COA ADVANCED PRACTICE PROVIDER CALL

Tuesday, August 15th, 12:30 pm ET
CAPP Co-Chairs:

Sara Pearce, NP-C, Cancer Care of WNC
spierce@cancercareofwnc.com

Diana Youngs, ANCP, North Shore Hematology Oncology dyoungs@nshoa.com
The call will be recorded. Access to the recording will be placed on the CAPP website.

Callers will be muted once the call begins. Toggle between mute and unmute with *6.

Please do not place our call on “hold” while unmuted.
Genetic Testing: The ACCE Framework
Analytic Validity
Clinical Validity
Clinical Utility
Ethical, Economic, Social Impact

Robert L. Nussbaum, MD
Chief Medical Officer
Invitae Corporation
Oncology testing menu

| Guidelines-based panels                        |
| Cross cancer panels                          |
| Breast and Gynecologic cancer panels          |
| Gastrointestinal cancer panels                |
| Genitourinary cancer panels                   |
| Endocrine cancer panels                       |
| Hematologic malignancy panels                 |
| Nervous system/brain cancer panels            |
| Sarcoma panel                                 |
| Skin cancer/Melanoma panel                    |
| Pediatric oncology                            |

Massively parallel sequencing (NGS) makes it cost effective to

1. Offer panel testing at one low price regardless of the number of genes in the panel
2. Allow flexible panel design customized by the ordering provider
3. Re-requisition to larger panel at no additional charge.
“Next Generation Sequencing (NGS)”
Massively Parallel Sequencing

- Current, most widely-used NGS technology provides ~200-300 bp of sequence from many millions of DNA fragments at the same time, in parallel, from a single flow cell.
- Use software to line up the billions of bases of sequence and examine the segments of interest.
Pre-Next Generation Sequencing: One Gene at a Time

DNA → Gene of interest → Amplify → Sequence → Compare

Reference Sequence

TTCTCTAGCTCAGGCTTA------------------------TACCAGTAGATT
TCGGCTCGGATGTTTCTCTTT------------------------CTAGGCTGACTGACTGA
GCTAGGCTAGCTTTGCA--------------------------CGGATCGGATCGG
TAGCTTAGCTAGGCTTAGGTTT----------------------ATCGATTGCGAT
GTCGCTCGGATGTTTCTTT------------------------CTAGGCTGACTGACTGA
CTGACTAAATCGATAC--------------------------GCTACCGAGACTGG
Panel Sequencing

ADD SHORT SYNTHETIC DNAS COMPLEMENTARY TO EXONS IN GENE OF INTEREST. ATTACH THEM TO MAGNETIC BEADS

Align each segment to Reference Sequence
# ACCE Framework

<table>
<thead>
<tr>
<th>Category</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td><strong>Analytic Validity</strong></td>
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<td><strong>Clinical Utility</strong></td>
<td>Test results are “useful” to patient and doctor</td>
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<td>Test results “make a difference”</td>
</tr>
<tr>
<td><strong>Ethical, Economic, Legal, Social Implications</strong></td>
<td>There is value to society in having test results</td>
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CDC Office of Public Health Genomics
Analytic Validity: True and False Positives

Did the right test on the right patient? Find an abnormality it when it is there and not when it is not?

Sensitivity: In people at risk or with the Disease ➔ How often is the test positive?

Specificity: In people not at risk or without the Disease ➔ How often is the test negative?
“Hard-to-Do’s”

30,000 sequential cases with clinical testing at Invitae across over 1,000 known disease genes

Of the last 5,000 patients with pathogenic findings…

<table>
<thead>
<tr>
<th>Percentage</th>
<th>Pathogenic Variant Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.9%</td>
<td>Single-exon del/dups (CNVs)</td>
</tr>
<tr>
<td>1.8%</td>
<td>Large indel (deletion≥10bp, insertion≥5bp) or complex sequence changes**</td>
</tr>
<tr>
<td>5.8%</td>
<td>In high-GC or difficult to map regions of the genome</td>
</tr>
</tbody>
</table>

