Don’t Believe Everything You Hear: Variant Classifications for BRCA1 and BRCA2 are Highly Concordant Across Major Clinical Testing Laboratories

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Disclosure

- I am an employee and shareholder in Invitae, a clinical diagnostic testing laboratory
Comparison of locus-specific databases for BRCA1 and BRCA2 variants reveals disparity in variant classification within and among databases

P. J. Vail (✉) · B. Morris · A. van Kan · B. C. Burdett · K. Moyes · A. Theisen · I. D. Kerr · R. J. Wenstrup · J. M. Eggington
Myriad Genetic Laboratories, Inc., 320 Wakara Way, Salt Lake City, UT 84108, USA

Finding: 3-14% disagreement rate between databases (BIC, HGMD, LOVD, UMD, and ClinVar)

Vail et al., J Community Genet 2015
The disagreement in public databases “precludes their wider use in clinical practice”

“Public databases [are] fraught with errors”

“Interpretation accuracy impossible with public databases”

Analyst Day Presentation, September 2015
investor.myriad.com > Events & Presentations > Financial Events
Accessed 2/1/2016

Vail et al., J Community Genet 2015

This issue can be misrepresented to clinicians…

• Experienced and responsible lab directors never simply copy variant classifications from any public database

• Instead, they critically evaluate underlying evidence and report classifications following established guidelines

Question: What is the clinical impact of discordance in public databases?

Per ACMG guidelines: Richards et al., Genet Med 2015
Clinical study

- Multisite prospective recruitment

- Over 1000 patients who:
  - Met NCCN criteria for hereditary breast/ovarian cancer evaluation
  - Not referred because of a mutation identified in a relative
  - Representative cohort in both demographics and indications

- Primary focus:
  - Patient management implications of panel testing compared to traditional BRCA1/BRCA2 testing

Desmond et al., JAMA Oncol 2015
Lincoln et al., J Mol Diag 2015

Clinical study of panel testing for HBOC

**JAMA Oncology**

Original Investigation

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

Andrea Desmond, BS; Allison W. Kuran, MD; Nischal M. Sabnis, MS, CGC; Meredith A. Miles, BA; Michael J. Anderson, PhD; Yuya Kobayashi, PhD; Nora Honick, 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

Invited Commentary

**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
A systematic comparison of traditional and multigene 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. Mills1, Geoffrey B. Nilsen1, Kevin B. Jacobs1, Federico A. Monzon1, Allison W. Kurian2, James M. Ford2, Leif W. Ellisen2,4

1. Invitae, San Francisco, CA
2. Massachusetts General Hospital Cancer Center, Boston, MA
3. Stanford University School of Medicine, Palo Alto, CA
4. Harvard Medical School, Boston, MA

Lincoln et al., J Mol Diag 2015

BRCA1/2 variant 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>Our test</td>
</tr>
<tr>
<td>99.8%</td>
<td>4.1%</td>
</tr>
<tr>
<td>Disagree</td>
<td>Traditional test</td>
</tr>
<tr>
<td>0.2%</td>
<td>3.2%</td>
</tr>
</tbody>
</table>

975 patients with both Myriad Genetics and independent test results. Variants uncovered by the independent test were blindly classified:

- Using only publicly available resources (literature and databases)
- Following a system based on the ACMG/AMP 2015 guidelines

All amended reports provided to clinical sites were incorporated, so the Myriad variant classifications were up to date

Lincoln et al., J Mol Diag 2015
Positive vs. not positive results

Differences that could substantially change patient management decisions

<table>
<thead>
<tr>
<th></th>
<th>Pathogenic (P)</th>
<th>Likely Pathogenic (LP)</th>
<th>VUS</th>
<th>Likely Benign (LB)</th>
<th>Benign (B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Discordant</td>
</tr>
<tr>
<td>VUS</td>
<td>Discordant</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LB</td>
<td>Discordant</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Discordant</td>
</tr>
</tbody>
</table>

Examining the larger data set in ClinVar

- **Inclusion Criteria** were ClinVar submitters that:
  - Are an established and licensed **diagnostic laboratory**
    - or are submitting data from such a lab
  - Submitted at least **200 classified variants** in BRCA1/2
  - Most classifications were from the last 5 years
    - Using “last evaluation date”, not “submission date” in ClinVar

- Thus we **excluded** data from:
  - Research projects, software vendors
  - Smaller labs, consortia (e.g. ENIGMA)
  - Old sources (e.g. BIC)
    - Mostly pre-2007 Myriad Genetics data

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ClinVar based data set

<table>
<thead>
<tr>
<th>Name</th>
<th>Classified Variants</th>
<th>Comparable Variants</th>
<th>Submitter Name in ClinVar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambry</td>
<td>2793</td>
<td>1502</td>
<td>AMBRY GENETICS</td>
</tr>
<tr>
<td>Myriad</td>
<td>2067</td>
<td>1184</td>
<td>SHARING CLINICAL REPORTS PROJECT (SCRP)</td>
</tr>
<tr>
<td>Invitae</td>
<td>1479</td>
<td>1082</td>
<td>INVITAE</td>
</tr>
<tr>
<td>GeneDx</td>
<td>1214</td>
<td>937</td>
<td>GENEDX</td>
</tr>
<tr>
<td>Counsyl</td>
<td>272</td>
<td>256</td>
<td>COUNSYL</td>
</tr>
<tr>
<td>CHEO</td>
<td>257</td>
<td>216</td>
<td>MOLECULAR GENETICS DIAGNOSTIC LABORATORY, CHILDREN’S HOSPITAL OF EASTERN ONTARIO</td>
</tr>
<tr>
<td>Emory</td>
<td>203</td>
<td>183</td>
<td>EMORY GENETICS LABORATORY</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>4725</strong></td>
<td><strong>1800</strong></td>
<td>Non-redundant list from the above</td>
</tr>
</tbody>
</table>

