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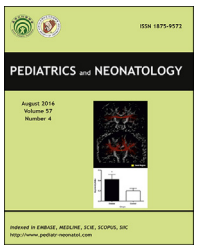

Mini Review Article

The incorporation of next-generation sequencing into pediatric care

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Abstract Genetic condition is one of the major etiologies causing morbidity and mortality in infants and children. More and more etiologies can be solved using next-generation sequencing (NGS) as it develops. Currently, whole-exome sequencing (WES) and whole-genome sequencing (WGS) have been highly integrated into clinical practice. The average diagnostic yield of WES/WGS in pediatric patients with genetic condition was around 40% (range: 21%—80%), with acceptable turnaround time and cost. The higher diagnostic yield categories are deafness, ophthalmic, neurological, skeletal conditions, and inborn error of metabolism. Positive results provide benefit with those for actionable diseases, next pregnancy planning, and family members. For those in critical condition, with the emergence of sequencing technology and bioinformatics analysis tools, provisional diagnosis can be made as short as 13.5 h using ultrarapid WGS. We believe this powerful tool has changed pediatric daily practice.

Pediatric onset diseases are highly related to genetic etiology

The genetic conditions and congenital anomalies have been estimated to affect 3%—6% of live births.[1] These conditions may result in 10%—30% of newborns being admitted to neonatal intensive care unit or even lead to early mortality.[2] No matter the incidence of these diseases is rare or not, they may still cause significance healthcare burden; 80% of rare diseases belong to genetic conditions, and 75% of them affect children that may cause severe morbidity requiring hospitalization and lead to mortality.[3] Moreover, 30% of children born with a rare disease may not live to see their fifth birthday,[4] highlighting the devastating condition of some genetic diseases. To provide these patients better care, understanding the molecular basis of the causative etiology is essential. Although chromosomal anomalies and copy number variations (CNVs) account for certain numbers (~35%) of birth defects, single-gene diseases also possess significance proportions of causes (~20%).[5] With the increasing number of new genes identified, the importance of single-gene etiology is apparently increasing.

Abbreviations: WES, whole exome sequencing; WGS, whole genome sequencing; NGS, next generation sequencing.

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Exome and genome sequencing are useful tools to diagnose pediatric genetic conditions

In the past fifteen years, WES and WGS have been successfully applied to pediatrics population in a variety of disease groups as well as severely ill infants. The diagnostic yield is 21%–80% with changes in clinical management in 42%–78% of patients.[3,6,7] There is no doubt that this tool is the most powerful tool in the diagnosis of pediatric patients with genetic condition.

WES/WGS initially only can detect single nucleotide variation and small indels in the exonic region. With the progress of bioinformatics tools, in the current era, not only CNVs and splicing variants, but also several other structural variations (trinucleotide repeats) can be identified in short-read sequencing.[8] Although there are limitations in trinucleotide repeat detection for those longer segments, this is still a huge progress.

The reported diagnostic yield of WES/WGS is between 21% and 80%, although the majority is reported to be around 40%–60%.[9,10] Compared with other disease categories, such as ophthalmological, auditory, craniofacial, metabolic, and neurological conditions, patients presenting with endocrine, hematological, renal and cardiac condition have relatively lower diagnostic yield.[11,12] Regarding WES, WGS can detect variants outside the exonic region, but the diagnostic yield is not significantly different.[10] Currently, applications in several disease categories have been applied:

1. Congenital anomaly/developmental delay/intellectual disability (CA/DD/ID)

To understand the clinical utility for WES and WGS in pediatric population with CA/DD/ID, American College of Medical Genetics and Genomics (ACMG) systematically reviewed the evidence base of the utilization of WES/WGS in this filed.[13] The analyzed yield of diagnosis is 38% (WES 34% vs. WGS 43%). The results revealed that the information provided from WES/WES informs clinical and reproductive decision-making, which could lead to improved outcomes for patients and their family members.[14] Based on above-mentioned information, in 2021, ACMG announced the guidelines of strongly recommending that WES/WGS be considered a first- or second-tier test for patients with congenital anomaly/developmental delay/intellectual disability.[13]

2. Inborn error of metabolism

Inborn error of metabolism (IEM) belongs to a heterogeneous group of diseases, including 23 categories, 767 genes with 1450 diseases.[15] This group of diseases mainly belongs to single-gene diseases with genetic heterogeneity. For example, mucopolysaccharidoses have seven main types and eleven genes with somewhat overlapping ocular, skeletal, cardiac and presentations.[16] Mitochondrial diseases can be due to mitochondrial genome defects or nuclear gene mutation, consisting of 1136 protein-coding genes.[17] The WES diagnostic yield of IEM is around 40%–70%.[11,18] With the increasing newly developed therapies, accurate diagnosis, even by newborn screen, is crucial to improve outcome.

