REVIEW ARTICLE

Prenatal Diagnosis of Birth Defects by Exome Sequencing

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ABSTRACT

Prenatal screening and diagnosis are increasingly becoming a part of medical practice. Prenatal screening can reduce the incidence of birth defects which cause morbidity and mortality in newborns. With emerging technologies, now it is possible to diagnose the genetic basis of birth defects more accurately in the prenatal period for early management. Different approaches are available for detecting genetic defects at different levels like karyotyping, chromosomal microarrays and Sanger sequencing. However, many cases still remain undiagnosed. Next generation sequencing has revolutionized the field of genetics that can detect genetic defects at the level of a single base pair. It includes both whole exome sequencing (WES) and whole genome sequencing (WGS). WES has particularly accelerated the discovery of disease-causing variants in many monogenic anomalies postnatally. Research is being conducted on the use of whole exome sequencing in the prenatal diagnostics of genetic anomalies detected by ultrasound. It is a more efficient way of getting an insight into the molecular basis of birth defects compared with conventional genetic approaches. However, technical and ethical issues need to be addressed before introducing this technique into routine prenatal clinical practice. Fetal cell sampling is done by invasive medical procedures like amniocentesis or chorionic villus sampling. However, noninvasive strategy of collecting fetal DNA from maternal plasma is an exciting and emerging domain. It is evident that in the coming years, we shall be able to use these techniques in the routine clinical setting and to improve the diagnosis and management of birth disorders during prenatal period.

Key Words: *Birth Defect, Prenatal Testing, Next Generation Sequencing, Exome Sequencing, Congenital Anomalies, Hereditary Defects, High Throughput Methods*.

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Introduction

Birth defects or congenital malformations are anomalies which originate during the developmental period and present at birth due to environmental or genetic insults. They are a serious public health issue globally, however, their prevalence is particularly high in low-income countries. It is estimated that each year 4-6% of the newborns have some kind of genetic birth defects globally, early diagnosis of such disorders is critical for timely management and in some cases for treatment.[1] Prenatal screening and diagnosis should be present in every antenatal unit. Many structural birth defects can be detected by prenatal ultrasound and then confirmed by molecular testing. However, the most effective prenatal screening methods were those that were used for screening of Down syndrome by maternal serum and ultrasound markers. Prenatal diagnosis by using techniques like karyotyping, Polymerase Chain Reaction (PCR) and more recently chromosomal microarray are being used for the detection of underlying genetic defects.[2] Monogenic disorders are diagnosed by sequencing particular those genes associated with that disease. However, these approaches are not always effective and many cases remain undiagnosed. In high risk pregnancy, if a genetic test confirms a lethal anomaly or a condition that causes lifelong disability, parents can choose to terminate a pregnancy.[3] If the disease is hereditary in nature, accurate diagnosis is also useful to access the risk of recurrence in a future pregnancy.

High throughput genomic sequencing especially whole exome sequencing (WES) is a likely solution to this problem in the present scenario. It has been

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used effectively in finding genetic defects in several types of genetic disorders. It is advantageous over conventional genetic testing because it can be used to find a molecular defect in any genetic disease, it is not locus-specific and all known protein-coding exons can be sequenced in a single experiment. Efforts should be made to apply exome sequencing for the prenatal diagnosis. However, it should be kept in mind that DNA sequencing result is often complicated and challenging to interpret. It needs specialized expertise to draw a conclusion from sequencing data.[4]

Another important point that needs more work is fetal cell sampling. Conventionally, fetal cells are sampled by chorionic villus sampling (CVS), amniocentesis (AC) or fetal blood. However, these techniques have some disadvantages like pregnancy loss and birth defects like limb amputations.[5] But at present these are only standard clinical tests.

Nowadays, researchers have discovered an entirely new technique called noninvasive prenatal test (NIPT) that detect cell-free fetal (cffDNA) in maternal circulation.[6] But it still needs a series of testing and clinical trials before it adopted into routine clinical practice.[7,8] It has the potential to minimize adverse events associated with an invasive procedure.

