Patient-Specific Probes for Preimplantation Genetic Diagnosis of Structural and Numerical Aberrations in Interphase Cells

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Purpose: Our purpose was to evaluate the utility of translocation breakpoint-spanning DNA probes for prenatal genetic diagnosis of structural and numerical chromosome aberrations in interphase cells.

Methods: Breakpoint-spanning translocation probes were isolated from large insert DNA libraries and labeled so that the breakpoint regions were stained in different colors. Hybridization conditions were optimized using blastomeres biopsied from donated embryos. Probes were then applied to analyze patient blastomeres.

Results: We prepared translocation breakpoint-specific probes for 18 in vitro fertilization patients. Here, we describe the preparation of probes for two patients carrying balanced translocations involving chromosome 11 [$t(11;22)(q23;q11)$, $t(6;11)(p22.1;p15.3)$]. The breakpoint cloning procedure could be accomplished in about 3–5 weeks. Additional time was needed to optimize probes. Application of probes demonstrated numerical as well as structural abnormalities.

Conclusions: Breakpoint-spanning probes allow chromosome analysis in interphase cells as required for preimplantation genetic diagnosis screening of blastomeres.

KEY WORDS: translocation; infertility; in vitro fertilization; multicolor in situ hybridization; DNA probe; interphase cells.

INTRODUCTION

Balanced chromosomal rearrangements may exist unrecognized in as much as 0.1% of the general popu-

lation (1). Often unaware of their cytogenetic abnormality, many carriers reach reproductive age, experience a high rate of reproductive failures, and seek help in professional, assisted reproduction. In vitro fertilization (IVF) programs therefore support a higher-than-average number of women who either are themselves a translocation carrier or are the partner of a carrier. In most cases, the women report a history of repeated or habitual spontaneous abortions. The cause of these miscarriages is most likely a compromised homologue pairing in meiosis, which may lead to severe disturbances during meiotic cell divisions and karyokinesis and, in many instances, aneuploid germ cells (2). Most chromosomal imbalances are incompatible with fetal development and are thus found frequently in first-trimester spontaneous abortuses (3,4). Although only sparse information is available, it appears that IVF success rates for couples with one spouse carrying a translocation fall below the average. This may very well reflect the lack of effective means for selection of fertilized oocytes and embryos prior to transfer.

Pre-selection of normal oocytes for in vitro fertilization and/or diploid embryos for transfer is expected to increase the chances of successful conception and implantation (5–7). Since most blastomeres obtained by embryo biopsy will be in interphase, the PGD procedure has to be performed on interphase cell nuclei (8). The exception is when the translocation is maternal in origin, in which case, preimplantation genetic diagnosis (PGD) can be performed in polar bodies at the metaphase stage (7,9). Fluorescence in situ hybridization (FISH) with chromosome- or locus-specific probes has been applied extensively for the analysis of interphase cells derived from solid tumors (10,11). Applications of FISH for enumeration of multiple chromosomes in cells from preimplantation embryos have been described recently (5,8,12–14). A method to detect Robertsonian translocations with generic

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probes to analyze the composition of first polar bodies has recently been developed (7,9). Within a few hours after oocyte harvest, the chromosomal DNA in first polar bodies is found in a somewhat condensed state so that individual chromosomes can be delineated by chromosome painting and/or spectral imaging (15,16). However, the complex organization of interphase chromatin and lack of chromosome domain definition invalidate the use of whole-chromosome painting probes for detection of chromosome rearrangements in interphase nuclei. Thus, in male carriers, for a reliable detection of structural abnormalities in interphase, including deletions and inversions, DNA probes flanking or spanning the breakpoints must be developed for each translocation (11).

Since 1992, we have developed rapid and inexpensive procedures for prenatal diagnosis and PGD of germ cells or embryos based on FISH (17–22). More recently, we focused our research effort on the preparation of patient- or case-specific probes to analyze structural abnormalities (23). Selecting yeast artificial chromosomes (YAC) (24) as DNA probes, our approach relies on overlapping translocation-specific probe contigs that span individual breakpoints (25). Labeling DNA probes with distinct reporter molecules and detecting them in fluorescent colors specific for either breakpoint, we now score normal and derivative chromosomes in individual nuclei. As demonstrated in the examples below and in a recently published report (26), the inclusion in the hybridization reaction of chromosome-specific centromeric probes labeled in a third color allows us to identify further the origin of derivative chromosomes in interphase cell nuclei.

