The challenges of variant classification – a snapshot of reality through proficiency testing

PB1264

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Introduction

Clinical genomic testing requires the classification of the pathogenicity of sequence variants, and the assessment of their clinical impact. As more guidance is generated to aid this work, there is a challenge to standardise approaches across variant types and in different clinical settings.

GenQA, the only end-to-end External Quality Assessment (EQA)/Proficiency Testing provider in genomics, has globally delivered laboratory proficiency testing (PT)/external quality assessments (EQAs) for variant interpretation since 2013. The ISO15189[1] standard requires laboratories to demonstrate the competency of individuals to perform specific tasks. Therefore, GenQA has adapted this approach to provide a mechanism for **individuals to demonstrate their competency**, and to support the education of the scientific and clinical workforce through the **GenQA Genomic Individual Education (GENie) platform**.

Methods

- Modules for classification of single nucleotide variants (SNVs) and copy number variants (CNVs) were provided online through the Genomics Individual Education (GENie) platform for a period of six weeks.
- SNVs were classified according to the ACMG[2] and ACGS[3] guidelines by a panel of expert advisors during February and March 2022.
- CNVs were classified according to the ACMG/ClinGen guidelines[4] by a panel of expert advisors during the period January 2022 to April 2023.
- Individual participants were provided with details of the three variants and the clinical setting and were expected to classify them along with documenting the evidence used to obtain the classification.
- The variants were randomised for classification each time a participant accessed GENie. These were generated from a bank of variants (25 SNVs and 21 CNVs) and included prenatal, postnatal, diagnostic, and predictive clinical scenarios.
- Real time assessment was applied through the platform and participants received a summary of their performance and the expected classification detailing the evidence applied.

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Participation

Over six weeks, 295 individuals completed the SNV module and 334 individuals completed the CNV module. Figure 1 summarises the number of times participants completed a set of variant classifications. Figure 2 shows the number of sets of variants participants completed. The majority completed one set for both the SNV and CNV modules.

Figure 1 – Number of times participants completed a set of variants

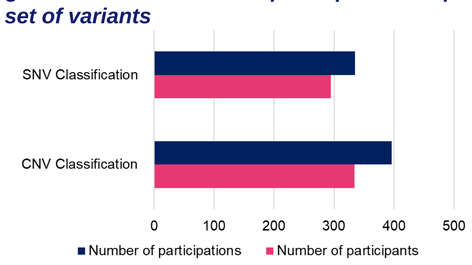
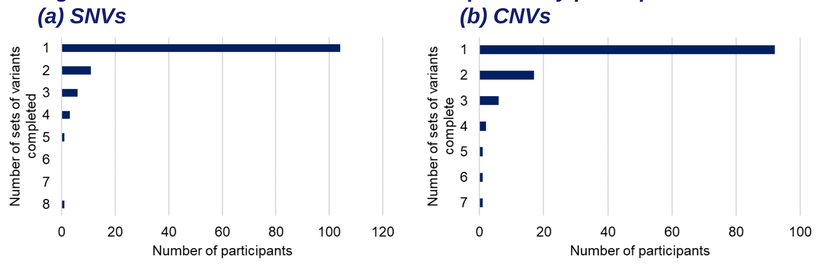


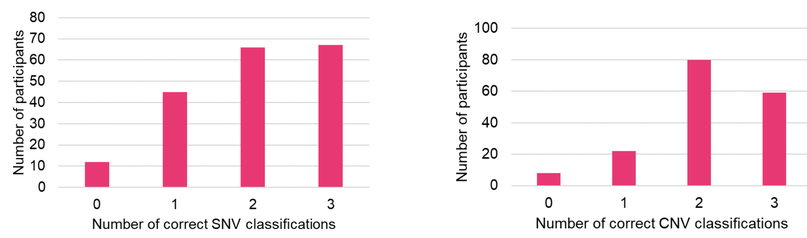
Figure 2 – Number of sets of variants completed by participants



Performance

The majority of individuals correctly classified 2 or 3 out of the 3 variants in the SNV module. The majority of participants classified only 2 out of the 3 CNVs (Figure 3).

