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Review

Whole Genome Sequencing in Era of Newborn Screening

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Abstract

After the completion of the human genome project, there have been many advances in the field of genetics. With next generation sequencing, patients can undergo genomic analysis through whole exome or whole genome testing. These comprehensive tests can shorten the diagnostic odyssey and guide medical management and thereby potentially reduce mortality and morbidity. To date, parents and physicians have reported positive perceptions of using these genomic testing even when a diagnosis is not made. Remaining challenges include reimbursement, access to testing and trained genetics professionals, and overall healthcare costs. Despite these challenges, potential role of genomic sequencing being incorporated into newborn screening due to its diagnostic yield and clinical utility seems plausible. This article reviews whole exome and whole genome sequencing use within neonatal and pediatric settings and provides a perspective for the future potential of whole genomic sequencing in newborn screening in the United States.

Keywords
Whole genome sequencing; whole exome sequencing; newborn screening; long reads; short reads; inborn errors

1. Introduction

Approximately 3% of babies in the United States and Europe are born with a birth defect and only around 10-20% of these have an identifiable syndrome [1], a portion of which is genetic. In the United States and Europe, an estimated 60 million people, predominantly children, have a genetic condition. Approximately 30% (45 million) die before the age of 5 [2]. Genetic testing can help identify such patients early, provide informed care, and potentially reduce mortality rates and healthcare costs.

Many molecular technological advances within the field of genetics are changing the way that we provide testing. Genomic scale genetic testing is now clinically available due to the advent of next generation sequencing (NGS) which are mostly short reads and is increasingly used to help find diagnoses [3]. Prior to clinical NGS availability, providers made a differential of the most likely gene(s) causing the patient's symptoms because only Sanger sequencing was available. Sanger sequencing, which sequences one gene at a time, is expensive and time consuming. In contrast NGS can analyze multiple genes or the entire human genome at once. The first draft composite human genome reported in April 2003; ushered in a new era of precision medicine with whole exome sequencing (WES), and whole genome sequencing (WGS) [2]. And the Human Genome Project, helped transform our understanding of the genetic components of disease through discovery of candidate disease genes and atypical presentations of known diseases. NGS is now routinely used clinically as it is faster, cheaper, and more comprehensive than Sanger sequencing. The implementation of NGS has an approximate diagnostic yield of 25% for many phenotypes [4, 5].

Within the clinical setting, genetic testing options include chromosomal microarray analysis (CMA) for copy number variants (CNVs) and regions of homozygosity (ROH); targeted NGS panels for single nucleotide variants (SNVs) and small insertions and deletions (indels) based on a phenotype [6]; and comprehensive WES and WGS. Exome sequencing profiles the protein coding regions and interrogates for approximately 80% of currently known genetic disorders, while WGS profiles the whole genome. Currently, in the United States, WES and WGS are harder to order than traditional testing like CMA, Sanger and NGS due to the interpretation costs and insurance coverage. Though, with increasing insurance coverage of these comprehensive tests, clinical utilization has also increased.

Genetic testing is a process that takes trained healthcare professionals to consent the patient, perform the testing, and interpret the information. Interpretation can be constrained by our ability to decipher a variant in a landscape of thousands of unique variants. In other words, everyone has variations within their genome, and to judge the likelihood that a variant alters physiology sufficiently to cause disease without functional or genetic evidence can be difficult. And, subjectively makes connecting a genotype to a particular phenotype with a high level of confidence difficult [7]. Unlike conventional genetic testing, WGS and WES annotation is driven by clinical phenotypes to

prioritize which genes are analyzed. This requires accurate and comprehensive phenotypic information on patients [6] and the onus often falls on the ordering provider.

The interpretation of the potential pathogenicity of some variants can be assisted by integration of functional data such as RNA sequencing and profiling of DNA methylation. For the latter, current methodologies profile methylated cytosine across the genome using bisulfite conversion of DNA and subsequent NGS or array. Certain patterns of altered DNA methylation are pathognomonic of DNA imprinting disorders and disorders of chromatin remodeling pathways [8, 9]. Detection of these patterns or epigenetic signatures can often assist in the classification of variants of uncertain significance [10, 11]. Additionally, detection of such a pattern in the absence of a reported DNA variant and in the context of the appropriate phenotype diagnostic of the disease and engenders further analysis of the DNA locus to identify the pathogenic variation.