100% Sensitivity and Specificity orthogonally confirmed
Distribution of Hard-to-Dos in different Clinical Areas

Among Invitae’s cohort of over 80,000 tested patients, pathogenic, medically important variants of technically challenging comprised

• 9% of pathogenic findings in hereditary cancer genes,
• 10% in cardiology,
• 12% in neurology, and
• 19% in pediatric metabolic disorders.
The spectrum of pathogenic variants in cancer genes that are hard for NGS to handle

- **BRCA2**: c.9342_9343insAlu - Novel Alu insertion

- **BRCA2**: c.9203_9328del126 - 126 bp deletion

- **MSH2**: c.942+3A>T - Splicing-altering mutation next to a 25 bp homopolymer-A

- **MSH2**: inv exons 1 - Breakpoint detection in intronic regions

- **CDKN2A**: c.9_32dup24 - Third copy of a wild-type tandem duplication in 5’ CpG island

- **PMS2, CHEK2**, and other examples - Portions of the gene are present elsewhere but are non-functional and not associated with disease
### ACCE Framework

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CDC Office of Public Health Genomics
Variant classification for germline test results

- Variant classification is the first pillar of clinical genetics

- Variants are either **Pathogenic, Likely Pathogenic, Likely Benign** or **Benign**...**BUT**

- Sometimes the effect of the variant is **Uncertain** given the current state of information available

- Interpretation process should
  - **Accurately**, **efficiently**, and **transparently** assess evidence for and against pathogenicity
  - Provide **reproducibility** over a large group of experts
  - When there is insufficient information, **confidently** conclude that the nature of the variant is uncertain
ACMG STANDARDS AND GUIDELINES

Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology

Sue Richards, PhD¹, Nazneen Aziz, PhD²,¹⁶, Sherri Bale, PhD³, David Bick, MD⁴, Soma Das, PhD⁵, Julie Gastier-Foster, PhD⁶,⁷,⁸, Wayne W. Grody, MD, PhD⁹,¹⁰,¹¹, Madhuri Hegde, PhD¹², Elaine Lyon, PhD¹³, Elaine Spector, PhD¹⁴, Karl Voelkerding, MD¹³ and Heidi L. Rehm, PhD¹⁵; on behalf of the ACMG Laboratory Quality Assurance Committee

Pub March 2015 Online

Uses 24 pieces of data (Frequency in unaffected populations, association with affected people, inheritance in families with multiple affects, etc.)
Sherloc: a comprehensive refinement of the ACMG–AMP variant classification criteria

Keith Nykamp, PhD, Michael Anderson, PhD, Martin Powers, MD, John Garcia, PhD, Blanca Herrera, PhD, Yuan-Yuan Ho, PhD, Yuya Kobayashi, PhD, Nila Patil, PhD, Janita Thusberg, PhD, Marjorie Westbrook, PhD, The Invitae Clinical Genomics Group and Scott Topper, PhD, FACMG
SHERLOC: Variant classification evidence types based on >100 discrete types of evidence

<table>
<thead>
<tr>
<th>Evidence Type</th>
<th>Benign</th>
<th>Likely</th>
<th>Uncertain Significance</th>
<th>Likely</th>
<th>Pathogenic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population Data</td>
<td>very HIGH 0.5% 1.0%</td>
<td>HIGH 0.1% 0.3%</td>
<td>within pathogenic range</td>
<td>absent from ExAC</td>
<td></td>
</tr>
<tr>
<td>Type of Variant</td>
<td>synonymous non-conserved intronic</td>
<td>missense</td>
<td>AG/GT dinucleotide</td>
<td>nonsense frameshift</td>
<td></td>
</tr>
<tr>
<td>Clinical Findings</td>
<td>Dom: co-occurrence in trans</td>
<td>Dom: co-occurrence phase unknown</td>
<td>2 cases 1 family</td>
<td>4+ cases 2 families</td>
<td></td>
</tr>
<tr>
<td>Experimental Studies</td>
<td>neutral STRONG</td>
<td>neutral WEAK</td>
<td>disrupted WEAK</td>
<td>disrupted STRONG</td>
<td></td>
</tr>
<tr>
<td>Indirect and Computational</td>
<td>suggest benign</td>
<td>suggest disrupted</td>
<td>essential AA</td>
<td></td>
<td></td>
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</table>
ClinGen Lists Labs Meeting Requirements for Quality Data Sharing on Genetic Variants

