REVIEW ARTICLE

Preimplantation Genetic Diagnosis

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Abstract

Preimplantation genetic diagnosis (PGD) or preimplantation genetic screening (PGS) is an early form of prenatal diagnosis which allows that, before the pregnancy has begun, embryos can be tested for genetic disorders.[1] The rationale behind the method lies in the removal of cells from early embryos and genetic analysis of these cells before being transferred to the uterus. This procedure offers an advantage for those couples having genetic disorders whose offspring has an increased risk of a specific genetic condition by helping in the delivery of a healthy baby or prevention of repeated spontaneous abortions.[2] As a result, PGS has developed as a valuable tool for enhancing pregnancy success with assisted reproductive technologies.[3] In fact, PGD may possibly be suggested for any disorder for which molecular testing can be performed.

Keywords: Embryo, Microarray, Next generation sequencing, Preimplantation genetic diagnosis.

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Biopsy Techniques

The diagnostic material which will be used for preimplantation genetic diagnosis (PGD) is generally collected at the three different stages of embryo development: (1) first and second polar body biopsy, (2) blastomere biopsy at the cleavage stage, and (3) trophectoderm tissue biopsy at the blastocyst stage.[2] Each of these approaches has some advantages and limitations.[1] For example, biopsies of the first and second polar bodies nowadays are not common practice as only a maternally-inherited disease can be analyzed, and the obtained genetic material is small, which increase possibilities of amplification errors in polymerase chain reaction (PCR) experiments, contaminations, and allelic dropout (ADO) during the PCR. However, polar body biopsy has no or minimum risk effect on embryo development and the lack of mosaicism.[2,4] Cleavage stage embryo biopsy, whereby one or two blastomeres are removed from a six-cell to eight-cell embryo, retains some advantages over polar body biopsy as maternally and paternally-inherited disorders can be diagnosed. Nevertheless, disadvantages remain, such as a small amount of the genetic material and the high risk of mosaicism exists, which appears to be a possible cause of misdiagnoses.[2,4] In the blastocyst stage, multiple cells can be biopsied, which leads to decreased amplification errors and an improvement of the result accuracy. Development of sequential culture medium facilitated application of TE biopsy in clinical practice by enabling a successful culture of embryos to the blastocyst stage and improving pregnancy outcome after the transfer of blastocyst.[4] However, mosaicism also occurs in blastocysts but to a lesser extent than in cleavage stage embryos, and the rate of aneuploidy is significantly lower in this stage in comparison with early-stage embryos.[2] Despite its significance and contribution to routine clinical procedures, PGD requires testing in an aggressive manner, which can disturb the embryo and compromise the clinical outcome. Therefore, the development of potential noninvasive approaches that in the future might play an important role is of interest and require further investigation.[2]

Molecular genetics and chromosomal analysis methods have also developed and currently include the PCR, fluorescence in situ hybridization (FISH), and comprehensive chromosome screening platforms such as the array comparative genomic hybridization (aCGH), single nucleotide polymorphism (SNP) microarray as well as next generation sequencing (NGS)[4] (Table 1).

The use of Polymerase Chain Reaction

The PCR protocol was used for the first clinical application of PGD in 1990 for gender determination of embryos for X-linked diseases.[5] Accordingly, the PCR method has become one of the most important molecular diagnostic techniques, especially useful for monogenic diseases. The first reports of PCR used for single gene defects were

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Table 1: Comparison of PGD methods

Method	*Diagnostic capabilities and strengths*	*Limitations*
PCR	Single gene defects	Contaminations Amplification failure ADO
FISH	Initially, used for sex determination Identification of aneuploidy Translocations	Limited number of chromosomes can be evaluated
qPCR	Whole chromosome aneuploidy Allows carrying out of genotyping Very sensitive and diagnostically accurate	Unable to detect segmental aneuploidy Cannot detect mosaicism or translocations
aCGH	Whole chromosome aneuploidy Translocations	Cannot detect mosaicism Might lead to false positives
SNP microarray	Whole chromosome aneuploidy Uniparental disomy Translocations More complex and with higher resolution then aCGH	Unable to detect balanced chromosomal rearrangement Unable to detect mosaicism
NGS	Whole chromosome aneuploidy Translocations Mosaicism Mitochondrial copy number Single gene disorders High resolution and complexity	Limited in detection of balanced chromosome translocations

