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# Preimplantation diagnosis for immunodeficiencies

Dr Yury Verlinsky is a graduate, post-graduate and PhD of Kharkov University of the former USSR. His research interests include cytogenetics, embryology and prenatal and preimplantation genetics. He introduced polar body testing for preimplantation genetic diagnosis and developed the methods for karyotyping second polar body and individual blastomeres. He has published over 100 papers, as well as three books on preimplantation genetics.

*Dr Yury Verlinsky*

Yury Verlinsky, Svetlana Rechitsky, Tatyana Sharapova, Katya Laziuk, Irina Barsky, Oleg Verlinsky, Ilan Tur-Kaspa, Anver Kuliev[1]
Reproductive Genetics Institute, 2825 North Halsted Street, Chicago, IL 60657, USA
[1]Correspondence: e-mail: anverkuliev@hotmail.com

## Abstract

Preimplantation genetic diagnosis (PGD) has become an established procedure for the detection of single gene disorders, and has recently been performed together with human leukocyte antigen (HLA) typing for couples with children affected by genetic disorders that require HLA-identical stem cell transplantation therapy. For these couples, PGD can ensure the birth of an unaffected child, and because HLA-matched stem cell transplantation improves or completely restores the immune system, this child may also serve as a potential stem cell donor for affected siblings. This paper presents the first cumulative experience (18 cycles) of PGD for detection of the following immunodeficiencies: Wiscott–Aldrich syndrome, X-linked hyper-IgM syndrome (HIGM), X-linked hypohidrotic ectodermal dysplasia with immune deficiency (HED-ID), ataxia telangiectasia and Omenn syndrome, resulting in the transfer of unaffected embryos in 13 cycles and the birth of seven unaffected children, with one healthy pregnancy ongoing. HLA-identical stem cells from some of these children have been used for transplantation therapy, resulting in the restoration of normal function in siblings with HIGM and HED-ID.

***Keywords:*** *congenital immunodeficiencies, HLA-identical stem cell transplantation, preimplantation genetic diagnosis, preimplantation HLA typing*

## Introduction

Preimplantation genetic diagnosis (PGD) has been performed for more than 100 different conditions, resulting in the birth of at least 1000 healthy children free of genetic disorder (Verlinsky and Kuliev, 2006). PGD is presently also used together with preimplantation human leukocyte antigen (HLA) typing for treatment of affected siblings with genetic and acquired disorders, requiring HLA-matched stem cell transplantation (Verlinsky *et al.*, 2001, 2004; Kuliev and Verlinsky, 2004; Rechitsky *et al.*, 2004; Van deVelde *et al.*, 2004; Kahraman *et al.*, 2005; Kuliev *et al.*, 2005). The first successful treatment using this approach was achieved for Fanconi anaemia (Verlinsky *et al.*, 2001); subsequently, the procedure has been used for siblings with Blackfan–Diamond anaemia (DBA) and thalassaemia (Verlinsky *et al.*, 2004; Kahraman *et al.*, 2005; Kuliev *et al.*, 2005).

PGD is an obvious way to avoid inherited forms of severe combined immunodeficiency (SCID), and because HLA-matched stem cell transplantation improves or completely restores the immune system PGD can ensure the birth of unaffected children who may then also serve as potential stem cell donors for affected siblings. The feasibility of PGD has been previously demonstrated for a few cases of SCID, such as Wiscott–Aldrich syndrome (WAS), X-linked hyper-IgM syndrome (HIGM), and X-linked hypohidrotic ectodermal dysplasia with immune deficiency (HED-ID) (Rechitsky *et al.*, 2004). This study describes the accumulated experience of PGD for SCID, including PGD for ataxia telangiectasia (AT), and Omenn syndrome (OMS) and additional cases of PGD for WAS, X-linked HIGM, and X-linked HED-ID. While the latter three conditions have been described in detail earlier (Rechitsky *et al.*, 2004), brief descriptions of AT and OMS are presented below.

Both AT and OMS are extremely rare autosomal recessive diseases with a prevalence of 1 in over 50,000, for which there is still no available cure, other than stem cell transplantation. OMS is an early-onset fatal immunodeficiency with the absence of B cells and excess production of highly restricted T lymphocytes. It is caused by mutation in recombinase-activating genes *RAG1* and *RAG2* located on chromosome 11p, coding the lymphoid specific proteins responsible for the process of variable, diversity and joining [V(D)J] segment recombination required for generation of the T- and B-cell repertoire (MIM 603554; www.ncbi.nlm.nih.gov/omim). This severe primary immunodeficiency disease is characterized by generalized erythroderma, protracted diarrhoea, repeated infections, hepatomegaly and leukocytosis with eosinophilia and elevated immunoglobulin E. The wide phenotypic variability of patients may be determined by different mutations in *RAG1* and *RAG2* genes, involving missense and splice mutations or deletions. Despite specific therapy for dermatitis and lymphadenitis, using immunosuppression or replacement therapy with intravenous immunoglobulins, persistent viral and bacterial infections and chronic diarrhoea resulting in inanition may be responsible for death, with the only cure being HLA-identical bone marrow transplantation.

AT is a progressive, neurodegenerative childhood disease that affects the brain and other body systems (MIM 208900; www.ncbi.nlm.nih.gov/omim). A weakened immune system makes patients susceptible to recurrent respiratory infections. The disease presents at between 1 and 4 years of age, as delayed development of motor skills, poor balance and slurred speech. Telangiectasias appear in the corners of the eyes or on the surface of the ears and cheeks. Patients with AT may develop cancer, such as acute lymphocytic leukaemia or lymphoma. Other features may include mild diabetes mellitus, premature greying of the hair, difficulty in swallowing, and delayed physical and sexual development. Although the currently used symptomatic and supportive treatment, including high-dose vitamin regimens, physical and occupational therapy and gamma-globulin injections to supplement a weakened immune system, may be helpful, the prognosis is very poor, with many patients dying in their teens. More than 500 unique mutations are known in the ATM gene associated with AT, resulting in the absence of serine protein kinase coded by the ATM gene located on chromosome 11q22.3 (MIM 607585; www.ncbi.nlm.nih.gov/omim). Sequence analysis detects as much as 90% of mutations, and others may be identified by linkage analysis, which is extremely accurate, based on testing for intragenic markers presently available.

