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Advances of Genetic Testing Technology in Etiology Diagnosis of Recurrent Spontaneous Abortion

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Abstract

Recurrent spontaneous abortion (RSA) is a complex and heterogeneous disorder with multiple etiologies. Genetic factors are thought to play an important role in the etiology of RSA. With recent advances in genetic testing technologies, there has been an increasing interest in using these tools to diagnose the etiology of RSA. This review discusses the different types of genetic testing methods, such as karyotyping, chromosomal microarray analysis, next-generation sequencing, and their applications in the diagnosis of the etiology RSA. The use of genetic testing in the diagnosis of RSA has the potential to improve the accuracy of diagnosis and the understanding of the underlying mechanisms of the disorder, which could lead to better management and treatment of affected individuals.

Keywords

Recurrent Spontaneous Abortion, Etiology, Genetic Testing Technology

1. Introduction

Abortion is the most common complication of human pregnancy, accounting for approximately 10% - 15% of clinically confirmed pregnancies [1]. According to the definition of American Society for Reproductive Medicine (ASRM) and European Society of Human Reproduction and Embryology (ESHRE), recurrent spontaneous abortion (RSA) refers to at least two spontaneous abortions occurring before 20 weeks of gestation. It is a common human reproductive disorder

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affecting about 3% of couples of reproductive ages [1] [2] [3] [4] [5]. The risk of miscarriage in RSA patients is relatively high in re-pregnancy. Finding the potential cause of miscarriage is an efficient method to estimate the recurrence risk and take preventive management measures.

The etiology of RSA is extremely complex, with significant heterogeneity [6]. It includes reproductive tract anatomy, genetics, endocrinology, immunology, coagulation, masculinity, and a number of additional unknown causes. Scholars believe chromosomal abnormalities or genetic imbalances in embryos or fetuses are the major cause of abortion [7]. Genetic analysis of miscarriage and stillbirth tissue is of great value in analyzing the causes of miscarriage and stillbirth, assessing recurrence risk, and prenatal diagnosis. This paper will review the application of genetic testing techniques to the etiological diagnosis of RSA to serve as a reference for the selection of clinical diagnosis and treatment.

2. Genetic Testing Technology

2.1. G-Banding Chromosome Karyotype Analysis

G-banding chromosome karyotype analysis has been the most common method for diagnosing chromosomal abnormalities [8]. This method involves culture of cells or tissues under sterile conditions, and chromosome specimens are prepared by a series of operations such as trypsin digestion and Giemsa staining, and chromosome morphology can be observed under a microscope for karyotype analysis.

2.1.1. Karyotype Analysis of Couple's Peripheral Blood

Couples with chromosomal abnormalities, known as carriers, account for 2% - 5% of the RSA population [9]. In the study of peripheral karyotype analysis in RSA couples, the detected chromosomal abnormalities included abnormal chromosome number and structural abnormalities, of which structural abnormalities are the most common, including chromosomal equilibrium translocations, Robertsonian translocations, inversions, duplications, deletions [10] [11]. Chromosomally balanced translocations are highest in the carrier population [12], which are characterized by the exchange of DNA segments between non-homologous chromosomes, with no DNA gain or loss at breakpoints, and thus a balanced rearrangement; individuals with balanced translocations are usually phenotypically normal unless the translocation breakpoint disrupts a dominant gene or the exchange of chromosomal segments has an effect on the expression of nearby genes [13]. Carriers commonly seek medical attention after pregnancy loss, and chromosomal abnormalities are detected through peripheral blood karyotyping.

2.1.2. Products of Conception (POC) Karyotyping

The G-banded karyotype analysis of POC has been used by numerous scholars to study the causes of abortion. POC includes aborted villi tissue and amniotic fluid cells. Compared with peripheral blood, the karyotype analysis of POC requires higher culture conditions, and there is inevitable maternal material con-

tamination. Due to the need for *in vitro* culture, POC tissues or cells are required to maintain favorable biological activity. In the karyotype analysis of POC, the detected chromosomal abnormalities include aneuploidy, polyploidy, deletion, duplication, translocation, etc, among which aneuploidy is the most common [14] [15]. Aneuploidy results from the nondisjunction of homologous chromosomes during germ cell meiosis, resulting in an abnormal number of chromosomes in the zygote.

