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Application of preimplantation genetic diagnosis at disorders of human haemoglobin

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Haemoglobin disorders are among the most frequent indications for preimplantation genetic diagnosis (PGD), introduced as an important option to couples at risk for producing offspring with thalassaemia and sickle cell disease. Previous experience mainly included PGD for α-thalassaemia, while PGD for α-thalassaemia resulting in an unaffected pregnancy has not been reported. This study presents the results of the world's largest experience of 197 PGD cycles for haemoglobin disorders, which includes PGD for α-thalassaemia, resulting in 53 clinical pregnancies and birth of 45 healthy children, with five still ongoing. Fifty-four of these cycles were performed in combination with HLA typing, allowing the birth of thalassaemia-free children who were also HLA identical to the affected sibling, with successful stem cell transplantation in one case. As an increasing proportion of patients requesting PGD with HLA typing are of advanced reproductive age, aneuploidy testing was performed simultaneously with PGD. The results show that PGD has now become a practical approach for prevention of haemoglobin disorders, and is gradually being used also for improving access to HLA compatible stem cell transplantation for this group of diseases.

Key words: *aneuploidy testing, PGD for* α*-thalassaemia, PGD for* β*-thalassaemia, preimplantation HLA typing, stem cell transplantation.*

Использование предимплантационной генетической диагностики при патологиях гемоглобина человека

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Патологии гемоглобина – одно из наиболее частых показаний для предимплантационной генетической диагностики (ПГД), рекомендуемой для супружеских пар с риском рождения детей с талассемией и серповидно-клеточной анемией. До настоящего времени ПГД предусматривала главным образом диагностику бета-талассемии, сообщения о ПГД альфа-талассемии, обеспечивающей благополучную беременность, отсутствуют. В настоящей работе представлены результаты самого крупномасштабного исследования в мире, в ходе которого было проведено 197 циклов предимплантационной генетической диагностики гемоглобинопатий, включающей ПГД альфа-талассемии, результатом которой стали 53 случая клинической беременности с рождением 45 здоровых детей; в 5 случаях срок разрешения беременности еще не наступил. 54 цикла из этой группы были проведены в комбинации с HLA-типированием; это обеспечило возможность рождения детей, не имеющих талассемии, которые по HLA были идентичны сибсам с дефектами – в одном случае с успешной трансплантацией стволовых клеток. В связи с тем, что среди желающих провести ПГД в комбинации с HLA-типированием появляется все больше пациентов пожилого репродуктивного возраста, одновременно с ПГД проводилось выявление анеуплоидии. Результаты показывают, что ПГД на сегодняшний день стала практическим подходом для предотвращения гемоглобинопатий и постепенно начинает использоваться также для улучшения доступа к HLA-совместимой трансплантации стволовых клеток при заболеваниях этой группы.

Ключевые слова: *выявление анеуплоидии, ПГД альфа-талассемии, ПГД бета-талассемии, предимплантационное HLA-типирование, трансплантация стволовых клеток.*

Introduction

Although preimplantation genetic diagnosis (PGD) for thalassaemias was introduced only a few years ago (Kuliev et al., 1998), haemoglobin disorders are presently one of the major indications for PGD (IWGPG, 2001; ESHRE PGD Consortium, 2002). Available experience involves PGD for α-thalassaemias (haemoglobin-β locus, HBB, localized on chromosome 11p15.5) performed mainly in Mediterranean populations, and currently also introduced in South East Asia, where these conditions are also prevalent (Kuliev et al., 1998, 1999; Kanavakis et al., 1999; Jiao et al., 2003). Initially, PGD was offered to at-risk couples who had undertaken prenatal diagnosis but had to terminate a pregnancy with an affected fetus in repeated attempts. However, PGD was then offered as the primary option to the patients with infertility problems, and to those who could not accept the risk for prenatal diagnosis and termination of pregnancy.

Most recently, PGD was applied to couples with existing thalassaemic children requiring HLA compatible bone marrow transplantation (Kahraman et al., 2004; Rechitsky et al., 2004; Van Den Velde et al., 2004). These couples requested testing primarily to have an unaffected (thalassaemia-free) child, but hoped that the resulting baby might also serve as an HLA compatible donor of stem cells for the affected sibling. Because in most of these cases the couples were already 35 years or older, age-related aneuploidy testing was also introduced in an attempt to increase the chances of pregnancy.

The present paper describes the world's largest experience of PGD for haemoglobin disorders, which also reports PGD for α-thalassaemia (haemoglobin-alpha locus 1, HBA 1, localized on chromosome 16pter-p13.3), and presents PGD for thalassaemia combined with HLA and aneuploidy testing.

