Standardising the variability of prenatal variant classification

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GenQA Genomics Quality Assessment

Member of UK NEQAS consortium

Introduction

GenQA (previously UK NEQAS for Molecular Genetics and CEQAS), has provided **external quality assessment** (EQA) for genomic testing since 1982. GenQA offers a suite of **online variant classification EQAs** encompassing single nucleotide variants (SNVs) and copy number variants (CNVs) for prenatal, postnatal and somatic referrals.

Expansion of genome-wide testing in prenatal samples requires a consistent approach to the **classification of CNV pathogenicity**. This can assist with accurate predictions of the impact of the CNVs detected and enable informed decision-making during pregnancy and beyond.

GenQA has provided **global laboratory EQAs for prenatal CNV** testing since 2015 and a tailored EQA to aid standardisation of the classification of CNVs in the prenatal setting was introduced in 2021.

Methods

- Two EQAs were offered during 2021 and 2022.
- Online clinical scenarios and results were provided and participants were expected to classify the copy number variants (CNVs) according to the clinical context.
- Participant results were submitted by proforma, detailing classifications with supporting evidence.
- Classifications using either the ACMG/ClinGen[1] guidelines and/or local justifications were permitted.
- Assessment based on correct CNV classification and clinical significance for the pregnancy, suitability of evidence provided and, where appropriate, the reporting decision and/or follow up testing recommended as determined by a panel of assessors.

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Results

Participation and cases

Each of the 2021 and 2022 pilot EQA rounds offered three clinical case scenarios (Table1), with 18 and 34 participating laboratories, respectively. Participants were from a total of 19 countries.

Submissions

Submissions were of a high standard given the challenging cases provided. Overall, there have been two classification errors to date which were considered to be critical (Table 1).

The majority of participants consistently used either their own justification, or the ACMG/ClinGen guidelines[1], to support all submitted classifications.

It was apparent that there was considerable variability in the application of the ACMG/ClinGen guidelines[1], partially explained by the difficulty in applying this framework to prenatal cases with limited phenotypic information.

Figure 1 – Summary of the classifications assigned to each CNV

The larger the bubble, the increased number of participants using that classification.

