Response to Shah *et al*: Using high-resolution variant frequencies empowers clinical genome interpretation and enables investigation of genetic architecture

Nicola Whiffin,[1,2,3] Angharad M Roberts,[1,2] Eric Minikel,[4,5] Zach Zappala,[4,5] Roddy Walsh,[1,2] Anne H O'Donnell-Luria,[4,5] Konrad J Karczewski,[4,5] Steven M Harrison,[6,7] Kate L Thomson,[8,9] Helen Sage,[8] Alexander Y Ing,[6,7] Paul J R Barton,[1,2] Birgit Funke,[6,7] Stuart A Cook,[1,2,10] Daniel G MacArthur,[4,5,11] and James S Ware[1,2,3,5]*

[1]Cardiovascular Genetics and Genomics, National Heart and Lung Institute, Imperial College Centre for Translational and Experimental Medicine, London, W12 0NN, UK
[2]NIHR Royal Brompton Cardiovascular Research Centre, Royal Brompton & Harefield NHS Foundation Trust, London, SW7 2AZ, UK
[3]MRC London Institute of Medical Sciences, Imperial College London, London, W12 0NN, UK
[4]Analytic & Translational Genetics Unit, Massachusetts General Hospital, Boston, MA 02114, USA
[5]Program in Medical and Population Genetics, Broad Institute of MIT & Harvard, Cambridge, MA 02142, USA
[6]Laboratory for Molecular Medicine, Partners HealthCare Personalized Medicine, Cambridge, MA 021319, USA
[7]Department of Pathology, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114, USA
[8]Oxford Medical Genetics Laboratory, Oxford University Hospitals NHS Foundation Trust, Oxford, OX3 7LJ, UK
[9]Division of Cardiovascular Medicine, Radcliffe Department of Medicine, University of Oxford, Oxford,OX3 9DU, UK
[10]National Heart Centre Singapore, Singapore, 169609
[11]Department of Medicine, Harvard Medical School, Boston, MA 02115, USA

*Correspondence should be addressed to Dr James Ware: j.ware@imperial.ac.uk

Main text

To the Editor: Recent work by Shah and colleagues[1] demonstrated that many variants in the ClinVar database[2] are misclassified, and that disease-specific allele frequency (AF) thresholds can identify wrongly classified alleles by flagging variants that are too prevalent in the population to be causative of rare penetrant disease. While we agree with the main conclusions of this work, the authors compare their AF filtering approach to our recently published method[3], concluding that the method we advanced "may be prone to removing potentially pathogenic variants". This is incorrect. Here we demonstrate that our approach is robust, and further illustrate the power of disease-specific AF thresholds for investigating the genetic architecture of disease.

Both methods compare the population frequency of a variant with the prevalence of a disease. Whilst this simple approach might be sufficient in some circumstances, such as when considering a fully penetrant disease with only a handful of causative variants, we advocate considering a fuller definition of disease architecture that explicitly incorporates penetrance and genetic heterogeneity. Using these parameters, we define the maximum AF at which a variant can be observed in the general population to be a credible candidate to cause a defined disease, under the specified genetic architecture. Importantly, we consider each ethnic sub-population separately, and account for sampling variance in reference datasets[4]. We previously demonstrated that our framework markedly improves signal:noise for identification of penetrant Mendelian variants, without loss of sensitivity[3].

It is worth restating some important caveats of filtering variants using AF. We must be vigilant for population-specific founder variants, especially in populations where the disease architecture may be distinct, such as the Finnish and Ashkenazi Jewish populations in the Genome

Aggregation Database (gnomAD). Also, we do not filter variants observed as singletons due to the stochastic nature of population sampling.

Shah *et al.* report that, in their hands, our method inappropriately filters 15 “high confidence” Pathogenic/Likely Pathogenic ClinVar variants across five cardiac phenotypes (dilated cardiomyopathy [PS115200, MIM 115200], hypertrophic cardiomyopathy [PS192600, MIM 192600], arrhythmogenic right ventricular cardiomyopathy [PS107970, MIM 107970], long QT syndrome (LQT [PS192500, MIM 192500]), and Brugada syndrome [MIM 601144]). The work of Shah *et al.* cannot be directly replicated as their methods and reference dataset are not fully available. Therefore, we have assessed these 15 variants using the larger and more comprehensive gnomAD dataset. We calculated a maximum credible population AF for a variant causative of each disease (defined as in Table 1 of Whiffin *et al.* - see Table S1) given a minimum penetrance of 50%.