Materials and methods

A total of 197 PGD cycles were performed for 114 couples at risk of producing children with haemoglobin disorders. This included six PGD cycles for α-thalassaemia, 54 in combination with HLA typing and three combined with aneuploidy testing.

PGD cycles were performed using a standard IVF protocol coupled with micromanipulation procedures for polar body (PB), or blastomere sampling, described in detail elsewhere (Verlinsky, Kuliev, 2004). The PB approach was used in the majority of cases, as testing for maternal mutations may be sufficient for preselection of unaffected embryos for transfer, possibly avoiding further testing for paternal mutation. In these cases, the first (PB1) and second (PB2) PB were removed following maturation and fertilization of the oocytes, and tested by the multiplex nested polymerase chain reaction (PCR) analysis, involving the mutation testing simultaneously with different linked markers (Kuliev et al., 1998, 1999; Piyamongkol et al., 2001; Rechitsky et al., 2001; Hellani et al., 2005). Mutation analysis involved the detection of thalassaemia mutations shown in Figure 1. At least three linked markers were tested simultaneously with the mutation and normal gene, which have been selected from those listed in Figure 1.

There are three genetic possibilities for the PB1 genotype originating from a heterozygous mother. PB1 may be homozygous normal or homozygous mutant, if no crossover occurs, and accordingly, heterozygous in the event of a crossover. The most accurate and reliable prediction of the embryo genotype was obtained when PB1 was heterozygous, due to the obvious detection of both normal and mutant genes, excluding any possibility for allele specific amplification failure or allele dropout (ADO), which is the major limitation of single cell PCR. The extensive experience of PB testing described in this paper provides strong evidence that embryos resulting from the oocytes with heterozygous PB1 and mutant PB2 are free of maternal mutation, and may be transferred with no risk for misdiagnosis. However, in the absence of a sufficient number of oocytes with heterozygous PB1, those embryos originating from oocytes with homozygous mutant PB1 may be transferred, if ADO of normal gene can be reliably excluded. This has been done by testing at least three linked markers simultaneously with the gene, which may have been present in PB1 but not detected because of ADO (Rechitsky et al., 2001; Verlinsky, Kuliev, 2004). Additional confirmation of diagnosis in such cases was achieved by the identification of the genotype of the extruded PB2, which should be opposite to the genotype of homozygous PB1 and, accordingly, identical to the oocyte genotype. Blastomere biopsy of embryos resulting from oocytes containing thalassaemia mutations was also performed, to identify heterozygous embryos acceptable for transfer. Testing at the cleavage stage was performed in all cases of PGD combined with HLA typing, in order to identify the embryos containing the maternal and paternal chromosomes 6 identical to the sibling with thalassaemia, as described in detail elsewhere (Rechitsky et al., 2004).

Blastomere analysis was also used for aneuploidy testing, in addition to aneuploidy analysis in PB1 and PB2, to identify chromosomal errors originating from maternal and paternal meiosis, as well as from the cleavage stage. For this purpose, primers for chromosome specific microsatellite markers were added to the multiplex PCR protocols worked out for thalassaemia testing, which included primers for chromosome 13 (D13S1493; D13S284; D13S1303; D13S1317; D13S800; D13S317; D13S631; D13S159; D13S174), chromosome 16 (D16S521; D16S3024; D16S3024; D16S3134; D16S423: D16S3021; D16S520), chromosome 18 (D18S1104; D18S66; D18S57; D18S1145; D18S1127; D18S1144; D18S386; D18S61), chromosome 21 (D21S1899; D21S1914; D21S1910; D21S1888; D21S267; D21S268; D21S1411; D21S1890; D21S1903; D21S11), and chromosome 22 (D22S1158; D22S277; D22S283; D22S423; D22S1157; D22S282), as described elsewhere (Verlinsky, Kuliev, 2004).

Because of the high prevalence of mosaicism at the cleavage stage, the copy number of the chromosome 11 was also assessed, in order to exclude the lack of mutant allele due to a possible monosomy 11 in the biopsied blastomeres. The copy number of chromosome 6 was tested in PGD cycles combined with HLA typing.

As mentioned, the preselection of mutation-free oocytes was performed based on the simultaneous mutation detection and linked marker analysis, involving three strongly linked markers from those shown in Figure 1. In some cases, prior to PGD a single sperm testing was also performed to identify the paternal haplotypes, while the maternal haplotypes were established based on PB analysis. Primer sequences and reaction conditions used for PGD of α-thalassaemia, which were not reported earlier, are presented in Table 1 and illustrated in Figure 2.

