Abstract

Agilent Technologies provides the hypertrophic cardiomyopathy (HCM) MASTR assay. This assay is based on next generation sequencing technology and analyzes the mutational status of five genes from DNA extracted from whole blood.

In this application note, the performance of the HCM MASTR is evaluated with the Seraseq® Cardiomyopathy Reference Material (SeraCare Life Sciences).

The Seraseq® Cardiomyopathy Reference Material was designed to present a range of prevalent variants in five genes implicated in cardiomyopathy including four of the genes covered by the HCM MASTR assay. The TPM1 is not within scope of the design of the HCM MASTR assay. The reference material, therefore, provides a valuable tool for the evaluation of the HCM MASTR assay.

The HCM MASTR assay successfully covered all relevant variants (i.e. except for TPM1) reported within the Seraseq® Cardiomyopathy Reference Material and no drop out was observed. Additional variants, including 3 variants in MYL2, a gene in scope of the HCM MASTR, were called. MYL2 is however not claimed or characterized in the Seraseq® Cardiomyopathy Reference Material.

We conclude that the HCM MASTR assay identifies accurately all relevant variants at correct allele frequencies present in the Reference Standards.

Introduction

Hypertrophic cardiomyopathy (HCM) is a common inherited heart disease with a predominantly autosomal dominant inheritance pattern and a prevalence of 1 in 500 individuals. It is defined by thickening (hypertrophy) of the ventricular septum of the heart coupled with the absence of other cardiac or systemic conditions or diseases that can account for the observed hypertrophy.

Hypertrophy in HCM, despite the morphological changes i.e. hypertrophy of the left ventricle and the intraventricular septum, does not typically result in decrease in the efficiency of circulation.

The clinical symptoms of HCM are widely variable and include dyspnea, chest pain, tachycardia and syncope. In addition, HCM has been highly associated with increased risk for heart atrial and ventricular arrhythmias, heart failure and sudden cardiac death. The risk of sudden cardiac death due to HCM is particularly high in adolescent or young adults and remains the primary/leading cause of sudden death in young competitive athletes.

Mutations in several genes have been documented to be responsible for or associated with HCM. Other genes, including some that have not been identified, may also be involved in this condition. Eleven genes encoding for cardiac protein important for muscle contraction, have been associated with HCM. A subset of these genes comprise of sarcomeric cardiac protein encoding genes.

The HCM MASTR assay is designed as a Research Use Only assay (Not for use in diagnostic procedures) to detect variants (CNVs, SNVs and Indels) in the entire coding regions in five genes encoding for sarcomeric proteins relevant to HCM i.e. MYH7, MYBPC3, TNNT2, TNNI3 and MYL2.

In one study conducted by Richard et al, 2003, 63% of patients were shown to carry mutations based on
On average, the mutation detection rate for the five top candidate genes observed in sporadic and familial cases ranged from 40-41% for MYBPC3 and MYH7 and 4%-10% for TNNT2, TNNI3 and MYL2.

The Seraseq® Cardiomyopathy Reference Material addresses the growing need for a multiplexed reference materials by presenting a range of prevalent pathogenic variants (SNVs and indels) in cardiomyopathy.

This application note, therefore, shows the relevant for the use of the Seraseq® Cardiomyopathy Reference Material in the evaluation of the HCM MASTR assay.

**Materials and Methods**

The commercially available Seraseq® Cardiomyopathy Reference Material and four DNA samples were run using the HCM MASTR assay.

This standard contains different mutations (including SNVs, an insertion, a large deletion and a deletion in a highly repetitive region) with target variant allele frequency of 50% for every documented variant (Table 1).

The HCM MASTR assay consists of five plexes. Per plex, 20 ng of DNA from the Seraseq® Cardiomyopathy Reference Material was used. Next generation sequencing analysis including variant calling was done using the SeqNext® Module of the Sequence Pilot software (JSI medical systems). The libraries were sequenced on a MiSeq Reagent Kit Regular v2 run with read length of 2x251bp. The data were analyzed using JSI SeqNext® v4.1.

All identified variants (CNVs, SNVs and indels) related to the variants in the Seraseq® Cardiomyopathy Reference Material are reported and described in Table 1.

