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ARTICLE

Successful haematopoietic stem cell transplantation in 44 children from healthy siblings conceived after preimplantation HLA matching

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Semra Kahraman is Professor of Obstetrics and Gynecology. Professor Kahraman is Director of Istanbul Memorial Hospital, ART and Genetics Centre, the largest such unit in Turkey, with more than 3000 ART cycles performed annually. The centre is also a national ART training centre. Professor Kahraman has published numerous articles in peer-reviewed journals. She has pioneered ICSI, embryo freezing and PGD for single gene disorders and HLA typing in Turkey. She and her team were the first in Turkey to isolate and establish embryonic stem cell lines. Dr. Kahraman is currently the President of the Preimplantation Genetic Diagnosis International Society.

Abstract Haematopoietic stem cell transplantation (HSCT) remains the best therapeutic option for many acquired and inherited paediatric haematological disorders. Unfortunately, the probability of finding an HLA matched donor is limited. An alternative technique is PGD combined with HLA matching, which offers the possibility of selecting unaffected embryos that are HLA compatible with the sick child, with the aim of possible use of stem cells from the resulting baby in future. Since the first successful report for Fanconi anaemia a decade ago, the therapeutic success of this technique was reported in a few cases and for a limited number of disorders. Here, we report full recovery of 44 sick children who received HSCT from healthy infants conceived after

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pre-implantation HLA matching for the following 10 indications; beta-thalassaemia, Wiskott–Aldrich syndrome, Fanconi anaemia, sickle cell anaemia, acute myeloid leukaemia, acute lymphoblastic leukaemia, Glanzmann's thrombasthaenia, Diamond–Blackfan anaemia, X-linked adrenoleukodystrophy and mucopolysaccharidosis type I. No serious complications were observed among recipients and donors. Graft failure occurred in four children with beta-thalassaemia where a second HSCT was planned. Preimplantation HLA matching is a reliable technique and provides a realistic option for couples seeking treatment for an affected child when no HLA-matched donor is available.

KEYWORDS: cord blood, hematopoietic stem cell transplantation (HSCT), human leukocyte antigen (HLA) matching, inherited and acquired hematological disorders, in vitro fertilization (IVF), preimplantation genetic diagnosis (PGD)

Introduction

Heamatopoietic stem cell transplantation (HSCT) remains the only curative treatment for many inherited and acquired paediatric haematological disorders, such as haemoglobinopathies, primary immunodeficiencies, metabolic disorders and bone marrow failure disorders.

The success of HSCT depends on how well HLA types of recipient and the donor match to each other. Unfortunately, the probability of finding an HLA matched donor is limited. The chance of having an HLA identical sibling is about 15% (Pennings et al., 2002), and the chance of finding an unrelated donor through national and international registries is extremely small. Despite recent technical improvements (Yi et al., 2012), a high risk of severe complications still exists. These include graft versus host disease (GVHD) and unsuccessful engraftment in cases where HSCT from an unrelated donor is carried out (Gaziev et al., 2000; Lucarelli et al., 2012; Orofino et al., 2003).

The technique of PGD, coupled with HLA matching, emerged as a therapeutic tool in 2001, when it was first used for Fanconi anaemia to select mutation-free and HLA identical embryos generated through IVF techniques (Verlinsky et al., 2001). In addition to allowing couples the opportunity of having an unaffected child, the technique also allows a potential donor for stem cell transplantation for the affected sibling to be selected (Grewal et al., 2004). HLA matching without mutation analysis has also been used for acquired diseases, such as acute myeloid leukaemia, acute lymphoblastic leukaemia, and chronic myeloid leukaemia (Goussetis et al., 2011; Rechitsky et al., 2004).

Although the number of reports involving HLA matching in embryos is increasing worldwide (Fiorentino et al., 2004; Goossens et al., 2012; Kuliev et al., 2011; Rechitsky et al., 2004; Van de Velde et al., 2009), data on the therapeutic outcome of treatments indicating the presence of successful HSCTs are limited (Bielorai et al., 2004; Goussetis et al., 2010, 2011; Grewal et al., 2004; Kuliev et al., 2005a; Reichenbach et al., 2008; Yesilipek et al., 2011). The follow up of families and siblings born after preimplantation HLA matching is crucially important for demonstrating the feasibility and success of this technique.

The primary purpose of this study was to report 44 successful HSCT from HLA identical siblings conceived after preimplantation HLA matching, the largest case group ever reported from one centre. In addition to this, research on the therapeutic outcomes of preimplantation HLA matching will also be reviewed.

Materials and methods

Characteristics of couples requesting IVF–PGD for HLA matching

Between 2003 and 2013, 242 referred couples, with at least one child in need of HSCT, underwent PGD for HLA matching at Istanbul Memorial Hospital, ART and Reproductive Genetics Centre. None of the sick children had an HLA-compatible donor among relatives, nor could an unrelated donor be found through national and international registries. The referred couples comprised 170 (70.2%) for haemoglobinophathies, 48 (19.8%) for haematological malignant disorders, nine (3.7%) for bone marrow failure disorders, seven (2.9%) for metabolic diseases, five (2.1%) for immunodeficiencies, and one for Glanzmann's thrombasthaenia (0.4%), and two couples for hyperimmunoglobulinaemia (0.8%) (**Table 1**). Although the indications were diverse, the major indication for preimplantation HLA matching was beta-thalassaemia (69.8%). All patients were examined by a clinical geneticist and a genetic consultant. The genetic diagnosis of the disorders in children had been completed before their application to Istanbul Memorial Hospital IVF and Reproductive Genetics Centre.

