Walsh et al., PNAS 2011
### Frequency of Mutations in Individuals With Breast Cancer Referred for *BRCA1* and *BRCA2* Testing Using Next-Generation Sequencing With a 25-Gene Panel

Nadine Tung, MD\(^1,2\); Chiara Battelli, MD\(^1\); Brian Allen, MS\(^3\); Rajesh Kaldate, MS\(^3\); Satish Bhatnagar, PhD\(^4\); Karla Bowles, PhD\(^5\); Kirsten Timms, PhD\(^6\); Judy E. Garber, MD\(^2,7\); Christina Herold, MD\(^1,2\); Leif Ellisen, MD, PhD\(^2,8\); Jill Krej dovsky, MS\(^9\); Kim DeLeonardis, MS\(^9\); Kristin Sedgwick, MS\(^9\); Kathleen Soltis, MA\(^9\); Benjamin Roa, PhD\(^5\); Richard J. Wenstrup, MD\(^10\); and Anne-Renee Hartman, MD\(^3\)

- 2,158 patients with breast cancer referred for genetic testing
  - Some also had ovarian or pancreatic cancer
- 38% from high risk families; median age at Dx ≈ 46
- 25-gene germline test

- **9.3% BRCA1/2 positive**
- **4.3% non-BRCA1/2 positive**
  - ATM, BARD1, BRIP1, CHD1, CDKN2A, CHEK2, MSH2, MSH6,
  - MUTYH (biallelic), NBN, PALB2, PMS2, TP53

*Tung et al., Cancer 2014*
Pilot clinical utility study

Clinical Evaluation of a Multiple-Gene Sequencing Panel for Hereditary Cancer Risk Assessment

Allison W. Kurian, Emily E. Hare, Meredith A. Mills, Kerry E. Kingham, Lisa McPherson, Alice S. Whittemore, Valerie McGuire, Uri Ladabaum, Yuya Kobayashi, Stephen E. Lincoln, Michele Cargill, and James M. Ford

Findings:
- Non-BRCA1/2 deleterious mutations were prevalent (4.5% - 9%)
- Many non-BRCA results were potentially clinically actionable

Limitations:
- Small patient population (n=198)
- Research panel included genes with varying levels of evidence
- Predates current interpretation and management guidelines
Key questions about panel tests

- **Analytic Validity**
  - Can the new tests detect everything the old, gene by gene, tests did?
  - Including technically difficult genes and mutation types?

- **Clinical Validity**
  - Interpretation/classification of DNA alterations
  - Clinical relevance of findings

- **Clinical Utility**
  - What is the clinical impact of using these new tests?
Key questions about panel tests

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▪ **Clinical Utility**
  – What is the clinical impact of using these new tests?
Laboratory technical study

A systematic comparison of traditional and multi-gene panel testing for hereditary breast and ovarian cancer in more than 1000 patients

Stephen E. Lincoln¹, Yuya Kobayashi¹, Michael J. Anderson¹, Shan Yang¹, Andrea J. Desmond², Meredith A. Mills³, Geoffrey B. Nilsen¹, Kevin B. Jacobs¹, Federico A. Monzon¹, Allison W. Kurian³, James M. Ford³, Leif W. Ellisen²,⁴

1. Invitae, San Francisco, CA
2. Massachusetts General Hospital Cancer Center, Boston, MA
3. Stanford University School of Medicine, Stanford, CA
4. Harvard Medical School, Boston, MA
Key questions about panel tests

▪ **Analytic Validity**
  – Can the new tests detect everything the old, gene by gene, tests did?
  – Including technically difficult genes and mutation types?

▪ **Clinical Validity**
  – Interpretation/classification of DNA alterations
  – Clinical relevance of findings

▪ **Clinical Utility**
  – What is the clinical impact of using these new tests?
Analytic validity of NGS in N=1105 individuals

NGS vs. Traditional Methods In 1105 Individuals

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>100.0%</td>
</tr>
<tr>
<td>Specificity</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

To achieve this, **specialized NGS methods**, biochemical and bioinformatics, are required.

The most challenging classes of variation tend to be **not well represented** in other validation studies.

750 Comparable Variants (Pathogenic or Otherwise)

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sequence Changes</td>
<td>721</td>
</tr>
<tr>
<td>Del/dups (CNVs)</td>
<td>29</td>
</tr>
<tr>
<td>Single Nucleotide</td>
<td>549</td>
</tr>
<tr>
<td>Small Indel</td>
<td>156</td>
</tr>
<tr>
<td>Large Indel*</td>
<td>13</td>
</tr>
<tr>
<td>Complex**</td>
<td>6</td>
</tr>
</tbody>
</table>

* Large Indel is deletion \( \geq 10 \text{bp} \), insertion \( \geq 5 \text{bp} \)

** Complex includes homopolymer associated variants, indels in low-complexity sequence, short range haplotypes, etc.

Lincoln et al., *J Mol Diag* 2015
Copy number del/dup detection by NGS

1. Biochemical methods to make data highly callable for CNVs
   - Nord et al. (U. Washington) - BMC Genomics 2011

2. Read depth analysis
   - Jacobs et al., CSHL 2013
   - Jacobs et al., ASHG 2013

3. Combined with split-read analysis
A different study: Analytic validity in 250 individuals

<table>
<thead>
<tr>
<th>NGS vs. Traditional Methods in 250 Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
</tr>
<tr>
<td>Specificity</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>3025 Variants Appropriate to Measure Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sequence Changes</td>
</tr>
<tr>
<td>Del/dups (CNVs)</td>
</tr>
</tbody>
</table>

Note: These numbers assume that Table 2 includes all of the indels and del/dups, and that the remaining variants are benign SNPs. This could have been worded more clearly in the paper.

