Review

Current status of preimplantation diagnosis for single gene disorders

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Dr Anver Kuliev received his PhD in Clinical Cytogenetics from Moscow Research Institute of Human Morphology in 1969. In 1979 he took the responsibility for the World Health Organization (WHO)'s Hereditary Diseases Program in Geneva, where he developed the community-based programmes for prevention of genetic disorders and early approaches for prenatal diagnosis. He moved to the Reproductive Genetics Institute in 1990, where he heads the WHO Collaborating Center for Prevention of Genetic Disorders, and scientific research in prenatal and preimplantation genetics. He is an author on more than a hundred papers and nine books in the above areas, including three books in the field of Preimplantation Genetics.

Abstract

Preimplantation genetic diagnosis (PGD) has become an established procedure for avoiding the birth of affected children with single gene disorders. PGD is performed through polar body or blastomere biopsy, which has no deleterious effect on pre- and post-implantation development. This review describes the most recent developments and current changes in the spectrum of conditions for which PGD has been applied. The most recent applications of PGD include congenital malformations, blood group incompatibility and an increasing number of late onset disorders with genetic predisposition, all of which have not previously been diagnosed using PGD. Despite ethical concerns, PGD has also been used for preselection of unaffected and HLA matched embryos, and recently for preimplantation HLA matching without testing for the causative gene. This extends the practical value of PGD, with its utility being no longer limited to prevention of single gene disorders, by expanding it to treatment of siblings requiring stem cell transplantation.

Keywords: blastomere biopsy, congenital malformations, ethics, HLA matching, late onset disorders, PGD

Introduction

Preimplantation genetic diagnosis (PGD) is a relatively new approach to detect genetic disorders before pregnancy, avoiding the need for prenatal diagnosis and abortion. It is based on testing of oocytes or embryos to pre-select and transfer only normal embryos back to the patient, achieving an unaffected pregnancy and the birth of a healthy child. PGD is performed either by testing single blastomeres removed from 8-cell preimplantation embryos, or by testing female gametes (Verlinsky and Kuliev, 2000). The latter is based on the removal and genetic analysis of the first and second polar bodies (PB1 and PB2), which represent the by-products of the first and second meiotic divisions. Because neither PB1 nor PB2 has any biological significance for embryo development, they may be removed and tested for their genetic content, in order to predict the resulting maternal contribution to the embryo. However, this approach cannot be applied to testing for paternally derived abnormalities and gender determination, which may be detected by genetic analysis of single blastomeres removed from cleavage stage embryos. Both methods, therefore, are complementary and may be applied...
More than half of PGD cases for single gene disorders, from the very beginning, have involved gender determination for X-linked conditions, using either polymerase chain reaction (PCR) or fluorescence in-situ hybridization (FISH) (IWGPG, 2001; ESHRE PGD Consortium, 2002). This was not only because the sequence information was not always available, but also because it was technically easier to identify female embryos by DNA analysis or FISH, despite the obvious cost of discarding 50% healthy male embryos. On the other hand, testing for X-linked genetic disorders may be entirely limited to the most recent developments and current changes in the spectrum of conditions for which PGD has been applied.

Specific diagnosis for X-linked diseases

More than half of PGD cycles have been performed, resulting in the birth of well over 1000 children (Verlinsky et al., 2003a). The overall experience of using PGD for single gene disorders presently exceeds 1000 cases, showing that it is an established alternative to traditional prenatal diagnosis, and may be reliably used as an integral part of genetic practices (IWGPG, 2001; Kuliev and Verlinsky, 2002; ESHRE PGD Consortium, 2002).

