Hereditary cancer testing: current and future challenges

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Kennedy Krieger Institute
Objectives

- To assess current challenges clinical GCs face in terms of test selection, patient volume, and clinical management; NSGC survey results to be presented and incorporated into the discussion.

- To teach appropriate questions to ask when comparing and selecting genetic testing laboratory partners.

- To assess the emerging challenges and opportunities for genetic counselors in clinical practice.
In summer 2016, we surveyed the NSGC on genetic testing preferences. The 457 respondents self-identified as:

**Clinical setting**
- Academic/university hospital or medical center
- Community hospital-based practice
- Private Practice
- Industry
- Other

**Clinical specialties**
- Prenatal
- Pediatrics
- Neurology
- Metabolic disorders /
- Hereditary cancer
- Hematology
- Cardiology
HEREDITARY CANCER TESTING: CURRENT AND FUTURE CHALLENGES

Andrea Forman, MS, LCGC
Fox Chase Cancer Center
Disclosures

- Invitae
  - Member of the Invitae National Genetic Counselor Advisory Board and have been compensated for my time

- Opinions are my own
Evolution of the next-gen genetic counselor
Growth
How many patients do you typically see in a month?

- 25 or fewer
- 26–40
- 41–50
- 51 or more
On average, how much do you expect the level of testing in your practice will change over the next 12 months?

- I expect it to stay about the same.
- I expect it to increase up to 25%.
- I expect it to increase by 25%-50%.
- I expect it to decrease.
- I expect it to increase by 50% or more.
15.2% increase in intake calls
21.1% increase in new genetic counseling patients seen
  - New patient appointment wait went from three weeks to three months.
16.8% increase in patients seen within the department
  - Counseling
  - Follow-up
  - High-risk screening clinics
Modification
## Time management?

**Time spent in face-to-face interaction with the patient**

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>2010 PSS</th>
<th>2016 PSS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>Less than 15</td>
<td>11</td>
<td>1</td>
</tr>
<tr>
<td>15–30</td>
<td>84</td>
<td>9</td>
</tr>
<tr>
<td>31–45</td>
<td>248</td>
<td>28</td>
</tr>
<tr>
<td>46–60</td>
<td>288</td>
<td>32</td>
</tr>
<tr>
<td>61–75</td>
<td>129</td>
<td>15</td>
</tr>
<tr>
<td>76–90</td>
<td>77</td>
<td>9</td>
</tr>
<tr>
<td>91–105</td>
<td>21</td>
<td>2</td>
</tr>
<tr>
<td>106–120</td>
<td>20</td>
<td>2</td>
</tr>
<tr>
<td>More than 120</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>887</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>
Increased potential for variants of unknown significance (VUS)
  – Plan for re-contact regarding updates to VUS findings

Limitations:
  – Cancer risk estimates for moderate-risk genes
  – Medical management options for moderate-risk gene mutations and VUS
  – Result interpretation for family members

Potential to identify mutations conferring risk for unexpected cancer syndrome in family

Potential for recessive disorders
Development of a tiered and binned genetic counseling model for informed consent in the era of multiplex testing for cancer susceptibility

Angela R. Bradbury, MD1,3, Linda Patrick-Miller, PhD4,5, Jessica Long, MS, CGC1, Jacquelyn Powers, MS, CGC1, Jill Stopfer, MS, CGC1, Andrea Forman, MS, CGC6, Christina Rybak, MS, CGC6, Kristin Mattie, MS, CGC1, Amanda Brandt, MS, CGC1, Rachelle Chambers, MS, CGC6, Wendy K. Chung, MD, PhD6,9, Jane Churpek, MD4, Mary B. Daly, MD, PhD9, Laura Digiovanni, MLA1, Dana Farengo-Clark, MS, CGC7, Dominique Fetzer, BA1, Pamela Ganschow, MD10, Generosa Grana, MD, FACP1, Cassandra Gulden, MS, CGC1, Michael Hall, MD6, Lynne Kohler, BA1, Kara Maxwell, MD, PhD1, Shana Merrill, MS, CGC1, Susan Montgomery, BSN, OCN6, Rebecca Mueller, MS, CGC1, Sarah Nielsen, MS, CGC6, Olufunmilayo Olopade, MD, FACP4,5, Kimberly Rainey, MS, CGC6, Christina Seelaus, MS, CGC10, Katherine L. Nathanson, MD1,11, and Susan M. Domchek, MD1,3

Tiered/binned model

- Prioritizing the most essential information
  - e.g., high VUS rates, limited-evidence genes, limited medical management guidance, potential for surprises

- Binning similar information
  - high vs. moderate vs. unclear-risk genes
Choices
Evolution of the next-gen genetic counselor
Andrea, the fickle genetic counselor...

