Clinical testing of five hereditary hemochromatosis-related genes: Preliminary evidence for the benefit of Next Generation Sequencing

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Objectives

- Hereditary hemochromatosis (HH) is a genetic form of iron overload. In cases of excessive iron deposition, serious clinical manifestations may occur, such as liver damage, cardiomyopathy, diabetes, and arthritis.
- First described in 1996, the HFE gene leads to autosomal recessive HH with reduced penetrance.
- In the last 15 years, 4 additional genes were discovered that cause HH: HAMP, HFE2, SLC40A1, and TFR2.
- In hereditary HH, mutations in these genes cause a severe, early-onset form of HH.

Methodology

- Methods: Patients were referred for full gene sequencing of HFE, HAMP, HFE2, SLC40A1, and TFR2 using Next Generation Sequencing (NGS).
- Our sequencing analysis covers clinically-validated genes in the last 2014 guidelines.
- The diagnostic yield of sequencing for all five HH genes was determined.
- Patients who had been sequenced for a subset of the five genes were analyzed separately.

Assay Design: Invitae is a CLIA-certified clinical diagnostic laboratory performing full gene sequencing and deletion/duplication analysis NGS. Our sequencing analysis covers clinically-important regions of each gene including coding exons, at least +/- 10 base pairs of intronic sequence, and known pathogenic variants in non-coding regions.

Results

- There were 15 additional patients who had sequencing of 1-3 of the available genes.
- Results for those patients consisted of 1 p.H63D HFE homozygote, 3 HFE heterozygotes (2 p.H63D and 1 p.C282Y) and 1 novel homozygous pathogenic mutation in TFR2 c.1409G>T (p.Ser470Ile).

Table 1. Summary of results among 41 patients who had testing for 5 genes

<table>
<thead>
<tr>
<th>Results of Pathogenic Mutations</th>
<th>No. of Patients</th>
<th>Sex</th>
<th>Ethnicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>HFE pathogenic mutations</td>
<td>6 total</td>
<td></td>
<td></td>
</tr>
<tr>
<td>c.845G&gt;A (p.C282Y) homozygote</td>
<td>1 &lt;10</td>
<td>M</td>
<td>Caucasian</td>
</tr>
<tr>
<td>c.187C&gt;G (p.H63D) c.845G&gt;A (p.C282Y) compound heterozygous</td>
<td>1 late teens</td>
<td>F</td>
<td>unknown</td>
</tr>
<tr>
<td>c.187C&gt;G (p.H63D) homozygous (low penetrance)</td>
<td>4 Avg. 36</td>
<td>M: F; 2</td>
<td>1 Caucasian 2 unknown 1 Hispanic</td>
</tr>
<tr>
<td>SLC40A1 pathogenic mutations</td>
<td>2 total</td>
<td></td>
<td></td>
</tr>
<tr>
<td>c.430A&gt;T (p.N141Y) heterozygous</td>
<td>1 late teens</td>
<td>M</td>
<td>Caucasian</td>
</tr>
<tr>
<td>c.533G&gt;A (p.R178Q) heterozygous (likely pathogenic)</td>
<td>1 30’s</td>
<td>M</td>
<td>Caucasian</td>
</tr>
<tr>
<td>HFE2 pathogenic mutations</td>
<td>1 total</td>
<td></td>
<td></td>
</tr>
<tr>
<td>c.959G&gt;T (p.G320V) homozygote</td>
<td>1 20’s</td>
<td>M</td>
<td>Caucasian</td>
</tr>
<tr>
<td>HFE carriers (7 p.H63D and 2 p.C282Y)</td>
<td>10 total</td>
<td>Avg. 41</td>
<td>M: F 6</td>
</tr>
</tbody>
</table>

- No mutations found 18 total

Conclusions

- The sequencing technology of NGS makes it possible to test multiple genes at the same time.
- In this cohort, sequencing of HFE, HAMP, HFE2, SLC40A1, and TFR2 genes resulted in an additional diagnostic yield compared to HFE C282Y and H63D testing alone.
- In patients who have a genetic explanation for their HH, management can be personalized based on genotype-phenotype correlation (e.g. N141Y SLC40A1 mutations may lead to reduced phlebotomy tolerance) and at-risk family members can be screened.
- Accurate risk assessment provides information about recurrence risk and family members (ex. SLC40A1 has dominant inheritance, and therefore much higher risks to family members than hemochromatosis caused by HFE, which is autosomal recessive).
- In addition, all patients in this sample with non-HFE positive results were reportedly Caucasian, highlighting the benefit of sequencing multiple genes regardless of ethnic background. Choosing which genes to test based on age of onset or ethnicity can be difficult, as there is substantial overlap in phenotype. By testing all five genes the yield can be maximized.
- This preliminary study is an important step toward gaining a better understanding of the genetics of HH. Ultimately, NGS data may make it possible to update current clinical guidelines for HH.

References


Notes:

1. Pathway NGS results are presented on all patients who were tested for HH at a different laboratory, and are intended for use in guiding the future hemochromatosis gene analysis (HAMP, HFE2, SLC40A1, TFR2).
2. Four patients who were heterozygous for SLC40A1 c.959G>T (p.G320V) were also identified to have a VUS (heterozygous TAFIR1 c.1009C>T (p.Y337fsC)).