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Spontaneous Abortions Are Reduced After Preconception Diagnosis of Translocations

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Purpose: *Preimplantation genetic diagnosis of translocations has seldom been attempted. Recently, a genetic test based on analyzing polar bodies at the methaphase stage, following fluorescent in situ hybridization with commercially available whole-chromosome painting DNA probes has been presented. Here we report the use of this method in seven couples in whom the female was a carrier of one of these balanced translocations: 45,XX,der (13q;14q)(q10;q10) (two cases), 46,XX,t(4;14)(p15.3;q24), 45,XX,der(14q;21q) (q10;q10), 46,XX,t(7;20)(q22;q11.2), 46,XX,t(9,11)(p24;q12), 46,XX,t(14;18)(q22;q11), and 46,XX,t(3;8)(q11;q11).*

Methods: *The original method was improved in two ways. First, centromeric probes for one or both chromosomes involved in the translocation were added to avoid misdiagnosis caused by possible confusion of first polar body monovalent chromosomes (with two chromatids each) with single chromatids. Second, for cases with terminal translocations where commercially available probes do not cover telomere sequences, a telomere probe labeling the translocated fragment was added.*

Results: *A total of 26 abnormal, 18 balanced, and 22 normal eggs was detected. Nine normal and seven balanced embryos were transferred, resulting in eight (50%) implanting, of which one spontaneously aborted. To date, the remainder have produced karyotypically normal or balanced babies and ongoing pregnancies. The rate of spontaneous abortions after preimplantation genetic diagnosis (12.5%) was significantly reduced ($P < 0.001$) compared to natural cycles in the same patients (95%).*

Conclusions: *With the above improvements, the test can characterize any translocation of maternal origin and produce a high pregnancy rate and an apparently low frequency of spontaneous abortion.*

KEY WORDS: 1:3 segregation; t(4;14); t(7;20); t(9;11); t(14;18); t(13;14); t(14;21).

INTRODUCTION

Translocation carriers have a higher incidence of infertility, may produce chromosomally abnormal offspring, and often experience repeated spontaneous abortions. Reproductive problems associated with translocations can be alleviated through preimplantation genetic diagnosis (PGD). Healthy normal babies have been born after PGD of X-linked genetic diseases (1,2), single-gene defects (3), aneuploidy (4,5), and recently PGD of translocations (6).

Two approaches for PGD of translocations have been used. One is to develop specific probes for each translocation type, which is time-consuming and expensive but nevertheless feasible (7,8). An exception within this approach is the use of enumerator probes in Robertsonian translocation carriers to prevent trisomic offspring (9). However, enumerator probes cannot differentiate normal from balanced embryos, the latter being less desirable for transfer because they can perpetuate the genetic disease in the family and tend to spontaneously abort more often.

The second approach, useful only for female carriers of translocations, is based on the observation that first polar body (PB) chromosomes are at metaphase stage immediately after oocyte retrieval for a limited period. Therefore, whole chromosome painting probes can be used to characterize the translocation and differentiate between unbalanced, balanced and normal oocytes (6).

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The present study describes seven clinical cases in which the latter approach was used and two changes made to improve the original protocol. First, commercially available probes usually do not cover telomeric regions and therefore specific probes for those regions had to be added. Second, enumerator probes were used to visualize individual chromatids, which therefore enabled us to differentiate them from monovalent chromosomes (with two chromatids).

MATERIALS AND METHODS

Source of Oocytes for Control Studies

Oocytes were obtained from the in vitro fertilization (IVF) program of The Institute for Reproductive Medicine and Science of Saint Barnabas Medical Center in accordance with guidelines set by the their internal review board, with written patient consent for each case.

Oocytes that failed to fertilize after IVF (aged mature oocytes) or fresh PBs from matured in vitro immature oocytes (at the germinal-vesicle or metaphase-I stage were used as fluorescent in situ hybridization (FISH) controls. Immature oocytes were cultured with inactivated follicular fluid from mature oocytes supplemented with 20% heat-inactivated human serum. They were checked for the presence of a first PB early on the next day and every 4 hr thereafter. These PBs were fixed shortly after extrusion.

