Preimplantation diagnosis: efficient tool for human leukocyte antigen matched bone marrow transplantation for thalassemia

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Abstract

Thalassemia is among the most frequent indications for preimplantation genetic diagnosis (PGD) to allow at risk couples reproducing without fear of having an affected child. In addition, those already having the affected child, have also the option to produce an unaffected offspring that may be also a complete human leukocyte antigen (HLA) match to affected child to ensure successful bone marrow transplantation. We present here the results of retrospective analysis of 293 PGD cycles for thalassemia, including 144 cases of simultaneous HLA typing, resulting in birth of 70 thalassemia-free children and 12 unaffected HLA matched ones, providing their cord blood and/or bone marrow for transplantation treatment of their affected siblings. The present overall experience includes successful cord blood or bone marrow transplantation in more than three dozens of cases with HLA matched stem cells obtained from children born after PGD, demonstrating that PGD is an efficient approach for improving success of bone marrow transplantation treatment for thalassemia.

Introduction

Thalassemia is presently one of the major indications for preimplantation genetic diagnosis (PGD).¹ PGD for thalassemia was first performed in Cyprus for at risk couples, who have previously undertaken prenatal diagnosis but had to terminate the pregnancy with the affected foetus in repeated attempts.² Then, it was offered as primary option to the patients with infertility problems, and to those who could not accept the risk for prenatal diagnosis and termination of pregnancy.^{3,4} This followed by PGD for the couples with existing thalassemic children, requiring human leukocyte antigen (HLA) compatible bone marrow transplantation.⁵⁻⁷ The objective of PGD in these cases was not only to have a thalassemia-free child, but also to ensure that the resulting baby could serve as an HLA compatible donor for

bone marrow transplantation for the affected siblings. The present paper describes the retrospective analysis of available experience of PGD for HLA typing with the purpose of bone marrow transplantation for siblings with thalassemia.

Materials and Methods

A total of 293 PGD cycles were performed for 161 couples at risk for producing offspring with thalassemia. PGD cycles were performed using a standard in vitro fertilization (IVF) protocol coupled with micromanipulation procedures for the polar body (PB), or blastomere sampling, described in detail elsewhere.⁸

Polar body sampling

The first PB (PB1) and second PB (PB2) were removed sequentially following maturation and fertilization of the oocytes. PB1 and PB2 were removed following stimulation and oocyte retrieval using a standard IVF protocol. In brief, following extrusion of PB1, the zona pellucida was opened mechanically using a microneedle, and PB1 aspirated into a blunt micropipette. Although mechanical method is safe and highly efficient for PB removal, laser assisted techniques may also be applied. The oocytes were then inseminated with motile sperm, or using intracytoplasmic sperm injection (ICSI), and examined for the presence of pronuclei and extrusion of PB2, which were removed in the same manner as PB1. The biopsied oocytes were then returned to culture, checked for cleavage and transferred, depending on the genotype of the corresponding PB1 and PB2.

It is understood that the PB approach allows testing only for maternally derived mutations, making possible to preselect unaffected embryos without a further testing for paternal mutations. However, HLA typing cannot be done on PB, as HLA typing information from both parents is required.

Blastomere biopsy

Blastomere biopsy was performed to identify thalassemia mutations of paternal origin, heterozygous embryos originating from oocytes with maternal mutations, and for HLA testing, in order to identify the embryos containing the maternal and paternal chromosomes 6 identical to the sibling with thalassemia, as described in detail elsewhere.^{1,5}

Blastomere biopsy was performed as soon as the embryo reached a minimum of 6 cells or more so as not to cause a considerable decrease in cell number at later stages of development. A mechanical opening of zona pellucida is performed, allowing the creation of

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a triangular or square flap opening, sufficient in size for a micropipette to pass through in order to remove blastomere(s). Then the micropipette was inserted and gentle suction applied to slowly aspirate the blastomere into the pipette to avoid breakage and disruption of the surrounding blastomeres. Once the blastomere was aspirated, pressure within the pipette was equilibrated and the micropipette was withdrawn.

DNA analysis

To perform genetic analysis, the biopsied PB1, PB2 and blastomeres were placed directly into a lysis solution, consisting of 0.5 mL 10 x PCR buffer, 0.5 mL 1% Tween 20, 0.5 mL 1% Triton x 100, 3.5 mL H₂O and 0.05 mL Proteinase K (20 mg/mL in a 0.5 mL PCR tube). After spinning down at a low speed in a microfuge for a few seconds, the samples were covered with 1 drop of mineral oil and incubated at 45°C for 15 min in a thermal cycler. Proteinase K was then inactivated at 96°C for 20 min, which was also the beginning of the hot start of the first round PCR.