3. Deafness and ophthalmic diseases

Deafness and inherited retinal disease (IRD) are two well-recognized disease categories with high diagnostic yield in WES/WGS testing; both can be up to 50%–70%.[11,19] In addition to molecular diagnosis in symptomatic children, another benefit of early screening for deafness genes is preventing hearing loss. For example, if knowing a baby carrying m.1555 A > G, avoiding prescribing aminoglycosides may prevent ototoxicity.[20] At least 270 genes are known to cause IRD with diverse clinical presentations, making NGS a cost-effective tool for first line molecular diagnosis.[19]

4. Epilepsy and neurogenetic disorders

Epilepsy and neurogenetic disorders have emerged in clinical practice more and more closely to genetic testing. Currently, more than a hundreds of genes related to these phenotypes have been reported, such as SCN1A, KCNQ2, PCDH19, CDKL5, SCN2A, SCN8A, and so forth.[21] The diagnostic yield is around 25% (10%–60%) in this field.[22,23] With the development of precision medicine, targeted treatment with underlying genetic etiology, such as sodium or potassium channelopathies, is possible, making the genetic diagnosis more important.

Neuromuscular disorders (NMDs) are also a group of heterogeneous diseases with complex genetic etiologies. Since the diagnostic yield is high (49%–83%),[24,25] WES/WGS should be considered due to the extensive phenotype overlapping and potential treatments. In addition, because of this powerful tool, the diagnostic algorithm for NMDs is changing, considering postponing muscle biopsy only for those who remain undiagnosed after molecular testing.[24]

5. Skeletal dysplasia

Skeletal dysplasia encompasses 42 groups, 461 disorders, and 437 genes.[26] This group of diseases has variable age of onset, variable location of involvement, and variable severity. After WES/WGS testing, around 20%–46% patients with skeletal dysplasia can have a confirmed diagnosis, avoiding the need for further investigations.[12,27]

6. Cardiovascular diseases

The diagnostic yield of WES/WGS in cardiovascular disease seems relatively low compared with other disease categories, ranging from 9.7% to 28%.[11,12] This is because nonsyndromic congenital heart disease is a multifactorial group of diseases that may not fit the single-gene disease bioinformatics analysis algorithm. However, the detection rate is higher in cardiomyopathy (44%–69%) and channelopathies, such as long QT and Brugada syndromes.[28] Since some channelopathies and cardiomyopathies are familial, several genes have been added in ACMG actionable gene list that recommend reporting even in asymptomatic children.[29]

7. Renal diseases

Renal disease is also another disease category having relative low diagnostic yield (23%–32%) in WES/WGS.[12] Since part of the renal conditions are due to immune reaction, only those with polycystic kidney disease/ciliopathies, nephrolithiasis/nephrocalcinosis (17%–29%), steroid resistant nephrotic syndrome (11%–29%), and Alport syndrome (21%–25%) have higher detection rate, but lower in CAKUT (2.5%–11.5%).[30]

8. Primary immunodeficiency (PID)

PID has been reported to have diagnostic yield of 29% (range 10%–40%).[12,31] For the best possible outcome, it is important to diagnose patients with PID before recurrent infection occurs. Positive WES/WGS results provide an accurate diagnosis that can benefit a treatment plan.[31] However, sometimes, the regular turnaround time (2–3 months) is too long for these patients. Therefore, a rapid/ultrarapid WES/WGS may help.

Roadmap of ultrarapid exome/genome sequencing in pediatric patients

In the past decade, initiatives to facilitate the molecular diagnosis of pediatric patients in the intensive care unit have been made (Table 1). In 2012, Professor Kingsmore demonstrated a rapid WGS workflow started the era of 50-h provisional diagnosis of genetic disorders.[32] They reduced the sample preparation from 16 to 4.5 h by target enrichment, single run model of 26 h in Illumina HiSeq 2500, bioinformatics processing (base calling, alignment, and variant calling) for 15 h, and used symptom- and sign-assisted genome analysis focusing on 591 well-established pediatrics-onset recessive diseases for interpretation. In 2015, his group further developed a 26-h system of WGS by implementation of adjusting the sequencing mode and DRAGEN pipeline.[33] Since then, some laboratories have adopted this system for a rapid WGS diagnosis.