Comprehensive genomic sequencing tests can produce data from genomic regions with minimal clinical information and correlation. When laboratories analyze genes and variants without clinical correlation, results can include variants of unknown significance (VUS) and genes of unknown disease significance (GUDS) and neither are clinically actionable [7]. Such results create confusion for healthcare providers and patients and increase the labor of explaining results. To address these challenges, the framework and governing principles of genomic medicine has been re-defined [12] and the classification of genomic variants has been standardized. Following the filtering and prioritization of the approximately 5-6 million variants identified by WGS per genome, variants are classified based on American College of Medical Genetics and Genomics criteria (pathogenic, likely pathogenic, uncertain, likely benign, benign). Although unable to quantify probability, the intent of this classification system is that subjectively a pathogenic variant has a greater than 99% probability of causing human disease, a likely pathogenic variant a 90-99% probability, an uncertain variant a 10-90% probability, a likely benign a 1-10% probability, and a benign variant a less than 1% probability.

Encompassing the majority of known genetic conditions outside of chromosomal aberrations, comprehensive genomic tests reduce the diagnostic odyssey of patients by bypassing the step-wise genetic testing that has been done traditionally. This review article looks at how genomic sequencing, especially WGS is being used in infants and pediatric patients and summarizes the benefits and challenges.

2. Use of WGS Clinically

The adaption of an organism to its ecological niche determines its health. This adaptation is a representation of the interplay between the genome and the environment; consequently, disease arises either from changes within the genome inducing maladaptation or from environmental changes reducing fitness of the genome. Within the pediatric population, the former largely manifest as rare diseases with strong monogenic or oligogenic etiologies, whereas the latter largely manifest as common diseases with polygenic etiologies.

Aside from teratogenic disorders, most severely maladaptive newborn and pediatric disorders are rare diseases with strong monogenic or oligogenic etiologies.

Heterogeneous presentation of genetic conditions, apart from many rare genetic variants within the causative genes increase the utility for genomic sequencing of WES or WGS [13]. Timely determination of a molecular etiology allows for specific interventions improving outcome [3].

Limitations of phenotypic driven panels can include missing diagnoses because the panel is not updated as rapidly as disorders are discovered or because of atypical presentations of conditions that were not included on the panel. Although the rate varies depending on the standards for diagnosing rare disease regionally, approximately 50% of individuals with a rare disease do not receive a diagnosis overall [14].

A study of pediatric patients without a known molecular cause for their phenotype found that conventional genetic testing methods had a diagnostic rate of 24%, whereas WGS not only detected most variant types found conventionally but had overall diagnostic yield of 41% [6]. Similarly, in a second study, trio WGS provided a genetic diagnosis for 57% of NICU patients, whereas conventional genetic testing had a diagnostic yield of 9% [2]; interestingly, 45% of the 57% had a diagnosis that was not previously considered because of an absence of classic phenotypic findings. In a third study, 13% of enrolled NICU and 25% of enrolled PICU patients (21% overall) received a diagnosis from WGS [13]. The disparity in diagnostic rate between the studies might represent either patient selection or patient description because when the second study is limited to patients enrolled by neurology or genetics, diagnostic yield increased to 39% [13].

For patients with intellectual disabilities, WGS detected a causal variant in 27%-50% compared to 11% for molecular karyotyping [15-19] suggesting potential role of WGS and WES as first tier testing [15-19].

Pediatric disorders arising from environmental changes reflect an evolutionarily abrupt change creating a mismatch between the genome of many exposed individuals and the new environment. Comparing the genomes of affected and unaffected individuals identifies highly penetrant variants and susceptibility variants for disease. Such diseases are polygenic and the genotypes across associated loci can be used to calculate Polygenic Risk Scores (PRS) [14]. Understanding the complexities of the genetic interactions with disease can allow medical providers and the patient and their families to make decisions to help lower the risk of disease through lifestyle changes and improve surveillance. In the current landscape of PRS there is a bias towards European populations as there is a more robust genomic database for that ancestry leading to approximately 63% of variant associations with disease [7] and relatively little data on PRS of relevance to the pediatric population and newborn screening.

In summary, understanding and diagnosing rare disease have many positive clinical outcomes. Besides diagnostic closure, these can include improved management, treatment/surveillance, understanding of disease progression, and recurrence counseling [14]. In addition to these direct clinical benefits, genomics studies contribute to the understanding the underlying pathophysiology of disease by defining variation associated with disease phenotypes [7]. In contrast, polygenic risk scores are in their infancy and have not yet achieved much clinical impact or wide adoption as part of newborn screening.