Ethical approval

Ethical committee approval was not considered necessary for this study, as it was a retrospective study involving data analysis and clinical outcomes of well-established treatment cycles, carried out in accordance with IVF guidelines and genetic analysis protocols. Patients were informed about the treatment and procedures, and written informed consent was obtained from all patients before starting IVF treatment, embryo biopsy genetic analysis and transfer procedures. Patients were informed about the possibilities of misdiagnosis and the cancellation of embryo transfer in the absence of HLA-matched embryos.

Preclinical set-up and PGD study

A preclinical set-up study was first conducted. Before preimplanation HLA matching, HLA haplotype analysis was carried out on peripheral blood samples of the mother, father and child of each family. When available, samples from other family members were obtained, such as healthy siblings or grandparents.

Table 1 Indications of couples with PGD for HLA typing.

Disease category	*Disease*	*Number of couples*	*Number of cycles*	*Coupled with mutation testing*
Haemoglobinopathies	Beta-thalassaemia	169	337	Yes
Haemoglobinopathies	Sickle cell anaemia	1	1	Yes
Malignancies	Acute lymphoblastic leukaemia	24	39	No
Malignancies	Acute myelogenous leukaemia	11	20	No
Malignancies	Anaplastic large-cell lymphoma	1	3	No
Malignancies	Aplastic anaemia	3	3	No
Malignancies	Burkitt's lymphoma	1	2	No
Malignancies	Chronic myelogenous leukaemia	4	6	No
Malignancies	Histiocytosis	1	3	No
Malignancies	Juvenile myelomonocytic leukaemia	1	1	Yes
Malignancies	Myelodysplastic syndrome	1	2	No
Malignancies	non-Hodgkin lymphoma	1	1	No
Bone marrow failure disorders	Diamond–Blackfan anaemia	1	1	Yes
Bone marrow failure disorders	Diamond–Blackfan anaemia	3	11	No
Bone marrow failure disorders	Fanconi anemia	4	5	Yes
Bone marrow failure disorders	Shwachman–Diamond syndrome	1	1	Yes
Inborn errors of metabolism	Alpha mannosidosis	1	4	Yes
Inborn errors of metabolism	Gaucher disease	1	4	Yes
Inborn errors of metabolism	Hurler syndrome	2	4	Yes
Inborn errors of metabolism	X-linked adrenoleukodystrophy	3	3	Yes
Immunodeficiency syndromes	Cd3 protein deficiency	1	1	Yes
Immunodeficiency syndromes	Haemophagocytic lymphohistiocytosis	1	1	Yes
Immunodeficiency syndromes	Wiskott–Aldrich syndrome	3	4	Yes
Other	Hyperimmunoglobulinaemia D syndrome	1	1	Yes
Other	Glanzmann's thrombasthaenia	2	3	Yes
Total		242	461	

A panel of 50 different short tandem repeat (STR) markers on HLA Class I, II and III genes were tested on genomic DNA to ensure the presence of enough informative markers to aid the identification of monosomy, trisomy, recombination, allele-drop-out and uniparental disomy of the analysed chromosomes and HLA regions. For each family, at least 12 informative STR markers were selected for PGD Study. The set-up studies were carried out at the Genoma Molecular Genetics Laboratory, Rome, Italy (2002–2007), the Reproductive Genetics Institute, Chicago, USA (2007–2010), and the Istanbul Memorial Hospital Reproductive Genetics Centre from 2010 till present (Kahraman et al., 2011). For couples with a low number of fully informative markers, the semi-informative markers were added to the study to increase the reliability of the test and to detect allele-drop-out. Only two cases with recombination in the HLA area were rejected, as probability of finding a matched embryo was extremely low.

IVF and embryo biopsy procedure

The stimulation protocols have been outlined previously (Kahraman et al., 2011). For ovarian stimulation, GnRH analogue suppression (short or long), GnRH antagonist protocol and HMG, or recombinant FSH was used. Oocyte retrievals were carried out 36 h after the injection of 10,000 IU HCG by transvaginal ultrasound guidance. About 3 ± 1 h after oocyte retrieval, cumulus cells were enzymatically removed. To avoid sperm DNA contamination, ICSI was applied to metaphase II oocytes (Palermo et al., 1992). Between 2003 and 2005, injected oocytes were placed in G1 and G3 media (Vitrolife, Gothenburg, Sweden) and between 2005 and 2013 to KSOM media (Life Global, Belgium), respectively. One blastomere was removed from cleavage stage embryos (De Vos and Van Steirteghem, 2001) from an opening made using laser (Saturn 3; Research Instruments, UK). Unless there was no diagnosis due to amplification failure, biopsy of a second blastomere was strictly avoided to prevent causing harm to the embryo through removing a considerable amount of embryonic volume (Cohen et al., 2007). Subsequently, embryo transfer was carried out usually on day 4 but rarely on day 5. Trophectoderm tissue biopsies have also been carried out since 2009. Blastocyst-stage biopsy involved making a hole in the zona pellucida on day 3 of embryonic development, which allowed the developing trophectoderm cells to protrude after blastulation, facilitating the biopsy. On day 5 after fertilization, between two and five cells were excised using laser energy, without loss of inner cell mass. After diagnosis, the embryos were transferred during the same cycle, on days 5 or 6. Pregnancy was first evaluated by serum HCG concentrations assay, 12 days after embryo transfer, and clinical pregnancy was diagnosed by ultrasonographic visualization of one or more gestational sacs or definitive clinical signs of pregnancy (Zegers-Hochschild et al., 2009). Implantation rate was defined as the number of gestational sacs with fetal heartbeat observed divided by the number of embryos transferred.