This paper presents the first cumulative experience of PGD for SCID, performed together with preimplantation HLA typing in families with previous siblings requiring HLA-identical bone marrow transplantation, resulting in unaffected pregnancies and birth of healthy children free of SCID.

## Materials and methods

A total of 18 PGD cycles for 10 couples who had produced progeny with the above conditions were performed, including cycles for HIGM, 3 for AT, 2 for WAS, 6 for HED-ID and 1 for OMS (**Table 1**). While, as mentioned, the details of PGD for HIGM, HED-ID and WAS, as well as the methods for preimplantation HLA typing, have been described earlier (Rechitsky *et al.*, 2004), PGD for AT and OMS will be presented in more detail.

A couple at risk of producing a child with OMS had two previous children, including a younger daughter with severe OMS resulting in death (**Figure 1**). The child was double heterozygous, with the inherited paternal R396C mutation, representing a sequence change from arginine to cysteine at amino acid position 396, caused by a single C→T sequence change (CGG→TGG) in codon 396 of the *RAG1* gene, and maternal mutation c.256_57delAA within the lysine 86 codon, causing a frameshift mutation that results in a premature termination signal at 32 codons downstream. The older heterozygous unaffected daughter inherited only the paternal A396C mutation in the *RAG1* gene.

The paternal mutation was tested by *Aci*I digestion, which creates two fragments of 64 and 109 bp in the polymerase chain reaction (PCR) product of normal *RAG1*, leaving the mutant allele uncut. The two 'A' nucleotide deletion in codon 86 of the maternal allele of *RAG1* was detected by capillary electrophoresis of the fluorescent-labelled PCR product, visualizing the 114-bp fragment versus the 116-bp fragments in the normal allele (**Figure 2**).

The couple at risk of producing a child with AT had one affected child who died in early infancy and one spontaneous abortion (**Figure 3**). The mother was a carrier of two ATM sequence changes, involving exon 38 (5419A→G, K1807E), and exon 48 (6784G→C, A2262P) (**Figure 4**). As it is not known which of these two mutations is responsible for AT, both were tested in polar body (PB)1 and PB2 and blastomeres. In all cases, a single blastomere was biopsied, using a mechanical method (Verlinsky and Kuliev, 2005). As shown in **Figure 4**, maternal mutation K1807E was identified by *Bsm*AI digestion, creating two fragments of 96 and 92 bp in the PCR product of the mutant gene. In contrast, maternal mutation A2262P was not cut by *Hae*III restriction digestion, but created two fragments of 35 and 99 bp in the normal gene.

The paternal mutation in the ATM gene was not identified, and had to be traced using four closely linked markers, listed in **Figure 3A**. Sequential analysis of PB1 and PB2 removed following maturation and fertilization of oocytes (Verlinsky and Kuliev, 2005) was therefore performed to identify the mutation-free oocytes, as shown in **Figure 4IIB**, and the resulting embryos then tested for paternal mutation by linkage analysis, simultaneously with testing for aneuploidy (**Figure 3C**).

In addition to the couple with HIGM with C218X mutation in exon 5 of CD40 ligand gene (CD40LG) described previously (Rechitsky *et al.*, 2004), PGD for three more couples with maternal mutations C218X exon 4 c.437_38insA, and exon 4 c.397insT were tested using the primers listed in **Table 2**. The maternal mutations were analysed by PB1 and PB2, followed by HLA and aneuploidy testing in biopsied blastomeres.

As the testing of a couple at risk for producing a progeny with HED-ID with L153R mutation has been described previously (Rechitsky *et al.*, 2004), **Table 2** presents only primers for testing of the couples with the maternal mutations Q348X and D113N. The method of choice for mutation testing was again

**Table 1.** Results and outcomes of preimplantation genetic diagnosis for immunodeficiencies.

| *Disease/gene/mutations* | *Patient/ cycle* | *Cell type tested* | *No. of embryos total/ amplified* | *No. of normal embryos* | *No. of abnormal embryos* | *No. embryos transferred/ /no. of embryos* | *Pregnancy/ birth* |
|---|---|---|---|---|---|---|---|
| HIGM/TNFSF (CD40L) gene/C218X, exon 4 c.437–438insA, exon 4 c.397insT | 4/6 | PB and BL; BL only | 46/43 | 31 | 12 | 6/9 | 2/2 |
| OMS/RAG1 gene/R 396C; 1/1 L86AAdel | PB and BL | 5/5 | 3 | 2 | 1/2 | 1/2 | |
| HED-ID/IKBKG(NEMO) gene/D113N; Q348X; L153R | 2/6 | PB and BL | 36/35 | 24 | 11 | 3/4 | 1/1 |
| AT/ATM gene/K1807E; A2262P; unknown | 1/3 | PB and BL | 20/20 | 10 | 10 | 2/4 | 1/1 |
| WAS/WASP gene/L39P; Nt. 361 (-2) A→→G | 2/2[a] | PB and BL; BL only | 10/10 | 6 | 4 | 1/2 | 1/1 |
| Total | 10/18 | – | 117/113 | 74 | 39 | 13/21 | 6/7[a] |

HIGM: X-linked hyper-IgM syndrome; OMS: Omenn syndrome; HED-ID: X-linked hypohidrotic ectodermal dysplasia with immune deficiency; AT: ataxia telangiectasia; WAS: Wiscott–Aldrich syndrome; PB: polar bodies; BL: blastocyst.
[a]One ongoing pregnancy.

sequential PB1 and PB2 analysis, followed by aneuploidy and HLA typing in an embryo biopsy.

Haplotype analysis of the father, mother and affected child was performed for each family prior to preimplantation HLA typing. This allowed the detection and avoidance of misdiagnosis due to preferential amplification and allele drop-out (ADO), thereby improving the diagnostic accuracy of PGD and HLA typing. A multiplex heminested PCR was used, with the first round of PCR requiring similar annealing temperatures for the outside primers.

Aneuploidy testing was performed in patients of advanced reproductive age by adding primers for chromosome specific microsatellite markers to the multiplex PCR protocols worked out for a specific genetic disorder or HLA typing, as described elsewhere (Rechitsky *et al.*, 2006).