• Initially excited about offering panels
  – Broader range of genes
  – “One-stop shopping”

• Challenges quickly became apparent!
  – Patient confusion
  – Crazy VUS rates (25% Caucasian, 66% African American)
  – Unexpected or non-actionable results
Evolution in options

• Experimented with different labs
  – Easiest (turnaround time, patient materials, test reports, billing, paperwork, follow-up)
• More-tailored panels becoming available
  – Syndrome-specific
  – Choose-your-own-genes adventure
  – How will insurance cover retesting?
• Too many choices
  – BRCA1/2 vs. breast-specific vs. pan-cancer vs. guidelines-based
• Now a fan of “baby bear” testing
  – Guidelines-based testing or big for the motivated patient.
  – Warning! Large, pan-cancer panels can increase VUS rates and lead to “surprise” results
• Rarely offer single-gene testing
• Appreciate labs with billing clarity
  – Patient explanation of benefits greater than $15,000
  – Time wasted in sessions and with panicked phone calls
What tests tend to be ordered?

For a new patient, how large a test do you most often order?

There’s a wide variability, but we tend to go big.
Ever-increasing test options

65,450 clinical genetic testing products available in US

- 58,491 singles
- 6,737 panels
- 176 exome/genome
- 46 NIPT

Ten new tests added every day!

April 1, 2016
NextGxDx, Inc.
Which of the following are most important to you when selecting a lab?

- Clinical support services: 137
- Ease of use: 144
- Turnaround time: 151
- Validation: 173
- Cost to patient: 231
What was the reason for shifting some of your genetic testing orders to a new laboratory?

- GC support
- Test catalog
- Lab quality
- Turnaround time
- Peer-to-peer support
- Patient resources
- Marketing/sales support
- Other
## Multi-Gene Panels: HBOC

<table>
<thead>
<tr>
<th>Panel Name</th>
<th>Ambyr-CA</th>
<th>Calico Genomics</th>
<th>GenetiDX-NC</th>
<th>GenetiDX-MD</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRCA1 plus V2 (11.114 to present)</td>
<td>High Risk, Breast</td>
<td>Breast</td>
<td>Breast Cancer</td>
<td>Breast High Risk Panel (10.94 to present)</td>
</tr>
<tr>
<td>ATM plus (12.134 to present)</td>
<td>3-4 weeks</td>
<td>6-8 weeks</td>
<td>2 weeks</td>
<td>2 weeks</td>
</tr>
<tr>
<td>ATM</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>BAP1</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>BAP1</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>BARD1</td>
<td></td>
<td></td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>BLM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BRCA1</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>BRCA2</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>BRIP1</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>CDH1</td>
<td>x</td>
<td></td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>CCR4</td>
<td>x</td>
<td></td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>CHEK2</td>
<td>x</td>
<td></td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>CTNMA1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
ASKING THE RIGHT QUESTIONS AND TESTING YOUR TEST PROVIDER

Steve Lincoln
Invitae
Disclosures

• I work for Invitae, a genetic-testing laboratory.
• I own stock in Illumina, a maker of DNA-sequencing instruments (among other things).
• I am a member of the Association for Molecular Pathology (AMP) workgroup on analytic validation standards.
• I am on the steering committee for the Genome in a Bottle validation standard, run by the National Institute for Standards and Technology (NIST).
• But my opinions expressed here do not necessarily reflect those of the larger groups.
Key considerations in genetic-test quality

• Billing practices
• Support
• Report clarity
• Turnaround time
• Specimen requirements
• Ordering logistics
• Technical considerations
Evaluating lab quality can be hard

- Rapidly changing technology and science
- Lots of technical detail
  - Only some of which is directly relevant to you
- Information *and misinformation* is widely available
Systematic detection of errors in genetic linkage data

Stephen E. Lincoln, Eric S. Lander
Whitehead Institute for Biomedical Research and Department of Biology and Center for Genome Research, Massachusetts Institute of Technology, Cambridge, Massachusetts 01242, USA
1. Sequencing methods vary among labs

2. Validation studies also vary among labs

3. Variant classification fundamentally involves expert judgment
1. Sequencing methods vary among labs

2. Validation studies also vary among labs

3. Variant classification fundamentally involves expert judgment
Similarities and differences among NGS labs

Generally standardized processes

Processes that can vary considerably between labs

DNA extraction and quality control
Target Prep
Next-generation sequencing
Informatics
Variant classification

Validation

Gene Selection
Coverage

Richards et al., Genet Med 2015
Nykamp et al., ACMG 2015
NGS coverage (a.k.a. read depth)

- Relatively high-coverage exons
- Low-coverage exons ("gaps") requiring fill-in
- Coverage plot
- NGS reads
- Exon targets
- Yet more coverage if you scroll down

Data courtesy Broad Institute (not Invitae)
Is higher coverage always better?

