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 (hepcidin), HFE2 (hemojuvelin), SLC40A1 (ferroportin), and TFR2 (transferrin receptor 2). HAMP, HFE2 and TFR2 mutations are inherited in a recessive pattern, whereas SLC40A1 mutations are inherited in a dominant pattern. HAMP and HFE2 mutations cause a severe, early-onset form of HH. There is some evidence that sequence changes in HAMP, HFE2 and TFR2 may interact with homozygous HFE mutations, causing a more severe phenotype.
- Current HH testing guidelines only exist for the most common HFE 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 HFE 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 and can detect rare and novel HH-causing mutations that are not typically assayed 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 abstract 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 clinical full gene sequencing of HFE, HAMP, HFE2, SLC40A1, and/or TFR2 using NGS (Illumina MiSeq) to a CLIA certified laboratory. Results from patients with a clinical indication of iron overload or HH who were tested from 9/2013 - 7/2014 were reviewed. 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 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

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. 40.9yrs). 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 homozygous or compound heterozygous for the c.187C>G (p.H63D) or c.845G>A (p.C282Y) HFE mutations.
    - Three (7.3%) had mutations in non-HFE genes: SLC40A1 c.430A>T (p.N144Y) pathogenic heterozygous, SLC40A1 c.533G>A (p.R178Q) likely pathogenic heterozygous, and HFE2 c.959G>T (p.G320V) pathogenic homozygous.
  - Ten patients (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.

Results (continued)

- 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 the 41 patients who had testing for 5 genes

Results	# of Patients	Age (years)	Sex	Ethnicity
HFE pathogenic mutations	6 total			
c.845G>A (p.C282Y) homozygous[1]	1	<10	M	Caucasian
c.187C>G (p.H63D) c.845G>A (p.C282Y) compound heterozygous	1	late teens	F	unknown
c.187C>G (p.H63D) homozygous (low penetrance) [1]	4	Avg. 36 (16-57)	M:2 F: 2	1 Caucasian 2 unknown 1 Hispanic
SLC40A1 pathogenic mutations	2 total			
c.430A>T (p.N144Y) heterozygous[2]	1	late teens	M	Caucasian
c.533G>A (p.R178Q) heterozygous (likely pathogenic)	1	30's	M	Caucasian
HFE2 pathogenic mutations	1 total			
c.959G>T (p.G320V) homozygous	1	20's	M	Caucasian
HFE carriers (7 p.H63D and 2 p.C282Y) [3]	10 total	Avg. 41 (16-52)	M:4 F:6	9/10 Caucasian
VUS findings in patients with no pathogenic mutations	4 total			
HFE2 c.512G>A (p.Gly171Glu) homozygous	1	20's	M	Hispanic
TFR2 c.1682+9G>C (p.) intronic heterozygous	1	30's	F	Asian
SLC40A1 c.626C>T (p.Ser209Leu) het (SLC40A1 is AD form of HH)	1	30's	F	Asian
TFR2 c.2389A>G (p.Ile797Val) het	1	50's	M	African American
VUS findings in patients with other pathogenic mutations[2,3]	2 total			
No mutations found	18 total			

[1] Positive HFE results are presented on two patients who were tested for HFE at a different laboratory, and had testing for the other four HH-causing genes at Invitae (HAMP, HFE2, TFR2, SLC40A1).
[2] One patient, who was heterozygous for SLC40A1 p.Asn144Tyr, was also identified to have a VUS, heterozygous HFE2 c.1004G>A (p.Arg335Gln).
[3] One patient, who was heterozygous for HFE p.H63D, was also identified to have a VUS, heterozygous TFR2 c.2269G>A (p.Gly757Arg).

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 (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.

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