Whole-exome sequencing for research in complex pediatric-onset disorders

"With whole-exome sequencing there is less data to transfer, analyze, and interpret, which is an important consideration, and it's more cost-effective with a faster turnaround time."

Dr. Christian Marshall

Introduction

The search for rapid, cost-effective genetic testing solutions

Genetic testing is now available for more than 2,000 rare and common diseases, and more tests are being adopted as more genetic markers are discovered [1]. Many of these assays are based on single-gene analysis or microarrays, and can cost up to $10,000 per test. The Hospital for Sick Children in Toronto is conducting several research projects to test the utility of whole-genome and whole-exome sequencing (WES). Dr. Christian Marshall is leading the hospital's research efforts to evaluate the Ion Proton™ System for cost-effectiveness in operations, concordance with gene panels, and the yields of whole-exome sequencing in studying complex disorders like autism.

Fast facts:

Fields of research:
Complex pediatric-onset diseases, autism

Applications/techniques:
Sanger sequencing, next-generation sequencing

Featured products:
Ion Proton™ System, Ion Reporter™ Software

Customer profile

The Hospital for Sick Children adopts next-generation sequencing (NGS)

Dr. Christian Marshall is a research associate in genetics and genome biology at the Hospital for Sick Children in Toronto, Ontario, Canada. He evaluates new genomics technologies and applies those technologies to disease gene discovery. The Genomics Center at the hospital has been shifting away from running about 10,000 microarrays a year to using the Ion Proton™ System with Ion AmpliSeq™ technology to evaluate whole-exome sequencing for clinical research.

The challenge

Proving the utility of whole-exome sequencing

Although the exome comprises less than 1% of the genome, it contains more than 85% of known disease-causing mutations [1]. Until the cost and complexity of whole-genome sequencing is reduced, whole-exome sequencing provides a cost-effective alternative to traditional methods for identifying single nucleotide variants (SNVs), small indels, and copy number variations. This may, in the future, help determine the cause of monogenic Mendelian diseases or even complex conditions like autism spectrum disorder.

life technologies

"With the trio analysis feature of Ion Reporter™ [Software], we discovered a mutation we weren't really looking for, helping us revisit the phenotype from a different perspective."

Dr. Christian Marshall

However, whole-exome sequencing increases the amount of data a laboratory has to analyze. While not as much data are produced as with whole-genome sequencing, exome sequencing does place an increased demand on the bioinformatics pipeline and the amount of analysis necessary to understand any given disease type. On the other hand, as new genes are discovered, it becomes challenging to scale up genetic testing with traditional panel sequencing and microarrays. Shifting to whole-exome sequencing enables labs to focus on running just a single assay.

Solution

Putting WES to the test with the Ion Proton™ System

To test the utility of whole-exome sequencing, Dr. Marshall and his colleagues looked at 25 samples from subjects with a range of disorders, including focal segmental glomerulosclerosis (FSGS), cone-rod dystrophy, ocular albinism, Stargardt disease, dystonia, cerebellar atrophy, glycosylation disorder, epilepsy, cardiology, hypertrophic cardiomyopathy, and period fever syndrome. With this project, whole-exome sequencing was tested for its concordance with traditional methods and for its ability to find mutations that could not be found using traditional gene panels. In a separate study, Dr. Marshall looked at samples from a subject previously diagnosed with a very rare disorder, Adams-Oliver Syndrome (AOS), using whole-exome sequencing data to find mutations in the four genes believed to be implicated in the disorder.

Finally, the team looked at data and archived samples from subjects previously diagnosed with autism, and sequenced the exomes of more than 600 samples. For all exome studies, the team used the Ion Proton™ System and Ion AmpliSeq™ exome workflow to sequence exomes in a rapid, cost-effective fashion.

Results

Concordance with traditional gene panel sequencing

Dr. Marshall's group found very high concordance of calls between gene panel testing and whole-exome sequencing on the Ion Proton™ System. In the case of an archived sample from a subject previously diagnosed with focal segmental glomerulosclerosis (FSGS), whole-exome sequencing discovered a mutation in a gene that was not on the FSGS gene panel—a variant in *PLCE1* that explained the phenotype. Furthermore, whole-exome sequencing identified additional causal variants in other genes that were not on the various gene panels.

Discovering a mutation in the Adams-Oliver syndrome (AOS) case

For the AOS case, whole-exome sequencing did not identify variants in the four known genes that could explain the phenotype. However, the team was able to get retrospective samples from the affected proband's parents, enabling trio analysis to identify inherited variants. The team used the trio analysis workflow in Ion Reporter™ Software and uncovered a new mutation in exon 8 of the gene *ACVR1*, which is associated with fibrodysplasia ossificans progressive (FOP), a disorder similar to AOS.

Autism Genome Project

For the Canadian Autism Genome Project, Dr. Marshall and his team are using microarrays, whole-exome sequencing, and whole-genome sequencing for a combined high-resolution genome analysis. This analysis has included working with over a million SNP microarrays, and comparing copy number variation across the different techniques. One of the objectives of adding WES to the study is to develop a clinical research exome report for the Autism Genome Project.

Autism has complex causes; the team at the Hospital for Sick Children uses a list of approximately 125 candidate genes known to be associated with autism. Not all 125 genes need to be mutated in autism; in many cases, it may be caused by one or two variants. The team looks at *de novo* variants in addition to these candidate genes. They found that typically about 25% of the cases have a variant, either a loss-of-function mutation in the 125 candidate genes or a *de novo* mutation that may be related to autism.

The Ion Torrent™ WES advantage

Dr. Marshall found the Ion AmpliSeq™ exome sequencing workflow to be a simple and reliable solution, requiring less than 60 minutes of hands-on time. The team currently sequences two exomes with an Ion PI™ Chip (v1 or v2), providing coverage of over 90% of bases at over 20x depth, quickly providing annotated filtered variants. The AmpliSeq™ Exome Kit contains about 300,000 primer pairs across 12 pools, and requires a very low amount of input DNA (usually ~50 ng). The team has found that constructing the library, preparing the template, sequencing, and analyzing data can be performed in just a few days, a remarkable improvement over traditional methods.

Conclusion

Whole-exome sequencing offers tremendous potential in clinical research labs

The ongoing research efforts at the Hospital for Sick Children have underscored the Ion Proton™ System's utility for performing whole-exome sequencing in the clinical research setting. The cost of whole-exome sequencing is quickly becoming lower than that of standard genetic tests. The team found very high concordance with gene panel testing, as well as the ability to discover new variants in a number of rare or complex diseases, including copy number variation. In addition, these studies highlight the importance of whole-exome trio analysis involving archived samples from subjects diagnosed with various disorders. Such expanded research studies may show how disease-causing variants may be inherited from two unaffected parents.

Reference

1. NIH Genetic Testing Registry, http://www.ncbi.nlm.nih.gov/gtr/

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