Following informed consent, approved by the Institutional Review Board, embryos free of haemoglobin disorders, based on the information about the mutation testing and polymorphic markers, and HLA matched or aneuploidy free when requested, were preselected for transfer back to patient, while those predicted to be mutant or with insufficient marker information were exposed to confirmatory analysis. Unaffected embryos that were not HLA matched were frozen for future use by the couples.

Results

The results of PGD for haemoglobin disorders are presented in Table 2 currently representing the largest world experience available. As can be seen from the list of thalassaemia mutations presented in Figure 1, approximately 24 different β-globin gene mutations were tested. At least half (52.6%) of these cycles were performed in Cyprus, where it has become a routine procedure for those couples who cannot accept prenatal diagnosis and termination of pregnancy, as partially described elsewhere (Kuliev et al., 1999). At the present time, PGD for haemoglobin disorders represents more than a quarter (26.1%) of the overall experience of 753 PGD cycles performed for single gene disorders.

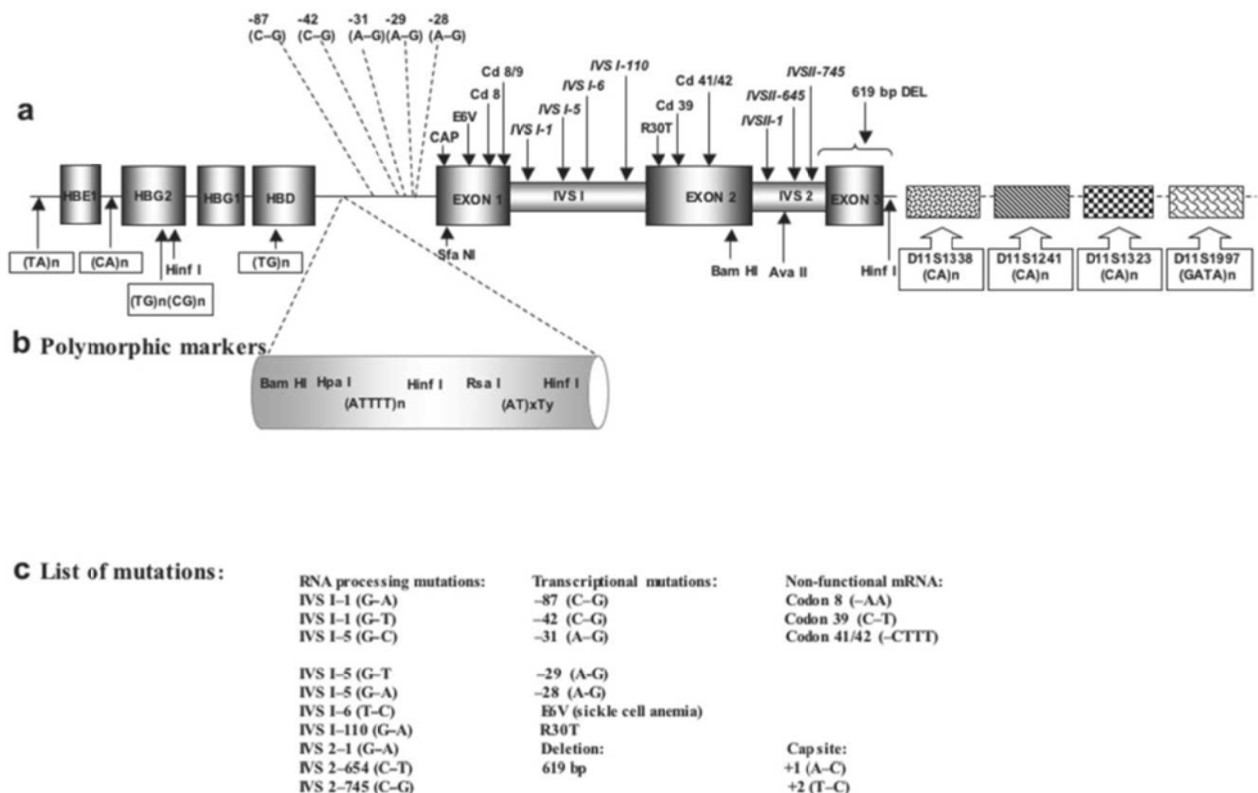

Figure 1. Mutations in β-globin gene for which preimplantation genetic diagnosis was performed and polymorphic markers used in multiplex polymerase chain reaction analysis

*(**a**) Map of human β-globin gene, showing sites and location of mutations.*

*(**b**) Linked polymorphic markers used for avoiding misdiagnosis. (**c**) List of mutations.*

Of 197 clinical cycles performed, unaffected embryos for transfer were available in 164 (83.3%) cycles, resulting in 53 (32.3%) clinical pregnancies and the birth of 45 healthy children, with five pregnancies still ongoing. Because the majority of cases, as mentioned, came from Cyprus, 74 (37.6%) were performed

for IVS I-110 mutation, which is the most common thalassaemia mutation in the Mediterranean region (Table 2).