Informed consent was given by the patients involved in the study. This was based on the Institutional Review Board criteria that unaffected HLA embryos would be preselected for transfer back to the patients, while unaffected but non-HLA-matched embryos would be frozen for future possible use by the couple. Mutant embryos would undergo confirmatory analysis using their genomic DNA to evaluate the accuracy of the single cell based PGD.

## Results

The results of PGD for immunodeficiencies are presented in **Table 1** and **Figures 1–4**. Of 117 oocytes and embryos tested in 18 PGD cycles performed for 10 couples carrying the abovementioned mutations, 74 unaffected embryos were detected, of which only 21 were acceptable for transfer in 13 of 18 PGD cycles, yielding six unaffected pregnancies (26.6% per transfer) and the birth of seven healthy children, with one pregnancy still ongoing. The low average number of embryos transferred in these cycles (1.6 embryos per transfer) is due to the simultaneous preselection of HLA type, performed in 12 of 18 cycles (one for WAS, five for HIGM and six for HED IP), resulting in only 13 unaffected HLA-identical embryos transferred (e.g. a single embryo transfer on average). Overall, 76 embryos were tested in combination with HLA typing, 13 (17.1%) of which were identified to be unaffected and HLA-identical to siblings. Twelve of these embryos were transferred in 9 cycles, resulting in the birth of two healthy HLA-identical children, whose stem cells were transferred to the siblings with HIGM and HED IP, resulting in the restoration of normal function in the affected siblings.

The other factor affecting the number of transferred embryos per cycle was simultaneous aneuploidy testing performed in 7 of 18 cycles, including 3 PGD cycles for HIGM with HLA typing, 1 for OMS, and 3 for AT (**Figures 1** and **3**). Overall, 45 embryos were tested for aneuploidy, of which 11 were found to be aneuploid, including four affected and seven unaffected embryos. Double monosomy 18 and 22 is shown in **Figure 3** (embryo 10), illustrating the results of PGD for AT, performed by sequential PB1, PB2 and blastomere analysis, which resulted in birth of an AT-free child. As seen from **Figure 1**, no embryos with aneuploidy were detected in PGD for OMS, allowing the transfer of two unaffected, aneuploidy free embryos, which resulted in the birth of healthy twins, one mutation free, and the other an unaffected carrier of the paternal mutation (**Figure 1**).

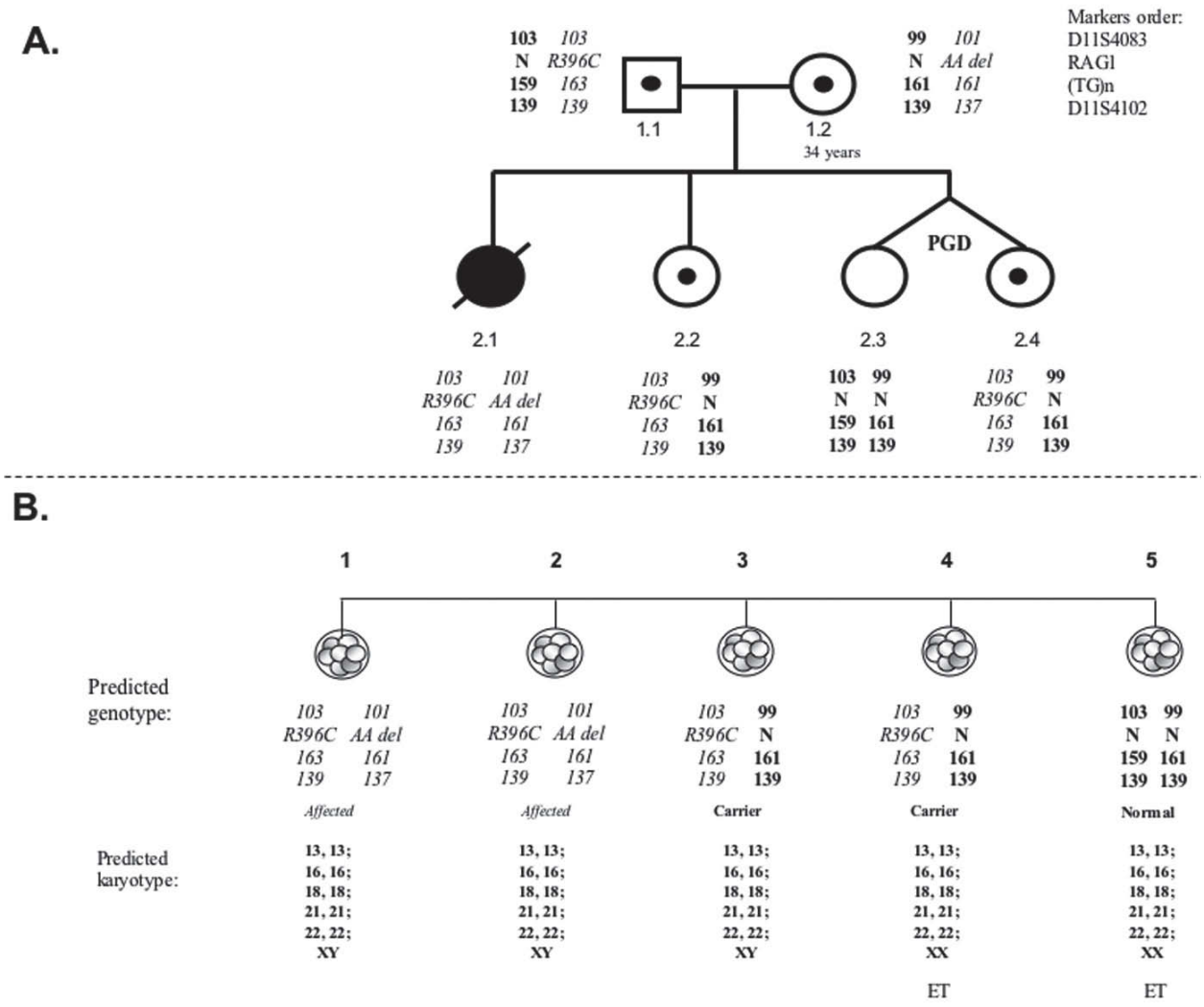


**Figure 1.** Preimplantation genetic diagnosis (PGD) for Omenn syndrome with aneuploidy testing. (**A**) Family pedigree with mutation and haplotype analysis of parents (1.1 and 1.2) and children (2.1, affected; and 2.2, healthy heterozygous carrier of paternal mutation). (**B**) Top: blastomere analysis involving mutation analysis of five embryos, including two affected (embryos 1 and 2), two carriers of paternal mutation (embryos 3 and 4) and one free of both paternal and maternal mutations (embryo 5). Bottom: blastomere analysis for aneuploidy, showing normal chromosomal sets for all five embryos, two of which were transferred (embryos 4 and 5) resulting in birth of healthy twins (2.3, normal, and 2.4, heterozygous carrier of the paternal mutation). ET = embryo transfer.