- Minimum coverage drives sequencing accuracy
- Average coverage is not highly relevant* (for constitutional tests) but can sound impressive.

<table>
<thead>
<tr>
<th>Example</th>
<th>Invitae\textsuperscript{3}</th>
<th>Ambry\textsuperscript{4}</th>
<th>Myriad\textsuperscript{5}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimum</td>
<td>&gt;50x</td>
<td>&gt;50x</td>
<td>&gt;50x</td>
</tr>
<tr>
<td>Average</td>
<td>&gt;350x</td>
<td>500x–1000x</td>
<td>&gt;1000x</td>
</tr>
</tbody>
</table>

*Coverage at any position over ~30x has limited value. Coverage over ~50x has essentially no value.\textsuperscript{1} Indeed, excessive coverage can create false positives.\textsuperscript{2}

\textsuperscript{1}Judkins et al. \textit{BMC Cancer} 2015
\textsuperscript{2}Ajay et al. \textit{Genome Research} 2011
\textsuperscript{3}Invitae Technical Specifications 2016
\textsuperscript{4}Ambry Cancer Panels Fact Sheet v9 2016
\textsuperscript{5}Wall et al., \textit{Genome Research} 2014
Coverage variability is the key issue

Normalized coverage data from patients with no actual copy number changes

Low Variability

High Variability

Substantial lab-specific research and development needed to achieve this.

The Problem: “Off the shelf” target prep methods produce data like this.

Del/dup
Copy number measurement

Low Variability

Del/dup
Low Variability

Comparing data from patient with a 2 exon deletion to controls.

We can measure variability with a goodness of fit test. If so we see cleaner data than comes from microarrays.

Del/dup
Approaches must be used in combination

1. Read-depth
   Also called “dosage” analysis

2. Split-read analysis
   Also paired-end analysis

3. Special-case methods
   For “complex” variants:
   - Inversions (copy neutral)
   - Large insertions (e.g. Alu)
   - High-homology (e.g. PMS2)
   - Homopolymer-associated

4. Confirmation
   Using an “orthogonal” method
   - Microarray
   - MLPA
   - Breakpoint sequencing
   - Long-read sequencing
Pathogenic variants not detected by standard NGS techniques are prevalent.

- Any alteration in PMS2 exons 12–15
  - High-homology (specifically, pseudogene associated) region

- BRCA2: c.9342_9343insAlu
  - Novel Alu insertion (at the time we first saw it)
  - Note: This is NOT the Portuguese founder mutation (that’s c.156_157insAlu).

- BRCA2: c.9203_9328del126
  - Very large sequence indel (or a very small del/dup)

- MSH2: inv exon 1-7
  - Requires breakpoint detection in specific intronic regions

- MSH2: c.942+3A>T
  - Homopolymer (25 A’s) associated splice-affecting mutation

- CDKN2A: c.9_32dup24
  - Third copy of a tandem duplication in 80% CG region (variant from hell)

Many more examples across oncology, cardiology, neurology, pediatrics, etc.

<table>
<thead>
<tr>
<th>%</th>
<th>“Hard” Pathogenic Variant Type Reported</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.9%</td>
<td>Single-exon copy number change (del/dup)</td>
</tr>
<tr>
<td>1.8%</td>
<td>Large indel or complex sequence change</td>
</tr>
<tr>
<td>5.8%</td>
<td>In “hard” regions of the genome</td>
</tr>
<tr>
<td><strong>10.5%</strong></td>
<td><strong>Total</strong></td>
</tr>
</tbody>
</table>

Lincoln et al., J Mol Diag 2015
Lions and tigers and bears, oh my!
Confirmation changes everything

Sensitivity

Initial detection

Specificity

Confirmation by an orthogonal method

Overall accuracy

Reporting

i.e., using a completely different technology:
- Microarray
- MLPA
- Breakpoint sequencing
- Long-read sequencing
### The data (Invitae)