Table 1.

Primers for preimplantation genetic diagnosis of α-thalassaemia

Gene /polymorphism	Upper primer	Lower primer	Annealing temperature
Hemoglobin-alpha locus 1; (HBA1), hemi-nested, or nested PCR	Outside: Alpha 1 5'AAATGGATGAGGACGGAGC3'	Alpha 4: 5'CACGCTTCCAATACGCTTAGTG3' Alpha C1: 5'TGAACTCCTGGACTTAAGTGA3'	62–45°C
	Outside: Alpha 5: 5'GGGTTGCGGGAGGTGTAGC3'	Alpha 6: 5'CCGCCCACTCAGACTTTATTC3'	
	Inside: Alpha 3: 5'CGCAGGAACTCGGTCGTCC3'	Alpha 4: 5' CACGCTTCCAATACGCTTAGTG 3'	60°C
	Inside: Alpha 7: 5'AAGCCACTGCCTGCTGGTG3'	Alpha 6: 5'CCGCCCACTCAGACTTTATTC3'	60°C
	Inside: Alpha 3: 5'CGCAGGAACTCGGTCGTCC3'	Alpha C1: 5'TGAACTCCTGGACTTAAGTGA3'	60°C
D16S3399, hemi-nested PCR	Outside: 5'GCACGATCCTAATTTTCATAATACA3'	5'GTTACAAGACTTCAAACACTACACA3'	62–45°C
	Inside: 5'GCACGATCCTAATTTTCATAATACA3'	5'FamAGAAAGTTCCCACAGTCCCAT3'	55°C
D16S521, hemi-nested PCR	Outside: 5'GGGCGACAGAGCGAGACTC3'	5'GCCTTACAAATGTCGTATTCACCAT3'	62–45°C
	Inside: 5'GGGCGACAGAGCGAGACTC3'	5'HexAACGGAGTTTACAACCAAAATGC3'	55°C
D16S423, hemi-nested PCR	Outside: 5'CTTCATACAAAACAGGCTTGAAAG3'	5'TTCTTTTTGTAGCATGTATGTGAAA3'	62–45°C
	Inside: 5'CTTCATACAAAACAGGCTTGAAAG3'	5'FamCTGTTTGCCTGCCTATTTGATA3'	55°C
D16S3134, hemi-nested PCR	Outside: 5'GTAACCCAGTCTTGACCAATGAG3'	5'GGGTGGCCAAGGTGTTTG3'	62–45°C
	Inside: 5'FamGGAAAGATGTCTCCTTCCTCATAG3'	5'GGGTGGCCAAGGTGTTTG3'	55°C
D16S3024, hemi-nested PCR	5'AAGACACTCCTTCATCCATATTTTG3'	5'AGGAGCCTGCTGGCAACA3'	62–45°C
	5'HexAAGTGGCTAAGTGGCCCCTT3'	5'AGGAGCCTGCTGGCAACA3'	55°C

