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External Quality Assessment for hereditary amyloidosis: Assuring test standards to support the identification of patients eligible for treatment

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Background

Hereditary amyloidosis is a group of rare autosomal dominant conditions characterised by amyloid accumulation impairing organ function. Hereditary transthyretin amyloidosis (ATTRv) - the most common type - is caused by >130 pathogenic variants in the *TTR* gene and presents with neurological and/or cardiac manifestations. Several disease modifying therapies for ATTRv are now available, making a proficient genetic diagnosis crucial for improved patient outcomes[1,2,3]. We have established a global external quality assessment (EQA) scheme for *TTR* testing to assure diagnostic service quality.

Aims and Objectives

The aim of this project was to establish an external quality assurance (EQA) scheme for hereditary amyloidosis to ensure consistency and accuracy of reporting across laboratories undertaking *TTR* gene testing.

Methods

- The scheme was designed to assess the entire diagnostic pipeline of a laboratory, including sample receipt and processing, analytical processing (genotyping), and reporting (biological and clinical interpretation of the test result) in the context of a clinical referral, as well as reporting clarity, content and clerical accuracy.
- A survey to express interest to participate in the pilot was shared with the EMQN network and the International Society of Amyloidosis (ISA) network. Thirty laboratories were selected.
- Three validated DNA samples with corresponding mock clinical referrals were provided to each participating laboratory for *TTR* gene analysis using their routine strategy (Table 1).

Table 1: EQA Sample details and validated genotype results

Case	Mock Clinical Scenario	Validated Result
1	Request from a Consultant Cardiologist for *TTR* testing in a 63-year-old female with a clinical picture suggestive of ATTR amyloidosis. Genetic testing was recommended to elucidate if it was hereditary.	**Heterozygous for *TTR* variant:** NM_000371.4:c.349G>T p.(Ala117Ser)
2	Request from a Genetic Counsellor for predictive *TTR* testing in an asymptomatic 34-year-old female with family history of hereditary ATTR amyloidosis on the paternal side. Genetic testing was requested to find if she is at risk developing a disease.	**Heterozygous for *TTR* variant:** NM_000371.4:c.148G>A p.(Val50Met)
3	Request from a Consultant Neurologist for *TTR* testing in a 78-year-old male with a clinical picture suggestive of ATTR amyloidosis. Genetic testing was recommended to elucidate if it was hereditary.	**Heterozygous for *TTR* variant:** NM_000371.4:c.238A>G p.(Thr80Ala)

- Clinical reports were returned and assessed anonymously by two experts for ability to:
  - Correctly genotype cases suspected of having hereditary amyloidosis against the validated result (Table 1),
  - Interpret the results in response to the clinical referral in a clear and concise format,
  - Correctly use internationally accepted standard nomenclature,
  - Provide appropriate and accurate patient and sample identifiers.
- Participants were awarded with 2.00 marks for each category and deductions were applied during assessment using pre-defined criteria.
- Marking of all reports was then moderated and any discordant marking was discussed by the expert panel to reach a final agreement.
- Participants received an individual report on performance including educational advice and a scheme report summarising performance of all participants in the scheme was also provided to each participating laboratory. This report included additional information from the cohort of participants e.g. geographical spread, methodologies employed, common errors, learning points and scheme statistics allowing participants to benchmark their performance.

Results

Participation

- Fifty-one laboratories applied to participate in the scheme and filled out an expression of interest survey.

Figure 1. Survey results

Figure. 1A Laboratory accreditation status

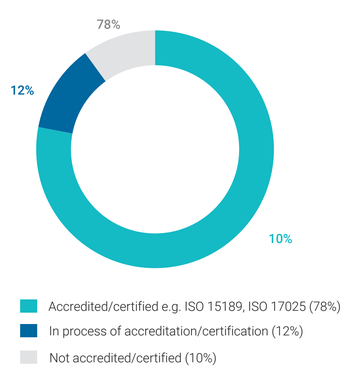


Figure.1B In what context, if at all, do you offer molecular testing for hereditary amyloidosis?