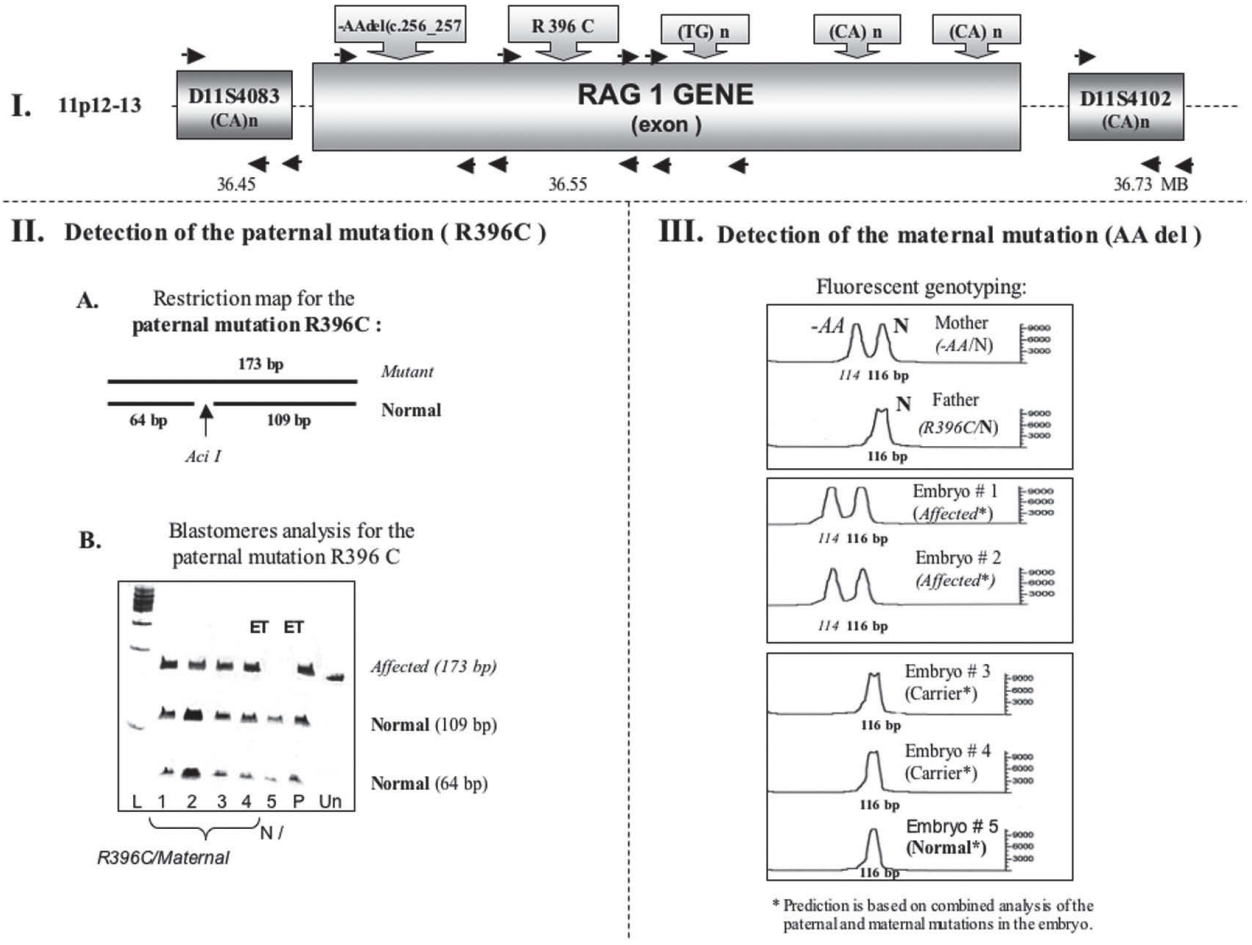


**Figure 2.** Preimplantation genetic diagnosis (PGD) for Omenn syndrome by blastomere analysis of paternal and maternal mutations. (**I**) Position of parental mutations and informative linked polymorphic markers used in PGD. (**IIA**) Restriction map of the paternal mutation R396C, following *Aci*I digestion, creating two fragments of 64 and 109 bp in polymerase chain reaction (PCR) product of the normal gene. (**IIB**) Polyacrylamide gel electropherogram of the Aci I–digested PCR products of biopsied blastomere from five embryos for the paternal mutation R396C, showing that only embryo 5 was free of paternal mutation, while the remaining embryos contained the paternal mutation. (III) Capillary electropherogram of fluorescently labelled PCR products of RAG1 gene obtained from mother, father and five embryos, showing normal fragment of 116 bp for father, two fragments of 114 pp (AAdel) and 116 bp for mother, same two fragments, including AAdel, for embryos 1 and 2, and one normal 116 bp fragment for embryos numbers 3, 4 and 5, the latter two of which were transferred, resulting in the birth of healthy twins.

