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Review Article

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Recent Advances in the Clinical Application of Next-Generation Sequencing

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The author have no financial conflicts of interest.

ABSTRACT

Next-generation sequencing (NGS) technologies have changed the process of genetic diagnosis from a gene-by-gene approach to syndrome-based diagnostic gene panel sequencing (DPS), diagnostic exome sequencing (DES), and diagnostic genome sequencing (DGS). A priori information on the causative genes that might underlie a genetic condition is a prerequisite for genetic diagnosis before conducting clinical NGS tests. Theoretically, DPS, DES, and DGS do not require any information on specific candidate genes. Therefore, clinical NGS tests sometimes detect disease-related pathogenic variants in genes underlying different conditions from the initial diagnosis. These clinical NGS tests are expensive, but they can be a cost-effective approach for the rapid diagnosis of rare disorders with genetic heterogeneity, such as the glycogen storage disease, familial intrahepatic cholestasis, lysosomal storage disease, and primary immunodeficiency. In addition, DES or DGS may find novel genes that that were previously not linked to human diseases.

Keywords: Next-generation sequencing; High-throughput nucleotide sequencing; Diagnostic exome sequencing; Diagnostic genome sequencing

INTRODUCTION

A pediatrician requested a genetic test for a patient suspected of having congenital chloride diarrhea (CLD). A PubMed search confirmed that *SLC26A3* is the only causative gene of CLD. After extracting the DNA from the blood sample of the patient, Sanger sequencing on all coding exons of *SLC26A3* was performed, which revealed two disease-related variants. Next, the two variants were examined in the parents, and it was confirmed that each variant was inherited from one parent; therefore, the genetic diagnosis has been made successfully [1].

This example illustrates the traditional genetic diagnosis process. In other words, in order to perform a genetic diagnosis in a patient suspected of having a genetic disease, the gene that causes the disease must be first identified. If the causative gene is identified, a genetic test is performed using Sanger sequencing; if a disease-related variants are found, a genetic diagnosis is reached (**Fig. 1A**).

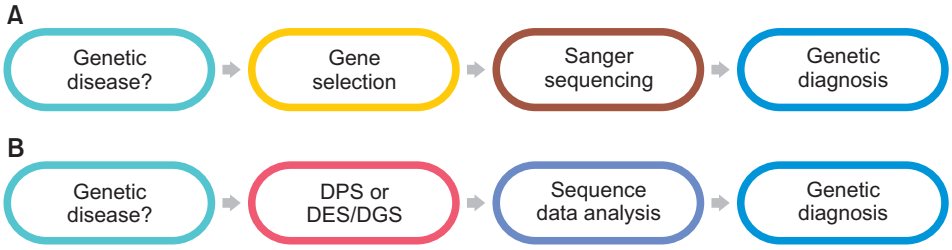

Fig. 1. Paradigm change of genetic diagnosis. (A) Traditional gene-by-gene approach using Sanger sequencing. (B) Syndrome-based genetic diagnosis process by diagnostic gene panel sequencing (DPS), diagnostic exome sequencing (DES), or diagnostic genome sequencing (DGS) using next-generation sequencing.

In Turkey 2009, a 5-month-old boy suffered from failure to thrive and dehydration, and various diseases such as the Batter syndrome were suspected, considering that the base was wet despite the presence of dehydration. Since the diagnosis was unclear, he was referred for whole exome sequencing (WES), which was emerging as a new technology at the time. WES is a test method that analyzes the coding exons of more than 20,000 human genes at once by next-generation sequencing (NGS). In this child, a disease-related variant was unexpectedly found in the *SLC26A3* gene, and thus, the patient was diagnosed with CLD [2].

This case is very important from several perspectives. First, it shows that despite the unclear clinical diagnosis, genetic testing can lead to an accurate diagnosis. Second, it proves that WES can be used for clinical diagnosis of rare diseases. Third, it has been suggested that genes may be identified using WES in diseases where the causative gene has not yet been identified.