G-banding karyotype analysis can detect a variety of chromosomal abnormalities, which provides a great reference value for the diagnosis of RSA. However, there are myriad factors involved in the whole culture and analysis process, such as culture environment, colchicine concentration and action time, hypotonicity, fixation, drip, trypsin digestion, Giemsa staining, etc. Currently, there is no unified quality control for experimental manipulation of chromosome preparation. At the same time, this method has elevated requirements for laboratory technicians, and karyotype reading has a certain subjectivity. Due to the limitation of band resolution, conventional G-banding karyotyping can only identify the abnormal fragments larger than 5 Mb, and cannot judge the deletion, duplication and structural abnormality of minor fragments.

2.2. Chromosomal Microarray Analysis (CMA)

CMA, known as "molecular karyotype technology", is a high-throughput gene detection technology developed in recent years. For RSA patients, CMA plays a more prominent role in detecting pregnancy products. Compared to conventional karyotyping, CMA has the advantage of higher resolution, shorter detection time, and the absence of tissue cell culture. CMA can also detect clinically significant genomic copy number variants (CNVs). In addition to numerical chromosome abnormalities, newborn fetuses may also inherit CNVs or acquire absence of heterozygosity (LOH) from their parents' genomes, which may be harmful to embryonic or fetal development. Recurrent CNVs result from DNA nonallelic homologous recombination repair in regions with low copy repeat sequences [16]. Fetal CNVs may be inherent in the parental genome or may be newly formed in the parental gametes. LOH in the reproductive system may be generated by close marriage or by abnormal gene repair early in embryonic development [17].

As abortion tissue is often old samples, CMA provides a more suitable choice for RSA patients to find the cause of abortion.

2.2.1. Array Comparative Genomic Hybridization (aCGH)

ACGH is created in an ordered fashion using a small number of DNA fragments, with probe sizes ranging from a few tens to 2 hundred thousand base pairs, DNA samples were denatured, mixed DNA hybridized with probes in the array, and various fluorescence signals were emitted according to copy number amplification, gain, loss, or deletion to generate fluorescence intensity maps to identify CNVs present in the test DNA [18]. Clinicians regard CMA as an essen-

tial tool for screening and diagnosing genetic diseases. It has been found that about 1.6% - 1.8% of RSA cases reported clinically significant CNVs by aCGH. ACGH improved the detection efficiency and detected CNVs that could not be identified by conventional karyotype analysis [19]. Studies have shown that aCGH is effective in identifying common genetic aberrations, submicroscopic genomic rearrangements, and genes whose mutations cause miscarriage [15] [20] [21] [22]. However, aCGH probes cannot cover all chromosome segments, nor detect polyploidy and low-proportion mosaicism.

2.2.2. Single Nucleotide Polymorphism Array (SNP-Array)

SNP-array hybridizes the tested samples to a set of normal genomic controls [23]. Compared with aCGH, SNP-array has the advantage of detecting long extension homozygote, which can detect not only uniparental disomy (UPD) but also LOH. Arrays based exclusively on SNP probes are biased to include common certain genomic segments, and an early SNP detection array can only detect about 26% of the CNVs detected by the phosphor-terminal sequence mapping strategy; Newer SNP detection arrays also contain non-polymorphic oligonucleotide probes designed for copy number testing to provide more reliable and uniform coverage [24]. Overall, all current array platforms are capable of providing sufficient sensitivity for clinical CMA testing due to sufficiently dense probe coverage. CMA detects genomic imbalances in clinical settings with higher resolution and less subjectivity. Most current clinical CMA platforms can detect copy number changes in the whole genome with a lower limit of resolution of about 400 kb, which is more than 10-fold higher than G-banding karyotype analysis. This level of resolution will provide a broad genomic survey and reliably identify all known recurrent microdeletion and microduplication syndromes mediated by segmental repeat structures, as well as the majority of non-recurrent pathogenic imbalances that are clearly pathogenic. In the diagnosis of the etiology of RSA patients, CMA is more commonly used in the detection of pregnancy products. In a study of 5003 miscarriage samples, 309 genes were identified as potential miscarriage candidates, and three recurrent submicroscopic CNVs (22q11.21, 2q37.3, and 9p24.3p24.2 microdeletions) were found to be significantly more common in miscarriage cases [25]. Existing studies shown that in CMA detection of pregnancy products, the incidence of aneuploidy is the highest, followed by chromosomal structural abnormalities, triploidy [9]. However, while obtaining higher resolution, many CNVs of unknown clinical significance will also be detected, which brings certain difficulties to clinical consultation. According to the latest technical standards for CNVs interpretation and reporting, CNVs are divided into five categories: Pathogenic (P), Likely pathogenic (LP), Uncertain Significance (VUS), Likely benign (LB), Benign (B) [26]. The practicability of clinical consultation will be enhanced by a standard approach to the interpretation of CNVs applicable to all technology platforms and a widely accessible database of CNVs.