In this communication, we demonstrate the application of our techniques in the mapping of translocation breakpoints in two phenotypically normal males carrying rearrangements involving chromosome 11. Furthermore, a review of translocation cases for which probes were prepared between 1996 and 1998 demonstrates the spectrum of breakpoint locations found in our patients.

MATERIALS AND METHODS

Metaphase Spreads and Blastomere Preparations

The two specific examples described in this communication required preparation of case-specific probes for two male patients, with the karyotypes 46,XY,t(11;22)(q23;q11) and 46,XY,t(6;11)(p22.1;p15.3),

respectively. Results of karyotyping analysis by G-banding were provided to us by the referring hospitals. Both spouses were karyotypically normal, had experienced several spontaneous abortions, and reported no previous live births.

Metaphase spreads were prepared from short-term lymphocyte cultures of the patients. Cells were arrested in mitosis and fixed in acetic acid:methanol (1:3, vol:vol) (27). The fixative was changed twice and cells were dropped on ethanol cleaned slides inside a CDS-5 Cytogenetic Drying Chamber (Thermotron Industries, Holland, MI) at 25°C and 47.5% humidity. Slides were stored for at least 2 weeks in ambient air at room temperature, then placed at –20°C in sealed plastic bags filled with nitrogen until use.

Blastomeres used for probe optimization were obtained from embryos donated for research. These embryos were obtained from the IVF program of the Institute for Reproductive Medicine and Science of Saint Barnabas Medical Center. In accordance with guidelines set by the internal review board of Saint Barnabas Medical Center, written consent was obtained from the patients in each case. Only monospermic embryos developing from bipronucleated zygotes were used for this study. Embryo biopsies and blastomere fixations were carried out as described (14).

Clone Selection, Probe Preparation, and FISH

We selected YAC clones based on sequence-tagged site (STS) markers that mapped close to the estimated breakpoint regions and information provided in the database of the Whitehead Institute for Biomedical Research/MIT Center for Genome Research (<http://www-genome.wi.mit.edu/>) (28). This database integrates various sources of genetic and physical mapping data. In addition, we used physical mapping data from the bacterial artificial chromosome (BAC) clones of human chromosome 22 (29,30). Most BAC probes have STSs attached to them, so they can be integrated easily into the Whitehead YAC map.

For the initial mapping of YAC clones, YAC DNA was isolated using a DNA miniprep protocol (31). The total amount of DNA prepared per clone was about 12–20 µg. The BAC DNA was purified from overnight bacterial cultures by an alkaline lysis protocol (32). The DNAs from YAC and BAC clones were labeled by random priming incorporating biotin-14-dCTP, digoxigenin-11-dUTP, or fluorescein-12-dUTP (32).

The hybridization mix contained 1–2 µl of each probe, 1 µl of human COT1 DNA (1 mg/ml; GIBCO/

LTI, Gaithersburg, MD), 1 μ l of salmon sperm DNA (3'-5'; 20 mg/ml), and 8 μ l of the hybridization master mix [78.6% formamide (FA); GIBCO/LTI], 14.3% dextran sulfate in 2.9 $\times$ standard saline citrate (SSC), pH 7.0 (2 $\times$ SSC is 300 mM NaCl, 30 mM Na citrate). During probe development using carrier lymphocytes, we denatured the hybridization mix at 76°C for 7 min and allowed it to preanneal at 37°C for 60 min. The slides were denatured for 3.5 min at 76°C in 70% FA/2 $\times$ SSC, pH 7.0, dehydrated in 70, 80, and 100% ethanol for 2 min each step, and allowed to air-dry. When tested on blastomeres, 6 μ l of the hybridization mix was applied to the slides and covered with a 9 $\times$ 9-mm coverslip. Then denaturation was done at 78°C for 3 min on a heat block (14). Finally, the hybridization was allowed to proceed overnight in a moisture chamber at 37°C.

