

# Medical and social perspectives of PGD for single gene disorders and human leukocyte antigen typing



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## Abstract

Preimplantation genetic diagnosis (PGD) for single gene disorders combined with human leukocyte antigen (HLA) typing has recently emerged as a therapeutic tool. For couples who are at risk of passing on a genetic disease to their offspring, preimplantation embryos can be selected according to their genetic status as well as a possible HLA matching with the affected sibling. Stem cells from the resulting baby's umbilical cord blood have, therefore, a great therapeutic value for haematopoietic and other life threatening diseases, as stem cells in the cord blood from a HLA-compatible newborn can be used for transplantation without graft rejection, thus saving an affected child's life. However, apart from being a valuable treatment option, there exist several medical and social aspects that should be evaluated and discussed. From the ethical and the social aspects, although PGD for single gene disorders is well defined and accepted, application of PGD combined with HLA typing is less obvious and under extensive debate. This article is aimed at summarizing the current results and limitations of PGD with HLA typing that are related to the successful medical outcome. It further discusses the ethical and social issues that have recently been raised on the application of this technique.

**Keywords:** genetic disease, HLA-typing, PGD, single gene disorders, stem cells

## Introduction

For couples at high risk of pregnancies with genetic abnormalities, preimplantation genetic diagnosis (PGD) is currently being offered as an alternative method to prevent the birth of an affected sibling (Shenfield *et al.*, 2003). From this perspective, it creates the possibility of avoiding the need to terminate affected pregnancies since it allows the selection of unaffected embryos for transfer. In fact, PGD is the early form of prenatal diagnosis, not an alternative. Besides preventing the birth of a child or another child with a genetic disorder, it further minimizes the probability of applying invasive diagnostic procedures such as chorionic villous sampling and amniocentesis, as well as the possibility of an established pregnancy termination and a risk of miscarriage due to the genetic disorders. The procedure would bring an additional

benefit for the latter, by decreasing the risk of future fertility problems that can be associated with multiple or repeated abortions (Verlinsky *et al.*, 2005).

Currently, more than 2000 healthy children have already been born after PGD and the expanding indications include chromosomal abnormalities, human leukocyte antigen (HLA) tissue typing of the embryos, predisposition of adult disorders, Rhesus incompatibility, special medical conditions such as congenital deafness, and sex-linked disorders (Simpson, 2001; Fiorentino *et al.*, 2004, 2006; Kuliev and Verlinsky, 2005; Seeho *et al.*, 2005). Nowadays, PGD can be carried out for any disorder for which molecular testing has been developed. Although the majority of these disorders are due to rare genetic

defects, the incidence of some, such as  $\beta$ -thalassaemia, sickle cell anaemia and cystic fibrosis, are very common in certain parts of the world, such as the Mediterranean region which includes Italy, Greece, Cyprus and Turkey. For the latter,  $\beta$ -thalassaemia carrier frequency is around 4%; however, this rate can go up to 14% in some areas where consanguineous marriage is common in Turkey.

Especially for blood-borne disorders, allogeneic haematopoietic stem cell transplantation has been used as the ultimate treatment modality. Thalassaemic patients can be effectively cured by this method if hepatomegaly and portal fibrosis have not developed. There is a 25% chance that an HLA-matched donor sibling can be found among siblings and genetic relatives. Therefore, haematopoietic stem cells from HLA-identical siblings provide the highest success rate according to Pesaro low-risk score (Lucarelli *et al.*, 1998), and current results indicate that about 90% of the cases can be cured successfully after haematopoietic stem cell transplantation (Gaziev and Lucarelli, 2005). Use of cord blood as a stem cell source also results in reduced incidence of graft rejection and other serious complications associated with bone marrow transplantation. However, in most cases a suitable donor cannot be found in the family and the patient requires allogeneic stem cell sources. Furthermore, the use of transplants from non-identical-HLA donors is associated with higher morbidity and poorer survival. Also, there is limited availability of unrelated HLA-matched donors in national or international registries.

