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Learnings from five years of External Quality Assessment (EQA) for porphyrias

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Introduction

Porphyria (POR) represents a group of disorders characterised by the accumulation of porphyrins or their precursors. Variants affecting haem biosynthesis can cause different genetic disorders, these can be autosomal dominant (AD), autosomal recessive (AR) or X-linked[1] (Table 1). Affected individuals develop severe cutaneous photosensitivity and/or suffer from attacks of severe abdominal pain. Proficient diagnosis is required for effective clinical management.

Table 1. Porphyrias, associated genes and inheritance[2]

Porphyria type	Gene	Inheritance
Aminolaevulinic acid dehydratase porphyria	ALAD	AR
Acute intermittent porphyria	HMBS*	AD
Congenital erythropoietic porphyria	UROS*	AR
Porphyria cutanea tarda	UROD*	AD
Hepatoerythropoietic porphyria	UROD*	AR
Hereditary coproporphyria	CPOX*	AD
Variegate porphyria	PPOX*	AD
Erythropoietic protoporphyria	FECH*	AR
X-linked erythropoietic porphyria	ALAS2	X-linked

External quality assessment (EQA), also referred to as proficiency testing, provides an independent, external measure of the quality of a laboratories service. It can identify opportunities for improvement of services and may help detect problems before patients are affected.

EMQN established a global EQA scheme in 2005, aimed at ensuring the proficiency of genetic testing for POR. We share findings from the last five years (2020-2024) of the POR EQA in which 31 laboratories participated (Figures 1&2).

* EQA samples are currently only available for these genes. Please contact office@emqn.org if you are able to donate cell lines, blood or DNA to support the POR EQA.

Figure 1. Heat map for participation in POR EQA scheme (2020-2024)

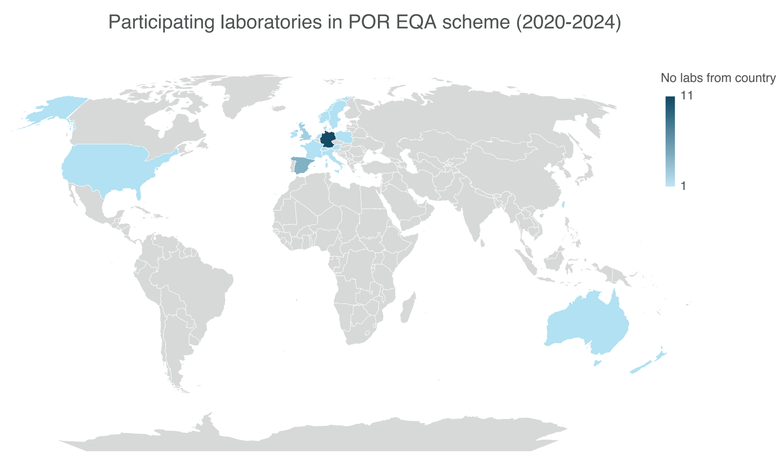
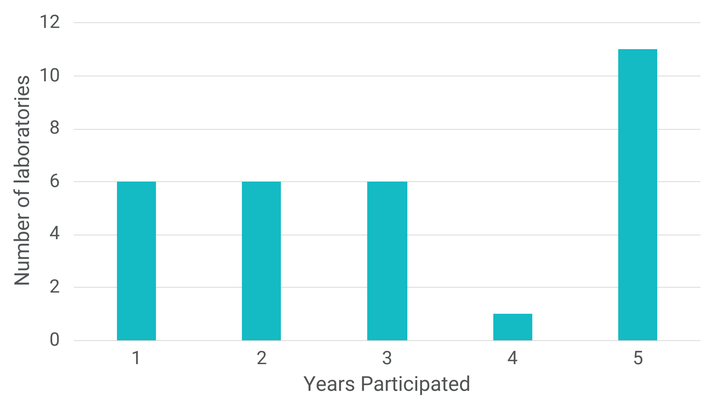


Figure 2. Laboratories years of participation (2020-2024)



Aims

The aim of the POR EQA is to assess the entire genetic diagnostic pipeline of a laboratory, including sample receipt and processing, genotyping, and reporting (biological and clinical interpretation of the test result) in the context of mock clinical referrals as well as reporting clarity, content and clerical accuracy. Through assessment and feedback in individual laboratory reports and summary scheme reports, laboratories are able to improve their practices.

