Enabling high quality hypertrophic cardiomyopathy genetic testing using multiplexed patient-like reference material

Zandra C. Deans¹, Farrah Khawaja¹, Fiona Moon¹, Dan Brudzewsky², Catherine Huang², Ram Santhanam²

1. UK NEQAS for Molecular Genetics/GenQQA, Department of Laboratory Medicine, Royal Infirmary of Edinburgh, Little France Crescent, Edinburgh, UK
2. SeraCare Life Sciences, Inc., 37 Birch Street, Milford, MA 01757, USA

INTRODUCTION

The UK National External Quality Assessment Service (NEQAS) for Molecular Genetics promotes high quality accurate genetic testing through external quality assessment (EQA) and educational exercises.

The detection and classification of sequencing variants can be challenging and in recognition of the heavy workload required to test multiple genes for the presence of many variants, UK NEQAS for Molecular Genetics distributed a single commercially available multiplexed reference sample (SeraCare) with known pathogenic and non-pathogenic variants associated with hypertrophic cardiomyopathy (HCM) in the MYBPC3, MYH7, TNNT3, TNNI2 and TPM1 genes.

Participants were required to test the sample and report all detected variants as well as their variant classification.

METHODS

- Laboratories registered in the UK NEQAS HCM 2017 EQA were invited to participate in this educational exercise.
- Laboratories received the reference sample and applied their routine HCM testing strategy. Any variants detected were to be classified for pathogenicity and those classified as either pathogenic (Class 5) or likely pathogenic (Class 4) were to be reported.
- The sample contained a range of variant types comprising of substitutions and small insertions to large deletions within repetitive regions and all variants were obtained from clinical sequencing data and present at an allelic frequency of 50% (see Table 1).
- The expected variant classifications were provided by SeraCare and assigned using a modified version of the ACMG guidelines¹ according to Alfares et al. 2015².
- This was an educational exercise and no scores were assigned to the laboratory results.

RESULTS AND DISCUSSION

Variant classification

- The majority of participants reported the expected classification for six of the ten variants (Variants 1, 2, 3, 5, 7 and 9).
- For variant 4, 13 out of 16 participants that identified the variant classified it as pathogenic (Class 5), however, the expected classification was Class 4, likely pathogenic.
- Out of the 15 participants that identified variant 6, just over half (eight) identified it as the expected classification: likely pathogenic: Class 4 (4), with the other seven participants classifying it as Class 5, pathogenic.
- Additionally, 10/15 participants who identified variant 8 classified it as Class 4, however the expected classification was Class 5, pathogenic.
- The range of variant classifications are shown as Graph 1.

Graph 1: Variant classifications

The size of the bubble represents the proportion of laboratories that reported that classification. Blue bubbles show the expected classification and orange bubbles are where a classification different to that expected was reported. Classes are according to a modified version of ACMG guidelines².

CONCLUSIONS

The standard of testing for this EQA was generally very high with 16/17 laboratories detecting a high proportion (≥70%) of the variants included in this reference sample. Variant 10 was detected the least, with only four laboratories reporting it, however the intronic, likely pathogenic variant was predominantly either not being included in the assay or not being within the region of interest for reporting.

The majority of laboratories reported classifications as expected for most of the variants.

The use of reference samples containing variants obtained from clinical sequencing data to identify areas of improvement in clinically assays has been demonstrated.

The large number of interested participants indicates that laboratories recognise the value of EQA educational exercises and use the results to amend their testing practice.

REFERENCES


RESULTS

The reference sample was distributed to 17 laboratories from eight countries; the majority were located in the United Kingdom (53%), followed by Australia (12%).

The known variants present in the reference sample are summarised in Table 1.

Table 1: Class 4 and 5 variants confirmed to be present in the reference sample

<table>
<thead>
<tr>
<th>Variant number</th>
<th>Gene</th>
<th>Variant Type</th>
<th>Nucleotide Amino acid</th>
<th>Expected Class</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MYBPC3</td>
<td>Substitution</td>
<td>c.1504C&gt;T p.Arg502Trp</td>
<td>Class 5-Pathogenic</td>
</tr>
<tr>
<td>2</td>
<td>MYH7</td>
<td>Substitution</td>
<td>c.1995G&gt;A p.Glu665Lys</td>
<td>Class 5-Pathogenic</td>
</tr>
<tr>
<td>3</td>
<td>TNNI3</td>
<td>Substitution</td>
<td>c.1357C&gt;T p.Arg453Cys</td>
<td>Class 5-Pathogenic</td>
</tr>
<tr>
<td>4</td>
<td>TNNI3</td>
<td>Substitution</td>
<td>c.198C&gt;G p.Glu66R</td>
<td>Class 5-Pathogenic</td>
</tr>
<tr>
<td>5</td>
<td>TPM1</td>
<td>Substitution</td>
<td>c.575G&gt;A p.Glu192Lys</td>
<td>Class 5-Pathogenic</td>
</tr>
</tbody>
</table>

A summary of the testing performed and the variants detected is shown in Table 2. A next generation sequencing (NGS) approach was used by all but one laboratory who performed Sanger sequencing. All laboratories specified the number of genes tested and there was considerable variation, ranging from 4 to 141 genes.

Table 2: Summary of the variants identified and methods performed

<table>
<thead>
<tr>
<th>Laboratory code</th>
<th>Variant number</th>
<th>Methodology</th>
<th>Number of genes tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓</td>
<td>Ampliseq</td>
<td>16</td>
</tr>
<tr>
<td>O</td>
<td>✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓</td>
<td>Sanger</td>
<td>4</td>
</tr>
<tr>
<td>P</td>
<td>✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓</td>
<td>HCM gene panel</td>
<td>10</td>
</tr>
<tr>
<td>Q</td>
<td>✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓</td>
<td>SmMIP &amp; NGS</td>
<td>7</td>
</tr>
</tbody>
</table>

Key: ✓ Variant reported  Green cell = Expected classification reported  X Variant not reported  Orange cell = Classification reported different to expected  # Gene not tested by laboratory  * Variant reported as an insertion or duplication  ? Codon position reported was not as expected

CONCLUSIONS

- Three variants were detected by all laboratories (Variants 1, 2 and 3).
- Three variants were detected by 94% of laboratories (Variants 4, 7 and 9). The remaining laboratory did not detect any of these three variants.
- The majority (88%) identified Variants 5, 6 and 8.
- Only three laboratories (18%) reported Variant 10 which was a large deletion in the repetitive region of MYBPC3.

Failure to report MYBPC3 large deletion (Variant 10)

Thirteen laboratories (76%) did not report the presence of the MYBPC3 c.3628-41,3628-17del variant. Further investigation determined (see Figure 1):

- Approximately did detect the variant but was not reported due to being out with their region of interest (ROI). These laboratories have now extended their ROI to include this variant.
- The region or copy number variants were not tested by two laboratories.
- One laboratory resolved the failure to detect the variant by using a different variant caller (HaplotypeCaller), so it was an informatics problem.
- No response was received from the remaining laboratories.

Extra variants

Three laboratories each reported an ‘extra’ variant; two of these were confirmed to be present by SeraCare (ACTN2c.959delC and DSG2c.2234C>T). The third laboratory identified a variant of uncertain significance in PIRK42 but did not validate the presence by Sanger Sequencing. This variant was not detected by SeraCare.

Figure 1: Reasons for not reporting Variant 10

The authors would like to thank the UK NEQAS participants for taking part in this educational exercise and the EQA Specialist Advisory Committee. For further information please contact Dr Sarah Down (D.S DOWN@UCL.AC.UK).