

Birth of Healthy Children After Preimplantation Diagnosis of Thalassemias

A. KULIEV,¹ S. RECHITSKY,¹ O. VERLINSKY,^{1,2} V. IVAKHNENKO,¹ J. CIESLAK,¹ S. EVSIKOV,¹ G. WOLF,¹ M. ANGASTINIOTIS,¹ G. KALAKOUTIS,¹ C. STROM, and Y. VERLINSKY¹

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Background: Preimplantation genetic diagnosis (PGD) allows couples at risk of having children with thalassemia to ensure the healthy outcome of their pregnancy.

Methods: Seventeen PGD clinical cycles were initiated for Cypriot couples at risk of having children with different thalassemia mutations, including IVS1-110, IVS1-6, and IVS II-745. Unaffected embryos for transfer were selected by testing oocytes, using first and second polar body (PB) removal and nested polymerase chain reaction analysis followed by restriction digestion.

Results: Unaffected embryos were selected in 16 of 17 PGD cycles. Of 166 oocytes studied from these cycles, 110 were analyzed by sequential analysis of both the first and the second PB, resulting in preselection and transfer of 45 unaffected embryos. This resulted in seven pregnancies and in the birth of five healthy thalassemia-free children. The embryos predicted to have inherited the affected allele were not transferred. Analysis of these embryos confirmed the PB diagnosis.

Conclusions: Sequential first and second PB testing of oocytes is reliable for PGD of thalassemia and is a feasible alternative to prenatal diagnosis in high-risk populations.

KEY WORDS: preimplantation genetic diagnosis; thalassemia; multiplex nested PCR; allele dropout.

INTRODUCTION

Programs for prevention of thalassemia are presently based on prospective carrier screening and prenatal diagnosis (1). Although these programs have been highly effective around the world, the fact that affected

pregnancies must be terminated to avoid the birth of affected children makes it desirable to develop methods for pre-pregnancy testing, with the purpose of preventing the establishment of pregnancy with an affected fetus (2). Initial results of the application of pre-pregnancy testing for the purpose of preimplantation genetic diagnosis (PGD) of thalassemias were reported earlier, demonstrating the feasibility of offering PGD in the framework of an ongoing preventive program for thalassemia (3). A favorable attitude and a high acceptance of PGD were demonstrated in couples at risk of having children with thalassemia in different ethnic groups (1,4).

The present paper describes the current experience with PGD of thalassemias in a Cypriot population, resulting in seven clinical pregnancies and the birth of the first five, thalassemia free children.

MATERIALS AND METHODS

Of 17 PGD cycles performed for 9 couples at risk of having children with thalassemia, 12 were for IVS1-110, 4 for IVSII-745, and 1 for IVS1-6 mutations. The methods for the biopsy of oocytes and DNA analysis of the biopsied material were described elsewhere (2,3,5). The genotype of the oocytes obtained after ovarian stimulation was tested using both the first (I) and the second (II) polar body (PB), removed from the oocytes following their maturation and fertilization. DNA analysis of IPBs and IIPBs was performed using nested PCR to amplify the affected exon from the IPB and IIPB, followed by restriction digestion (3). Depending on the thalassemia mutation studied, different primer systems were used (3). To avoid misdiagnosis due to preferential amplification or allele dropout (ADO),

¹ Reproductive Genetics Institute, 836 West Wellington Avenue, Chicago, Illinois 60657.

² To whom correspondence should be addressed.

Table I. Results of Two-Step Polar Body Analysis in Predicting the Genotype of Oocytes in Preimplantation Diagnosis of Thalassemias

Locus	Total oocytes amplified	Oocytes with IPB data		Oocytes with IIPB data	Oocytes with IPB and IIPB data		Resulting embryos	
		Heteroz.	Homoz.		Total	ADO	Studied	Confirmed
IVSII-745	54	42	12	47	38	1	23	23
IVSI-110	102	67	35	67	65	9	28	28
IVSI-6	10	7	3	8	7	0	6	6
Total	166	116	50	122	110	10	57	57

a multiplex polymerase chain reaction (PCR) was performed, using two linked polymorphic markers, a short tandem repeat (STR) 5' to the β -globin gene, and HUMTH01. To detect contamination, three other nonlinked STRs located on different chromosomes were used (3). This multiplex PCR analysis made it possible to follow up the genotype of each embryo not only to confirm the PB diagnosis, but also to establish the origin of each embryo that implanted.