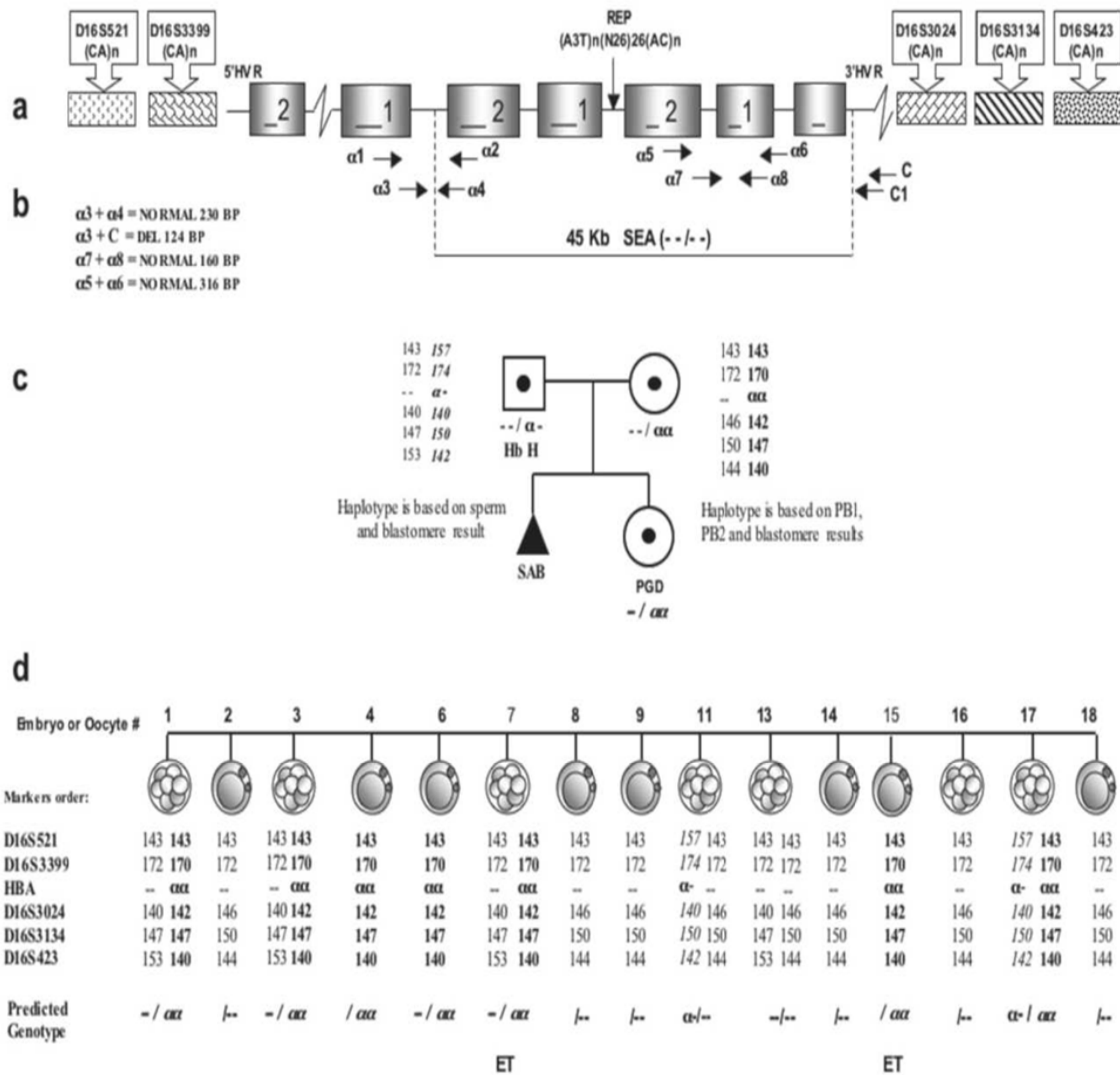

Figure 2. PGD for alpha-thalassaemia (SEA deletion)

*(**a**) Map of human α-globin gene, showing the position of 45 kb SEA deletion and polymorphic markers used in multiplex PCR analysis.*

*(**b**) Size of fragments.*

*(**c**) Family pedigree showing both parents carrying SEA deletion, the father also having haemaglobin H (HbH) disease; parental haplotypes are also shown, with paternal haplotypes obtained from sperm testing, and maternal haplotypes obtained from polar body (PB)1 and PB2 analysis. SAB = spontaneous abortion.*

*(**d**) Results of testing 15 oocytes and embryos showing the presence of seven with normal globin gene (normal allele shown in bold), of which two were transferred back to patients (ET = embryo transfer), resulting in birth of an unaffected child.*

Table 2.

Results of 197 PGD cycles for haemoglobin disorders and their clinical outcome

Mutation	No. of patients	No. of cycles	No. of transfers	No. of embryos transferred	Pregnancy	Birth / (ongoing pregnancy[a])
IVS I – 1	6	9	6	7	2	1
IVS I – 5	3	3	2	3	1	1
IVS I – 6	11	17	16	33	7	8 (1)
IVS I – 110	42	74	64	154	24	20 (1)
IVS II – 2	1	2	1	3	1	2
IVS II – 745	5	11	9	22	3	2
Codone 6	1	1	1	3	0	0
Codone 8	6	7	7	12	2	2
Codone 39	8	13	11	21	3	4
Codone 41/42	3	5	2	6	0	0
–29 (A–G)	1	1	1	1	0	0
–87	1	2	2	2	0	0
Cap 1	1	2	1	1	0	0
R 30 T	1	10	6	9	0	0
Deletion 619 bp	2	6	5	8	1	0 (1)
Deletion 45 kb HBB	1	1	0	0	0	0
HBS (sickle cell anaemia)	16	24	23	51	7	4 (1)
HBE	1	2	1	1	0	0
HBC	1	1	1	2	1	0 (1)
Alpha thalassaemia (45 kb deletion)	3	6	5	9	1	1
Total	114	197	164 (83.3%)	348	53 (32.3%)	45 (5)

