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EDITORIAL

Application of next generation sequencing in the screening of monogenic diseases in China, 2021: a consensus among Chinese newborn screening experts

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Introduction

Newborn screening (NBS) refers to a maternal and newborn healthcare technology, in which special examinations of congenital and genetic diseases that could seriously impact the health of newborns, are implemented during the neonatal period to provide early diagnosis and treatment [1]. With a history of more than 60 years, NBS has advanced greatly due to technological progress resulting in significant improvement in the number of diseases covered by NBS and in screening efficiency [2–7]. Decades of use have shown that NBS has effectively prevented the death or disability of most patients improved the prognosis of patients with certain diseases, improved the quality of life of the patients and their family, and brought significant benefits to families and society [2–7]. However, there are limitations to traditional NBS including: (1) there are only a small number of diseases covered by NBS; (2) the screening efficiency for some diseases is still low. Despite the fact that the positive predictive value of screening has been greatly improved thanks to the efforts of researchers from various countries and regions, the problem of false negative results in some cases with normal biochemical markers during preliminary screening has not been resolved [8–10].

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In recent years, the advent of next generation sequencing (NGS) has led to significant breakthroughs in the diagnosis of genetic diseases. Improvement in analytical accuracy, reporting turn-around-time and standardization of result interpretation as well declining sequencing costs have led to rapidly increased adoption of NGS-based genetic testing in regular clinical practice by domestic and overseas experts [11–23]. In addition, the widespread application of various molecular diagnostic technologies, especially NGS, in the diagnosis of genetic diseases has helped discover a large number of disease-causing variants. Accurately evaluating and analyzing the clinical significance of these variants have become huge challenges in the field of molecular diagnostics, which prompted the need for standardization of analysis and interpretation of the pathogenicity of genetic variants.

Studies exploring the use of genetic testing technologies in NBS or genetic diagnosis of high-risk children, along with rapidly accumulating genetic and phenotypic data from clinical studies, have provided an evidence-based foundation for newborn genetic screening [24–34]. Compared with traditional NBS, genetic screening has obvious advantages including screening for diseases with no reliable biochemical markers as well as higher throughput. It has the potential to provide fast, accurate diagnoses which could lead to treatment changes and earlier prevention and surveillance of genetic diseases [24–34].

However, researchers have identified important concerns with newborn genetic screening: (1) it is difficult to interpret the unidentified loci of disease-causing genes; (2) based on the current level of awareness of human genetic diseases, the advantages of genome sequencing technology for newborn screening are yet to be identified; (3) there are still issues concerning social ethics [25–35]. The number of pilot genetic NBS projects has been on the rise in China. To protect the interests of newborns to be screened and their families to the greatest extent, the Newborn Inherited Metabolic Disease Screening Group, Specialized Committee for Birth Defect Prevention and Control, Chinese Preventive Medicine Association, and the Neonatology Group, Pediatrics Branch, Chinese Medical Association gathered experts to discuss the development of a consensus statement on China's NBS for monogenic diseases (referred to as genetic NBS). The group developed this consensus through an ethics-first, forward-looking lens, with the hope that it will further standardize the genetic screening system for newborns and provide guidance on the application of NGS in genetic NBS.

Basic requirements for genetic newborn screening

Requirements for facility

Facility site, personnel, equipment, reagents, and management procedures for genetic NBS shall comply with the current regulations related to NBS [1, 35]. Meanwhile, a screening facility shall be qualified to perform nucleic acid amplification analysis, and shall comply with the Management Regulations on Clinical Gene Amplification Laboratories of Medical Facilities 2010 or provide testing service jointly with a facility that complies with the above requirements.

Requirements for personnel

On the basis of complying with the requirements for personnel in relevant technical specifications on newborn screening, the head of a laboratory shall have a doctoral degree or a senior technical title, and have work experience in genetic testing. All personnel shall strictly fulfill their duties and comply with technical regulations on diagnosis and treatment required by relevant regulations [1, 35].

Requirements for equipment

A laboratory shall meet the requirements for conventional equipment in traditional NBS and have laboratory diagnosis equipment for molecular genetics.

Requirements for information and data management

NBS is a social health project integrating organizational management, laboratory technologies, clinical diagnosis and public education, which involves multiple disciplines and shall be equipped with a sophisticated data analysis and information management system. A screening facility shall establish and partner with other organizations such as hospitals with clinicians in genetics specialty to develop a comprehensive referral mechanism to meet the needs of affected newborns identified with genetic screening (e.g., treatment options, follow-up visits), treatment, and follow-up visit. The cooperative facility should sign agreements with the newborn screening facility to define their respective responsibilities and obligations, and provide subsequent testing and diagnostic services. Relevant medical facilities must strictly follow the principles of medical ethics, actively take measures to safeguard the rights and interests of newborns, and protect the privacy of individuals and their families.