In conclusion, more studies are warranted to bring noninvasive prenatal testing (NIPT) and exome sequencing into clinics. These techniques have opened a new horizon to solve undiagnosed cases and to provide timely management.

Different methods of prenatal diagnosis

1. Conventional methods

The detection of birth defects in the growing fetus was initiated over 50 years ago. It was started by detecting neural tube defect markers in maternal serum.[6] Subsequently, many approaches were presented for prenatal screening of various medical conditions. However, Down syndrome screening methods were most effective. Ultrasound and maternal serum markers were used to screen Down syndrome. Prenatal diagnosis by using techniques like karyotyping, Polymerase Chain Reaction (PCR) and more recently chromosomal microarray are being used for the detection of underlying molecular defects. Monogenic disorders are diagnosed by sequencing a single gene or a panel of genes. However, these approaches are not always effective and many cases remain undiagnosed. Diagnostic odyssey for rare birth defects can badly disturb the lives of patients and their families both mentally and financially.[9] So there is a need for current and advanced diagnostic approaches for timely management of birth defects.

2. Latest method

Next-generation sequencing (NGS) is the latest technique for deep DNA sequencing. It is more robust than conventional methods. It can be used to dissect the genetic architecture of a variety of diseases in clinical practice by one of the following methods.

(1) Whole exome sequencing (WES) or exome sequencing (ES), contains all protein-coding genes.

(2) Whole genome sequencing (WGS), contains the complete genome including both the coding regions (genes) and non-coding regions.

Almost 85% of reported disease-associated human mutations are in either coding region or intergenic splice site and can be detected through WES.[10,11] Additionally, WES is five times more cost-effective and produces 20-fold less raw data in comparison to WGS and hence is mainly used in prenatal diagnosis.[12,13] It has also been reported that WES can detect some additional small variants which might be missed by whole genome sequencing.[14] For pathogenic variant detection DNA is obtained from the affected fetus and both parents. Parental DNA is important for prioritizing pathogenic variants and also for confirming the mode of inheritance.[15]

Success of whole exome sequencing in prenatal diagnosis

The main advantage of WES is the identification of underlying molecular defects in patients where conventional methods fail to give an accurate diagnosis. The result can be used for genetic counseling of the parents. In the case of pathogenic variant detection in the fetus, parents can make a decision about the termination of pregnancy. The clinical effectiveness of exome sequencing has been established.[3] However, there is limited data available in the use of WES in the prenatal diagnosis of genetic defects. There are only a few studies that describe the use of WES in prenatal diagnosis. For instance, in a study, 24 fetuses with abnormal ultrasound findings were recruited for amniocentesis or chorionic villi sampling to extract DNA. After whole

exome sequencing (WES), a definite diagnosis was made for 21% cases.[16] Another study was conducted to identify genetic defects in fetuses with congenital anomalies of the kidney and urinary tract. Thecord blood of 30 fetuses eas sampled and whole exome sequencing (WES) was performed. In four cases, mutations were found while in two cases likely pathogenic variants were identified. Moreover, a large clinical study was conducted on 610 fetuses with structural anomalies.[17] WES was used for molecular diagnosis of structural anomalies and 8.5% of the cases were solved at the molecular level. These studies showed that structural defects can be diagnosed more accurately with WES. It can help the genetic counseling of couples and facilitates decision making.[18]

Whole exome sequencing steps

The typical workflow of WES can be divided into following main steps:

Library preparation and exome capture methodology

These steps are generally performed by commercial companies and include 1) DNA fragmentation using mechanical or biological enzymatic digestion 2) ligation of blunt ends with adequate adaptors 3) target enrichment to capture exome. After the washing of non-targeted sequences, the targeted region is amplified. Library concentration and fragment size distribution are evaluated using instruments like bioanalyzer by the commercial venders.[17,19]

Though many techniques have been described for targeted capture, only two have been extended to capture the entire exome.[6] The first is the array-based hybrid capture method and a more recent in-solution capture method. As the name suggests probes are immobilized in array-based platforms while probes are free in solution-based capture. Amplification and enrichment of captured exons is done by polymerase chain reaction (PCR).[20]

