Detecting Novel Variants in Alpha Thalassemia Carriers

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Presented by Genevieve Gould, PhD

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Disclosure Slide

Financial Disclosure for:
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Employee and share holder
Background

- Alpha thalassemia is caused by the loss of alpha globin chains encoded by HBA1 and HBA2.

- Alpha Thalassemia carrier screening is recommended for all women who are pregnant or planning a pregnancy.¹

<table>
<thead>
<tr>
<th># of HBA copies</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Normal</td>
</tr>
<tr>
<td>3</td>
<td>Carrier, no α-thalassemia</td>
</tr>
<tr>
<td>2 (cis)</td>
<td>“α-thalassemia trait” → mild anemia</td>
</tr>
<tr>
<td>2 (trans)</td>
<td>“HbH disease” → moderate-severe anemia</td>
</tr>
<tr>
<td>1</td>
<td>“Hb Bart’s hydrops foetalis” → lethal</td>
</tr>
<tr>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>
Background

• Determining alpha thalassemia carrier status via NGS is technically challenging because of high homology between \( HBA1 \) and \( HBA2 \).

• We previously developed a hybrid capture-based NGS assay that detects common copy number variants (CNVs) and the Constant Spring variant\(^2\), resulting in a 90% detection rate for alpha thalassemia in high-risk ethnicities.\(^3\)

• Here we present an improvement to the assay to identify novel variants (both single nucleotide variants (SNVs) and insertions/deletions (indels)), resulting in a >99% detection rate in high-risk ethnicities.
Methods

Hybrid Capture

• Our previously established hybrid-capture assay was updated to detect novel SNVs and indel via tetraploid calling.

• 259 patient samples were analyzed with the improved assay.

• Long range PCR (LR-PCR), utilizing unique regions in the genome, was also performed on all samples and served as an orthogonal truth dataset.
• 79 SNVs and 10 indels were identified in the set of 259 samples.

• The improved alpha thalassemia hybrid capture (HC) assay achieved 100% concordance with the LR-PCR data.

• No FNs or FPs were identified.
Conclusions

• These results demonstrate that the improved NGS can be used to detect novel SNVs and indels in the *HBA1* and *HBA2* genes.
References