After hybridization, the slides were washed three times in 50% FA, 2 $\times$ SSC for 10 min, followed by two washes in 2 $\times$ SSC for 10 min and one wash in 0.4 $\times$ SSC for 5 min at 43°C. Bound probes were detected either with fluorescein-avidin DCS [20 μ g/ml in PNM (33); Vector Laboratories, Burlingame, CA] or antidigoxigenin-rhodamine [20 μ g/ml in PNM; Boehringer Mannheim (B.M.)]. The slides were then washed three times in 2 $\times$ SSC for 10 min each. Finally, the slides were mounted in 8 μ l of 0.05 μ g/ml 4,6-diamino-2-phenylindole (DAPI; Calbiochem, La Jolla, CA) in antifade solution [0.1% *p*-phenylenediamine dihydrochloride (Sigma), 0.1 $\times$ PBS (GIBCO/LTI), 45 mM NaHCO₃, 82% glycerol, pH 8.0] to counterstain nuclei and chromosomes.

Fluorescence microscopy was performed on a Zeiss Axioskop microscope with a filter set for simultaneous observation of Texas Red/rhodamine and FITC and a separate filter for DAPI detection (ChromaTechnology,

Brattleboro, VT). Images were collected using a cooled CCD camera (CH-250; Photometrics, Tucson, AZ) and a SUN Sparc station (SUN Microcomputers, Mountain View, CA) (30).

Optimization of DNA Probes

Once we decided which YAC clones to include in the final probe set, probe optimization was carried out by using pulsed-field gel electrophoresis (PFGE) and degenerate oligonucleotide-primed PCR (DOP-PCR) (33,34). We prepared probe DNA from PFGE-purified YAC DNA with a 42-cycle DOP-PCR using primer JUN1 (5'-CCCAAGCTTGCATG-CGAATTCNNNN-CAGG-3'; *N* = ACGT), with an initial seven cycles of primer annealing at 37°C and extension by T7 DNA polymerase (23). The PCR products were further amplified at a higher annealing temperature (50–55°C) using primer JUN15 (5'-CCCAAGCTTGCATGCG-AATTC-3') and Taq polymerase for 35 cycles. The PCR products were then purified by chloroform extraction, precipitated in ethanol, and resuspended in 1 $\times$ TE buffer, pH 7.2. Then, probe labeling was performed by random priming (32).

Blastomere Analysis

Blastomere hybridization and analysis followed our previously described protocol (14). The probes spanning the breakpoint on 22q11 were labeled with FITC, which emits green fluorescent light. The probe DNA spanning the breakpoint on 11q23 (Patient A) was labeled with digoxigenin and detected with rhodamine-labeled anti-digoxigenin antibodies (B.M.) (red fluorescence). To distinguish the derivative chromosomes der (11) from the der (22), we included a blue fluores-

Fig. 1. (opposite) Development and application of breakpoint-spanning DNA probes. (A) Idiograms of normal and derivative chromosomes 11 and 22 of Patient A showing the color scheme used for breakpoint-spanning probes (red, green). The blue probe specific for centromeric DNA repeats of chromosome 11 was used only in blastomere analyses. (B) A simplified physical map of the breakpoint region on chromosome 11 (top) and chromosome 22 (bottom) showing some of the markers used for clone selection. Clones used in optimization and blastomere analysis are shown below the respective maps. (C) An interphase lymphocyte cell of Patient A hybridized with the breakpoint-spanning YAC probes. A blue-red (labeled CEP11, LSI11) and a green hybridization domain (labeled LSI 22), corresponding to the normal homologues of chromosomes 11 and 22, respectively, and two domains with colocalizing red and green signals, corresponding to the derivative chromosomes der (11) and der (22), respectively, are clearly visible. (D) Hybridization result using an unrelated, normal blastomere showing two signal pairs of red-blue fluorescence (chromosome 11) and two green signals (LSI 22). (E) Hybridization result using an unrelated, abnormal blastomere, showing three signal pairs of red-blue fluorescence (chromosome 11) and three green signals (LSI 22). (F) Probe labeling scheme to detect structural alterations in cells derived from Patient B carrying a balanced translocation t(6;11)(p22.1;p15.3). (G) Hybridization result using an unrelated, normal blastomere, showing two signal pairs, each corresponding to normal chromosomes 6 and normal chromosomes 11, respectively. (H) Analysis of a blastomere biopsied from embryo 3 of Patient B's spouse. There is a clear indication of chromosomal imbalance by signals derived from two copies of normal chromosome 11 in the presence of one normal homologue of chromosome 6 and the der (6) chromosome.