Figure 3 – Number of correct classifications submitted by participants

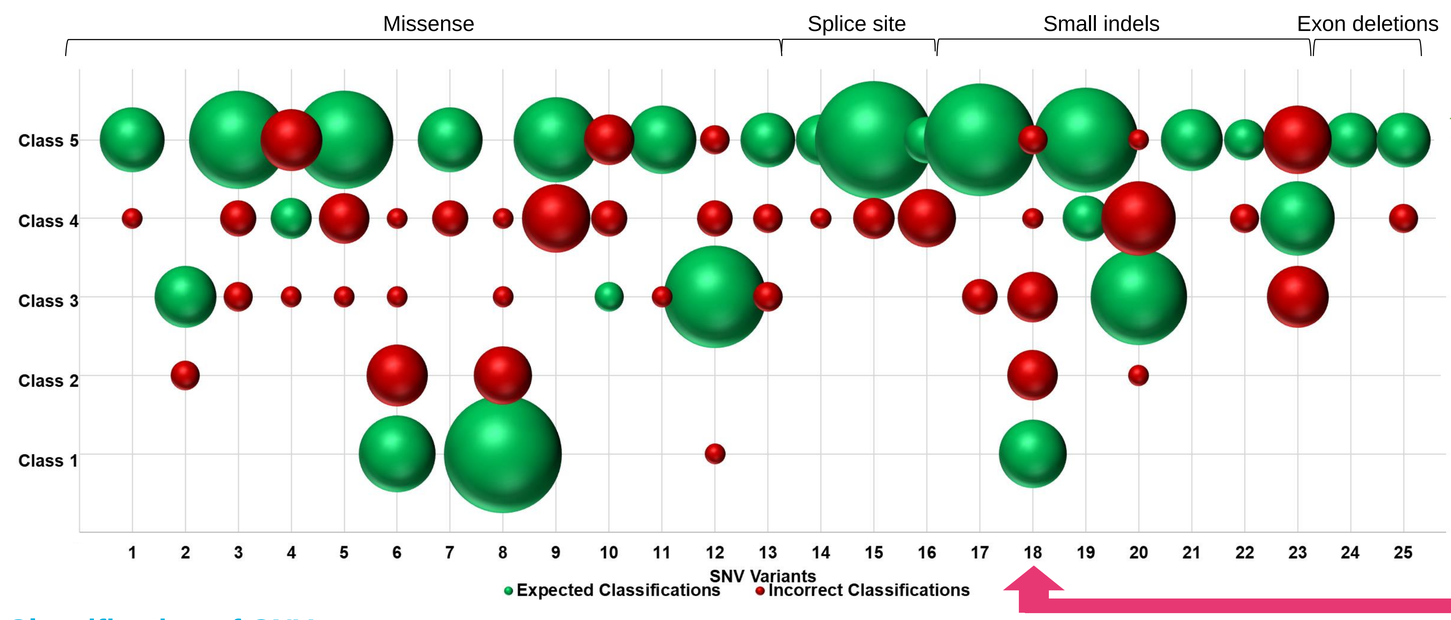


Results

Classification of SNVs

The classification submitted by the participants for each SNV is represented by bubbles (Figure 4), the size of which corresponds to the percentage of participants who reported the expected classification (green) or incorrect classification (red). The classification of 11 variants (1, 3, 7, 9, 15, 16, 19, 21, 22, 24 and 25) were predominantly aligned with the expected classification. However, a considerable number of variants were classified with a wide range of precited pathogenicity which would change the clinical management of the patient. Variant 18 is described in more detail below and in Table 1.

Figure 4 – Classifications submitted by participants for the SNV module



Classification of SNV 18
BRCA1 NM_007294.4:c.1106_1108del p.(Asp369del)

✓ This SNV was expected to be classified as benign (Class 1) according to the expert review (Table 1) and **42% of participants agreed** with this classification. Also 23% classed it as likely benign (Class 2) which was also accepted.

✓ A further 23% classified it as a VUS (Class 3) citing that the variant had been reported in a number of probands but there was insufficient number of controls to determine the frequency in the population.

✗ **4% incorrectly assigned it as likely pathogenic** (Class 4) By applying PVS1, PS1, PM1 and PP1.

✗ 8% incorrectly classed the SNV as pathogenic (Class 5) by applying PVS1 and PM2.