Changing spectrum of PGD application

The list of disorders, presently comprising more than 50 different conditions, for which PGD has been applied is being extended beyond the indications for prenatal diagnosis, although the most frequent ones are still cystic fibrosis and haemoglobin disorders (IWGPG, 2001; ESHRE PGD Consortium, 2002). Of approximately 400 PGD cycles for single gene disorders, almost half were performed for cystic fibrosis and haemoglobin disorders, followed by myotonic dystrophy and X-linked mental retardation (XMR1) (Verlinsky et al., unpublished data), similar to experience in other active centres (ESHRE PGD Consortium, 2002). As there have been many previous reports on PGD for different single gene disorders, as well as extensive reviews on the subject (Handyside et al., 1992; Sermon et al., 1997, 1999, 2000; Kuliev et al., 1998, 1999; Xu et al., 1998; Kanavakis et al., 1999; De Vos et al., 2000; Goossens et al., 2000; Carvalho et al., 2001; Daniels et al., 2001; Georgiou et al., 2001; Harper et al., 2002; Hellani et al., 2002; Stern et al., 2002), this review will be limited to the most recent developments and current changes in the spectrum of conditions for which PGD has been applied.

Couples with homozygous affected partner

PGD has also been successfully used in couples with one homozygous or compound-heterozygous affected partner with thalassaemia or phenylketonuria (PKU) (Verlinsky and Kuliev, 2000; Verlinsky et al., 2001a). Although the risk of producing an affected child in such couples is 50%, irrespective of maternal or paternal affected status, the strategy of PGD in such cases will depend on whether the father or mother is affected. In one such case when the father was affected, PGD was concentrated on the preselection of mutation free oocytes, while in the other, with the mother affected, a cleavage stage PGD was required to identify those few embryos containing the normal gene.

Testing is particularly complicated if the parents are carrying different mutations. In one such case performed for thalassaemia, the affected mother was double heterozygous (IVS I-110; IVS I-6), while the male partner was a heterozygous carrier of the third mutation (IVS II-745) (Verlinsky and Kuliev, 2000). This required a complex PGD design to exclude preferential amplification of each of the three alleles tested in biopsied blastomeres, as described also in PGD for PKU (Verlinsky et al., 2001a). In this case, the affected father was compound heterozygous for R408 and Y414C mutations in exon 12 of the phenylalanine hydroxylase (PAH) gene, and the carrier mother heterozygous for the R408W mutation in the same exon. PGD strategy was based on the pre-selection of the mutation-free oocytes using sequential PB1 and PB2 DNA analysis. Based on multiplex hemi-nested PCR analysis, four embryos resulting from the zygotes predicted to contain no mutant allele of the PAH gene were transferred, resulting in an unaffected twin pregnancy and the birth of the healthy twins.

Cancer predisposition

Cancer predisposition has not traditionally been considered as an indication for prenatal diagnosis, as this would lead to pregnancy termination, which is scarcely justifiable on the basis of genetic predisposition alone. On the other hand, the possibility of choosing embryos free of genetic predisposition for transfer would obviate the need for considering pregnancy termination, as only potentially normal pregnancies would be established. PGD for such conditions appears acceptable on ethical grounds, because only a limited number of the embryos available from hyperstimulation are selected for transfer.

The first PGD for inherited cancer predisposition was performed for couples carrying p53 tumour suppressor gene depending on the PGD objectives in each patient.

With improvements in treatment and extended life expectancy for many genetic disorders, an increasing number of couples with affected maternal or paternal partners may require PGD in order to have unaffected children.
mutations, known to determine a strong predisposition to the majority of cancers. It resulted in a singleton pregnancy and the birth of a healthy child free from the mutation predisposing to Li–Fraumeni syndrome (Verlinsky et al., 2001b). The couple presented with the paternally derived missense mutation due to transversion of G to A in exon 5 of the p53 tumour suppressor gene. The carrier was a 38-year-old proband with Li–Fraumeni syndrome (LFS), diagnosed with rhabdomyosarcoma of the right shoulder at the age of 2 years, followed by right upper extremity amputation. At the age of 31 years, he was also diagnosed with a high-grade leiomyosarcoma of the bladder and underwent a radical cystoprostatectomy. His mother was diagnosed with leiomyosarcoma at age 37 years.