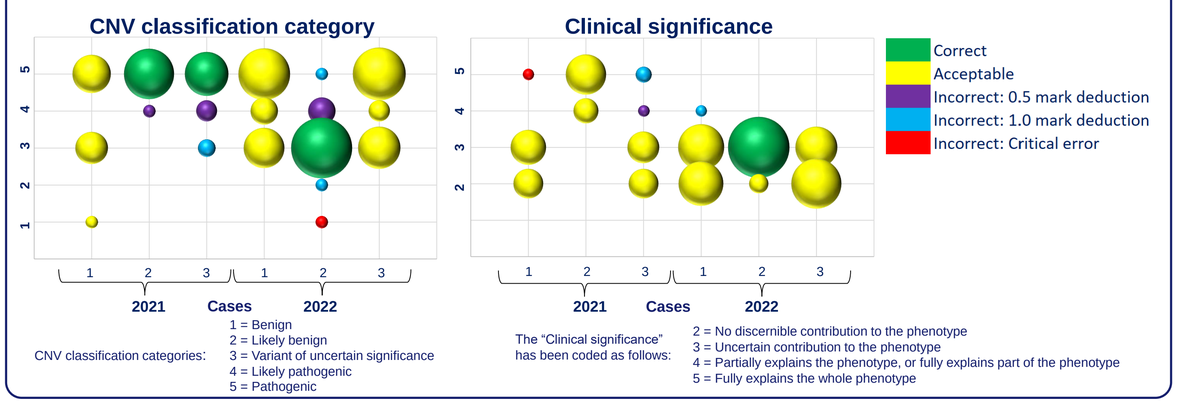

Table 1 – Summary of EQA case scenarios and critical errors

Year	Case	Fetal sex	Referral reason	Gestation	CNV	CNV size	Critical errors
2021	1	Male	IUGR, microcephaly, ventriculomegaly, increased nuchal translucency.	22 weeks	arr[GRCh38] 15q11.2(22693148_23088545)x1 mat	395kb	1
	2	Female	Cleft lip, brain anomalies (?agenesis of corpus callosum), small for dates.	19 weeks	arr[GRCh38] 18q22.1q23(65485559_80255845)x1	14.77Mb	0
	3	Male	Increased nuchal translucency, small for dates.	14 weeks	arr[GRCh38] Xq28(154890368_155331063)x2 mat	440kb	0
2022	1	Female	Increased nuchal translucency 3.9mm	14 weeks	arr[GRCh38] 1q21.1(145635445_146019823)x1 dn	384kb	0
	2	Male	Small/absent cavum septi pellucidi and head circumference on the 3rd centile	22 weeks	arr[GRCh38] 5q22.2(112750708_112780733)x3	30kb	1
	3	Female	Mother carries a balanced translocation t(3;15)(q29;q25)	12 weeks	arr[GRCh38] 17q12(36792631_37854407)x3 dn	1Mb	0

Challenges to accurate and standardised CNV classification

These EQA exercises demonstrate how uncoupling evidence-based classification from potential implications for an individual can assist in standardisation of variant classification. However, there are significant challenges with application of the ACMG/ClinGen guidelines[1] in the prenatal setting, which are highlighted by these EQA scenarios.

Example 1: 2021 Case 3 – Incidental finding unrelated to referral reason

Summary

- **Incidental finding:** This CNV would be likely to cause a postnatal phenotype unrelated to the current reason for referral;
- Errors possibly occurred by a failure to uncouple the CNV classification from the clinical significance:
  - The CNV should be classified as **pathogenic (class 5)**;
  - However, there is currently **uncertain or no discernible contribution to the phenotype**.

Evidence

- This region of Xq28 has a ClinGen triplosensitivity score of 3[2] and is associated with syndromic X-linked intellectual disability and distinctive facies[3];
- The CNV shows high penetrance for a neurodevelopmental phenotype in males[4] but carrier females have a milder phenotype or are clinically unaffected;
- There is no published evidence of a direct link between the CNV and the non-specific fetal anomalies in this case;
- 28% of participants used the ACMG/ClinGen guidelines[1] and all correctly applied 2A ('*overlaps with an established triplosensitivity region*') with a score of 1.

Submissions

- A correct pathogenic CNV classification using evidence-based scoring was given by 72% of participants;
- A correct interpretation of the clinical significance was given by 83.5% of participants;
- All participants correctly stated that they would report this CNV to allow further follow up of the fetus.

Example 2: 2021 Case 1 – Neurosusceptibility locus

Summary

This maternally inherited CNV overlaps the 15q11.2 recurrent region (BP1-BP2), a **susceptibility locus for neurodevelopmental phenotypes[2,5,6,7]**.

Critical error

- One participant submitted 'fully explains the whole phenotype'.

Challenges

- **Limited prenatal case reports** with structural anomalies detected by ultrasound scanning[8,9] but no clear association and the non-specific fetal anomalies in this case cannot be confidently attributed to this CNV;
- **Low penetrance:** The expression of any phenotype associated with this deletion has been estimated to be between 8-10%[2].

Example 3: 2022 Case 2 – Limitations of microarray technique for gains

Summary

Both breakpoints of this gain are within *APC,* a haploinsufficient gene[2] and a high actionability cancer susceptibility gene associated with adenomatous polyposis coli conditions[4].

Critical error

- One participant did not consider the potential **positional effect of the gain** and incorrectly classified the CNV as benign instead of as a variant of uncertain significance.

Challenges

- The positional effect of the gain is uncertain:
  - Is it a **tandem duplication** or an **insertion** elsewhere in the genome?;
  - Gains of the region have been observed in unaffected individuals[10];
  - **The technique used cannot predict whether a truncated APC protein is produced.**
- Further testing (RNA testing or long read sequencing) would be required to resolve the uncertainty.

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Conclusion

Accuracy of variant classification in the prenatal setting is essential to ensure the correct result is reported and appropriate genetic counselling is offered. The EQA submissions demonstrated variability in the use and application of classification guidelines. Further integration of sequence variant[11] and CNV classification frameworks[1] for intragenic variants is required. There is a continued need for laboratory-based EQAs to educate and promote standardisation, and for individual competency assessments to identify training requirements.