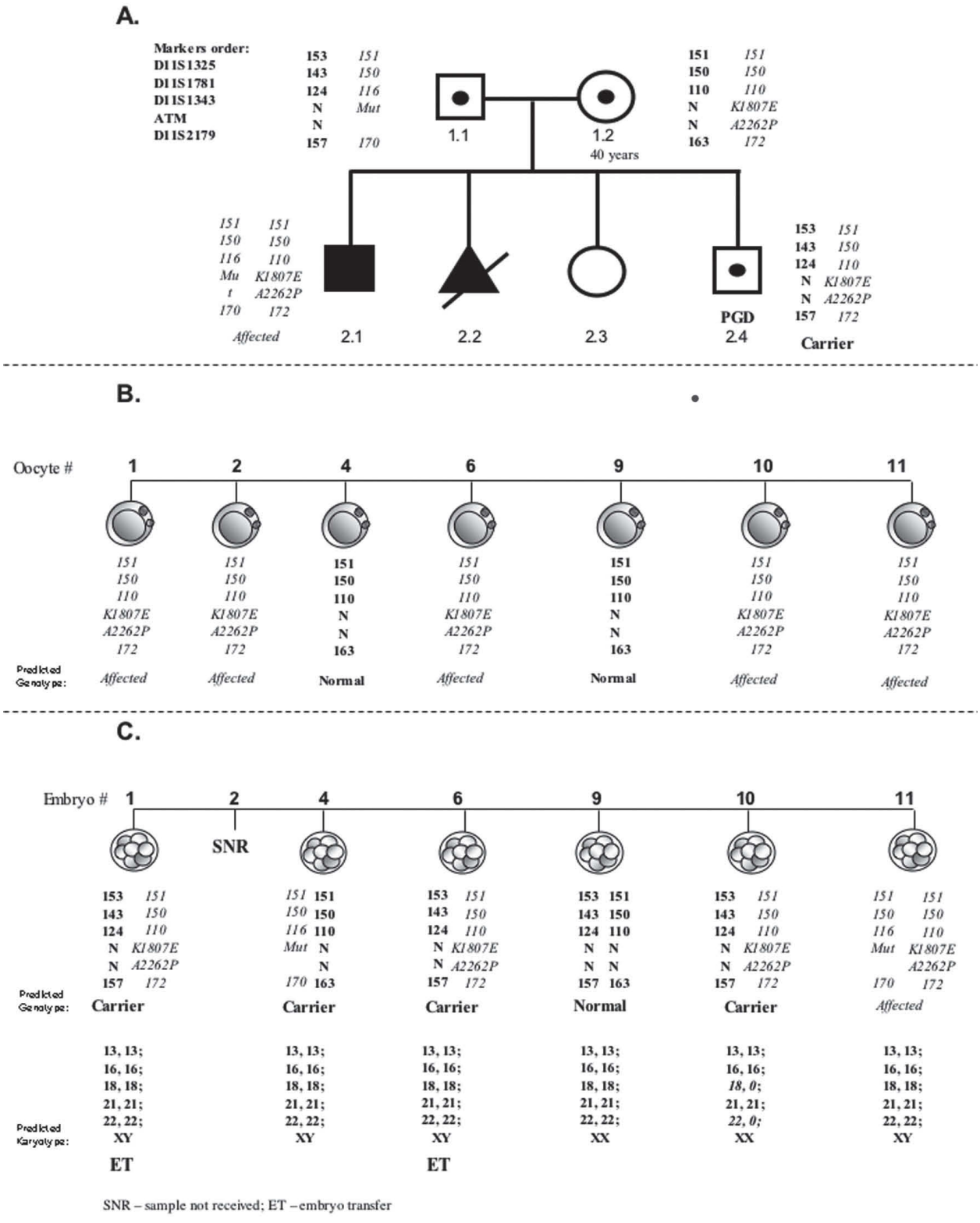


**Figure 3.** Preimplantation genetic diagnosis (PGD) for ataxia telangiectasia with aneuploidy testing. (**A**) Family pedigree showing the results of mutation and haplotype analysis in parents (1.1 and 1.2) and affected child (2.1). (**B**) Results of polar body analysis of seven oocytes, only two of which (oocytes 4 and 9) were free of mutation, based on mutation and marker analysis. The remaining five oocytes were affected, containing both maternal mutations tested. (**C**) Upper panel: results of mutation and linked marker analysis of six embryos originating from the above oocytes (no sample was available from the embryo originating from oocyte 2). Five of these six embryos were either normal (embryo 9) or carriers (embryos 1, 4, 6 and 10), while the remaining embryo (embryo 11) was affected, inheriting both maternal and paternal mutations. Bottom panel: results of aneuploidy testing for chromosomes 13, 16, 18, 21, 22, X and Y, showing one double monosomy 18 and 22 in heterozygous unaffected embryo number 10.