Cell lysis and PCR reactions and mutation analysis

All PGD studies and evaluations were conducted at the Istanbul Memorial Hospital Reproductive Genetics Unit. Both alkaline lysis (Fiorentino et al., 2006) and proteinase K (Verlinsky et al., 2001) lysis methods were used according to the time period as stated previously (Kahraman et al., 2011). DNA testing involved two rounds of polymerase chain reaction (PCR) reactions; in the first round using multiplex PCR, which allows simultaneous amplification of HLA regions (**Figure 1**) and mutation-linked markers, and in the second round using single-plex which is a fluorescent PCR with semi- or heminested primers (Fiorentino et al., 2004; Rechitsky et al., 2004). HLA matching was carried out alone for acquired haematological disorders and simultaneously with mutation analysis for inherited haematological disorders (**Figure 2A and B**). Both minisequencing and restriction fragment length polymorphism methods were used for mutation detection according to preference. Fanconi anaemia is a difficult disease to manage, and the work-up period is long compared with other diseases. At first, a complementation test, which significantly increases the work-up period, should be conducted to identify the gene that has the mutation. Time is then needed for the gene to be sequenced to find the exact position of the mutation. On the basis of these data, a set-up study was carried out with a panel of 10–12 STR markers for Fanconi anaemia, and about 50 STR markers for HLA region so that a sufficient number of informative markers were available for each condition. The time period for the identification of the position of the mutation is expected to decrease with the implementation of new techniques, such as next-generation sequencing, as it might eliminate the need for complementation test.

Results

Outcome of IVF–PGD cycles for HLA matching

A total of 461 cycles were carried out for 242 couples; 192 couples (Group 1) underwent 371 cycles (80.5%) for HLA matching plus mutation testing, and 50 couples (Group 2) underwent 90 cycles (19.5%) for HLA testing only (**Table 2**). A total of 3973 embryos were biopsied, of which (3556) 89.5% were diagnosed successfully. For the HLA-only testing group (Group 2), 17.0% of analysed embryos were found as HLA-compatible and transferrable, whereas, in Group 1 in which mutation testing was also carried out, 11.9% of analysed embryos were found as both disease-free and HLA compatible. In 275 (59.6%) of cycles, at least one suitable embryo could be found and transferred, resulting in an ongoing pregnancy rate of 29.8% per transfer. Ninety-four healthy babies were born from 80 births, and three pregnancies were ongoing at the time of writing this manuscript. Ninety babies were HLA compatible with their siblings. The remaining four babies were HLA non-identical. Three babies were born as a result of transfer of HLA non-identical embryos in two families upon their consent. Lastly, one HLA non-identical baby was born as a result of a misdiagnosis out of 94 babies (1.06%).

Haematopoietic stem cell transplantation outcome

It was possible to carry out HSCT in 48 families where both recipients and donors met the appropriate criteria for transplantation and maintained a good state of health. Eleven families were awaiting HSCT from a donor who was not eligible to donate bone marrow because of low body weight, and therefore postponed. Unfortunately, five children with acute forms of leukaemia died while awaiting HSCT, either before or immediately after the sibling was born. The average time period between diagnosis and first consultation at the IVF–PGD centre was 1.5 years for patients with leukaemia. As 10 children are in remission, HSCT is not planned for the time being (**Table 3**).

Sixteen different institutions carried out HSCT. Most were conducted in Turkey ($n = 35$), and the rest in Italy ($n = 9$), Israel ($n = 2$) and Germany ($n = 2$). The engraftment parameters, chimerism values, transplant-related mortality, relapse- and disease-free survival time were used as assessment criteria on the outcome of each HSCT.

A total of 48 HSCT were carried out for the following indications; beta-thalassaemia ($n = 37$), sickle cell anaemia ($n = 1$), Wiskott–Aldrich syndrome ($n = 3$), Fanconi anaemia ($n = 1$), Glanzmann's thrombasthaenia ($n = 1$), X-linked adrenoleukodystrophy ($n = 1$), acute myeloid leukaemia ($n = 1$), acute lymphoblastic leukaemia ($n = 1$), Diamond–Blackfan anaemia (sporadic) ($n = 1$) and mucopolysaccharidosis type I (Hurler syndrome) ($n = 1$).

The procedure was successful in 44 children whose post-transplantational clinical conditions demonstrate hematopoietic and immunologic reconstitution. A complete list of diseases cured by HSCT after IVF–PGD for HLA typing in both this study and previous studies is presented in **Table 4**. According to the follow-up data available from haematology institutes, the median engraftment time was 15 days and median platelet engraftment time was 23 days after transplantation. Chimerism tests revealed values in the range of 84–100%, indicating successful transplantation in 44 patients (**Table 5**). Unfortunately, in four cases with beta-thalassaemia, graft failure occurred after HSCT. This was explained by the high number of previous blood transfusions, which negatively affect transplantation outcome in those children who are also older than average (11.7 versus 7.0 years) in our case group (personal communication with haematologists). In those unsuccessful cases, the rates of donor–recipient chimerism were continuously decreasing, favouring the recipient's cells and necessitating post-transplantation blood transfusions.

Source of stem cells

For three HSCT, umbilical cord blood (UCB) was used as the sole source despite the collection of UCB at birth from most babies. As a result of the low volume or the low number of $CD34^+$ cells in the cord blood samples collected, either bone marrow cells-only ($n = 12$) or in combination with frozen and thawed UCB cells ($n = 33$) were used in the rest (**Table S1** in the online version at doi:10.1016/j.rbmo.2014.05.010).