## Preimplantation HLA matching in clinical practice

Preimplantation HLA matching is one of the most recent applications in reproductive medicine. PGD is used not only to avoid the birth of an affected child but also to conceive healthy children who may also be potential HLA-identical donors for haematopoietic stem cell transplantation. At delivery of the newborn, cord blood haematopoietic stem cells can be collected and later used to treat the affected sibling. The first application was performed in United States for Fanconi anaemia in 1999 (Verlinsky *et al.*, 2000). Since then, according to the current literature, worldwide experience of preimplantation HLA typing includes more than 300 cycles for life-threatening disorders such as  $\beta$ -thalassaemia, Fanconi anaemia, Wiskott-Aldrich syndrome, Diamond-Blackfan anaemia, X-linked hyperimmunoglobulin M syndrome, X-linked adrenoleukodystrophy, X-linked hypohidrotic ectodermal dysplasia with immune deficiency and aplastic anaemia, which have been performed in centres located in Australia, Belgium, the United States, Italy and Turkey (Fiorentino *et al.*, 2004, 2005, 2006; Kahraman *et al.*, 2004; Kuliev and Verlinsky, 2004; Marshall *et al.*, 2004; Van de Velde *et al.*, 2004; Kuliev *et al.*, 2005). Apart from the cases listed above, for diseases such as acute lymphoid leukaemia, HLA matching becomes the primary indication because, in this case, there is no need for the detection of a genetic disorder.

## Worldwide policies on PGD for HLA typing

A large spectrum of different approaches and attitudes currently exist even for the PGD procedure itself, including PGD for

HLA typing. The general scheme has recently been reviewed by several authors (Boyle and Savulescu, 2001; Edwards, 2003, 2004; Knoppers and Isasi, 2004). As examples of both extremes, in countries such as United States, Spain, Belgium and the UK, PGD is already in clinical practice, whereas in countries such as Germany, Italy and Austria, the application of the procedure is prohibited. In the UK, where both IVF and PGD were pioneered (Stephens and Edwards, 1978; Handyside *et al.*, 1990), PGD is allowed and regulated with well-defined guidelines and regulations in such a way that licensed centres are required to obtain permission from Human Fertilization and Embryology Authority (HFEA) for each case. In the United States, there are no federal and state restrictions, therefore IVF clinics that are capable of performing PGD set individual policies, guided by the recommendations of several relevant societies including American Society for Reproductive Medicine (ASRM). In developing countries and some others, no definite guidelines and regulations exist yet for PGD. However, this situation is currently changing and usually similar applications and regulations established in other countries are taken as models (Kahraman and Findikli, 2005).

In nearly all countries in which assisted reproductive techniques can be provided, legislation regarding the procedures is based largely on the social, cultural and religious stature of the society. In Turkey, IVF procedures are regulated by Assisted Human Reproduction High Council, which is localized in the body of Turkish Ministry of Health according to the 'Bylaw for Centres Providing Assisted Reproductive Techniques' which was issued on 19 November 1996 and recently modified in 2001. Although the bylaw covers the services provided only for infertility treatment, there is no specific objection to PGD together with HLA typing, in which ovarian stimulation is needed to provide oocytes (mostly) from fertile females. The general requirements for centres aiming to perform genetic diagnosis is, on the other hand, outlined in the bylaw issued on 10 January 1998, but it does not specifically describe the type of genetic services that can be provided. Therefore a draft version, which outlines the procedures and indications of preimplantation genetic testing, has also been prepared by the Turkish Ministry of Health, the final version of which is expected to be announced soon. Currently PGD practice in Turkey depends on the clinic's available technical infrastructure. However, the application of PGD necessitates having the permission from the local ethics committee. Turkey, where 98% of the population is Muslim, represents an ideal model country in which the legal framework is being shaped according to the secular governmental protocols, established socio-cultural values as well as contemporary medical advances.