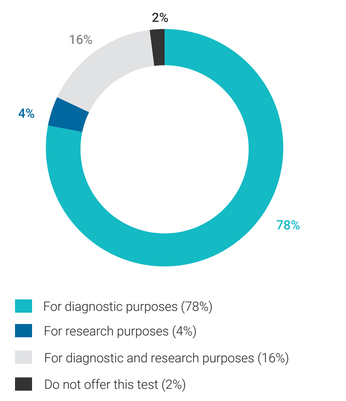


Figure.1C Do you partner with another organisation in order to deliver this service, and if so what parts of the service do you outsource?

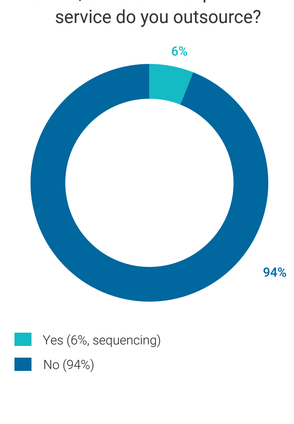
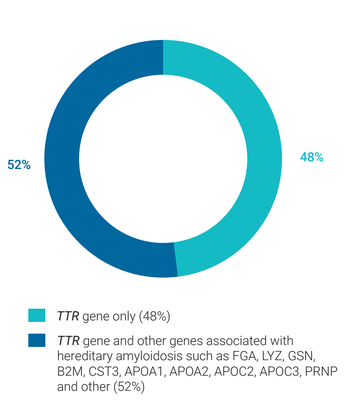


Figure.1B Which genes associated with hereditary amyloidosis do you currently offer testing for?



- Thirty laboratories from 21 countries were selected to participate in the pilot EQA based on experience of providing molecular testing for hereditary amyloidosis (Figure. 2).

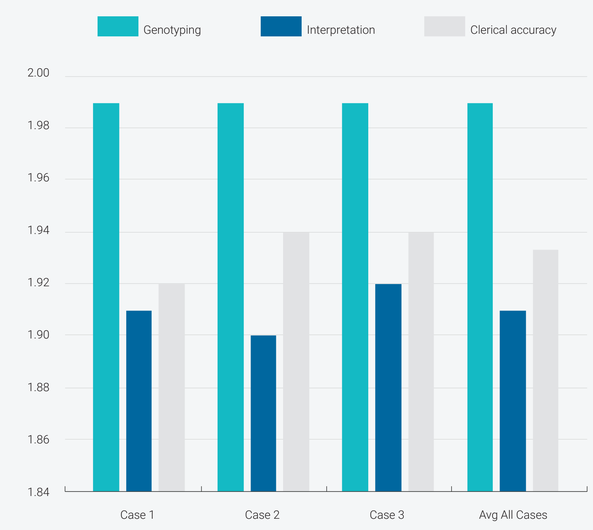
Australia	**1**	**Italy**	**3**
Austria	**1**	**Japan**	**1**
Belgium	**1**	**Netherlands**	**1**
Brazil	**1**	**Portugal**	**2**
Bulgaria	**1**	**Spain**	**2**
Denmark	**1**	**Sweden**	**2**
France	**2**	**Switzerland**	**1**
Germany	**4**	**United Kingdom**	**1**
Greece	**1**	**United States**	**1**
Ireland	**1**		

Figure 2. Participation by Geographical Location of the Hereditary Amyloidosis Pilot EQA scheme; blue intensity indicates number at the given location.

Scheme outcomes:

- Twenty-eight laboratories returned clinical reports. One laboratory did in the end not register for the scheme while another one did not submit the results.

Figure 3. Mean scores per case and category and average score across all cases (out of a maximum of 2.00).

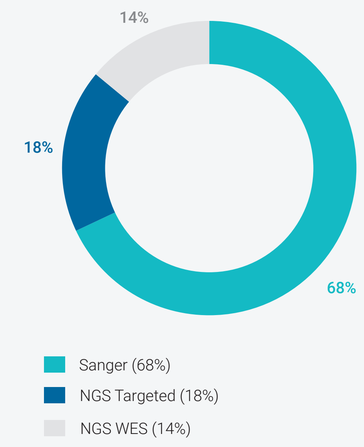


Genotyping

All cases:

- All laboratories correctly reported the expected genotype.
- The average genotyping score was 1.99 (Fig.3).
- 93% of laboratories received full marks for genotyping.
- 7% laboratories failed to include or included incorrect gene transcript or transcript version in their reports.