In our patient group, no reports were received from haematologists about any discomfort or risks experienced by

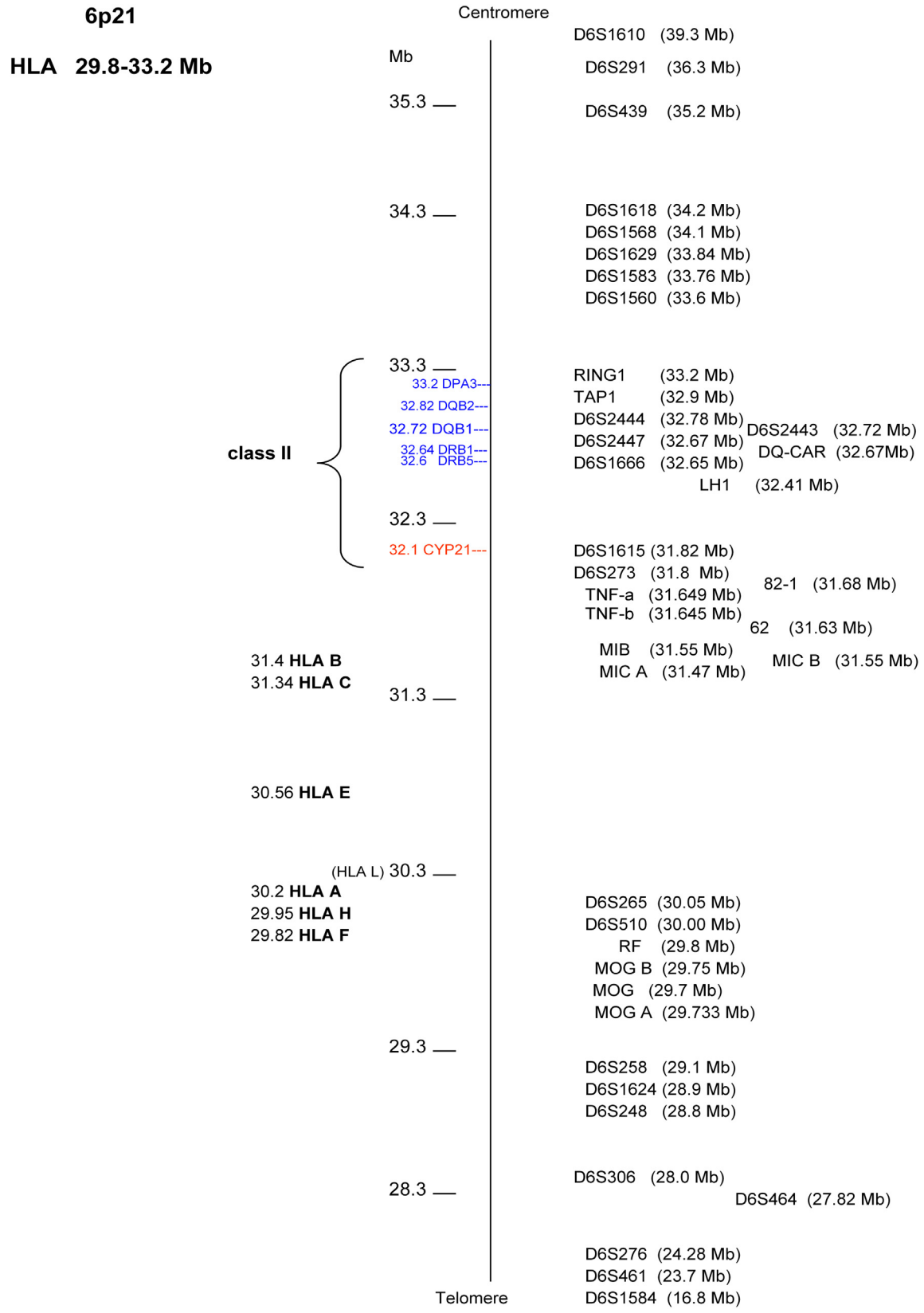

Figure 1 The short tandem repeat markers and their positions on the HLA genes on 6p21 locus.