<table>
<thead>
<tr>
<th>Sensitivity</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Events independently detected by non-NGS method in another lab</td>
<td>150</td>
</tr>
<tr>
<td>Also detected by NGS methods</td>
<td>150 (100%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Specificity</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Candidate Events from NGS</td>
<td>1151</td>
</tr>
<tr>
<td>Confirmed by non-NGS method</td>
<td>1126 (98%)</td>
</tr>
<tr>
<td>Specificity with confirmation</td>
<td>100%</td>
</tr>
</tbody>
</table>

99.2% of exons in over 980 genes achieve single exon resolution by NGS (single to noise analysis)

There usually is no comparable measurement for microarrays

All of the 25 (2%) were considered low confidence by the NGS methods
### Published validation studies

<table>
<thead>
<tr>
<th>Type of Variation</th>
<th>Chong 2014</th>
<th>Judkins 2015</th>
<th>Kang 2016</th>
<th>Lincoln 2015</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single nucleotide</td>
<td>3010 (^a)</td>
<td>3884 (^a)</td>
<td>536 (^{a,b})</td>
<td>501 (^a)</td>
</tr>
<tr>
<td>Small indel</td>
<td>11 (0.4%)</td>
<td>39 (1.0%)</td>
<td>? (^b)</td>
<td>156 (22%)</td>
</tr>
<tr>
<td>Multi-exon CNV (del/dup)</td>
<td>2 (0.07%)</td>
<td>41 (1.0%)</td>
<td>? (^c)</td>
<td>16 (2.3%)</td>
</tr>
<tr>
<td>Single exon CNV (del/dup)</td>
<td>2 (0.07%)</td>
<td>8 (0.2%)</td>
<td>? (^{b,c})</td>
<td>13 (1.8%)</td>
</tr>
<tr>
<td>Large indel, complex change</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>? (^b)</td>
<td>19 (2.7%)</td>
</tr>
</tbody>
</table>

- **a.** The vast majority (95%) of SNVs in these studies 1-3 were benign polymorphisms.
- **b.** Variant types are not separated clearly, but the number of “hardest” events is likely to be very small based on the study design.
- **c.** 60 altered exons summed across a much smaller number of events/individuals, although the number of these is not stated.

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Chong et al., *PLOS One* 2014  
Judkins et al., *BMC Cancer* 2015  
Kang et al., *PeerJ* 2016  
Lincoln et al., *J Mol Diag* 2015
“There’s lies, damned lies, and statistics”
- Mark Twain
“There’s lies, damned lies, and statistics”
- Benjamin Disraeli, as quoted by Mark Twain
Questions you can ask (analytical)

• **What methods do you use?**
  – But... You may get a pile of uninterpretable jargon as the answer.

• **What is your minimum NGS coverage? How do you handle gaps?**
  – **How often** do you have coverage gaps in gene X, Y, or Z?
  – **WARNING:** 99% coverage is not as good as it sounds!

• **Which technically challenging variant types can you detect?**
  – 1. Single exon del/dups. 2. Previously unseen Alu insertions. Indels >40bp in size. 3. Known inversions. 4. Homopolymer-associated events. 5. Events in homology regions (like PMS2). Etc...
  – How many of these variants were in your validation studies?

• **If you’re told by lab X that lab Y can’t do ____ by NGS.**
  – Lab X should speak for themselves.
KEEP CALM AND SHOW ME THE DATA
Technical considerations

- Sequencing methods do vary among labs
- Validation studies also vary among labs
- Variant classification fundamentally involves expert judgment
Human genome trivia

• How many genetic alterations does an average person have?
  – Out of our 3 billion base germline genome...
  – 3-4 million

• How many alterations does a person have that are:
  – Rare, Protein altering, VUS
  – In one of the ≈3000 genes currently known to be medically relevant
  – Roughly 200

• Of those, how many have any substantial impact on our disease risk or medical care options?
  – Exclude carrier screening, drug metabolism, low penetrance risk
  – Generally ZERO. Sometimes ONE or TWO. Rarely a few more...

Most VUS are actually benign, but lack evidence to prove it.
Most VUS are actually benign

• Reclassification rates in ClinVar show it
  – 90-95% of all VUS, when reclassified, become benign or likely benign
  – Lincoln et al., manuscript in review

• Reclassification of traditional BRCA1/2 test results shows it
  – Murray et al. Genetics in Medicine, 2011

• Mutagenesis studies show that most protein alterations have no phenotype
  – Or they are embryonic lethal, and are immediately selected out

• Population genetics studies are consistent with this
Variant classification guidelines, guidelines, and guidelines

- **2007/2008 ACMG Guidelines**¹
  - Lay out the general concept well
  - Fairly vague and not very strict about evidence