It can be commented that, from the medical standpoint, several limitations of PGD with and without HLA typing exist. Of course maternal age and ovarian reserve are the most important factors because of the difficulty in retrieving a high number of good quality oocytes from patients with advanced maternal age or with diminished ovarian reserve. As shown in **Table 1**, 120 cycles for PGD with HLA typing were evaluated in terms of clinical outcome. In such a large series, there was no misdiagnosis: all pregnant cases were evaluated by prenatal diagnosis to verify the PGD diagnosis. Since it is generally known that maternal age also affects

**Table 1.** Clinical outcome of 120 preimplantation genetic diagnosis (PGD) cycles for human leukocyte antigen typing.

	All cases	<37 years	≥37 years	P-value
No. cycles (n)	120 <sup>b</sup>	88	32	-
No. patients	65	47	17	-
Maternal age (years) <sup>a</sup>	32.9 ± 5.4	30.5 ± 3.4	39.8 ± 2.2	NS
Maternal age range (years)	21–44	21–36	37–44	-
Oocytes retrieved <sup>a</sup>	16.8 ± 9.8	18.0 ± 9.5	12.8 ± 7.2	NS
Embryos analysed <sup>a</sup>	9.4 ± 5.7	10.3 ± 5.8	7.6 ± 5.3	NS
No. embryo transfer cycles (%)	79 (65.8)	57 (64.9)	22 (66.7)	NS
Transferred embryos <sup>a</sup>	1.6 ± 0.7	1.6 ± 0.8	1.6 ± 0.7	NS
No. of pregnancies	31	24	42.1	-
Pregnancy rate/transfer (%)	39.0	41.1	31.8	<0.05
Implantation rate/embryo transfer (%)	15.7	15.4	14.7	NS

NS = not significant.

<sup>a</sup>Mean ± SD.

<sup>b</sup>Data updated from Kahraman *et al.*, 2004.

the chromosomal constitution of the developing embryos, the technique can be combined with aneuploidy screening in cases with advanced maternal age in order to eliminate the chromosomally abnormal pregnancies (Fiorentino *et al.*, 2006; Rechitsky *et al.*, 2006; S Kahraman *et al.*, unpublished).

## Ethical and social perspectives on PGD for HLA typing

Besides the accuracy and safety issues and the therapeutic potential for an affected sibling, PGD for HLA typing has created numerous ethical and social dilemmas and arguments, the most important of which is the ‘slippery slope argument’, a fear that comes from the lack of limits and controls which could lead eventually to eugenic applications and designer babies. The possibility of instrumentalization of a child for curing another sibling and whether it is against human dignity are other arguments that should be assessed well (Pennings *et al.*, 2002).

Apart from the moral perspectives of conceiving a child to save another child, the topic is further expanded with questions regarding psychosocial risks for the family and the risk of undermining the child’s self-esteem? According to Pennings and de Wert, parents applying for PGD with HLA typing show the same intention to love and care for the sibling as much as they do for the affected brother or sister and there are no indications that they are likely to behave differently in the future (Pennings and de Wert, 2003). However, proper and detailed genetic and psychological counselling is important before and during treatment. From the family perspective, the issue of whether curing an existing child is the only motive for having another child should be carefully assessed. In any case, the use of a child as a donor should not be considered disrespectful towards him or her. However, in accordance with the HFEA guidelines, in terms of patient selection, the condition of the sick child needs to be sufficiently severe or

life threatening and there should be a realistic chance of the treatment being successful. All realistic, alternative treatments and sources of the tissue should be investigated with the couple.

Parents should also be given clear and accurate information about the treatment procedure and possible outcome, regarding not only the genetic possibilities but also the risks and benefits of undergoing an IVF and intracytoplasmic sperm injection procedure. Although ovarian stimulation is relatively easy and less invasive than many other medical interventions, it should be mentioned that it is not totally free of complications. Despite the fact that complications are rare, treatments can be associated with severe situations such as ovarian hyperstimulation syndrome, infection and intraabdominal bleeding, which may require hospitalization. Counselling becomes more important in these cases since the couple is more focused on the outcome rather than the complications that the procedure may bring. Moreover, it is likely that the treatment will not be successful in treating the affected sibling, and the parents’ motivation and attitude in handling this possibility should be determined before starting treatment, although such assessments are extremely difficult to perform.

