What's in a VUS rate? Simulated VUS calculations for hereditary cancer genes in a general population using population frequency data and ClinVar submissions

K. Eerik Kaseniit MEng, Megan Judkins MS CGC, Rebecca Mar-Heyming PhD, H. Peter Kang MD, Eric A. Evans PhD, Kaylene Ready MS CGC, Dale Muzzey PhD

All authors are employees of Counsyl Inc., South San Francisco, California

The rate of reports with variants of uncertain significance ("VUS rate") can be different across laboratories offering inherited cancer screening even if they have the same classifications for all variants.

Introduction

A common metric by which inherited-cancer tests are compared is the likelihood of a patient receiving a report with a variant of uncertain significance ("VUS"). This "reported-VUS likelihood", "VUS report rate", or "VUS rate" metric has questionable value, however, because multiple factors influence it: the number of genes tested, the particular assayed regions (e.g., exons, introns), the allele frequency threshold for benign variants, and the patient ethnicity. By permuting these various determinants for panels of hereditary cancer genes that have specific medical management guidelines, we demonstrate that the VUS report rate value can vary greatly.

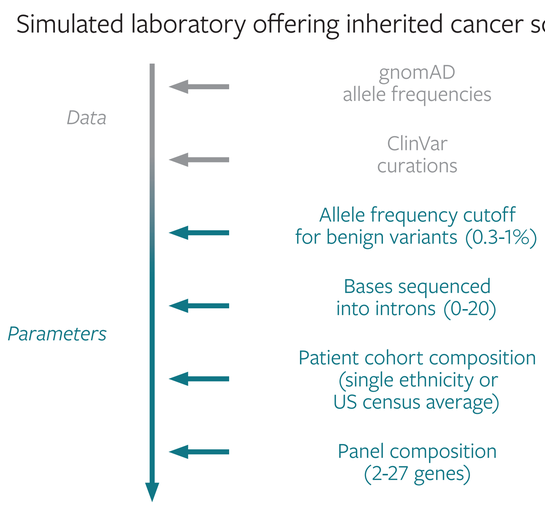


Figure 1: Methods overview. Hundreds of thousands of patient reports from a hypothetical laboratory were simulated based on indicated data and parameters.

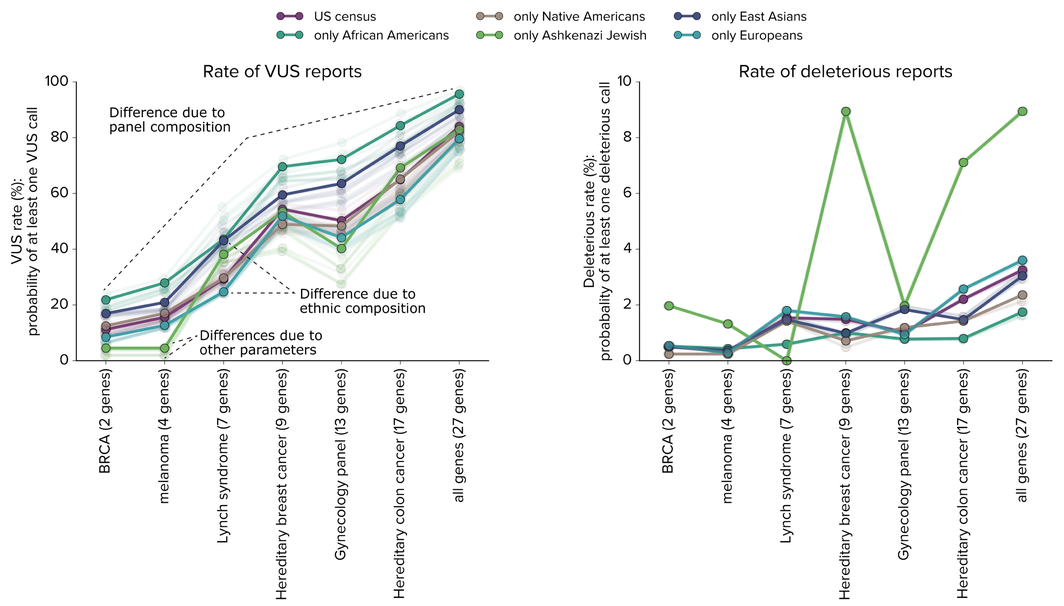
Methods

We simulated hypothetical laboratories offering inherited cancer screening by permuting four parameters that could easily differ among commercial laboratories. The first parameter was the stringency in variant interpretation: we used weighted March 2017 ClinVar classifications and applied different frequency cutoffs (0.3-1%) for designating a high-frequency variant—not already classified in ClinVar—as benign. Second, to represent the impact of ostensibly subtle differences in the parts of genes covered by a particular laboratory (its "region-of-interest"), we varied the number of bases sequenced into intronic regions (0-20). Third, to evaluate the impact of ethnicity, we considered a US-population weighted patient cohort, as well as cohorts comprised of each gnomAD ethnicity individually. Finally, different panels of genes were analyzed. All simulations modeled patients' variants using the ethnic-specific gnomAD allele frequencies. Variants not in ClinVar were designated as VUS, unless determined benign based on allele frequency. Family history and variants not observed in gnomAD were not modeled. The presence of pathogenic variants was not considered in the VUS rate calculation.