3. Bridging the Gap of Clinical and Research Based Genomic Sequencing

Genomic sequencing's ability to detect common and rare variants across the genome improves understanding of the underlying genomic contributors to disease. And, enables research into genetic causality, treatments, and gene/disease discovery [20]. For each meiosis 50-100 new variants occur; the majority have no known functional significance [20]. These de novo and rare familial variants create an interpretative burden that often require resolution through translational

research. For successful genomic sequencing we need to allow for variant discovery and prediction models to help clarify VUS and GUDS results [20, 21], and understand potential role of PRS contributing to higher recurrence of neurodevelopmental disorder (NDD) within a family [21].

4. WES VS WGS

WES targets approximately 1-2% of the genome and can identify 98% of known disease-causing variants [7], whereas WGS examines approximately 90% of the genome [22]. WES is designed to sequence regions of the genome encoding proteins (exons), and evaluates the intronic regions (noncoding regions) immediately adjacent to the exon, i.e., typically the 10-20 intronic bases adjacent to the exon [22]. Generally, WES has high sequencing depth but more bias in overall coverage than WGS. Whereas WGS has less bias and reduced the laboratory processing time, detects variants outside of targeted exons, has better coverage of the exons including exon variants missed by WES, and identifies noncoding variants that can alter gene expression, splicing, and chromosome replication [23]. The downside for this extra coverage is the higher sequencing and data storage costs and the limited understanding of noncoding regions. [7]. While the limitations of WES include poor detection of indels, structural variants, intronic variants, and inadequate coverage of some exons essential to analysis [6, 24-27].

Compared to a diagnostic yield of 12% for CMA, WGS found an actionable diagnostic variant or secondary variant in 38% of children undergoing a CMA, with a diagnostic yield of 33% [28], despite the limited ability of short read WGS to detect triplet repeat expansions or variants in genes with highly homologous paralogues or pseudogenes [2].

A randomized control trial of ill infants comparing ultra-rapid or rapid WGS and rapid WES found that WGS analytics out-performed WES in detecting variants likely to affect protein function, and ClinVar pathogenic/likely pathogenic variants; however the diagnostic yield were similar at 19% and 20%, respectively [22]. Another study found increase of 4%-7% in diagnostic yield for WGS compared to WES when reanalyzing WES raw data on a WGS platform [29].

A study of patients with early infantile epileptic encephalopathy found that WES and WGS overlapped for most reported SNVs or indels [30]. For three patients who previously had a negative WES commercially, WGS detected a diagnostic variant and two others without prior testing had diagnostic variants only detectable by WGS.

The CAUSES study, which performed WES or WGS on 500 families as trio testing and followed these families for 5.1 years after initial sequencing to allow for reanalysis and reinterpretation of results, found that the initial test's diagnostic yield seen in 43% of families increased to 52.2%, an increase of 4.8% per year. Though, found no significant difference between the diagnostic rates of WGS and WES, 51.8% and 52.3%, respectively. Reanalysis increased the rate of diagnosis in families with previously uncertain or uninformative results by 17.2% and that 1.9% of families had a diagnosis rescinded. And, concluded that a multidisciplinary team approach including genetic counselors and medical geneticists improved the clinical value of genomic sequencing [31].

WES provides a molecular diagnosis for approximately 25% of individuals with rare diseases. WGS can resolve protein coding variants at similar or higher rates than WES, and with the theoretical decrease in cost, clinical transition to WGS seems preferred [7, 32-34].

5. Benefits to Genomic Sequencing

There are several benefits to WES or WGS. As a single test, they more rapidly define a diagnosis with a diagnostic yield of 25% to 57% [23] based on phenotypic presentation, reduce healthcare costs [13, 35], and can improve of ongoing clinical management, initiation of new specialist care, outcomes, modified medical management decisions/treatment options, and conversations on palliative care for those with poor prognoses [13]. A genetic diagnosis also provides a foundation for long-term care management and coordination for clinicians decision making [27]. A molecular diagnosis and defined inheritance help reproductive plans and facilitate closure by providing a rational explanation [13].

Genomic sequencing can decrease infant mortality in the ICU due to genetic condition. Earlier identification with WGS or rapid WGS can help implement management and treatment guidelines known in 70% of genetic conditions. Understanding the contribution of genetic diseases to infant mortality could provide valuable information for clinical and public health initiatives [36]. Given that only 19% of infant mortality is attributable to the trisomies (21, 18, and 13) and 22q11 deletion syndromes [36], WGS and WES can help screening for other genetic etiologies.

A cohort study of critically ill neonates showed a diagnostic rate of 37.5% for WGS [37]. Indicative of a diagnostic limitation, WGS did not detect mosaic monosomy on whole blood, whereas fluorescence in situ hybridization of buccal cells did. This shows that WGS is unlikely to replace all genetic tests although its ability to detect many types of genomic variation positions it as first-tier test, not as a last resort.

