CURRENT GENETIC TESTING TOOLS IN NEONATAL MEDICINE

Dr. Bahar Naghavi

Assistant professor of Basic Science Department, Shahid Beheshti University of Medical Sciences, Tehran, Iran
Introduction

- Over 4000 Mendelian disorders are known to have a genetic etiology at present, and a significant fraction of these present in the perinatal period with one or more of these clinical presentations.
• The incidence of genetic disorder was higher in newborns if all \textit{congenital anomalies} were included (7.94\%). It is remarkable that over 800 genetic disorders have been catalogued in the Online Mendelian Inheritance in Man presenting in the newborn period.
In these instances, a molecular diagnosis has a significant impact not only on the recurrence risk for families, but also on providing appropriate medical care and health intervention for the newborn.

Therefore, it is critical that healthcare providers understand the basics of the multitude of genetic tests that are currently in place, and that they be familiar with the fundamental genetic concepts to assess children suspected to have inherited disorders.
Newborns with congenital malformations, syndromic conditions, and inherited disorders often undergo an extensive, expensive, and long diagnostic process, often without a final diagnosis resulting in significant health care, societal, and personal costs.
• With the growing understanding of the magnitude of genetic diseases in newborns and equally rapid advancement of tools used for genetic diagnoses, healthcare providers must have a sufficient knowledge base to both recognize and evaluate genetic diseases in the neonatal period.
Newborn screening

• The most successful application of genetic testing has undoubtedly been in the field of newborn screening, aimed at identifying treatable conditions. Every year, millions of pre-symptomatic newborns are tested for critical genetic, endocrine, metabolic, and hemoglobin disorders using a single 3-mm dried blood spot sample.

• Early detection of metabolic diseases has assisted in reducing mortality, morbidity, and disabilities in at-risk newborns.
newborn screening system

. The newborn screening system consists of 5 parts: (1) newborn testing; (2) follow-up of abnormal screening results to facilitate timely diagnostic testing and management; (3) diagnostic testing; (4) disease management, which requires coordination with the medical home and genetic counseling; and (5) continuous evaluation and improvement of the newborn screening system.
Chromosomal abnormalities constitute a major category of genetic diseases, which involve the entire chromosome, causing either trisomy or monosomy (aneuploidy) as a consequence of nondisjunction.

These can also be seen as structural rearrangements, observed as insertions, translocations, inversions, deletions, duplications, and complex rearrangements. The effects of chromosomal imbalance are largely due to altered dosage of genes that are important for various cellular functions.
Genetic Tests

• Chromosomal microarray analysis

• For the past five decades, **G-banded karyotype analysis** has been used for the evaluation of birth defects in children, offering resolution of genetic imbalance of over 5–10 Mb in size.

• **G-banded karyotype analysis** continues to be the mainstay of the evaluation of aneuploidies in present day, including trisomy 21, trisomy 18, trisomy 13, and Turner syndrome (45,X).
For the first time, fluorescence in situ hybridization allowed detection of disease-causing submicroscopic events that were often below the detection threshold of G-banded karyotype analysis. Fluorescence in situ hybridization (FISH) is available for rapid diagnosis of these critical aneuploidy syndromes.

The characterization of these submicroscopic events in human diseases, also called structural variations or DNA copy number variations (CNVs), has been revolutionary in the field of human genetics.
• CNVs involving all chromosomes have been identified in the past decade due to the growing use of array-comparative genomic hybridization (aCGH) [chromosomal microarray analysis (CMA)]. The higher sensitivity of array-comparative genomic hybridization, often within 10–30 kb resolution, offers advantages over routine G-banded karyotype analysis when identifying genomic imbalances that are submicroscopic.
Clinical characteristics of patients are not always related to specific syndromes. **Array-comparative genomic hybridization (aCGH)** is used to detect submicroscopic copy number variants within the genome not visible by conventional karyotyping.

The clinical application of **aCGH** has helped the genetic diagnosis of patients with unexplained 1) developmental delay/intellectual disability, 2) autism spectrum disorders, 3) with or without multiple congenital anomalies.