Lower stringency and longer annealing time were used in the first round PCR, with the introduction of the mixture of all outside primers for both mutant genes and polymorphic markers. Following the first round PCR, separate aliquots were amplified in the second round PCR with specific inside primers for each site, using a higher stringency.

To avoid misdiagnosis due to preferential amplification, multiplex nested PCR was performed, involving a simultaneous detection of the mutant gene together with up to three or more highly polymorphic markers, closely linked to the gene tested. Only when the polymorphic sites and the mutation agreed, were embryos transferred. So multiplex amplification allowed detecting allele-drop out (ADO) and prevented the transfer of misdiagnosed affected embryos.⁹

Human leukocyte antigen typing

The method for HLA typing, to identify the embryos containing the maternal and paternal chromosomes 6 identical to the sibling with thalassemia, was described in detail elsewhere.⁵ In brief, HLA genes were tested simultaneously, using the short tandem repeats in the HLA region, by applying a multiplex hemi-nested PCR system, involving only closely linked polymorphic short tandem repeat (STR) markers located throughout HLA region (D6S426, D6S291, Ring 3 CA, TAP1, G51152, D6S2447, LH1, DN, D6S273, 9N-2, TNF a,b,c,d; 62, MIC A, MIB, D6S276, D6S439, D6S1624, D6S265, D6S510; D6S248, RF, MOG a,b,c,d, D6S 258, D6S306, D6S464, D6S299, D6S461). The choice of alleles and markers was based on the information they provide about the presence of maternal and paternal matching or non-matching chromosomes. For each family heterozygous alleles and markers were selected not shared by the parents. Such markers provided the information about the origin of chromosome.⁶ A haplotype analysis for father, mother and the affected child was performed for each family prior to preimplantation HLA typing. This allowed detecting and avoiding misdiagnosis due to preferential amplification and allele drop out (ADO), exceeding 10% in PCR of single blastomeres, potential recombination within the HLA region, and a possible aneuploidy or uniparental disomy of chromosome 6, which may also affect the diagnostic accuracy of HLA typing of the embryo. Multiplex nature of the first-round of PCR required similar annealing temperature of the outside primers. Thirty cycles of PCR were performed with denaturation step at 95°C for 20 sec, annealing at 62-50°C for a min and elongation at 72°C for 30 sec. Twenty min of incubation at 96°C were performed before starting cycling. After cycling ten min. of elongation at 72°C were performed. Annealing temperature for the second-round was programmed at 55°C. The applied strategy provided a 100% HLA match, because the embryos with the same paternal and maternal chromosome 6 as in the affected siblings were preselected.

The chances to identify unaffected embryos fully matched to thalassemic siblings is 18.75%, based on 25% chance of HLA match and 75% chance of having unaffected embryo.

The HLA matched and thalassemia free embryos, based on the information about the mutation testing and polymorphic markers, were pre-selected for transfer back to patient, while those predicted to be mutant or with insufficient marker information were exposed to confirmatory analysis. Non matched unaffected embryos were frozen for future use by the couple.

Results and discussion

Overall, 293 PGD cycles were performed for two dozens of different β -globin gene mutations (Table 1). The most frequent ones were IVS1-110 mutation -100 cases (33%), followed by IVS I-6 -39 cases, IVSII-745 -23 cases, Codon 8 -20 cases, IVS1-1 -18 cases, and codon 39 and IVS1-5 -16 cases each. Among other mutations were IVSII-2, Codon 5, Codon 6, Codon 41/2, E121K, -29 (A-G) -87, R30T, Cap 1, deletion 69 kb and deletion 13.4 kb (Table 1). This resulted in detection and transfer of 476 unaffected embryos (approximately, 2 embryos per transfer) in 240 (81.9%) of 293 clinical cycles, yielding 67 (27.9%) unaffected pregnancies and birth of 70 thalassemia-free children. PGD for thalassemias currently represents 15% of our overall experience of 2028 PGD cycles performed for single gene disorders.¹⁰

A total of 144 PGD cycles were performed for

HLA typing, which allowed detecting and transferring unaffected HLA matched embryos in 78 of them (Table 2). Of a total 824 embryos with conclusive results for testing of beta-globin gene mutations and HLA type, 602 (73.0%) were predicted to be unaffected carriers or normal, of which 126 (15.3%) appeared to be HLA identical to the affected siblings, not significantly different from the expectation. As many as 123 of these embryos developed appropriately to be acceptable for transfer, resulting in 18 unaffected HLA identical pregnancies and birth of 12 healthy children. Umbilical cord blood was collected at birth of these children and transplanted or pending, resulting in a successful hematopoietic reconstitution. The Figure 1 demonstrates the case of PGD for HLA typing for a couple with two thalassemic children, resulting in preselection and transfer of unaffected embryos matched to each of the affected children.

