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

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Background

• 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. In 1996, HH gene mutations can lead to autosomal recessive HH.

• In the last 15 years, 4 more genes were discovered that cause HH: HAMP (hepcidin), HFE2 (hemoglobin), SLC40A1 (ferroportin), and TFR2 (transferrin receptor 2). There is some evidence that sequence changes in HAMP, HFE2, and TFR2 may interact with homologous HFE mutations, causing a more severe phenotype.

Table 1. Summary of the five hereditary hemochromatosis (HH)-causing genes

<table>
<thead>
<tr>
<th>Gene</th>
<th>Inheritance</th>
<th>Penetration</th>
<th>Phenotype</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>HFE</td>
<td>Recessive</td>
<td>Incomplete</td>
<td>Severe, early onset (&gt;30yr); hypogonadism and cardiomyopathy more prevalent</td>
<td>Multiple mild and severe mutations</td>
</tr>
<tr>
<td>HFE2</td>
<td>Recessive</td>
<td>Incomplete</td>
<td>Severe, early onset (&lt;30yr); hypogonadism and cardiomyopathy more prevalent</td>
<td>May have lower phenotypic expression</td>
</tr>
<tr>
<td>TFR2</td>
<td>Recessive</td>
<td>Incomplete</td>
<td>Similar symptoms as HFE type, but intermediate age of onset (young adulthood)</td>
<td></td>
</tr>
<tr>
<td>SLC40A1</td>
<td>Recessive</td>
<td>Incomplete</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SLC40A1</td>
<td>Recessive</td>
<td>Incomplete</td>
<td>May have lower phenotypic expression</td>
<td></td>
</tr>
<tr>
<td>HFE2</td>
<td>Dominant</td>
<td>Complete</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Current HH testing guidelines only exist for the most common HH mutations (C282Y and low penetrance H63D), with no specific recommendations regarding full gene sequencing for any of the HH genes.

Recent research suggests that sequential sequencing may be beneficial in patients who test negative for the most common HH mutations, exhibit a more severe or early-onset phenotype compared to what is normally seen in HFE-related HH, and/or are of non-Caucasian ethnicity.

Next Generation Sequencing (NGS) is a new high-throughput sequencing technology that allows testing of multiple genes concurrently. It detects rare and novel HH-causing mutations that are not typically assessed using targeted methods. However, sequencing can also identify sequence changes known as variants of uncertain significance (VUS) - changes that have not yet been characterized as disease-causing or benign.

This poster summarizes the results of clinical NGS for the five HH-related genes, and shows preliminary evidence as to its increased diagnostic yield for HH diagnosis.

Methodology

Methods: Patients were referred for full clinical gene sequencing of HFE, HAMP, HFE2, SLC40A1, and/or TFR2 using NGS (Illuama MiSeq) to a CLIA certified laboratory (Invitae, San Francisco, CA). Results from patients with a clinical indication of iron overload or HH who were tested from September 2013 through July 2014 were analyzed. The diagnostic yield of sequencing for all five HH genes was determined. Patients who only had sequencing for a subset of the five genes were analyzed separately. Patients who had testing for a familial mutation were excluded from the review.

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

Results

• Patient demographics: In total, 56 patients underwent HH-related NGS, of which 41 patients had testing for all 5 genes. Of the total 56, 35 (62.5%) were males and 21 (37.5%) were females. Ages ranged from 3-77yrs (avg. 40.5yrs), Fifty-one percent were Caucasian, 9% Hispanic, 4% African American, 16% Asian, and 20% not specified.

• Forty-one patients were tested for all five genes.

• HH-causing mutations were found in 9 patients (21.9%):
  - Six (14.6%) were either hemoglobinopathies or compound heterozygotes for the c.187C>G (p.H63D) or c.845G>A (p.C282Y) HFE mutations.
  - Ten (24.4%) were heterozygous carriers of an HFE mutation.
  - Six patients (14.6%) were identified to have a VUS.
  - Four patients (9.8%) had VUSs and no other findings.
  - Two VUSs were found in patients who had another pathogenic mutation.
  - In 18 patients (43.9%), no pathogenic mutations or VUSs were found.
  - There were 15 additional patients who had sequencing of 1-3 of the available genes. Results for those patients included 1 p.H63C homozygote, 3 p.H63D and 1 p.C282Y and 1 novel homologous pathogenic mutation in TFR2 c.1429G>T (p. Ser470Ile).

Table 2. Summary of results among the 41 patients who had testing for 5 genes

<table>
<thead>
<tr>
<th>Gene</th>
<th>% of Patients</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Ethnicity</th>
<th>Indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>HFE</td>
<td>c.845G&gt;A (p.C282Y)</td>
<td>1</td>
<td>&lt;10 M</td>
<td>Caucasian</td>
<td>Severe hyperferritinemia, clinical hemochromatosis</td>
</tr>
<tr>
<td>HFE2</td>
<td>c.187C&gt;G (p.H63D) compound heterozygous</td>
<td>1</td>
<td>late teens F</td>
<td>unknown</td>
<td>Polycythemia, hypermobile joints, hemochromatosis NGS</td>
</tr>
<tr>
<td>SLC40A1</td>
<td>(low penetrance)</td>
<td>30% F</td>
<td>Caucasian</td>
<td>Iron overload</td>
<td></td>
</tr>
<tr>
<td>HFE2</td>
<td>c.845G&gt;A (p.C282Y)</td>
<td>30% M</td>
<td>Hispanic</td>
<td>Non-HFE iron overload syndrome: Advanced liver dz, portal hypertension, liver bx and MELD</td>
<td></td>
</tr>
<tr>
<td>SLC40A1</td>
<td>homozygous</td>
<td>50% M</td>
<td>unknown</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>No mutations found</td>
<td>18 total</td>
<td>Avg. 46 (16-77)</td>
<td>M: 13; F: 5</td>
<td>7/18 Caucasian, 5/18 Asian</td>
<td>Various indications, including clinical hemochromatosis, hemochromatosis, iron overload</td>
</tr>
</tbody>
</table>

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. N144Y 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 risk to family members (e.g. 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