- **Specialized guidelines**
  - For example: IARC guidelines for BRCA1/2²

- **2015 ACMG/AMP Guidelines**³
  - Less vague and more strict about evidence
  - Multiple rounds of review from 2013 to 2015
  - Still just a guideline; ongoing improvements in process

- **Lab-specific SOPs (standard operating procedures)**
  - For example: Invitae Sherloc framework⁴

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2. Plon et al., Human Mut. 2008
4. Nykamp et al., ACMG 2015
Evidence types in 2015 ACMG Guidelines

Nykamp et al., ACMG 2015
Variant classification process

The literature

Public databases
- Including ClinVar
- Unconvincing “evidence”
- Classifications without evidence for review

Evidence evaluation, QC, and variant classification by lab directors (LDs) and specialist PhD scientists

Final report text from LDs, PhDs, and GCs

Clinical report

Richards et al. Genet Med. 2015
Nykamp et al., ACMG 2015
Variant classification and reporting: It takes a village (example of Invitae)

Robert Nussbaum
M.D., FACP, FACMG
Chief medical officer

Clinical expertise
• Oncology
• Cardiology
• Neurology

Scientific expertise
• Molecular genetics
• Statistical genetics
• Protein structure
• Cell biology
• Functional assays

11 Lab directors (ABMG, MGP)
23 Clinical genomics scientists (PhD)
22 Genetic counselors (CGC, LCGC)
21 Bioinformatics +43 software eng.
Variant classification process

- The literature
- Public databases
  - Including ClinVar
- Evidence evaluation, QC, and variant classification by lab directors (LDs) and specialist PhD scientists
- Final report text from LDs, PhDs, and GCs
- Data submission including evidence for peer review
- Clinical report
- ClinVar

Unconvincing reports
Classifications without evidence for review

Richards et al., Genet Med. 2015
Nykamp et al., ACMG 2015
Data sharing: The new gold standard

ClinVar submission is a mark of being both confident and receptive to critique.

Data sharing through ClinVar allows ongoing inter-laboratory quality control and detailed peer review of every variant classification by the global community of experts, per recommendations of the

- National Society of Genetic Counselors (NSGC)
- American Medical Association (AMA)
- And others

AMA Resolution #519, 2013
NSGC Statement, 2015
What we (all) can do with ClinVar

• Ongoing inter-laboratory quality control
  – The literature does not and can not accomplish this

• Leverage each other’s expertise
  – All of us are smarter than any one of us

• Keep up to date
  – Evidence for/against pathogenicity evolves over time

• Dig into details
  • Of things people say...
Conflicting Interpretation of Genetic Variants and Cancer Risk by Commercial Laboratories as Assessed by the Prospective Registry of Multiplex Testing


- 70 variants representing 155 findings (26% of 603 total) had a interpretation discordance
- 19 variants representing 57 findings (9.5% of 603 total) had a clinically significant interpretation discordance
  - e.g. pathogenic or LP vs. VUS, benign or LB

Interpretations in PROMPT reports and in ClinVar
### Significant Discordances in Balmana et al.

<table>
<thead>
<tr>
<th>#variants</th>
<th>#patients</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>19</td>
<td>57</td>
<td><strong>Total clinically significant discordances in the paper</strong></td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>Not discordant in current ClinVar</td>
</tr>
<tr>
<td>4</td>
<td>9</td>
<td>The one “outlier” classification is an non-clinical lab and non-expert panel submission to ClinVar (e.g. OMIM).</td>
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<tr>
<td>3</td>
<td>21</td>
<td>Substantial agreement among 4 to 8 clinical labs with a single outlier. 18 of these findings are CHEK2:c.470C&gt;T (7 labs P/LP, 1 lab VUS), a pathogenic (low penetrance) variant in a low penetrance gene.</td>
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<tr>
<td>1</td>
<td>2</td>
<td>Pathogenic (low penetrance) or risk factor variant in APC, similar to CHEK2 variant.</td>
</tr>
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<td>2</td>
<td>3</td>
<td>The one oldest or newest classification is the only outlier. Possible new evidence must be evaluated.</td>
</tr>
<tr>
<td>6</td>
<td>7</td>
<td>Unexplained discordance in current ClinVar.</td>
</tr>
</tbody>
</table>
8 variants representing 10 findings (1.7% of the 603) show a clinically significant discordance among the majority of clinical labs in current ClinVar.