<table>
<thead>
<tr>
<th>Type</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single Nucleotide</td>
<td>3010</td>
</tr>
<tr>
<td>Small Indel</td>
<td>11 ?</td>
</tr>
<tr>
<td>Large Indel *</td>
<td>0</td>
</tr>
<tr>
<td>Complex **</td>
<td>0</td>
</tr>
</tbody>
</table>

* Large Indel is deletion ≥ 10bp, insertion ≥ 5bp
** Complex includes homopolymer associated variants, indels in low-complexity sequence, short range haplotypes, etc.

Chong et al., PLOS One 2014
A significant fraction of the pathogenic variants in clinical cases are technically challenging.

Total: 48 of 260 pathogenic variants (13.4%) in study

Lincoln et al., J Mol Diag 2015
Bioinformatics screen: Sequencing reads from both PMS2 and PMS2CL are aligned to PMS2 only.

Non-benign variants detected

Sequence variants

Deletion/duplication variants (CNVs)

MLPA confirmation of deletion/duplication variants

Sanger sequencing of LR-PCR products of PMS2 and PMS2CL is performed to determine the location of variants.
Key questions about panel tests

- **Analytic Validity**
  - Can the new tests detect everything the old, gene by gene, tests did?
  - Including technically difficult genes and mutation types?

- **Clinical Validity**
  - Interpretation/classification of DNA alterations
  - Clinical relevance of findings

- **Clinical Utility**
  - What is the clinical impact of using these new tests?
BRCA1/2 classification concordance

<table>
<thead>
<tr>
<th>Positive vs. not positive result for BRCA1/2</th>
<th>% of patients with one or more VUS in BRCA1/2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agree</td>
<td>Panel test</td>
</tr>
<tr>
<td>99.8%</td>
<td>4.1%</td>
</tr>
<tr>
<td>Disagree</td>
<td>Previous test</td>
</tr>
<tr>
<td>0.2%</td>
<td>3.2%</td>
</tr>
</tbody>
</table>

Positive result = pathogenic or likely pathogenic variant
Not positive = VUS, likely benign, or benign variants only

VUS rate = % patients with any variant of uncertain significance, regardless of pathogenic variants present in the same patient
Key questions about panel tests

- **Analytic Validity**
  - Can the new tests detect everything the old, gene by gene, tests did?
  - Including technically difficult genes and mutation types?

- **Clinical Validity**
  - Interpretation/classification of DNA alterations
  - Clinical relevance of findings

- **Clinical Utility**
  - What is the clinical impact of using these new tests?
Clinical actionability study

Clinical Actionability of Multigene Panel Testing for Hereditary Breast and Ovarian Cancer Risk Assessment

Andrea Desmond, BS; Allison W. Kurian, MD, MSc; Michele Gabree, MS, CGC; Meredith A. Mills, BA; Michael J. Anderson, PhD; Yuya Kobayashi, PhD; Nora Horick, MS; Shan Yang, PhD; Kristen M. Shannon, MS, CGC; Nadine Tung, MD; James M. Ford, MD; Stephen E. Lincoln, BS; Leif W. Ellisen, MD, PhD

Usefulness of Multigene Testing
Catching the Train That’s Left the Station

Elizabeth M. Swisher, MD

Desmond et al., JAMA Oncol. 2015
Swisher, JAMA Oncol. 2015
Clinical study patient population

- Multisite *prospective* recruitment

- N=1046 patients referred to Genetics who:
  - *Met NCCN criteria* for hereditary breast/ovarian cancer evaluation
  - Not BRCA1/2 positive
  - Not referred because of a mutation identified in a relative
  - Tested with a multigene panel (25 to 29 genes)
  - Representative cohort (in both demographics and indications)

- **63** patients with non-*BRCA1/2* mutations
  - 40 of 1046 (3.8%)
  - 23 additional patients from clinical practice (who met same criteria)

Desmond *et al.*, JAMA Oncol 2015
Key questions about panel tests

- **Analytic Validity**
  - Can the new tests detect everything the old, gene by gene, tests did?
  - Including technically difficult genes and mutation types?

- **Clinical Validity**
  - Interpretation/classification of DNA alterations
  - Clinical relevance of findings

- **Clinical Utility**
  - What is the clinical impact of using these new tests?
Clinical relevance of 63 non-\textit{BRCA1/2} findings

- 74\% of the cancer-affected probands have a \textit{syndromic cancer} for the gene they were found to carry (26\% do not)

- In 92\% of cases, the patient’s \textit{personal and/or family history was consistent} with the syndromic effects of the gene they carry

- Nevertheless, in most of these cases the proband \textit{would not have been eligible for testing} for the gene they carry under indication-based guidelines

Desmond \textit{et al.}, \textit{JAMA Oncol} 2015
Key questions about panel tests

- **Analytic Validity**
  - Can the new tests detect everything the old, gene by gene, tests did?
  - Including technically difficult genes and mutation types?

- **Clinical Validity**
  - Interpretation/classification of DNA alterations
  - Clinical relevance of findings

- **Clinical Utility**
  - What is the clinical impact of using these new tests?
Clinical actionability of non-\textit{BRCA1/2} findings

- For patients with non-\textit{BRCA1/2} mutations we compared pre-test management recommendations to post-test recommendations, in both cases following current consensus management guidelines.