Jul 17, 2017 | Turna Ray

Invitae was one of only two commercial laboratories receiving the highest recognition for data sharing from ClinVar
Collaborative study demonstrates very high concordance for Actionable BRCA1/2 Variants between Clinical Diagnostic Laboratories

Per-variant concordance for **actionability** was 98.5% (CI, 97.9% to 99.0%).

Per-patient concordance for **actionability** is higher (99.7%).

ClinVar facilitated resolution of many of the discordant variants, and concordance increased to 99.0% per variant and 99.8% per patient when reclassified variants and submission errors were addressed by professional consultation. Includes >2000 Myriad reports put into Clinvar by Providers.

**Methods**

ClinVar contains more than 2,000 BRCA1/2 variants in approximately 22,000 patients who were dichotomized as clinically actionable compared among as many as ten laboratories. The properties of classification differences were investigated in detail.

**Results**

Per-variant concordance was 98.5% (CI, 97.9% to 99.0%). Per-patient concordance was estimated to be higher as resolution of many of the discordant variants, and concordance increased to 99.8% per patient when reclassified, but not yet resubmitted, variants were addressed. Most of the remaining discordances seemed to reflect differences in expert judgment regarding particular scientific evidence or the availability of important scientific evidence.

**Conclusion**

Significant classification disagreements among providers represented in ClinVar are infrequent yet important. Unrestricted submissions allow detailed inter-laboratory quality control and peer review, as well as dissemination of high-quality clinical data.

**Acknowledgments**

We are deeply grateful to the laboratories and other groups who are working to advance medical care by submitting data to the ClinVar database. We particularly thank the staff at SCRP, Ambry Genetics, GeneDx, Counsyl, the CHEO Molecular Genetics Laboratory, and the Emory Genetics Laboratory for their hard work on data submissions. We thank George Riley (National Institutes of Health/National Center for Biotechnology Information) and Salina Chan (University of California, San Francisco) for providing updated SCRP data. We thank Olga Jarinova and Hussein Daoud (CHEO Molecular Genetics Laboratory), Jill Dolinsky and Tina Pesaran (Ambry Genetics), Peter Kang (Counsyl), and Kathryn Garber (Emory Genetics Laboratory) for clarifying aspects of their data in ClinVar. We thank Linda Robinson (University of Texas Southwestern) for bringing the changes to Myriad’s terms and conditions into effect. We thank Nancy Jacoby (Invitae) for help with the manuscript and John Garcia and Tim Chiu (Invitae) for assistance with ClinVar. We thank our colleagues who volunteered anonymized BRCA1 and BRCA2 test reports to the SCRP project for submission to ClinVar. We especially thank Laura Swaminathan, who generously volunteered her time to make the SCRP project possible, with the assistance and encouragement of Danielle Azzariti (Harvard), George Riley, and Heidi Rehm (Harvard).

**J of Clinical Oncology Precision Oncology 2017 (ASCO)**
Source of Most Discordances: Older research literature. Reputable clinical laboratories submitting clinical reports to CLINVAR show remarkable concordance >98% among themselves.
Variant Interpretation in Pathology

VARIABILITY IN PATHOLOGISTS’ INTERPRETATION OF INDIVIDUAL BREAST BIOPSY SLIDES: B-PATH STUDY

- 115 practicing pathologists reviewed 240 breast biopsy slides: 30% benign, 30% atypia, 30% DCIS, 10% Invasive Ca
- Interpretation of the same slide by a reference group of 3 “expert” pathologists = “Consensus-derived reference”

Compared with the consensus-derived reference, overall concordance per slide was 75.3%(95% CI, 73.4%-77.0%)

Compared with the consensus-derived reference, overall concordance per patient was 92.3%(95% CI, 73.4%-77.0%)

What is “The Gold Standard”?  