Clinical Cases

Cases A and B, with a 45,XX,der(13;14)(q10;q10) karyotype, and case C, with a 46,XX,t(4;14)(p15.3;q24) karyotype, were described previously (6).

Patient D was a 33-year-old female carrier of a balanced reciprocal translocation with a 45,XX,der (14;21)(q10;q10), ascertained after a spontaneous abortion that was karyotyped as being balanced for the translocation.

Patient E was a 28-year-old female carrier of a balanced reciprocal translocation 46,XX,t(7;20)(q22;q11.2), ascertained after four pregnancy losses. One of the spontaneous abortuses could be karyotyped as being balanced for the translocation.

Patient F was a 37-year-old female carrier of a balanced reciprocal translocation 46,XX,t(9,11)(p24;q12). Her brother had the same translocation and she experienced five pregnancy losses. They have a healthy baby from a natural cycle.

Patient G was a 36-year-old female carrier of a balanced reciprocal translocation with a 46,XX,t(14;18) (q22;q11). She experienced five pregnancy losses prior to the PGD procedure.

Patient H was a 37-year-old female carrier of a balanced reciprocal translocation with a 46,XX,t(3;8) (q11;q11) and had previously experienced three spontaneous abortuses.

Oocyte retrieval, PB biopsy, in vitro fertilization, and embryo transfer were performed as described previously without modification (6, 10).

FISH Chromosome Painting on First PBs

The probe solutions for A, B, and C were described previously (6). For the remaining cases, an enumerator probe for each chromosome was added with the exception of case G, in which only one enumerator was available. In addition, the hybridization solution for case F had a telomeric probe for the region not covered by painting probes, which also worked as an enumerator. The labeling of the probes and the source and amount of each probe used per assay are shown in Table I. For each assay (10 μL), the probes described in Table I were concentrated to 3 μl using a Speed-Vac centrifugal evaporator (DyNA VAP, National Labnet Company) and added to 7 μl of WCP hybridization buffer (Vysis).

The hybridization solutions were codenatured with the corresponding slides by placing the mounted slides on a hotplate at 78°C for 3 min. The slides were then sealed with rubber cement and placed in a moist chamber at 37°C for overnight hybridization. Afterwards, all slides were washed in 0.4× SSC for 2 min, at 72°C for lymphocytes and 71°C for PBs, and counterstained with DAPI in antifade. Slides treated with hybridization solutions containing digoxigenin- or biotin-labeled probes were demonstrated as described previously without modification (Munné *et al.*, 1998). The slides were observed under a fluorescence scope (Olympus BX60) with a triple-band pass filter to visualize simultaneously Spectrum Green or fluorescein isothiocyanate (FITC), Spectrum Orange or rhodamine, and Spectrum Aqua and the images recorded with an image analysis system (Metasystems, Belmont, MA).

RESULTS

Control Results

The probes were assessed in at least 20 patient- and 20 control- lymphocyte metaphases. In addition,

Table I. Source and Labeling of Probes Used[a]

