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LITERATURE REVIEW

Perspective of Fetal Medicine in the era of prenatal genomics Tania

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Abstract

In recent decades, prenatal medicine has progressively incorporated different diagnostic technologies that have been able to complement existing methods. Cytogenetic techniques such as karyotyping have been complemented with novel high-resolution molecular techniques, allowing the identification of genomic changes with single nucleotide resolution. Some of these techniques incorporated into the evaluation of prenatal cases are QF-PCR, comparative genomic hybridization (CGH array), different methods of massively parallel sequencing, among others. Currently these molecular technologies for prenatal diagnosis are being implemented in our region since the last decade. Every implementation process brings with it advantages and challenges intrinsic to each technology, and the multidisciplinary team must clearly manage the indications for its use and the implications after the generation of results. In this paper we present some of the considerations by the American College of Genetic and Genomic Medicine and the International Society for Prenatal Diagnosis regarding the indications for these molecular tests and post-test counseling. This will allow the health personnel involved in these tests to implement them effectively, and to obtain a greater benefit for the patient.

INTRODUCTION

Congenital anomalies are conditions that cause a high rate of infant mortality and disability [1-3]. Worldwide, there are 3.2 million children with disabilities per year and 270,000 newborns die each year due to congenital anomalies. Most of these cases are born without a specific prenatal genetic diagnosis [4,5].

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All patients in whom a fetal anomaly is diagnosed should be offered genetic counseling including a description of the various existing genetic tests with advantages and disadvantages [5]. This group of tests in prenatal genetic includes targeted testing (QF-PCR and gene panels) and genomic studies at different levels (karyotyping, microarrays, exome sequencing and whole genome sequencing) (Table 1) [6].

Historically, G-banded karyotyping (resolution between 5-10 Mb) has focused on the detection of chromosomal abnormalities. Its major disadvantage has been the time to obtain results [4-7]. The combined use of other molecular techniques, such as fluorescence in situ hybridization (FISH) and QF-PCR (quantitative fluorescent polymerase chain reaction) has allowed rapid detection (2-3 days) of the most common fetal aneuploidies (trisomy 21,18,13 and numerical alterations of the sex chromosomes) [6]. Around 2010, microarray (0.2 Mb resolution) was implemented in prenatal diagnosis, detecting DNA gains and losses in the genome. Its importance lies in the fact that it can detect additional findings to traditional methods such as duplications, deletions, aneuploidies and other complex chromosomal aberrations. Among these we

Table 1. Prenatal genetic diagnostic methods.

Method	What we detect	Advantages	Disadvantages	Prenatal Application
Karyotype	Chromosomal abnormalities. Numerical (polyploidies or aneuploidies). Size:5-10Mb.	Detects chromosomal structural abnormalities. economic technique	Late result, between 12-15 days. Submicroscopic abnormalities are undetectable.	Subgroup of patients with high-risk first trimester or second trimester screening. Normal structural ultrasonography.
Array-AA	Chromosomal abnormalities. Numerical (polyploidies or aneuploidies). CNV. ROH	It detects chromosomal abnormalities that the karyotype does not find. It can be performed on non-viable tissue.	Does not detect most of single gene disorders. Limitations to detect mosaicism.	First-line study indicated when structural abnormalities are found on ultrasound. Recommended as first line when there is nuchal translucency >3.5mm.
Sequencing Directed Panels	Comprehensive analysis of selected genes. Deletions and duplications. CNVs.	Cheaper if we compare them with exome sequencing and complete sequencing.	It is difficult to define phenotypes with all their characteristics prenatally. It depends on gestational age, fetal position, available imaging studies, and maternal BMI. There is a difference between the prenatal and postnatal phenotype of a genetic disorder.	Panels described for skeletal dysplasia.
Exome sequencing	Exon analysis.	Identifies new variants (present in the fetus and absent in the parents). Inheritance of recessive variants.	The increase in test performance depends on management with an expert geneticist. Variants in non-coding regions, indels, rearrangements or translocations are not detected. It does not detect CNVs.	30% diagnosis of RASopathies in Non-immune Hydrops. It could be considered second line when there are multiple anomalies in various systems.
Whole genome sequencing	Analysis of intron and exon regions. NVCs	Time delivery of results is fast.	Encoded sequences only, not all genes are captured equally. Expensive	Situations in which single gene disorders are suspected.