**aCGH** is proving to be a powerful tool for the identification of novel chromosomal syndromes, thus allowing accurate prognosis and phenotype-genotype correlations.
Advantages of the array-CGH

- Microscopic chromosomal rearrangements
  - Aneuploidy (with limited mosaicism detection)
  - imbalanced rearrangements
  - Marker chromosomes
- Submicroscopic alterations
  - Subtelomeric imbalanced rearrangements
  - Micro-deletion/duplication syndromes

Disadvantages and limitations

- Balanced rearrangements
  - Reciprocal translocation
  - Inversions
  - Robertson translocations
- Imbalanced rearrangements below the diagnostic resolution
  - Point mutations
  - Three nucleotide expansions
  - Deletions / Duplications in not covered regions
- Limited ability to detect polyploidy
- Limited ability to detect mosaicism
- The method needs a great quantity of DNA
The microarray technology is based on hybridization of patient's labeled DNA against a healthy reference control. The measurement of signal intensity ratio of patient's DNA to reference DNA allows identification of gains or losses of chromosomal material.

These CNVs involve deletion or duplication of genomic segments responsible for genomic disorders. Genomic disorders, usually affecting dosage-sensitive gene(s), are increasingly being recognized as important players in human birth defects, as shown by the increasing use of CMA in clinical practice.
• Offering an increasing detection rate of about 15–20%, CMA is now considered as a first-tier test in the evaluation of children with multiple congenital anomalies.

• Undoubtedly, CMA undertaken for evaluation of dysmorphic features and congenital anomalies in the newborn period often uncovers these relevant genetic disorders that require prolonged multidisciplinary care. In many tertiary care centers, CMA is now routinely performed for congenital cardiovascular malformations, with diagnostic yield as high as 20% in children with syndromic cardiac defects.
While CMA is a powerful tool in identifying the genetic cause of multiple congenital anomalies, it is important to note that not all structural variations observed on CMA are pathogenic. Some are benign and largely characterized as copy number polymorphisms.

In other instances, CNVs may be involved in complex disease traits, with incomplete penetrance and variable expressivity. Since CNVs are common in the genomes of healthy individuals, it can often be challenging to attribute pathogenicity to loci that are frequently involved in structural rearrangement in the unaffected population.
Much progress has been made in recent years in utilizing **massively parallel sequencing** for rapid diagnosis of genetic conditions in neonates.

**Next-generation sequencing** is increasingly being used for noninvasive prenatal diagnosis, and it may become an essential component of newborn screening.
Rapid whole genome sequencing (WGS) is imperative in light of growing evidence of its utility in acute care, such as in diagnosis of genetic diseases in very ill infants, and genotype-guided choice of chemotherapy at cancer relapse. In such situations, delayed, empiric, or phenotype-based clinical decisions may meet with substantial morbidity or mortality.
While the application of cytogenetics and molecular cytogenetics has been pivotal in the evaluation of newborns with suspected genetic diseases, DNA-based sequencing studies have been equally important with ground breaking and innovative utility in newborn evaluation. Mendelian disorders, such as congenital muscular dystrophy, ...in newborns, have traditionally been evaluated by single-gene DNA sequencing studies or gene-panel evaluation. Massive parallel sequencing or next-generation sequencing is now increasingly being utilized in neonatal intensive care setting for rapid genetic diagnosis. Collectively, the 180,000 exons (termed exome) only account for about 1.5% of the human genome, but they contribute to 80–85% of all the known disease-causative variants.
What can exome sequencing do for you?

• Recent advances in next-generation sequencing technologies have brought a paradigm shift in how medical researchers investigate both rare and common human disorders. While whole genome sequencing remains prohibitively expensive for most applications, exome sequencing—a technique which focuses on only the protein-coding portion of the genome—places many advantages of the emerging technologies into researchers' hands. Recent successes using this technology have uncovered genetic defects with a limited number of probands regardless of shared genetic heritage, and are changing our approach to Mendelian disorders where soon all causative variants, genes and their relation to phenotype will be uncovered. The expectation is that, in the very near future, this technology will enable us to identify all the variants in an individual's personal genome and, in particular, clinically relevant alleles. Beyond this, whole genome sequencing is also expected to bring a major shift in clinical practice in terms of diagnosis and understanding of diseases, ultimately enabling personalised medicine based on one's genome.
Exome sequencing has recently been elevated to the standard of care for genetic diagnostic testing, particularly for genetically diverse and clinically heterogeneous disorders. Overall, the widespread adoption and use of exome sequencing in routine clinical practice is expected to improve diagnosis rates and reduce test costs, while leading to improvements in patient outcomes and a renewed emphasis on disease management.