## Indications for PGD with HLA typing

The indications for PGD with HLA typing is also under extensive discussion in many societies in which PGD is technically applicable. Is it only for cases of a genetic indication for PGD? Only for the treatment of terminal or lethal conditions? Only for the treatment of a sibling? Only with the aim of obtaining haematopoietic stem cells? These are the questions that are frequently asked for PGD and HLA typing. Although they are usually underestimated by the legal authorities, such questions usually put the clinician in a primary target position, in front of a patient asking for a

medical treatment alternative. Therefore it is a daunting task for a clinician to advise them of the best treatment modality, especially in a country where legal issues are not well established. On the other hand, in some countries, such cases can have an important effect on the regulative authorities. Very good examples initially came from the UK, in which three English families (Hashmi, Whitaker and Fletcher families), by creating an intense public debate, have pushed the legal limits and regulations and resulted in a change in the policy of the HFEA. Current debates indicate that these discussions will continue as the available technology expands towards the acceptable limits of the public (Simon and Schenker, 2005). However, when establishing the guidelines and the limits, the case usually moves from the clinicians' and patients' perspectives to the values of the society. As frequently experienced, the debate then becomes a religious, financial and civil problem, obscuring the medical option that the patients or their sick children might benefit. A recent well-known example was the establishment of an extremely restrictive law in Italy in 2004. People requiring such medical services are forced to look to other countries in which these are available, thus creating another controversy in which such options can only be available for the wealthy individuals, violating the equity principle.

## The fate of the unselected embryos

Another intense ethical argument related to HLA typing is that considerable numbers of embryos are necessary to find a disease-free, as well as HLA-compatible, embryo for transfer, and once suitable embryos are found, there is a probability that other, possibly healthy, embryos are destined for destruction after treatment. Moreover, in order to increase the likelihood of finding suitable embryos, there is a general trend to produce more oocytes and embryos by ovarian stimulation.

It is the discarding of these disease-free embryos, due to a lack of a specific genetic make-up rather than a disease-causing mutation or defect, which creates the ethical and public debate. Unlike some who consider this procedure as a criminal act to human beings that violates the principle 'do not harm', from the clinicians' perspectives and medical ethics standpoint, it can more likely be considered as a medical intervention aimed at preventing harm to the sick sibling. Moreover, if one considers other available alternative options, it can easily be seen that various protocols exist in which the 'leftover' embryos can be utilized for the benefits of 'human beings': embryos which are unaffected but non-HLA matched, are not transferred to the patients and could be frozen for future possible use, although there is no legal status of the unaffected embryos to say whether they should be kept or discarded. The couples may wish to have more unaffected and HLA-compatible children. Disease free embryos can be frozen in storage for a future use, such as donation to infertile couples, although this is not possible for Turkey at least, or to stem cell research. For the disease-carrying embryos, they can also be donated to research or discarded and, although in the authors' current practices, carriers in the transfer cohort are mostly included in cases where normal embryos are found, some clinics prefer not to do so. Parents are therefore required to consider the disposition of the remaining non-HLA-compatible healthy embryos.

## Conclusion

As with the other indications for PGD, proper and accurate genetic counselling has key importance for HLA typing. Genetic counselling consists of reviewing the couples' genetic history and motivation, reasons for requesting PGD with or without HLA typing and extensive explanation of the PGD process. It should obviously include the relevant information regarding the success rates, the risk of misdiagnosis and possible genetic, clinical and social outcomes. Obtaining signed informed consent, in which a possible risk of misdiagnosis is specified and confirmatory prenatal diagnosis is recommended, for any pregnancy is a must.

In conclusion, worldwide experience has demonstrated that the procedures of PGD and HLA typing are reliable and can provide a realistic option for the couples desiring to have an HLA-compatible child for the treatment of an affected sibling. Being the second largest series in the world, the data demonstrate that, once a mutation-free and HLA-compatible embryo is found, acceptable pregnancy rates can be obtained with this approach. However, there exist certain medical, ethical and social parameters that should be considered and extensively discussed with the couple before starting the treatment. Special attention should be given to the interest of the prospective child and follow-up of the psychosocial environment.

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