<table>
<thead>
<tr>
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</tbody>
</table>
ClinVar entry for PALB2:c.3113G>A

NM_024675.3(PALB2):c.3113G>A (p.Trp1038Ter)

Variation ID: 126711
Review status: ★ ★ ★ ★ criteria provided, conflicting interpretations

Interpretation

Clinical significance: Conflicting interpretations of pathogenicity, risk factor
Likely pathogenic(1);Pathogenic(7);Uncertain significance(1)

Last evaluated: May 2, 2016
Number of submission(s): 10
Condition(s):
- Familial cancer of breast [MedGen - Orphanet - OMIM]
- Breast cancer, susceptibility to [MedGen]
- Hereditary cancer-predisposing syndrome [MedGen]

See supporting ClinVar records
<table>
<thead>
<tr>
<th>Date</th>
<th>Submission Info</th>
<th>Criteria Provided</th>
<th>Clinical Testing</th>
<th>Inheritance</th>
<th>Source</th>
<th>PubMed</th>
<th>PMID</th>
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<tr>
<td>Nov 19, 2015</td>
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<td>PubMed, PALB2 DATABASE (PALB2_10150), Other citation</td>
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</table>

This pathogenic variant is denoted... Full description
This sequence change creates a... Full description
This sequence change creates a premature translational stop signal at codon 1038 (p.Trp1038*). It is expected to result in an absent or disrupted protein product. Truncating variants in PALB2 are known to be pathogenic. This particular truncation has been reported in patients and families affected with breast cancer, with evidence of co-segregation (PMID: 17200668, 21182766, 21285249, 23471749). Experimental studies have shown that this variant caused altered splicing, creating different PALB2 transcripts in lymphoblastoid cells derived from breast cancer patients (PMID: 21285249, 23448497). For these reasons, this variant has been classified as Pathogenic.
This pathogenic variant is denoted PALB2 c.3113G>A at the cDNA level and p.Trp1038Ter (W1038X) at the protein level. This is a nonsense variant, changing a Tryptophan to a premature stop codon. In an mRNA-based assay, Casadei et al. (2011) showed that the expected transcript, an immediate stop codon, was not the only aberrant transcript produced by this variant; two other alternate transcripts, an in-frame deletion of exon 10 and an out-of-frame deletion of 31 base pairs in exon 10, were also identified and are a result of the proximity of the variant to the splice donor site. Additionally, Teo et al. (2013) confirmed the presence of these two alternate transcripts but failed to identify the expected nonsense transcript in an mRNA-based assay. The in-frame deletion of exon 10 is located within a region of interaction with BRCA2 which is thought to be important to PALB2 protein function, and both the expected transcript and the deletion of 31 base pairs are predicted to cause loss of normal protein function through either protein truncation or nonsense-mediated mRNA decay (Casadei 2011, Teo 2013). Lastly, this variant has been reported in several individuals with a personal and/or family history of breast cancer (Rahman 2007, Southey 2010, Wong 2011, Teo 2013) and is considered a pathogenic founder variant in the British population (Hartley 2014). Based on currently available evidence, we consider this variant to be pathogenic.
ClinVar entry for PALB2:c.3113G>A (Invitae)

This sequence change creates a premature translational stop signal at codon 1038 (p.Trp1038*). It is expected to result in an absent or disrupted protein product. Truncating variants in PALB2 are known to be pathogenic. This particular truncation has been reported in patients and families affected with breast cancer, with evidence of co-segregation (PMID: 17200668, 21182766, 21285249, 23471749). Experimental studies have shown that this variant caused altered splicing, creating different PALB2 transcripts in lymphoblastoid cells derived from breast cancer patients (PMID: 21285249, 23448497). For these reasons, this variant has been classified as Pathogenic.
What to watch out for in ClinVar

• Old data

• Data from sources other than clinical labs
  – Usually not classified to clinical standards

• Classifications without detailed evidence
  – Providing details for public scrutiny is a sign of confidence
  – An it’s a sign of collaboration: Only with these details is the entry useful to another lab director
CinVar enables transparency
Example: BRCA1/2

<table>
<thead>
<tr>
<th>Name</th>
<th>Classified Variants</th>
<th>Comparable Variants</th>
<th>Evidence Provided**</th>
<th>Last update (months ago)</th>
<th>Note</th>
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<td>Ambry</td>
<td>2792</td>
<td>1613</td>
<td>NO</td>
<td>16</td>
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<tr>
<td>Myriad (via SCRP*)</td>
<td>2327</td>
<td>1351</td>
<td>NO</td>
<td>0</td>
<td>Benign and Likely Benign variants are under-represented.</td>
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<tr>
<td>Invitae</td>
<td>1998</td>
<td>1367</td>
<td>Yes</td>
<td>3</td>
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<td>GeneDx</td>
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<td>957</td>
<td>Yes</td>
<td>8</td>
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<td>Counsyl</td>
<td>272</td>
<td>256</td>
<td>NO</td>
<td>16</td>
<td>No VUS submitted.</td>
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<td>CHEO (Children’s Hospital of Eastern Ontario)</td>
<td>257</td>
<td>220</td>
<td>NO</td>
<td>n/a</td>
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<tr>
<td>Emory Genetics Lab</td>
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<td>183</td>
<td>NO</td>
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<tr>
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<td><strong>5124</strong></td>
<td><strong>2006</strong></td>
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</table>