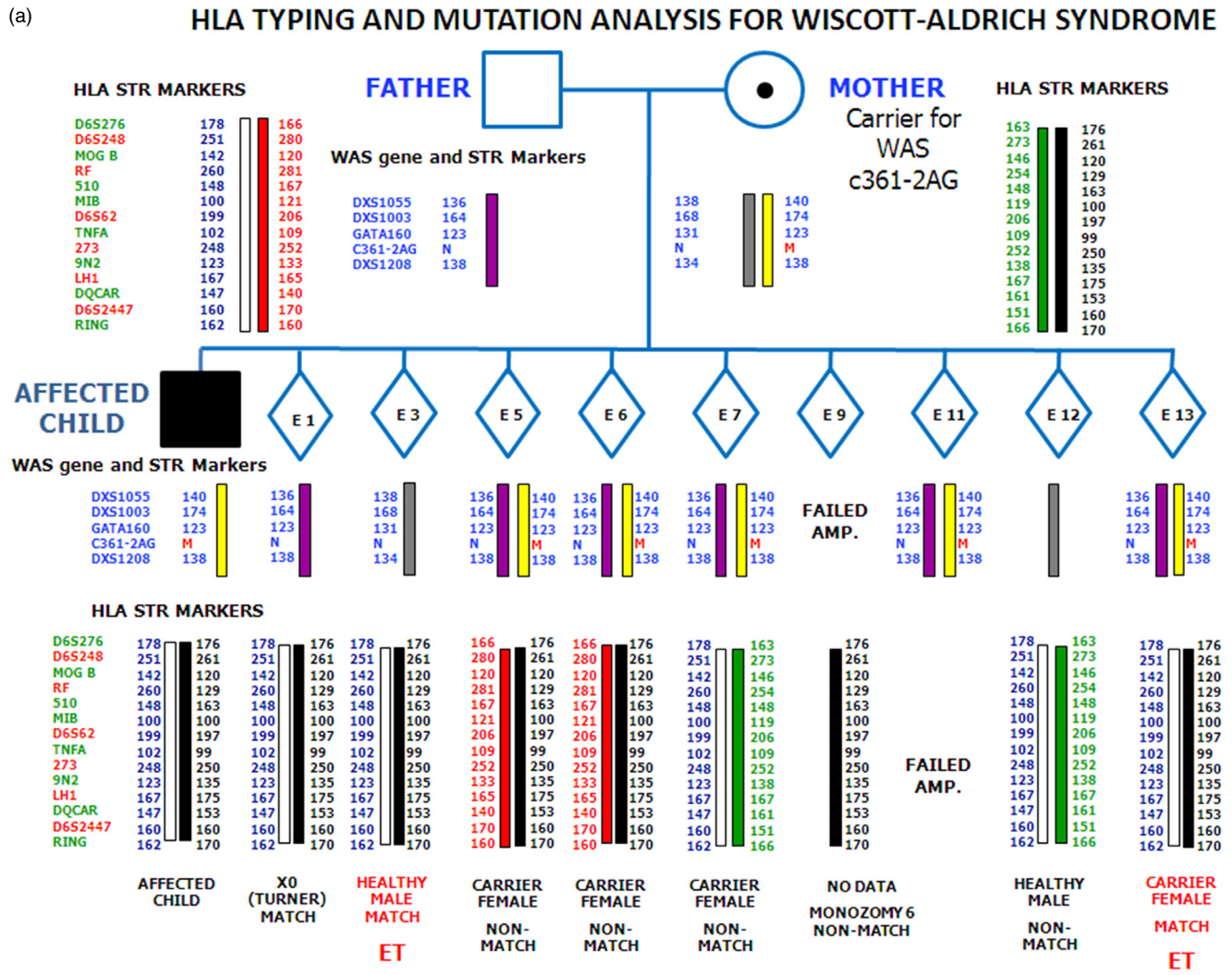

Figure 2 (a) Linked short tandem repeat (STR) marker analysis for Wiscott–Aldrich syndrome and HLA typing simultaneously. Four informative STR markers (three upstream and one downstream) were used for detecting mutation c361-2AG for the Wiskott–Aldrich gene located on X chromosome. Fourteen informative STR markers were used for HLA typing. For mutation detection, restriction fragment length polymorphism technique was used. Eight embryos were amplified successfully out of the nine biopsied embryos at cleavage stage. Two HLA-matched embryos (one healthy and one carrier for the mutation) were transferred, resulting in the birth of a healthy singleton; (b) HLA typing and mutation analysis in Fanconi anemia. In their first attempt, the couple had nine embryos suitable for biopsy in cleavage stage. According to the seven informative STR markers and mutation testing for Fanconi anaemia; out of nine embryos, two were healthy, three were carrier, one was affected, two were monosomic for chromosome 16, and one produced no result. According to the 14 informative markers for HLA region, two were HLA matched with the affected sibling and seven were non-matched; in one of them, a recombination event was detected which originated from the mother. As a result, only one embryo was both unaffected and HLA matched. With the transfer of this embryo, the couple achieved a pregnancy, which resulted in the birth of healthy and HLA-matched baby. The sick child was recovered from disease after successful transplantation. ET, embryo transfer; Failed amp, failed amplification, no result available; STR, short tandem repeat.

the donor child during and after transplantation. The bone marrow samples were harvested once the donor had reached an appropriate weight to donate a sufficient volume of bone marrow, posing no threat to the donor's health and well-being. Children's health status was monitored continuously by paediatric haematologists and oncologists before and after HSCT, ensuring no harm had been caused from the harvesting.

Discussion

Haematopoietic stem cell transplantation still remains the only curative treatment for many disorders, including haemoglobinopathies, bone marrow failure disorders and hematological malignancies. Combining PGD with HLA matching is a therapeutic option that allows couples to have an unaffected child but also enables a potential donor to be