for well-known monogenic diseases such as cystic fibrosis and thalassemia.[6] In general, PCR is a very powerful tool for the exponential amplification of short deoxyribonucleic acid (DNA) fragments starting with a very small quantity, which opens up the possibilities for analyzing single-cell genetic content. However, dealing with a limited DNA amount in a PCR reaction is challenging and leads to a number of hitches, including contaminations, amplification failure, and ADO (extreme preferential amplification of one allele or complete absence of one allele) in heterozygous samples.[1] Source of contamination are numerous, and commonly used precautions can be undertaken to overcome this problem. On the other hand, ADO can lead to misdiagnoses, especially for compound heterozygous or autosomal dominant conditions, while ADO should not lead to the transfer of an affected embryo in autosomal recessive disorder if both partners carry the same mutation. Therefore, for monogenic diseases, ADO appeared to be a complication for accurate PGD.[1] Accordingly, several variants and improvements of PCR have developed in an attempt to overcome these problems. For instance, to improve sensitivity and specificity, nested PCR was introduced where two consecutive rounds of PCR are applied. A more sensitive detection method, fluorescent PCR, was also developed and has helped in ADO detection because of its higher resolution and higher accuracy. In particular, fluorescent PCR is very precise in fragment sizing because it uses a laser system for automated fragment analysis with different fluorescent molecules.[1,6] Another PCR strategy, multiplex PCR, has been adopted for simultaneous amplification of two or more DNA templates by using combinations of unrelated primer sets in a single PCR assay in the attempt to overcome the limitations of single-cell PCR. In addition, linked markers can be used for ADO detection as there is a low possibility of ADO occurring at a series of different adjacent loci.[1] Microsatellite markers or SNP markers may well be used for linked markers. In multiplex PCR, both the mutation and the polymorphic markers are amplified together to increase the diagnostic accuracy.[7] Moreover, a substantial benefit of multiplex PCR is the possibility of using linked markers for diagnosis, and this strategy was applied in cystic fibrosis, fragile X syndrome, and Duchenne muscular dystrophy deletion carriers. Besides, in some of these cases, ADO might be detected as well.[6] Consequently, multiplex PCR with linked markers in combination with fluorescent PCR has become a method of choice in the diagnosis of different ailments.[6] For instance, a fluorescent one-step multiplex PCR technique based on the co-amplification of CAG repeats and three different polymorphic microsatellites was developed at the single-cell level for PGD of Huntington disease.[8] Moreover, the authors have used the same approach for PGD for other genetic disorders, like cystic fibrosis, spinal muscular atrophy, and Duchenne muscular dystrophy. Interestingly, this methodology can be considered for PGD cycles in which the use of numerous markers is required.[8] For a comprehensive overview of PCR based strategies used in PGD and a summary of the methods which were used for the reduction and detection of ADO, please refer to related literature[1,6]

Quantitative real-time PCR (qPCR or RT PCR) proved to be a highly sensitive technique in which the fluorescent reporter molecules are used for the quantification and monitoring of amplicon accumulation during each cycle of the PCR reaction. Moreover, the technique permits carrying out of genotyping, and even a single nucleotide can be detected, because fluorescent probes can be directed to either a wild-type or mutant sequence showing the potential of the implementation of this method in PGD.[1] Furthermore, a qPCR assay was established for identification of whole chromosome aneuploidy through detection of the copy number of each examined chromosome. The method relies on the comparison of several locus-specific amplified sequences of each chromosome to a reference gene from the

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same chromosome.[3,4] The technique is highly diagnostically accurate and completed in approximately 4 hours; however, it is unable to detect segmental aneuploidy as well as translocations or mosaicism.[4]

Microarray Platforms

Initially, the FISH was used for sex determination, soon after that for aneuploidy identification through visualization of chromosomal regions, and later in PGD for translocations.[6] However, the FISH allows for a limited number of chromosomes to be evaluated,[3,9,10] therefore, more comprehensive chromosomal screening techniques have been recently developed. For example, aCGH microarray technology is able to test whole chromosome aneuploidy as well as translocations, but its limitation is its inability of mosaicism detection.[4] The method utilizes a PCR library-based whole genome amplification followed by fluorescent DNA labeling, hybridization, and array screening. The amplified DNA from blastomere biopsy is compared to karyotypically normal reference DNA, and both are then hybridized to a microarray with around 3000–4000 human DNA fragments probes. The experimental procedure is complete for about 12 hours.[4,7] The method might lead to false positives; nevertheless, trophectoderm biopsy with aCGH appeared to be highly sensitive and specific for aneuploidy screening.