DISCREPANCIES IN INTERPRETATION ARE PART OF MEDICINE AND ARE BEST RESOLVED COOPERATIVELY, NOT COMPETITIVELY

- The claim that one laboratory is “The Gold Standard” is no more valid than the claim that one pathology department in one hospital has a monopoly on the ability to interpret surgical biopsy specimens.
- As in other parts of medicine, the correct “Gold Standard” is a consensus that comes from sharing among clinicians to reach consensus.
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CDC Office of Public Health Genomics
Managed Care Objections to Panels:
We spend too much testing the wrong people to obtain results that we either cannot interpret or won’t change management
More specifically…

1. Genetic testing is already very expensive – larger panels mean higher bills to insurance companies.
2. Testing outside of guidelines increases costs by testing people who are less likely to yield a positive, increasing the number required to test to detect a positive.
3. Increasing size of panels identifies mutations in genes for which there are no or only guidelines are expert opinion (NCCN).
4. Increasing size of panels increases the number of VUS, which clinicians misinterpret, leading them to make inappropriate or harmful interventions.
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Do Guidelines Work?

- 6125 individual HBOC tests for Medicare eligible patients
- Patients who met criteria (4433, 72.4%)
- Patients who do not meet criteria (1692, 27.6%)

We performed testing for EVERYBODY and ate the cost if Medicare declared the tests were out of guidelines.
Test Outcome for Patients in and out of Medicare Criteria

<table>
<thead>
<tr>
<th>Test Result</th>
<th>% Meet</th>
<th>% not Meet</th>
</tr>
</thead>
<tbody>
<tr>
<td>negative</td>
<td>60.1%</td>
<td>59.4%</td>
</tr>
<tr>
<td>Uncertain (VUS)</td>
<td>29.1%</td>
<td>31.6%</td>
</tr>
<tr>
<td>positive</td>
<td>10.8%</td>
<td>9.0%</td>
</tr>
</tbody>
</table>
Comparing two panels in patients meeting or not meeting guidelines

- Tested just for BRCA1 and 2 or with 19 Gene Breast/Gyn Guidelines Panel

<table>
<thead>
<tr>
<th>Test Result</th>
<th>BRCA 1 or 2 only</th>
<th>Breast/Gyn cancer guideline genes (19 in total)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pct Meet Guideline</td>
<td>pct not Meet Guideline</td>
</tr>
<tr>
<td>negative</td>
<td>93.5%</td>
<td>95.0%</td>
</tr>
<tr>
<td>uncertain</td>
<td>3.2%</td>
<td>3.0%</td>
</tr>
<tr>
<td>positive</td>
<td>3.3%</td>
<td>2.0%</td>
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4. Increasing size of panels increases the number of VUS, which clinicians misinterpret, leading them to make inappropriate or harmful interventions.
Invitae’s Data: Almost half of patients referred for HBOC testing had mutations in a gene on our 80 gene panel other than BRCA1/2

Total cohort: 20,592 women with breast cancer

- No P/LP variants identified
  - 89.8% (n = 18,487)
    - No variants
      - 72% (n = 13,298)
    - ≥1 VUS
      - 28% (n = 5189)

- P/LP variants identified
  - 10.2% (n = 2105)
    - P/LP variants in other genes*
      - 5.27% (n = 1085)
        *excludes BRCA1/2
    - P/LP variants in BRCA1/2
      - 4.95% (n = 1020)
Pathogenic/Likely Pathogenic Mutations in Genes Other than *BRCA1/2*

Most have NCCN Management Guidelines

![Bar chart showing the number of variants identified in genes with and without NCCN management guidelines.](chart.png)

- Blue bars: Variants identified in genes with no management guidelines.
- Yellow bars: Variants identified in genes with management guidelines.