6. Parent and Clinician Perceptions of WGS

Parents are often motivated to have WGS to find a cause for their child's illness, to rule out a genetic condition, or to support improvement in healthcare for other families. Those who declined WGS stated that they were not ready for a genetic diagnosis in the neonatal period because they were overwhelmed with the current critical illness or believed that their child's symptoms were not genetic. Some families had concerns over a 'genetic label' for their child [13] The option to use genomic sequencing as a tool should involve parental consent and should be obtained by professionals who have thorough and current knowledge.

The NSIGHT2 group investigated parents' feelings regarding genomic sequencing, with 97% of parents reporting that the genomic sequencing was at minimum somewhat helpful to their infant even though only 23% of infants received a diagnosis. Without any demographic correlation, 50.3% of the parents did not have decisional regret over the testing, suggesting that half had some regrets. Most parents, irrespective of results findings, reported that genomic sequencing provided a better perspective on their infant's future and management, and on their reproductive risks. And, 81% of parents and clinicians agreed that the genomic results were useful [38]. Overall families have a positive outlook on genomic sequencing, although the regret requires further study.

A retrospective study of clinicians' perceptions observed that 77% of clinicians found genomic sequencing useful. 93% thought that positive results had clinical utility and 72% thought that negative results had clinical utility. Clinicians reported that genomic sequencing changed management for 28% and improved outcomes for 15% of infants. Clinicians also reported improved communication with families regarding outcomes, expectations, and prognosis after genomic sequencing. Overall, clinicians felt positive about the implementation of genomic sequencing [39].

In summary, both parents and clinicians have a favorable outlook toward genomic sequencing. And, an informed consent process to genetic testing by a medical geneticist or genetic counselor can help reduce parental decisional regret.

7. Cost Effectiveness and WGS as First-Tier Genetic Testing

WES or WGS as a first-tier test rather than a 'last resort' can reduce the time to diagnosis or diagnostic odyssey, impact the availability and the effectiveness of clinical interventions for infants and pediatric patients [3], improve outcomes for patients needing a rapid diagnosis and reduces the longer-term healthcare costs [40]. Higher diagnostic yield of WGS compared to CMA, WES, and other standard methods for patients with intellectual disabilities, neurodevelopmental disorders, developmental delays, critical illness, and early onset epileptic encephalopathy [16, 17, 40-42], can potentially reduce the health disparities perpetuated by diagnostic inequity [27]. Though, WGS cannot replace conventional tandem mass spectrometry's specificity and rapid turnaround of results in detection of many IEMs [2].

Project Baby Bear rapid WGS for California's Medicaid patients admitted to intensive care units, had a diagnostic yield of 43% and change in care for 31% of those patients, with an approximately $2.5 million in healthcare savings. The success of this project led to the 'Ending the Diagnostic Odyssey Act 2021.' [42].

A systematic review found that globally the cost of WES ranges from approximately $467 to $5169 and of WGS ranges from $1604 to $24810 [43, 44]. There is evidence for better genomic coverage, number of variants detected, a simpler laboratory workflow, better turnaround times, increased yield from initial testing or reanalysis, and overall impact on healthcare [40].

A review of 31 clinical studies using first tier WGS (2012 to 2021) found that WGS was a cost-effective way to make a genetic diagnosis that alters medical management through refined prognosis and alternate therapeutic strategies. It also helped in the estimation of recurrence risks for families and reduced mortality rates within ICUs [44].

A prospective study found that 39.8% of patients with a diagnosis from WGS led to an average cost reduction of $100, 440 per person totaling $6.53 million in savings on 65 patients. And concluded that WGS was cost effective, reduced the diagnostic odyssey, and increased the diagnostic yield compared to more traditional approaches [45].

8. Challenges

Barriers to implementation of genomic testing include cost, availability of trained providers, the bioinformatic workload, time of interpretation, and storage of patient data [4]. Clinical judgement necessary in providing genomic testing to patients, requires time and expertise and increasing demand on a constrained and limited workforce to successfully implement WGS [4]. WGS needs to be offered by trained professionals who understand genomic testing and have the skills to guide dialog and obtain informed consent and return of results [4, 41]. Paucity of genetic workforce and access to genetic specialist or genetic counselor limits ability of many medical centers to pursue much needed comprehensive genetic testing resulting in healthcare inequity.

