Editorial

Newborn Screening: To WES or not to WES, that is the question.

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Routine screening of all newborns for inherited disorders began in the 1960s after the American microbiologist Robert Guthrie, M.D., Ph.D., developed a simple test to identify neonates with phenylketonuria[1]. Neonates who tested positive could receive treatment before they became symptomatic. Since then, newborn screening (NBS) has become the standard approach to screening populations for several rare disorders, including inherited metabolic diseases. However, as more and more conditions have been added, priority has been given to improving test performance and reducing false-positive results. False positives can disrupt parent-child bonding during the critical first weeks of life, and can cause lasting distress for parents[2]. Therefore, NBS must ideally have high sensitivity and specificity, and minimal need for manual review. Quality control is an essential component of NBS; for example, the Newborn Screening Quality Assurance Program, administered by the Centers for Disease Control and Prevention, studied samples submitted by 648 laboratories in 85 different countries in 2019.[3] NBS has been recommended for 34 health conditions in the U.S. (although the exact number varies by state and country) and is performed with tandem mass spectrometry (MS/MS)[4]. NBS by MS/MS has ~99% sensitivity and almost 100% specificity[1,4]. For some disorders, MS/MS has low positive predictive value and results may be nonspecific. It is imperative that NBS minimizes false positives while identifying true positives.

Rapid advances in next generation sequencing technology and computing power have led to the widespread adoption of whole exome sequencing (WES) in clinical practice within the last decade. In some countries, WES is now commonly used for rapid diagnosis of seriously ill children expressing a disease phenotype[5]. Although this application presents the opportunity to collect and analyze large amounts of DNA sequence data in the newborn period, there is a significant knowledge gap regarding population-wide performance characteristics, predictive value, and utility of newborn genomic sequencing.

Now, Adhikari et al[6] report on the first comprehensive comparison of WES and established screening technology, MS/MS. In California, between July 2005 and December 2013, the Genetic Disease Screening Program screened dried blood spots from nearly 4.5 million neonates using a multiplex MS/MS platform. The authors obtained a set of 1,728 residual, de-identified, archived dried blood spots representing all cases with inborn errors of metabolism. They also obtained selected blood spots that initially screened positive from neonates who were later found to be unaffected.

As a primary screen they analyzed an "exome slice" of 78 genes associated with the 48 inborn errors of metabolism ascertained by NBS in California. Their pipeline correctly identified 571 of 647 IEM-affected infants as having a potentially pathogenic IEM genotype revealing an overall sensitivity of 88%. In the clinically confident subgroup of individuals, their pipeline achieved 93.7% overall sensitivity. Wider WES analysis identified eleven exome-positive infants for genes unrelated to their IEM. This produced an overall specificity of 98.4%. This would extrapolate to ~8,000 false positives among the half million annual births in California alone. This is far more than the actual 1,367 MS/MS false positive cases in 2015. Collectively, these data show that when used alone, sequencing underperforms the classical MS/MS pipeline, misses some affected babies, while identifying many healthy neonates as "positive" and targeting them for unnecessary follow-up testing.

One limitation to the report by Adhikari et al. is the relatively low number of cases studied (1,728 vs ~half million NBS per year in California)[6]. The sensitivity of their screen varied largely by disorder, and performed better for more prevalent IEMs reaching close to 100% sensitivity. Statistical confidence for very rare IEMs would require larger cohort sizes and more data before definitive conclusions can be drawn about the utility of newborn genomic sequencing in NBS.

Abnormal results trigger second-tier testing critical to distinguishing a false positive from a true positive result. Second-tier tests are typically more sensitive and specific than the primary

newborn screening assay, but for various reasons, including cost, time, and complexity, they are not suitable to be used as primary screening assays.

Performing newborn genomic sequencing as a second tier test when the primary screening results are abnormal could be a cost effective alternative to second-tier biochemical testing. Adhikari et al., therefore, considered WES as a reflex follow-up test for MS/MS positive individuals before conducting second-tier biochemical/clinical studies[6]. They found that WES could facilitate rapid and precise clinical resolution for neonates with positive MS/MS on NBS and propose that sequencing can still be useful in cases that look suspicious but were not clearly identified by MS/MS. One has to note, however, that cost and turn-around time, critical concerns in NBS, have not been considered in their study. They found that turn-around time of WES for critically ill infants ranged from 2 to 3 week to less than 24 hours. This finding, and the relatively modest caseload of positive NBS for IEM (~0.3% of births), suggest that WES could become an economical and cost-effective second-tier test after a positive MS/MS result. Nonetheless, clinical consideration for individuals with IEM should dictate whether urgent referral after positive MS/MS is required, or it could await sequencing results.

As metabolic specialists we must emphasize the importance of biochemical testing; elevated metabolites detected by MS/MS are the result of a functional deficit in a pathway regardless of the genes involved, whereas WES, at best, identifies known pathogenic mutations or variants of unknown significance, but provides no data on their functional relevance. This gives the classic methodology a superiority over genetic techniques, which are currently also slower, and more expensive.

In 2020, NBS is mostly focused on inherited metabolic diseases, an emphasis which might change in the near future. If WES becomes the method of choice for other disorders included in NBS, outside the MS/MS panel and performed in every newborn, a combination of the two methods (WES and MS/MS) seems to be a logical option.

In summary, WES alone may not meet standard criteria for NBS yet, but sequencing could be used as a second-tier test for positive MS/MS results and could reveal a gene variant that provides us with a definite diagnosis, if testing is fast enough and cost effective. Several Mendelian conditions, such as neuro-genetic disorders with upcoming treatment options, are not amenable to MS/MS and currently go unrecognized until it is too late for optimal intervention. In these cases, NBS by WES could potentially offer early definitive diagnosis.

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