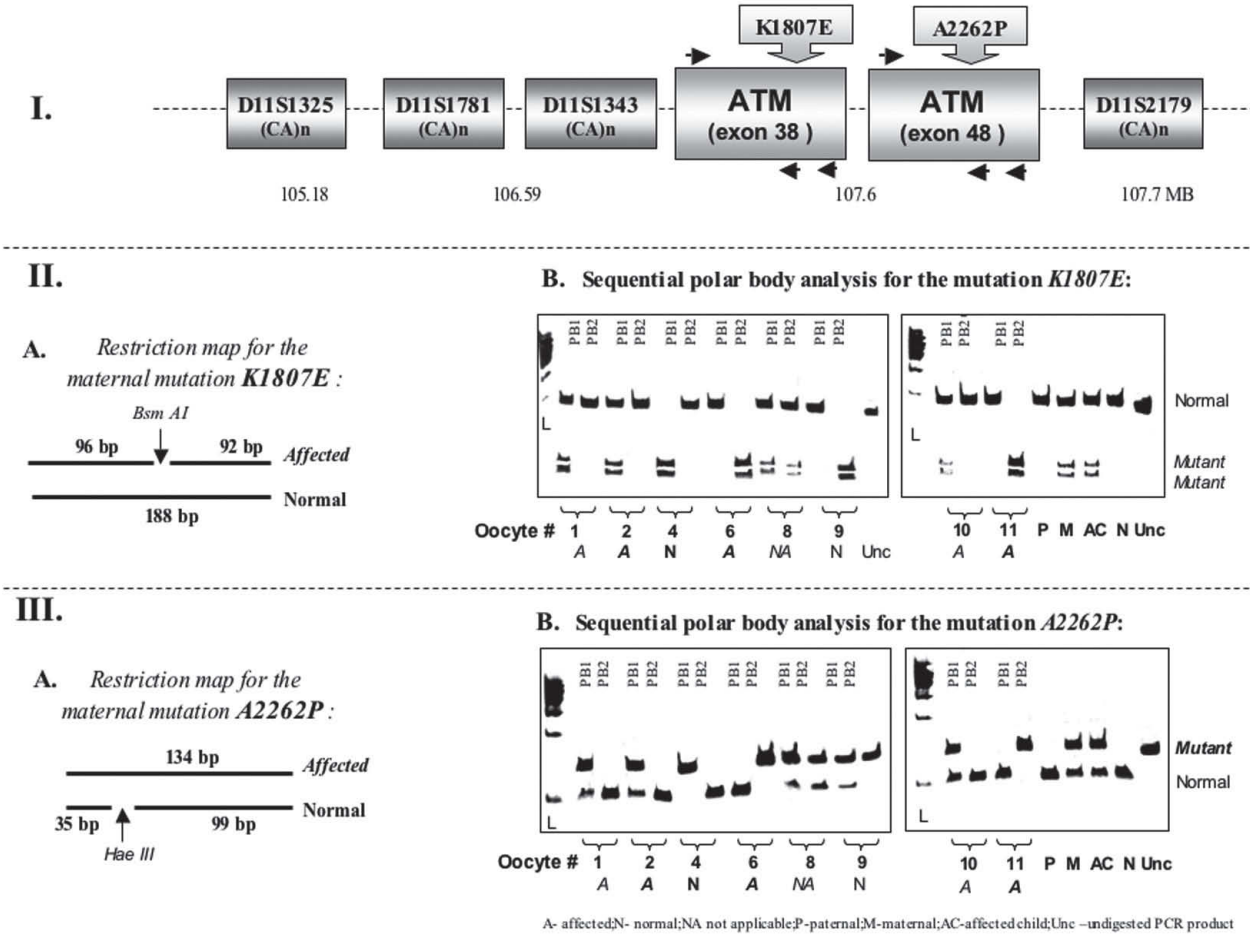


**Figure 4.** Preimplantation genetic diagnosis (PGD) for ataxia telangiectasia (AT) by sequential polar body 1 (PB1) and PB2 analysis of maternal mutations. (**I**) Position of two different mutations and informative linked polymorphic markers used in PGD. (**IIA**) Restriction map of maternal mutation K1807E, following *Bsm*AI digestion, which creates two fragments in the polymerase chain reaction (PCR) product of the mutant gene. (**IIB**) Polyacrylamide gel electropherogram of *Bsm*AI–digested PCR products of PB1 and PB2 from eight oocytes, of which only two (oocytes 4 and 9) were free of maternal mutation. Five oocytes were mutant, and one with the heterozygous status of both PB1 and PB2, excluded from the further study, due to possible DNA contamination. (**IIIA**) Restriction maps for the maternal mutation A2262P following *Hae*III restriction digestion, which creates two fragments of 35 and 99 bp in normal allele, leaving the mutant allele uncut. (**IIIB**) The polyacrylamide gel electropherogram of the *Hae*III-digested PCR products of PB1 and PB2 from eight oocytes, of which two (oocytes 4 and 9) were free of maternal mutation, five oocytes mutant (oocytes 1, 2, 6, 10 and 11), and one with the heterozygous status of both PB1 and PB2, excluded from the further study, due to possible DNA contamination, as in the analysis for K1807E mutation.

**Table 2**. Primers and reaction conditions for PGD of immunodeficiencies.

| *Gene/polymorphism* | *Accession no.* | *Hetero-zygosity index* | *No. of alleles* | | *Upper primer* | *Lower primer* | *Annealing temp (ºC)* |
|---|---|---|---|---|---|---|---|
| ATMK1807E | AH004875 | NA | NA | Outside | 5'AGTTTTTAGAAGTACCCAGATTTGA3' | 5'TAGATAAACAGGTCATAAACAAGGA3' | 62–45 |
| (Heminested) *Bsm*AI | | | | Inside | 5'AGTTTTTAGAAGTACCCAGATTTGA3' | 5'TCTTCTTACTTCACACATTGGCT3' | 55 |
| cuts mutant sequence | | | | | | | |
| ATMA2262P | AH004875 | NA | NA | Outside | 5'AAGGAAATGGACAACTCACAAAG3' | 5'CCCTCAGGCTTTCTGTTTTTTA3' | 62–45 |
| (Heminested) *Hae*III | | | | Inside | 5'TCTCACCAAACACCTTGTAGAACTC3' | 5'CCCTCAGGCTTTCTGTTTTTTA3' | 55 |
| cuts normal sequence | | | | | | | |
| D11S1325 | Z23828 | 0.52 | 3 | Outside | 5'AACATCAAATGGTTCTTGCTTC3' | 5'TTTTATCTCTTTTTCAATACAATGC3' | 62–45 |
| (Heminested) | | | | Inside | 5'AACATCAAATGGTTCTTGCTTC3' | 5'*Fam*GGGATTCTGCTTTTTTCCTTTA3' | 55 |
| D11S1781 | Z52108 | 0.34 | 4 | Outside | 5'GGGGATGAGTAATGATATAAGACAA3' | 5'ACTTCTACTGTGTATATTTACGGCA3' | 62–45 |
| (Heminested) | | | | Inside | 5'GGGGATGAGTAATGATATAAGACAA3' | 5'*Fam*CGGCATATAACATAGTGTTATTTTG3' | 55 |
| D11S1343 | Z24175 | 0.56 | 5 | Outside | 5'CTCCTTCCCAAACAATCCACT3' | 5'CCTGGTTCATGTAGCAGTTCCT3' | 62–45 |
| (Heminested) | | | | Inside | 5'CTCCTTCCCAAACAATCCACT3' | 5'*Fam*CCCCTACTGTTTTATGACCCA3' | 55 |
| D11S2179 | AF119249 | NA | 8 | Outside | 5'CTCCTCATTCTAAACAACAACTG3' | 5'GCTTGCAACATCTACTATATATTTT3' | 62–45 |
| (Heminested) | | | | Inside | 5'*Fam*TTCTCTTCTATGAATATAACAGGAG3' | 5'GCTTGCAACATCTACTATATATTTT3' | 55 |
| RAGI R396C | NT_009237 | NA | NA | Outside | 5'CCACATCTCAAGTCACAAGGAA3' | 5'GCCAGCAGGAACAAGGTCAT3' | 62–45 |
| (Heminested) *Aci*I | | | | Inside | 5'CCACATCTCAAGTCACAAGGAA3' | 5'ACTTCACATCTCCACCTTCTTCT3' | 55 |
| cuts normal sequence | | | | | | | |
| RAG1 c.256_57 | NT_009237 | NA | NA | Outside | 5'GAAACCCTCTCTGGAGCAATCT3' | 5'GCTCTAAAAGAATTCCCACAGA3' | 62–45 |
| del AA (Nested) | | | | Inside | 5'ACAAGGCTGATGGTCAGAAG3' | 5'*Fam*TTGGCTTGATGGATCGCTT3' | 55 |
| D11S4083 | Z52164 | 0.87 | 15 | Outside | 5'GGACTCTTGGAACTCTGGACT3' | 5'TTGGGGATCATGTGTACCC3' | 62–45 |
| (Heminested) | | | | Inside | 5'GGACTCTTGGAACTCTGGACT3' | 5'*Fam*AGGGCAGAGTATTTACAAAGAAG3' | 55 |
| RAG1 (TG)n | NT_009237 | NA | 4 | Outside | 5'AGAAGTTTGTTGGTTTCATTTA3' | 5'GTATCCAGCAGAGTGCCTAGT3' | 62–45 |
| (Heminested) | | | | Inside | 5'*Fam*CCTTGCTTCTTTAGTTGCTTT3' | 5'GTATCCAGCAGAGTGCCTAGT3' | 55 |
| D11S4102 | Z52543 | 0.77 | 10 | Outside | 5'ATCCTCACCTTATTCACCCTG3' | 5'AATCCTGGAAAGCCCTGG3' | 62–45 |
| (Heminested) | | | | Inside | 5'ATCCTCACCTTATTCACCCTG3' | 5'*Hex*TAGGGATTTTAGGAGGGATGACT3' | 55 |