In the view of the high cost of WGS, alternative methods use WES. Because the size of the data output of WES is smaller than that of WES, the Illumina NextSeq 500 sequencer followed by Agilent exome kits had been tried.[34] However, the long DNA extraction (4 days), library preparation and sequencing (25 days), bioinformatics analysis plus interpretation (4 days), and Sanger confirmation (7 days), make the turnaround time still longer than expectation.[34] In addition to Illumina system, Elloitt et al. demonstrated rapid trio WES using Ion Proton system (Thermo Fisher Scientific, USA) with the mean turnaround time of 7.2 days.[35] Currently, several clinical groups provide rapid WGS or trio WES as with a turnaround time approximately 1–2 weeks as a clinical diagnostic service.[8,9]

Table 1 Landmarks of rapid exome/genome sequencing.

Year	Study	Target	Sequencing platform (run mode)/kit	Pipeline	Median turnaround time
2012	Saunders et al.,[32]	WGS	Illumina HiSeq 2500 (26 h mode)	CASAVA, RUNES, SSAGA	50 h
2015	Miller et al.,[33]	WGS_trio	Illumina HiSeq 2500 Rapid SBS v1 (Rapid run mode, SBS26/SBS18)	DRAGEN	26 h
2017	Bourchany et al.,[34]	WES	Illumina NextSeq 500/ SureSelect Human All Exon V5/XT Clinical Research Exome kit (Agilent)	BWA, GATK, SeattleSep SNP annotation 138, in-house pipeline	Mean 40 d (25–100)
2019	Elliott et al.,[35]	WES_trio	Ion Proton System/ AmpliSeq Exome Kit	Torrent Suite™ Software (mapping, variant calling) and in-house pipeline	7.2 d (mean)
2020	Wang et al.,[36]	WES_trio	Ion S5XL/AmpliSeq HiFi Mix and AmpliSeq Exome pool kit	Torrent Suite™, Fudan pipeline	24 h (22–27)
2021	Bamborschke et al.,[37]	WGS_trio	Illumina HiSeq 4000/ Nextrera DNA Flex Kit	DRAGEN	17 h
2022	Gorzynski et al.,[38]	WGS	PromethION (48 flow cells)/DNA Flex library Prep Kit (Nextera)	Guppy v4.2.2, PEPPERMargin-DeepVariant	11.3 h (7.3–18.7)
2022	Owen et al.,[8]	WGS	NovaSeq 6000 (SP flow cell)/Illumina DNA PCR-Free Prep	DRAGEN v.3.7	13.5 h

D, days, H, hours, PE, pair-end; RUNES, Rapid Understanding of Nucleotide variant Effect Software; SSAGA, symptom- and sign-assisted genome analysis; TAT, turnaround time.

However, for critical patients, waiting for 1–2 weeks to have molecular diagnosis is still a lengthy journey. In 2020, Wang et al. established a 24-h trio-exome sequencing using the Ion Torrent S5 XL system (Life Technologies, USA).[36] Since then, rapid WGS with provisional diagnosis provided within 24 h could be achieved with various systems. Bamborschke et al. published a 17 h ultrarapid WGS achieved by the Illumina HiSeq 4000 and DRAGEN pipeline.[37] This progress was enabled by facilitated the DNA extraction (2 h), library preparation (3 h), sequencing (10 h), and bioinformatics analysis (2 h). Nevertheless, a recent publication from Professor Kingsmore's group established an automated 13.5-h system for ultrarapid WGS.[8] With the implementation of DRAGEN, automated diagnosis modules (GEM, Mon, TruSight) and the Genome-to-Treatment (GTRx) open the era of ultrarapid WGS.

In addition to short-read sequencing, nanopore first demonstrated ultrarapid long-read sequencing system in 2022.[38] Using 48 flow cells simultaneously, PromethION (Oxford Nanopore, Oxford, UK) accelerated the sequencing with real-time multiple cloud computing system to achieve the fastest run time (7 h 18 min). This enables the feasibility of long-read WGS in critical care diagnosis.

Compared with traditional method, the advantage of rapid/ultrarapid WES/WGS is the short turnaround time that can have the molecular diagnosis faster. However, the diagnostic yield is similar.[9] The downside of rapid/ultrarapid WES/WGS is the high cost. Compared with WGS, WES is more cost-effective, with lower cost and similar diagnostic yield.[13,39] However, the cost of rapid trio WES is three times more than that of singleton regular WES; this hampers its usage. However, with the rapidly decreasing sequencing cost, from $108,065 USD per WGS in 2010 to $200 USD per WGS by NovaSeq X in 2022,[8,40] this has become more and more affordable. Nevertheless, more and more evidence support of incremental net benefit of WES/WGS after cost-effectiveness analysis,[39] means that WES/WGS will be the first line molecular test in the diagnosis of pediatric genetic diseases.

Declaration of competing interest

The author has no conflicts of interest to declare.

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