Table I. Steps Involved in Isolation of Breakpoint-Spanning Large Insert DNA Clones

I.	Cytogenetic data
II.	Interval definition
III.	Clone selection
IV.	FISH
V.	Spans breakpoint?
	If so, continue
	If not, note map position and repeat from II
VI.	Probe optimization
	PFGE, labeling, FISH
VII.	PGD

cent α -satellite probe for the centromeric region of chromosome 11 (D11Z1, CEP-11 Spectrum Aqua; Vysis, Downer Grove, IL). The labeling scheme applied in the case of t(11;22) (Patient A) is shown schematically in Fig. 1A.

The probe mixture for analysis of blastomeres biopsied from embryos from patients t(6;11)(22.1;p15.3) was 1.5 μ l of 6p digoxigenin-labeled probe, 4 μ l of biotin-labeled 11p15 probe, 1 μ l of Spectrum Aqua-labeled D11Z1 probe, 1 μ l of Spectrum Aqua-labeled CEP-6 probe, 1 μ l of Spectrum Orange-labeled CEP-6 probe (CEP-6 Spectrum Orange and Spectrum Aqua; Vysis), 1 μ l of COT1 DNA, and 1 μ l of salmon sperm DNA. This mixture was concentrated to 5 μ l and added to 7 μ l of WCP hybridization buffer (Vysis). Therefore, a normal chromosome 6 displayed a red and a pink signal, while a normal 11 appeared as a green and a blue signal (Fig. 1F). On the other hand, a derivative 6 chromosome produced an association between a red and a green signal plus a pink signal, while the der (11) showed overlapping red and green signals plus a separate blue domain (Fig. 1F).

RESULTS

Clone Selection

The major steps for the rapid cloning of translocation breakpoints in YAC clones are summarized in Table I. All probes selected from the YAC database must meet the requirements of the correct STS content, no signs of chimerism, and an insert size of at least 300 kb. Clone selection and mapping were done in cycles to narrow the region of interest. The selected probes were hybridized to the translocation carrier metaphase spreads, allowing us rapidly to classify clones as either proximal or distal to the breakpoint and to define the genomic interval containing the breakpoint. The next

cycle of clone selection was then based on these results and mapped additional clones in this interval. Each cycle took about 10–14 days. We continued to select and map YACs in increasingly smaller intervals until clones were found that either spanned or closely flanked the breakpoint. We then mapped closely flanking clones to prepare a high-resolution map of the breakpoint region. For probe optimization, YAC insert DNA was purified by pulsed-field gel electrophoresis (PFGE) and then amplified by polymerase chain reaction (PCR) using mixed-base oligonucleotide primers (22,31).

Mapping Translocation Breakpoints in Patient A Carrying t(11;22)

Figure 1 shows an idiogram of the t(11;22). Arai and co-workers (35) published a physical map spanning a candidate tumor suppressor gene region at 11q22.2–q23.3. Four YACs between D11S1885 and D11S29 (Fig. 1B) that mapped approximately in the breakpoint region of a constitutional translocation t(11;22)(q23;q11) (35) were selected in the first cycle to localize the chromosome 11-specific breakpoint in our patient. FISH results indicated that clone 939b12 mapped proximal to the breakpoint, while clones 785e12 and 911f2 spanned the breakpoint and the probe prepared from clone 822g8 failed to give a hybridization signal. When hybridizing the probe prepared from YAC clone 911f2 on to patient metaphase spreads, three strong green signals were found, on the normal chromosome 11, the der (11), and the der (22), respectively (data not shown). Thus, utilizing published physical maps, YAC clones spanning the chromosome 11 breakpoint were found in the first cycle. It took only 10 days to localize the breakpoint on chromosome 11 in patient A and to identify large insert DNA clones suitable for PGD.