With comprehensive genomic sequencing, more VUS and GUDS are often reported. Currently, there are approximately 4,700 clinically relevant genes in OMIM (www.omim.org), whereas in 2018

approximately 3,400; an increase of 1,300 clinically relevant genes identified in short four years [46-48].

Genomic variation is common. Each person's genome differs by approximately 4-5 million base pairs from the reference genome that is used for clinical comparison [49, 50]. This variation leads to uncertain findings with a need for a larger bioinformatic infrastructure to decipher the 'noise.' It can also cause confusion and uncertainty for healthcare professionals and patients. Medical management changes should not be based on uncertain findings, with the risk of errant recommendations, over medicalizing, and inappropriately stopping the search for a diagnosis. Although multiple tools have been developed to improve the variant call [51], more resolution of such uncertainty through functional testing is needed to aid in the classification and reclassification of genomic variants [7].

Another challenge to the implementation genomic testing is equity of care. Ensuring equitable access is necessary if providing WGS as first tier testing. Trio WGS theoretically increases the diagnostic yield but can also affect the equity of the testing for those for whom trio collection is not possible. For an inpatient infant cohort, however, trio testing only increased the diagnostic yield by 1% over proband only WGS although trio testing did decrease the turnaround time [22]. Similarly, a comparison of trio and proband only WES found that trio sequencing enabled one additional diagnosis [52]. This suggests that starting with proband only sequencing and reflexing to trio could reduce costs for families and extend limited healthcare resources to achieve greater equity.

'Ending the Diagnostic Odyssey Act 2021' allows all 50 states' Medicaid programs to cover rapid WGS for eligible individuals [42], although private insurances still need to follow for equitable access.

The ethical implications of implementation of genomic sequencing needs careful consideration especially for infants and pediatric patients. A central tenant of pediatric ethics is that the best interest of the child is first and foremost, but when it comes to severely ill infants, this determination can be a challenge [47]. Those favoring genomic sequencing find a benefit in the avoidance of high intensity treatments and risky interventions and unnecessary suffering when palliative care is more appropriate [53-57]. With very limited information about rare genetic disorder management, lack of clear guidance towards any medical care changes is debatable to be in the best interest of the child is debatable [47]. The clinical utility of genomic sequencing should be assessed not only in the context of a diagnosis, but also on the ethical, legal, and social implications. It is not as simple as overall healthcare dollars and diagnostic odysseys. Is there utility in discovering an adult-onset condition in a severely ill neonate? Are we taking away the child's autonomy to make future decisions? Lastly, personal and familial utility should be a part of the discussion [58-62].

9. Where WGS is Going in the Future

With the completion of the first partial draft of the human genome in 2003 and the subsequent advent of next generation sequencing, access to genomic medicine has increased. DNA sequencing by synthesis has introduced precision medicine into medical decision making. As understood from studies beginning in the early 20th century, the expressivity of phenotype related to a variant is often a continuum, and the phenotype associated with any given variant can often be suppressed to effect incomplete penetrance or enhanced to cause more severe disease. By its very nature, short read sequencing by synthesis lacks the information content to dissect this complexity and enable more nuanced or precise pursuit of genome driven precision medicine. The dawning of long read

sequencing of native DNA enables a new and more thorough understanding of DNA structure and its covalent modifications; this insight, which can be correlated with the metabolic state, chromatin territories, and environmental impacts to which the genome was and is exposed, opens a new universe of precision medicine in which variable expressivity and incomplete penetrance will eventually be quantified.

Long read sequencing of native DNA defines a future state for detection of many covalent modifications of native DNA such as glycation, acylation, alkylation, oxidation, and methylation [63, 64]. Besides evidence of imprinting and chromatin remodeling disorders, profiling of these modifications across the genome will provide insights into the metabolic and physiologic state as well as a history of environmental interactions. Thus, we foresee a future potential of WGS in which screening programs can quantify the risk of diseases from prenatal exposures, another major public health issue that probably affects as many babies as all IEMs combined.

10. Conclusions

Genetic conditions have clinical overlap, heterogeneity, and variable presentations. Genomic sequencing has accelerated the delineation of molecular diagnoses and shows promise for reducing and targeting healthcare expenditures. Genomic sequencing brings a larger burden of consenting, interpretation, and follow up. Patients who undergo genomic sequencing could have harm from incidental findings such as non-paternity or risk for adult-onset conditions. The ethical implications of comprehensive genomic testing extend beyond the cost and clinical utility to include personal utility. Clinical genomic testing is optimally provided by a team knowledgeable in genetics and the social, ethical, and medical implications of genetic testing.

Author Contributions

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Competing Interests

The authors have declared that no competing interests exist.

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