A comprehensive low-cost diagnostic test for hundreds of inherited conditions

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Abstract

Background:
Historically, diagnostic tests using DNA sequencing have only been offered for a limited set of genes to patients with specific clinical indications. The high cost of de-facto-standard assays (Sanger sequencing, MLPA, etc.), and more importantly, the high cost and challenges in clinical data interpretation have been cited among the reasons for this. Thus, many genetic diseases and clinically important genetic conditions often go undiagnosed.

Methods:
We have developed an in-house infrastructure for NGS-based diagnostic assay development, validation, and operation in a CLIA environment. To date, we have conducted a thorough scientific review of the literature for over 500 genes and their associated conditions, storing validated gene sequences, transcripts, risk models, and over 32,000 clinically characterized variants in a database used both to optimize assay design and to help interpret results. We have a hybrid calling and data QC pipeline employing GATK, Freebayes, and custom algorithms for different variant classes. Preliminary reports for known and novel SNVs, indels, and CNVs are automatically generated for review, and a team of medical specialists then classifies variants according to ACMG guidelines given the patient's indication and signs-out finalized clinical reports.

Results:
CLIA validation of our test menu was completed in 2012. Large-scale clinical and validation studies are in process for our upcoming update, from which preliminary results are presented below.

Conclusions:
In collaboration with other labs and patient advocates, we have launched an effort to expand the publicly available set of unpublished clinical variants that we believe will be critical in diagnostic settings. Most importantly, we believe these processes are highly scalable, allowing the assay to grow to report on the vast majority of gene-conditions with high accuracy.

Gene Curation & Assay Design

Curation and Review:
Extensive review of the available literature and public database entries for each gene to catalog:
- Characteristics of genetic disorder(s) such as disease penetrance, inheritance, and known pathogenic variants.
- Molecular characteristics of the gene including known isoforms and their detailed coding and non-coding gene structure. Identify technically “tricky” regions to assay.

After review, genes are organized into curated test panels by condition.

Assay Design:
INVITAE is a CLIA-certified clinical diagnostic laboratory performing full gene sequencing using next-generation sequencing technology.

Clinically important regions of each gene are targeted:
- Coding exons
- Splice sites: at least ±10 base pairs at intron/exon junctions
- Regions with known pathogenic variants.
- Deletion/duplication (copy number variant) detection—Coming this Fall

Case Study: Broad Cancer Testing

Background:
As part of a research collaboration with Stanford University, we applied an early version of our hereditary cancer multi-gene panel to bio-banked samples from consented breast and ovarian cancer patients with family histories of cancer.

Patient:
Female, diagnosed with unilateral breast cancer in her mid40s.

Previous Results:
At the time of diagnosis, the patient received a negative BRCA1/2 test report from an independent laboratory.

New Results:
The patient was found to have a pathogenic variant in the gene MLH1 associated with Lynch syndrome.

Follow-up:
Following IRB-approved protocol, the patient was re-contacted and the MLH1 results independently confirmed and communicated to her.

Outcome:
In the time between the BRCA1/2 and gene panel tests, the patient had been diagnosed with endometrial cancer. Additionally, a baseline colonoscopy had been performed at age 50 with negative results, and she had been told to come back at age 60. Following communication of her MLH1 status, she underwent an early second colonoscopy and a polyp was found and removed. She is currently 53 years old, so this tubulovillous adenoma was caught 7 years earlier than if no broad genetic test had been performed.

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Open Access:
INVITAE is a supporter of Free the Data, a grassroots movement where patients, physicians, and researchers are contributing their genetic variant data to support genetics research, advance understanding of genetic disease, and enhance treatment options.

The largest source of medically relevant information on human genetic variation lies in diagnostic labs. However, most of these data are never published or released.

With patient consent, INVITAE is committed to the ongoing public release of de-identified clinical variants.

Learn more at www.freethe-data.org and clinvar.invitae.com