At present, PGD is also being used for other cancers, including familial adenomatous polyposis coli, Von Hippel–Lindau syndrome, retinoblastoma, neurofibromatosis type I and II and familial posterior fossa brain tumour (Rechitsky et al., 2002). Overall, 20 PGD cycles have been performed for 10 couples, resulting in preselection and transfer of 40 mutation-free embryos, which yielded five unaffected clinical pregnancies and four healthy children born to date. Despite the controversy surrounding PGD use for late onset disorders, the data demonstrate the usefulness of this approach as the only acceptable option for at-risk couples to avoid the birth of children with inherited predisposition to cancer, and to have a healthy child.

**Other late-onset disorders with genetic predisposition**

One of the first experiences of PGD for late-onset disorders was for genetic predisposition to one of the forms of Alzheimer’s disease (AD) (Verlinsky et al., 2002b). This condition is caused by an autosomal dominant familial predisposition to a presenile form of dementia, determined by a nearly completely penetrant autosomal dominant mutation in the amyloid precursor protein (APP) gene, for which no treatment is available despite a possible predictive diagnosis. The 30-year-old woman had no signs of AD, but was a carrier of the V717L mutation, resulting from a G to C substitution in exon 17 of the APP gene. Predictive testing in the patient was performed because of early onset AD in her sister, who carried this mutation and developed symptoms of AD at the age of 38 years. This sister is still alive, but her cognitive problems progressed to the point that she was placed in an assisted living facility. Her father had died at age 42 years and had also a history of psychological difficulties and marked memory problems. V717L mutation was also detected in one of her brothers, who experienced mild short-term memory problems as early as 35 years of age, with a moderate decline in memory, new learning and sequential tracking in the next 2–3 years. The other family members, including one brother and two sisters, were asymptomatic, although predictive testing was performed only in the sisters, who appeared to be free from a mutation in the APP gene.

PGD was performed by DNA analysis of PB1 and PB2, resulting in a singleton clinical pregnancy and birth of an unaffected child. It may be argued that PGD should not be provided for a patient who might not be able to raise her child into adulthood, but it was the patient’s decision to undertake PGD, and it is arguably a better option than to reproduce without testing, with a high risk of having an affected child.

PGD therefore provides a further option for patients who may wish to avoid transmission of the mutant gene predisposing to late onset disorders in their potential children. Because such diseases present beyond early childhood, and even later may not be expressed in 100% of cases, the application of PGD for this group of disorders is still controversial. However, for diseases with no current prospect for treatment, arising despite pre-symptomatic diagnosis and follow-up, PGD may be offered as the only solution for at-risk couples.

One example of the application of PGD in a late onset disorder is that of Huntington disease (HD). Non-disclosure PGD has been considered for at-risk couples, and involves the transfer of disease-free embryos, while the prospective parents do not learn their own status (Stern et al., 2002). Parents receive no information about number of oocytes obtained after hormonal stimulation, number of embryos developed, and the number of embryos available for transfer. However, there might be no unaffected embryos for transfer, or no affected embryos can be found, suggesting that the parent is genetically normal. The other alternative in HD is exclusion-PGD testing, but embryos with a detected grandparental allele would be excluded from transfer, notwithstanding that only half of these embryos would contain the affected allele. However, some European centres prefer performing direct testing of embryos from known disease gene carriers (Sermon et al., 2001).

**Blood group incompatibility**

The first PGD for materno–fetal incompatibility resulting in a healthy pregnancy was performed for Kell (K1) genotype, which is one of major antigenic systems in human red blood cells. It is comparable in importance to Kell (K1) antigen. Blood group incompatibility or neonatal death. PGD is therefore a possible option for identification of pregnancies at risk for HDN, this may not always prevent potential complications for the fetus, stillbirth or neonatal death. PGD is therefore a possible option for preventing both Kell and Rhesus haemolytic diseases.

PGD for Kell disease was performed for two at-risk couples with a history of neonatal death in previous pregnancies due to HDN. The preselection and transfer of embryos free from the K1 allele of the KEL gene was possible in each case, yielding a clinical pregnancy and the birth of healthy twins, confirmed to be free of the K1 allele.