Case	Karyotype	Probes (label, source, μl/assay)[b]
A	45XX,der(13;14)(q10;q10)	WCP13 (SpG, Vysis, 1.5 μl), WCP14 (SpO, Vysis, 1.5μl)
B	45XX,der(13;14)(q10;q10)	WCP13 (SpG, Vysis, 1.5 μl), WCP14 (SpO, Vysis, 1.5μl)
C	46XX,t(4;14)(p15.3;q24)	WCP4 (SpG, Vysis, 0.7 μl), WCP14 (dig, Oncor, 0.3 μl), 4ptel (D4F26)(Bio, Oncor, 5 μl)
D	45XX,der(14;21)(q10;q10)	WCP21 (SpG, Vysis, 1.5 μl), WCP14 (Dig, Oncor, 3 μl), LSI21 (spO, Vysis, 2 μl), LSI14 (FITC, Jingli Fung of UCSF, 2 μl)
E	46XX,t(7;20)(q22;q11.2)	WCP7 (Spg, Vysis, 1 μl), WCP20 (SpO, Vysis, 1.5 μl), CEP7 (SpA, Vysis, 1.5 μl), CEP20 (Bio, Oncor, 4 μl)
F	46XX,t(9,11)(p24;q12)	WCP9 (SpG, Vysis, 1 μl), WCP11 (SpO, Vysis, 1 μl), CEP9 (SpA, Vysis, 1 μl), 11qtel (Bio, Jingli Fung of UCSF, 3 μl)
G	46XX,t(14;18)(q22;q11)	WCP18 (SpG, Vysis, 4 μl), WCP14 (Dig, Oncor, 1.5 μl), CEP18 (SpA, Vysis, 1.5 μl)
H	46XX,t(3;8)(q11;q11)	WCP3 (SpO, Vysis, 2 μl), WCP8 (SpG, Vysis, 1 μl), CEP3 (SpA, Vysis, 1.5 μl), CEP3 (SpA, Vysis, 2 μl)

[a] SpG, labeled with Spectrum Green; SpO, labeled with Spectrum Orange; SpA, labeled with Spectrum Aqua; Dig, labeled with digoxigenin; Bio, labeled with biotin; LSI, locus-specific probe; WCP, whole-chromosome painting probe; CEP, centromeric or paracentromeric probe.
[b] For each assay of 10 μl, the amount of probe shown in the table was concentrated to 3 μl and added to 7 μl of WCP hybridization buffer (Vysis).

a minimum of 10 inseminated but unfertilized oocytes was analyzed per case as controls. The control results are presented in Table II.

Case Results

The PGD results of first PBs for cases A–G are shown in Table III. As yet, case H has not started her IVF cycle. A total of 87 first PBs were biopsied, of which 21 (24%) had no results because 10 did not produce clear signals, 8 were damaged during biopsy, 2 were lost during fixation, and 1 was DNA-free. Usually, PB chromosomes that did not spread during biopsy and clumped together did not produce clear signals. On the other hand, a good part of the PBs that were damaged during biopsy had cytoplasmic bridges, indicating egg immaturity. Figure 1 shows a chromosomally abnormal PB from case G, with well-spread chromosomes showing each chromosome with two chromatids.

Table II. Control Results

Case	Karyotypes[a]	Blood control	Blood case	Oocyte controls
A, B	13, 14, der (13, 14)	0	38	0
	13, 13, 14, 14	40	2	0
	13u, 14u	0	0	30
	14u	0	0	1
C	4, der 4, 14, der 14	0	50	0
	4, 4, 14, 14	20	0	0
	4u,14u	0	0	10
D	14, 21, der (14, 21)	0	24	0
	14, 14, 21, 21	30	1	0
	14u,21u	0	0	10
E	7, der 7, 20, der 20	0	20	0
	7, 7, 20, 20	20	0	0
	7u, 20u	0	0	9
	chtd 7, 7u, 20u	0	0	1
F	9, der 9, 11, der 11	0	20	0
	9,9,11,11	20	0	0
	9u, 11u	0	0	10
G	14, der 14, 18, der 18	0	20	0
	14, 14, 18, 18	20	0	0
	14u, 18u	0	0	9
	14u, 14u, 18u, 18u	0	0	9
H	3, der 3, 8, der 8	0	45	0
	3, 3, 8, 8	25	0	0
	3u, 8u	0	0	22
	8u	0	0	1

[a] der, derivative chromosome; u, univalent (two chromatids); chtd, single chromatid.

The efficiency of the analysis could be obtained by comparing unfertilized oocytes, good-quality second PB methaphases, or blastomeres arrested at metaphase with antimitotics (Fig. 2) to the first PB results from the corresponding oocyte. In total, 21 first PBs had their corresponding oocytes; second PBs, or blastomeres analyzed, of which 2 (9.5%) did not complement. These errors both occurred in case D, where two PBs were nullisomic for chromosome 21, yet oocytes were normal. Chromosome 21 from those PBs was likely lost during fixation.

