Analysis of Sex Chromosomes in Preimplantation Genetic Diagnosis for X-Chromosome-Linked Disorders

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Preimplantation genetic diagnosis (PGD) is diagnostic tool to avoid inheritance of genetic disease by transferring unaffected embryos. Recently, PCR and FISH have been mainly applied to the diagnosis of single gene disorders and chromosomal abnormalities, respectively. Since with PGD, only a few cells are available for genetic tests, both gene and chromosomes analysis have to be obtained from the same, limited material. Cell recycling makes it possible to obtain the information on genes as well as chromosomes from the same cells. Therefore cell recycling is an acceptable strategy where in PGD targets large proportions of embryos severe chromosomal abnormalities. The responsible genes of the X-linked disorder and numerical abnormalities of sex chromosomes should be analyzed simultaneously. Gender information is definitely useful because only male affected embryos should be avoided for transfer.

KEY WORDS: Preimplantation genetic diagnosis (PGD); X-linked disorder; cell recycling.

Preimplantation genetic diagnosis (PGD) is diagnostic tool to avoid inheritance of genetic disease by transferring unaffected embryos. The initial application of PGD was gender determination for X-chromosome-linked disorders (X-linked disorder) by polymerase chain reaction (PCR). Although PCR is able to detect the existence of target chromosomes, it is impossible to specify their number. Therefore, the application of fluorescence in situ hybridization (FISH) for gender determination was recommended to substitute for PCR. Recently, PCR and FISH have been mainly applied to the diagnosis of single gene disorders and chromosomal abnormalities, respectively.

The advantage of PGD over classical methods of prenatal diagnosis (PND), such as chorionic villi sampling (CVS) and amniocentesis, is that genetic information can be obtained before conception. If PND reveals that a genetic disease affects a fetus, only the

termination of pregnancy is an option. The principal disadvantage of PGD is that only very few cells are available for the necessary genetic tests, while with PND, almost unlimited numbers of cells are available. PND, therefore, allows the reexamination of results to assure their accuracy.

Typically, PGD using individual blastomeres is applied to embryos at 4–8 cell stage. CVS is applied to fetuses at 10–12 weeks of gestation and amniocentesis at 16–18 weeks. Recently, it was reported that amongst preimplantation embryos many show severe chromosomal abnormalities. Munne *et al.* (1) reported that chromosomal abnormalities were detected in 71.4% of arrested embryos, 50.8% of slow developing and fragmented embryos, and 41.3% of embryos with good morphology. It can, therefore, be assumed that severe chromosomal abnormalities may be the cause of developmental arrest, implantation failure, and spontaneous abortions. Until the end of the first trimester, most of fetus with severe chromosomal abnormalities would then disappear by natural selection. Consequently, the genetic character of PGD targets is completely different from those of classical PND targets.

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The fact that preimplantation embryos include a large proportion of embryos with severe chromosomal abnormalities suggest that the number of X-chromosome is not always one or two in blastomeres from male and female embryos, respectively. In analyzing day 3 embryos by FISH, using probes for chromosome 13, 16, 18, 21, 22, X and Y, extensive mosaicism was noted. When single genes of X-linked disorders are analyzed by PGD, the numerical analysis of sex chromosomes is necessary and gender information is important because not only the existence of X-chromosome (1) but also their number directly affects the results of gene analysis. For example, in PGD cases for Duchenne muscular dystrophy (DMD), the deletion of exons in the dystrophin gene can be detected by multiple-nest PCR from a single blastomere. When a particular DNA fragment is amplified, it signifies only the existence of the exon. The number of X-chromosomes could be three or four, because of polyploidy or aneuploidy, or it even could be the product of DNA contamination. PGD for X-linked disorders by gene analysis alone, without a numerical analysis of sex chromosomes may, therefore, lead to misdiagnosis and is not acceptable as a diagnostic strategy. The results of any gene analysis has, therefore, to be confirmed be consistent with the number of sex chromosomes.

Since with PGD, only a few cells are available for genetic tests, both gene and chromosome analysis have to be obtained from the same, limited material.

This is done by using PCR primer sets to specify the X-chromosome and to analyze genes together. This strategy determines whether X-chromosomes exist at all on the blastomeres, but it is useless in detecting the number of X-chromosomes. A second strategy involves the biopsy of two blastomeres from cleavage stage embryos. One blastomere is then used for gene analysis and the other for chromosome analysis, especially sex chromosomes. When this strategy is applied to PGD cases, mosaicism can be the cause of inconsistencies between gene and sex chromosome analyses.

A third strategy is "cell recycling," which combined PCR and FISH on the same blastomeres (3). This technique makes it possible to obtain the information on genes as well as chromosomes from the same cells. The biopsied blastomeres are fixed on the tips of miniature slides, designed to be inserted into PCR tubes. The first step of cell recycling is PCR. Then, the miniature slides are used for FISH, while PCR products are used for genetic diagnosis. If the amount of PCR products is not enough for analysis, nest PCR can be applied. The genomic DNA, fixed on the miniature slides, can be a template of amplification and can then also be a target of hybridization. We developed this technique for Duchenne muscular dystrophy (DMD). The deletion of multiple exons in the dystrophin gene could be detected by PCR and the gender and/or numeric chromosomal abnormality by FISH (Fig. 1). When this strategy is

Fig. 1. Cell recycling.

applied to PGD cases, allele dropout (ADO) has to be considered (4).

Munne *et al.* (5) reported that aneuploidy screening reduces embryo loss after conception. The number of embryos to be transferred into the uterus should be limited. Therefore simultaneous aneuploid screening is recommended in PGD cases. Only genetically unaffected embryos should be selected for transfer.

In conclusion, cell recycling is an acceptable strategy where in PGD targets large proportions of embryos with severe chromosomal abnormalities. The responsible genes of the X-linked disorder and numerical abnormalities of sex chromosomes should be analyzed simultaneously. Gender information is definitely useful because only male affected embryos should be avoided for transfer. If possible, simultaneous aneuploid screening is recommended.

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