Figure 4. Reported testing methods used for all three cases



Interpretation

All cases:

- The overall quality of interpretation was very good.
- The mean score was 1.91 across all three cases (Figure.3).
- 87% of laboratories received full marks for interpretation (86% of laboratories for case 1 and 2 and 89% of laboratories for case 3).
- 69% of laboratories failed to recommend that the results of genetic testing should be interpreted in the clinical context. Educational comment shown below was included in the summary scheme report explaining that, while the results of genetic testing can be supportive of a diagnosis of a hereditary amyloidosis, these results should be interpreted within the clinical context and integrated with clinical data, laboratory test results and other clinical information, to establish a definitive diagnosis. Also, referral to a clinical expert in the field of systemic amyloidosis should be advised in the genetic report.

  "Systemic amyloidosis is remarkably diverse in terms of cause, clinical manifestation, anatomic distribution, progression, and prognosis and can be both hereditary and acquired. Thus, establishing a definitive diagnosis of systemic amyloidosis critically depends on the integration of several sources of clinical data, including laboratory tests, imaging, genetic testing and eventually histologic and/or proteomic data on amyloid-laden tissue samples.

  Clinical information accompanying a request for genetic testing is often insufficient to establish a definitive diagnosis of systemic amyloidosis and should only be used to guide the choice of gene(s) to be analysed. Likewise, the results of genetic testing should not be used to establish the diagnosis without proper clinical assessment".

  Points were not deducted for omitting this information in the clinical reports in pilot scheme; however, laboratories were informed that the points will be deducted in future schemes in such instances.

Other identified issues:

- Failure to state the risk of an affected child applies to each pregnancy: 21% of laboratories in case 1 and 3, 14% in case 2.
- Using the term "carrier" for autosomal dominant disorder: 11% of laboratories in case 1, 14% in case 2 and 7% in case 3.
- Failure to provide adequate details/limitations of test performed: 7% of laboratories in all cases.
- Failure to suggest genetic screening in close family members: 7% of laboratories in case 2 and 4% in cases 1 and 3.
- Failure to recommend counselling and/or follow-up: 4% of laboratories in all cases.

Case specific comments:

Case 1:

- 4% of laboratories incorrectly stated the pathogenicity as likely pathogenic instead of pathogenic.

Case 2:

- 4% of laboratories incorrectly stated in the report that the result was diagnostic for an asymptomatic individual in a frame of a predictive test.
- 4% of laboratories failed to suggest genetic counselling and/or follow up, which is important for asymptomatic individuals with a positive test result.

Clerical Accuracy

- 85% of laboratories received full marks for clerical accuracy.
- Mean score across all three cases for clerical accuracy was 1.93 (Fig.3).
- One laboratory incorrectly re-stated the patient's name.
- The most common errors/inaccuracies detected were:
  - Spelling and typographic error,
  - No evidence that the report was authorised by two people,
  - Incorrect or no pagination,
  - Five reports were too long (over two pages).

Conclusions

This pilot EQA scheme has established that the standard of *TTR* testing for hereditary amyloidosis is good. However, areas of improvement, particularly around the accuracy of interpretation of the genotype result, were identified. To date, there are no standardised criteria for genetic analysis and reporting of *TTR* variants in hereditary amyloidosis. This lack of guidance might compromise patient care. We anticipate continued provision of this EQA scheme will improve testing and reporting standards, whilst simultaneously facilitating the development of best practice guidelines.

Next steps

The scope of this EQA scheme will be extended and we aim to establish best practice guidelines for genetic testing in this rare disease ensuring appropriate patient care.

References

1. Rowczenio D, Quarta CC, Fontana M, et al. Analysis of the TTR gene in the investigation of amyloidosis: A 25-year single UK center experience. Hum Mutat. 2019;40(1):90-96. doi:10.1002/humu.23669. 2. Obi CA, Mostertz WC, Griffin JM, Judge DP. ATTR Epidemiology, Genetics, and Prognostic Factors. Methodist Debakey Cardiovasc J. 2022;18(2):17-26. Published 2022 Mar 14. doi:10.14797/mdcvj.1066. 3. Ando Y, Adams D, Benson MD, et al. Guidelines and new directions in the therapy and monitoring of ATTRv amyloidosis. Amyloid. 2022;29(3):143-155. doi:10.1080/13506129.2022.2052838

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