Basic principles for newborn genetic screening

Principles for disease and gene selection

The following criteria shall be considered when establishing which diseases will be part of newborn genetic screening: (1) diseases that may result in serious consequences

and cause disability or death; (2) diseases with a relatively high incidence and well-defined pathogenesis; (3) diseases that have no special symptoms in the early stage but can be confirmed/clarified by the results of laboratory testing; (4) diseases that can be screened with an accurate and reliable method suitable for large-scale screening in newborns, have relatively low false positive and negative rates and the cost can be easily accepted by parents; (5) diseases that can be reversed or mitigated by effective treatment methods and whose prognoses can be improved due to early treatment; (6) diseases with reasonable cost–benefit ratio; (7) diseases that have no effective treatment or an actionable suggestions, but comply with other aforesaid principles and can inform family planning; (8) monogenic diseases with a well-established genetic etiology (i.e., causative genes).

On the basis of compliance with the above standards, it is highly recommended to select monogenic inherited diseases that have well-established disease-causing genes and well-defined genotype–phenotype correlation. Special consideration should be given to diseases that can be diagnosed in the neonatal period for which effective treatments are available and/or early interventions would be beneficial or diseases that meet the requirements of Principle (7). Genes associated with adult onset diseases or susceptibility are not recommended. For disease selection by making full use of the advantages of NGS and considering the prevalence/morbidity of local genetic diseases, and in principle. And genes covered by genetic NBS shall meet the confirming grading criteria of Clinical Genome Resource (ClinGen). It is recommended to include genes that relative high penetrance with majority of the known pathogenic variants detectable by the selected methodology. Finally, the sensitivity of the genetic testing technology and the cost–benefit ratio of the entire screening project shall be carefully considered.

Highly recommended diseases to be covered by newborn screening

Based on the aforesaid two principles and the collective experiences of the NBS for monogenic diseases expert panel, the following diseases are highly recommended (see Supplementary Table 1). The list may be expanded or narrowed based on local conditions, or a panel including different numbers of genes may be prepared, from which parents providing informed consent may select genes based on their own needs.

Combining genetic newborn screening with traditional newborn screening

If newborns have both traditional biochemical/tandem mass spectrometry screening (referred to as traditional NBS) and genetic screening with overlap of the target diseases their combined results will be more effective and accurate to clarify the disease status in the newborn. The traditional NBS result can be used to supplement the interpretation of the genetic screening result, while the genetic screening result will help to detect a false positive or false negative result of the traditional NBS. Efficient combined analysis of traditional NBS and a genetic NBS will require the developing and application of infrastructure that transmits results and data in a timely and accurate manner.

Newborn genetic screening workflow

Notification and signing of informed consent before screening

Medical staff should strictly follow the principles of medical ethics, and fully inform guardians of newborns of the target diseases, significance, cost, operation process, detection risks and other issues; the limitations of the NBS methodology and the possibility of false negative and false positive results; and the NBS screening and follow-up visit processes. Guardians of newborns should sign the informed consent after being informed of all relevant information. The informed consent must be signed prior to specimen collection and testing. The informed consent should be made in duplicate, with one copy kept by the screening facility and the other one by the guardian.

Collection of basic information and transportation of newborn samples

For the collection of basic information, sample collection and transportation, the Technical Specifications for Newborn Disease Screening 2010 and the Expert Consensus on the Collection, Delivery and Preservation of Blood Spot Card for Newborn Disease Screening 2019 should be followed. It should be noted that the quantity of samples collected must meet the requirements of the genetic screening project.

Next generation sequencing

NGS is characterized by: (1) massively parallel sequencing, high throughput, and high analytical sensitivity and specificity; (2) the quantitative function (i.e., the abundance of a gene in a sample is represented by the number of times a certain gene sequence is sequenced which allows for genome copy number analysis; (3) a greatly reduced cost of single base extension compared with first-generation sequencing. NGS has obvious advantages in

molecular diagnosis of birth defects and genetic diseases involving multiple genes, multiple variant types, and rare variants, which is also very effective in identifying new disease-causing genes [36, 37]. NGS can be categorized by detection range: disease-targeted gene package panel sequencing, whole exome sequencing, and whole genome sequencing (WGS).

Laboratory testing process and quality control of screening

DNA extraction

Upon samples receipt the laboratory should complete DNA extraction and DNA quality inspection following established protocols in the laboratory. The quality of DNA can be evaluated with the absorbance ratio method (A260/A280 value 1.6–2.2), and the integrity of DNA can be checked by agarose gel electrophoresis when necessary. DNA samples maybe stored at 4 °C for a short term and at − 20 °C or below for long-term storage, during which unnecessary repeated freezing and thawing should be avoided.

