Variant Classifications for BRCA1 and BRCA2 are Highly Concordant Across Major Clinical Testing Laboratories

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De-identified data from this study have been deposited into the public ClinVar database hosted by the National Institutes of Health (NIH).

www.clinvar.com http://www.free-the-data.org

Background

- Public databases of clinically observed variants are a rapidly growing and valuable resource. However, variant classification differences between public databases have been raised as a concern by at least one commercial laboratory who suggested that "widespread disagreement" should "preclude their wider use in clinical practice" [1].
- The clinical impact of these disagreements is not clear. Experienced lab directors never simply copy classifications from any public database. Instead, they critically evaluate evidence and determine classifications rigorously following established guidelines.
- Appropriate practices for the use of public databases have been established in the clinical genetics community for years. The need for, and methods for the integration and quality control of databases by their users are well-understood.
- Both our prior studies and our clinical experience show that expert BRCA1/2 variant classifications, appropriately utilizing public data (including the literature), are highly concordant with classifications that utilize non-public, proprietary information.
- Here we sought to measure BRCA1/2 classification concordance in a large multi-laboratory public data set.

[1] Vail *et al., J. Community Genetics,* 2015

Prior Research (Validation Study)

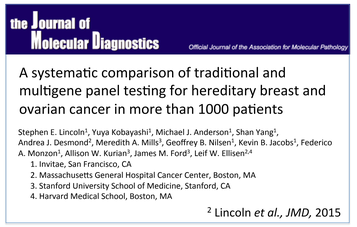
- Our recent study[2] observed high (99.8%) concordance of 975 BRCA1/2 tests classified following current guidelines using only publicly available data, compared to tests that also utilized non-public information. The study was a blinded analysis in a prospectively accrued, clinically representative patient population (see Reference 2, Methods).
- Our companion clinical utility study[3] incorporated additional data in which no classification differences were observed. VUS (variant of uncertain significance) rates were comparable.

Concordance of BRCA1/2 tests, N=975	
Agree	99.8%
Disagree	0.2%

Table 1A. Concordance data from reference 2.

% of patients with one or more VUS in BRCA1/2	
New test	4.1%
Previous test	3.2%

Table 1B. VUS rate data from reference 2.

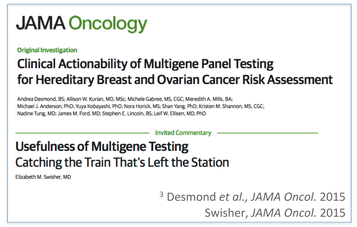

the Journal of Molecular Diagnostics — Official Journal of the Association for Molecular Pathology

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

Stephen E. Lincoln[1], Yuya Kobayashi[1], Michael J. Anderson[1], Shan Yang[1], Andrea J. Desmond[2], Meredith A. Mills[1], Geoffrey B. Nilsen[1], Kevin B. Jacobs[1], Federico A. Monzon[1], Allison W. Kurian[3], James M. Ford[3], Leif W. Ellisen[2,4]

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

[2] Lincoln *et al., JMD,* 2015


JAMA Oncology

Original Investigation

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. Ellsen, MD, PhD

Invited Commentary

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

Elizabeth M. Swisher, MD

[3] Desmond *et al., JAMA Oncol.* 2015
Swisher, *JAMA Oncol.* 2015

Classification Concordance, Per-Variant

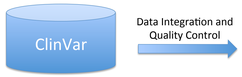
Table 2. Classification concordance between laboratories on a per-variant basis.

	Ambry	Invitae	GeneDx	Counsyl	CHEO	Emory
Myriad via SCRP	98.7% 939/951	99.2% 619/624	99.5% 569/572	99.4% 171/172	99.5% 139/142	97.2% 103/106
Ambry		99.2% 860/867	99.6% 780/783	99.6% 223/224	98.3% 176/179	98.8% 161/163
Invitae			99.8% 593/594	99.1% 214/216	98.2% 161/164	99.3% 144/145
GeneDx				99.5% 221/222	97.9% 138/141	99.3% 149/150
Counsyl		Concordance Concordant/All			100% 82/82	100% 105/105
CHEO						98.3% 57/58

- Only 27 variants with any discordant classifications were observed out of 1800 reported by more than one lab.
- Counting each variant separately, concordance between pairs of labs is high: 97.2% to 100.0%.
- All of the discordant classifications were in rare variants that, by definition, are present in very few patients.
- Thus, this calculation **greatly underestimates** the much higher concordance observed on a per-patient basis.
- Reports from other labs that pre-date SCRP releases had comparable concordance to those that post-date SCRP, suggesting that the SCRP data did not bias the other labs.

Methods

I. Data Sources



1. BRCA1/2 data were collected for these 6 clinical labs from ClinVar. Available submissions pending release were included.
2. Data integration was improved by standardizing variant nomenclature.
3. Data were quality controlled manually and computationally. Clearly erroneous records were repaired or removed.

Key Steps

Table 3. Data used to compare classifications

Source	Total Variants	Reported by Multiple Labs*
Ambry Genetics	2793	1502
Myriad Genetics via SCRP	2067	1184
Invitae	1479	1082
GeneDx	1214	937
Counsyl	272	256
CHEO Molecular Genetics Lab	257	216
Emory Genetics	203	183

SCRP = Sharing Clinical Reports Project (at UCSF). Most SCRP reports are from 2011 or later. The older Myriad BIC data were not used.