**Genes:**
- CHEK2
- MUTYH
- PALB2
- ATM
- TP53
- BRIP1
- MSH6
- NBN
- BARD1
- RAD51C
- MLH1
- PTEN
- CDH1
- MSH2
Increased Yield of Pathogenic/Likely Pathogenic Variants in Breast and Ovarian Cancer, Colorectal Cancer and Prostate Cancer Using Expanded Panels

Indication for Testing → HBOC | Colorectal Cancer | Prostate Cancer

- BRCA1/2 19 Gene Panel
- BRCA1/2 11 Gene Panel
- 80 Gene Panel
- Lynch 17 Gene Panel
- 80 Gene Panel

Frequency Among Patients Tested for Indication Shown

- All P/LP
- Moderate risk alleles removed
Genes associated primarily with breast cancer (ATM, BARD1, BRCA1, BRCA2, BRR1, CDH1, CHEK2, FANCC, MRE11A, NBN, NF1, PALB2, PTEN, STK11, TP53)

All the genes in group A plus genes associated with other commonly assessed cancer types (APC, AXIN2, BMPR1A, CDKN2A, Dicer1, EPCAM, GREM1, KIT, MENT, MLH1, MSH2, MSH6, MUTYH, PDGFRA, PMS2, POLD1, POLE, RAD51C, RAD51D, SDHA, SDHB, SDHC, SDHD, SMAD4, SMARCA4, TSC1, TSC2, VHL)

Comprehensiv e panels including all the genes in groups A and B plus an expanded list of genes for other tumor types up to 79 genes
## Invitae: Yield of Pathogenic/Likely Pathogenic (P/LP) Variants in Less Frequent Cancers

<table>
<thead>
<tr>
<th>Cancer Type</th>
<th>Total Patients</th>
<th>Positive Patients (%)</th>
<th>Total P/LP Variants</th>
<th>P/LP variants in genes within guidelines panels</th>
<th>P/LP variants in genes outside guidelines panels</th>
<th>P/LP variants outside guidelines panels that would change management</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melanoma</td>
<td>933</td>
<td>115 (12%)</td>
<td>122</td>
<td>69 (57%)</td>
<td>53 (43%)</td>
<td>50 of 53 (94%)</td>
</tr>
<tr>
<td>Paraganglioma</td>
<td>223</td>
<td>67 (30%)</td>
<td>71</td>
<td>50 (70%)</td>
<td>21 (30%)</td>
<td>20 of 21 (95%)</td>
</tr>
<tr>
<td>Pancreatic Cancer</td>
<td>693</td>
<td>96 (14%)</td>
<td>101</td>
<td>83 (82%)</td>
<td>18 (18%)</td>
<td>18 of 18 (100%)</td>
</tr>
<tr>
<td>Renal Cancer</td>
<td>949</td>
<td>266 (28%)</td>
<td>294</td>
<td>204 (69%)</td>
<td>90 (31%)</td>
<td>89 of 90 (99%)</td>
</tr>
<tr>
<td>Sarcoma</td>
<td>423</td>
<td>68 (16%)</td>
<td>71</td>
<td>39 (55%)</td>
<td>32 (45%)</td>
<td>30 of 32 (94%)</td>
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Provider Ordering Behavior is Rapidly Moving Beyond Narrowly Defined Panels

1. Providers value increased sensitivity for pathogenic variants in genes outside guidelines that would alter management

2. Providers have developed a new appreciation of the complexity of cancer genetics
   1. We do not always know which genes or which hereditary cancer syndrome is responsible for a constellation of cancers in a family
   2. Family history is insensitive
   3. Testing more genes does not have to cost more
   4. Providers are becoming more comfortable with Variants of Uncertain Significance