At the present time PGD for HLA typing is

Table 1. Clinical outcome of preimplantation genetic diagnosis for thalassemias.

Disease/ Mutation	No. patient	No. cycle	No. transfers	No. embryos transferred	Pregnancy No. embryos	Birth / (ongoing pregnancy)
IVS I - 1	9	18	13	20	2	3
IVS I - 5	10	16	11	17	3	2
IVS I - 6	21	39	35	71	17	19
IVS I - 110	61	100	88	202	26	24
IVS II - 2	4	9	5	8	1	2
IVS II - 745	9	23	20	42	3	2
Codone 6	1	1	1	3	0	0
Codone 5	3	3	2	3	1	1
Codone 8	12	20	17	26	3	4
Codone 39	10	16	14	26	6	7
E121K	1	1	1	2	0	0
Codone 41/42	7	11	4	10	1	1
-29 (A-G)	1	1	1	1	0	0
-87	1	2	2	2	0	0
Cap 1	2	3	2	2	0	0
R 30 T	1	12	9	13	0	0
Deletion 619 bp	6	13	11	19	2	3
Deletion 13.4 Kb	1	4	4	9	2	2
Deletion 45 kb HBB	1	1	0	0	0	0
TOTAL	161	293	240 (81.9%)	476 (2.0)	67 (27.9%)	70

Table 2. Results of preimplantation diagnosis together with preimplantation human leukocyte antigen testing.

Patient/ Cycle	No. embryos total / amplified	No. normal embryos		No. transfers / No. embryos	Pregnancy/ Birth
		Non-match	Match		
52/144	907/824	746	126	78 / 123	18 / 12
	91%			1.57	23%

Figure 1. Preimplantation genetic diagnosis (PGD) for human leukocyte antigen (HLA) typing in couple with 2 thalassemic children, requiring HLA matched bone marrow transplantation. Upper Panel: Family pedigree with HLA haplotype analysis based on parental (1.1 and 1.2) and affected children's (2.1; 2.2) genomic DNA testing. HLA markers order is presented on the upper left for father and right for mother. Paternal and maternal matching HLA haplotypes to the affected children (2.1; 2.2) are shown in different colors. Maternal and paternal mutations and the linked markers are also presented accordingly. Lower Panel: HLA typing by short tandem repeats (STRs) along with mutation analysis was performed on blastomeres from seven embryos, one of which (No. 4) was predicted to be carrier and HLA mach to the affected sibling 2.2, and another (No. 9) also carrier and matched to the affected sibling 2.1. Three others (embryos No. 6-8) were non matched, while embryo #2 was matched but has inconclusive results of mutation testing due to lack of maternal chromosome 11. Both carrier matched embryos were transferred, but singleton pregnancy was obtained with birth of thalassemia free child matched to one of the affected siblings.

applied also for other conditions, including congenital and acquired disorders, requiring HLA matched stem cell transplantation. According to the two world's largest series, each of over 300 PGD cycles,^{1,10,11} approximately 100 HLA matched healthy babies were born, with almost half of them already transplanted or pending, resulting in successful hematopoietic reconstitution in all the cases,^{1,10-12}

With the current progress in treatment of haemoglobin disorders, PGD may have an increasing impact on the decision of the well-treated patients to reproduce. In fact, the life expectancy of the patients with haemoglobin disorders has recently been dramatically improved with the increasing success rate of radical treatment by stem cell transplantation.¹³ However, the further impact of this treatment will depend on the availability of HLA identical donors.

Conclusions

As seen from the above experience, PGD for HLA typing is an efficient tool for couples at risk to ensure having thalassemia-free children HLA identical to the affected siblings, to serve a potential donor for stem cells for transplantation treatment. This currently is available for a wider application in those communities where thalassemia is highly prevalent, and will improve the access to HLA matched bone marrow transplantation of thalassemia.

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