Although it is extremely powerful, there is a concern of uncovering incidental findings (unrelated to the reason for testing of the newborn) that can be relevant for child's future health, or can impact the well-being of parents and other family members. For this consideration, it is highly recommended that WES be performed with detailed pretest counseling, generally offered by genetic counselors or clinical geneticists skilled in addressing these concerns related to genomic testing.
Massively parallel DNA-sequencing systems provide sequence of huge numbers of different DNA strands at once. These technologies are revolutionizing our understanding in medical genetics, accelerating health-improvement projects, and ushering to a fully understood personalized medicine in near future.

Whole-exome sequencing (WES) is application of the next-generation technology to determine the variations of all coding regions, or exons, of known genes.

WES provides coverage of more than 95% of the exons, which contains 85% of disease-causing mutations in Mendelian disorders and many disease-predisposing SNPs throughout the genome. The role of more than 150 genes has been distinguished by means of WES, and this statistics is quickly growing.
Background: exome-seq

- Main application of exome-seq
  - Find disease causing mutations in humans

- Advantages
  - Allows investigate all protein coding sequences
  - Possible to detect both SNPs and small indels
  - Low cost (compared to WGS)
  - Possible to multiplex several exomes in one run
  - Standardized work flow for data analysis

- Disadvantage
  - All genetic variants outside of exons are missed (~98%)
Mitochondrial disease in newborns

- The increasing awareness of the presentation of mitochondrial disease in neonatal period has resulted in systematic evaluation of this disorder in the newborn critical care units. Mitochondria are energy-generating power plants of the cell, having their own small genome containing 37 genes that encode 13 proteins, 22 tRNAs, and two rRNAs. Inherited as both an autosomal recessive and an autosomal dominant disease due to nuclear DNA mutation, mitochondrial disease is also caused by sporadic or maternally inherited mitochondrial DNA changes, leading to dysfunction of the mitochondrial respiratory chain. A disruption of this function can virtually involve any organ system in the body, seen clinically as lactic acidosis, encephalopathy, skeletal myopathy, cardiomyopathy, liver disease, respiratory difficulties, swallowing dysfunction, sensorineural deafness, and/or ocular disease. Electron transport chain studies have traditionally been the mainstay in the diagnosis of mitochondrial diseases, although with the emergence of high-throughput technologies, next-generation sequencing is increasingly being used for the diagnosis of such diseases.
Conclusion

• In summary, the phenomenal advancement of molecular technologies in the diagnosis of genetic disease in recent years has dramatically changed the landscape of neonatal medicine.

• While the utility of whole genome sequencing is being deliberated currently for its inclusion in the newborn screening program, the implementation of this technique in the clinical evaluation of critically ill neonates is imminent. It is vital for pediatric healthcare providers to be cognizant of the available diagnostic tools for providing the best clinical care to their patients with genetic disorders.
A molecular diagnosis for a patient with genetic disease can provide information regarding a patient's prognosis, management and reproductive risk, and identify molecular targets for treatment. However, genomic testing frequently identifies variants of uncertain significance.
• **Whole-genome and whole-exome sequencing** for clinical applications is now an integral part of medical genetics practice. The term newborn screening refers to public health programs designed to screen newborns for various treatable metabolic conditions, by measuring levels of circulating blood metabolites.

• The availability and significant decrease in sequencing costs has raised the question of whether metabolic newborn screening should be replaced by whole-genome or whole-exome sequencing.

• While newborn genome sequencing can potentially increase the number of disorders identified by newborn screening, the generalization of its practice raises a number of important ethical issues.
Thanks for your attention