*Continued on page 222*

**Table 2**. *continued from page 221.*

| *Gene/polymorphism* | *Accession no.* | *Hetero-zygosity index* | *No. of alleles* | | *Upper primer* | *Lower primer* | *Annealing temp (°C)* |
|---|---|---|---|---|---|---|---|
| WAS Nt.361 A--G | AF115548, | NA | NA | Outside | 5'AGGAGATGGGAAAGTTGCGG3' | 5'CCAACTTCCTTTCCTCCCCTG3' | 62–45 |
| (Heminested) | AF115549 | | | Inside | 5'AGGAGATGGGAAAGTTGCGG3' | 5'GATTCCTTTTTTGTATCTTCTCCTG3' | 55 |
| WAS L39P | AF115548, | NA | NA | Outside | 5'TCAGCAGAACATACCCTCC3' | 5'AGAGAGAGAAGGAGGAGAGG3' | 62–45 |
| (Heminested) *ScrFI* | AF115549 | | | Inside | 5'TCAGCAGAACATACCCTCC3' | 5'GAAGAAACGGTGGGGAC3' | 55 |
| cuts mutant sequence | | | | | | | |
| DXS1003 | Z17201 | 0.8 | 11 | Outside | 5'AGAAGCCGTTATTGGTGGACTC3' | 5'ACACTGCTACTCCTTGGGAAATC3' | 62–45 |
| (Heminested) | | | | Inside | 5'AGAAGCCGTTATTGGTGGACTC3' | 5'*Hex*CATTCCTCACTGGCAAGTTTTA3' | 55 |
| GATA160B08 | G10694 | 0.71 | 5 | Outside | 5'CCAATTGCCTACTGGATATTACCAA3' | 5'TGGGAACAAAACAGGCAAAGTC3' | 62–45 |
| (Heminested) | | | | Inside | 5'CCAATTGCCTACTGGATATTACCAA3' | 5'*Fam*TTTGCCCTCATGGAGTGCC3' | 55 |
| DXS1208 | Z23944 | 0.54 | 8 | Outside | 5'TCTAAAGCCCAGGACCCCG3' | 5'TGGTTAAAGGATTTGGGAGGC3' | 62–45 |
| (Heminested) | | | | Inside | 5'*Hex*TCAGGGCTCCAACTCCAGG3' | 5'TGGTTAAAGGATTTGGGAGGC3' | 55 |
| DXS1039 | Z23372 | 0.56 | 9 | Outside | 5'CCCTCTTCACTTTTCCAGTCAAT3' | 5'GGAAAGGGAAGAAGAATGCC3' | 62–45 |
| (Heminested) | | | | Inside | 5'*Fam*TGTTCCTGGTATGTGACAATGC3' | 5'GGAAAGGGAAGAAGAATGCC3' | 55 |
| DXS8023 | Z52342 | 0.57 | 9 | Outside | 5'GTGCAAACTGTTCCACCTGG3' | 5'CTCAAAGAATGAAGTAGAATAAGGATA3' | 62–45 |
| (Heminested) | | | | Inside | 5'GTGCAAACTGTTCCACCTGG3' | 5'*Fam*TTGATAAAGTAGTCAGGAAAGGCT3' | 55 |
| HED-ID (IKBKG | NT_025965 | NA | NA | Outside | 5'GGGAGTACAGCAAACTGAAGGC3' | 5'CCCTAACCCAGAACACCAGG3' | 62–45 |
| gene), Q348X *Hpy188* III | | | | Inside | 5'GGGAGTACAGCAAACTGAAGGC3' | 5'CCATCCGTCTCCTGTGGTC3' | 55 |
| cuts normal sequence | | | | | | | |
| HED-ID (IKBKG | NT_025965 | NA | NA | Outside | 5'AGGAGTTCCTCATGTGCAAGTT3' | 5'CCTTGTGGAACACTGGCG3' | 62–45 |
| gene), D113N *Mbo*I | | | | Inside | 5'AGGAGTTCCTCATGTGCAAGTT3' | 5'GTTTTCAGAACCTGGCCCTG3' | 55 |
| cuts normal sequence | | | | | | | |
| TNFSF (CD40 gene), | D31796 | NA | NA | Outside | 5'TTTGGTTCCATTTCAGGTGATC3' | 5'AACATGACTTCGGCATCCCA3' | 62–45 |
| Exon4C. | | | | Inside | 5'TTTGGTTCCATTTCAGGTGATC3' | 5'*Fam*CGCTCAGATGCTGTGTGACTTAC3' | 55 |
| 397insT, Exon4C. | | | | | | | |
| 437_38insA, (Heminested) | | | | | | | |