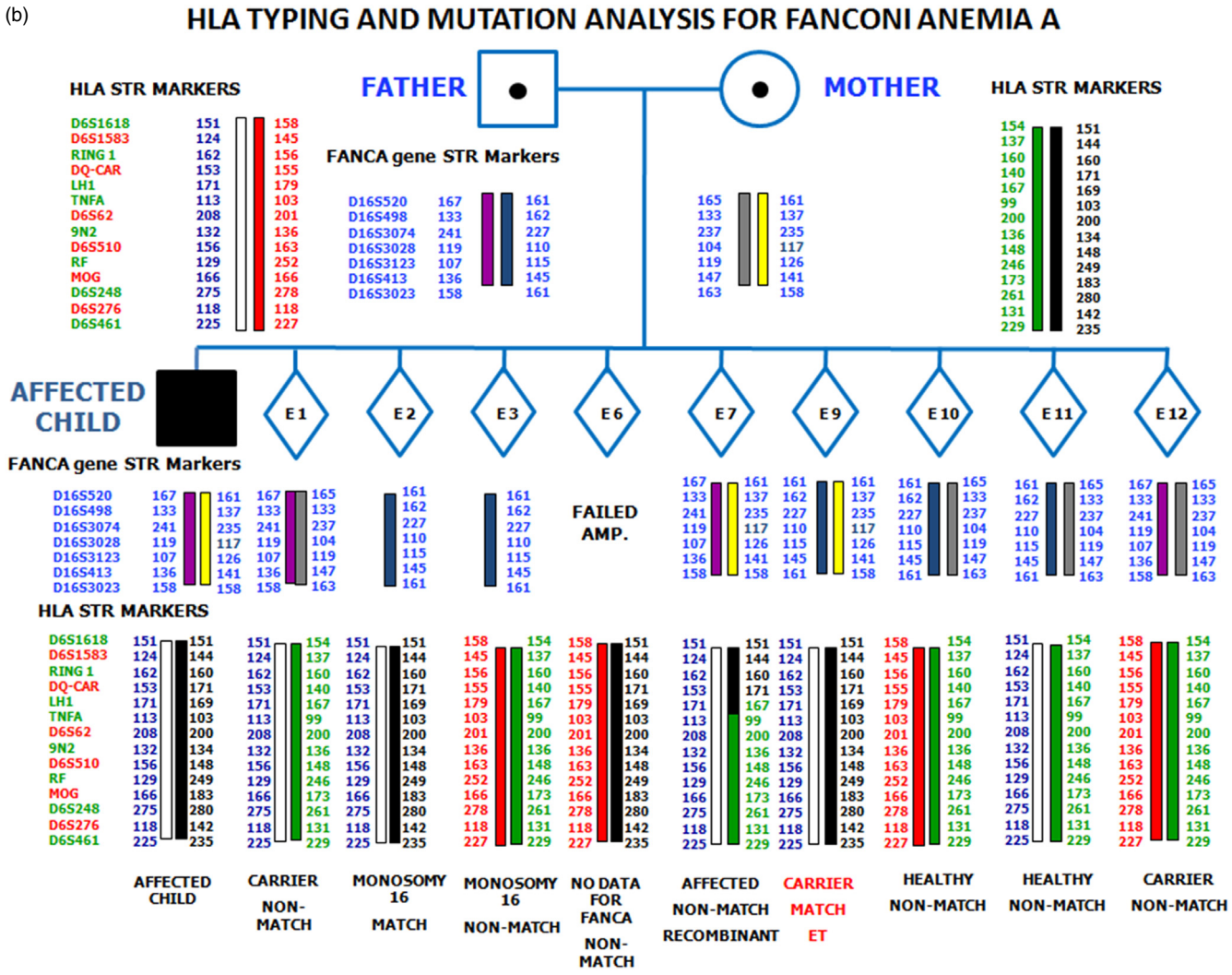

Figure 2 Continued.

selected for stem cell transplantation for the affected sibling (Kahraman et al., 2011; Kuliev et al., 2011; Verlinsky et al., 2001).

The follow up of families and siblings born after preimplantation HLA matching is crucially important for demonstrating the feasibility and success of this technique. Although the number of IVF cycles initiated primarily to select HLA identical embryos is increasing worldwide (Goossens et al., 2012), data on outcome of treatments and follow up of those families are limited (El-Toukhy et al., 2010). To date, preimplantation HLA matching has been shown to be a successful therapeutic option for children with beta-thalassaemia (Kahraman et al., 2011; Kuliev et al., 2011; Milachich et al., 2013; Qureshi et al., 2005; Van de Velde et al., 2009; Yesilipek et al., 2011), Fanconi anaemia (Bielorai et al., 2004; Grewal et al., 2004), X-linked hypohidrotic ectodermal dysplasia with immune deficiency (Verlinsky et al., 2007), X-linked hyperimmunoglobulinaemia syndrome (Verlinsky et al., 2007), X-linked chronic granulomatous disease (Goussetis et al., 2010; Reichenbach et al., 2008), chronic myeloid leukaemia (Goussetis et al., 2011), Diamond–Blackfan anaemia (Kahraman et al., 2011; Kuliev et al., 2005b; Verlinsky et al., 2004), Wiskott–Aldrich syndrome (Kahraman et al., 2011), acute myeloid leukaemia (Kahraman et al., 2011), acute lymphoblastic leukaemia (Van de Velde et al., 2009), X-linked adrenoleukodystrophy (Kahraman et al., 2011), and Glanzmann's thrombasthaenia (Kahraman et al., 2011). This study extends the list of disorders that have been cured via preimplantation HLA matching with the addition of sickle cell anaemia and mucopolysaccharidosis type I (also known as Hurler Syndrome) (**Table 4**). Another important implication of this study is to present the successful recovery in two acute forms of haematological malignancies (acute myeloid leukaemia and acute lymphoblastic leukaemia).

One of the most important limitations of this technique is the low probability of identifying matched embryos for transfer, and these limitations should be clearly highlighted during counseling. Although the chance of finding a suitable embryo for transfer was 70.0% in cases of HLA matching only, the chance of finding a suitable embryo for transfer was only 57,1% in cases requiring HLA matching and mutation testing. Furthermore, the achievement of embryo transfer is highly dependent on the number of eggs retrieved; therefore, the chances of success are significantly lowered for women with

Table 2 Outcome of IVF cycles with PGD for HLA typing.

	HLA + mutation testing (Group 1)	*HLA only (Group II)*	*P-value*	*Total*
Number of couples/cycles	192/371	50/90		242/461
Maternal age (mean ± SD)	31.9 ± 4.7	34.1 ± 5.7	<0.05	32.2 ± 5.0
Oocytes collected (mean ± SD)	14.8 ± 8.3	15.8 ± 7.6	NS	15.1 ± 8.3
Matured oocytes injected (mean ± SD)	11.3 ± 6.5	12.1 ± 6.0	NS	11.4 ± 6.4
Oocytes fertilized (%)	86.8 ± 12.2	84.1 ± 14.6	<0.05	86.6 ± 12.8
Embryos biopsied (*n*)	3185	788		3973
Embryos diagnosed (%)	89.6	89.2	NS	89.5
Embryos transferable[a] (%)	11.9	17.0	<0.001	12.9
Cycles with transfer % (*n*)	57.1 (212)	70.0 (63)	<0.001	59.6 (275)
Transfered embryos (mean ± SD)	1.5 ± 0.6	1.5 ± 0.6	NS	1.5 ± 0.6
Implantation[b] rate (%)	95/321 (29.6)	27/94 (28.7)	NS	29.7
Clinical pregnancy rate/ transfer % (*n*)	37.7 (80)	36.5 (23)	NS	37.5 (103)
Live birth rate/transfer (%)	60/212 (28.3)[c]	20/63 (31.7)	NS	29.1
Number of live births/ babies born (*n*)	60/70	20/24		80/94

Statistical analysis was done using chi-aquare test.
[a]Transferable refers to unaffected, euploid and HLA compatible for group 1 and HLA compatible and euploid for group 2.
[b]Fetal heart beat positive.
[c]Three pregnancies out of the 212 transfers are still ongoing.