Types of gamete segregation varied greatly between patients, with cases B and F producing only alternate segregation products, case C producing mostly alternate and adjacent-1 products, and case G producing mostly alternate and adjacent-2 segregations (Figs. 1 and 2). Interestingly, patients A and B, carriers of the same translocation, produced diverse segregation products. Due to the small sample of gametes per patient, statistical analysis of these differences was meaningless.

Table III. Case Results

Case	Karyotype	Cycle	Oocyte diagnosis[a]							
					Unbalanced					
			Normal	Balanced	A1	A2	3:1	O	NR	Error (b)
A	45XX,der(13;14)(q10;q10)	1st	6(3T)	1	4	0	0	0	4	0/0
B	45XX,der(13;14)(q10;q10)	1st	5(2T)	1(1T)	0	0	0	0	0	0/0
C	46XX,t (4;14)(p15.3;q24)	1st	0	2(2T)	3	0	0	0	1	0/0
		2nd	0	1	3	0	2	0	1	0/1
D	45XX,der (14;21)(q10;q10)	1st	3	5(2T)	4	0	0	1(a)	5	1/8
		2nd	4(3T)	4	3	0	0	0	3	1/8
E	46XX,t (7;20)(q22;q11.2)	1st	0	3(2T)	1	0	2	0	2	0/0
F	46XX,t (9,11)(p24;q12)	1st	3(1T)	0	0	0	0	0	2	0/1
G	46XX,t(14;18)(q22;q11)	1st	1	1	0	3	0	0	3	0/3

[a] Oocyte diagnoses were inferred from PB results where a PB karyotype was assumed to be the complementary karyotype of the oocyte. Example: If a PB was found to be normal, the oocyte was diagnosed as balanced. T, embryo transferred; A1, segregation adjacent-1; A2, segregation adjacent-2; 3:1, segregation 3:1, O, other segregations; (a) a PB with a missing chromatid 21; (b) based on the analysis of second PBs, unfertilized oocytes, or blastomeres at metaphase stage. Both errors were PBs with only a 14 chromosome, while the oocyte was normal.

Pregnancy Outcome

Table IV summarizes the PGD pregnancy outcomes. Embryos diagnosed as karyotypically normal with optimal development and morphology were preferentially selected for transfer. This was the case for patients A, D (second cycle) and F, who had only karyotypically normal embryos transferred (see Table IV). However, for the rest of the cases, a combination of normal and balanced embryos (case B) or only balanced embryos [cases C (first cycle) D (first cycle), and E] were replaced. Transfers did not occur at all in patients C (second cycle) and G due to a combination of karyotypically and developmentally abnormal embryos.