- Similar review of first-degree female family members

---

Desmond \textit{et al}., \textit{JAMA Oncol} 2015
<table>
<thead>
<tr>
<th>Intervention</th>
<th>Recommend MRI(^c) (&gt;20% risk of breast cancer(^d))</th>
<th>Recommend RRSO</th>
<th>Discuss Option of RRM</th>
</tr>
</thead>
</table>
| Warranted based on gene and/or risk level | ATM  
  BRCA1  
  BRCA2  
  CDH1  
  CHEK2  
  PALB2  
  PTEN  
  STK11  
  TP53 | BRCA1  
  BRCA2  
  Lynch syndrome\(^e\) | BRCA1  
  BRCA2  
  CDH1  
  PTEN  
  TP53 |
| Insufficient evidence for intervention\(^b\) | BARD1  
  BRIP1 | BARD1  
  BRIP1  
  PALB2  
  RAD51C  
  RAD51D | ATM  
  BARD1  
  CHEK2  
  PALB2  
  STK11 |

\(^a\)Other genes may be included in multi-gene testing.

\(^b\)Intervention may still be warranted based on family history or other clinical factors.

\(^c\)See NCCN Guidelines for Breast Cancer Screening and Diagnosis.

\(^d\)May be modified based on family history or specific gene mutation.

\(^e\)See NCCN Guidelines for Genetic/Familial High-Risk Assessment: Colorectal.

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**Note:** All recommendations are category 2A unless otherwise indicated. Clinical Trials: NCCN believes that the best management of any cancer patient is in a clinical trial. Participation in clinical trials is especially encouraged.
Genes with established links to breast cancer

The NEW ENGLAND JOURNAL of MEDICINE

SPECIAL REPORT

Gene-Panel Sequencing and the Prediction of Breast-Cancer Risk

Douglas F. Easton, Ph.D., Paul D.P. Pharoah, Ph.D., Antonis C. Antoniou, Ph.D., Marc Tischkowitz, M.D., Ph.D., Sean V. Tavtigian, Ph.D., Katherine L. Nathanson, M.D., Peter Devilee, Ph.D., Alfons Meindl, Ph.D., Fergus J. Couch, Ph.D., Melissa Southey, Ph.D., David E. Goldgar, Ph.D., D. Gareth R. Evans, M.D., Georgia Chenevix-Trench, Ph.D., Nazneen Rahman, M.D., Ph.D., Mark Robson, M.D., Susan M. Domchek, M.D., and William D. Foulkes, M.B., B.S., Ph.D.
Eleven genes with established links to breast cancer

**High-Risk Panels**
- BRCA1
- BRCA2
- TP53
- CDH1
- PALB2
- PTEN *
- STK11 *

**Moderate-Risk Panels**
- ATM
- CHEK2
- NBN °
- NF1 °

° Not mentioned in 1.2015 NCCN HBOC guidelines
* Relative risk is not yet precisely estimated (per Easton et al.)
+ Typically included on panels offered by multiple laboratories

Easton et al., NEJM 2015 (Table 3)
Clinical management recommendations changed

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Positive genes</th>
<th>Potential change</th>
<th>Patients</th>
<th>Family members</th>
</tr>
</thead>
<tbody>
<tr>
<td>High-risk genes</td>
<td>CDH1, TP53, PTEN, MLH1, MSH2, MSH6, PMS2, APC, BMPR1A, MUTYH (biallelic)</td>
<td>Guidelines-based surveillance/prevention</td>
<td>20 / 20</td>
<td>19 / 19</td>
</tr>
<tr>
<td>1.2015 NCCN management guidelines</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;40% breast cancer risk (and &lt;40% pre-test risk)</td>
<td>PALB2</td>
<td>Surgical prevention candidate</td>
<td>5 / 8</td>
<td>7 / 7</td>
</tr>
<tr>
<td>&gt;20% breast cancer risk (&lt;20% pre-test risk)</td>
<td>ATM, CHEK2, NBN, RAD51C, BRIP1</td>
<td>Enhanced breast screening candidate</td>
<td>5 / 32</td>
<td>13 / 29</td>
</tr>
<tr>
<td>NCCN 1.2015 guidelines</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other cancer risks (pancreas, melanoma)</td>
<td>CDKN2A</td>
<td>Pancreas screening candidate</td>
<td>3 / 3</td>
<td>3 / 3</td>
</tr>
</tbody>
</table>

- 52% of non-BRCA1/2 positive probands (33/63)
- 72% of family members (42/58) if found to also be positive

Desmond et al. JAMA Oncology 2015
Case study: MSH6 positive

- Maternal Fx: colon, bladder, ovarian, breast cancers
- Screening for colorectal, gynecologic, and urologic cancers would be indicated.
- RR gynecologic surgery could be considered.
- Additional family member testing recommended.

Desmond et al. JAMA Oncology 2015
Case study: PALB2 positive

- Proband was already a candidate (based on Fx alone) for enhanced breast screening.

- Given PALB2 finding, risk-reducing surgery could be considered.
- Additional family member testing recommended.

