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GenQA Genomics Quality Assessment

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An eight-year review of the accuracy of genetic testing in Fabry disease.

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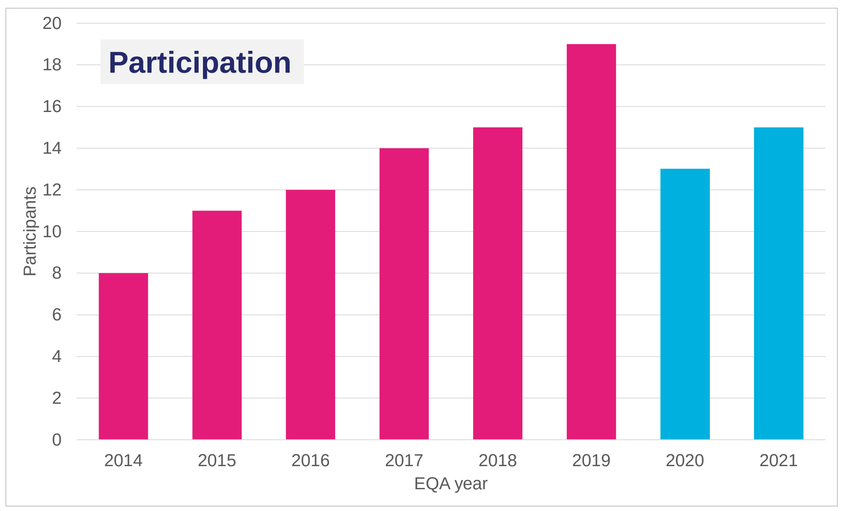
Introduction

Fabry disease, an X-linked disorder due to deficiency of the enzyme alpha-galactosidase, has traditionally been one of the more studied and tested lysosomal storage disorders. GenQA (a member of the UK NEQAS consortium) has been providing external quality assessment (EQA) for Fabry disease since 2014 to determine the accuracy of testing for disease causing variants in the *GLA* gene, including variant classification and clinical interpretation of results. Participation provides laboratories with objective review and advice to improve and maintain a high-quality service which can then be demonstrated to service users and regulatory bodies, leading ultimately to optimal patient care.

Methods

DNA samples are sent to laboratories annually with assessment of both genotyping and interpretation including variant classification (using ACMG[1] or alternative guidelines). For each EQA run, laboratories are provided the same samples for testing to enable benchmarking and performance evaluation.



Fabry disease was offered as a standalone EQA from 2014 to 2019 comprising 3 cases per year. The number of participants is shown in Figure 1.

From 2020 a single Fabry disease case has been included in a broader Inborn Errors of Metabolism EQA.
2020: included medium chain acyl-CoA dehydrogenase deficiency (MCADD)
2021: included Tay-Sachs disease, Gaucher disease, MCADD
The number of participants is shown in Figure 1.

Figure 1: EQA participation by year.

Results

A range of *GLA* genotypes have been provided across 20 EQA cases to date. Table 1 illustrates selected cases. 6 cases with no pathogenic variants were also distributed.

Cases were set to cover common scenarios encountered in the diagnostic laboratory, for example:

- Female patients with normal plasma and/or leucocyte α-galactosidase activity.
- Females patients with no pathogenic variants detected.
- Male patients with classic Fabry disease symptoms.
- Patients with non-classic symptoms such as isolated hypertrophic cardiomyopathy.
- Patients with equivocal enzyme results e.g. from dried blood spot analysis.
- Referrals where enzyme analysis had not been carried out.

Performance was assessed against marking criteria set by expert advisors. Marks were deducted where participants made errors. Critical errors would include incorrect genotyping or errors in interpretation that could lead to serious clinical consequences. See Figure 2 for the number of critical errors reported over 8 years.

Interesting variants provided

2015 case 1: c.937G>T p.(Asp313Tyr) was initially reported as pathogenic however the current weight of evidence indicates that the variant does not cause Fabry disease. This highlights the importance of reviewing all relevant publications and of publishing results wherever possible to add to the available evidence base.

2015 case 2: Although the **c.801G>A** variant is predicted to result in the missense change p.(Met267Ile) the majority of participants correctly suggested a likely affect on splicing (the variant being present at the last base of an exon).

2015 case 3: The **c.-30G>A** variant is not pathogenic however it has been reported to increase plasma α-galactosidase activity and therefore should be reported where relevant. Although this finding was not useful in a female patient with no enzyme results reported (this case) it may be relevant for a male patient with low enzyme activity or normal activity in association with a variant of uncertain significance.

2019 case 3: The **c.679T>C p.(Arg227Ter)** variant was mistakenly reported as an exon 5 deletion by one participant as the variant is situated within the MLPA probe binding site. This highlights the importance of confirming MLPA findings.

