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Next Generation Sequencing in New Born Screening - Current Insights

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Abstract

Newborn screening (NBS) program refers to a nation-wide or state-wide program that identifies and treats newborns with rare congenital conditions before the onset of symptoms, preventing premature death and serious disability in thousands of newborns. Following the great success of Next Generation Sequencing (NGS) technology in the clinical diagnosis of genetic disorders, a lot of expectations have been raised among researchers, clinicians and the public for its implementation in the newborn screening program (NBS). But in view of the ethical, legal and social issues revolving around the use of genome sequencing approaches in health-care and public health programs it is necessary to address these issues beforehand to avoid its long term failure. This review will focus on the realized and expected benefits of using NGS in state NBS program and will also highlight the major hurdles and practical difficulties that have to be considered for materialization of such a program.

Introduction

Till date Sanger sequencing has been the gold standard for DNA sequencing. Using this technology a major foray called the Human Genome project started in 1990 and lasted for 13 long years wherein $3 billion was expended to determine the whole human genome sequence. But in spite of the known usefulness of DNA sequence analysis at that time it was beyond imagination for the clinicians to think about sequencing every patient's genome to find possible variants underlying the concerned disease due to practical limitations of this technology i.e. being expensive and time consuming. Thereafter, with continuous advancement in the research methodology and scientific aptitude, DNA sequencing underwent major improvements making it possible to sequence a large number of samples in parallel which was not quite possible by Sanger sequencing. The emergence of Next generation sequencing (NGS) in 2005 met the key shortcomings of Sanger sequencing in being more cost effective, rapid, and requiring lesser amount of DNA. Clinical implementation of NGS for disease characterization in individual patients was found to be highly fruitful (Worthey et. al., 2011; Lupski et. al., 2010; Liew et. al., 2013).

NGS, in view of its present achievements in the field of diagnosis, has heightened the expectations of the scientific community and clinicians in incorporating it in routine clinical practice and more recently in mass screening programs like NBS (new born screening).This review will focus on the realized and expected benefits of using NGS in the state NBS program and will also highlight the major limitations that have to be considered for materialization of such a program.

Extension of NBS in the Genomic Era

NBS is an essential, preventive public health program established internationally in order to identify disorders in newborns that was started almost 55 years ago. It began as a method for pre-symptomatic diagnosis and preventive treatment for one disorder Phenylketonuria (PKU) in newborns and later, in the 1990s, with the introduction of a much cheaper and reliable analytical technique Tandem mass spectrometry, more than 30 different metabolic disorders were added to the screening panel in neonates which led to a significant expansion of NBS. The initial guidelines followed for including any disorder in the neonatal screening program were based on the Wilson and Jungner criteria (Laine et. al., 2013) that emphasized on conditions that are considered as

an important health problem with well understood natural history and requiring immediate medical intervention in order to prevent serious and permanent illness, and for which there is an available treatment. Currently with expanded NBS most babies are screened at birth for between 30 and 50 genetic disorders, primarily by using tandem mass spectrometry (MS/MS) and many of these disorders do not fit into the classical paradigm of NBS. In this way, newborns are being screened even for conditions that do not present as emergencies and may not be immediately life-threatening, but could benefit from treatment with prophylactic antibiotics or if their screening might have additional benefit to parents for reproductive purposes (Grosse et. al., 2006). Thus, right from the beginning, expansion of NBS has been an attempt to provide maximum benefit to the child and the family, through use of the growing knowledge about genetic disorders and technology. Now when we are in the Genomic era where progress is taking place in an ever quickening pace, many of the seemingly unrealistic visions are beginning to materialize. The recent reduction in the cost and time required for sequencing the whole genome and the promise it holds both for research and health care have drawn a significant momentum around the idea of using NGS in a state-run mass screening program for NBS. But before this thought could be objectified we must recognize all the challenges that might interfere in attaining this vision.

Challenges to be Faced Before the Establishment of NGS–NBS Program

NBS, as discussed before, is a state-run mass screening program that aims to identify serious, treatable disorders in asymptomatic newborns that require immediate medical intervention. To carry out newborn screening NGS can be used in a better way. Being a high throughput technology it can scan the entire sequence of the newborn's genome to produce a huge amount of information about target and off target disorders, information of which may or may not be desired, and may not require clinical intervention, but it stands as a

Table 1 Challenges to be addressed before implementation of Next generation sequencing in newborn screening.