For the first cycle of breakpoint mapping on chromosome 22, we selected four YAC clones mapping in intervals of about 10 Mbp along the proximal long arm of chromosome 22. Three YACs (848d2, 765e2, 966a8) failed to give hybridization signals, and clone 944c4 did not map to chromosome 22. More YACs were selected in the second cycle. While clones 925g12 and 742b5 did not produce FISH signals, clones 809b10 and 803g9 mapped proximal to the breakpoint, and clone 881h10 spanned it. However, the probe prepared from clone 881h10 was not suitable for interphase PGD, because less than half of the interphase cells showed the expected three signals. In the third cycle, we therefore selected an additional six

YAC and five BAC clones. Of these, YAC 874a4 did not give a hybridization signal, YAC 908g9 did not map to chromosome 22, and three YACs (872f9, 884e1, 904b10) and four BACs (453h4, 254b1, 489c8, 528c8) mapped distal of the breakpoint on chromosome 22. Only YAC 849e9 and BAC 563b9 were found spanning the chromosome 22 breakpoint and three signals were found, on the normal chromosome 22, the der (22), and the der (11), respectively (data not shown). The relative locations of the breakpoint spanning YAC and BAC clones are shown in Fig. 1B.

Mapping Translocation Breakpoints in Patient B Carrying a t(6;11)

Tables IIA and B list the YAC clones selected for the mapping the breakpoints on 6p22 and 11p15, respectively. Two clones (901a10 and 753h12) selected in the initial cycle appeared to map distal to the breakpoint on 6p. In the second cycle, clone 875f4 mapped distal to the breakpoint, while clone 963f2 mapped proximal. Four clones chosen in the third cycle (YAC 755c6, 808h5, and 819a10) mapped proximal to the breakpoint, and the YAC 923b5 clone spanned the breakpoint. However, the probes prepared from 923b5 gave a weak signal, and unfortunately, all the

other nearby clones tested gave either weak signals or no signals. We thus decided to optimize the distal probes. Hybridization of the labeled DOP-PCR products revealed that YAC clone 753h12, which was mapped distal to the 6p22 breakpoint in the first cycle, actually spanned this breakpoint. These results might be due to clone instability and partial deletion of the insert in the clone used initially or, more generally, to low hybridization efficiency. We often found intensity differences between probes prepared from DNA isolated using a miniprep protocol and probes made from the DNA isolated by PFGE. In most instances, probes made from PFGE bands produced brighter and more specific signals. The YAC clone 753h12 was chosen for blastomere analysis.

Table IIB shows the YAC clones used for localization of the breakpoint on chromosome 11p15. In the first cycle, four clones (967a5, 847a12, 954f4, and 814a4) mapped distal and one clone (776e7) mapped proximal to the breakpoint. Clone selection in the second cycle was based on the results obtained in the first cycle indicating a breakpoint location between 24cM and 25cM or 46cR and 59cR. Three clones (933b8, 763a10, and 791g9) were found to be distal, two clones (803f11 and 953d9) mapped proximal, and clone 808b11 spanned the breakpoint. Finally, YAC clones

Table II. YAC Clones Selected for Localization of the Breakpoint on Chromosomes (A) 6p22 and (B) 11p15^a

Cycle No.	Clone	Position (cM)	Position (cR)	FISH results
A				
1	901a10	42, 43	135	Distal
1	753h12	44	149, 171	Distal
2	875f4	45	187, 196	Distal
2	963f2	54	219, 252	Proximal
3	923b5	—	—	Spanning (weak)
3	755c6	49	202	Proximal
3	808h5	50	—	Proximal
3	819a10	54	238, 241	Proximal
4	753h12	44	149, 171	Spanning
B				
1	967a5	13	—	Distal
1	847a12	—	43, 44	Distal
1	954f4	16	46	Distal
1	814a4	22, 24	—	Distal
1	776e7	25	59, 60, 65	Proximal
2	933b8	19	49	Distal
2	763a10	20	49	Distal
2	791g9	22, 24	—	Distal
2	808b11	22, 24	55	Spanning
2	803f11	25	58, 62, 65	Proximal
2	953d9	25	60, 62, 65	Proximal

^a Clones shown in boldface were selected for optimization and blastomere analysis.

814a4 and 808b11 were selected for optimization and blastomere analysis.

Probe Optimization

For each of the breakpoints involved in any translocation, it is crucial to obtain probes with the highest hybridization efficiency for interphase analysis. After the identification of suitable clones, either spanning or flanking a breakpoint, probe optimization is the next important step. For analysis of cells derived from Patient A, clones 939b12, 785e12, and 911f2 were optimized for the breakpoint on chromosome 11q. For chromosome 22q11, we optimized only the YAC probes, because the probes prepared from YAC clones 881h10 and 849e9 gave brighter signals than the probe made from BAC clone 563b9. For the interphase cell analysis of blastomeres in the case of Patient B, we optimized one YAC clone (753h12) for chromosome 6p and two YAC clones (814a4 and 808b11) for chromosome 11p.