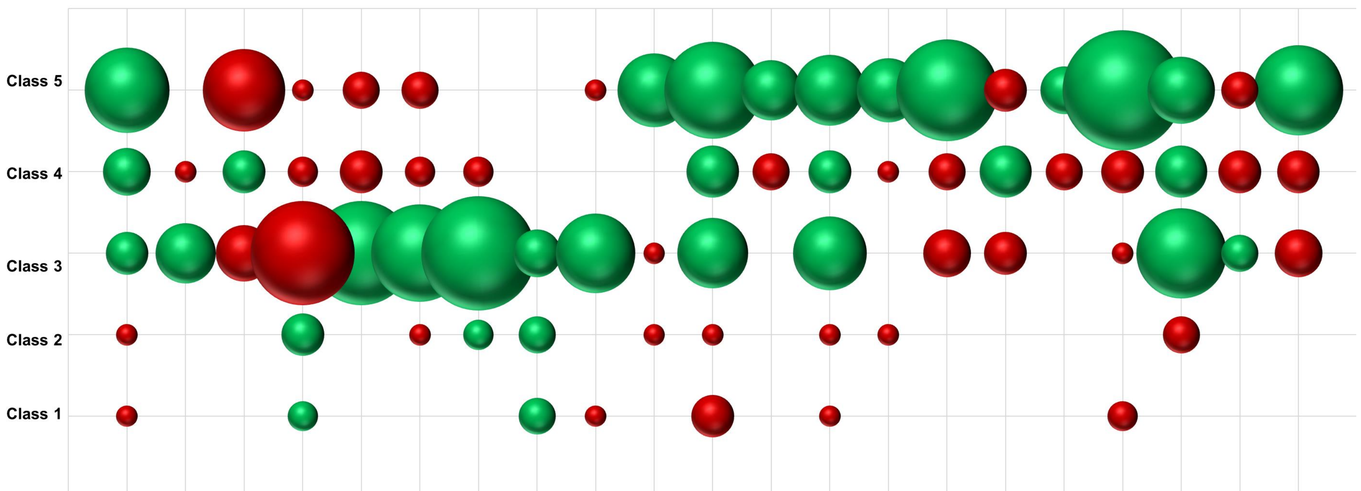
Table 1 – Evidence used by expert panel for SNV18

Criteria	Evidence/justification
BP6_STR	IARC Combined LR[5] (segregation, pathology, co-occurrence, family history) = $1.94x10^{-5}$ Converts to: ACMG evidence points: <-4 = Strong for benignity
BS3_STR	Functional study validated for PS3/BS3 application at a strong level is supportive of the variant having no functional effect (neutral)[6,7]
BP3_STR	In-frame deletion within poorly conserved region; with no predicted splicing impact
Predicted	**Benign SNV**

Classification of CNVs

The classification submitted by the participants for each CNV is represented by bubbles (Figure 5), the size of which corresponds to the percentage of participants who reported the expected classification (green) or incorrect classification (red). The classification of three variants (2, 12 and 17) were predominantly aligned with the expected classification and a considerable number of variants were classified with a wide range of precited pathogenicity, more so than the SNV classification. However, it must be noted that the ACMG/ClinGen guidelines[4] recommend uncoupling the evidence-based variant classification from the clinical significance in the context of an individual patient's diagnosis. This exercise only assessed the classification of the CNV and did not take into account the clinical significance for the proband. Variant 14 is described in more detail below and in Table 2.

Figure 5 – Classifications submitted by participants for the CNV module



Classification of CNV 4
arr[GRCh37] 1q44 (247,815,979_ 248,609,997)x1

✓ This CNV should be classified as likely benign (Class 2), see Table 2, and **12% of participants correctly did so**. A further 6% classed it as benign (Class 1) and this was also accepted.

✗ **The majority (73%) incorrectly classed it as a VUS**, 6% as likely pathogenic and 3% as pathogenic. The evidence submitted was the same as that used by the participants classifying it is benign/likely benign, indicating that the application of the guidelines[4] was variable, not the evidence reviewed.

Table 2 – Evidence used by expert panel for CNV 4

Section	Description	Score applied	Evidence/justification
1	Initial assessment of genomic content	1A: 0	Olfactory receptor gene cluster
2	Overlap with established/predicted haploinsufficiency (HI) or benign genes/genomic regions	-	Not applicable – no overlap with these types of genes/regions
3	Evaluation of gene number	3A: 0	
4	Detailed evaluation of genomic content using cases from published literature, public databases, and/or internal laboratory data	4N (-0.90) or 4O (less than -1)	
5	Evaluation of inheritance pattern/family history for patient being studied	5F: 0	
		Predicted	**Likely benign, Benign CNV**

References: 1. ISO 15189:2012. Medical laboratories – requirements for quality and competence.
2. Richards *et al.,* 2015 PMID: 25741868
3. Ellard *et al*., 2020 (https://www.acgs.uk.com/quality/best-practice-guidelines/)
4. Riggs *et al*., 2020. PMID: 31690835
5. Parsons *et al*., 2019 PMID: 31131967
6. Brnich *et al*., 2019 PMID: 31892348
7. Bouwman *et al*., 2020 PMID: 32546644

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Conflicts of interest: The authors have no conflicts of interest to declare.

Conclusion

There continues to be variability in the classification of SNVS and CNVs. There is merging consensus in the application of the SNV gudielines[2,3] however, this CNV classification assessment has demonstrated the need for further education and standardisation of how guidance is applied[4]. Educational modules such as GENie EQA can deliver assessment for individuals to promote good practice and standardisation.