Includes all BRCA1/2 data from ClinVar (ncbi.nlm.nih.gov) in June 2016 XML release in over 200 BRCA1/2 variants submitted, where most (>50%) of the classifications are <5 years old

Criteria:
- Clinical labs with over 200 BRCA1/2 variants submitted, where most (>50%) of the classifications are <5 years old

* Data submitted by clinicians and patients through the Sharing Clinical Reports Project
** For Pathogenic, Likely Pathogenic and VUS entries

Approximately 20,000 patients
ClinVar BRCA1/BRCA2 comparison results

• Inter-lab concordance varies: 97%–100%

• For example: Invitae vs. SCRP (Myriad): 99.4% per variant concordance
  – Per patient concordance is even higher: 99.8 – 99.9%
  – Consistent with our prior study

• We investigated classification differences in detail
  – Some data in ClinVar comes from non-clinical labs
  – Most appear to be a simple function of date
  – Sometimes expert judgment differences re: certain evidence
  – Occasionally all labs may not have incorporated certain evidence
  – Rarely proprietary evidence

1. In terms of actionable vs. not actionable (i.e., Pathogenic or LP vs. VUS, LB, or Benign) After all updates applied (e.g., from Rosenthal et al., 2015)

2. Lincoln et al., J Mol Diag 2015
A case study (not from Invitae)

Lab 1
Themselves

Negative result

Likely benign (favors polymorphism) based on a proprietary computer program

BRCA2

Lab 2
Not named

Positive result

Likely pathogenic based on no evidence (ClinVar entry)

Unnecessary procedures

Source: Myriad Analyst Day Presentation, September 2015; available from www.myriad.com

Lab 1

BRCA2
c.9006A>C

Lab 2

Not named
All ClinVar data for BRCA2:c.9006A>T

- All classifications in ClinVar for this variant predate this case study presentation by between 9 months and 2 years
- None of these classifications are Likely Pathogenic (or Pathogenic)
- ...except BIC entry, which is old Myriad data, since reclassified downward
Questions you can ask (variant classification)

• How do you classify variants?
  – What guidelines do you follow? Who does it? What are their areas of expertise and qualifications (note the plurals)?
  – How to you handle ambiguities and limitations in the current ACMG guidelines (are you even aware of them)?
  – How and when do you reclassify and how is that reported back?

• Do you deposit data in ClinVar for ongoing peer review?
  – With evidence descriptions? Remember: Providing an “assertion method” is not good enough.
  – How up to date do you keep ClinVar? Remember: We can check!
  – Do you submit all observed variants? If not, which ones get submitted (ask for exact details on this)?

• If you do not, why not?
  – BRCAShare is not an open alternative – it comes with substantial use restrictions (and fees)
  – PROMPT is great, but it is not a variant sharing resource
  – Publications are not a alternative nor an excuse (no journal considers ClinVar submission a prior publication)
  – ClinGen has an IRB approved, HIPAA-compliant protocol that any laboratory can join which allows sharing of de-identified variants.
References and acknowledgements

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

- Leif Ellisen
- Andrea Desmond
- Kristen Shannon

- Jim Ford
- Allison Kurian
- Meredith Mills

- Melissa Cline
- Molly Zhang
- David Haussler
- Benedict Paten

- Steve Lincoln
- Shan Yang
- Yuya Kobayashi
- Michael Anderson
- Swaroop Aradhya

- Bob Nussbaum
- Scott Topper
- Rebecca Truty
- Keith Nykamp
- Geoff Nilsen

steve.lincoln@invitae.com
Thank you!
EMERGING CHALLENGES IN GENETIC COUNSELING

Julie S. Cohen, ScM, CGC
Kennedy Krieger Institute
• I have received compensation as an advisor to and speaker for Invitae.
Ever-increasing test options

65,450 clinical genetic testing products available in US

58,491 singles

6,737 panels

176 exome/genome

46 NIPT

Ten new tests added every day!

