PERSPECTIVE

Next generation sequencing for newborn screening: are we there yet?

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Screening programs for asymptomatic newborns (newborn screening – NBS) have increasingly been implemented in many westernized countries since the end of the 20th century (Wilson et al., 2010). The major goal of these programs is to unselectively screen all newborns for a well defined group of severe, rare, clearly identifiable and actionable conditions. These conditions should be diagnosed and treated in a timely fashion to ensure short and long term health of the newborn as an infant and an adult. As such, NBS programs are one of the pivotal public health achievements of the past decade (Centers for Disease Control and Prevention, 2011) that have led to the saving of lives and improving quality of life as well as posing less financial burden on the health care system. Technically the currently practiced screening process is performed 48 hours after birth, using a minute amount of blood collected on a dried blood spot card, which is subsequently subjected to biochemical analysis predominantly using mass spectrometry assays.

The overwhelming majority of conditions covered by NBS have a strong genetic component, and in fact represent classic Mendelian disorders, with most of the relevant genes already identified. Thus, a plausible alternative approach to 'biochemical based' NBS would be applying next generation sequencing (NGS) technologies to screen for mutations in the relevant disease predisposition genes. This concept of 'targeted NBS panels' is indeed a viable possibility now offered in the USA (reviewed in Howard *et al.*, 2015).

A major driving force for this alternative approach is the rapidly decreasing price of massively parallel sequencing coupled with the vastly improved read depths and accuracy of the sequencing platform. In

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fact, extrapolating from past and current trends it should be feasible and clinically applicable to perform whole exome sequencing (WES) or even whole genome sequencing (WGS) on all newborns at a cost that would be competitive with current NBS prices (estimated at \$45 in Israel: http://www.health.gov.il/Subjects/Genetics/newborn_neonatal_screening/Pages/default.aspx). These novel sequencing and bioinformatics technologies have successfully been applied over the past decade to diagnosing complex clinical phenotypes in newborns and infants, for both monogenic and polygenetic traits (Saunders *et al.*, 2012).

Thus the possibility of newborn infants undergoing WES/WGS within the first few days of birth seems closer and more feasible than ever. This prospect raises a whole host of issues encompassing several disciplines that need to be discussed and fully addressed before we embark on this path.

Data generation and interpretation

Applying WES/WGS to any human genome generates hundreds of thousands of peri-gene sequence variants. A subset of these variants is detected in several hundreds of genes associated with relevant childhood disorders. Additionally, a substantial number of genetic variants cluster within genes that herald adult onset disorders or predisposition genes. One major challenge is to accurately interpret the clinical significance of these variants. There is still a lack of sufficiently large ethnic specific genetic datasets for an accurate evaluation of the possible pathogenicity or the benign nature of some of these variants to be carried out. These uninterpretable variants, collectively called variants of unknown significance (VUS), pose the dual danger of risk underestimation (where the variants are pathogenic and disease associated and are

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interpreted as benign) and risk overestimation (where benign variants are misinterpreted as pathogenic). This hurdle needs to be resolved by a combined international effort to generate large freely available datasets, better prediction algorithms, as well as developing reliable fast lab based technologies that could resolve data interpretation.

Reported information

Genetic data generated by applying WES/WGS results in data regarding a host of genetic disorders spanning a wide range of disorders: monogenic to polygenic, childhood onset to adult onset, carriership of autosomal recessive disorders and autosomal dominant predisposition genes with variable penetrance as well as pharmacogenomics data (Wade et al., 2013). These genetic disorders can either be treatable (or actionable) or untreatable and either require immediate action (e.g., avoiding exposure to phenylalanine) or a delayed action (e.g., breast cancer surveillance for BRCA mutation carriers). Noteworthy, genetic testing for minors is currently applied exclusively for existing or imminent medical conditions where the genetic information may help in clinical decision making (Dondorp & de Wert, 2013). Should the data generated at NBS be reported for all disorders regardless of actionability? Could the parents conceivably choose a 'treatable disorders gene panel' to be reported exclusively? How should carrier status of autosomal recessive and dominant disorders be reported? At what age? What about the newborn autonomy and the right not to know his or her genetic status (United Educational, Scientific and Cultural Organization, 1997)? Another major obstacle that needs to be resolved is 'incidental findings'. These can range from adult onset diseases that have a delayed clinical implication that are not apparent from the family history (e.g., ovarian cancer risk in BRCA1 mutation carriers originating on the paternal lineage) to cancer predisposition that has an immediate clinical impact (e.g., total thyroidectomy in RET gene mutation carriers) to nonpaternity, consanguinity and pharmacogenetic information. One possibility is to have an agreed upon list of childhood onset actionable disorders to be reported immediately to the parents with the additional data reported at a later stage either when the clinical significance of the findings becomes clear (e.g., resolution of VUS) or when the existing data has any clinical value later in life. Indeed, the ACMG recommendations specify a panel of 56 condition genes where mutations detected should be reported to the parents, irrespective of age of tested infant, since they are deemed 'actionable' (Green et al., 2013). Similarly the Public Population Project in Genomics and Society (P3G), suggested the disclosure of any WGS results that are "scientifically valid, clinically useful, and reveal conditions that are preventable and actionable during child-hood" (Knoppers *et al.*, 2013). While a 'staggered genetic test disclosure' approach is a feasible solution, it places a burden on the parents and the health care provider to disclose the information to the tested infant and provide him or her with the correct interpretation and consequences.