[a] – *values in parentheses are ongoing pregnancies.*

While PGD cycles were mainly performed for heterozygous carriers, eight cycles were performed for three couples with homozygous or compound heterozygous male or female patients at 50% risk of producing an affected child. In these couples, PGD involved testing for three different mutations, while in the majority of cases two different mutations (97 cycles) or the same maternal and paternal mutation were analysed either simultaneously or in sequence by testing of the maternal mutation in PB1 and PB2, and the paternal mutation in blastomeres. In the case of a male affected partner, 25 embryos were available for testing, allowing preselection of nine unaffected embryos, and sufficient number of embryos for transfer, compared

with cycles with a female thalassaemic partner, in which blastomere biopsy resulted in preselection of no, or only a single unaffected embryo for transfer in each cycle, due to a limited number of oocytes obtained from thalassaemic females.

While mainly β-globin mutations were tested, α-thalassaemia mutations have been tested in six cycles performed for three couples. One of these cycles is presented in Figure 2, which is the world's first PGD for α-thalassaemia 45K deletion, resulting in a clinical pregnancy and birth of an unaffected child. Both parents were carriers of this mutation, with the father also having haemoglobin H disease. The couple had one previous pregnancy resulting in spontaneous abortion, caused by hydrops fetalis. To avoid misdiagnosis, haplotype analysis for five polymorphic markers was performed. Of 15 oocytes and embryos tested, eight were predicted to be affected, with the remaining seven carrying one copy of the normal α-globin gene, of which two (embryos 7 and 15) were transferred; the mutant embryos were subsequently confirmed to be affected, showing the reliability of the approach.

Of 54 PGD cycles performed simultaneously with HLA typing, 35 resulted in transfer of 55 unaffected HLA-matched embryos (this was partially reported previously by Rechitsky et al., 2004). Of a total of 466 embryos tested, 435 showed conclusive results, of which 135 (31%) were predicted to be abnormal and 300 (69%) unaffected carriers or normal (Table 3). The HLA typing revealed 84 (19%) embryos to be identical to the affected siblings, although only 65 (15%) were also unaffected, of which 55 developed appropriately to be acceptable for transfer in 35 PGD cycles, resulting in seven unaffected HLA-identical pregnancies. One of these pregnancies resulted in a stillbirth, the unaffected HLA-matched status of which was confirmed, and five in the birth of unaffected HLA-matched children, with one pregnancy still ongoing at this time. Umbilical cord blood was collected from these children at birth and transplanted to one of the affected siblings, resulting in successful haematopoietic reconstitution.

Table 3.

Combined β-globin and human leukocyte antigen DNA testing

Patient/cycle	No. embryos total/amplified	No. normal embryos		No. abnormal embryos		No. transfers/ no. embryos	Pregnancy/ birth[a]
		Non-match	Match	Non-match	Match		
27/54	466/435	235	65	116	19	35/55	7[b]/5 (1)

[a] – value in parentheses is number of ongoing pregnancies;
[b] – one pregnancy resulted in a stillbirth.

Three cycles involved simultaneous aneuploidy testing, one of which involved PGD for thalassaemia combined with HLA typing, performed due to advanced reproductive age of the mother, who carried the Cd8 mutation, and IVSI-1 in the father (Figure 3). The couple had one affected child requiring HLA identical bone marrow transplantation, so they requested PGD to conceive an unaffected child, who might be also a potential stem cell donor for the affected sibling. Of four embryos available for testing, only one appeared to be an unaffected carrier of Cd8, and also full HLA matched and euploid for chromosomes 13, 16, 18, 21 and 22. This embryo was transferred, resulting in birth of an unaffected HLA-matched child. Although the remaining three embryos were chromosomally normal, one was compound heterozygous Cd8/IVSI-1 affected and a paternal HLA mis-match, and the other two were either maternal or paternal HLA mis-match, despite being unaffected carriers of Cd8 or IVSI-1 mutations. In addition to testing for the most common age-related aneuploidies, the copy number of chromosomes 11 and 6 was routinely tested in PGD for β-thalassaemia combined with HLA typing, in the attempt to improve the accuracy of diagnosis. Of 435 embryos tested overall, 49 (11.3%) had abnormal copy numbers of these chromosomes, 38 (8.6%) of which were monosomies [15 with monosomy 6 (3.4%), 6 (1.4%) with monosomy 11, and 17 (3.9%) with double monosomies], equally of maternal (18 embryos) or paternal (20) origin (Table 4). Only nine (2.1%) of 435 embryos had trisomies, all of maternal origin, and two (0.5%) uniparental disomies were of maternal origin.

The information of the copy number of these chromosomes was essential for pre-selection of unaffected and HLA-identical embryos for transfer.

