Lessons from the 2022 GenQA 'variant validation' external quality assessment for combined reporting of single nucleotide and copy number variants.

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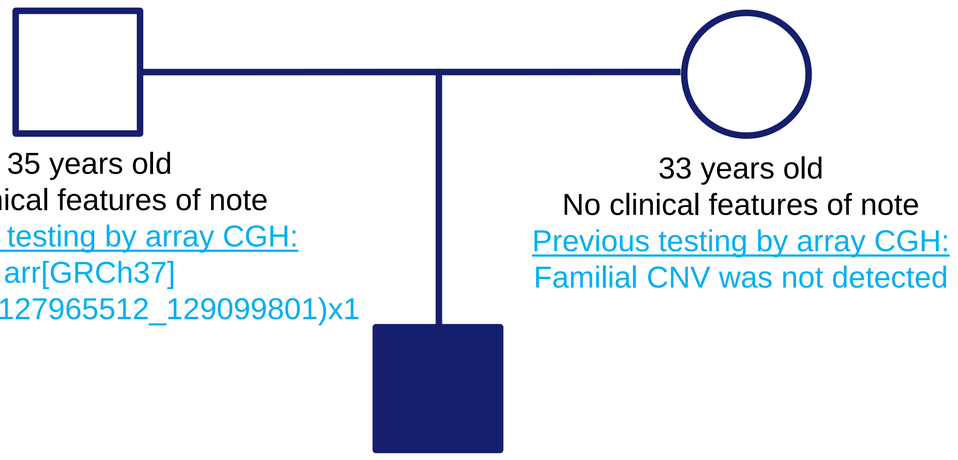
Introduction

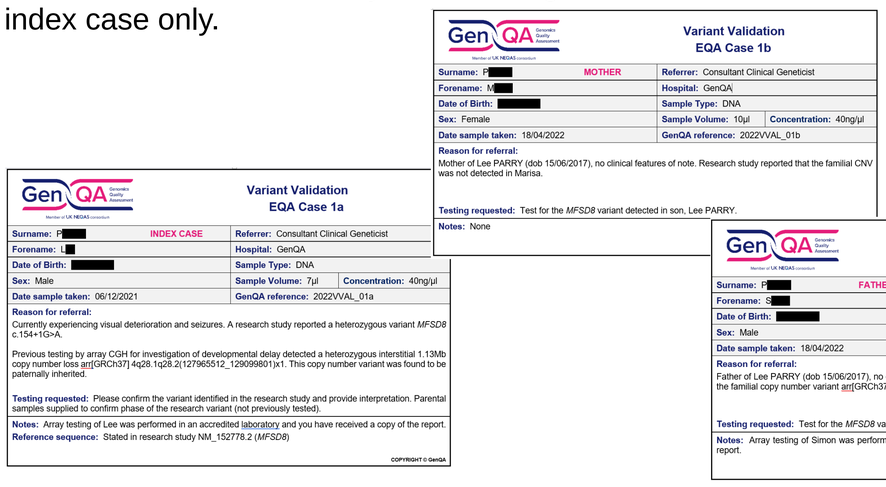
Diagnostic genomic laboratories routinely confirm the results of research testing and whole exome / genome analysis. Genomics Quality Assessment (GenQA) has therefore provided an external quality assessment (EQA) for 'variant validation' since 2019 to assess the quality of testing, interpretation and clinical reporting. From 2019 to 2021 a single nucleotide variant (SNV) had been provided to validate; in 2022 a copy number variant (CNV) was also included to reflect the ongoing consolidation of genomic services.

Figure 1: Summary of information provided to participants



Methods

Participants were provided with DNA samples from a trio (index case and parents) and the results of previous array CGH testing and recent research testing carried out in the index case only.



Variant Validation EQA Case 1a

Surname: P[redacted]	INDEX CASE	**Referrer:** Consultant Clinical Geneticist	
Forename: L[redacted]		**Hospital:** GenQA	
Date of Birth: [redacted]		**Sample Type:** DNA	
Sex: Male		**Sample Volume:** 7µl	**Concentration:** 40ng/µl
Date sample taken: 06/12/2021		**GenQA reference:** 2022VVAL_01a	

Reason for referral:
Currently experiencing visual deterioration and seizures. A research study reported a heterozygous variant *MFSD8* c.154+1G>A.