Desmond et al. JAMA Oncology 2015
Patients with no management change in this study

1. Patient has or had breast cancer
   – Breast screening recommendations were not considered

2. Patient already had bilateral mastectomy
   – RRM recommendation was not considered

3. Action would already be indicated based on personal or family history alone

4. Lifetime risk was not likely to be >20%

- We also did not evaluate management changes for patients with negative results (potentially downgrading risk)

- Thus, we likely underestimated actionability in this study
Important notes on this study

1. We used current gene-specific risk estimates.

2. We used current consensus guidelines.

3. We had extensive family histories on all patients.

4. We did not examine which of these management changes were or would be implemented.

5. We measured actionability in appropriately referred patients meeting current guidelines for evaluation.

Desmond et al. JAMA Oncology 2015
Conclusions

- **Analytic Validity**
  - NGS-based tests can provide **equivalent performance** to traditional genetic tests
    - *Includes CNVs (del/dups) and complex classes of sequence variation*
    - *But only with specialized NGS biochemistry and bioinformatics methods*

- **Clinical Validity**
  - Interpretations for BRCA1/2 can be **highly concordant**
    - *Following ACMG 2015 ISV guidelines and using public resources*

- **Clinical Utility**
  - Findings from gene panels are often **clinically actionable**
    - *Under current guidelines and over and above any actions based on personal and family history alone*
Conclusions

- **Analytic Validity**
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  - Findings from gene panels are often *clinically actionable*
    - *Under current guidelines and over and above any actions based on personal and family history alone*
Desmond et al., 2015 (open access)
Swisher, 2015 (commentary)

Lincoln et al., 2015 (open access)

www.invitae.com > Science
– copy of this presentation, posters, whitepapers
Email me:
– steve.lincoln@invitae.com

• Leif Ellisen
• Andrea Desmond
• Michelle Gabree
• Kristen Shannon

• Jim Ford
• Allison Kurian
• Meredith Mills

• Nadine Tung
A systematic and accurate approach to variant classification

Scott Topper, PhD, FACMG
Head of Clinical Genomics, Invitae
Variant Classification

- Variant classification is the first pillar of clinical genetics.

- Variants are either **Pathogenic** or **Benign**.

- Sometimes the nature of the variant is **Unknowable** given the current state of information.

- Goals:
  - **Accurately, efficiently, and transparently** assess pathogenicity
  - When there is insufficient information, **confidently** conclude that the nature of the variant is unknowable
Challenge 1: Abundance

- Many variants
- Many labs
- Many opinions
- Extensive literature
- Conflicting literature
- Redundant literature
- Extensive direct and indirect evidence

**ClinVar submissions**

This page summarizes the number of genes and distinct variant locations currently represented in ClinVar from in submissions. A gene is reported if a variant in ClinVar is either found within, or includes, that gene. Thus the nomenclature is because of the structural variants in the database that span many genes.

This page lists all submitters and the summary of their contributions. We acknowledge their support.

**Submission overview**

<table>
<thead>
<tr>
<th>Category of analysis</th>
<th>Current total (Apr 27, 2015)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total accessioned submissions</td>
<td>172043</td>
</tr>
<tr>
<td>Total genes represented</td>
<td>26391</td>
</tr>
<tr>
<td>Total genes, in submissions with assertions, with variants in one gene</td>
<td>7406</td>
</tr>
<tr>
<td>Total genes, in submissions with assertions, with variants in multiple genes</td>
<td>26204</td>
</tr>
<tr>
<td>Total variations represented</td>
<td>145314</td>
</tr>
<tr>
<td>Total variations, in submissions with assertions</td>
<td>118174</td>
</tr>
<tr>
<td>Total submitters</td>
<td>312</td>
</tr>
</tbody>
</table>
Challenge 2: Too little, too late

**Mutation of MEF2A in an Inherited Disorder with Features of Coronary Artery Disease**

- 21bp in-frame deletion; conserved AAs
- Segregates in one large family (LOD 4.1)
- del7 AA disrupts protein function in vitro
- Variant is not present in 119 normal individuals

**Possible Conclusion:** causes disease

**Research article**

Lack of *MEF2A* mutations in coronary artery disease

- 0/300 CAD patients had any likely causative variants in MEF2A
- 3/1821 (0.2%) controls had the MEF2A del7 variant
- No segregation with disease in 3 families with del7 variant

**Possible Conclusion:** does NOT cause disease
Challenge 3: Passion

- We **want** to find an answer, and sometimes the answer isn’t there

- Reasonable people disagree

What we need:

- A system and infrastructure that…
  - Encourages different people to come to the same conclusion
  - Protects against the overuse of types of evidence
  - Supports effective discussion, disagreement and debate
Standardization efforts

ACMG/AMP ISV Guidelines

Draft

Aug 2013

The Scoring Rules for Classification

Pathogenic
a) 1 Very Strong (PVS1) AND
   a) 1 Strong (PS1-PS4) OR
      b) ≥1 Moderate (PM1-PM6) AND 1 Supporting (PP1-PP5) OR
      c) ≥2 Supporting (PP1-PP5)
   b) 2 Strong (PS1-PS4)
   c) 1 Strong (PS1-PS4) AND
      a) ≥3 Moderate (PM1-PM6) OR
      b) ≥2 Moderate (PM1-PM6) AND 2 Supporting (PP1-PP5) OR
      c) ≥1 Moderate (PM1-PM6) AND 4 Supporting (PP1-PP5)

Likely Pathogenic
a) 1 Strong (PS1-PS4) AND
   a) ≥1 Moderate (PM1-PM6) OR
   b) ≥2 Supporting (PP1-PP5)
   b) ≥3 Moderate (PM1-PM6)
   c) ≥2 Moderate (PM1-PM6) AND 2 Supporting (PP1-PP5)
   d) ≥1 Moderate (PM1-PM6) AND 4 Supporting (PP1-PP5)

Benign
a) 1 Stand-Alone (BA1) OR
   b) ≥2 Strong (BS1-BS4)

Likely Benign
a) 1 Strong (BS1-BS4) and ≥1 Supporting (BP1-BP6) OR
   b) ≥2 Supporting (BP1-BP6)

*Variants should be classified as Uncertain Significance if other criteria are unmet.
Expertly trained doctorate-level scientists

A large team of doctorate-level scientists with years of research training and a deep understanding of molecular and human genetics, led by Keith Nykamp and Michael Anderson.
A strong genetic counseling team

A large team of board-certified genetic counselors with years of experience in both laboratories and the clinic, led by **Amy Fuller** and **Christy Hartshorne**
Renowned medical and clinical genetics experts

Strong lineup of board-certified professionals and genomics scientists, led by chief medical officer Dr. Robert Nussbaum, a pioneer in medical genetics

Robert Nussbaum, M.D.
Chief Medical Officer

Adam Rosendorff
M.D.

