

Preimplantation genetic diagnosis in the prevention of the haemoglobin disorders

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Abstract

Preimplantation Genetic Diagnosis (PGD) is currently an alternative for couples with high risk of pregnancies with genetic anomalies; it offers the possibility of avoiding the need to terminate affected pregnancies, since it allows the selection of unaffected embryos for transfer. PGD for inherited disorders has become extremely accurate (99.5%), and may currently be performed for any single gene disorders in which mutation is identified. PGD has been performed for more than 100 different conditions resulting in the birth of at least 1000 healthy children free of genetic disorder. PGD is presently also used together with preimplantation HLA typing for treatment of affected sibling with genetic and acquired disorders requiring HLA matched stem cell transplantation. This is not only to allow couples to have an unaffected child but also to select a potential donor progeny for stem cell transplantation. In Turkey, thalassemia is the most commonly seen genetic disorder the rate of thalassemia carriers is about 3 - 4% in Turkey. The majority of our PGD cases are thalassemia carriers. They do not only require thalassemia mutation analysis but also HLA typing for their affected child. In this study PGD results of 236 Turkish couples with or without HLA typing will be presented and discussed. A full diagnosis was achieved in 91.0% of the biopsied samples. In Group I, 17.8% of the analyzed embryos were found to be HLA compatible. HLA compatible and disease free embryos were 12.9% of all diagnosed embryos. In group II, 17.2% of embryos were found to be HLA matched and 71.4% HLA non-matched. The majority of our HLA typing combined with PGD cases were β -Thalassemia carriers (87.9%). The mutations analyzed have high heterogeneity, the most frequent mutation was IVS-I-110 G-A and comprised 46.2% of all mutations. To date, 70 healthy and HLA compatible children have been born. Twenty-five sick children have already been cured with cord

blood cell and/or bone marrow transplantation. Twenty-one children are waiting for their newborn siblings to gain sufficient weight and maturity for the donation of stem cells. The successful transplantations have been performed for the following indications: β -Thalassemia (n=19), Wiskott Aldrich syndrome (n=2), Glanzmann Disease (n=1), X-Adrenoleukodystrophy (n=1) and acute myeloid leukemia (n=1) and Diamond Blackfan anemia (n=1). This data presents one of the world's largest experiences on preimplantation HLA typing, and the outcome of stem cell transplantation is the largest number available from one center. Our results indicate HLA typing with or without mutation analysis is a promising and effective therapeutic tool for curation of an affected sibling.

Introduction

Preimplantation Genetic Diagnosis (PGD) is currently an alternative for couples with high risk of pregnancies with genetic anomalies. PGD offers to these couples the possibility of avoiding the need to terminate affected pregnancies, since it allows the selection of unaffected embryos for transfer. PGD for inherited disorders has become extremely accurate (99.5%), and may currently be performed for any single gene disorders in which mutation is identified.

PGD has been performed for more than 100 different conditions resulting in the birth of at least 1000 healthy children free of genetic disorder. PGD is presently also used together with preimplantation HLA typing for treatment of affected sibling with genetic and acquired disorders requiring HLA matched stem cell transplantation (Fiorentino *et al.*, 2006; Kahraman *et al.*, 2004, 2007; Verlinsky *et al.*, 2001, 2004; Van de Velde *et al.*, 2004, 2009). This is not only to allow couples to have an unaffected child but also to select a potential donor progeny for stem cell transplantation.

Due to a small number of children per family, only one third of patients are able to find an HLA identical sibling. This may further be improved by 3% using an extended family research for a matched related donor. In the remaining patients the only resort is the identification of a matched unrelated donor, which may be maximized by establishing national registries.

It is therefore of a great value for hematopoietic and other life threatening diseases as stem cells in the cord blood and bone marrow from an HLA compatible newborn can be used for transplantation without graft rejection, thus saving an affected children's life. PGD for HLA matching has also been used as a primary indication in cases not requiring mutation testing (Verlinsky *et al.*, 2004) (*i.e.* leukemia). Considering that the theoretical probability of finding HLA compatible and mutation free embryos is about 18%, obtaining a sufficient number of suitable embryos as well as good quality necessitates a higher number of oocytes to be fertilized and embryos to be biopsied for a given cycle. Apart from being a valuable treatment approach, there may exist several patients or cycle-specific limitations and it seems that not all couples can benefit from the present procedures.

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In Turkey, thalassemia is the most commonly seen genetic disorder the rate of thalassemia carriers is about 3 - 4% in Turkey (Basak *et al.*, 2007). The majority of our PGD cases are thalassemia carriers. They do not only require thalassemia mutation analysis but also HLA typing for their affected child. In this chapter, PGD results of 236 Turkish couples with or without HLA typing will be presented and discussed.