With proper application of our approach in this reference population, four (of 15) variants flagged by Shah *et al.* are not filtered (Table 1). We curated the remaining 11 variants according to contemporary ACMG/AMP guidelines[5] using cardioclassifier.org[6], ClinVar, and the published literature. Five did not reach a Pathogenic/Likely Pathogenic classification (Table 1; Table S3).

The remaining six variants did have sufficient evidence to be classified as (Likely) Pathogenic. For four of the variants, where ethnicity-specific case AFs were available, we estimated their penetrance by comparing the case AFs to gnomAD, as previously described[7]. The penetrance of these variants ranged from 1.1% to 12% (Table 1). Crucially, the upper confidence intervals of all six penetrance estimates are well below the pre-specified 50% threshold. In other words, our approach appropriately filters these variants as incompatible with the specified genetic architecture.

We extended our analysis to evaluate all Pathogenic/Likely Pathogenic ClinVar variants for these five cardiac phenotypes. Starting with the same data (clinvar_20170905.vcf.gz), we annotated variants reported to cause the specified diseases with the tiering strategy outlined by Shah *et al.*[1] To identify variants above the maximum credible AF for each disease, we used the highest filtering allele frequency across all gnomAD populations ("popmax") for each variant represented, as described previously[3]. These data, and the code to reproduce this analysis, are available for download (see Web Resources).

47 additional variants, previously reported as Pathogenic/Likely Pathogenic, were flagged as "insufficiently rare" by this analysis. We reassessed the clinical interpretation using contemporary ACMG/AMP[5] guidelines. 45/47 (95.7%) were classified as VUS (Table 2; Table S2), and two were classified as (Likely) Pathogenic, but with low penetrance, clearly below our defined genetic architecture (Table 1).

Across both analyses, we identified eight (Likely) Pathogenic, low-penetrance variants (Table 2), two of which recapitulate a known mechanism of low-penetrance. These variants are reported as disease-causing for Jervell Lange-Nielsen syndrome (LQT & deafness [PS220400, MIM 220400]) in biallelic states, but have low-penetrance for dominant LQT in heterozygous relatives[8]. As these examples demonstrate, our AF filtering framework effectively discriminates alleles with lower penetrance that require tailored counselling.

Within the ACMG/AMP framework, frequency evidence favouring a benign interpretation (BS1) is not sufficient for a (Likely) Benign classification in the absence of further supporting evidence. Similarly, activating BS1 does not preclude a (Likely) Pathogenic classification overall. For example, a common low-penetrance variant may be seen recurrently in cases, showing statistical enrichment in cases over controls (PS4). This contradictory evidence should trigger closer inspection and lead to consideration of a low-penetrance architecture. In other contexts, a more conservative low-penetrance architecture may be specified from the outset.

It is worth highlighting a key limitation of the ACMG/AMP variant interpretation guidelines[5]: that they were not intended to be applied to variants of very low penetrance. However, with no clear guidance on where to "draw the line", the terminology that evolved for highly penetrant disorders is commonly also applied to low penetrance alleles, where it may be more appropriate to describe variants as "risk factors" rather than "pathogenic".

In conclusion, we previously introduced a statistically-robust, disease-specific framework to leverage reference population AF for variant assessment. We show that this method does not remove true pathogenic variants, provided that they fall within the pre-defined genetic architecture. Although a specified architecture may lead to some low-penetrance variants being flagged with evidence in favour of benign status (BS1), this does not prevent them achieving an actionable ACMG/AMP classification in combination with other lines of evidence. Indeed, flagging this group of variants for in depth review enables more nuanced reporting and counselling around low-penetrance variation.

Supplemental Data description

Supplementary Data include three tables.

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Declaration of Interests

The authors declare no competing interests.