References
1. Richards S.*et al.* (2015). Genetics in Medicine;17(5):405-23
2. Deans Z. *et al*. (2022). Eur J Hum Genet; Apr 1. doi: 10.1038/s41431-022-01091-0

Table 1: Cases included

Year & Case	Patient sex & age	Referral reason provided	Validated *GLA* genotype	ACMG classification[1]
2014 case 1	Female, 24	No obvious clinical symptoms. Father (deceased) affected with classical Fabry with deficient plasma and leucocyte α-galactosidase. No molecular testing carried out.	c.831G>A heterozygote p.(Trp277Ter)	5
2014 case 2	Male, 20	Severe pain in extremities and hypohidrosis. Undetectable plasma and leucocyte α-galactosidase.	c.613C>A hemizygote p.(Pro205Thr)	4 or 5
2015 case 1	Female, 38	Hypertrophic cardiomyopathy. Plasma α-galactosidase was low, leucocyte activity normal	c.937G>T heterozygote p.(Asp313Tyr)	2 or 3
2015 case 2	Female, 84	Apparently unaffected. Son died from classical Fabry. Deficient α-galactosidase in plasma and leucocytes but no molecular testing carried out.	c.801G>A heterozygote p.(Met267Ile)	5
2015 case 3	Female, 29	Severe pain in extremities. Enzyme results not yet available.	No pathogenic variant detected c.-30G>A heterozygote	N/A
2016 case 1	Female, 78	Isolated angiokeratoma. Plasma and leucocyte α-galactosidase slightly below normal range. Son died from renal failure.	c.496_497delinsTC heterozygote p.(Leu166Ser)	4 or 3
2017 case 1	Male, 55	Hypertrophic cardiomyopathy. Dried blood α-galactosidase clearly deficient.	c.644A>G hemizygote p.(Asn215Ser)	5
2017 case 3	Female, 48	Left ventricular hypertrophy and hypohidrosis since childhood. Deficient plasma α-galactosidase.	c.476T>C heterozygote p.(Phe159Ser)	4 or 3
2018 case 2	Female, 38	Multiple angiokeratoma and hypohidrosis.	c.274G>C heterozygote p.(Asp92His)	4 or 5
2018 case 3	Male, 32	Exercise-induced pain and angiokeratoma. Corneal opacity, proteinuria with end stage renal disease. No α-galactosidase activity in plasma and leucocytes.	c.1118G>A hemizygote p.(Gly373Asp)	5
2019 case 1	Female, 24	Fabry disease confirmed on renal biopsy.	c.1087C>T heterozygote p.(Arg363Cys)	4 or 5
2019 case 3	Male, 14	Angiokeratoma, hypohidrosis, corneal opacity, renal disease.	c.679C>T hemizygote p.(Arg227Ter)	5
2020	Female, 50	Left ventricular hypertrophy and cardiomyopathy.	c.644A>G heterozygote p.(Asn215Ser)	5
2021	Male 25	Angiokeratoma, corneal clouding. Enzyme analysis consistent with Fabry disease.	c.748_801del hemizygote p.?	5

Critical errors:

Five critical genotyping errors and one critical interpretation error were reported in 265 reports (6/265, 2%) spanning 20 cases over eight years, which is extremely low.

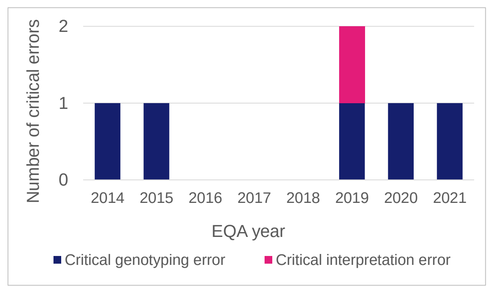


Figure 2: Critical errors reported by year.

ACMG classification[1] key

5	Pathogenic
4	Likely pathogenic
3	Uncertain significance
2	Likely benign
1	Benign

Recurrent issues and errors encountered

- **No clear clinical conclusion:** This was the most frequently encountered issue across all years and cases. A summary statement within the report is recommended e.g. 'diagnosis of Fabry disease confirmed' or 'unlikely to be affected with Fabry disease'. The conclusion should link back to the original referral reason and/or clinical features e.g. symptoms of classical Fabry disease, isolated hypertrophic cardiomyopathy, confirmatory testing of a biochemically diagnosed patient vs. excluding a diagnosis for a patient with possible symptoms etc.
- **No evidence provided for variant classification** e.g. previously reported in other affected patients, expression studies etc.
- **Not linking the results of biochemical tests to the genetic result** e.g. patients with deficient enzyme activity already have a diagnosis of Fabry disease
- **Not stating the implications to other family members** e.g. daughters of affected males will inherit the causative variant
- **Not stating test sensitivity or limitations** e.g. has MLPA testing for whole exon or gene deletions or duplications been carried out?
- **Inappropriate use of the word carrier** This is potentially misleading for female patients with heterozygous pathogenic variants detected.

See Deans *et al.* 2022 EJHG[2] for further reporting recommendations.

Conclusion

EQA participation is important to demonstrate the standard of genomic testing for a wide range of disease-causing variants. Performance was generally excellent for this EQA which may reflect the specialist nature of the participating laboratories. Fabry disease will continue to form part of the widened 'Inborn Errors of Metabolism' EQA which has been further broadened in 2022 to include mucopolysaccharidosis and galactosaemia.

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