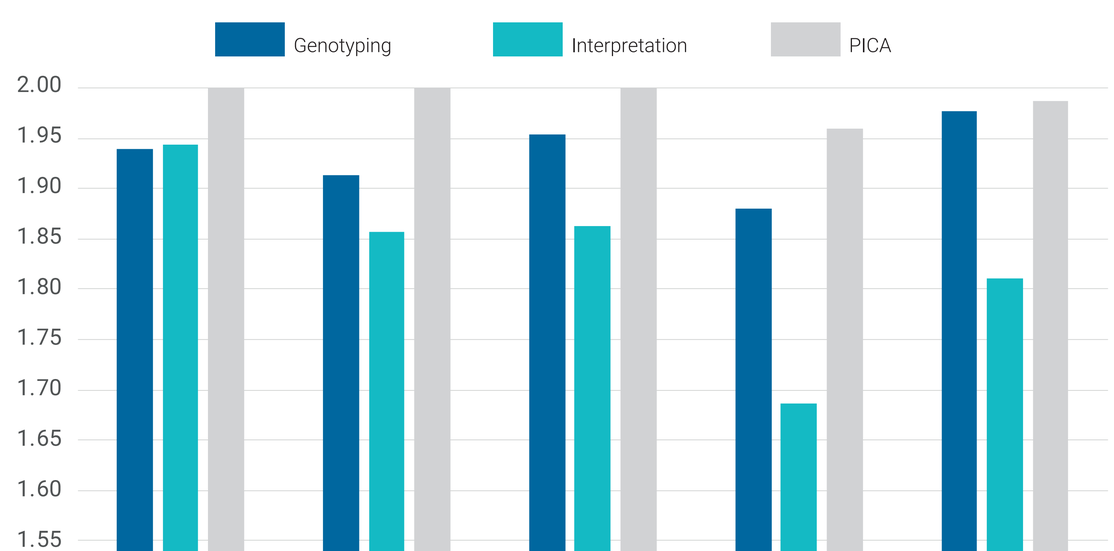
Methods

Participants received three or four DNA samples** with corresponding mock clinical referrals for testing for each round of EQA which runs annually. Laboratories were instructed to use their routine testing methodologies and submit their results in the form of their routine clinical report format. The anonymised reports were assessed for genotyping, interpretation, and clerical accuracy (maximum score per category = 2.00). Participant performance was analysed over a 5-year period (2020-2024) to establish trends and understand the impact on reporting quality from continued EQA provision.

**from Coriell Institute for Medical Research or manufactured at the Genomics Diagnostic Laboratory, Manchester Centre for Genomic Medicine, UK.

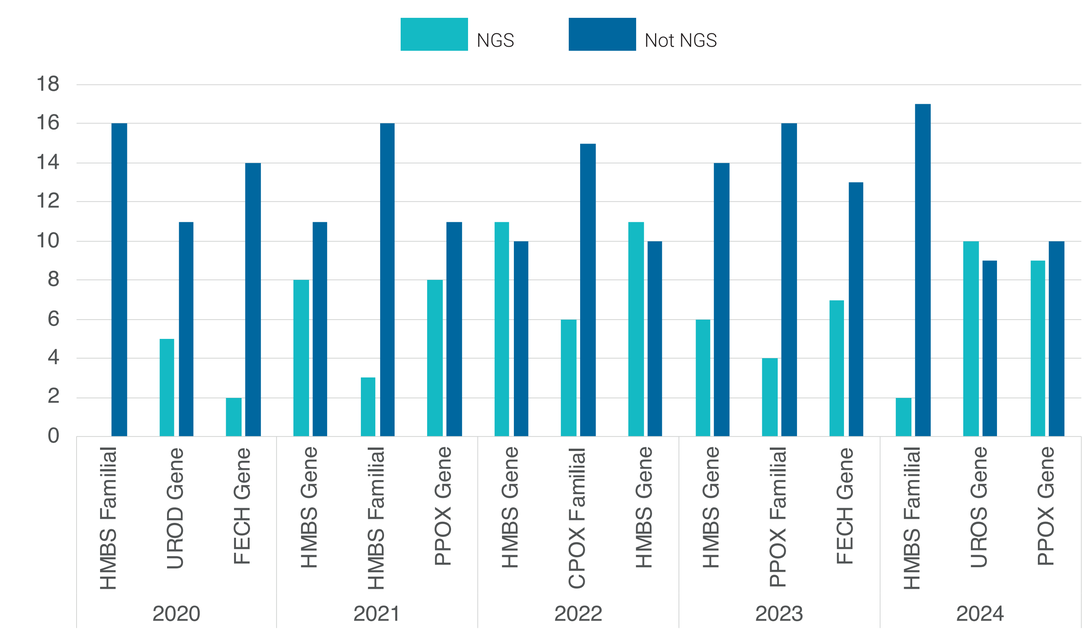
Discussion

Figure 3. POR EQA Scheme - Mean scores 2020-2024



Ninety-six participations have been assessed from 31 laboratories in 17 different countries. There were no withdrawals or failures to submit results, and 35.5% (11/31) laboratories have participated in all five rounds of EQA (Figure 3).

Figure 4. Number of participants using NGS for whole gene testing (2020-2024)



Laboratories typically use Sanger sequencing for the POR EQA, however, more laboratories are starting to use NGS for cases that require whole gene testing in the referral (Figure 4).

