Genetics inMedicine | COMMENTARY

Newborn testing and screening by whole-genome sequencing

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Over the course of the next few decades, the availability of cheap, efficient DNA sequencing technology will lead to a medical landscape in which each baby's genome is sequenced, and that information is used to shape a lifetime of personalized strategies for disease prevention, detection, and treatment.

—Francis Collins[1]

When the helmsman of the world's leading supporter of biomedical research declares that large-scale newborn genome sequencing is on the horizon and will transform medicine, it behooves health-care researchers, practitioners, and leaders to pay close attention. Thus, to introduce this themed issue on newborn diagnostic testing and newborn screening (NBS) by next-generation sequencing (NGS), I briefly summarize progress and trends in this area of imminent great change.

In the past 18 months there have been remarkable improvements in methods for whole-genome sequencing (WGS). Most notable was the introduction of a new range of Illumina sequencing instruments with patterned flow cells. These instruments allow 18,000 genomes per year to be robustly sequenced at a cost of $1,000 each.[2] Together with improved software for read alignment and variant calling, these instruments have increased the analytic sensitivity and specificity of WGS for nucleotide variants to greater than 99.5%.[3] In parallel, Complete Genomics is in the process of a commercial launch of a sequencing system that enables WGS of 10,000 genomes per year, at a similar cost.[4] Concomitantly, there have been significant advances in the quality, functionality, and availability of commercial software and freeware for WGS analysis and interpretation, and of reference and pathogenic variant databases, such that interpretation and reporting are now scalable to large numbers of patients. Thus, for the first time, WGS of the 3.9 million babies born in the United States each year will be technically feasible in 2016. It is likely to be Qatar, and not the United States, however, that will be the first to undertake population-scale newborn WGS.[5]

Single-gene diseases, which now number an astonishing 8,042, are the leading cause of death in infants and in those in neonatal (NICUs) and pediatric intensive care units (PICUs) in the United States.[6] Thus, it is not surprising that one of the first applications in which WGS has shown diagnostic utility is in NICU infants.[7,8] For acute clinical utility in the NICU or PICU, however, WGS must be capable of returning results much faster than in a typical research or reference laboratory setting. Indeed, in a recent case series, 57% of regional (level IV) NICU babies diagnosed with genetic diseases had died by day of life 100.[7] In response, rapid WGS methods have been developed specifically for timely genetic disease diagnosis in the NICU and PICU.[3,9–11] The current speed record is 26 h from neonatologist referral to return of a provisional diagnosis.[3] In the same case series, the diagnostic rate of WGS was 57%, a figure higher than that obtained in other clinical settings.[7,8,12–15] To date, the clinical utility of rapid WGS has been examined in only one retrospective case series in which molecular diagnoses helped guide level IV NICU management of 65% of infants, with a significant beneficial impact on outcome in 20%.[7,8] However, at least two prospective studies of cost-effectiveness and clinical utility of WGS in NICU patients are currently in progress.[16,17] Given the economics of level III and IV NICU care and the potential for 78 added quality-adjusted life years per NICU infant's life saved, it is likely that the first medical indication in which WGS is adopted as standard of care will be the ~25% of the newborns in level III and IV NICUs for whom the etiologic diagnosis is unclear at admission.

Although technologically indistinguishable, NBS for genetic disorders by WGS differs markedly from newborn diagnostic testing by WGS. The pretest probability of a genetic disorder in a NICU infant in whom genetic etiology is undergoing consideration is high—57% in the series mentioned above. Thus, the analytic metric to which WGS is attuned has high sensitivity. The clinical features of disease in the affected infant act as powerful secondary filters for genetic variants with regard to causality in individual patients. Furthermore, given the high acute morbidity and mortality in NICU infants with symptomatic genetic diseases absent treatment, the relative risk of benefits and harms of WGS is heavily weighted toward beneficence.

By contrast, the pretest probability of a genetic disorder in healthy newborns is low—less than 4% in families without consanguinity or geographic isolation. Thus, the analytic metric to which WGS must be attuned is avoidance of false positives (high specificity). In healthy newborns, because there are no clinical features with which to triage genetic findings prior to reporting, interpretation of pathogenicity must be highly conservative.

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Furthermore, the likelihood of morbidity and mortality in healthy newborns is very low, so the relative risk of potential benefits and harms of WGS is more weighted toward concerns with regard to false-positive results, psychosocial impacts, privacy, and genetic discrimination in this highly protected population.