A number of attempts have also been undertaken to perform PGD for Rhesus disease, although they have not yet resulted in
a clinical pregnancy (Van Den Veyver, 2000). Both Kell disease and Rhesus incompatibility are quite prevalent (approximately 15% frequency for RhD and 9% for KEL antigen), presenting a risk for alloimmunization that may lead to HDN in some at-risk couples. Therefore, PGD may be a useful option for these couples to avoid the establishment of a RhD or K1 pregnancy in sensitized mothers.

Although at-risk pregnancies detected by prenatal diagnosis may be treated by intrauterine transfusion, potential complications for the fetus cannot be completely excluded even after this procedure. Pregnancy termination in such cases will also be unacceptable, as the antibodies to K1, for example, are developed in only 5% of persons exposed to incompatible blood. On the other hand, some at-risk couples have had such an unfortunate experience of HDN, resulting in neonatal death, that they regard PGD as their only option to plan another pregnancy. This makes PGD attractive for patients at risk for alloimmunization, although such conditions have rarely been an indication for prenatal diagnosis.

**Congenital malformations**

Congenital malformations are highly prevalent (29.3/1000 live births) and are usually sporadic. However, with progress of the Human Genome Project, an increasing number of inherited forms are being described, which, therefore, may be avoided through PGD. For example, Sonic Hedgehog (SHH) gene mutation, for which the first PGD was recently performed (Verlinsky et al., 2003b), causes failure of the cerebral hemispheres to separate into distinct left and right halves and leads to holoprosencephaly (HPE), which is one of the most common developmental anomalies of forebrain and mid-face. Although the majority of HPE are sporadic, familial cases are not rare, with clear autosomal dominant inheritance.

Significant intrafamilial clinical variability of HPE, from alobar HPE and cyclopia, to cleft lip and palate, microcephaly, ocular hypertelorism, and even normal phenotype, suggests interaction of the SHH gene with other genes expressed during craniofacial development and the possible involvement of environmental factors. This may explain the fact that almost one-third of carriers of SHH mutations may be clinically unaffected. Therefore, even in familial cases, the detection of SHH mutations in prenatal diagnosis might not justify pregnancy termination, making PGD a more attractive option for couples at risk for producing children with HPE.

In the case described (Verlinsky et al., 2003b), the couple presented for PGD because of the previous birth of two children with clinical signs of HPE. One, a female with severe HPE and cleft lip and palate, died shortly after birth. Both the child and the parents were chromosomally normal, but DNA analysis in the child’s autopsy material demonstrated the presence of SHH nonsense mutation due to GAG→TAG sequence change, leading to premature termination of the protein at position 256 (Glu256→stop). The same mutation was found in their 5-year-old son, who was born after a full-term normal pregnancy. This child had less severe facial dysmorphisms, which included microcephaly, Rathke’s pouch

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**Figure 1.** PGD for Sonic Hedgehog (SHH) mutation: family pedigree. Upper panel: the father (I-1) has a gonadal mosaicism for SHH mutation, which is linked to 156 bp dinucleotide C-A repeat allele of D7S550 polymorphic marker, while the mother (I-2) is normal, with one normal allele linked to 158 bp repeat and the other to 138 bp repeat alleles. Lower panel: reproductive outcomes of this couple, including three previous pregnancies, one resulting in the birth of affected child with holoprosencephaly, carrying the mutant gene (lower left) (II-1), one in perinatal death (II-2), also carrying the mutant gene [lower middle (circle)], and one in a spontaneously aborted fetus with Turner syndrome (II-3), free from SHH mutation [lower middle (triangle)]. The lower right open circle (II-4) represents the outcome of preimplantation diagnosis, resulting in an unaffected clinical pregnancy and birth of a healthy child, following confirmation of mutation-free status by amniocentesis. Reprinted with permission, from Verlinsky et al. (2003b).
cyst, single central incisor and choanal stenosis. There was also cliniodactyly of the fifth fingers and incurred fourth toes bilaterally. The child’s growth was slow in the first 2 years, but since then, he has been maintaining reasonably good growth.