Single nucleotide polymorphism (SNP) microarrays appeared to be more complex and time-consuming than aCGH, even though they have a higher resolution. The SNP array offers genotype information for each sample analyzed in comparison to the human reference genome in the assessment of roughly 300,000 SNPs spaced throughput the genome. The array allows the identification of whole chromosome aneuploidy, uniparental disomy, plus approximately 250 common structural chromosome aberrations. Large deletions or duplications, bigger than 50 Mb can be detected as well.[3,4] However, the technique is limited in the detection of balanced chromosomal rearrangements and is unable to detect mosaicism. In addition, in the case of a consanguineous couple, genetic anomalies might not be detected due to the possibility of SNPs being homozygous at every locus.[4]

Interestingly, karyomapping was also developed to screen and compare the genotype of embryos with a reference genome, naturally, that of an affected family member. Therefore, karyomapping is typically used to assess embryos for single conditions that affect essentially their family. The technique utilizes genome-wide linkage analysis for the comparison of mother and father SNPs with those of the family members of a known genetic status in order to identify the SNPs alleles' combination linked to a chromosome which carries a gene mutation.[4] In addition, the method appears to be very accurate, with no need of design patient/disease-specific tests.[4] Furthermore, the karyomap gene chip has been used in PGD to avoid monogenic disease as well as chromosomal anomalies simultaneously.[11,12] Recently, the karyomap gene chip was used for monogenic disease PGD and PGS for exploring aneuploidy incidence in embryos from couples carrying monogenic diseases and the effect of embryo aneuploidy screening in monogenic disease PGD.[11] In particular, blastocysts were analyzed using the karyomap gene chip technique and, among embryos diagnosed as normal for monogenic diseases, 26.5% (approximately 1–4) were found to be aneuploidy and could not be transferred, demonstrating the requirement and importance of embryo aneuploidy screening in PGD for monogenic diseases. Therefore, the advantage of the karyomapping technology-based monogenic PGD is the ability to simultaneously implement embryo aneuploidy screening.[11]

Next Generation Sequencing

Next generation sequencing (NGS) is generally a massive parallel sequencing that has significantly reduced the cost of human genome sequencing. Accordingly, in the field of PGD and PGS, this method might have certain contributions to the improvement of the genetic assessment of embryos before their transfer to the uterus.[13] NGS assay for PGS utilizes whole-genome DNA amplification followed by DNA fragmentation and library preparation, where DNA fragments are fused to designated adapters.[3,4] Library preparation is followed by emulsion PCR or bridge PCR steps, depending on the NGS platform that has been used.[3] Two main NGS platforms are currently in use for PGS, Thermo Fisher Ion PGM from Thermo Fisher Scientific and Illumina MiSeq, both offerings targeted clinical applications however employing different sequencing techniques.[3,4,14] In general, NGS is highly complex, possesses high resolution, and testing takes less than 24 hours to perform. MiSeq can detect whole chromosome aneuploidy, mosaicism, mitochondrial copy number, as well as single-gene disorders and translocations.[3,4] However, the MiSeq platform is not designed to detect large deletions or duplications (>50 Mb)[3] but rather segmental imbalances of around 14 Mb and more.[4] PGM also allows the identification of whole chromosome aneuploidy, mosaicism, mitochondrial copy number, and single-gene mutations. PGM, in contrast to MiSeq, is able to identify large deletions or duplications, and clinically significant deletions or duplications to a resolution of approximately 800 kb–1 Mb.[3,4] NGS is a powerful technique for comprehensive chromosome screening, though the platform has limited ability to detect balanced translocations of the chromosome. Nevertheless, NGS-based platforms are to become the standard of care due to high accuracy and throughput.[4]

For example, the recent applicability of a commercial NGS-based workflow (MiSeq system from Illumina) was evaluated for preimplantation genetic testing for chromosomal structural rearrangement.[15] Indeed, the study demonstrated the ability of NGS to diagnose unbalanced reciprocal

translocation/inversion products with the same efficiency as aCGH. In addition, using the karyotype of reciprocal translocation/inversion carriers, the size of predicted segmental aneuploidies could be calculated and used for the prediction of NGS implementation before treatment proceeding, which is important in counseling couples before beginning the treatment.[15] The potential of NGS was also shown in analyzing couples with an increased risk of autosomal recessive disorders.[16] Targeted NGS was undertaken for carriers screening of autosomal recessive lethal disorders in consanguineous and non-consanguineous couples with one or more affected children. The study has shown that NGS-based gene panel sequencing of selected genes involved in lethal autosomal recessive disorders appeared to be a valuable tool to detect the carrier status in families that had early child death and/or multiple abortions experience. Therefore, these might now be used for prenatal and PGD for families in whom causative variants could be identified.[16]

The PGD methods will surely continue to develop in favor of improved diagnostic precision and affordable costs for patients. However, before proceeding with PGD, patients need proper genetic counseling on the possible risks and clinical outcomes. Namely, each diagnostic platform might have some error rate, and not all available technologies offer equal diagnostic capabilities. Choosing the diagnostic platform needs to be personalized to fit both patients' as well as clinical needs.