Swaroop Aradhya
Ph.D., FACMG

Scott Topper
Ph.D., FACMG

Martin Powers
M.D.

Anne Deucher
M.D., Ph.D.

Karen Ouyang
Ph.D., FACMG

Ed Esplin
M.D., Ph.D., FACMG

Eden Haverfield
Ph.D., FACMG

Britt Jhpson
Ph.D., FACMG

Tom Winder
Ph.D., FACMG
We have an outstanding team

Expertise with a diverse array of diagnostic areas and platforms

Expertise with a wide range of clinical diseases and areas
Standardization efforts

ACMG/AMP ISV Guidelines

Draft

Aug 2013

The Scoring Rules for Classification

Pathogenic
a) 1 Very Strong (PS1-PS4) AND
   a) 1 Strong (PS1-PS4) OR
   b) ≥1 Moderate (PM1-PM6) and 1 Supporting (PP1-PP5) OR
   c) ≥2 Supporting (PP1-PP5)

b) 2 Strong (PS1-PS4)
   a) 1 Strong (PS1-PS4) AND
   b) ≥3 Moderate (PM1-PM6) OR
   c) ≥3 Moderate (PM1-PM6) and 2 Supporting (PP1-PP5) OR
   d) ≥1 Moderate (PM1-PM6) and 4 Supporting (PP1-PP5)

Likely Pathogenic
a) 1 Strong (PS1-PS4) AND
   a) ≥1 Moderate (PM1-PM6) OR
   b) ≥2 Supporting (PP1-PP5)

b) ≥3 Moderate (PM1-PM6)
   a) ≥2 Moderate (PM1-PM6) AND 2 Supporting (PP1-PP5)
   b) ≥1 Moderate (PM1-PM6) AND 4 Supporting (PP1-PP5)

Benign
a) 1 Stand-Alone (BA1) OR
b) ≥2 Strong (BS1-BS4)

Likely Benign
a) 1 Strong (BS1-BS4) and ≥1 Supporting (BP1-BP6) OR
b) ≥2 Supporting (BP1-BP6)

*Variants should be classified as Uncertain Significance if other criteria are unmet.
Standardization efforts

ACMG/AMP ISV Guidelines

Draft → Final

Aug 2013 → Mar 2015

"consensus document"

Table 5: Rules for combining criteria to classify sequence variants

<table>
<thead>
<tr>
<th>Pathogenic</th>
<th>Likely pathogenic</th>
<th>Likely benign</th>
<th>Benign</th>
</tr>
</thead>
<tbody>
<tr>
<td>(i) Very strong (PM1–PM3) OR (a) 3 strong (PM1–PM3) OR (b) 2 moderate (PM1–PM3) OR (c) 1 moderate (PM1–PM3) and 1 supporting (PP1–PP3) OR (d) 2 supporting (PM1–PM3) OR (e) 2 strong (PS1–PS4) OR (f) 1 strong (PS1–PS4) AND (g) 2 moderate (PM1–PM3) OR (h) 1 moderate (PM1–PM3) and 1 supporting (PP1–PP3) OR (i) Moderate (PM1–PM3) and 1 supporting (PP1–PP3) OR (j) Other criteria shown above that are not met OR (k) the criteria for benign and pathogenic are contradictory</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(i) Very strong (PM1–PM3) AND</td>
<td>(a) 3 strong (PM1–PM3) OR (b) 2 moderate (PM1–PM3) OR (c) 1 moderate (PM1–PM3) and 1 supporting (PP1–PP3) OR (d) 2 supporting (PM1–PM3) OR (e) 2 strong (PS1–PS4) OR (f) 1 strong (PS1–PS4) AND (g) 2 moderate (PM1–PM3) OR (h) 1 moderate (PM1–PM3) and 1 supporting (PP1–PP3) OR (i) Moderate (PM1–PM3) and 1 supporting (PP1–PP3) OR (j) Other criteria shown above that are not met OR (k) the criteria for benign and pathogenic are contradictory</td>
<td></td>
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<tr>
<td>(i) Strong (PS1–PS4) OR (b) Moderate (PM1–PM3) OR (c) 1 moderate (PM1–PM3) and 1 supporting (PP1–PP3) OR (d) 2 supporting (PM1–PM3) OR (e) 2 strong (PS1–PS4) OR (f) 1 strong (PS1–PS4) AND (g) 2 moderate (PM1–PM3) OR (h) 1 moderate (PM1–PM3) and 1 supporting (PP1–PP3) OR (i) Moderate (PM1–PM3) and 1 supporting (PP1–PP3) OR (j) Other criteria shown above that are not met OR (k) the criteria for benign and pathogenic are contradictory</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(i) Strong (PS1–PS4) OR (b) Moderate (PM1–PM3) OR (c) 1 moderate (PM1–PM3) and 1 supporting (PP1–PP3) OR (d) 2 supporting (PM1–PM3) OR (e) 2 strong (PS1–PS4) OR (f) 1 strong (PS1–PS4) AND (g) 2 moderate (PM1–PM3) OR (h) 1 moderate (PM1–PM3) and 1 supporting (PP1–PP3) OR (i) Moderate (PM1–PM3) and 1 supporting (PP1–PP3) OR (j) Other criteria shown above that are not met OR (k) the criteria for benign and pathogenic are contradictory</td>
<td></td>
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</tr>
<tr>
<td>(i) Strong (PS1–PS4) OR (b) Moderate (PM1–PM3) OR (c) 1 moderate (PM1–PM3) and 1 supporting (PP1–PP3) OR (d) 2 supporting (PM1–PM3) OR (e) 2 strong (PS1–PS4) OR (f) 1 strong (PS1–PS4) AND (g) 2 moderate (PM1–PM3) OR (h) 1 moderate (PM1–PM3) and 1 supporting (PP1–PP3) OR (i) Moderate (PM1–PM3) and 1 supporting (PP1–PP3) OR (j) Other criteria shown above that are not met OR (k) the criteria for benign and pathogenic are contradictory</td>
<td></td>
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</table>