RESULTS AND DISCUSSION

The results of PCR analysis of 166 oocytes tested by sequential analysis of the IPB and IIPB are presented in Table I. Only 50 of these oocytes were homozygous following the first meiotic division (MI). Because both mutant and normal genes were present in the IPB of the rest of the oocytes, the embryos resulting from these oocytes would have been discarded, without sequential analysis of the corresponding IIPB. The results for the IIPB were available in 122 of 166 oocytes studied, with the data for both the IPB and the IIPB available in 110 oocytes. Overall, 49 mutation-free oocytes were predicted. Ten oocytes had homozygous mutant IPB and hemizygous normal

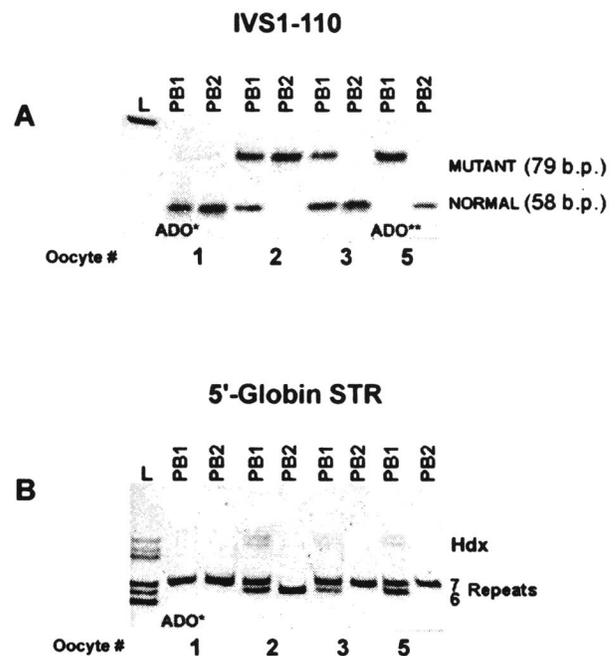


Fig. 1. (A) ADO detection by sequential polar body analysis in preimplantation diagnosis of an IVS1-110 mutation. (B) ADO detection by linked marker analysis. PB1, first polar body; PB2, second polar body; L, size standard; b.p., base pairs; Hdx, heteroduplex; ADO, allele dropout; * ADO of both the mutant allele and the six-repeat allele; ** ADO of the normal allele.

Table II. Results of Two-Step Polar Body Analysis for Thalassemia Mutations

Loci	Oocytes with IPB1 + IIPB	Homozygote after IPB		Hemizygote after IIPB		Oocytes with predicted genotypes		
		N	M	N	M	N	M	Total
IVSII-745	38	1	6	12	16	13	22	35
IVSI-110	65	8	7	24	23	32	30	62
IVSI-6	7	1	1	3	2	4	3	7
Total	110	10	14	39	41	49	55	104

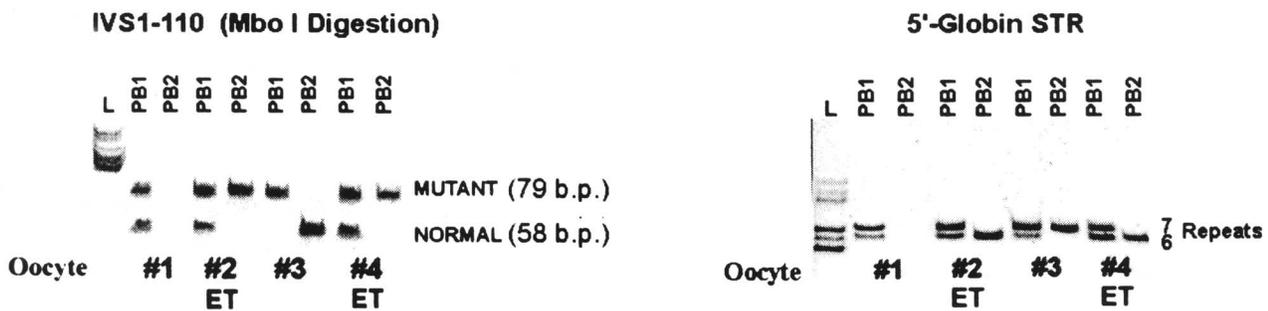
Table III. Preimplantation Diagnosis for Thalassemia by Sequential First and Second Polar Body Analysis