ISSUES	KEY QUESTIONS
DATA STORAGE	Who will be responsible for maintenance and governance of large amount of data generated?
REAL COST	Keeping in mind the costs for data analysis, family notification, follow ups and confirmatory testing, what should be the cost per test and the total cost for screening?
ETHICAL CONSIDERATION	How to report uncertain results to parents and how to do follow up once the newborn is discharged from the health center? Whether to report back results for late onset disorders to parents as it hampers the child's autonomy to know or not to know? Who will be responsible for the privacy of stored data and results until maturation of the child? How to settle the issue of sharing results regarding serious, preventable genetic disorders with at-risk close relatives, if parents decline to give consent? Will the test information result in prejudice against individuals with genetic disorders?
INSURANCE DISPUTES	Will test information make it difficult for an individual to obtain health insurance as predisposition to a genetic disease might be considered as a pre-existing condition?
MEDICAL MALPRACTICE	Whether clinicians are properly trained to interpret data generated by genome sequencing? Will hospital administrators be sued over testing errors?

modern choice over existing technology. In view of the significant demands in achieving the NBS objectives, NGS is expected to bring both these ends (NBS with modern technology) together. However, this definitely calls for making changes in the current policy, ethics and legal considerations (Table 1).

• NGS-NBS: Whole Genome, Exome or Gene Panel: At the most basic level, before establishment of NGS-NBS, the first thing to be sorted out is how is NGS to be used in a screening program? NGS being a most versatile tool can be implemented in different ways to provide information about the entire genome or only the gene coding region or certain target genes in a selected panel known to be involved in disease pathogenesis.

Generally exome sequencing has an edge over sequencing of the entire genome, owing to its low cost and easy interpretation in terms of disease. But the exome covers only 1% of the human genome: thus any DNA variation in the non-protein coding region will obviously be missed. Furthermore, exome capturing by hybridization can introduce substantial amount of coverage variability that will have impact on comparative analyses. Also, copy number and structural variations (CNVs and SVs), as well as some insertions, deletions and block substitutions are difficult to detect in exome capture data (Belkadi et al., 2015; Meynert et. al., 2014). These studies highlight the technical upper hand of whole genome sequencing over exome sequencing in providing an intrinsically richer data of polymorphisms outside the coding region and disclosing genomic rearrangement. With the steep reduction in the cost of DNA sequencing in recent years (Wetterstrand, 2013; Young S, 2014), the main economic benefit of using exome sequencing is nullified as more sequence information now could be obtained by using WGS, thereby cost effectively supporting the use of WGS in NBS. To explore the possibility of establishing WGS-NBS a study was performed recently, which compared the screening results of 1,696 infants by the state-run NBS program and whole genome sequencing for 27 disorders. Though WGS yielded fewer false positive results as compared to TMS-based NBS, the frequency of results with uncertain significance was quite high. The conclusion of the study was that WGS might be used in complementation with the present TMS-based NBS assays (Dale et al., 2015). Conversely, a recent online survey to analyze the professional opinion of genetic counselors about the use of whole genome sequencing in the newborn period identified that majority of the respondents felt that presently WGS should not be used in NBS and if it were to be used, it should not be mandatory. They considered that accurate interpretation of the result, more extensive consent process, pre and post-test counselling, comparable cost and turnaround time must be achieved before using NGS in NBS (Ulm et al., 2015). Howard et al. in 2015 have suggested to perform targeted analysis of genes that are clearly involved in a specific disease with effective and accepted preventive or therapeutic intervention (Howard et al., 2015). However the choice of panel of genes to be tested will depend upon the epidemiological prevalence which is not uniform across the world.