Thus, the results of the study by Bodian et al.,[18] reported herein, will be of broad interest to readers of *Genetics in Medicine*. In the first prospective study of its kind, they compared the relative diagnostic yield of traditional NBS with NBS-by-WGS for 65 genes in 1,696 predominantly healthy newborns. The results were somewhat surprising. Traditional NBS identified 34 true-positive results and NBS-by-WGS identified 32. Thus, traditional NBS missed one diagnosis, whereas NBS-by-WGS missed three diagnoses. The authors' conclusion was that a combination of methods was highly synergistic, both in terms of diagnostic yield and in refuting false-positive results. A key unanswered question to date is the diagnostic yield of NBS-by-WGS for treatable genetic diseases that are not screened by traditional NBS. This will require additional studies, which will be forthcoming from both the Inova Health System group and the four groups funded through the National Institutes of Health NSIGHT program.[16]

Similarly, Baker et al.[19] report herein that NGS improved the diagnostic yield and provided timely refutation of false-positive results from traditional NBS for cystic fibrosis. They retrospectively examined the diagnostic utility of targeted NGS for 162 well-established cystic fibrosis–causing mutations in 165 dried blood spots from infants with abnormal immunoreactive trypsin values and with a single, non-diagnostic, heterozygous mutation upon testing with the American College of Medical Genetics and Genomics panel of 23 *CFTR* mutations. In 91.5% of these indeterminate samples by traditional NBS, no second mutation was detected, which was consistent with clinical determination of carriers by sweat chloride and other testing (true negatives). In 5.5%, a second likely pathogenic variant was detected. Clinical assessment indicated that 4.2% were true positives and 1.2% were carriers (false positives). Finally, 3% of true positives were missed by the targeted panel. However, at least 1.2% of these false negatives would have been detected by NGS-by-WGS. These results are important because there is great concern that variants of uncertain significance will overwhelm interpretation of WGS. Our knowledge of pathogenic variation, at least for *CFTR*, is sufficiently mature for adoption of NBS-by-WGS. This study emphasizes the importance of efforts to catalog comprehensively the effects of all nucleotide variants on function of prominent genetic diseases and polymorphic disease genes, as has been accomplished for *CFTR* and, more recently, titin.[20]

A third article, by Tang et al.,[21] reports that blood spots collected between 12 and 23 h after birth performed as well in traditional NBS as the California standard, which is 24–48 h after birth. The rationale for delay in testing had been that metabolic maturation and feeding were necessary to unveil certain metabolic conditions. This has relevance to NBS-by-WGS because it has no such temporal sample constraints. Indeed, NBS-by-WGS would ideally be performed on cord blood. Time to diagnosis is critical for certain NBS conditions, such as maple syrup urine disease, and accelerated time to diagnosis over current NBS ranges may be associated with improved outcomes. This hypothesis is worthy of testing.

Finally, in this themed issue, Dr Kerruish reports long-term parental attitudes with respect to NBS for risk of development of type I diabetes mellitus (T1D).[22] Specifically, 15 of 41 mothers of newborns who had received a T1D risk result of 1 in 16 were interviewed to elicit descriptions of their experiences. Of note, none of the children had developed T1D at the time of interviews, which was when they were 11–13 years old. NBS for risk of common, complex genetic disorders is very different from NBS for rare, highly penetrant, treatable, monogenetic conditions because results are not deterministic and evidence for the effectiveness of preventative measures may be lacking. Thus, there is even greater concern that the potential psychosocial and economic harms of testing may outweigh the medical benefits. However, NBS-by-WGS for common, complex disorders is very much the stuff of Dr Collins' prognostication.[1] Notably, the author did not find adverse psychosocial effects of positive tests in mothers or in at-risk children upon disclosure. These results generally concur with the limited literature to date.[23]

The results of additional such studies will be forthcoming from the four groups that are funded through the National Institutes of Health NSIGHT program.[16]

In summary, the rapidly declining cost of WGS, gains in our knowledge of causative variation underpinning genetic diseases, and improvements in WGS analysis and interpretation methods are converging to make broad NBS-by-WGS feasible both analytically and diagnostically. The success of traditional NBS programs, recent publications of high diagnostic yield of WGS in the NICU, and strong global societal desire for improved outcomes of childhood genetic diseases are combining to drive implementation of WGS in medicine. Possibly Dr Collins will be proven right.[1] Again.

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DISCLOSURE

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