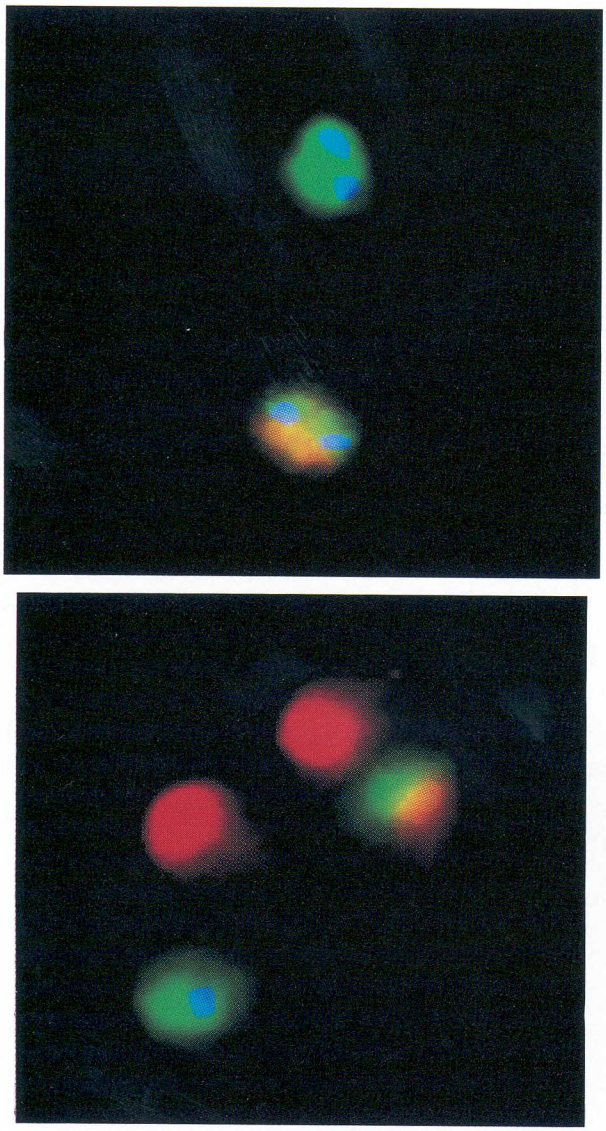
In total, 16 embryos were transferred, 9 normal and 7 balanced. Of those, 8 (50%) implanted, resulting in 9 babies or fetuses, since one implantation produced identical twins. Of the 9 embryos that implanted, 5 resulted in karyotypically normal babies after birth, 3 were balanced after birth or prenatal diagnosis, and 1 could not be diagnosed because it spontaneously aborted at 7 weeks of pregnancy. Assuming that the last embryo was balanced, normal and balanced embryos seem to implant at equal frequencies, 44% (4/9) and 57% (4/7), respectively.

The frequency of spontaneous abortions per embryo transferred in the group that became pregnant was significantly reduced ($P < 0.001$, F test), from 95% (A, 1/1; B, 5/5; C, 3/3; E, 4/4; F, 5/6) in natural cycles to 12.5% (1/8) after PGD. The single spontaneous abortion after PGD resulted from an embryo expected to be balanced according to PGD results.

DISCUSSION

The analysis of first PBs has been applied to PGD of monogenic diseases (11), aneuploidy (5,12), and recently translocations of maternal origin (6). The latter application is based on the observation that first PB chromosomes are mostly at metaphase shortly after oocyte retrieval, and therefore FISH analysis with chromosome-painting probes can easily be performed. However, the original protocol had several limitations that were partly overcome in the present study.

One of the improvements was the addition of telomere probes in those cases with terminal breakpoints (cases C and F), because commercially available probes usually do not cover that region. Another improvement was the addition to the hybridization solution of enumerator probes to ascertain predivision of chromatids. This phenomenon has been described as one of the mechanisms that cause aneuploidy (13,14) and also as an artifact produced by oocyte aging and PB degeneration (12,14). Because first PB chromosomes usually inflate and lose their original morphology after FISH, it is difficult to differentiate a single chromatid from a univalent chromosome. The problem of predivision can be solved, by adding enumerator probes. We detected 10 PBs where the analysis could not be performed because of overlapping or unclear results. Of those, 4 were from case A (no enumerators), 4 from case D (2 enumerators but of the same color as the printing probes and 1 as big as one of the chromosomes), and 2 from case G (1 enumerator only). Although other factors, such as fixa-



Figs. 1 and 2. Chromosome painting of a PB (Fig. 1) and a blastomere (Fig. 2) from case G, carrier of a 46XX,t(14;18)(q22;q11) translocation. The probes used were WCP-18 (green), WCP-14 (red), and an enumerator probe for chromosome 18 (blue). Figure 1 shows an unbalanced first PB with a derivative 18 and a normal 18 chromosome, each one with two chromatids, as expected. Figure 2 shows the metaphase of a blastomere from an unbalanced with a 46,XX,t(14;18), 18,+der(14)t(14;18).ish.

tion, can influence the ability to analyze a PB, the presence of two enumerator chromosomes labeled in aqua (such as in case H) should facilitate clear analysis.