The limitation of CMA is that it cannot detect polyploidy, balanced transloca-

tions and low proportion mosaicism, and economic benefits are also part of the consideration due to the high chip cost.

2.3. Next-Generation Sequencing (NGS)

In recent years, with the rapid development of NGS, it has the advantage of more comprehensive genetic testing coverage and lower cost, and is widely used for disease diagnosis, especially prenatal diagnosis, providing additional options for the diagnosis of miscarriage in RSA patients.

2.3.1. Copy Number Variation Sequencing (CNV-Seq)

CNV-seq is the discovery of CNVs through bioinformatics analysis by sequencing samples and comparing the sequencing results with human reference genomes. It can detect CNVs of different sizes by adjusting the sequencing depth and changing the resolution. Numerous studies have reported the clinical use of CNV-seq to analyze the relationship between CNVs and miscarriage [25] [27] [28] [29] [30]. In a prospective chromosomal analysis of 3429 amniocentesis samples, the detection rate of pathogenic and potentially pathogenic CNVs increased from 1.8% to 2.8% using CNV-seq compared with karyotyping [31].

2.3.2. Whole Genome Sequencing (WGS)

WGS extracts DNA from the test sample, maps the sequenced reads to the reference genome and assigns them to a 20 kb sequencing box with a 5 kb slide to obtain higher resolution CNVs. In a study of 2186 pregnancy products with CNVs detected by WGS [32], chromosomal abnormalities were consistent with CMA; they found developmental genes that can be used to effectively identify pregnancy loss or congenital abnormal phenotypes. These genes were rich in genes related to embryonic development, especially neuronal development and differentiation.

2.3.3. Whole Exome Sequencing (WES)

WES is a gene detection technology for exon sequencing in protein coding regions, which is commonly used to detect the role of gene mutations in the mechanism of clinical diseases. In terms of prenatal diagnosis, if karyotype testing and CMA cannot determine the underlying cause of fetal malformations and structural abnormalities, WES can provide relevant information to aid in current pregnancy management. In the latest studies, many gene variants related to embryo abortion have been found by WES, suggesting that gene variants may be the potential etiology of RSA [33] [34] [35] [36] [37]. Future enriched WES results help to create a comprehensive database of genetic information containing mutations in genes that cause embryonic death, which will facilitate a broader understanding of the etiology of RSA and the development of strategies.

At present, NGS has been widely used in non-invasive prenatal screening, and it also plays an important role in the study of the etiology of RSA. Although NGS has many advantages in the detection of pregnancy products, its own limitations, such as the failure to detect UPD, balanced structural translocations and polyp-

loidy, should not be ignored. Due to the higher resolution of NGS, the interpretation of the results may increase the difficulty of genetic counseling.

2.4. Other Detection Techniques

In the process of using genetic testing technology to study the etiology of RSA, it is sometimes necessary to use some technical methods to support verification or technical exclusion of the detected abnormal results. Maternal cells may be involved in the collection of abortion tissue, which may affect the accuracy of the results. In addition, the next step of parental verification is required when chromosomal abnormalities are detected.

2.4.1. Fluorescent in Situ Hybridization (FISH)

FISH is a technique that uses a known nucleotide sequence labeled with fluorescence as a probe to hybridize with the target sequence in the chromosome to be tested, and then the fluorescence signal is observed under a fluorescence microscope to analyze the chromosome of the specimen to be tested. The detection efficiency of FISH is more dependent on the design of the probe and the corresponding detection. The chromosomal abnormalities of the abortion products detected by the above techniques can be verified by FISH for the parental chromosomes [38] [39], determining whether the mutation is new or inherited from the parents. The economic cost of using FISH probes is high because of the variation detected.

2.4.2. Quantitative Fluorescent Polymerase Chain Reaction (QF-PCR)

QF-PCR uses multiple pairs of fluorescent labeled primers for specific PCR amplification, and then the products are subjected to capillary electrophoresis, the chromosomal abnormalities are diagnosed according to the fluorescence signal intensity of the primers. In the detection of pregnancy products, QF-PCR is often used to exclude maternal cell contamination (MCC) in the sample to be tested [27] [40] [41]. If the specimen to be examined has a significant MCC, it should be excluded from the study.