Previous testing by array CGH for investigation of developmental delay detected a heterozygous interstitial 1.13Mb copy number loss arr[GRCh37] 4q28.1q28.2(127965512_129099801)x1. This copy number variant was found to be paternally inherited.

Testing requested: Please confirm the variant identified in the research study and provide interpretation. Parental samples supplied to confirm phase of the research variant (not previously tested).

Notes: Array testing of Lee was performed in an accredited laboratory and you have received a copy of the report.
Reference sequence: Stated in research study NM_152778.2 (*MFSD8*)

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Variant Validation EQA Case 1b

Surname: P[redacted]	MOTHER	**Referrer:** Consultant Clinical Geneticist	
Forename: M[redacted]		**Hospital:** GenQA	
Date of Birth: [redacted]		**Sample Type:** DNA	
Sex: Female		**Sample Volume:** 10µl	**Concentration:** 40ng/µl
Date sample taken: 18/04/2022		**GenQA reference:** 2022VVAL_01b	

Reason for referral:
Mother of Lee PARRY (dob 15/06/2017), no clinical features of note. Research study reported that the familial CNV was not detected in Marisa.

Testing requested: Test for the *MFSD8* variant detected in son, Lee PARRY.

Notes: None

Variant Validation EQA Case 1c

Surname: P[redacted]	FATHER	**Referrer:** Consultant Clinical Geneticist	
Forename: S[redacted]		**Hospital:** GenQA	
Date of Birth: [redacted]		**Sample Type:** DNA	
Sex: Male		**Sample Volume:** 7µl	**Concentration:** 40ng/µl
Date sample taken: 18/04/2022		**GenQA reference:** 2022VVAL_01c	

Reason for referral:
Father of Lee PARRY (dob 15/06/2017), no clinical features of note. Research study reported that Simon has the familial copy number variant arr[GRCh37] 4q28.1q28.2(127965512_129099801)x1.

Testing requested: Test for the *MFSD8* variant detected in son, Lee PARRY.

Notes: Array testing of Simon was performed in an accredited laboratory and you have received a copy of the report.

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The participants were provided with the array results (*Figure 1*) and were asked to test the DNA samples for the research finding, a *MFSD8* c.154+1G>A variant, and provide interpretation and a clinical report(s).

It was expected that participants would classify both the SNV and CNV, and provide a diagnosis of neuronal ceroid lipofuscinosis 7 (CLN7) due to biallelic pathogenic variants in the *MFSD8* gene.

The genotyping results, clinical interpretation in the context of the cases provided, and clerical accuracy of the reports was assessed by a panel of assessors against peer-reviewed marking criteria and current best practice guidelines.

Results

SNV reporting

Individual	Expected result
Index case	Hemizygous *MFSD8*:c.154+1G>A
Mother	Heterozygous *MFSD8*:c.154+1G>A
Father	MFSD8 familial variant not detected

All 27 participating laboratories correctly genotyped the SNV, provided classification and gave the correct clinical conclusion i.e. the combined CNV and SNV results are consistent with a diagnosis of CLN7.

The SNV results were combined well with the CNV results for the index case.

Three participants (11%) classified the variant as likely pathogenic despite there being sufficient evidence to classify as pathogenic[1] (*Figure 2*). However, it should be noted that this classification would not alter the clinical conclusion.

Appropriate classification criteria according to Richards, *et al*. (2015)[1]:

PVS1
Strong clinical validity (ClinGen HI 30, DDG2P definitive, PanelApp green gene). LOF variants described. Variant in canonical splice site.

PM2 moderate
Not present in gnomAD

PM3 moderate
Detected in *trans* with a pathogenic variant (this patient)

CNV reporting

Most participants merely stated that the CNV encompassed the entire *MFSD8* gene with only 11 (41%) specifically classifying the variant as pathogenic.

In light of new phenotypic features and the newly detected SNV in *MFSD8*, it is good practice to re-classify the CNV and provide up to date interpretation for the patient.

It is recommended that CNVs are classified using appropriate guidelines such as Riggs, *et al.* (2020)[2] or internal laboratory protocols and the classification provided in the report. All those participants that provided classification for the CNV stated it was pathogenic (*Figure 2*).

Appropriate classification criteria according to Riggs, *et al*. (2020)[2]:

1: contains protein coding genes.