References

1. Thornhill AR, Snow K. Molecular diagnostics in preimplantation genetic diagnosis. J Mol Diagn 2002;4(1):11–29. DOI: 10.1016/S1525-1578(10)60676-9
2. Milachich T. New advances of preimplantation and prenatal genetic screening and noninvasive testing as a potential predictor of health status of babies. Biomed Res Int 2014;2014:306505. DOI: 10.1155/2014/306505
3. Brezina PR, Anchan R, Kearns WG. Preimplantation genetic testing for aneuploidy: what technology should you use and what are the differences? J Assist Reprod Genet 2016;33(7):823–832. DOI: 10.1007/s10815-016-0740-2
4. Sullivan-Pyke C, Dokras A. Preimplantation genetic screening and preimplantation genetic diagnosis. Obstet Gynecol Clin North Am 2018;45(1):113–125. DOI: 10.1016/j.ogc.2017.10.009
5. Handyside AH, Kontogianni EH, Hardy K, et al. Pregnancies from biopsied human preimplantation embryos sexed by Y-specific DNA amplification. Nature 1990;344(6268):768–770. DOI: 10.1038/344768a0
6. Sermon K. Current concepts in preimplantation genetic diagnosis (PGD): a molecular biologist's view. Hum Reprod Update 2002;8(1):11–20. DOI: 10.1093/humupd/8.1.11
7. Lee VCY, Chow JFC, Yeung WSB, et al. Preimplantation genetic diagnosis for monogenic diseases. Best Pract Res Clin Obstet Gynaecol 2017;44:68–75. DOI: 10.1016/j.bpobgyn.2017.04.001
8. Peciña A, Lozano Arana MD, García-Lozano JC, et al. One-step multiplex polymerase chain reaction for preimplantation genetic diagnosis of Huntington disease. Fertil Steril 2010;93(7):2411–2412. DOI: 10.1016/j.fertnstert.2009.01.120
9. Jobanputra V, Sobrino A, Kinney A, et al. Multiplex interphase FISH as a screen for common aneuploidies in spontaneous abortions. Hum Reprod 2002;17(5):1166–1170. DOI: 10.1093/humrep/17.5.1166
10. Northrop LE, Treff NR, Levy B, et al. SNP microarray-based 24 chromosome aneuploidy screening demonstrates that cleavage-stage FISH poorly predicts aneuploidy in embryos that develop to morphologically normal blastocysts. Mol Hum Reprod 2010;16(8):590–600. DOI: 10.1093/molehr/gaq037
11. Li G, Niu W, Jin H, et al. Importance of embryo aneuploidy screening in preimplantation genetic diagnosis for monogenic diseases using the karyomap gene chip. Sci Rep 2018;8(1):3139. DOI: 10.1038/s41598-018-21094-6
12. Handyside AH, Harton GL, Mariani B, et al. Karyomapping: a universal method for genome wide analysis of genetic disease based on mapping crossovers between parental haplotypes. J Med Genet 2010;47(10):651–658. DOI: 10.1136/jmg.2009.069971
13. Martín J, Cervero A, Mir P, et al. The impact of next-generation sequencing technology on preimplantation genetic diagnosis and screening. Fertil Steril 2013;99(4):1054–1061.e3. DOI: 10.1016/j.fertnstert.2013.02.001
14. Liu L, Li Y, Li S, et al. Comparison of next-generation sequencing systems. J Biomed Biotechnol 2012;2012:251364. DOI: 10.1155/2012/251364
15. Chow JFC, Yeung WSB, Lee VCY, et al. Evaluation of preimplantation genetic testing for chromosomal structural rearrangement by a commonly used next generation sequencing workflow. Eur J Obstet Gynecol Reprod Biol 2018;224:66–73. DOI: 10.1016/j.ejogrb.2018.03.013
16. Komlosi K, Diederich S, Fend-Guella DL, et al. Targeted next-generation sequencing analysis in couples at increased risk for autosomal recessive disorders. Orphanet J Rare Dis 2018;13(1):23. DOI: 10.1186/s13023-018-0763-0