Regardless of the fact that PB metaphases degenerate soon after oocyte retrieval (12), the small size of the PB makes it difficult to fix. Of 87 fixed PB, 10 could not be read, mostly because the chromosomes were too closely grouped together, or clumped, and 2 were FISH errors probably caused by the loss of chromosomes during fixation. This is caused by insufficient or excessive spreading. Fixation parameters of PBs in order to obtain metaphases requires improvement.

The analysis of PBs in translocation carriers will provide further information about gamete segregation in female translocation carriers. All the information thus far is based on spontaneous abortions and live births, but some segregation products may be lethal and not detected as clinical conceptions. As discussed previously (6), cases A, B, and C produced segregations in agreement with the data obtained from clinical conceptions (15). Case E is the only one that produced 3:1 segregations, and its karyotype coincides with the clinical conception data of translocation cases in which one break is near the centromere and the participating chromosomes are very unequal in size (15). Case G, which produced unbalanced embryos resulting only from adjacent-II segregation, fits the description of translocations with one interstitial segment so short that it is unable to form a chiasma, which produce unbalanced clinical conceptions resulting mostly from adjacent-II segregations (15). The present data, although limited, suggest that the unbalanced segregation products in clinical pregnancies are similar to the ones found in the female gametes, indicating a low periimplantation mortality rate.

Similarly, the present results indicate that there are no differences in the implantation rates of embryos with balanced and normal karyotypes. Our data, however, are still too limited to know whether balanced embryos spontaneously abort more often than karyotypically normal ones, a phenomenon described previously (16).

PGD of translocations using PB biopsy seems to provide a number of benefits for affected couples. One is to reduce the risk of trisomic and unbalanced offspring. The test has been able to prevent the transfer of unbalanced embryos, which account for more than one-third of oocytes produced, with about a 90% efficiency. So far all the babies born have been chromosomally normal, or if balanced embryos were transferred, balanced. In addition, for those couples of advanced maternal age, PBs could be reanalyzed by FISH to screen for the most common trisomies (17), or PGD of translocations and aneuploidy could be done simultaneously using spectral imaging (18). The reanalysis of the PBs to screen for aneuploidy could also be important if an interchromosomal effect increasing aneuploidy rates in translocation carriers is confirmed (19)

Table IV. Pregnancy Outcome

Case	Karyotype	Cycle	Embryos transferred[a]	Outcome	Karyotype
A	45XX,der(13;14)(q10;q10)	1st	3N	3 delivered (two identical)	3N
B	45XX,der(13;14)(q10;q10)	1st	2N,1B	2 delivered	1N, 1B
C	46XX,t(4;14)(p15.3;q24)	1st	2B	1 fetus spont. abort. (7 weeks)	???
		2nd	0	No transfer	
D	45XX,der(14;21)(q10;q10)	1st	2B	Not pregnant	
		2nd	3N	Not pregnant	
E	46XX,t(7;20)(q22;q11.2)	1st	2B	2 delivered	2B
F	46XX,t(9,11)(p24;q12)	1st	1N	1 ongoing	1N
G	46XX,t(14;18)(q22;q11)	1st	0	No transfer	

[a] N, chromosomally normal; B, balanced.

Another benefit of the present approach to PGD of translocations is to increase the couple's chances of sustaining a pregnancy. For instance, in the present study 50% (8/16) of the embryos have implanted, although translocations of maternal origin usually produce repeated spontaneous abortions, many of which show a balanced instead of an unbalanced translocation complement (16). The only spontaneous abortion, after PGD, within this group of patients was from a supposedly balanced embryo. These patients may have a higher chance of producing a viable pregnancy, by doing PGD and transferring normal embryos. Nevertheless, even after transferring balanced embryos, we have demonstrated a significant decrease in spontaneous abortions ($P < 0.001$), from 95% in natural cycles to 12.5% after PB biopsy and PGD of maternal translocations.

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