We prepared highly purified YAC insert DNA by PFGE separation of the YACs from the host DNA. Plug preparation was done from single colonies and PFGE conditions were adjusted according to the size of the clone. Following excision of the respective bands from the gel and β -agarase digestion of the gel slices (30), the purified YAC DNA was PCR amplified using mixed-base primers (31). The probes for chromosomes 22q (Patient A) were labeled with fluorescein-12-dUTP (green). The probes for chromosomes 11p (Patient B) were labeled with biotin-14-dCTP and detected with fluoresceinated avidin (green). The probes for chromosomes 11q (Patient A) and 6p (Patient B) were labeled with digoxigenin-11-dUTP and detected with an antidigoxigenin-rhodamine antibody (red). A typical hybridization result using lymphocytes carrying the t(11;22) is shown in Fig. 1C. As expected, one green, one red, two blue, and two red/green fusion signals were found in the carrier's interphase cells.

Preimplantation Genetic Diagnosis (PGD)

Before using the probes on the patient blastomeres, we tested them on unrelated blastomeres obtained from chromosomally normal embryos donated for research. This step is necessary to optimize the final hybridization procedure. For t(11;22)(q23;q11), the labeling scheme used for the blastomeres is shown in Fig. 1A. In the hybridization mixture, we included a commercially available centromeric probe for chromosome 11 (blue).

This differentiates the der (11) from the der (22) chromosomes in interphase nuclei. It produced an extra blue signal with the red/green or yellow fusion domain when a der (11) chromosome was present. Also shown are typical hybridization results obtained with a chromosomally normal unrelated blastomere (Fig. 1D). Figure 1E shows that hybridization results from an unrelated embryo likely to be triploid (Fig. 1E; three blue-red signals and three green signals).

Figure 1F shows the labeling scheme used for the blastomeres in the t(6;11) case. The FISH results using a normal blastomere are shown in Fig. 1G. Two pink-red signals indicate two normal copies of chromosome 6, and two blue-green signals indicate two normal copies of chromosome 11. The abnormal patient blastomere shown in Fig. 1H was biopsied from embryo 4 in cycle 2 and carried an unbalanced translocation with one der (6), in addition to one normal homologue of chromosome 6 and two normal copies of chromosomes 11.

A total of 17 embryos from Patient B was analyzed in two cycles. The results are summarized in Tables IIIA and B. To assess additional numerical chromosome aberrations, cells biopsied in the first cycle were re-hybridized with probes specific for chromosomes 13, 18, 21, X, and Y (36). Only 2 of the 17 embryos were found to be normal with respect to chromosomes 6 and 11. Three embryos were found to be arrested and thus were not biopsied. Nine embryos were found to be abnormal, one cell had no nucleus, and two cells biopsied in cycle 1 gave unclear results (Table III).

DISCUSSION

Access to on-line physical mapping databases and in-house resources such as yeast artificial chromosome libraries now allows us to determine the location of a translocation breakpoint in as little as 3 weeks. The process of breakpoint mapping is facilitated by lymphocyte metaphase spreads prepared from carriers of balanced translocations which contain the normal homologues plus the derivative chromosomes. Classifying probes as mapping either proximal or distal to the breakpoint becomes straightforward, since additional chromosome-specific probes such as centromeric repeat probes can be included in the hybridization mixture, if necessary. In most of the 18 cases that we have dealt with in the last 2 years (Table IV), however, the normal and derivative chromosomes were easily identifiable and centromeric probes were not needed during probe development.