Results

Generally, larger panels showed higher VUS and deleterious rates than smaller panels (**Fig 2**). For a 27-gene panel and a general population, the VUS rate values differed by up to **7.6 percentage points** based on the other parameters (**Fig 3**). Decreasing the panel size tended to restrict the range: a 6.1 percentage point spread for a hereditary breast cancer panel with nine genes, and a 3.4% spread for only BRCA1/2 (**Fig 3**). With a non-stringent benign allele frequency cutoff of >0.3%, the VUS rate differed by up to **2.8 percentage points** for a 27-gene panel, depending on whether the 20 intronic bases flanking exons were part of the assay (**Fig 3**). When stratified by ethnicity, the VUS rate for an African/African American cohort in one scenario was **11.6 percentage points higher**† than the US-population-weighted rate, while for an European cohort the rate was **4.4 percentage points lower**‡ (**Fig 4**). The largest difference for the 27-gene panel across all parameters was 28 percentage points (**Fig 2**).

View all posters and research at
research.counsyl.com



BRCA: *BRCA1, BRCA2*; **Melanoma:** *BRCA2, CDK4, CDKN2A, TP53*; **Lynch syndrome:** *APC, EPCAM, MLH1, MSH2, MSH6, MUTYH, PMS2*; **Hereditary breast cancer:** *ATM, BRCA1, BRCA2, CDH1, CHEK2, PALB2, PTEN, STK11, TP53*; **Gynecology panel:** *BRCA1, BRCA2, BRIP1, EPCAM, MLH1, MSH2, MSH6, PMS2, PTEN, RAD51C, RAD51D, STK11, TP53*; **Hereditary colon cancer:** *APC, BMPR1A, CDH1, CHEK2, EPCAM, GREM1, MLH1, MSH2, MSH6, MUTYH, PMS2, POLD1, POLE, PTEN, SMAD4, STK11, TP51.*

Figure 2: The VUS and deleterious rates as a function of the gene panel and patient cohort compositions. The solid lines are for a 1% allele frequency cutoff for benign variants and 3 bases sequenced into introns; other lines show variations of these parameters.

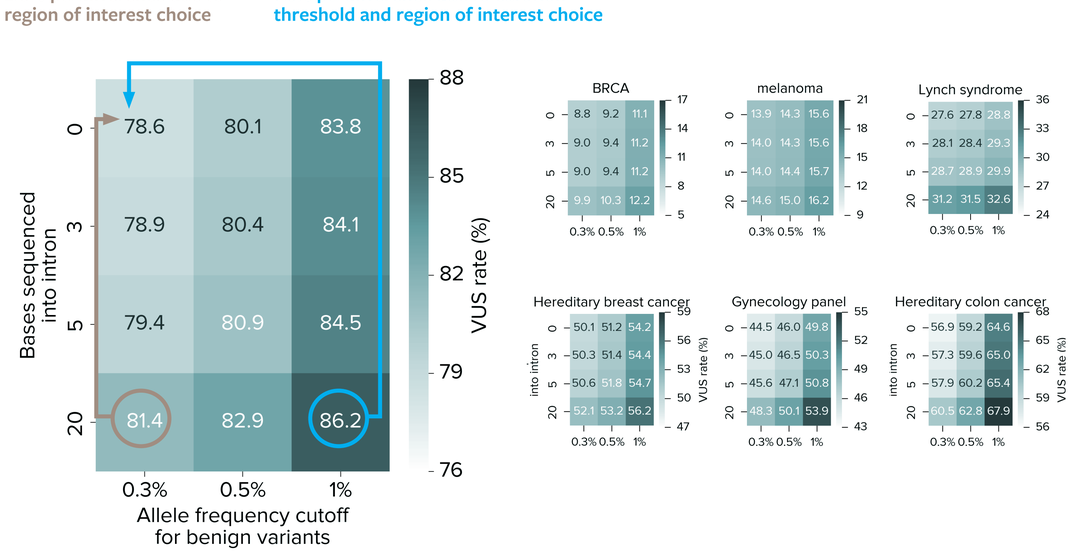


Figure 3: The VUS rate as a function of the bases sequenced into introns (region of interest choice) and the allele frequency cutoff for benign variants (curation threshold). Patient cohort composition (US census) was held fixed. Each colorbar shows a range of 12 percentage points. Left plot: all genes.

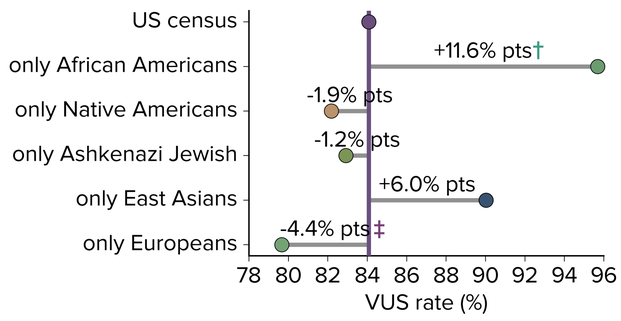


Figure 4: The VUS rate as a function of the patient cohort composition. Panel composition (27-gene panel), allele frequency cutoff (1%), and intronic bases sequenced (3) were held fixed. Real labs might have ethnic compositions different from the US census, e.g. having more European patients.

Discussion

Our simulations do not reflect any true diagnostic laboratory, yet they show that there can be a wide range of VUS report rates based on the type of test being ordered, the benign allele frequency threshold, the assay design, and the ethnic mix of the patient cohort. Our results indicate that the VUS rate in isolation is not a reliable measurement of quality, suggesting that multiple criteria should be considered when evaluating which genetic tests to offer patients.