Data from ≥2 labs

Shan Yang, Invitae

- Replicated our results
- Open-source code to help mine ClinVar
- Integrating many other sources of BRCA1/2 data

www.brcaexchange.org

Benedict Paten, Melissa Cline, Molly Zhang, David Haussler, *et al.*, UCSC
<table>
<thead>
<tr>
<th></th>
<th>Ambry</th>
<th>Invitae</th>
<th>GeneDx</th>
<th>Counsyl</th>
<th>CHEO</th>
<th>Emory</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Myriad via SCRP</strong></td>
<td>98.7% 939/951</td>
<td>99.2% 619/624</td>
<td>99.5% 569/572</td>
<td>99.4% 171/172</td>
<td>99.5% 139/142</td>
<td>97.2% 103/106</td>
</tr>
<tr>
<td></td>
<td>97.9% - 99.3%</td>
<td>98.3% - 99.7%</td>
<td>98.6% - 99.9%</td>
<td>97.3% - 100%</td>
<td>94.5% - 99.4%</td>
<td>92.6% - 99.2%</td>
</tr>
<tr>
<td><strong>Ambry</strong></td>
<td>99.2% 860/867</td>
<td>99.6% 780/783</td>
<td>99.0% - 99.9%</td>
<td>99.6% 223/224</td>
<td>98.3% 176/179</td>
<td>98.8% 161/163</td>
</tr>
<tr>
<td></td>
<td>98.4% - 99.6%</td>
<td>99.0% - 99.9%</td>
<td>97.9% - 100%</td>
<td>95.6% - 99.5%</td>
<td>96.1% - 99.7%</td>
<td></td>
</tr>
<tr>
<td><strong>Invitae</strong></td>
<td>99.8% 593/594</td>
<td>99.1% 214/216</td>
<td>99.1% 161/164</td>
<td>98.2% 138/141</td>
<td>99.3% 149/150</td>
<td>96.9% - 100%</td>
</tr>
<tr>
<td></td>
<td>99.2% - 100%</td>
<td>97.1% - 99.8%</td>
<td>95.2% - 99.5%</td>
<td>94.4% - 99.4%</td>
<td>99.9% - 100%</td>
<td></td>
</tr>
<tr>
<td><strong>GeneDx</strong></td>
<td>99.5% 221/222</td>
<td>99.7% 138/141</td>
<td>99.9% 149/150</td>
<td>99.9% 105/105</td>
<td>96.9% - 100%</td>
<td>100% 105/105</td>
</tr>
<tr>
<td></td>
<td>97.9% - 100%</td>
<td>94.4% - 99.4%</td>
<td>99.9% - 100%</td>
<td>96.9% - 100%</td>
<td>100% 105/105</td>
<td></td>
</tr>
<tr>
<td><strong>Counsyl</strong></td>
<td><strong>Concordance</strong></td>
<td><strong>Concordant/All</strong></td>
<td><strong>Conf. Int.</strong></td>
<td><strong>100%</strong></td>
<td><strong>100%</strong></td>
<td><strong>100%</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>100% 82/82</td>
<td>100% 105/105</td>
<td>97.6% - 100%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>97.0% - 100%</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>CHEO</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>98.3% 57/58</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>92.2% - 99.9%</td>
</tr>
</tbody>
</table>

On a per-variant basis:

Pairwise concordance: 97.2% - 100.0%

Overall concordance: 98.5%

Only 27/1800 variants have a significant classification discordance between any two reporting laboratories
How many individuals does this represent?

≈22,000 patients give
≈1800 non-redundant variants

Did SCRP data bias results from other labs?

Apparently not

<table>
<thead>
<tr>
<th>Other lab classifications</th>
<th>Concordance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-dating SCRP release</td>
<td>99.0%</td>
</tr>
<tr>
<td>Post-dating SCRP release</td>
<td>98.9%</td>
</tr>
</tbody>
</table>

Compare ClinVar submission date of Myriad data via SCRP to evaluation date of same variant from other labs
Most variants in ClinVar are rare
All of the discordant variants are rare

- All of the 27 variants with classification discordances:
  - Have population allele frequencies $\leq 0.05\%$ in all of:
    - ExAC
    - ESP
    - 1000 Genomes
  - Also have a patient prevalence $\leq 0.05\%$
    - Database of $n=20,000$ cases
- Only $16.6\%$ of patients carry any such rare variant(s)
- Most $(98.4\%)$ of these rare variants are completely concordant when seen by more than one laboratory

Per-variant and per-patient concordance are different

- Estimated per-patient concordance 2 orthogonal ways:
  - From population allele frequencies
  - From patient database ($n=20,000$) $99.8\%$ Concordant

- Patients with 1 or more rare variants, all concordant (16.4%)
- Patients with 1 or more rare variants having a discordance (0.2%)
- Patients with no rare variants (83.4%)
Do not over-generalize these results

- Discordance in other genes can be higher
  - e.g. some cardiovascular genes

- Discordance from other sources can be higher

Discussion

- While substantial disagreements in BRCA1/2 are infrequent, they are important
- We must resolve differences collaboratively, not competitively, to deliver the best patient care, as is done in other areas of medicine
- Labs that maintain data as a proprietary asset can make arbitrary and unverifiable claims regarding interpretation accuracy
- Data submission ClinVar facilitates peer review and interlaboratory quality control on a global scale, as exemplified by this study
For copy of slides:

steve.lincoln@me.com
or www.invitae.com

Desmond et al., 2015 (open access)
Swisher, 2015 (commentary)
Lincoln et al., 2015 (open access)