Description of high-throughput sequencing tests for genetic disorders offered by the Personalized Genomic Medicine Laboratory of Columbia University Medical Center

Overview

The human genome contains in excess of 20000 protein coding genes. In a constantly updated encyclopedia of mendelian human disorders, OMIM, as of October 16, 2012, there are about 3600 phenotypes described in which the molecular basis of the disease is known. There is also an additional 3600 phenotypes listed that have no known molecular basis to date. The number of genes described on the site is past 14,000, and all these genes are thought to be relevant to human disease, although maybe only half of them have a confirmed role in one or more genetic disorders.

The current paradigm for genetic diagnosis rests on PCR and microarray-based detection of specific mutations of known significance and/or Sanger sequencing that allows detection of previously described, as well as novel mutations, in genes that have been well established to play a role in a specific disease. These methods work well for screening for specific mutations or for demonstration of mutations in a small number of genes, if the gene(s) to test can be largely ascertained based on the clinical phenotype. However, they fall short in cases where the phenotype and genotype correlation is not strong enough to efficiently guide the decision-making process as to what mutation in which gene should be tested for. In cases like congenital hearing loss in which over 100 loci have been implicated, it is often impossible to establish in what order to perform the review of genes linked to the phenotype. Even if a more logical phenotype based approach is available, like in the case of muscular dystrophies, the diagnostic odyssey is often too painful and expensive to undertake. Since genetic information is changing at a rapid pace, clinicians may be unaware of all known genes and, therefore, fail to order the appropriate tests. NGS allows for simultaneous sequencing of large number of genes that might carry mutations causing the symptoms of a patient at an affordable price. This prevents individual clinician bias in testing decisions and improves diagnostic success rate.

We offer four clinical NGS tests at the Personalized Genomic Medicine Laboratories at Columbia University Medical Center. These are full mitochondrial genome sequencing (MGS), the Columbia Combined Genetic Panel (CCGP), whole exome sequencing (WES) and whole genome sequencing (WGS) tests. The MGS and CCGP tests are for patients whose disease shows a very characteristic phenotype strongly associated with mutations in the mitochondrial genome, or in a few dozen candidate genes. The WES and WGS tests are to be used in situations where one cannot generate a credible list of candidate genes to be tested. These tests interrogate all coding regions or the entire genome, respectively. In addition to identifying known disease causing mutations and probable disease causing mutations in disease associated genes they can also identify entirely new private mutations in genes previously not linked to the disease.

Confirmation of the disease causing nature of these novel mutations is based on segregation of the mutation in families and structural and functional characteristics of the mutation and the gene itself.

Indication for testing

- The presence of a congenital developmental abnormality of presumed genetic origin
- Development of symptoms that suggest the presence of a genetic disorder with mitochondrial or Mendelian inheritance

Methodology

- Hardware: Illumina sequencing instruments: MiSeq for MGS and CCGP tests and HiSeq2500 for WES and WGS tests.
- Capture reagents: We use PCR to capture the mitochondrial genome and Agilent Sureselect technology to capture the regions of interest (ROI) for the CCGP and WES studies. The WGS does not require capture, since the entire genome is sequenced.

Specimen requirements

- All specimens should carry two independent identifiers.
- Blood > 300microliters, should be anti-coagulated, preferably with citrate or EDTA and should be less than a week old.
- Muscle biopsy specimens (>50mg) should be refrigerated from the time of biopsy to arrival to the lab.
- DNA should be greater than 10kb median length. We optimally need 3 micrograms of genomic DNA at concentrations 50-200ng/microliter.