Variant classification in an unaffected population, an example from expanded carrier screening and a comparison to ClinVar classifications

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Background

The American College of Medical Genetics and Genomics (ACMG) has published standards for the interpretation of sequence variants, while cautioning that more evidence should be required to call pathogenic a variant identified in a healthy individual than a variant identified in an affected individual[1]. Here we present the variant classification criteria used at Counsyl for expanded carrier screening and compare the classifications of 3,303 variants to those in ClinVar[2].

Classification criteria in carrier screening

Counsyl's major classification criteria for variants detected in carrier screening of recessive conditions are listed below in abbreviated form. For the detailed classification rules see:

http://www.ncbi.nlm.nih.gov/clinvar/submitters/320494/.

These rules are adapted from the ACMG variant classification guidelines[1] for the purpose of testing a presumptively healthy population.

Known/likely/predicted deleterious

- Statistically significant association or segregation with disease
- Experimental evidence supports loss of function
- Loss of function variant

Variant of uncertain significance (VUS)

- Insufficient or conflicting evidence

Known/likely/predicted benign

- Population frequency is higher than expected given the disease incidence
- Silent or intronic variant with additional evidence for benign

Comparison to ClinVar classifications

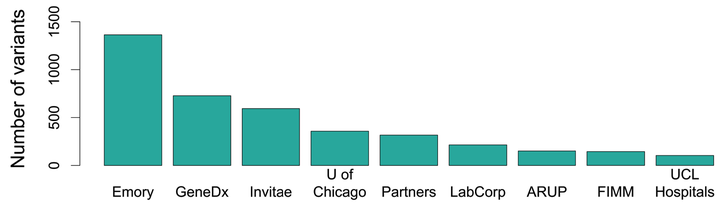
1. Dataset

Counsyl carrier screening and ClinVar variant classifications[2] were obtained from April 2016 releases. Of the 73,616 variants classified at Counsyl 5,442 had a ClinVar entry.
We excluded:

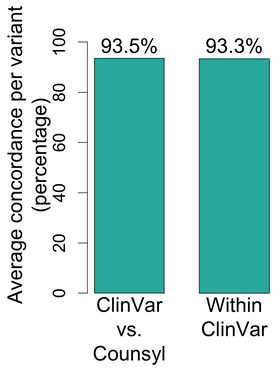
- Counsyl's own classifications deposited in ClinVar
- *ATM* and *NBN* classifications for hereditary cancer susceptibility
- "Other/not provided" classifications in ClinVar
- Non-laboratory submissions (e.g. OMIM)
- Clinical labs with less than 100 of the variants classified

In total, the classifications of 3,303 unique variants in 100 genes were compared with 3,967 ClinVar classifications submitted by one of 9 clinical labs.

Number of variants included in the comparison by ClinVar submitter



2. Concordance within ClinVar and ClinVar concordance to Counsyl is equally high

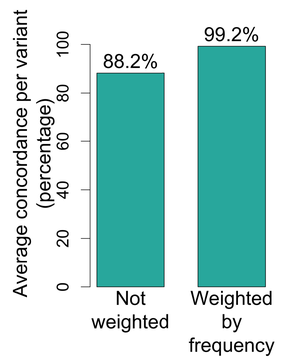


Variants were binned into two simplified categories according to medical management in carrier screening:

Classification	Simplified to classification
known/likely/predicted deleterious	pathogenic
VUS, known/likely/predicted benign	VUS/benign

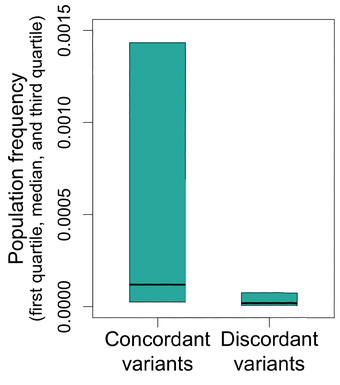
Concordance to Counsyl and among ClinVar submitters is similar: 93.5% vs. 93.3%, n=545 variants with ≥2 ClinVar classifications. For each variant, concordance was calculated as the proportion of concordant classifications among the relevant pairwise comparisons. Concordance per variant is averaged, thereby giving equal weight to each variant.

3. Discordant variants are more rare than concordant variants



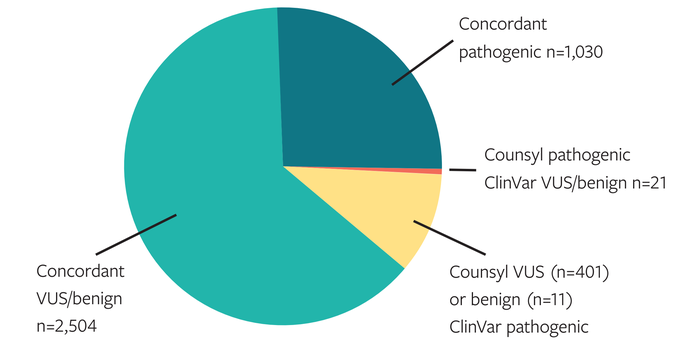
Concordance to Counsyl weighted by population allele frequency is 99.2%, while the unweighted concordance is 88.2% for the full dataset, demonstrating that discordant variants tend to be more rare than concordant variants.

The median population allele frequency of a variant with any discordance is 6.3 fold lower than that of a fully concordant variant ($p<0.0001$).

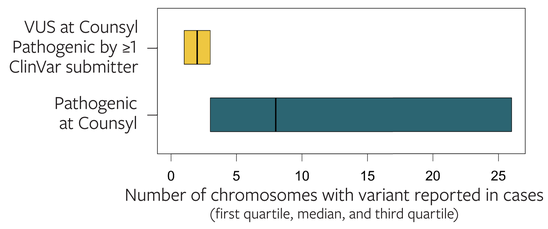


4. Pairwise comparison of Counsyl to ClinVar

Among the 3,967 pairwise comparisons to Counsyl, most discordances with Counsyl were variants classified as VUS at Counsyl and as pathogenic in a ClinVar submission, consistent with the need in carrier screening to favor specificity over sensitivity. At Counsyl, a novel discrepancy will trigger the review of the variant.



By classification design, VUSs have less known evidence of pathogenicity than do pathogenic variants. We illustrate this for one of the lines of evidence in the classification: the number of cases with disease and the variant published.



Discordant VUSs (VUS at Counsyl/pathogenic in ClinVar) (n=312) tend to have fewer affected cases described in the literature than do pathogenic variants (n=727) ($p<0.0001$, variants with a case count available from the manual curation pipeline and an available population frequency). This remains true of the number of chromosomes normalized by population frequency to avoid confounding by allele frequency.

Conclusions

- Overall concordance with ClinVar for carrier screening genes is high, concordance to Counsyl and among ClinVar submitters is similar, and discordant variants tend to be rare.
- The direction of the discordance to Counsyl is consistent with the need in population screening to favor specificity (low false positive rate) over sensitivity (low false negative rate). Other factors explaining classification discordance include labs internal data, out-of-date classifications, and differences in the interpretation of classification guidelines.
- ClinVar is a valuable resource to compare classifications from independent labs. While at Counsyl we do not make classification decisions based on ClinVar, ClinVar can be used as a quality control tool whereby a novel ClinVar discrepancy triggers variant review.

REFERENCES: **1.** Richards et al, Genetics in Medicine. 2015. May;17(5):405-24. PMID: 25741868 **2.** http://www.ncbi.nlm.nih.gov/clinvar/

View all posters and research at
research.counsyl.com