Reducing VUS rates among ethnic groups: Sequencing smarter not harder

Shan Yang, Yuya Kobayashi, Edward Esplin, Tom Callis, Robert L. Nussbaum

Invitae, San Francisco, CA

BACKGROUND

Advances in DNA sequencing have led to genetic testing of many patients with large gene panels. However, it has been reported that the rates of detecting a variant of uncertain significance (VUS) are lower in White/Europeans than in other ethnic groups. Two possible explanations have been proposed for this phenomenon.

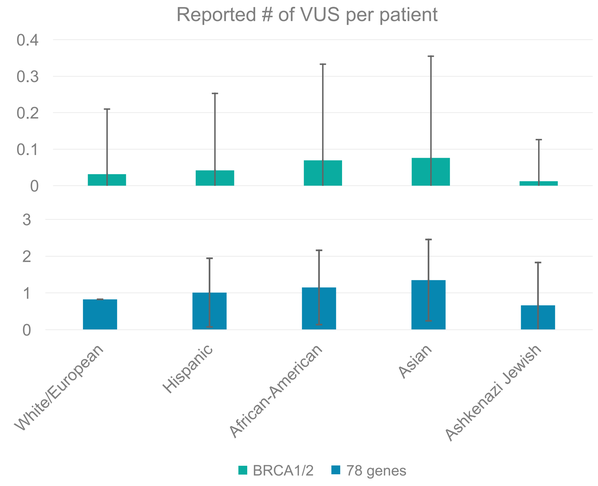
- Numerous studies based on White/European cohorts mean that we have more information about this population's variants.
- Non-White/European populations may carry more variants relative to the human reference genome.

To better understand this issue, we studied rare missense variants in *BRCA*1 and *BRCA2*, the most extensively studied hereditary cancer genes, and 78 other hereditary cancer genes.

METHODS

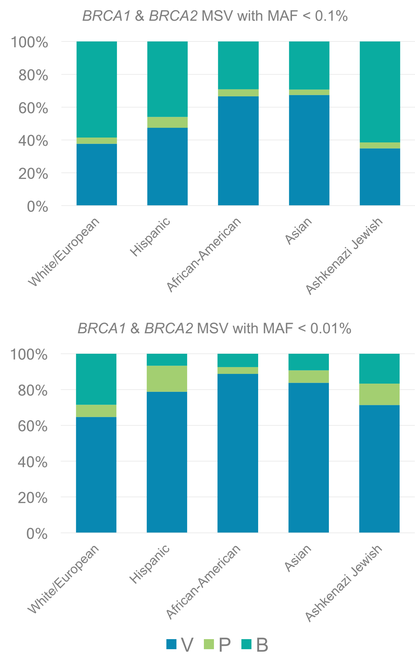
- We identified 123,251 patients for whom *BRCA1* and *BRCA2*, with (13,041) or without (110,210) an additional 78 hereditary cancer genes, were requisitioned at Invitae in 2016 and 2017. Self-reported ethnicity and identified variants were retrieved.
- For each missense variant identified in these patients, the classification and maximal minor allele frequency (MAF) among the 7 populations from gnomAD were recorded.
- MAF <0.1% and MAF <0.01% were used as cutoffs for considering a variant as rare or very rare.
- Differences in VUS rates among ethnic groups were assessed for significance by the chi-square test.

VUS per patient rates are low but differ among ethnic groups



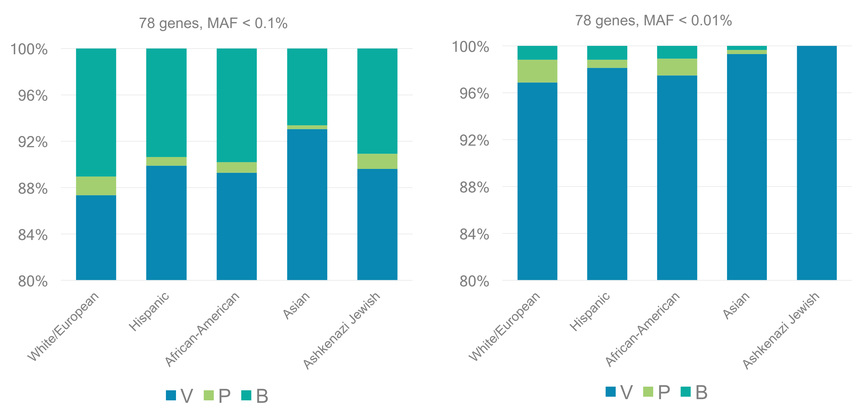
- The total number of VUS were reported per patient in *BRCA1* and *BRCA2* is low (0.01-0.08), with the lowest in Ashkenazi Jewish (AJ) patients then in White/Europeans and Hispanics, and the highest rate in AA and Asians.
- When the other 78 genes were considered, the average VUS rates were higher (0.7-1.3) with the same ethnicity-dependent trends observed.