2: Using clinical judgment, this is a whole gene deletion of an autosomal recessive gene known to be associated with LOF variants (ClinGen HI 30). Though the guidelines do not specifically include autosomal recessive genes (ClinGen HI 30) in this category, internal amendments to the guidelines include the use of DDG2P definitive genes with absence of gene product categorisation so that this can be used for AR genes.

3: 7 protein coding genes in Decipher.

4: Not applied, similar variants found but not identical with same phenotype to patient to justify use.

5: Inheritance not informative – parents are carriers which does not detract from pathogenic evidence in autosomal recessive cases. Phenotype specific and consistent with gene.

Variant Classification

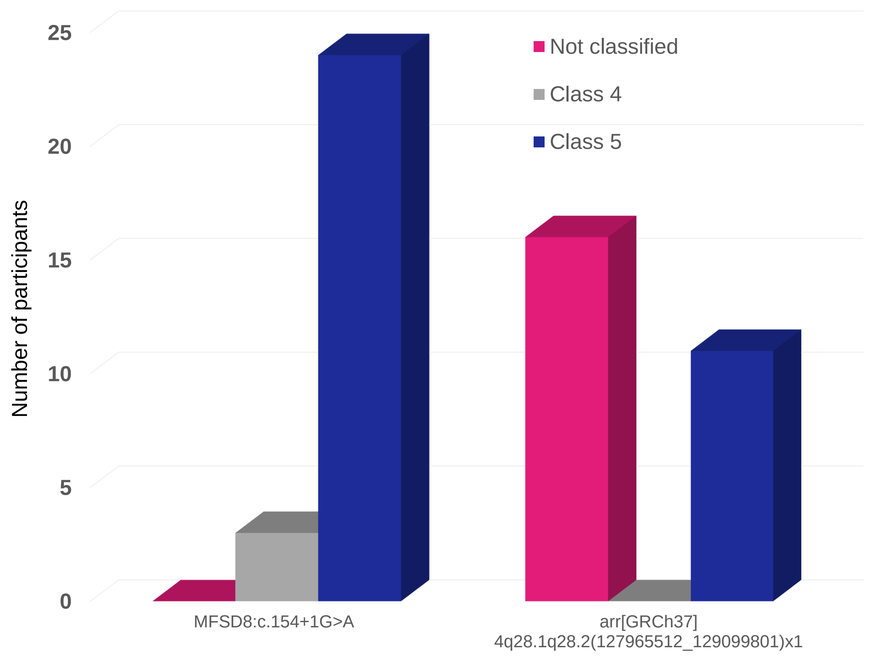

Figure 2: Variant classification provided by participants

Reporting Issues

The **maternal results** were generally reported well with some omissions regarding implications to family members and future offspring. The CNV was often not addressed specifically in these reports, at most being discussed in relation to future offspring.

The **paternal results** were less well reported with some participants omitting important information about the CNV:

- Two participants (7%) did not mention that the father is a carrier of the pathogenic CNV and there was no clinical interpretation or implications to other relatives or offspring provided.
- Despite mentioning the pathogenic CNV on the father's report, two participants (7%) did not clearly state that he is a confirmed carrier and the implications of this to other relatives or offspring.
- One participant stated in their report that the 'family screening result was negative' which is misleading (given the father is a carrier of the pathogenic CNV).
- Eleven participants (41%) did not state that molecular testing of relevant family members for the CNV is possible following appropriate genetic counselling.
- Three participants (11%) did not state that prenatal testing may be offered to this couple.
- Three participants (11%) did not state that that there is a risk to offspring of CLN7.
- One participant did not mention the disorder associated with the *MFSD8* variants.

Conclusion

Expansion of the 'variant validation' EQA to include both SNV and CNV results has highlighted that some laboratories are struggling to combine these results in their reports. Specifically, the CNV classification, carrier status and implications for other relatives and offspring were not well addressed by some participants. The educational aspect of this ongoing EQA will promote improvements to the reporting of SNV and CNV results together by laboratories as the capabilities of genomic testing expand in the diagnostic / clinical setting.

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

References

1. Richards S.e*t al.* (2015). Genetics in Medicine;17(5):405-23
2. Riggs ER, *et al*. (2020). Genetics in Medicine 22:245-257.

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