Preimplantation HLA typing for stem cell transplantation treatment of hemoglobinopathies

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Abstract

Preimplantation genetic diagnosis (PGD) for HLA typing is steadily becoming an option for at risk couples with thalassemic children, requiring HLA matched bone marrow transplantation treatment. The paper presents the world's largest PGD experience of 475 cases for over 2 dozens thalassemia mutations, resulting in birth of 132 unaffected children. A total of 146 cases were performed together with preimplantation HLA typing, resulting in detection and transfer of HLA matched unaffected embryos in 83 of them, yielding the birth of 16 HLA matched children, potential donors for their affected siblings. The presented experience of HLA matched stem cell transplantation for thalassemia, following PGD demonstrated a successful hematopoietic reconstitution both for younger and older patients. The data show that PGD is an efficient approach for HLA matched stem cell transplantation treatment for thalassemia.

Introduction

Thalassemia continues to be one of the major indications for preimplantation genetic diagnosis (PGD).^{1,2} Although it was originally offered for those at risk who could not accept the risk for prenatal diagnosis and termination of pregnancy,³⁻⁵ increasing number of at risk couples request also preimplantation HLA typing to allow performing HLA compatible stem cell transplantation for existing thalassemic siblings.⁶⁻⁹ We have previously reported the results of PGD for thalassemia, with simultaneous HLA typing, resulting in birth of thalassemia-free HLA matched children, as donors of cord blood and/or bone marrow for transplantation treatment for their affected siblings.^{10,11} The present paper describes the progress in PGD for hemoglobinopathies with preimplantation HLA typing, demonstrating that PGD has become an acceptable approach for HLA matched stem cell transplantation treatment for hemoglobinopathies.

Materials and Methods

A total of 475 PGD cycles were performed for 276 couples at risk for producing offspring with thalassemia and sickle cell disease (Table 1). PGD cycles were performed using a standard IVF protocol coupled with micromanipulation procedures for the polar body (PB) sampling, or embryo biopsy at cleavage or blastocyst stage, described in detail elsewhere.^{2,10,11} In brief, the oocytes were tested by sampling of the first and second polar bodies (PB1 and PB2), removed following maturation and fertilization of oocytes, using mechanical micromanipulation technique. The biopsied oocytes were then returned to culture, checked for cleavage and transferred, depending on the genotype of the corresponding PB1 and PB2, which, however, provide the diagnosis only for maternal mutations.

HLA typing or testing for paternal mutations was performed on embryos biopsy samples, obtained at the cleavage or blastocyst stage, in order to identify the embryos containing the maternal and paternal chromosomes 6, identical to the sibling with thalassemia, as also described in detail elsewhere.^{2,6,11} In brief, HLA haplotypes were tested simultaneously with mutations, using the short tandem repeats (STR) in the HLA region, by applying a multiplex hemi-nested polymerase chain reaction system, involving only closely linked STRs 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 linked 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 were selected to be not shared by the parents. Such markers provided the information about the origin of chromosome 6. The applied method provided the data for obtaining a 100% HLA match, because the embryos with the same paternal and maternal chromosome 6, as in the affected siblings, were preselected.

Results and Discussion

Our experience of PGD for hemoglobinopathies is presented in Table 1, representing the largest world's experience available in one center. At the present time, PGD for hemoglobinopathies is more than 15% of our overall experience of 2982 PGD cycles performed for single gene disorders, and involves testing of over two dozens of different HBB gene mutations.² Of 475 clinical cycles performed, unaf-

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affected embryos for transfer were detected in 372 (78.3%) of them, resulting in 133 (30.8%) clinical pregnancies and birth of 132 hemoglobinopathy-free children. As many as one quarter of PGD cycles were performed for IVS 1-110 mutation, which is the most common thalassemia mutation in the Eastern Mediterranean region, and approximately one fifth for sickle cell disease, prevalent in African Americans in the USA.

PGD with HLA typing for thalassemia and sickle cell disease was performed in 146 cases (137 for thalassemia and 9 for sickle cell disease), which resulted in transfer of 130 unaffected HLA matched embryos (121 and 9 respectively) in 83 cycles, yielding 21 clinical pregnancies and birth of 16 unaffected HLA match children. 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.

Figure 1 presents PGD for HLA with the birth of thalassemia free baby boy, who became a donor of HLA matched stem cells for his thalassemic sibling. Bone marrow transplantation was performed in the International Center for Transplantation in Thalassemia and Sickle Cell Anemia of the Mediterranean Institute of Hematology in Rome, Italy (Javid Gaziev, personal communication, 2008). At the time of transplantation, the patient was 14 years old, with regular blood transfusion and chelation therapy since he was diagnosed with double heterozygous (Cod39/IVS1-110) thalassemia major. Despite being at the class-3 risk,¹² transplantation with bone marrow obtained from the HLA matched sibling shown in Figure 1 resulted in a complete hematopoietic reconstitution.

The above experience is only a part of our overall world's largest experience of 474 cases of preimplantation HLA typing, which resulted in detection and transfer of HLA matched unaf-

Table 1. Preimplantation genetic diagnosis for hemoglobinopathies with HLA typing.

Disease	Gene	# Patient	# Cycle	# Transfers	#Embryo transferred	Pregnancy	Birth
Hemoglobin- α locus 1; HBA1	<i>HBA1</i>	148	237	206	437	78	86
Hemoglobin- β locus; HBB	<i>HBB</i>						
Sickle cell anemia	<i>HBB</i>	61	92	83	170	34	30
Subtotal		209	329	289	607	112	116
HLA + hemoglobin- β locus; HBB	<i>HBB</i>	61	137	78	121	20	15
Sickle cell anemia + HLA	<i>HBB</i>	6	9	5	9	1	1
Subtotal		67	146	82	130	21	16
TOTAL		276	475	372	737	133	132

fectured embryos in 230 of them, yielding 72 unaffected HLA matched pregnancies and the birth of 62 HLA matched children, potential donors for their affected siblings.

The other successful experience of 148 PGD cases performed for thalassemia with HLA typing has recently been reported from Istanbul Center.¹³ Of 44 transplanted children, 40 showed a total hematopoietic reconstitution, while the graft failure occurred only in 4 of them. With the current progress in treatment of hemoglobinopathies, PGD may have an increasing impact on the decision of the well-treated patients with a carrier partner to reproduce. In fact, the life expectancy of the patients with hemoglobin disorders has been dramatically improved in last 10 year, with the success of radical treatment by stem cell transplantation, so PGD for hemoglobinopathies is already a practical option, available for a wider application in those communities where these genetic diseases are prevalent. It may be expected that a combined PGD and preimplantation HLA testing will in future be more widely used for further improvements in radical therapy of hemoglobinopathies by stem cell transplantation.

Conclusions

Despite ethical issues involved in preimplantation HLA typing, there is an increase of the attractiveness of this option for the couples with affected children requiring HLA compatible stem cell transplantation, providing a practical

solution for those couples who would like to have another child anyway. This and other new indications make preimplantation testing for thalassemia a genuine alternative to conventional prenatal diagnosis, providing patients with important prospect not only to avoid an inherited risk without facing termination of pregnancy, but also to establish a pregnancy with particular genetic parameters, which may also benefit the affected member of the family.

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