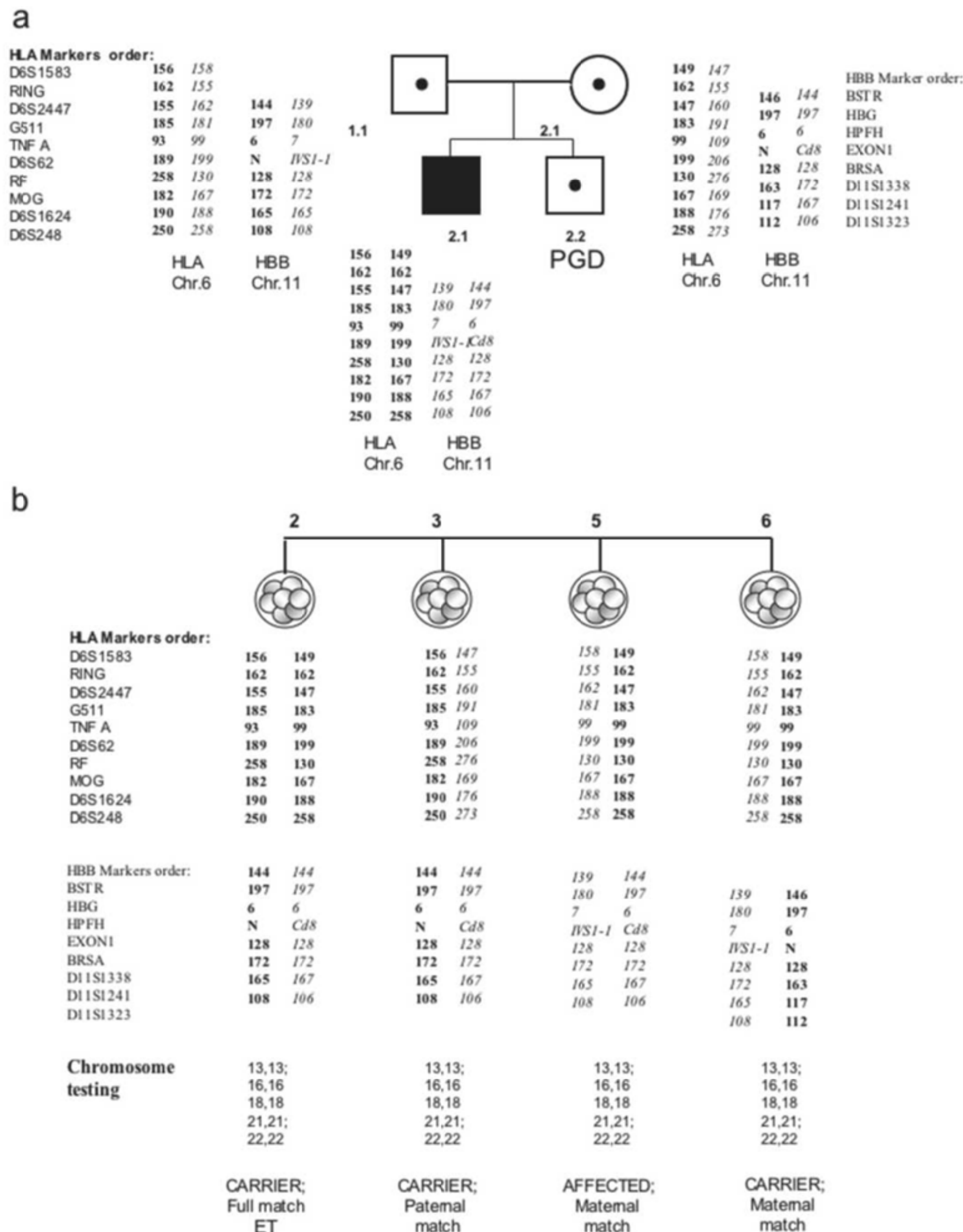

Figure 3. Preimplantation genetic diagnosis (PGD) with human leukocyte antigen (HLA) typing and aneuploidy testing for β thalassaemia

*(**a**) Family pedigree with HLA-haplotype analysis based on parental (1.1 and 1.2) and affected child's (2.1) genomic DNA testing. HLA markers order is presented on the upper left for father and upper right for mother. Paternal and maternal matching HLA haplotypes to the affected child (2.1) are shown in bold. Maternal and paternal mutations and the linked markers are also presented and shown in non-bold, while normal alleles and their linked markers are shown in bold. HBB = haemoglobin beta locus.*