Rare missense variants in *BRCA*1 & *BRCA2*: Significantly fewer VUS in White/European and AJ but Similar rate of pathogenic variants among groups



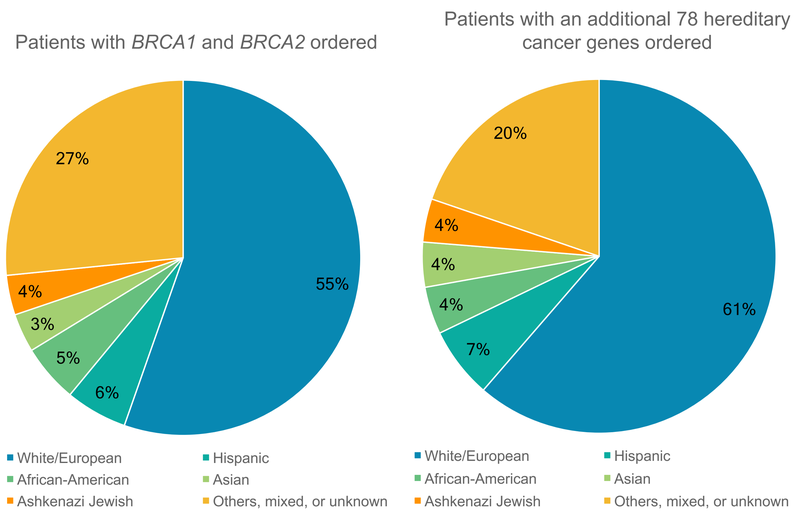
- Variants with high MAF or truncating variants are relatively easy to interpret as benign or pathogenic, respectively. We focused our analysis on rare (MAF<0.1%) and very rare (MAF<0.01%) missense variants (MSV) and calculated the frequency of variants of different interpretations over the total number of variants to remove total variant number effect in groups.
- For **rare** MSV in *BRCA1* and *BRCA2* (MAF<0.1%):
  - Frequency of VUS in White/Europeans and AJ was lower (38% and 35%) compared with Hispanic (47%), AA (67%), and Asian (67%) groups (**p<0.00001**).
  - The proportion of pathogenic variants was similar among all groups (**p=0.02**).
- For **very rare** MSV in *BRCA1* and *BRCA2* (MAF<0.01%):
  - White/Europeans had a significantly lower frequency of VUS (65%), and AJ had a relatively lower frequency of VUS (71%) compared with other ethnic groups: Hispanic 79%, AA 89%, and Asian 84% (**p<0.00001**).
  - The frequency of pathogenic variants among these groups are also significantly different (**p<0.00001**).

Rare missense variants in 78 hereditary cancer genes other than *BRCA1* & *BRCA2*



- For **rare** MSV (MAF<0.1%) in the other 78 genes:
  - The frequency of VUS was still the lowest for White/Europeans (87%) than other groups: Hispanic 90%, AA 89%, Asian 93%, and AJ 90%; but to a much lesser extent (**p=0.0002**)
- For **vary rare** MSV (MAF<0.01%) in other 78 genes:
  - The frequency of VUS was still the lowest for White/Europeans (87%) than other groups but the difference is not significant (**p=0.06**)
- We looked into some rare and very rare benign variants and found that in the White/European population, a small number of rare but recurring benign variants contributed greatly to lowering the frequency of VUSs. These variants were sufficiently rare in population databases to make it impossible to classify them as benign based on MAF alone, thereby resulting in what would ordinarily be a VUS classification; however, published experimental and/or clinical studies provided the additional evidence necessary for a benign classification.

Ethnic groups reported in patients



CONCLUSIONS

- Compared with other ethnicities, White/European or AJ patients have a similar proportion of rare pathogenic missense variants in hereditary cancer syndrome genes, but fewer VUS per patient. This pattern was observed in both well-studied genes (*BRCA1* and *BRCA2*) and in 78 other hereditary cancer syndrome genes. These ethnic differences in the number of VUS per patient were primarily the result of a few rare but recurring variants in White/European and AJ populations for which published studies made a benign interpretation possible.
- Large population databases, such as gnomAD, now provide us with allele frequencies of variants in previously undersequenced populations. In order to reduce VUS rates in non-White/European individuals, we recommend targeted functional and segregation analysis of rare but recurrent variants in these populations. We believe this strategy will be more effective than simply sequencing additional unselected individuals from these populations.

Disclosures: Authors are stockholders and employees of Invitae.