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Implementation, expert review and evolution

ACMG/AMP ISV Guidelines

Draft Aug 2013 v1

Final Mar 2015

22 CRITERIA
**Truncating Variants**

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

<table>
<thead>
<tr>
<th>Type of Variant</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Truncating Variant, Presumed loss of protein</td>
<td>Only use for genes with known loss-of-function mechanisms. Do not use if the premature stop codon is in the last exon or within the last 15 codons of the penultimate exon, unless there are proven LOF mutations farther downstream. Instead use “Truncating mutation, may escape NMD.” Do not use for start codon mutations if an alternate methionine may be present nearby.</td>
</tr>
<tr>
<td>Truncating Variant, may escape NMD</td>
<td>Only use for genes with known loss-of-function mechanisms. Used for truncating mutations when complete loss of protein function cannot be presumed. Examples include a premature stop codon in the last exon or within the last 15 codons of the penultimate exon, a truncating variant seen at a relatively high frequency in the general population, or a start codon substitution with a nearby alternative initiator methionine.</td>
</tr>
<tr>
<td>Truncating Variant, Molecular mechanism unknown</td>
<td>Use this if the molecular mechanism is unknown or complicated.</td>
</tr>
<tr>
<td>Truncating Variant, GOF mechanism</td>
<td>Use if gain-of-function has been established as the only disease mechanism</td>
</tr>
</tbody>
</table>
Case Reports

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

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.

4 unrelated case reports, w/ pathognomonic features

STRONG segregation w/ disease - (LOD>3, 2+ families)

Homozygous or hemizygous in an unaffected adult, recessive or X-linked, HIGH penetrance,

Co-occurrence in an affected individual w/ an alternate cause of disease
Implementation, expert review and evolution

ACMG/AMP ISV Guidelines

Draft
Aug 2013
v1

Oct 2013
v2

May 2014
v3

July 2014
v3.1

Mar 2015
v4

Final

22
CRITERIA

35

72

89

1100
VARIANTS

Invitae Clinical Genomics Team

- Add/remove criteria
- Change scores, adjust LB threshold
- Create priority groups
- Add usage notes
5 basic organizing principles in the schema

- **Points**: each EV criteria is given a preset number of points – meant to reflect the relative importance of each evidence type

- **Pathogenic vs. Benign**: each EV criteria supports either Pathogenic or Benign, and sums in both directions are assessed independently

- **Groups**: certain types of evidence contribute to the same basic argument, and these are grouped together with only the highest priority evidence contributing to classification

- **Additive**: multiple instances of the same evidence type, in some cases, can contribute to the final classifications – other types of evidence do not

- **Thresholds**: the number of points required for a classification is preset
Evidence Types

<table>
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<tr>
<th>Evidence Types</th>
<th>Benign</th>
<th>Likely</th>
<th>Uncertain Significance</th>
<th>Likely</th>
<th>Pathogenic</th>
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<td>Very HIGH 0.5%</td>
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<td>HIGH 0.1%</td>
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<td>0.3%</td>
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<tr>
<td>Within pathogenic range</td>
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<td>Absent from ExAC</td>
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<td>Synonymous</td>
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<td>Non-conserved intrinsic</td>
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<td>Missense</td>
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<td>AG/GT dinucleotide</td>
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<td>Nonsense* frameshift</td>
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<td>Clinical Findings</td>
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<td>Domain: co-occurrence phase unknown</td>
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<td>2 cases 1 family</td>
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<td>3 cases 2 families</td>
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<td>4+ cases 3+ families</td>
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<td>Experimental Studies</td>
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<td>Neutral STRONG</td>
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<td>Neutral WEAK</td>
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<td>Disrupted WEAK</td>
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<td>Disrupted STRONG</td>
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<td>Indirect and Computational</td>
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<tr>
<td>Suggest benign</td>
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<td>Suggest disrupted</td>
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<td>Essential AA</td>
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</table>
…with detailed usage rules