April 1, 2016
NextGxDx, Inc.
Ever-increasing patient volumes

• More patients need genetic testing.
• Testing is needed across more medical specialties.
• Tests are often ordered by non-genetics clinicians.
Balancing the needs and wants of multiple stakeholders

• The patient and their family
• The ordering provider
• The payer or insurance company
Experience at my own institute

• The majority of clinicians are not geneticists (neurologists/developmental pediatricians).
• A very large proportion of patients seen for medical evaluations have a genetic etiology underlying their disability.
Experience at my own institute

- Shift from testing done *always* with genetic counselor to testing ordered *without* a genetic counselor and only referred afterward if needed... and back again
  - Clinician comfort level, knowledge, education
  - Complexities and requirements of insurance companies

- Impact on access to genetic testing, on patient and family experiences with the testing process, and on how results are understood
Experience at my own institute

- It may be ideal for all patients to see a genetic counselor prior to genetic testing, but it’s not realistic.
- Each year, more than 2,000 patients with developmental delay, intellectual disability, and/or autism spectrum disorder are seen for medical evaluations at KKI, but there are only 2.4 full-time GCs.
How can we keep up?

• The obvious solution is more genetic counselors.

• Yes!

• However...
How can we keep up?

• The currently predominant model of one-on-one, face-to-face genetic counseling for every patient undergoing testing is not scalable.
• We’re going to need to get more creative.
The future of genetic counseling

How, do you feel, will the increasing need for additional genetic counseling most likely be managed in 2020?

- Increase in the number of GCs: 49 (17%)
- Remote counseling: 144 (49%)
- Shorter counseling sessions: 52 (18%)
- Restrictions on who is tested: 6 (2%)
- Other: 42 (14%)
Tele-genetic counseling

• Studied in cancer genetics, need studies in other specialties
• Labs offering genetic counseling services
  – Some insurance companies require genetic counseling before testing can be approved, but genetic counselor cannot be affiliated with lab.
• Billing and reimbursement challenges
• Telegenetics will increase access but does not address the issue of volume
Enhancing efficiency of genetic counseling

- Use of “genetic counseling extenders” (e.g., genetic counseling assistants) to handle certain tasks, freeing up time for a genetic counselor to spend with a patient
- Shorter genetic counseling sessions
- Videos and written support materials for some educational components of the session
- Group genetic counseling sessions
- NSGC efficiency task force
Genetic testing without a genetic counselor

Most frequent free response to survey question, many viewed negatively

“I think other providers will perform more education even if it’s not complete/accurate”

“Unfortunately, a bigger reliance on other healthcare professionals to handle genetic counseling”

“Less qualified providers filling the GC gap”
Genetic testing without a genetic counselor

• This is already happening!
• Rather than opposing collaboration with non-genetics providers, we need to *increase* collaboration with them.
GCs as experts, not gatekeepers

• Identify types of patients/indications/tests that don’t require a genetic counselor’s involvement from those that do.
• Educate providers on appropriate test ordering, pre-test counseling, and knowing when to refer.
Providing family guidance in rapidly shifting sand: informed consent for genetic testing

JoLIE COHEN | ALEC HOON | ANNA MARIA WILMS FLOET

Table 1: Basic components of informed consent

<table>
<thead>
<tr>
<th>Nature and scope</th>
<th>Background information</th>
</tr>
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<tbody>
<tr>
<td>Purpose of test</td>
<td>determine genetic cause</td>
</tr>
<tr>
<td>Possible result outcomes</td>
<td>(1) normal; (2) abnormal; (3) variant of unknown significance; (4) incidental/secondary</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Benefits</th>
<th>May identify the genetic cause/diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Medical and psychosocial benefits to diagnosis</td>
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<table>
<thead>
<tr>
<th>Limitations</th>
<th>Does not rule out all genetic conditions</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>May not lead to definitive cure or treatment</td>
</tr>
<tr>
<td></td>
<td>May require further testing</td>
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<table>
<thead>
<tr>
<th>Risks</th>
<th>Ambiguous results</th>
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<tr>
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<td>Unexpected/unrelated information</td>
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<tr>
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<td>Familial implications</td>
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<table>
<thead>
<tr>
<th>Costs</th>
<th>Check with insurance</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Advise on out-of-pocket expenses</td>
</tr>
</tbody>
</table>
• Payers may be moving in the direction of requiring genetic counseling (by a genetic counselor or a geneticist) prior to approving testing.
• Genetic counselor as consultant providing test review
What does genetic counseling in 2020 look like to you?
Thank you!