NGS has changed the paradigm of genetic diagnosis. In the past, the gene that caused the disease had to be identified for genetic diagnosis. Specific genes were selected and analyzed one after the other through the Sanger sequencing method. However, when a genetic disease is suspected as in the aforementioned case, it is possible to analyze dozens, hundreds, or 20,000 genes at once, find disease-related variants, and then confirm the clinical diagnosis (**Fig. 1B**).

In this review, the latest findings on the clinical application of NGS, which has changed the paradigm of genetic diagnosis is presented.

TYPES OF CLINICAL NGS TESTS

While NGS is inadequate for analyzing a single gene due to the nature of the test, it is useful to analyze tens to hundreds or thousands of genes simultaneously. If the Sanger sequencing and NGS methods are compared to means of transportation, Sanger sequencing is expensive, such as a taxi or a private plane, but can selectively and conveniently move a small number of passengers, whereas NGS is a method used to move a large number of passengers, such as a large commercial aircraft.

Clinical diagnosis methods using NGS can be divided into gene panel, exome, and genome according to the composition of the gene and the range to be analyzed [3]. Considering the purpose of genetic diagnosis, these methods are named diagnostic gene panel sequencing (DPS), diagnostic exome sequencing (DES), or diagnostic genome sequencing (DGS).

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Table 1. Comparison of clinical next-generation sequencing test

Charateristics	DPS	DES	DGS
Genome coverage	Low	Intermediate	High
Sequencing depth	High	Intermediate	Low
Number of genes	Up to thousands	More than 20,000	More than 20,000
Capture bias	Yes	Yes	No
Diagnostic yield			
SNV/INDEL	High	High to intermediate	High
Intron variant	Low	Low	High
CNV	Intermediate	Intermediate	High
Gene rearrangement	Low	Low	High
Re-analysis potential	Low	Intermediate to high	High
Cost	Low	Intermediate	High

DPS: diagnostic gene panel sequencing, DES: diagnostic exome sequencing, DGS: diagnostic genome sequencing, SNV: single nucleotide variant, INDEL: insertion and deletion, CNV: copy number variation.

The difference between DPS, DES, and DGS for clinical NGS testing may be expressed in terms of how many of the three billion nucleotide sequences of the human genome are analyzed (genome coverage) and the number of times a sequence is read and analyzed (sequencing depth). In the case of DGS, the genome coverage is 90–95% and the sequencing depth is approximately 30–60×; for DES, the genome coverage is approximately 1–2% and the sequencing depth is approximately 100–200×; and for DPS, the genome coverage is 0.01–0.1% and the sequencing depth is approximately 200–500× (**Table 1**).

DIAGNOSTIC GENE PANEL SEQUENCING

DPS is called targeted gene panel, targeted exome, and focused NGS panel. This test selects tens to hundreds of genes related to a specific disease or syndrome, capturing the coding exon and adjacent intron sites of the corresponding genes, and performing NGS analysis. DPS is the most widely used for clinical diagnosis.

DPS may show a wide variety of diagnostic yields depending on the disease group, the method of selecting a study subject, and the gene panel composition. In Korean patients, the diagnostic yields of DPS have been reported in disorders of sex development (29.5%) [4], syndromic growth disorder (46.2%) [5], inherited peripheral neuropathies (27.0%) [6], developmental and epileptic encephalopathy (EIEE) (37.1%) [7], intractable early onset epilepsy (37.8%) [8], developmental delay and/or intellectual disability (29%) [9], infantile nystagmus syndrome (58.3%) [10], autism spectrum disorder, and comorbid epilepsy (17.5%) [11].

