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Minireview

Next generation sequencing in endocrine practice

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ABSTRACT

With the completion of the Human Genome Project and advances in genomic sequencing technologies, the use of clinical molecular diagnostics has grown tremendously over the last decade. Next-generation sequencing (NGS) has overcome many of the practical roadblocks that had slowed the adoption of molecular testing for routine clinical diagnosis. In endocrinology, targeted NGS now complements biochemical testing and imaging studies. The goal of this review is to provide clinicians with a guide to the application of NGS to genetic testing for endocrine conditions, by compiling a list of established gene mutations detectable by NGS, and highlighting key phenotypic features of these disorders. As we outline in this review, the clinical utility of NGS-based molecular testing for endocrine disorders is very high. Identifying an exact genetic etiology improves understanding of the disease, provides clear explanation to families about the cause, and guides decisions about screening, prevention and/or treatment. To illustrate this approach, a case of hypophosphatasia with a pathogenic mutation in the *ALPL* gene detected by NGS is presented.

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1. Introduction

The diagnostic approach to endocrine diseases has traditionally been based on a constellation of physical findings, biochemical testing, and imaging studies. With advances in genomics and sequencing technologies, the role of genetic diagnosis in endocrinology has taken on greater importance. The etiology of endocrinopathies is multifactorial and, when genetically determined, is frequently multigenic [1]. Yet, a significant number of endocrine disorders demonstrate Mendelian transmission, suggesting disease-causing mutations. Identification of a mutation can complement biochemical studies and provide diagnostic clues for early detection and treatment.

Historically, the use of molecular testing has been sparse, due to its expense and technical difficulty. As recently as ten years ago, DNA sequencing for medical diagnosis was unusual, performed only when there was a very strong clinical rationale to seek a genetic diagnosis. Tests were difficult to obtain, turn-around was slow, and results were often difficult to interpret; many tests were only available on a research basis. However, the transition from Sanger sequencing to high-throughput next-generation sequencing (NGS) technologies has dramatically decreased the costs and time involved in obtaining high quality DNA sequence data.

Much of this transformation derives from the fact that NGS methods allow clinicians to investigate candidate genes "in parallel" rather than "in series". Due to its cost, Sanger sequencing typically involves a serial process of sequencing one candidate gene after another, starting with the highest probability genes and proceeding to lower-probability genes, in a process referred to as "a genetic odyssey". The genetic odyssey can involve months of frustrating and expensive searching. In contrast, NGS – in which the cost of adding additional genes to a sequencing panel is minimal – permits all genes-of-interest to be sequenced simultaneously. The parallel nature of NGS therefore allows for the rapid query of multiple loci at once, including an analysis as broad as that of the entire exome or genome [2–4].

In clinical practice, then, a managing physician can quickly analyze all of the genes in a cellular pathway. It is worth noting that the benefits of this kind of parallel analysis offer more than an increase in speed and decrease in cost. Indeed, evidence is emerging that multiple "hits" (mutations) in the same pathway or in related pathways are important disease modifiers, even in disorders with classical Mendelian inheritance [5]. In other words, since a "genetic odyssey" using Sanger sequencing typically ends once a genetic "hit" is found, it risks returning an incomplete picture of diseases even when successful. The unbiased NGS-based approach, in contrast, suffers from no such ascertainment bias. NGS has been rapidly adopted in the molecular genetics community and is widely available for clinical testing. Due to the quickly decreasing cost, marked increase in availability, and significant clinical utility, molecular sequencing as a clinical diagnostic test is fast moving from the purview of academic medical geneticists into the realm of multispecialty clinical care.

Targeted NGS promises to revolutionize the diagnosis of endocrine conditions. However, the utilization of NGS is hampered by a number of factors, including uncertainty about the selection of genes that could be tested for presence of mutations. Thus, the goal of this review is to provide clinicians with a guide to NGS-based genetic testing for endocrine conditions. To illustrate this approach, a patient presenting with clinical features of hypophosphatasia who underwent targeted NGS of the *ALPL* gene is presented.

2. Material and methods

2.1. Next-generation sequencing

Targeted NGS of *ALPL* gene was performed at the University of Minnesota Medical Center, Molecular Diagnostics Laboratory and the University of Minnesota Genomics Center. Genomic DNA was extracted from the blood sample. Sequencing libraries were prepared and sequence capture performed according to Illumina protocols utilizing the TruSight One Sequencing Panel, with one minor modification. DNA libraries from clinical samples were pooled for sequence capture in groups of 10 samples (9 clinical samples plus one control sample) rather than pools of 12 samples, as recommended in the standard Illumina protocol, in order to increase read depth per sample. The enriched DNA libraries were sequenced on an Illumina HiSeq 2500 instrument using paired-end 100-bp sequencing reads, generating ≥20 M reads (4 Gb) per sample. Raw sequencing reads were mapped to the reference genome using Burrows–Wheeler Alignment [2]. Raw alignment files were realigned in the neighborhood of indels, and recalibrated for base quality accuracy using the Genome Analysis Tool Kit (GATK) [3,4]. Point mutation and indel calls in exons and adjoining intronic regions were made using the GATK Unified Genotyper. Variants were interpreted according to guidance issued by the American College of Medical Genetics [6]. Known variants with a minor allele frequency >0.01 (1%) in the 1000 genomes dataset are considered unlikely to be the cause of rare Mendelian phenotypes. Coverage is excellent under our protocol, with >20× coverage at 100% of the loci in *ALPL*, yielding a sensitivity of >95% and a specificity of >99% for all subtypes of hypophosphatasia.

2.2. Methods of data acquisition

The list of endocrine disorders and selected syndromes with multiple endocrinopathies was limited to those that are due to mutations detectable by NGS that are included in the TruSight One Sequencing Panel. Disorders of bone and mineral metabolism were limited to vitamin D deficiency and resistance, parathyroid diseases, hypophosphatasia, and hypophosphatemic rickets due to elevated FGF23 levels. Gonadal disorders were limited to those that are caused by abnormal hormone synthesis or action (*CYP21A2* mutations were excluded due to presence of pseudogenes). Inclusion of an extensive and a rapidly growing number of disorders of sex development was deemed to be outside the scope of this review. Diseases caused by chromosomal abnormalities, disorders of glucose and insulin metabolism, obesity, and lipid disorders were excluded. Individual diseases were cross-referenced against Online Mendelian Inheritance in Man (OMIM) database (http://omim.org) to provide gene and disease identifiers. Tables were constructed and most salient clinical phenotypes were listed as a guide to clinical presentation.

3. Approach to disease process and clinical implications of genetic testing

3.1. Disorders of bone and mineral metabolism

Patients with parathyroid or vitamin D disorders may come to medical attention because of either acute symptoms such as hypocalcemic seizures or more chronic manifestations of hypo- or hypercalcemia, including paresthesias, renal calculi, weakness, anorexia, polydipsia or polyuria. Phenotypically, skeletal deformities (genu varum, epiphyseal widening) may point to the diagnosis of rickets. Characteristic facial appearance (micrognathia, short palpebral fissures, prominent nose with relatively deficient alae, smooth philtrum, small ears), and presence of congenital heart defects may trigger an evaluation for DiGeorge syndrome. Short stature, obesity, and short forth metacarpals in a patient with an elevated parathyroid hormone (PTH) level and low calcium level raises a possibility of pseudohypoparathyroidism. Confirmatory biochemical testing includes measurement of calcium, phosphorus, PTH, vitamin D, alkaline phosphatase and other related blood, urine, and imaging studies.