Data storage accessibility and future queries

WES/WGS for NBS generates a substantial amount of patient specific genetic data that could and should be stored primarily for the patient's own well-being. Future analyses may reveal information that can impact clinical management and possibly therapeutic decisions of the tested individual. Another benefit from such data storage is actually generating 'newborn biobanks' (Knoppers et al., 2012). Such biobanks seem invaluable and will certainly have an effect on future research that will incorporate the genetic data with environmental factors and exposures into a model that will expedite unravelling the pathogenesis of complex diseases. An unresolved issue is where to store the data: should it be stored in the newborn medical file (Dondorp & de Wert, 2013)? Should it be in the possession of the parents and then the adult testee? The family physician? Who will have access to the data? Who will pay for the storage? Should the existing data be revised and updated on an ongoing basis for defining novel 'actionable' genetic variants? Who is responsible for these queries?

Mandatory testing or consensual?

Currently, nongenetic NBS programs are offered at no cost and require no parental consent as they are intended primarily for the child's benefit, with a secondary aim of reporting information that may impact familial reproduction decisions. WES/WGS in NBS can possibly be objected to by the parents, requires specific consent, as all genetic testing does, and raises the concern that not all gathered information can be used for the immediate benefit of the infant. One way to circumvent these potential objections is to have mandatory NBS by WES/WGS for a specific set of disorders that could lead to immediate impact and benefit for the infant's health, and ask for consent for the additional diseases that may affect health later in life (Tarini & Goldenberg, 2012). An additional issue that relates to consent is the need to provide genetic counselling in order to obtain consent. Given the vast amount of information that could potentially be detected, the burden on the current genetic counsellor work force and the relevant physicians will increase to become an insurmountable task. One has to rethink the classical genetic informed consent paradigm in order to streamline the process, while maintaining professional integrity.

Other issues

In order to start the process of WGS for NBS at current day prices, should we first start with infants at high risk of having a serious detectable genetic disease (such as infants in intensive care units)? Who should prioritize these tests? What would be the criteria for such a prioritization?

Standardization of tests in terms of lab work (e.g., sequencing platforms, read depths, mean and minimum coverage) as well as reporting of these future tests should be considered of prime importance. In addition, it seems equally important to decide that these tests should not be performed as a direct to consumer for all to buy. It is crucial that these tests be developed in the academia context, namely within university affiliated medical centres so that it is possible to guarantee quality of health care, ensure adequate genetic counselling to parents and unselected accessibility.

Existing policies and guidelines

There is still a paucity of clear policies and guidelines by both professional organizations and policy makers. In 2005, the UK's Human Genetics Commission issued a report entitled 'Profiling the Newborn', that basically rejected using genetic testing for NBS based on the lack of evidence of the utility and benefit of this approach and costs (Human Genetics Commission, 2005). The American College of Medical Genetics and Genomics (ACMG) and the American Academy of Pediatrics issued a statement that infant genetic testing and screening "should be driven by the best interest of the child", and that testing for adult onset disorders should be delayed until later (ACMG Board of Directors, 2012; Committee on Bioethics et al., 2013). The ACMG further states that pediatric WES/WGS should not be used for NBS (ACMG Board of Directors, 2012) and should be considered as a diagnostic test with a potential impact on clinical management and decision making or in the context of an ethically approved research protocol (ACMG Board of Directors, 2013). A similar view was echoed by the European Society of Human Genetics (ESHG) (van El et al., 2013) and the Foundation for Genomics and Population Health (PHG Foundation, 2014). The paucity of policy directives and professional guidelines pertaining to utilizing WES/WGS in NBS programs emphasize the urgent need for an international effort to define 'actionable consensus' that is based on solid scientific evidence that considers and addresses the multiple aspects of utilizing these novel techniques to NBS (Evans et al., 2013). Indeed, the NIH has directed funds to studies on this issue (National Institutes of Health, 2013). Three major players [the Pediatric Platform of the P3G (www.p3g.org/p3g-internationalpaediatricresearch-programme), the Ethics Committee of the Human Genome Organization (www.hugointernational.org/comm hugoethicscommittee.php) and the Professional and Public Policy Committee of the ESHG (www.eshg.org/pppc.0.html)] have issued a scientifically based recommendation that states the "the primary objective of genome sequencing in NBS should be the identification of gene variants conferring a high risk of preventable or treatable conditions, for which treatment has to start in the newborn period or in early childhood At this time, we recommend a targeted sequencing or targeted analysis approach" (Howard et al., 2015).

So the answer to the question – next generation sequencing for newborn screening: are we there yet? Is not yet, but we are well on our way.

Declaration of Interest

None.

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