Next generation sequencing library construction

The construction of a target gene library is an important part of the entire NGS process and its quality will have a direct impact on the quality of subsequent sequencing data. The yield of DNA from one 8-mm blood spot rarely exceeds 200 ng, so capturing the target sequence through multiplex polymerase chain reaction amplification is an appropriate method for library construction. If enough DNA sample can be extracted from multiple blood spots, library construction can be carried out with the liquid-phase hybridization capture method, which is more commonly used. The concentration or the total amount, fragment length and distribution of a constructed library should be tested, and the specific standards should be defined based on different detection strategies.

Sequence generation

Currently NGS instruments of various throughput, read length, sequence accuracy and chemistries are available. A sequencing platform and pipeline maybe selected based on the sample size, quality metrics, running time and read length to ensure that output data meet the requirements for data quality and target area coverage. Sequence quality metrics such as Q30 scores should be established during test validation according to specific methods and sequencing platforms following current guidelines and standards [38, 39].

Bioinformatic analysis

The bioinformatic analysis process in NGS mainly includes sequence alignment, variant detection, variant annotation, variant filtering, and relevant quality control statistics. Each laboratory should define bioinformatic quality metrics including coverage of the target area, average coverage depth, mapping percentage, and the sequencing depth of each base in the target regions, etc. Genetic variant analysis involves multiple steps, such as preliminary variant screening, phenotype matching, and interpretation of the pathogenicity of variants. Each laboratory should establish standard operating procedures (SOPs) for data interpretation in accordance with relevant guidelines in the industry and require relevant personnel to receive sufficient training and assessment prior to data interpretation and report issuance.

Verification of testing results

Generally Sanger sequencing is performed to verify any suspected variations detected by NGS, such as single-nucleotide variant. If the laboratory has a well-established methods with a validated quality control system to reliably detect variations supported by previous verification results, those loci may be exempted from verification. This can help reduce the reporting time and testing cost. However, for some more complex types of variants, such as small insertions and deletions (Indel), or variants that are first identified in the laboratory, verification is still highly recommended. It is recommended that until the laboratory becomes experienced in testing and establishing a comprehensive quality control system, all suspected positive results should be verified.

Principles for interpretation of genetic newborn screening results

In 2015, the American College of Medical Genetics and the Association of Molecular Pathology jointly issued the Guidelines for the Interpretation of the Pathogenicity of Gene Sequence Variants based on the surveys on a number of clinical molecular diagnosis laboratories and expert advice [40]. The ClinGen expert group continuously supplements and optimizes the Guidelines, and has also provided a series of recommendations [41]. These guidelines, recommendations and consensus have been applied by many clinical molecular diagnostic laboratories, which has expedited the standardization of interpretation of the pathogenicity of gene variants to a great extent and improved the consistency of the results of locus interpretation. As for genetic variants detected by a genetic NBS, it is recommended that each laboratory refers to existing guidelines, consensus and technical standards on locus interpretation, and establish a

standard process for the interpretation of testing results, to ensure the consistency and accuracy.

Unlike general genetic disease diagnostic testings, newborn genetic screening mainly targets newborns without clinical presentations at the time of testing, so it is impossible to evaluate pathogenicity of any detected variants based on newborns' clinical phenotypes. Therefore, publicly available resources such as the published literature and public databases, as well as internal variant databases developed by individual laboratories and computational prediction results will serve as major sources of evidence for the interpretation of these loci. A carefully established SOP to collect available information thoroughly and analyze evidence systematically is crucial to full and accurate locus interpretation. In addition, some commercial databases can also provide more information [42, 43].

Principles of genetic newborn screening reporting

For the genes and loci of genetic NBS reports, the focus should be in the interests of the tested newborns and their families. Thus, every effort should be made to avoid causing unnecessary concern to the families and raising ethical issues. Therefore, suggestions related to the reporting of newborn genetic screening results are as follows: (1) reported genes shall be carefully selected and sufficient evidence will be required to demonstrate that the gene(s) can cause childhood diseases with high penetrance (onset before age 18); (2) genes with moderate evidence or moderate penetrance, for which intervention in childhood may prevent subsequent major diseases, may be considered for reporting [43]; (3) it is suggested that only variants with pathogenicity and suspected pathogenicity should be reported so as to avoid the uncertainty of report contents and reduce the difficulty of clinical consultation; (4) if only one pathogenic or likely pathogenic locus is identified in an autosomal recessive genopathy, it shall be reported as a likely carrier, but a follow up diagnostic testing is recommended if the gene associated phenotype is consistent with other clinical indications or family history, such as variant revalidation by higher resolution gene sequencing tech or functional studies; (5) if a locus causes mild symptoms or has a late onset (> 18 years old), or there is literature supporting the presence of overt insufficiency of the locus, it is advisable not to report it. It may be considered for reporting after comprehensive consideration of family history and other clinical indications; (6) it is recommended to report those variants with which female heterozygous carriers of X-linked gene disease-causing loci may have non-classical phenotype due to X chromosome inactivation preference. Monitoring and follow-up visits can significantly reduce the risk of disease; however, full genetic counseling by a genetic specialist is required.