CHEO = Children's Hospital of Eastern Ontario

*i.e. The number of variants classified by two or more labs on this list.

II. Comparison Methodology

Table 4. Comparisons in this study distinguish between potentially clinically actionable and not actionable findings.

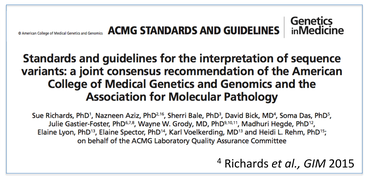
	Positive	Uncertain	Negative
Positive	✔	✖	✖
Uncertain	✖	✔	✔
Negative	✖	✔	✔

Positive = Pathogenic or Likely Pathogenic
Negative = Benign or Likely Benign

✖ = Considered discordant in this study
✔ = Considered concordant in this study

III. Variant Classification Process

Variants were classified at Invitae using a system (called **Sherloc**) that closely adheres to the 2015 ACMG guidelines[4] for the Interpretation of Sequence Variants.


American College of Medical Genetics and Genomics ACMG STANDARDS AND GUIDELINES Genetics inMedicine

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, PhD[1], Nazneen Aziz, PhD[2,15], Sherri Bale, PhD[3], David Bick, MD[4], Soma Das, PhD[5], Julie Gastier-Foster, PhD[6,14], Wayne W. Grody, MD, PhD[7,8,15], Madhuri Hegde, PhD[9], Elaine Lyon, PhD[3,], Elaine Spector, PhD[10], Karl Voelkerding, MD[11] and Heidi L. Rehm, PhD[12]; on behalf of the ACMG Laboratory Quality Assurance Committee

[4] Richards *et al., GIM* 2015

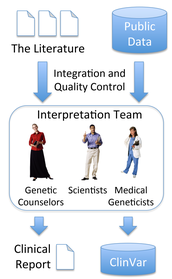


Figure 1. Classification Workflow

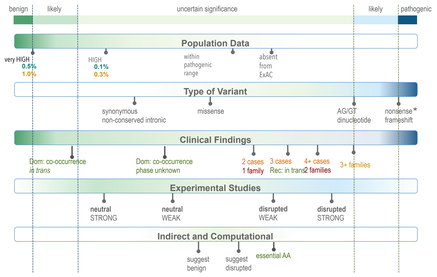
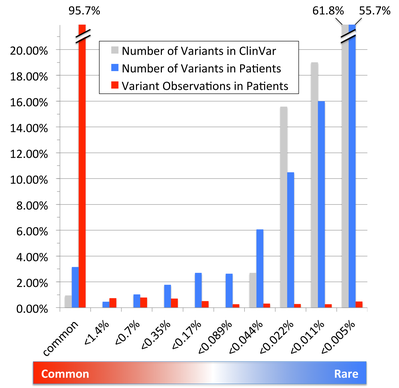


Figure 2. Evidence Types Used in Variant Classification

Classification Concordance, Per-Patient

Figure 3. Prevalence of variants. The blue and grey bars count each variant once regardless of how many individuals it is seen in, showing that the majority of variants are very rare. The red bars count each observation of any variant separately, showing that the majority of variants observed in patients are not the rarest ones. Common benign variants are frequently observed but are typically excluded from diagnostic test reports.



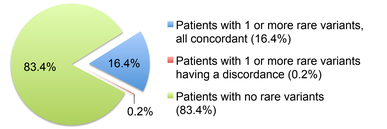
Analysis details: Prevalence of variants in patients was measured in a sequential series of over 15,000 unrelated individuals tested at Invitae.

Common variants are defined here as those that are either (a) seen in the general population at >2.0% frequency, or (b) have an prevalence in tested patients >1.4%. Rare variants have <0.05% population frequency and <0.044% prevalence.

Population frequency is the maximum of the minor allele frequency reported by ExAC (Broad Inst.), the 1000 Genomes Project (NIH), and the Exome Variant Server (U. Washington) following data integration and quality control. Ethnicity-specific rates are not shown here.

- Most variants (>90%) in public databases are quite rare. While there are many of these rare variants, few patients (≈16%) carry one or more of them.
- Classifications of most rare variants (98.4%) are concordant, whether positive or not positive.
- We calculate that the expected chance of a patient having a variant with a discordant classification is <0.2%, similar to our prior study's result (Reference 2, see panel at left).

Figure 4. Expected fraction of patients with discordant results based on the combination of prevalence and concordance data.



- Definitive classifications of rare variants are possible based on effect on the protein sequence or gene splicing, or alternatively by functional assays, co-occurrence or pedigree analysis. Others are VUS.
- Reclassifications of VUS in ClinVar (by any one of the labs) show them usually downgraded to benign or likely benign as further data emerge.

Conclusions

Classification concordance needs to be measured carefully in order to avoid over-counting differences and misinterpreting the implications for patient care. What matters most is the fraction of patients, not the fraction of variants in public databases, that show a discordance.

While discordances are infrequent, they are important and it is essential to resolve them collaboratively, not competitively, in order to deliver the best patient care, as is done in all other areas of medicine[5]. Independent peer review of classifications, such as this study, are enabled by public databases. Such analyses both aid laboratory quality control efforts and help improve clinical guidelines.

Even after detailed examination of the evidence underlying the few classification disagreements seen in this study, the maximally correct classification under current ACMG guidelines was sometimes still unclear. Most classification differences appear to be due to a difference in precise criteria used, not a difference in underlying data available to the lab.

[5] Rehm *et al., NEJM* 2015