PGD was performed by blastomere biopsy and multiplex nested PCR analysis, involving specific mutation testing simultaneously with linked marker analysis. Of nine tested embryos, four were free of the mutant gene, of which two were transferred back to the patient, resulting in a singleton pregnancy and birth of a healthy child following confirmation of the mutation free status by amniocentesis (Figure 1). A similar approach has been used for PGD of Crouzon syndrome (Abou-Sleiman et al., 2002).

The data suggest the clinical usefulness of PGD for familial cases of congenital malformations. Because of the high prevalence of congenital anomalies, the approach may have practical implications for at-risk couples as a preventive measure to be employed in genetic practice.

**Preimplantation HLA matching combined with causative gene test**

Preimplantation HLA matching was first introduced in combination with mutation analysis for Fanconi anaemia, with the objective of establishing an unaffected pregnancy yielding a potential donor progeny for transplantation in an affected sibling (Verlinsky et al., 2001c). This resulted in a clinical pregnancy and birth of an unaffected child, whose cord blood was transplanted to the affected sibling, saving her life.

This strategy is unlikely to be clinically acceptable through traditional prenatal genetic diagnosis, because of possible clinical pregnancy termination after HLA matching. However, PGD for such purposes should be acceptable, because only a limited number of the embryos are usually pre-selected for transfer, which in this case will represent unaffected embryos with a perfect match for affected siblings in need of a transplant. Since the multiplex single cell PCR used in PGD presents the opportunity for combined PGD and HLA testing, it has become a useful way to pre-select an embryo which may be an HLA match to the affected sibling requiring stem cell transplantation.

The method has currently been applied for HLA genotyping in two dozen cycles in combination with PGD for thalassaemia, Fanconi anaemia, hyper-immunoglobulin M syndrome, X-linked adrenoleukodystrophy and Wiscott–Aldrich syndrome. The results confirm the usefulness of preimplantation HLA matching as part of PGD, with the prospect of applying this approach to other inherited conditions, also requiring an HLA compatible donor for bone marrow transplantation (Verlinsky et al., unpublished data). This provides a realistic option for couples desiring to avoid the birth of an affected child, together with the establishment of a healthy pregnancy, potentially providing an HLA match progeny for treatment of an affected sibling.

**Ethical issues**

The increasing use of PGD for late onset disorders with genetic predisposition, and preimplantation HLA typing to produce an HLA compatible donor to treat a family member requiring stem cell transplantation, raises important ethical issues (see Edwards et al., 2003). Although there is no actual difference in the application of PGD for the latter conditions, the controversy can be explained by the fact that in traditional prenatal diagnosis, if the fetus is found to carry the gene predisposing to late-onset disorder or to be HLA unmatched, the couple would have to make an extremely difficult decision regarding pregnancy termination, which could hardly be justified by such findings. Alternatively, PGD technology allows genetic testing of human eggs and embryos before pregnancy is established, making it completely realistic to establish only HLA matched or potentially normal pregnancies without genetic predisposition to late onset disorders.

In any case, as seen from the above review, PGD is now becoming an established clinical option in reproductive medicine and is applied using separate consent forms and the research protocols approved by institutional ethics committees. The number of apparently healthy children born after PGD has passed its first thousand, showing that there is no evidence of any incurred adverse effect. However, these protocols would still require confirmatory chorionic villous sampling or amniocentesis and follow-up monitoring of safety and accuracy. Although PGD will help solve some longstanding ethical problems, such as the abortion issue (which will be avoided as a result of this new approach), others could become a serious obstacle, particularly those related to ‘designer babies’. These considerations are highly relevant to the subject of PGD, as well as to any other new methods, as further development of appropriate technology for controlling genetic disability takes place.

**References**


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