Table 3 Follow up of families with children born by IVF-PGD for HLA.

	n
Total number	80 family; 94 babies
Allogenic HSCT performed	48
Allogenic HSCT not performed	32
Awaiting[a]	11
Sick sibling passed away[b]	5
Not yet planned[c]	10
Lost to follow up[d]	2
Transplantation from an unrelated donor[e]	1
HLA non-identical[f]	3

[a]Not eligible because of low body weight.
[b]Five children with acute forms of leukaemia passed away while waiting or HSCT either before or after the sibling was born.
[c]In 10 cases, the children are in remission so haematopoietic stem cell transplantation is not planned for the time being.
[d]No information is available after the birth of two HLA-identical babies in two families.
[e]HSCT from an unrelated donor was carried out in a child with relapsed leukaemia when a donor was found after an HLA identical pregnancy was established.
[f]Four HLA non-identical babies were born in three families. Three babies were born as a result of non-identical embryo transfer with the consent of parents where no identical embryos were available according to PGD results, and one baby was born as a result of misdiagnosis.

diminished ovarian reserve. The couple should be emotionally ready for the possibility of repeating IVF–PGD cycles to conceive a matched and healthy child.

Another important issue to discuss with patients is the probability of a single treatment cycle progressing to harvesting stem cells and correcting the phenotype of the affected sibling at the end. An inference could be made on the basis of the results presented in this study; out of 461 cycles, embryo transfer was achieved in 275 (59.6%); 83 (30.2%)resulted in ongoing pregnancies and a total of 80 live births. So far, 44 successful HSCT have been achieved. On this basis, the current rate for successful transplantation seems to be 9.5% (44/461). This could, however, lead to an underestimation of the success rate, as some children are waiting for treatment, some have been lost to follow up, and some have not yet been scheduled for transplantation; all these factors combined are likely to contribute to the success data in the near future.

Although single-cell PCR technique is complex and challenging (Piyamongkol et al., 2003), the technique is reliable and misdiagnosis rates are low. In the present study, only one misdiagnosis out of 94 babies (1.06%) occurred in the diagnosis of HLA compatibility. The baby was a carrier for beta-thalassaemia, but HLA non-identical with the affected sibling. This rate is clinically acceptable and should be discussed and clearly explained to couples before obtaining consent.

Although the couples are fertile, the implantation rate and the live birth rate reported (Van de Velde et al., 2009) in cycles performed for HLA matching is lower (15%) compared with regular ICSI cycles, ranging from 20.7–47.4% (Gelbaya et al.,

Table 4 List of diseases cured by HSCT after IVF-PGD for HLA typing.

	Number of patients cured		*Total (n)*
	Present study (n)	*Previous studies (n)*	
Beta-thalassaemia	33	Qureshi et al., 2005 (1); Kuliev et al., 2011 (12); Yesilipek et al. 2011 (2)	48
Wiskott–Aldrich syndrome	3		3
Fanconi anaemia	1	Bielorai et al. 2004 (1); Grewal et al., 2004 (1)	3
Sickle cell anaemia[a]	1		1
Glanzmann's thrombasthaenia	1		1
X-linked adrenoleukodystrophy	1		1
Mucopolysaccharidosis Type I (Hurler syndrome)[a]	1		1
Acute myeloid leukaemia	1		1
Acute lymphoblastic leukaemia	1[b]	Van de Velde et al., 2009 (2)[b]	2
Diamond–Blackfan anaemia	1	Verlinsky et al., 2004 (1)	2
X-linked hypohidrotic ectodermal dysplasia with immune deficiency		Verlinsky et al., 2007 (1)	1
X-linked hyperimmunoglobulinaemia syndrome		Verlinsky et al., 2007 (1)	1
X-linked chronic granulomatous disease		Reichenbach et al., 2008 (1); Goussetis et al., 2010 (2)	3
Chronic myeloid leukaemia		Goussetis et al., 2011 (1)	1
Total (*n*)	44	25	69

[a]First reported by the present study.
[b]One case in common.

Table 5 Patient demographics and post-transplantation clinical outcomes of 48 haematopoietic stem cell transplantations after IVF–PGD for HLA.

Median recipient age, year (range)	7 (3–16.5)
Median donor age, month (range)	17 (6–72)
Median recipient weight, kg (range)	24.5 (12.6–60)
Median donor weight, kg (range)	13 (7–20)
Sex of recipient male/female	26/22
Sex of donor male/female	20/32
Stem cell source	
Umbilical cord blood	3
Bone marrow	12
Umbilical cord blood plus bone marrow	33
Successful engraftment and recovery	44
Neutrophil engraftment (>500\μl) day, median	15 (14–16)
Platelet engraftment (>20,000\μl) day, median	23 (14–35)
Chimerism rate,% range	96 (84–100)
Unsuccesful transplantation	4[a]
Major complications	0
Graft versus host disease	0

[a]Four out of the 48 haematopoietic stem cell transplantations (8.3%) failed, and the children are waiting for a repeated haematopoietic stem cell transplantation.

2010). This can be attributed to the selection of matched embryos for transfer rather than using best quality embryos. Furthermore, in one-third of couples, the woman was aged 37 years or older. This may have a negative effect on implantation given that embryological parameters, gonadal responses and the likelihood of chromosomal abnormalities are known to be severely affected by female age (Eichenlaub-Ritter et al., 2004; Kuliev et al., 2003; Munné et al., 2007).