Web Resources

Github repository: https://github.com/ImperialCardioGenetics/ResponseToShahEtAl

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Tables

Table 1: Curations and penetrance estimates of 15 variants flagged by Shah *et al*. and 2 additional candidate low-penetrance variants identified in this analysis. Full details can be found in Table S3. In the Shah et al analysis 15 variants with "high-confidence" pathogenic assertions in ClinVar were reported to exceed the maximum credible population allele frequency for a pathogenic variant defined by our framework. In our reanalysis, four of these are not filtered by our recommended application of this approach (using gnomAD reference populations), five do not have sufficient evidence for a pathogenic assertion, and six are likely pathogenic but with low penetrance.

Phenotype	Gene	cDNA	Penetrance	ACMG evidence	ACMG class	conclusion
HCM	*MYBPC3*	c.3330+5G>C	12.0% (3.0-45.0%)	PVS1, PS4, PP1_strong	Pathogenic	Likely pathogenic but with low penetrance
LQTS	*KCNQ1*	c.1588C>T	2.5% (0.64-9.8%)	PVS1, PS4_moderate	Likely Pathogenic	Likely pathogenic but with low penetrance
LQTS	*KCNQ1*	c.1781G>A	4.1% (1.0-16%)	PS4, PM1, PP1, PS3_supporting	Likely Pathogenic	Likely pathogenic but with low penetrance
Brugada	*SCN5A*	c.3956G>T	1.1% (0.010-12%)	PS3, PP2, PP3, PP1	Likely Pathogenic	Likely pathogenic but with low penetrance
Brugada	*SCN5A*	c.5129C>T	Ethnicity matched case data not available	PS3, PM1, PP3	Likely Pathogenic	Likely pathogenic but with low penetrance
Brugada/ LQTS	*SCN5A*	c.1099C>T	Ethnicity matched case data not available	PS3, PM1, PP3	Likely Pathogenic	Likely pathogenic but with low penetrance
LQTS	*SCN5A*	c.4877G>A	-	PS3_moderate, PP2, PP3	VUS	Insufficient evidence for a pathogenic assertion
LQTS	*KCNQ1*	c.364dupT	-	PVS1	VUS	Insufficient evidence for a pathogenic assertion
HCM	*MYL3*	c.170C>G	-	PS3_moderate, PP1_moderate, PP2	VUS	Insufficient evidence for a pathogenic assertion
LQTS	*SCN5A*	c.5872C>T	-	PVS1	VUS	Insufficient evidence for a pathogenic assertion
LQTS	*KCNQ1*	c.1085A>G	-	PM1, PP3	VUS	Insufficient evidence for a pathogenic assertion
DCM	*LMNA*	c.961C>T	-	PVS1, PP1_Strong, PM2, PS4_Moderate, PS3_Moderate	Pathogenic	Not filtered using gnomAD
Brugada	*SCN5A*	c.4885C>T	-	PVS1, PS3_moderate	Likely Pathogenic	Not filtered using gnomAD
LQTS	*KCNQ1*	c.573_577delGCGCT	-	PVS1, PP1_supporting, PS3_supporting	Likely Pathogenic	Not filtered using gnomAD
HCM	*MYBPC3*	c.3181C>T	-	PVS1, PP1_strong	Pathogenic	Not filtered using gnomAD
LQTS	*KCNE1*	c.226G>A	1.7% (0.58-5.2%)	PS4, PS3	Pathogenic	Likely pathogenic but with low penetrance[a]
LQTS	*KCNQ1*	c.1664G>A	1.6% (0.22-12%)	PM5, PM1, PS3_moderate, PP3	Likely Pathogenic	Likely pathogenic but with low penetrance[a]

[a]Variants identified by wider gnomAD analysis

Table 2: The final classifications of 15 variants flagged by Shah et al. and 47 additional variants identified in this analysis.

Variant class	Total variants	Insufficient evidence for a pathogenic assertion	Likely pathogenic but with low penetrance	Not filtered using gnomAD[a]
Flagged by Shah et al.	15	5 (33.3%)	6 (40.0%)	4 (26.7%)
Other set 1	10	8 (80.0%)	2 (20.0%)	-
Set 2	26	26 (100.0%)	0 (0.0%)	-
Set 3	11	11 (100.0%)	0 (0.0%)	-
	62	**50 (80.6%)**	**8 (12.9%)**	**4 (6.5%)**

[a]Either there is additional data in the unpublished cohort used by Shah et al that was not obtainable for this work, or the framework was applied incorrectly, for example by filtering singleton variants or known founder alleles