Mutation	Patients/cycles	Total No. of oocytes	No. of oocytes with I- + II PB data	No. of normal embryos transferred	No. of embryo transfers	Pregnancies/births
IVS II-745	1/4	54	38	12	4	1/1
IVS I-110	7/12	102	65	30	11	5/1
IVS1-6	1/1	10	7	3	1	1/3, Triplets (2-N/N 1-N/110)
TOTAL	9/17	166	110	45	16	7/5

IIPB. Thirty-nine oocytes had hemizygous mutant IIPB and heterozygous IPB (Table 2). Of 55 mutant oocytes, 14 were detected after MI, based on IPB analysis, and 41 based on both IPB and IIPB analysis. Therefore, of 110 oocytes with data on the IPB and IIPB, the genotype was predicted in 104 oocytes. Although ADO was observed in 10 of 110 oocytes with IPB and IIPB data, all of them were detected

either by sequential analysis of IPB and IIPB (Fig. 1A, embryo 1, and Fig. 1B, embryo 1) or by multiplex PCR (Fig. 1A, embryo 5, and Fig. 1B, embryo 5). Follow-up analysis of 57 embryos resulting from the oocytes predicted to contain an abnormal gene confirmed the PB diagnosis in all of them (Table I), demonstrating a high accuracy of sequential IPB and IIPB analysis by multiplex PCR.

A



B

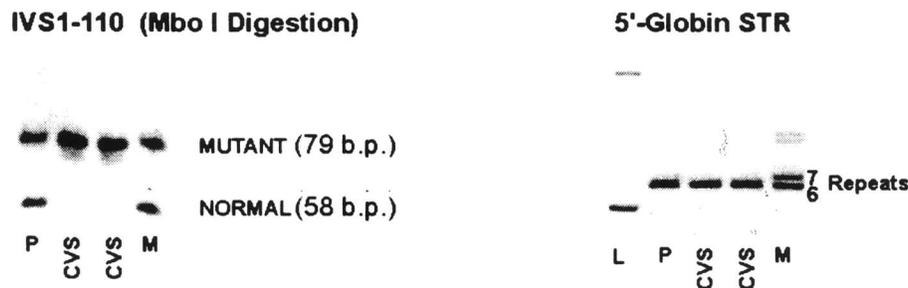


Fig. 2. (A) PGD by multiplex PCR analysis of the first and second polar bodies for thalassemia mutation IVS1-110 and for linked STR. (B) Prenatal diagnosis for thalassemia IVS1-110 by DNA analysis of CVS. PB1, first polar body; PB2, second polar body; L, size standard; b.p., base pairs; ET, embryo transfer; M, maternal genotype; P, paternal genotype; CVS, chorionic villi samplings.