Sequencing

Sequencing is done by using a next generation sequencing platform. There are multiple commercial platforms available with variable sequencing strategies. Agilent, Illumina, NimbleGen are the popular stakeholders in next generation sequencing platforms.[13,14]

Computational analysis of exome-sequencing data

Exome capturing amplification and sequencing generates raw sequencing data and next step is the analysis of this data. In spite of multiple open source and freely available packages, data analysis is still a challenge for biologists/clinicians due to optimization, standardization of existing pipelines along with the integration of different tools.[9,21] The computational power required for whole exome analysis depends on several parameters including sequencing technology, desired throughput and genome size. However, a recent protocol stated that 64GB RAM, 8 core CPUs and Linux based systems might be appropriate for exome analysis.[22]

The entire process of data analysis can be divided into two major categories Pre-Variant Calling and Post Variant Calling. The first category includes computational tools to generate pre-variant calling format (Pre-VCF) file. These tools are used for the alignment of the raw sequencing reads to a reference genome. The second category contains workflow and computational tools for Post-VCF file generation. This analysis includes the detection of chromosomal positions of variants and type of changes etc. It contains workflows and tools for mutation detection, pathway analysis, copy number alterations, INDEL identification, and driver prediction. In the prioritization of the candidate mutations, the variants with high coverage and high-quality scores as well as predicted damaging effects by online tools are given priority. However, all variants should be taken into consideration. It also includes methods that link selected variants to clinical data.[23,24]

Validation

Sanger sequencing is considered as the gold standard for the validation of pathogenic variants selected by WES data analysis.

Methodological Concerns

Some points that need to be considered about WES include:

1. Basic procedures like sample acquisition and DNA extraction is time-consuming and require a high level of quality control because there is a risk of contamination.
2. WES sample preparation is very challenging and requires proper handling of high-tech equipment and rigorously following optimized

lab protocols.

3. WES only includes coding regions of the genome and if a mutation is present in non-coding regions like in introns, it cannot be captured by WES. In some cases, it is possible that all coding exons of a gene are not captured correctly. This can also lead to the failure of mutation detection in coding regions.
4. Other obstacles in the use of WES are high cost and turnaround time. However, it is becoming more cost-effective with the passage of time. But data analysis and variant interpretation require very specialized knowledge and highly skilled manpower to accelerate the process of analysis by robust bioinformatics pipelines. Since it is directly related to patient care, therefore a very reliable interpretation of data is a crucial step for an accurate diagnosis.
5. A multidisciplinary team is required for the use of NGS in prenatal diagnosis. So, it is not suitable in resource-limited and small clinical settings.

Future directions

It is expected that in near future NGS will be part of routine practice. There is continuous research and improvement in these techniques. Many genetic disorders are yet to be diagnosed at the molecular level. It is most likely that prenatal diagnosis will move from WES to WGS for unsolved cases. This technique will be coupled with the development of techniques related to the extraction of cell-free fetal DNA from the maternal circulation. So, it can be hoped that in the future there will be no need for an invasive sampling of fetal cells. And with the advancement of technology, problems related to data interpretation and turnaround time will be solved.

Burden of genetic diseases and future of prenatal testing in Pakistan

The burden of genetic disorders is higher in Pakistan than in Western countries. The prevalence of cousin marriages in our society is an important reason for this high incidence of familial disorders. Families with hereditary disorders having many affected individuals can be easily found in the Pakistani population.[25,26] So, there is an urgent need to provide prenatal testing services in Pakistani hospitals. Presently prenatal testing for certain genetic conditions is being provided in hospitals of few major cities by conventional methods. However, the noninvasive method of collecting fetal DNA and WES is still not in practice. We propose that there is an immediate need for developing a noninvasive method of collecting fetal DNA from maternal blood along with NGS technique at an economic cost. It will solve many problems that are encountered in current diagnostic testing methods. It carries no risk of miscarriage and limb amputations. It can provide a timely and more accurate results to make informed decisions. It can solve many ethical issues related to the late diagnosis of congenital anomalies and termination of pregnancy. However, we also need to develop a system to address ethical, legal and social issues related to the use of this technology.

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