Pre-clinical work up

First, a haplotype analysis of mother, father and child, and when available of other family members, was performed for each family prior to preimplantation HLA typing. For this, genomic DNA is isolated from peripheral blood samples of father, mother and the affected child. A panel of 50 different short tandem repeat (STR) markers (Figure 1) were tested on genomic DNAs to ensure the presence of enough informative markers (Figure 2) to aid the identification of monosomy, trisomy, recombination, allele-drop out (ADO) and uniparental disomy (UPD) of the analyzed chromosomes and regions. For each family at least 12 heterozygous markers spanning the HLA-A, HLA-B, HLA-C, HLA-DR,HLA-DQ regions (HLA Classes I, II, and III) were selected for PGD Study.

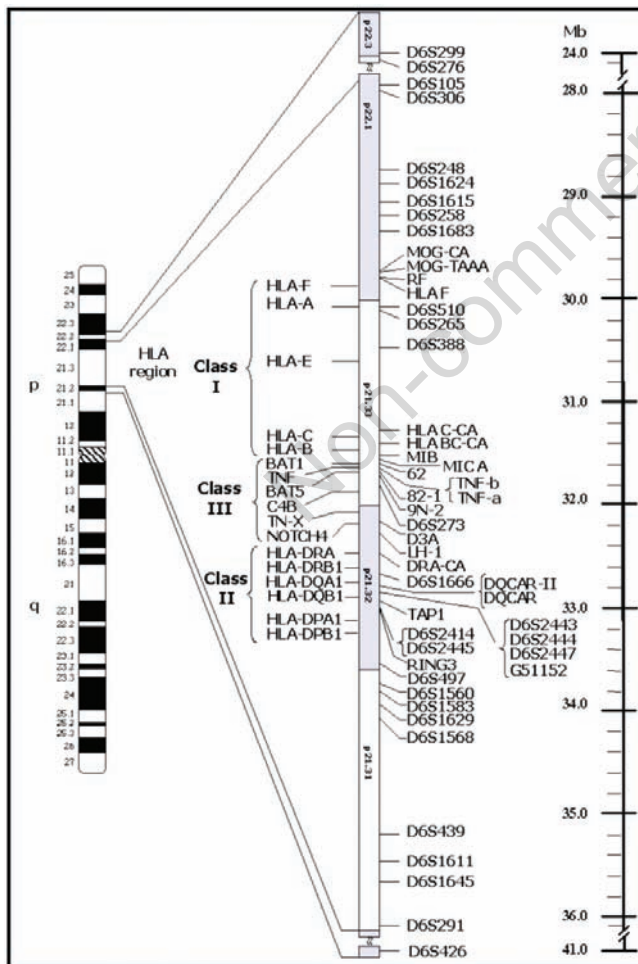


Figure 1. Polymorphic STR markers used in HLA typing of the embryos.

Preimplantation genetic diagnosis study

DNA testing was performed by two rounds of PCR (polymerase chain reactions): in the first round, using multiplex PCR which allows simultaneous amplification of HLA regions and mutation-linked markers and in the second round, using singleplex PCR which is a fluorescent PCR with semi or heminested primers. Primer sequences and polymerase chain reaction conditions used in this study have been reported previously (Verlinsky *et al.*, 2001; Fiorentino *et al.*, 2004; 2005; Rechitsky *et al.*, 2004; Verlinsky *et al.*, 2004).

IVF and embryo biopsy procedure

The stimulation protocols were as outlined previously (Kahraman *et al.*, 2004). Oocyte retrievals were performed 36 h after the injection of rhCG (ovitrel) by transvaginal-ultrasound-guidance. Approximately 2-3 h after oocyte retrieval, cumulus cells were enzymatically removed. Intracytoplasmic sperm injection (ICSI) was applied to metaphase II oocytes. One blastomere was removed from cleavage stage embryos (Figure 3a) from an opening made using laser (IodoLaser, Research Instruments). Subsequently, embryo transfer was performed usually on day-4 but rarely on day-5. Recently, since 2009, trophectoderm tissue biopsies have also been performed.

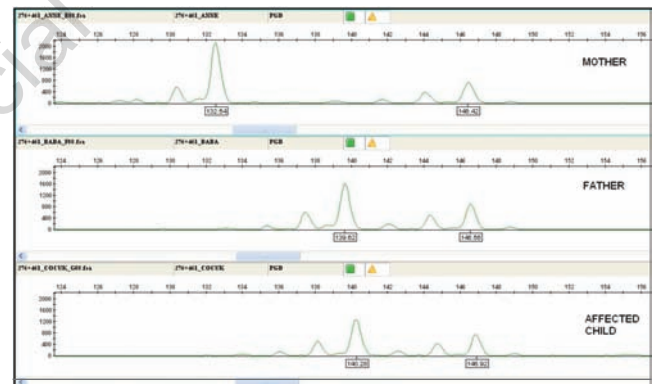
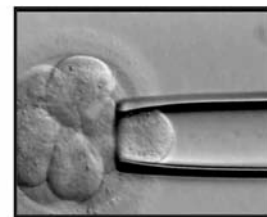


Figure 2. Identification of informative markers during HLA set up study.

a) Cleavage Stage Biopsy



b) Blastocyst Stage Biopsy

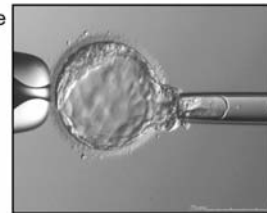


Figure 3. Biopsy Techniques.