• Data storage and retrieval: cost and privacy: Sequencing the genome of all the newborns will generate a huge amount of data and proper data analysis and storage will be required. The cost of the TMS-based NBS procedure (2011) in the European Union ranges from € 0.46 per newborn to € 43.24 which is much lower in comparison to the cost that will be needed for sequencing neonate genome and analyzing the data (Frank et al., 2013). The real budget for the entire process of screening by applying genomic sequencing is far more than the proposed $1000 as it does not include the cost of data analysis, family notification and follow-ups and confirmatory testing (Mardis et al., 2010). On an average 353,000 babies are born per day around the world and the economic feasibility of sequencing, analyzing and maintaining the vast amount of data generated is questionable. In the case of late onset disorders or a fatal disease the information regarding the result of sequencing must be given to the child after he becomes mature and decides to know his disease status. Fully guaranteeing the governance and privacy of this information until being disclosed is not possible (Chadwick et al., 2013). However, as the speed at which technology is progressing, in the near future it is likely that more advanced cost effective sequencing technology will emerge, thus there might actually be no need to store such information and sequencing could be done when desired at a later age. But this option will invalidate the much boasted utility of NGS at an early age and using the information generated for aiding personalized medicine in the future.

• Variants of unknown clinical utility: Incorporation of NGS in NBS will increase the number of uncertain variants simultaneously increasing the

burden on the parents and the care providers (Cooper and Shendure, 2011). Additionally, it will also lead to an upsurge in the pressure on the laboratory and clinicians to determine the clinical validity of the variant at the earliest. A combination of variants might be detected in some newborns which may never lead to occurrence of disease. This will cause consequences of over-treatment in an otherwise healthy child and will result in unnecessary psychological and financial burden on the family. On the other hand, as sequencing is not totally free from error and also there are chances that some variations might get missed depending on the sequencing platform used, an infant may get deprived of early diagnosis and ameliorative or preventive therapy (Knoppers et al., 2013; Clark et al., 2011). Interpretation of results might also vary among the laboratories and there might exist discrepancy in assigning a variation as pathogenic or inconsequential hence causing under-diagnosis or over-diagnosis of disease. Keeping all this in mind it is advisable to determine whether or not to return uncertain results to the parent.and whether to store the data until validation of these variants and to then notify it to the parents Also, as the status of variants of unknown significance keeps on changing and is mostly reclassified over time, it is important to plan follow-up procedures and family notification wisely without raising anxiety among the parents. The follow up procedure in a country like India will become even more problematic as most of the families come from remote areas and are often impossible to trace again.

- Unsolicited findings: Although the main emphasis of the NBS program is centered around what is most beneficial for the child and its expansion to NGS is expected to maximize the benefits to the child's health, exome or whole genome sequencing can often reveal probabilistic information about the relatives in the extended family also. The primary concern of the clinician is to decide on how to return these results to the parents. Though the information of an adult onset disease may not be required for the child, it might still have clinical implications for the parents or relatives. It is a conflicting situation for the clinician to decide whether to disclose it to the concerned at-risk individual as it may hamper the child's right to an open future. Many attempts have been made to categorize these unsolicited findings and decide which of these to be disclosed, but not in the screening context (Bredenoord et al., 2011; Berg et al., 2011). More recently the Public and Professional Policy Committee of the European Society of Human Genetics, the Human Genome Organization Committee on Ethics, Law and Society, the PHG Foundation and the P3G International Pediatric Platform have recommended that unsolicited findings which lead to a preventable or treatable health problem should be communicated (Howard et al., 2015). Such ethical issues need to be considered and it is advisable to counsel the parents about such consequences before the test is performed. In case of untreatable diseases, it is recommended that the information must not be given to the parents but to the child at the proper age after consent (Shannon, 2014).

- Need for clinicians trained in genetics: A large number of variants are identified in sequencing the newborn genome and the clinical relevance of most of them is not so straight forward. Relatively few doctors receive significant training in genetics and related molecular sciences, and thus lack the background needed to effectively interpret the results of a genetic test. What to report back to the parents is often a difficult judgment call for these clinicians and if this issue is not addressed before extending NBS to NGS, it may increase the number of cases of medical malpractice, where a physician can be held responsible for not being able to detect or disclose the genetic risks preceding the eventual manifestation of the genetic disorder. Though not in context of newborn screening, but a recent case in Connecticut in which a woman sued her physician for failing to warn her that her family history of breast cancer also implied a possible genetic risk for ovarian cancer (Downs v. Trias, 2012) provides some insight into the bigger picture of NBS upgradation and its pitfalls. One factor that can help reduce liability risks is to improve the knowledge and training of physicians on genetics-based healthcare. But unluckily, most medical schools have only recently started training students in genetics, and many physicians feel that they are not well trained to address genetic issues (Richard et al., 2011).On the other hand, the fear of missing important genetic information and being held for medical malpractice might force the physician and the policy makers to return more positive results to the parents for which the follow up results may be normal but still it can have negative psychological impact on the parents (Hewlett et al., 2006; Johanna et al., 2012).