CNVs, variation in copy number; AA: array analysis; ROH, regions of heterogenicity; indels: insertions and deletions.

have that these arrays can identify variants whose size is less than 5 Mb, and therefore are not recognized in conventional karyotyping.

Additionally, the arrays are capable of evaluating the entire genome in a single procedure [6]. Thus, these techniques began to be used to identify copy number change (CNVs) and microdeletions/microduplications that are not detectable by conventional lower resolution methods [6-8].

In 2012, a transition point in prenatal diagnosis was marked when Wapner et. al. [8], reported a 4-6% increase in diagnosis, using microarray, in fetuses with a structural anomaly that had normal karyotype. The microarray detected clinically relevant deletions and duplications in approximately 1 in 60 pregnancies without structural anomalies and in 1 in 17 pregnancies with a structural anomaly.

In clinical practice the microarray has several advantages: it does not require cell culture, so the turnaround time for results is shorter (3-4 days), it can be used in fetal loss samples (fetal death, recurrent gestational loss). The latter was demonstrated in a study where 532 fetal death samples were analyzed, the microarray researchers were able to find causal copy number variants in 87.4% of cases.

Accurate genetic diagnosis can help define fetal prognosis and improve prenatal care, as patients can make decisions that improve their reproductive outcome [6]. Knowing these diagnoses is extremely important for in utero therapy, birth planning, and neonatal management, as it can potentially decrease morbidity and mortality associated with genetic anomalies. It can also refine genetic counseling by better determining the risk of recurrence and allows subsequent reproductive choices to be made such as preimplantation genetic diagnosis or allows consideration of donated gametes or targeted genetic studies in future pregnancies [6,9].

In the following, we will describe the next generation sequencing (NGS) strategies that have been developed in the last ten years. Molecular prenatal diagnosis has been gradually introduced, initially "within the research setting" and

currently in specific clinical situations, within protocols established by multidisciplinary teams [10].

Next-generation sequencing

1. Targeted genetic panels (targeted panel sequencing)

When a particular clinical phenotype is identified, targeted sequencing of a group of genes responsible for causing a monogenic disorder is performed [4,11]. One of the most cost-effective examples of the use of panels is the study of skeletal dysplasia [12]. One of the limitations of this type of approach is that the most accurate identification of the observed phenotype is required. To achieve this, follow-up protocols for studies such as high-definition ultrasonography, fetal echocardiography and fetal magnetic resonance imaging must be performed, and there must be interdisciplinary management between fetal medicine, genetics and pediatrics.

Another important limitation in middle-income countries or countries with mixed health systems is the need to perform additional studies in addition to ultrasonography, since ultrasonography has low sensitivity for detecting minor dysmorphic features [13]. Therefore, the application of these panels should be individualized by the type of abnormality detected.

Finally, the limitation of selection bias occurs when using panels because there is a possibility that some disease-causing variant is located in another gene not included in the test. This limitation is overcome over time by identifying new genes involved in the pathology.

2. Whole Exome Sequencing (WES)

The use of this technology should supplement what is observed in a particular phenotype, allowing the clinic to target the search for variants in genes that have been previously associated with the phenotype. Exome sequencing is based on the analysis of protein-coding regions of the genome, known as exons.

There are more than 20,000 protein-coding genes, representing approximately 2% of the genome [9]. About 85% of the genetic variants known today to be associated with disease are found in the exome.

By performing a trio-based SE study, known as trio-based sequencing (including both parents and the fetus), it is possible to evaluate the segregation of the gene and phenotype and to determine whether the variant found is de novo or inherited [10]. This has relevance for the interpretation of the pathogenicity or not of such a variant.