Table 1. Cycle participations of patients and indications for HLA Typing.

	TOTAL	188 couples	362 cycles	
HLA + Mutation testing	β -thalassemia	131	258	
	Wiscott Aldrich	3	4	
	X-Adrenoleukodystrophy	3	3	
	Fanconi Anemia	3	4	
	α -Mannosidosis	1	4	
	Gaucher Syndrome	1	4	
	Hurler Syndrome	2	3	
	Hyper IgD	1	1	
	Glanzmann Trombasthenia	1	2	
	Sickle Cell Anemia	1	1	
	Diamond Blackfan Anemia	1	1	
	Cd3 Deficiency	1	1	
	HLA only	Acute Lymphoblastic Leukemia	17	30
		Acute Myeloid Leukemia	11	20
Diamond Blackfan Anemia		3	11	
Histiocytosis		1	3	
Chronic Myeloid Leukemia		1	2	
Burkitt's Lymphoma		1	2	
Aplastic Anemia		2	2	
Anaplastic Anemia		1	3	
Myelodysplastic Syndrome		1	2	
Non-hodglin Lymphoma		1	1	

Table 2. Overall clinical results of HLA typing.

	HLA+ mutation testing (Group I)	HLA Only (Group II)	TOTAL
No of patients/cycles	149/286	39/76	188/362
Maternal age, mean (min-max)	32.0 \pm 4.83	33.9 \pm 5.65	
Cycles with transfer, %	62.2	72.4	
No of embryos transfered, mean	1.55 \pm 0.71	1.56 \pm 0.72	
Clinical pregnancy rate per transfer, %	37.6	32.7	
Implantation rate, %	28.9	24.4	
Clinical miscarriages, n	14	3	
No of babies born, n	57	13	
No of successful transplantations, n	23	2	25*

* 21 children are awaiting an appropriate time for HSC transplantation.

Table 3. The most frequent β -Thalassemia mutations in our sample population.

Mutation	Frequency, %
IVS-I-110 G-A	46,2
Cod8 delAA	7,3
Cod39 C-T	6,5
IVS-II-745	5,7
IVS-II-1	4,6
IVS-I-1 G-A	3,8
TOTAL	74,0

Table 4. The list of diseases and the number of patients who have complete cure after HSCT.

Indication	Patients, n
β -Thalassemia	19
Wiscott-Aldrich	2
Glanzmann disease	1
X-Adrenoleukodystrophy	1
Acute Myeloid Leukemia	1
Diamond Blackfan Anemia	1
TOTAL	25

Results

In HLA+mutation testing group (Group I), and HLA-only group (Group II), 62.2% and 72.4% of the initiated cycles reached the stage of embryo transfer, respectively. The detailed distribution of indications and overall results for each group was shown in Table 1 and in Table 2.

A full diagnosis was achieved in 91.0% of the biopsied samples. In Group I, 17.8 % of the analyzed embryos were found to be HLA compatible. HLA compatible and disease free embryos were 12.9 % of all diagnosed embryos. In group II, 17.2% of embryos were found to be HLA matched and 71.4% HLA non-matched.

The majority of our HLA typing combined with PGD cases were β -Thalassemia carriers (87.9%). The mutations analyzed have high heterogeneity, the most frequent mutation was IVS-I-110 G-A and comprised 46.2% of all mutations. The total frequency of the most frequent 6 mutations were 74,0% (Table 3).

A total of 85 clinical pregnancies (36.5%) were achieved from 233 ET cycles. 5 pregnancies are ongoing. To date, 70 healthy and HLA compatible children have been born. 25 sick children have already been cured with cord blood cell and/or bone marrow transplantation. 21 children are waiting for their newborn siblings to gain sufficient weight and maturity for the donation of stem cells (Table 2). The successful transplantations have been performed for the following indications: β -Thalassemia (n=19), Wiskott Aldrich syndrome (n=2), Glanzmann Disease (n=1), X-Adrenoleukodystrophy (n=1) and acute myeloid leukemia (n=1) and Diamond Blackfan anemia (n=1) (Table 4).

Conclusion

This data presents one of the world's largest experiences on preimplantation HLA typing, and the outcome of stem cell transplantation is the largest number available from one center. To date 25 children have been cured with this approach and 21 children are awaiting appropriate time for transplantation. Our results indicate HLA typing with or without mutation analysis is a promising and effective therapeutic tool for curation of an affected sibling.

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