- Informed consent: NBS is usually conducted without an explicit consent because it is seen to be

in the best interest of the child's health. However, for genetic screening informed consent is the utmost requirement and the case is no different in genetic NBS also. The biggest concern in obtaining parental consent is that who should convey the complicated genetic counselling to the parents and get the informed consent? Are the nurse and physician well trained for this or a genetic counselor must be appointed for this purpose? Will it be possible to give such facilities in smaller hospitals and medical centers where most of the babies are born? Would an information brochure be sufficient for resolving parental queries regarding genetic screening and whether most of the parents, who have minimal genetic testing experience, can actually understand the complex genetic information (Harvey, 2014)? A survey was recently carried out on parents' opinion of whole-genome sequencing for newborns, if it were offered by newborn screening programs or pediatrician services. In both scenarios, 70% of parents expressed interest in whole-genome sequencing, citing test accuracy and the ability to protect a child from developing a disease as important factors in their decision-making process. But rest of the parents expressed no interest in newborn WGS and were concerned about the "privacy of results", "potential for results to be used to discriminate against their child," and that results could be used for research (Goldenberg et al., 2014). Thus the major threat for genetic NBS is that some parents might completely opt out of NBS due to fear that the detection of certain genetic variations in their newborn can jeopardize obtaining health or life insurance, or even school acceptance and future employment (Landau et al., 2014). This might have serious consequences for an infant who has a disorder that needs immediate medical intervention.

Testing the Ground Reality of NGS

For implementation of a robust technology like NGS in a mass newborn screening program, the main focus should not be just technologically biased; it should also be tested for its long and short term impact on the family and the child. The crucial question here is whether large-scale genomic sequencing can provide useful medical information beyond what current newborn screening is already providing and at what economical and emotional cost? To address these issues and to analyze the technical, clinical, practical and ethical aspects of genomics research in the newborn period, the NICHD (National Institute of Child Health and Human Development) and the NHGRI (National Human Genome Research Institute), both parts of the NIH (National Institutes of Health) had launched 4 pilot programs in the year 2013 and allotted a fund of $25 million to four grantees over five years. These grantees include: Brigham and Women's Hospital, Boston where the genome sequence-based screening for childhood risk and newborn illness will be studied in both sick and healthy infants by employing whole genome sequencing; Children's Mercy Hospital, Kansas city where the researchers are examining the benefits and risks of using rapid genomic sequencing technology in the NICU population and trying to return the results in 50 hours; University of California, San Francisco are conducting exome sequencing utilizing newborn blood spots for disorders that are or are not currently screened for in NBS with an agenda to improve and expand NBS; and University of North Carolina at Chapel Hill that is performing exome sequencing of healthy infants and infants with known disorders. These projects are presently ongoing and in the years ahead we may look forward to finding more realistic answers to the current ambiguity regarding the application of genomic sequencing in NBS.

Conclusion

Implementation of NGS in NBS would require stretching the benefits related to NBS i.e. from what is good for the infant, to what might be potentially good for the infant, to what might be good for the family (e.g., reproductive benefit or health benefits for family members), or to what might be beneficial for the society at large (research), thus in a way diluting the primary goal of the screening program. Presently, in view of the haze surrounding the use of WGS or WES in NBS, it seems not likely to fit within the available public health-care system due to the practical, financial and ethical challenges that are making this vision difficult to achieve. Though the outcomes of the pilot projects on large-scale assessments of the risks and benefits of genome sequencing for newborns will aid in designing the guidelines for NBS expansion in the near future, as of now the topic of newborn genome sequencing as a public health initiative remains contentious. It is recommended that such a program could be conducted but as a commercial supplement with consent.

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