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Enhancing Diagnosis Through RNA Sequencing 113

David R. Murdock

The diagnostic rate of comprehensive genomic sequencing remains only 25% to 30% due to the difficulty in interpreting variants of uncertain significance and noncoding mutations and in elucidating downstream effects of these and other genetic changes. Unlike DNA sequencing, RNA sequencing (RNAseq) reveals the functional consequence of genetic variation through the detection of abnormal gene expression levels, differences in gene splicing, and allele-specific expression. RNAseq can provide nearly 40% improvement in diagnostic rates depending on disease and tissue source. In this burgeoning era of precision medicine, RNAseq offers a powerful tool to improve diagnostic rates and understand disease mechanisms.

The Clinical Application of RNA Sequencing in Genetic Diagnosis of Mendelian Disorders 121

Sarah L. Stenton and Holger Prokisch

Molecular genetic approaches have evolved at an astonishing pace resulting in increasingly routine use of whole exome sequencing in Mendelian disorder diagnosis. After whole exome sequencing, 50% to 75% of patients remain without a genetic diagnosis, indicating limitations in variant calling and prioritization and a role for noncoding variants. Whole genome sequencing has the potential to reveal all genetic variants; however, it escalates the challenge of variant prioritization owing to the vast numbers called. Promising approaches to aid in variant interpretation include the integration of functional genomic data such as transcriptome sequencing, which achieves diagnostic yields of 10% to 35%. International-scale collaboration and establishment of data repositories are paramount in accelerating the diagnosis of Mendelian disorders.

The Evolution of Constitutional Sequence Variant Interpretation 135

Jessica Mester and Tina Pesaran

A combination of different types of evidence incorporating population data, functional studies, clinical data, and predictive tools is necessary for thorough, thoughtful variant classification. Variant classification criteria may be optimized in a quantitative, gene-specific manner using validated predictors of pathogenicity for genes or conditions with sufficient information. Large-scale data (genome sequencing of healthy and affected cohorts, high-throughput functional studies, and in silico metapredictors) increase the robustness of evidence used for variant classification and

lend themselves to incorporation in quantitative frameworks. Collaborative efforts by laboratories and disease-specific expert groups reduce variant classification discrepancies and improve the quality of variant interpretation information available to patients and researchers.

Clinical Bioinformatics in Precise Diagnosis of Mitochondrial Disease **149**

Lishuang Shen, Elizabeth M. McCormick, Colleen Clarke Muraresku, Marni J. Falk, and Xiaowu Gai

Clinical bioinformatics system is well-established for diagnosing genetic disease based on next-generation sequencing, but requires special considerations when being adapted for the next-generation sequencing-based genetic diagnosis of mitochondrial diseases. Challenges are caused by the involvement of mitochondrial DNA genome in disease etiology. Heteroplasmy and haplogroup are key factors in interpreting mitochondrial DNA variant effects. Data resources and tools for analyzing variant and sequencing data are available at MSeqDR, MitoMap, and HmtDB. Revised specifications of the American College of Medical Genetics/Association of Molecular Pathology standards and guidelines for mitochondrial DNA variant interpretation are proposed by the MSeqDr Consortium and community experts.

Bioinformatics in Clinical Genomic Sequencing **163**

Matthew S. Lebo, Limin Hao, Chiao-Feng Lin, and Arti Singh

Clinical bioinformatics encompasses generating raw sequence data from the machine through identifying reportable variants. Throughout the process, important quality control metrics are tracked based on the data, including the completeness of coverage across the region of interest for the assay. The process starts by taking raw sequence data, aligning it to a reference genome, and identifying variants based on the quality of the reads and the base pair calls. Variants are then annotated and filtered using a variety of features, including gene, transcript, Human Genome Variation Society nomenclature, population frequency, and presence in databases. In a clinical setting, a thorough validation of each of the components of the bioinformatics pipeline is critical, as is a detailed understanding of infrastructure, privacy, and security requirements.

Precision Therapy for Inherited Retinal Disease: At the Forefront of Genomic Medicine **189**

Nicole Koulisis and Aaron Nagiel

Inherited retinal diseases (IRDs) represent a diverse array of conditions characterized by dysfunction or loss of 1 or more retinal cell types. Next-generation sequencing has enabled rapid and relatively inexpensive genotyping, with more than 250 genes identified as responsible for IRDs. This expansion in molecular diagnostic accuracy, in combination with the retina's relative accessibility and immune privilege, has fostered the development of precision therapies to treat these myriad conditions. Novel techniques are being used in early trials. Precision molecular therapies for IRDs hold great promise as diagnostic and treatment strategies continue to expand.

Therapeutic Gene Editing with CRISPR: A Laboratory Medicine Perspective 205

Elan Hahn and Matthew Hiemenz

Therapeutic gene editing with the clustered regularly interspaced short palindromic repeat (CRISPR)–Cas system offers significant improvements in specificity and programmability compared with previous methods. CRISPR editing strategies can be used ex vivo and in vivo with many theoretic disease applications. Off-target effects of CRISPR-mediated gene editing are an important outcome to be aware of, minimize, and detect. The current methods of regulatory approval for personalized therapies are complex and may be proved inefficient as these therapies are implemented more widely. The role of pathologists and laboratory medicine practitioners is vital to the clinical implementation of therapeutic gene editing.

The Future of Clinical Diagnosis: Moving Functional Genomics Approaches to the Bedside 221

Rini Pauly and Charles E. Schwartz

Whole-genome sequencing (WGS) identifies critical alterations in the genome that are not present in the coding genes. Genome-wide methylation studies identify epi-signatures that allow clarification and proper classification of variants of uncertain significance. RNA-seq, both targeted and untargeted, allows diagnosis of human disorders, particularly those in patients with a suspicious phenotype and no obvious genomic alteration. Bioinformatics tools, and neural networks, allow for the association of apparently unrelated events. Multi-omic analysis—the integrated analysis of data from various omic studies (WGS, methylation, RNAseq)—identifies coordinated interaction of variants leading to a phenotype.