Standards for genetic newborn screening reports

It is recommended that a newborn genetic screening report, which is similar to that of a genetic disease test report in terms of format, includes the following information: (1) basic information: unique identifier of the subject, date of birth, sex, parents' information, specimen type, sampling date, testing date, report date, information on the submitting physician and hospital, and information on the test laboratory; (2) detection results: standard gene name (followed HGSV nomenclature), reference genome coordinate, transcript number, nucleotide change, amino acid change, heterozygosity, pathogenicity, introduction to related diseases and information, and genetic model; (3) other information: clinical and genetic consultation suggestions, technical quality control parameters, genes or variant loci covered by detection, clinical and technical limitations and references.

Clinical management after newborn genetic screening

Principles for patient recall

The following are scenarios in which recall should be performed: (1) newborn genetic screening cases with positive results; (2) newborn genetic screening cases with negative results in the combined genetic and traditional biochemical screening program but suspected positive with just the traditional biochemical screening result.

For diseases covered by both genetic NBS and traditional NBS, the negative genetic NBS result should not be considered as an indicator to rule outpatient recall, particularly when the traditional biochemical NBS result is positive, provided the technical limitation of genetic testing whereas true pathogenic variants may not be detected or reported as pathogenic due to limited evidence. The screening facility is responsible for the recall of positive/suspected positive cases.

Diagnosis and treatment of positive/suspected positive cases, genetic counseling, and follow-up visit

The above-mentioned cases need to be referred to consultants and specialists with genetics training for further verification of variant loci in parents and other auxiliary

examinations to assist in the diagnosis. Patient diagnosis should involve multidisciplinary medical staff, social service personnel, and charity organizations in treatment, genetic counseling, and follow-up visit to ensure that the patient and his/her family benefit from NBS.

Potential ethical issues

The ethical principles of seeking benefit, avoiding harm, autonomy and fairness must be followed in the process of genetic counseling. It is required to ensure the confidentiality of family medical history and data to reduce the psychological burden on patients and their families, and avoid possible social discrimination, which need more social propagate.

Limitations and prospect of newborn genetic screening

The technical limitations of NGS and variants information should be considered in the design of testing protocol, interpretation of results and reporting. Newborn genetic screening in different region need to inform the residual risks of screening diseases based on specific implementation conditions. For example, areas of highly repetitive sequences or homologous sequences, deletion or insertion variants of large fragments, and special complex variants (such as the deletion of the spinal muscular dystrophy gene *SMN1*, and the most common intron inversion variants of 1 and 22 in the hemophilia A gene F8) require special experimental method design for detection. However, because the special experimental method design will increase the complexity and cost of the experimental process, it is necessary to thoroughly consider the genetic characteristics upfront such as variant spectrum and incidence rate of the target population.

Newborn genetic screening has been proved to be successful when using single monogenetic disease or target genetic sequencing panels currently in China. The ultimate goal of newborn screening is to shift from monogenic to polygenic genetic disease screening and from disease diagnosis and treatment to health management, and to ensure equal access to genetic screening for every newborn. There are new NBS models being developed abroad, such as age-based genetic screening (ABGS) [44].With ABGS, genomic screening of newborns is performed in the neonatal period and subsequent genomic analysis is performed at different stages of childhood development according to various genetic disease characteristics, so that the corresponding preventive management measures can be taken to improve disease prognosis.

However, the pathogenesis of human genetic diseases is more complex than expected, although the genetic testing technologies fast developing, there are still about half of highly suspected genetic-related diseases that have not yet been diagnosed. WGS has shown certain advantages in the clinical diagnosis of genetic diseases, it has also laid the foundation for large population genome database generation. Some countries have begun to try to use WGS technology to accumulate genomic information in the neonatal genome project, to apply genetic data as personal medical data to guide medication, risk prediction and lifestyle interventions in the future. However, WGS technology is difficult to popularize at this stage due to its cost, data interpretation, storage and other issues. It can be expected along with the fast growth of genetic testing methods and large human genomic data accumulation, people can achieve above goals one day. For this reason, the consensus team hopes to improve the existing test abilities and accumulate operation experiences through the practice of newborn genetic screening projects, so as to help neonatologists using new technologies to carry out screening work with a practical and operative guidance, then, the efficiency of newborn screening can be greatly improved through guidance, so as to realize the proactive prevention and control of hereditary rare diseases in the neonatal stage. Newborn patients can be identified early and take interventions early, will improve the overall quality of life of citizens ultimately.

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