DIAGNOSTIC EXOME SEQUENCING

DES is a method of NGS analysis that captures coding exons and adjacent intron sites of all disease-related genes (Mendeliome) or all human genes (Whole Exome). DES has been clinically used before DPS. DES may include genes that are not related to genetic diseases; therefore, even if a gene causing disease is not identified in the current analysis, a reanalysis of data may be conducted to identify new causative genes [12].

When DES was initially used for genetic diagnosis, it was expected that the diagnosis rate of genetic diseases would be very high because all disease-causing genes or all human genes could be analyzed at once. However, when DES was conducted in 2013 in 250 patients with

genetic diseases, the diagnostic yield was only 24.8% (62/250) [13]. In a follow-up study, the researchers reported a 25.2% (504/2,000) diagnostic yield through DES in 2,000 patients with genetic disorders [14]. However, it was reported that the diagnostic yield was as high as 36.1% in the specific neurological disease group, while it was as low as 20.1% in the non-neuronal disease group. Another research team reported that 26% of patients with suspected genetic diseases were diagnosed through DES [15]. The Trio DES, which was performed simultaneously with parents and patients, resulted in a diagnostic yield of 31%. Since then, DES diagnostic yields ranging from less than 10% to more than 50% have been reported in various disease groups, showing a wide variety of diagnosis rates according to the disease group [16].

Even though DES is a very efficient method for genetic diagnosis, it has some limitations. DES may miss regions such as GC-rich regions, and copy number variation (CNV) such as large deletions or duplications difficult to detect [17].

DIAGNOSTIC GENOME SEQUENCING

DGS is the most comprehensive method to analyze entire human genomes, including not only genes, but also the intergenic region between genes and the intron region between exons. However, the data size is too big to handle, and the sequencing cost is too high. Recently, the cost of NGS analyses decreased, and the advantages of DGS are emphasized because the genome data analyzed through DGS have higher uniformity than DPS and DES, and most of the GC-rich regions can be analyzed. In addition, even with DES or DPS, a significant number of patients do not receive a genetic diagnosis. Therefore, DGS has been proposed as a first-tier genetic test [18].

DGS was performed as a first-tier test in 103 pediatric patients with suspected genetic diseases. The diagnostic yield was 41%, which was significantly higher than the 24% of the conventional method [19]. In another study, DGS was performed on 14 early infantile EIEE patients, and disease-related variants were found in all the patients [20]. Two of these patients were not diagnosed with DES and another three were previously tested through DPS with negative results.

DGS has several advantages over DPS and DES. First, the CNV, such as large deletions and duplications as well as genomic rearrangements can be detected. DPS and DES can also detect part of the CNV, but DGS can detect most types of CNV and genomic rearrangements [21]. According to the results of DGS as a first-tier test in 60 rare, undiagnosed, or genetic diseases, clinically important genomic findings were observed in 41 patients (68.3%), of which 20 were CNV or gross chromosome abnormalities [22]. Second, it is possible to detect a deep intronic variant. In some cases where disease-related pathogenic variants are not found in DPS or DES, the mRNA sequence may be affected by deep intronic variants [23]. For example, deep intron variants that were detected by DGS in patients with retinal dystrophy and nephronophthisis have been reported [24,25].

CONCLUSION

NGS-based DPS, DES, and DGS changed the paradigm of genetic diagnosis. DPS is most commonly used, and DES is used less frequently, but serves a prominent role in clinical

settings. DGS is rapidly approaching financial feasibility for use in everyday practice. However, even when these NGS-based genetic tests are used, approximately 50% of the patients do not receive a definite genetic diagnosis. There are many genetic reasons for this, including repeat expansion variants, somatic variants, and deep intron variants with indeterminate splicing effects, yet inadequate clinical information and the consequent inappropriate application of clinical NGS tests remain the most important cause of negative results. Therefore, the provision of detailed and accurate clinical information is a prerequisite for achieving the optimal diagnostic yield.

Clinicians and laboratory medicine doctors need to communicate closely and effectively in the process of selecting appropriate genetic tests and interpreting their results.

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