ABSTRACT  PgmNr 2551/T

Spinal muscular atrophy is a debilitating disease with a symptom of losing spinal muscle control and can cause infant mortality in the most extreme cases. Normal spinal nerve-muscle connections require the presence of protein produced by the SMN1 gene (Survival of Motor Neuron 1 Gene). Disease severity can be reduced by the presence of extra copies of the SMN2 gene, which produces a similar type of protein but with reduced activity. The SMN1 and SMN2 genes are nearly identical in sequence (99.6% identity). Accurate detection of the copies of SMN1 and SMN2 genes is critical to identifying the SMA carrier status of an individual. Approximately one in 6,000 babies are born with the disease and one in 40 individuals are carriers of a defective SMN1 gene.

For analytical verification, 386 DNA samples were characterized using two methods: the Applied Biosystems CarrierScan® Assay and droplet digital PCR (ddPCR). In this data set, 7 samples were identified as having SMA carrier status. 19 samples were identified as having the SMN1 g.27134T>G mutation that is in linkage-disequilibrium with the silent-carrier chromosome, a chromosome containing 2 copies of the SMN1 gene in cis in some populations.

The CarrierScan and ddPCR data were 100% concordant for SMA carrier state and g.27134T>G genotype. Concordance for SMN1 copy number was 94% and concordance for SMN2 copy number was 82%.

The results show that the highly-multiplexed CarrierScan® assay is very effective and accurate for detecting SMA carrier genotypes.

INTRODUCTION

Spinal Muscular Atrophy (SMA) is an autosomal recessive (Fig. 1) genetic disorder causing weakness and wasting of spinal skeletal muscles with various levels of severity from mild muscle atrophy to infantile death. In most cases, the disease is caused by a lack of a functional protein called Survival of Motor Neuron 1 (SMN1). The severity of the disease is associated with gene dosage for SMN1, a fully functional protein of unknown function and a pseudogene. Survival of Motor Neuron 2 (SMN2) with similar but lower activity: One copy of a normal, fully functional SMN1 gene is enough to restore spinal neuron function to a level where an individual might not be aware that they have only functional copy. Individuals with only one functional copy are an important target for carrier screening research.

The SMN1 and SMN2 genes are near identical with only 35 bp differences in the 10,000 bp each transcript (99.65% identity). The near identical sequences make it difficult to determine the exact gene copy number of the two genes.

The Applied Biosystems CarrierScan® Assay is a high-throughput, highly-multiplexed assay for about 6,000 genetic variants related to a large collection of genetic disorders and important for carrier screening research. With a special array design, CarrierScan® incorporates the accurate detection of SMA carriers. Proprietary software and algorithms have been developed to facilitate SMN gene copy number analysis and genotype calls. In this study we analyze 386 DNA samples with CarrierScan® to detect SMA carriers and compare results with those obtained by using droplet digital PCR (ddPCR).

RESULTS

Table 1: Concordance of CarrierScan SMN1 Copy Number Detection with ddPCR

<table>
<thead>
<tr>
<th>Ascertained by CarrierScan®</th>
<th>Ascertained by ddPCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMN1 CN1</td>
<td>7</td>
</tr>
<tr>
<td>SMN1 CN2+</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 2: Concordance of CarrierScan SMN1 g.27134T>G Detection with ddPCR

<table>
<thead>
<tr>
<th>Genotyping by CarrierScan®</th>
<th>Genotyping by ddPCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>With T&gt;G mutation</td>
<td>19</td>
</tr>
<tr>
<td>Without T&gt;G mutation</td>
<td>0</td>
</tr>
</tbody>
</table>

Both methods identify the same 7 samples as having SMA carrier status and the same 19 samples as having the SMN1 g.27134T>G mutation. The CarrierScan® and ddPCR data were 100% concordant for SMA carrier state and g.27134T>G genotype. Concordance for SMN1 copy number was 94% and concordance for SMN2 copy number was 82%.

CONCLUSIONS

The results show that the highly-multiplexed CarrierScan® assay is very effective and accurate for detecting SMA carrier genotypes and the SMN1 g.27134T>G mutation.

ACKNOWLEDGEMENTS

The authors acknowledge the contributions of Doron Behar in motivating and testing the inclusion of SMA testing for Expanded Carrier Screening (ECS) research.

TRADEMARKS/LICENSEING

CarrierScan® is a registered trademark of Thermo Fisher Scientific.

Figure 1. Inheritance of an autosomal recessive disorder

Figure 2. Applied Biosystems CarrierScan1S 96-Array Plate