Genotyping quality was consistently excellent with an average score of 1.93/2.00. However, several laboratories missed a known low expression allele intronic *FECH* variant: NC_000018.10(NM_000140.5):c.315-48T>C[3]. This well-known variant (rs2272783) has a high frequency in the general population and should be included in the scope of the chosen testing strategy. Frequency is considered to be 10%[4] in Caucasians, ranging from 4% in European (non-Finnish) to 38% in East Asian samples in gnomAD.

Critical genotyping errors (i.e. false positives/false negatives) have typically remained low with only 3 false negatives reported over the 5-year period (3/283 reports, 0.01%).

Critical interpretation errors (CIE) (i.e. misinterpretation of results that could cause patient harm) have also remained low with 4 reported (4/283 reports, 0.01%), however, Case 1 in 2022 accounted for 3 of these CIEs. The CIEs were assigned to laboratories that described the heterozygous HMBS variant NM_000190.4:c.254T>C p.(Leu85Pro) as a variant of uncertain significance (VUS) and therefore concluded that this does not explain the patient's acute intermittent porphyria (AIP) diagnosis. A classification of either likely-pathogenic or pathogenic was accepted by the assessors as both would confirm the diagnosis of AIP[5].

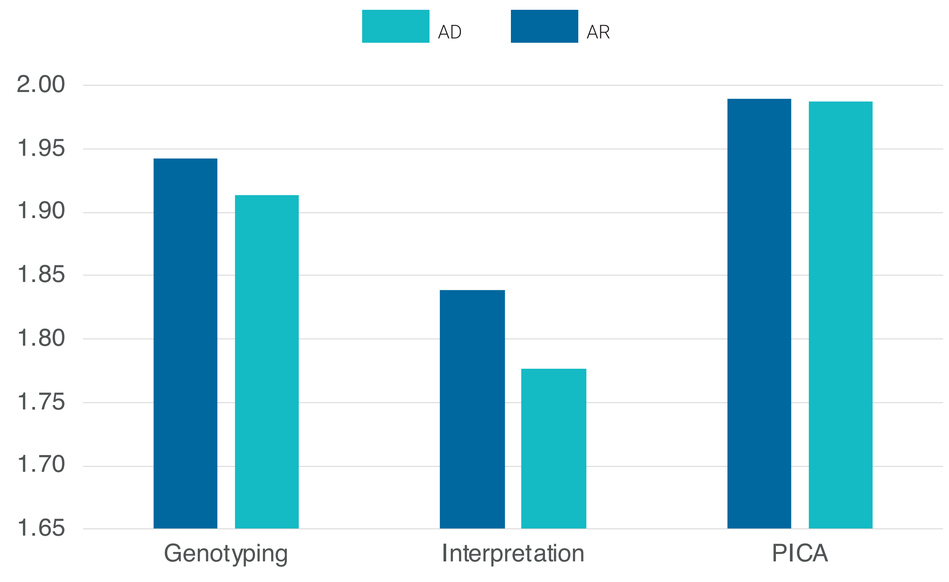
The quality of interpretation was also very good, averaging 1.83/2.00 over the period surveyed. In 2023, interpretation performance dropped to 1.63/2.00, primarily due to 55% of laboratories failing to interpret the genotyping result in the context of the clinical referral. The case was for a 16-year-old female with a recent prescription of contraceptive pills whose mother has variegate porphyria (PV). Laboratories failed to comment about the consequences of having PV and oral contraception and not providing a referral to a website about safe drug medications to avoid triggering factors for a PV attack.

Some other reasons for interpretation deductions in 2023 include:

- Failure to provide adequate details of test performed i.e. limitations of NGS methodology in context of test request (restricted testing to specific genes)
- Failure to suggest further testing to verify the phase of two heterozygous variants or provide interpretation with explicitly assuming the variants are in trans.
  - The case contained: two heterozygous *FECH* variants NC_000018.10(NM_000140.5):c.1078-2A>G p.? and NC_000018.10(NM_000140.5):c.315-48T>C p.?
- Stating erythropoietic protoporphyria is autosomal dominant.
- Insufficient evidence for classification of variants.

Given the different inheritance for the porphyrias, the EQA provides cases for different genes to ensure coverage of the different types. The autosomal recessive types appear to be more challenging for laboratories in the interpretation of results (Figure 5).

Figure 5. Means scores between autosomal dominant and autosomal recessive cases (2020-2024)



Conclusions

Data from the past five years of the POR EQA demonstrates that POR testing services are consistently of a good standard. However, improvements could be made in ensuring the scope of testing for rare causes and improving interpretation to ensure reports are informative for the recipient. We anticipate the continued provision of this EQA is crucial for improving testing and reporting standards for POR.

References

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