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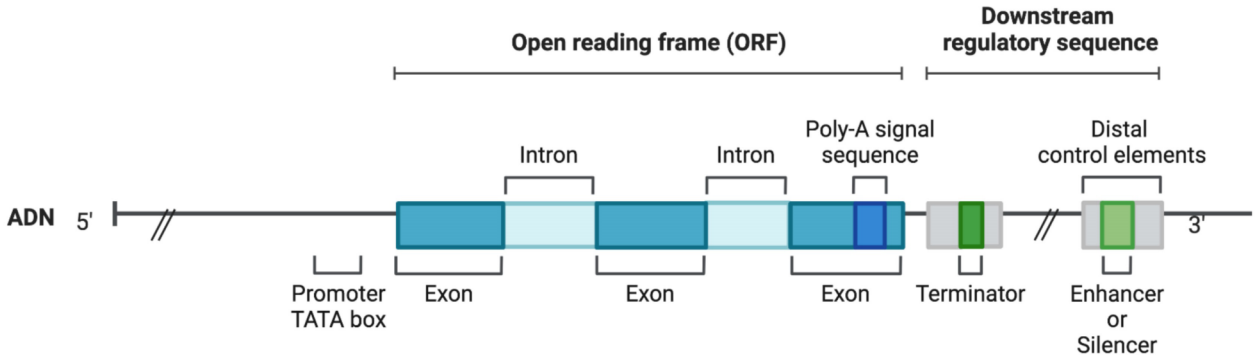
In a recently published meta-analysis, the diagnostic yield of SE, independent of the affected organ was 9-47%. Several studies have reported that in cases of central nervous system anomalies the diagnostic increase is 3-34% [16], recommending WES for those cases with karyotyping and array-based methodologies with negative results in cases of patients with central nervous system anomalies [17].

3. Whole genome sequencing (WGS)

Whole genome sequencing analyzes the entire genome, including intron and regulatory regions. These regions may contain regulatory domains important for correct gene transcription [4,5,11]. For example, a 2017 report, presents the use of WGS in the context of prenatal diagnosis for the detection of balanced chromosomal translocations, thus overcoming the limitations that other array techniques present [17].

Another important advantage is the ability to detect variants in non-coding regions. Although most of the disease-associated variants described so far are located within exons and exon-intron junctions, there are a considerable number of variants reported outside these regions; for example, in regulatory regions (such as promoters, enhancers, and transcription binding sites, etc). To understand this, it is important to keep in mind the structure of a gene and the regulatory elements (See Figure 1).

Figure 1. Structure of a gen con sus elementos of regulation.



Leyenda: DNA: Acido deoxyribonucleotide; Poly-A: poli-nucleotide signal; ORF: Marco de lectura abierto; Imagen bajo licencia de uso por Biorender.com

Some take-home aspects to consider are:

Both the American College of Genetic and Genomic Medicine and the International Society for Prenatal Diagnosis have published guidelines setting out the indications for each molecular test [5,10].

We have listed here some of the indications for the most common molecular tests in prenatal medicine:

Pre-test considerations:

1. SE could be considered for a fetus that presents with abnormalities by ultrasound, but microarray and karyotype are reported negative. During the multidisciplinary evaluation there is a high clinical suspicion of a genetic etiology (single-gene disorder).

2. A previous fetus (or child) with an anomaly or anomalies suggestive of a genetic etiology with an unexplained recurrence during the current pregnancy.

3. SE should not be offered as a routine study when there are no fetal anomalies.

4. Patients should be presented with the likely results that could be obtained including VUS, preferences in reporting incidental findings, unanticipated findings, turnaround time for results, and the likelihood of having to resample for reanalysis.

Post-test considerations [11]:

Follow what was established during initial counseling and respect decisions about which results are to be presented to patients and which are to be withheld:

1. A negative result does not necessarily mean that there is no genetic disorder in the fetus.

2. In most cases, uncertain results should not be used for pre-implantation testing or genetic testing in the next pregnancy. These results should be discussed and interpreted by an experienced geneticist.

3. Uncertain or negative cases may benefit from reanalysis if new clinical findings appear.

CONCLUSIONS

Molecular genetics has advanced greatly in Latin America. This implementation has become a very important tool for accurate prenatal diagnosis. As the complexity of prenatal genetic diagnostic options expands, the quality and quantity of genetic counseling services, based on adequate pre-test and post-test counseling, must also increase. The educational offer should be for both the health care provider and the patients, so that the best informed decision can be made. It is important that health professionals understand these new tools, and know their capabilities, limitations and indications for use.

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