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<tr>
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<td>path rules variant type: score: description:</td>
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<td>Major</td>
<td>Population</td>
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<td>VI/S</td>
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<td>Population data: BRCA1/2 - Very high MAF 0.4%</td>
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<td>Major</td>
<td>Population</td>
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<td>VI/S</td>
<td>4</td>
<td>Population data: BRCA1/2 - High MAF 0.1%</td>
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<td>Major</td>
<td>Population</td>
<td>Dominant</td>
<td>VI/S</td>
<td>1</td>
<td>Population data: BRCA1/2 - Somewhat high, 3 reports</td>
<td>20001</td>
<td>6</td>
<td>N</td>
<td>N0059, N0055</td>
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<td>EV0013</td>
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<td>Major</td>
<td>Population</td>
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<td>4</td>
<td>3+ homozygotes in public databases HIGH</td>
<td>20002</td>
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<td>N0047, N0066</td>
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<td>EV0090</td>
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<td>N0047, N0045</td>
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<td>24</td>
<td>EV0082</td>
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<td>Individual Observations</td>
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<td>CNV/MS/ID</td>
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<td>Novel homozygous variant in a classically affected individual</td>
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<td>EV0014</td>
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<td>Individual Observations</td>
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<td>ALL</td>
<td>2.5</td>
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<td>Population</td>
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<td>EV0016</td>
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<td>Major</td>
<td>Sequence Observations</td>
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<td>5</td>
<td>Truncating mutation, presumed loss of protein</td>
<td>20004</td>
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<td>EV0017</td>
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<td>Major</td>
<td>Sequence Observations</td>
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<td>CSS</td>
<td>4</td>
<td>Nucleotide in consensus splice site</td>
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<td>EV0018</td>
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<td>Sequence Observations</td>
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<td>MS/ID</td>
<td>4</td>
<td>Same AA change as a pathogenic missense</td>
<td>20004</td>
<td>3</td>
<td>N</td>
<td>N0031, N0054</td>
</tr>
</tbody>
</table>

**Evidence Type**

- Truncating mutation, presumed loss of protein
- Sequence Observations: Pathogenic
  - Truncating mutation, presumed loss of protein
  - Nucleotide in consensus splice site
  - Same AA change as a pathogenic missense
  - Truncating mutation, may escape NMD
  - Last nucleotide of exon
  - Small in-frame indel

**Curation DB**

- ARUP
- Invitae
- dbSNP
- OMIM
- ClinVar
- LOVD
- HGMD

**Pathogenic Score**

- 5

**Benign Score**

- 

**Notes**

EVO016 Pathogenic; 5.0 pts. Major. Truncating mutation, presumed loss of protein (mode of inheritance:ALL) (variant types:T)

Do not use if the premature stop codon is in the last exon or within the last 15 codons of the penultimate exon, unless there are proven LOF mutations farther downstream. Instead use "Truncating mutation, may escape NMD." Do not use for start codon mutations if an alternate methionine may be present nearby. Only use for genes with known loss-of-function mechanisms. The assumption is that these variants will be novel or very rare and additional points should not be given for being absent from the population (i.e. EVO014 or EVO015). Conversely, if the variant is present in the general population at a somewhat high (or higher) frequency, this criteria may not apply. Use family studies, patient observations and/or experimental data towards pathogenicity instead.
**Comprehensive literature searching**

A thorough evaluation of the available literature is **ESSENTIAL**.

**There are no shortcuts**

Evolving nomenclature standards re: gene names, transcripts, variant names....

**Literature searching is non-trivial**

<table>
<thead>
<tr>
<th>PMID</th>
<th>Library Status</th>
<th>PMID History</th>
<th>Date Reviewed</th>
<th>Reviewed By</th>
<th>Notes</th>
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<td>Found in a prostate cancer patient.</td>
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<td>10.24.2015 CP</td>
<td>CP</td>
<td>Observed in 1 Dutch family with history of HBOC. Table 5. Reports the variant as a deleterious change.</td>
</tr>
<tr>
<td>21305653</td>
<td></td>
<td></td>
<td>10.24.2015 CP</td>
<td>CP</td>
<td>Sequence technology (Table S1, as one of 14 variants in a high risk family)</td>
</tr>
<tr>
<td>21233401</td>
<td></td>
<td></td>
<td>10.24.2015 CP</td>
<td>CP</td>
<td>Study in frozen tumor sample found a hotspot mutation.</td>
</tr>
<tr>
<td>23242139</td>
<td></td>
<td></td>
<td>10.24.2015 CP</td>
<td>CP</td>
<td>Did not find the variant.</td>
</tr>
<tr>
<td>26315209</td>
<td></td>
<td></td>
<td>10.24.2015 CP</td>
<td>CP</td>
<td>Development of a variant database to find the variant.</td>
</tr>
<tr>
<td>24504028</td>
<td>⭐</td>
<td></td>
<td>07.20.2015 CROP</td>
<td>CP</td>
<td>Report signed out on 07.20.2015 CROP without any manually added notes.</td>
</tr>
<tr>
<td>24448499</td>
<td></td>
<td></td>
<td>07.20.2015 CROP</td>
<td>CP</td>
<td>Report signed out on 10.22.2015 without any manually added notes.</td>
</tr>
</tbody>
</table>
Pulling all available information into focus

- All publicly available information (but cleaned up, standardized and mapped correctly)
  - All Invitae information and variant classifications presented
    - Variant history
    - Links to quality threshold data
Evaluating the validity of our variant classification approach
There is no gold-standard data set for comparison

<table>
<thead>
<tr>
<th>Invitae</th>
<th>vs</th>
<th>Lab X</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Method 1</strong></td>
<td>Pathogenic</td>
<td>VUS</td>
</tr>
<tr>
<td>Classifications</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Invitae</th>
<th>vs</th>
<th>Lab 1</th>
<th>Lab 2</th>
<th>Lab 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pathogenic</td>
<td>Pathogenic</td>
<td>Pathogenic</td>
<td>VUS</td>
<td></td>
</tr>
<tr>
<td>Likely Pathogenic</td>
<td>Likely Pathogenic</td>
<td>Likely Pathogenic</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Do not use**
Method 1: We compared our results to Myriad’s

