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

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Background

- Public databases of clinically observed variants are a rapidly growing and valuable resource for laboratories, clinicians, and patients.[1,2]
- However, variant classification differences between public databases have been raised as a concern by at least one commercial laboratory who suggested that "widespread disagreement" in these databases should "preclude their wider use in clinical practice."[3]
- **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.
- Our prior studies show that expert BRCA1/2 variant classifications, appropriately utilizing public data (including databases and 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] Rehm *et al., NEJM,* 2015
[2] Pfimister, *NEJM,* 2015
[3] Vail *et al., J. Community Genetics,* 2015

Prior Studies

- Our recent study[4] observed high (**99.8%**) concordance of 975 BRCA1/2 reports 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 (for details see the Methods section of reference 4).
- Our companion clinical utility study[5,6] 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 4.

% 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 4.

the Journal of Molecular Diagnostics JAMA Oncology

[4] Lincoln *et al., J Mol Diag,* 2015
[5] Desmond *et al., JAMA Oncology,* 2015
[6] Swisher, *JAMA Oncology,* 2015

II. Variant Classification Process

Variants were classified at Invitae using a system (called **Sherloc**[7,8,9]) that closely adheres to the 2015 ACMG guidelines[10] for the Interpretation of Sequence Variants.

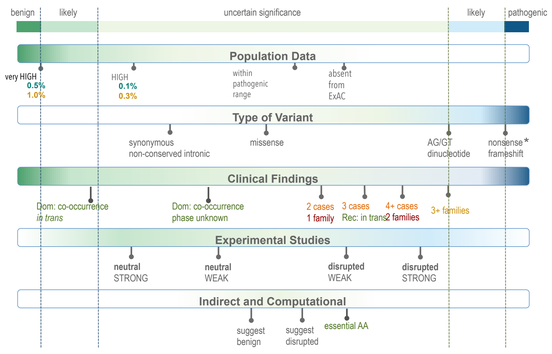


Figure 1. Evidence Types Used in Variant Classification

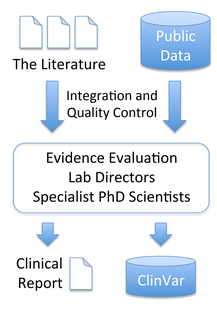


Figure 2. Classification Workflow

Classification Concordance Per-Variant

- Only **27 out of 1800 variants** with any discordant classifications between any two labs were observed.
- 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.

	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

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

Methods

I. Data Sources

Approx. 20,000 Patients Represented

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.

1. BRCA1/2 data were collected from ClinVar. Analysis was limited to clinically relevant data:
   - From established clinical labs (not research)
   - with at least 200 BRCA1/2 classifications in ClinVar
   - that were mostly recent (not >5 years old)
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 not done in the Vail paper

III. Comparison Basis

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

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

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

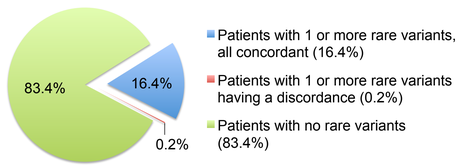
Table 4. Comparisons in this study distinguish between potentially clinically actionable and not actionable findings.

[7] Nykamp *et al., ACMG* 2015
[8] Nykamp *et al., ClinGen* 2015
[9] Kobayashi *et al., ASHG* 2015
[10] Richards *et al., Genet Med,* 2015

Classification Concordance Per-Patient

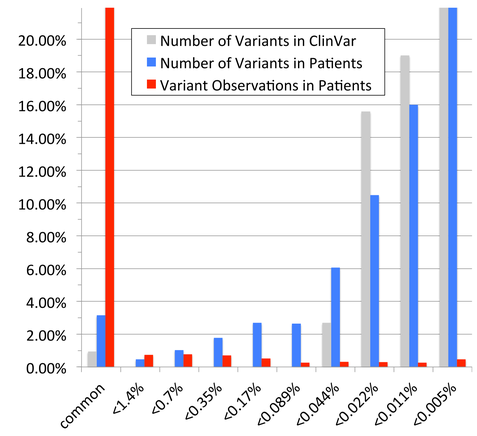
- Most variants (>95%) in public databases are very rare. Here we define rare as having <0.05% allele frequency in all available clinical and population databases.
- While there are many of these rare variants, few patients (≈16%) carry one or more of them.
- Classifications of most rare variants (98.4%) are still highly concordant.
- Thus, patients would be expected to have 99.8% classification concordance in clinical reports, similar to our prior study's result (Reference 4, see panel at left).

Figure 3. Expected fraction of patients with discordant results based is 0.2% 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 in ClinVar (by any of the labs) show VUS almost always are downgraded to benign or likely benign as further data emerge.

Figure 4. 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.



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 that show a classification discordance that would significantly change management.
- While such 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 in other areas of medicine.[1,2]
- Laboratories who do not contribute to public databases can make arbitrary and unverifiable claims regarding variant classification.
- Independent review of classifications, such as this study, are enabled by public databases. Such analyses both enhance laboratory quality control and help improve clinical guidelines.
- Even after detailed re-examination of all evidence underlying the few classification disagreements seen in this study, the maximally correct classification under current ACMG guidelines was 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.