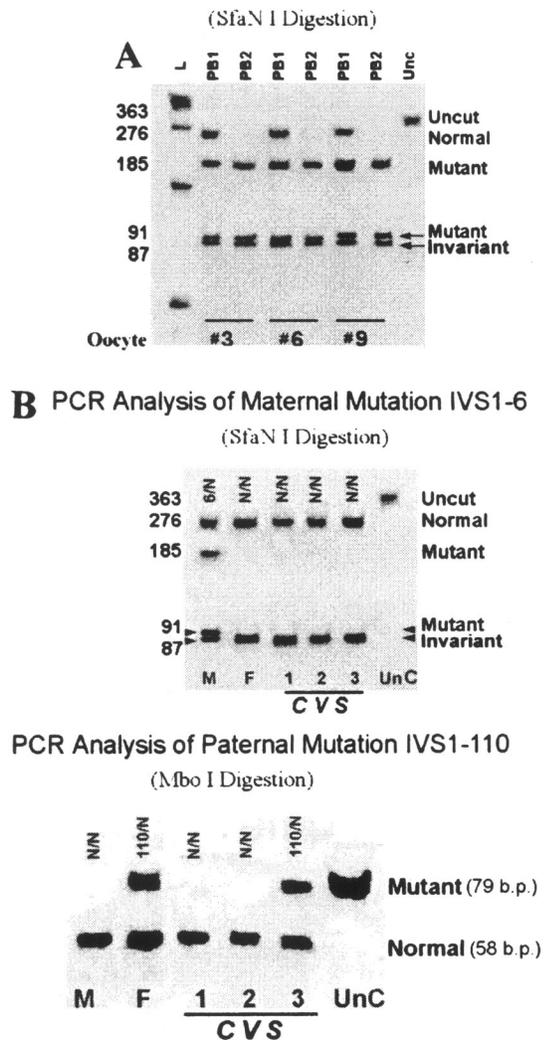


Fig. 3. (A) PGD by multiplex PCR analysis of the first and second polar bodies for thalassemia mutation IVS1-6. (B) Confirmation of PGD of thalassemia IVS1-6 by DNA analysis of CVS from the resulting triplet pregnancy. PB1, first polar body; PB2, second polar body; L, size standard; b.p., base pairs; M, maternal genotype; F, paternal genotype; CVS, chorionic villi samplings; UnC, undigested PCR product; N/N, homozygous normal; 110/N, heterozygous with a paternal mutation.

The application of multiplex marker analysis also makes it possible to identify which individual oocyte resulted in each pregnancy. This allowed us to demonstrate the noncorrespondence of a transferred embryo to a fetus in one of the established erroneous pregnancies (Fig. 2). The fetus in this pregnancy should have contained the normal allele together with the corresponding seven-repeat polymorphic marker similar to the oocyte that was transferred (Fig. 2A, embryos 2 and 4). On the contrary, the fetus showed

only a six-repeat marker strongly linked to the mutant allele in the mother (Fig. 2B, chorionic villi sampling). Because PGD usually involves fertile couples, in whom incidental pregnancy beyond IVF cannot be excluded, simultaneous mutation and linked polymorphic marker studies should be an integral part of PGD, to identify the origin of the resulting pregnancy and exclude the possibility of continuation of affected erroneous pregnancy.

As mentioned, in 16 of 17 cycles performed in Cypriot couples at risk for producing gametes with thalassemia genes, we were able to select unaffected embryos for transfer (Table III). Overall, 45 embryos were transferred, including 12 embryos in 4 cycles for PGD of IVSII-745, 30 in 11 cycles for IVSI-110, and 3 in a cycle for IVSI-6 (Fig. 3A, embryos 3, 6, and 9). The latter transfer led to a triplet pregnancy, resulting in the birth of three healthy children following confirmation by prenatal diagnosis. Two children were homozygous normal (Fig. 3B, CVS 1 and 2) and one was heterozygous, containing an IVSI-110 mutation contributed by the father (Fig. 3B, CVS 3).

The other two pregnancies, one in the case of an IVSII-745 mutation and the other in the case of an IVSI-110 mutation, have resulted in the birth of two healthy homozygous normal children. It is of interest that only one embryo was transferred in the latter case. The remaining four pregnancies were terminated either spontaneously (in three of them) or following a CVS procedure, although this pregnancy was confirmed to be thalassemia-free.

The results demonstrate that sequential IPB and IIPB analysis of oocytes is an efficient method for PGD of thalassemia, providing an acceptable number of mutation-free embryos for transfer. The follow-up studies of the resulting pregnancies, as well as the embryos resulting from affected oocytes, demonstrated a high accuracy of IPB and IIPB multiplex PCR analysis. The data might be of practical relevance, showing that PGD of thalassemias may be offered as a viable option for those couples who cannot accept prenatal diagnosis and termination of pregnancy for prevention of the birth of an affected child.

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