- Compared Invitae’s NGS test to traditional BRCA1/BRCA2 tests in more than 1000 patients
- 95% classification concordance along the 5-tier scale for all variants
- 100% analytic sensitivity and specificity for sequencing and CNV detection
- 99.8% report concordance (Pos, VUS, Neg) with Myriad Genetics reports
Method 2: Sherloc interpretations are consistent with ClinVar

Sherloc vs ClinVar Consensus

- 92.2% concordance with the consensus majority
- Almost all mismatches were P-LP or VUS-LB-B differences
- The single exception was a Sherloc VUS compared to a Consensus LP

<table>
<thead>
<tr>
<th>Consensus Interpretation</th>
<th>Pathogenic</th>
<th>LP</th>
<th>VUS</th>
<th>LB</th>
<th>Benign</th>
<th>Total</th>
<th>#Variants</th>
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</thead>
<tbody>
<tr>
<td>Pathogenic</td>
<td>99.1%</td>
<td>0.9%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>100%</td>
<td>117</td>
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<tr>
<td>LP</td>
<td>50.0%</td>
<td>50.0%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>100%</td>
<td>10</td>
</tr>
<tr>
<td>VUS</td>
<td>0.0%</td>
<td>0.4%</td>
<td>93.6%</td>
<td>5.6%</td>
<td>0.4%</td>
<td>100%</td>
<td>249</td>
</tr>
<tr>
<td>LB</td>
<td>0.0%</td>
<td>0.0%</td>
<td>11.0%</td>
<td>78.0%</td>
<td>11.0%</td>
<td>100%</td>
<td>82</td>
</tr>
<tr>
<td>Benign</td>
<td>0.0%</td>
<td>0.0%</td>
<td>2.5%</td>
<td>2.2%</td>
<td>95.2%</td>
<td>100%</td>
<td>356</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>814</td>
</tr>
</tbody>
</table>
…more so than the average clinical testing lab

**Sherloc vs ClinVar Consensus**

- 92.2% concordance with the consensus majority
- Almost all mismatches were P-LP or VUS-LB-B differences
- The single exception was a Sherloc VUS compared to a Consensus LP

**Other Labs vs ClinVar Consensus**

- Other laboratories had on average an 85.7% concordance
- The average lab had more clinically significant discordances
In conclusion…

“Nothing clears up a case so much as stating it to another person.”

-Silver Blaze, Sir Arthur Conan Doyle

- Sherloc is adaptable, efficient and transparent.
- Variant classifications are a direct result of thorough and reproducible evaluation of evidence.
- Your clients need to know the logic behind your conclusions.
Thank you
Appendix
**BRCA1** pathogenic mutation frequency spectrum

- **Red**: LoF-type mutations *(from ClinVar and/or ExAC)*
- **Orange**: P/LP missense, intronic or in-frame indels *(from ClinVar)*

**Allele Count in ExAC**

- c.1687C>T (Swedish Founder)
- c.4035delA (E. European Founder)
- c.181T>G (E. European Founder)
- c.5266dupC (Ashk. J. Founder)
- c.68_69delAG (Ashk. J. Founder)
BRCA1 pathogenic mutation frequency spectrum

Current Invitae “Likely Benign” cutoff (0.1%)

Old Invitae “Likely Benign” cutoff (0.25%)

c.68_69delAG (Ashk. J. Founder)
BRCA2 pathogenic mutation frequency spectrum

Number of unique pathogenic mutations

Allele Count in ExAC
PTCH1 pathogenic mutation frequency spectrum

Gorlin syndrome (ADOM)

Prevalence: 1 in 31,000 ~ 1 in 164,000
Penetrance: Complete

Allele Count in ExAC
MYBPC3 pathogenic mutation frequency spectrum

Hypertrophic Cardiomyopathy (ADOM)

- Prevalence: 1 in 500
- Penetrance: Incomplete

Allele Count in ExAC
CFTR pathogenic mutation frequency spectrum

Cystic fibrosis (AREC)

Prevalence: 1 in 3200 ~ 1 in 31,000
Penetrance: Variable

c.1521_1523del [deltaF508] (N Eur Founder)

Allele Count in ExAC
CFTR pathogenic mutation frequency spectrum

Cystic fibrosis (AREC)

Prevalence: 1 in 3200 ~ 1 in 31,000
Penetrance: Variable

c.1521_1523del [deltaF508] (N Eur Founder)

Green: CAVD or hereditary pancreatitis

Allele Count in ExAC
**BBS1** pathogenic mutation frequency spectrum

![Frequency Spectrum Diagram]

**c.1169T>G** (Famous Founder. 80% of BBS)

**Allele Count in ExAC**
BBS1 pathogenic mutation frequency spectrum

Number of unique pathogenic mutations

Allele Count in ExAC

c.271dupT (European Founder)
c.1736A>G (LabCorp LP. I didn’t evaluate merits)
*CEP290* pathogenic mutation frequency spectrum

Number of unique pathogenic mutations

Allele Count in ExAC

c.1666dup
DNAH5 pathogenic mutation frequency spectrum

Allele Count in ExAC
**DNAH11** pathogenic mutation frequency spectrum

Number of unique pathogenic mutations

Allele Count in ExAC
INVS pathogenic mutation frequency spectrum

Number of unique pathogenic mutations

Allele Count in ExAC
**TMEM67** pathogenic mutation frequency spectrum

- **Allele Count in ExAC**

- **Number of unique pathogenic mutations**

- **c.579_580delAG**
**TMEM216 pathogenic mutation frequency spectrum**

- **Allele Count in ExAC**
- **Number of unique pathogenic mutations**
- **c.432-1G>C**