COMMENT

Genome sequencing as a single comprehensive test in molecular diagnosis

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Exome sequencing has been implemented in molecular diagnostic laboratories for a decade as a first-tier test for developmental disorders. However, for some unique disease entities, complimentary molecular tests are often needed, such as chromosome microarray analysis (CMA), multiplex ligation-dependent probe amplification (MLPA), and droplet digital PCR (ddPCR) for copy number variations (CNVs), methylation assays for imprinting disorders (e.g., Pseudohypoparathyroidism, Silver-Russell syndrome, Beckwith–Wiedemann syndrome, and Angelman syndrome), repeat-primed PCR for repeat expansion diseases, mitochondrial genome sequencing for suspected mitochondrial disorders, and gene panel with deep sequencing for mosaicism. Current stepwise approach may be cost-effective, but time-consuming. With the molecular basis being identified for more than 6,100 Mendelian disorders (https://www.omim.org/statistics/geneMap) and the concomitant reduction in sequencing costs, genome sequencing (GS) has become as a rapid testing approach to enable early implementation of precision medicine [1]. As most rare Mendelian disorders are either congenital or childhood onset, early molecular diagnosis could shorten the diagnostic odyssey, inform the clinical management, reduce healthcare costs, facilitate genetics counseling regarding the long-term prognosis and recurrence risk.

In a recent study, van der Sanden et al. demonstrates the advantage of GS as a one-test approach [2]. The authors performed GS on 150 prospective patient-parent trios with neurodevelopmental disorders. In parallel, traditional clinical genetic testing, including exome sequencing, CMA, subtelomeric MLPA, single-gene methylation assay, and repeat expansion assay, was also preformed, allowing for a fair and direct comparison of the molecular diagnostic yield. GS revealed 3 relatively large CNVs ranging in size from 600 kb to 1.3 Mb that could be readily resolved by exome CNV analysis. In addition, GS successfully made two molecular diagnoses with one or two exons deletions that were beyond the resolution of CMA or exome CNV analysis. The authors demonstrated that apart from all small variants, including single nucleotide variants (SNVs) and small insertion/deletion (indels), GS could also reveal repeat expansions in the *FMR1* gene by incorporating short tandem repeats analysis. Furthermore, GS could resolve the complex rearrangement, a deletion-duplication event at 20p13, suggesting GS is superior to exome sequencing in profiling complex genomic rearrangements, also supported by previous studies [3].

These exemplify the astonishing progress made in its utility in pediatric diagnostics. Short-read GS in principle enables to assess multiple types of variants, small or large, coding or deep intronic variants, and even non-coding regulatory variants, including variants disrupting the translation of the upstream open reading frame [4], raising the potential to replace nearly all existing clinical genetic tests (CMA, exome, mitochondrial genome sequencing) with the exception of methylation assays and karyotyping for germline variants. In recent years, with reductions in sequencing costs and sequencing error rates, emerging long-read sequencing has, however, allowed for DNA methylation profiling and better resolution of repetitive and low complexity regions while generating sequencing reads from 1,000 to over 1 million base pairs in length, opening up the possibility of its utility in clinical diagnostics as another single test to uncover SNVs, indels, SVs, repeat expansions, and methylation changes. It is widely acknowledged that we have much more to learn about the genetic basis for molecularly undiagnosed individuals with a suspected genetic disorder, not to mention a variety of pathogenic variant types that are missed by currently existing tests and variant prioritization algorithms. Despite the fact that our ability to fully interpret and utilize GS data has lagged behind, the dataset generated now could be retrospectively analyzed when improved bioinformatic tools or additional data are available. For example, it could couple with RNA sequencing to better understand deep intronic variants [5] that lead to aberrant splicing (such as exon skipping or extension). Recent studies also suggested that functional genomics, e.g., metabolic and biochemical tests that are routinely used in clinical diagnostics, can guide the variant interpretation of how pathogenic variants cause human disease.

What is clear is that exome sequencing in clinical diagnostics alone is not sufficient. Although implementing clinical GS testing in many diagnostic laboratories still poses a number of challenges as discussed by the authors, such as substantially increased bioinformatic burden associated with analysis and elevated costs related to data storage, short-read or long-read GS, a comprehensive genetic test similar or superior to clinical genetic tests currently offered, would be anticipated to become a one-test diagnostic approach and a first-tier option in clinical genetic testing.

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COMPETING INTERESTS
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ADDITIONAL INFORMATION
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