**Results**

The HCM MASTR assay in combination with the JSI SeqNext® analysis software correctly identified all variants (9 variants in 4 genes MYBPC, MYH7, TNNI3 and TNNT2) in the Seraseq® Cardiomyopathy Reference Material. Moreover, the results obtained from the HCM MASTR showed the same variant heterozygosity as the Seraseq® Cardiomyopathy Reference Material and the variant allele frequency (VAF) detected in this study ranged between 43% and 58% (Table 1). No variants in the TPM1 gene (bearing 1 variant in the Seraseq sample) are called since the TPM1 gene is not covered by the HCM MASTR assay.

In addition to the variants reported in the Seraseq® Cardiomyopathy Reference Material product sheet, additional variants were identified. These included variants (VAF > 15%) in MYBPC3 (6), MYH7 (17), TNNT2 (7), TNNI3 (7) and MYL2 (3).

Additional variants were identified by the HCM MASTR assay and SeqNext analysis. These variants are related to the human cell line GM224385 background and fall outside the scope of this application note.

The calling of additional variants by HCM MASTR assay not documented in the Seraseq® Cardiomyopathy Reference Material product sheet, can be attributed to the fact that the reference sample was designed using the GM24385 human genomic DNA background cell line as ‘wild-type’ material.
Table 1: Comparison of variants detection using the Agilent Technologies’ HCM MASTR assay and known variants in the commercially available Seraseq® Cardiomyopathy Reference Material (SeraCare Life Sciences)

<table>
<thead>
<tr>
<th>Known variants in the Seraseq® Cardiomyopathy Reference Material</th>
<th>Variants detected using HCM MASTR</th>
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</thead>
<tbody>
<tr>
<td>Gene</td>
<td>HGVS nomenclature</td>
</tr>
<tr>
<td>------</td>
<td>-------------------</td>
</tr>
<tr>
<td>MYBPC3</td>
<td>c.1504C&gt;T</td>
</tr>
<tr>
<td>MYBPC3</td>
<td>c.2373_2374insG</td>
</tr>
<tr>
<td>MYBPC3</td>
<td>c.3628-1_3628-17del</td>
</tr>
<tr>
<td>MYH7</td>
<td>c.1357C&gt;T</td>
</tr>
<tr>
<td>MYH7</td>
<td>c.1750G&gt;C</td>
</tr>
<tr>
<td>MYH7</td>
<td>c.1988G&gt;A</td>
</tr>
<tr>
<td>TNNI3</td>
<td>c.532_534delAAG</td>
</tr>
<tr>
<td>TNNI3</td>
<td>c.575G&gt;A</td>
</tr>
<tr>
<td>TNN12</td>
<td>c.487_489delGAG</td>
</tr>
<tr>
<td>TPM1</td>
<td>c.574G&gt;A</td>
</tr>
</tbody>
</table>

*This information consists of VAF % observed in total number of reads (total number of reads = number of forward reads number of reverse reads)

** Variants of TPM1 are not called on the HCM MASTR as the gene is not in scope for the HCM MASTR assay.

*** No variants data analysis is available for MYL2 for Seraseq® Cardiomyopathy Reference Material as the gene is not in scope of the design for the reference standard.
Conclusion

In conclusion, the Seraseq® Cardiomyopathy Reference Material allows for the rapid evaluation of the HCM MASTR assay. All amplicons within the Seraseq® Cardiomyopathy Reference Material were successfully covered and no drop-out was observed. The HCM MASTR application is shown to correctly and accurately identify all covered variants at the expected allele frequency. No variants were identified for the TPM1 gene using the HCM MASTR since this gene is out of the scope of the assay. In addition to the reported variants present in the reference material, 3 variants in MYL2, in scope of the HCM MASTR, were called. These variants were not previously reported for the HCM Seraseq® Cardiomyopathy Reference since MYL2 is not within the scope for the HCM Seraseq® Cardiomyopathy Reference Material.

The data presented indicate that the Seraseq® Cardiomyopathy Reference Material is a valuable tool in the evaluation of the HCM assay and enables for easy and rapid implementation of the HCM MASTR assay in the research setting for routine monitoring of assay performance.

References


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