## Discussion

The presented data show the usefulness of PGD for SCID, as there is no effective treatment for these conditions other than stem cell transplantation. PGD provides couples at risk with the option of avoiding an affected pregnancy and having progeny free of SCID. In cases where there is already an affected child in the family, PGD with HLA typing also provides the possibility of having access to HLA-identical stem cell transplantation through selection and transfer of those unaffected embryos which are also HLA-matched to the sibling. Because selection of the HLA-identical stem cell donor is the key for achieving success in stem cell transplantation (Gaziev and Lucarelli, 2005), the restoration of normal function was observed in both cases of stem cell transplantation in siblings with HIGM and HED-ID.

The data further demonstrate the utility of detection and avoidance of transfer of chromosomally abnormal embryos, destined to be lost before or after implantation. Although simultaneous testing for the causative gene and aneuploidy with or without HLA typing reduces the average number of the embryos available for transfer (Rechitsky *et al.*, 2006), the transfer of even a single embryo appeared to result in an acceptable pregnancy outcome. Overall, embryo transfer was possible in 13 of 18 cycles, resulting in the birth of six unaffected children, including two who were suitable HLA-identical donors for affected siblings.

The presented case of OMS represents the first report of PGD for this condition; PGD for AT has been reported earlier (Hellani *et al.*, 2002). This earlier case was performed for a Saudi family with three affected children caused by a large deletion of more than two-thirds of the AT gene, using amplification of one of the deleted exons (exon 19). Of three embryos available for biopsy and testing, one was deletion-free and was transferred, resulting in an unaffected pregnancy. In the present case, the couple had one affected child who died in early infancy and one pregnancy that resulted in a spontaneous abortion. The mother was a carrier of two ATM sequence changes, involving exon 38 (5419A→G, K1807E), and exon 48 (6784G→C, A2262P), while the father's mutation was not identified. A sequential PB1 and PB2 analysis was performed to identify mutation-free oocytes, followed by testing of the resulting embryos for paternal mutation by linkage analysis, and aneuploidy. As shown in **Figure 3**, the transfer of two unaffected carrier embryos resulted in a singleton pregnancy and birth of healthy baby girl, confirmed to be an unaffected carrier of maternal mutations.

PGD for OMS was also performed together with aneuploidy testing, using PB1 and PB2 and blastomere analysis, and resulted in transfer of two unaffected aneuploidy-free embryos, resulting in the birth of healthy twins. Because in this case, as well as in the case of AT, the affected siblings died early in childhood, there was no need for HLA typing, but couples with previous AT and OMS children will definitely be potential candidates for PGD with HLA typing, with the aim of providing an identical HLA donor for stem cell transplantation.

As mentioned, stem cells from children free of HIGM and HEP-IP, obtained as a result of the presented PGD and preimplantation HLA typing, have already been used for transplantation treatment, resulting in restoration of normal function. Similar results have been demonstrated in PGD and HLA typing for Fanconi anaemia, thalassaemia, WAS and Blackfan–Dimond anaemia (Verlinsky *et al.*, 2002; Rechitsky *et al.*, 2004; Kahraman *et al.*, 2005; Kuliev *et al.*, 2005). Although ethical issues still present a barrier for a wider application of this technology, there has been an increase in referral for PGD with preimplantation HLA typing, with current overall experience of preimplantation HLA typing of more than 200 cases, more than half of which have been carried out within the last 2 years. The need for preimplantation HLA typing may be expected to increase in the future with progress in cellular therapy, requiring improvement of access to HLA-compatible stem cell transplantation.

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## References

Gaziev J, Lucarelli G 2005 Stem cell transplantation for thalassaemia. *Reproductive BioMedicine Online* **10**, 111–115.

Hellani A, Lauge A, Ozand P *et al.* 2002 Pregnancy after preimplantation genetic diagnosis for ataxia telangiectasia. *Molecular Human Reproduction* **8**, 785–788.

Kahraman S, Karlilaya G, Sertyel S *et al.* 2004 Clinical aspects of preimplantation genetic diagnosis of single gene disorders combined with HLA typing. *Reproductive BioMedicine Online* **9**, 529–532.

Kuliev A, Verlinsky Y 2004 Preimplantation HLA typing and stem cell transplantation. *Reproductive BioMedicine Online* **9**, 205–209.

Kuliev A, Rechitsky S, Verlinsky O *et al.* 2005 Preimplantation diagnosis and HLA typing for hemoglobin disorders. *Reproductive BioMedicine Online* **11**, 362–370.

Rechitsky S, Kuliev A, Sharapova T *et al.* 2006 Preimplantation HLA typing with aneuploidy testing. *Reproductive BioMedicine Online* **12**, 81–92.

Rechitsky S, Kuliev A, Tur-Kaspa I *et al.* 2004 Preimplantation HLA typing with preimplantation genetic diagnosis. *Reproductive BioMedicine Online* **6**, 210–221.

Van deVelde H, Georgiou I, De Rycke M *et al.* 2004 Novel universal approach for preimplantation genetic diagnosis of β-thalassemia in combination with HLA matching of embryos. *Human Reproduction* **19**, 700–708.

Verlinsky Y, Kuliev A 2006 *Practical Preimplantation Genetic Diagnosis*. Springer, London, p. 198.

Verlinsky Y, Kuliev A 2005 *Atlas of Preimplantation Genetic Diagnosis, Second Edition*. Taylor and Francis, London and New York, p. 288.

Verlinsky Y, Rechitsky S, Sharapova T *et al.* 2004 Preimplantation HLA typing. *Journal of the American Medical Association* **291**, 2079–2085.

Verlinsky Y, Rechitsky S, Schoolcraft W *et al.* 2001 Preimplantation diagnosis for Fanconi anemia combined with HLA matching. *Journal of the American Medical Association* **285**, 3130–3133.

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