3. Summary and Discussion

For pregnant couples, miscarriage can be a deeply distressing experience. Uncertainty about the cause of miscarriage and the concern that subsequent pregnancies may also fail gravely affect the physical and psychological well-being of RSA couples. Identifying the cause of a miscarriage can help predict the likelihood of continuing a pregnancy in the future and guide treatment. This is of great significance to couples who have experienced recurrent miscarriages.

This review discusses the application of chromosome karyotype analysis, CMA and NGS in RSA, and analyzes the advantages and limitations of each detection method by comparing the technical principle, type, distinguished and detection cost of different detection methods (**Table 1**), so as to provide certain guiding value for clinical selection of detection technology. Genetic testing is an

integral part of diagnosing the causes of abortion. A thorough understanding of the applicability of these testing methods and the selection of appropriate detection strategies will help to efficiently detect the etiology of RSA and provide more accurate information for subsequent genetic counseling. In addition, this paper provides a strategy for genetic testing of pregnancy products (**Figure 1**), and selects subsequent detection directions according to chromosome detection results to help determine whether chromosome abnormalities are original mutations or inherited from parents. Identification of specific diagnostic genetic variants can facilitate parental reproductive counseling and lead to improved management of future pregnancies by allowing prenatal or pre-implantation genetic diagnosis.

However, there are some limitations in this paper. Due to the rapid development of genetic testing techniques and the constant update of various testing methods, some of the contents of this paper may be partially biased and further literature reviews are needed to complement the relevant contents in the future.

Table 1. Advantages and limitations of various genetic testing methods.

	Aneuploid	Multiploid	Chimera	Microdeletion	Microdeletion, Microrepeat	Distinguishability	Cell culture	UPD	Cost	Others
G-banding chromosome karyotype analysis	√	√	√	√	×	Low	√	×	Low	Strong subjectivity
CMA	√	×	Low proportion of chimerism could not be detected	×	√	High	×	√	High	Uncertain significance CNVs
NGS	√	×	Low proportion of chimerism could not be detected	×	√	High	×	×	Relatively low	Uncertain significance CNVs

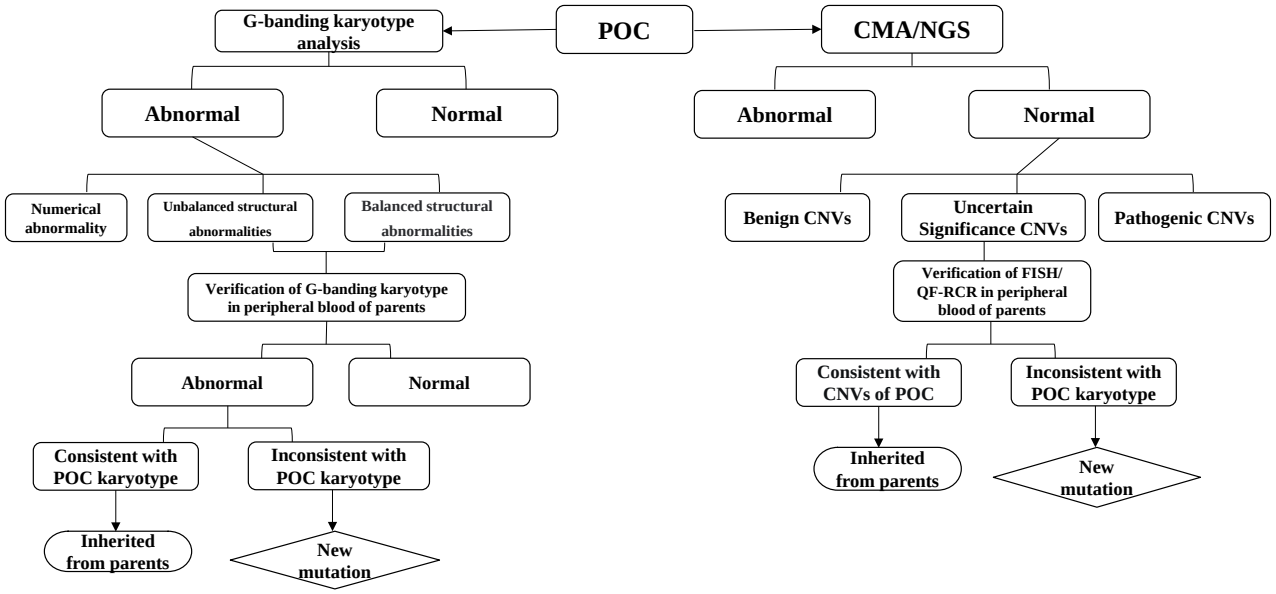

Figure 1. Strategies for genetic testing of pregnancy products.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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