*(**b**) Upper panel: HLA typing by short tandem repeats (STR) along with mutation analysis was performed on blastomeres from four embryos, one of which (no. 2) was predicted to be an HLA match to that of the affected sibling (2.1), although carrying the maternal mutation (also see below). Three others were not matched, one being only a paternal match (embryo no. 3), and the other two, only maternal match. Middle panel: maternal and paternal mutations and linked polymorphic markers, showing that, one embryo was affected (no. 5), two were carriers of maternal mutation Cd8 (nos 2 and 3) or one paternal (no. 6) mutations. As seen from HLA typing above (see upper panel), embryo no. 2 was also fully HLA matched to the sick sibling (**a**; 2.1). Bottom panel: Polymerase chain reaction-based aneuploidy testing for chromosomal 13, 16, 18, 21 and 22, showing the normal number of chromosomes in all four embryos. ET = embryo transfer.*

Table 4.
Detection of copy number of chromosomes 6 and 11 in preimplantation genetic diagnosis for β-thalassemia combined with human leukocyte antigen typing. Total number of embryos tested – 435

Abnormal	No. of embryos (%)	Parental origin	
		Maternal	Paternal
Monosomy 6	15 (3.4)	9	6
Monosomy 11	6 (1.4)	3	3
Monosomy 6 + 11	17 (3.9)	6	11
Subtotal monosomies	38 (8.6)	18	20
Trisomy 6	5 (1.1)	5	0
Trisomy 11	2 (0.5)	2	0
Trisomy 6 + 11	2 (0.5)	2	0
Subtotal trisomies	9 (2.1)	9	0
UPD	2 (0.5)	2[a]	0
Total	49 (11.3)	29	20

UPD = uniparental disomy;
[a] *– chromosome 6.*

Discussion

Haemoglobin disorders are presently among the most frequent indications for PGD. In some communities, PGD application is also due to the fact that prenatal diagnosis is not acceptable because of social and religious factors. As seen from the presented data of the largest series of 197 PGD cycles for haemoglobin disorders, 83.3% of these cycles resulted in transfer of at least two unaffected embryos, yielding a relatively high pregnancy rate of 32.3% and the birth of 38 unaffected children. The accuracy and reliability of PGD was achieved by using sequential PB1 and PB2 and embryo biopsy procedures, applying multiplex PCR analysis for a wide range of β-globin gene mutations and closely linked multiple markers, summarized in Figure 1, and also testing for copy number of chromosome 11 and chromosome 6, when combined with preimplantation HLA typing.

Presented data on PGD for α-thalassaemia may be of special relevance: now that the feasibility of PGD for this condition has been demonstrated, as described in Figure 2, PGD application may be expected to expand in South East Asia, where this condition is highly prevalent, leading not only to fetal death, but also to maternal mortality.

With current progress in treatment of haemoglobin disorders, PGD may have an increasing impact on the decision of patients whose disease is currently under control to reproduce. In fact, the life expectancy of patients with haemoglobin disorders has recently been dramatically improved with the success of radical treatment by stem cell transplantation (Lucarelli et al., 2002). The PGD strategy in such cases will depend on whether the ex-thalassaemic partners are males or females, because testing may be entirely based on oocytes if the male partner is affected, in contrast to embryo testing for female affected partners. However, the further impact of radical treatment of thalassaemia by bone marrow transplantation will depend on the availability of HLA-identical donors of stem cells. As seen from the presentation of the PGD experience of 54 cycles combined with HLA typing, it is presently feasible that couples at risk may utilize PGD to have thalassaemia-free children who may also be HLA identical to the affected siblings, to serve as potential donors for stem cells for transplantation treatment. At least in two such cases, cord blood stem cells were harvested from babies born after preimplantation HLA typing, and transplanted to the siblings, resulting in complete cure (Kahraman et al., 2004; Rechitsky et al., 2004).

Because the majority of couples requesting PGD for haemoglobin disorders combined with HLA typing may be of advanced reproductive age, contributing to a relatively low pregnancy rate, testing for aneuploidy in these cycles, simultaneously with mutation analysis and HLA typing, may improve the clinical outcome of the procedure. The data presented in this study show the feasibility of simultaneous testing for the above parameters in the same biopsied cell. The data also show the usefulness of obtaining information on the copy number of chromosome 11, in which the β-globin gene is located, and chromosomes 6, when HLA typing is performed. Although more data will be needed to understand the nature and origin of the detected abnormalities, currently available information suggests that the detection of possible abnormal copy numbers of these chromosomes may affect the appropriate selection of unaffected and HLA-identical embryos for transfer.

In conclusion, PGD for haemoglobin disorders has become a realistic option, available for wider application in those communities where these genetic diseases are highly prevalent. Combining PGD with HLA testing will also help in the improvement of thalassaemia therapy by stem cell transplantation.

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