So far, HSCT has only been carried out in 48 families (**Table 3**). In most, the insufficiency of cord blood volume collected at birth required haematologists to combine it with bone marrow, which increased the waiting period for HSCT. In this present study, the proportion of transplantation where cord blood was used as the sole source was low (3/48). Ninety-four per cent of the transplantations used bone marrow, either alone or together with cord blood. Although, UCB cells constitute a suitable source of stem cells with high concentrations of $CD34^+$ cells and low probability of graft failure and GVHD (Orofino et al., 2003), some problems are related to its use (Shahrokhi et al., 2012). Two reasons have been proposed to underlie these problems; first, donor T-lymphocytes may elicit a blunted allogeneic effect by the naïve immune system. Second, a reduced number of haematopoietic progenitor cells may cause graft failure and delayed haematopoietic recovery, which can further contribute to serious infections (Shahrokhi et al., 2012). In addition, in-vitro expansion techniques, despite promising to increase the content of the cord blood, are still being

considered as experimental, and no universally accepted protocol is in place for clinical use. The major limitation for our sample group was the cell dose, which was difficult to overcome, especially for the recipients with larger body mass. Therefore, the most common reason why HSCT was not carried out was the need for extra time for the child to gain sufficient weight before donating his or her bone marrow cells safely. The second most common reason was the postponement of transplantation as a result of the malignant disorder being in remission. For those patients, a possible transplantation was planned in the event of a relapse. Finally, HSCT could not be carried out in five children with acute leukaemia or myelodysplastic syndrome, as they died either before or soon after their matched siblings were born (**Table 3**).

The timing of IVF-PGD treatments for HLA matching is important, especially for malignant disorders such as leukaemia, where the time-frame for the technique to be applicable and beneficial is limited. Some guidelines have been suggested on when and in what conditions this technology should be offered to parents (Samuel et al., 2009; Tur-Kaspa, 2012). First, it should be considered when a related matched donor is not available. The need for HSCT should not be urgent, as the treatments last at least 9–12 months, and may require recurrent trials to find a suitable embryo and achieve a healthy birth. For that reason, even for the diseases with good prognosis, treatment should start as early as possible. Of course, the mother should of reproductive age to have a pool of embryos from which an HLA matched embryo can be found (Tur-Kaspa and Najeemuddin, 2010). Those conditions should be met before starting IVF–PGD treatment.

Out of 48 HSCTs, graft failure occurred in four cases with beta-thalassaemia (8.3%). A number of factors may contribute to the risk of graft failure in patients with beta-thalassaemia major. These include inadequate conditioning regimens, insufficiency of stem-cell source, previous number of blood transfusions, and the presence of damage in the internal organs of the recipient at the time of HSCT (Lucarelli et al., 1996). According to the Pesaro criteria (Lucarelli et al., 1990), which is based on the presence of portal fibrosis, hepatomegaly and a history of inadequate chelation therapy are risk factors; graft rejection creates a threat with a probability of more than 3% even for the class I (low risk) patients (Sodani et al., 2004).

A severe problem associated with allogeneic stem cell or bone marrow transplantation is GVHD. This may cause mortality in the acute form. In our patient group, neither acute nor chronic GVHD was observed in the recipients; this may be because appropriate GVHD prophylaxis regimens were given before HSCT from histo-compatible siblings.

In this present study, complications were observed in nine patients. Infections are important complications seen after HSCT. Three patients with cytomegalovirus reactivation and another three with bacteraemia were treated with antiviral and antibiotic therapies, respectively. The complications were not severe and the patients did not require hospitalization for more than 15 days. Neutropenic fever was observed in two patients. Veno-occlusive disease developed in one patient, and was successfully treated by defibrotide (**Table S1** in the online version at doi:10.1016/j.rbmo.2014.05.010).

Consistent side-effects of pre- and post-HSCT regimens also occur. In addition to their well-known transient side-effects, high-dose conditioning therapy and total body irradiation may cause gonadal toxicity, leading to infertility. Cryopreservation of ovarian tissue has been shown to have a high potential of maintaining endocrine function and preserving fertility after being replaced into its anatomical position in prepubertal girls (Fabbri et al., 2012). Preservation of fertility in prepubertal boys is more challenging. Although cryopreservation of sperm before gonadotoxic treatment is well established for adolescents, for young patients who are unable to provide sperm samples, cryopreservation of testicular tissue is the only option, albeit experimental (Ginsberg et al., 2010). Cryopreservation and in-vitro propagation of human spermatogonial stem cells may provide a new hope for fertility preservation in the future (Hwang and Lamb, 2010).

Conclusion

The follow up of families with affected children and siblings conceived after PGD is crucial. In the present study, full recovery was achieved after successful HSCT in 44 affected siblings with 10 different conditions: beta-thalassaemia, Fanconi anaemia, Diamond–Blackfan anaemia, sickle-cell anaemia, Wiskott–Aldrich syndrome, X-linked adrenoleukodystrophy, Glanzmann's thrombasthaenia, Hurler syndrome, acute myeloid leukaemia, and acute lymphoblastic leukaemia. Data from different international centres demonstrate the feasibility and success of this technique. Success has been achieved through multidisciplinary team work involving embryologists, geneticists, gynaecologists, paediatric haematologists, and oncologists. Developing clinical guidelines and efficient communication between these areas may significantly decrease the waiting period for couples in need of IVF-PGD for HLA. World-wide awareness should be increased, and the feasibility of this technique should be discussed with families as an alternative option. Preimplantation HLA matching, therefore, provides a realistic option for couples in the treatment of an affected sibling when no HLA-matched donor is available.

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Appendix: Supplementary material

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