Table III. Analysis of Embryos from Patient B Carrying a Balanced t(6;11)

A. Cycle 1 results											
Embryo	Translocation				Aneuploidy					Diagnosis	Rebiopsy
	6	der6	11	der11	XY	13	16	18	21		
1	Arrested embryo									Not biopsied	Abnormal
2	1	0	2	1	XY	1	3	2	2	Abnormal	Abnormal
3	2	0	1	1	XX	2	2	1	2	Abnormal	Abnormal
4	1	0	0	0	X	1	0	1	0	Abnormal	Abnormal
5	2	0	2	0	XY	2	2	2	2	Normal	
6	Arrested embryo									Not biopsied	Not analyzed
7	Arrested embryo									Not biopsied	Not analyzed
8	2	0	1	1	NA ^a	NA	NA	NA	NA	Abnormal	Abnormal
9	1	NA	1	NA	XX	1	2	2	2	Unclear	Abnormal
10	NA	NA	NA	NA	NA	NA	NA	NA	NA	Unclear	No result
B. Cycle 2 results											
Embryo	6	der6	11	der11	Diagnosis						
1	—	—	—	—	No nucleus						
2	2	0	1	1	Abnormal						
3	1	1	2	0	Abnormal						
4	2	0	2	0	Normal						
5	2	1	1	0	Abnormal						
6	2	0	1	1 (2 cells)	Abnormal						
7	2	1	1	0	Abnormal						

^a Not analyzable.

The objective of our probe development was the preparation of bright, breakpoint-spanning probes. The small number of cells and the brief time frame (hours) available for PGD required probes which performed in FISH experiments with virtually 100% efficiency. To ensure unambiguous identification of normal and derivative chromosomes in interphase cell nuclei, we therefore optimized all probes prior to use on the patient's blastomeres. The optimization process

involved PFGE and DOP-PCR as well as random priming in the presence of different reporter molecules. Occasionally, we had to combine several clones to obtain sufficient coverage. Finally, the probes were tested on unrelated blastomeres biopsied from embryos donated for research and the color labeling scheme was defined before the probes were used for preimplantation genetic diagnosis. This test was necessary, because cells fixed individually, such as blastomeres, typically have a higher hybridization efficiency than lymphocytes (12).

The total time to prepare an optimized case-specific probe set for interphase PGD of translocation is presently 7–8 weeks. The price associated with this procedure averages about \$5000 per case. However, we anticipate significant improvements in turnaround time and, thus, a lower cost, as new libraries (such as the BAC libraries) become available and the amount of physical mapping data increases. Further technical developments in this area combined with even better high-resolution integrated maps should make this technology affordable for the majority of translocation carriers.

It is interesting to note that the breakpoint locations among our 18 patients appear to be spread across the entire genome (Table III). Therefore, we could not take

Table IV. Cases Studied, 1996–1998

46,XX; t(5;11)(q31;q21-22)
 46,XX; t(12;20)(p13.1;q13.3)
 46,XX; t(7;20)(q22;q11.2)
 46,XX; inv(6)(p23;q23.1)
 46,XY; t(12;17)(q23;p12)
 46,XY; t(3;4)(p24;p15)
 46,XY; t(11;22)(q23;q11)
 46,XX; t(4;14)(p15.3;q24)
 46,XX; t(1;4)(p36.3;q31.3)
 46,XY; t(6;11)(p22.1;p15.3)
 46,XY; t(14;17)(p11.2;p11.1)
 46,XX; del(18)(p11)
 46,XY; t(4;10)(q33;p12.2)
 46,XY; t(7;16)(p13;q24)
 46,XY; t(10;18)(q24.1;p11.2)
 46,XY; t(5;6)(q33;p23)
 46,XY; t(17;20)(p13;p12)
 46,XY; t(6;10)(q23;q26)

advantage of probes prepared for previous cases. This is expected to change with time and an increasing number of probes and/or cases. The use of preexisting probes in the initial cycles will help to expedite breakpoint mapping and will further decrease the overall cost of probes preparation.

Only 2 of our 18 PGD patients (11%) carried intrachromosomal rearrangements: 1 patient presented with a pericentric inversion of chromosome 6 (karyotype: 46,XX, inv (6) (p23; q23.1) (23), while the second patient had an interstitial deletion on the short arm of chromosome 18 (karyotype: 46,XX; del (18) (p11)) (Table III). A meaningful statistical analysis of our patient group, however, is difficult, since these patients were selected for the preparation of case-specific probes, because simpler PGD schemes were not applicable. As mentioned before, polar body analysis with whole-chromosome painting probes is the technique of choice in cases, when the woman carries a balanced reciprocal translocation (6,7,9). For commercially available WCP probes to work reliably, a major chunk of DNA (typically at least several megabasepairs) needs to be translocated. Therefore, either most female patients in our group carried intrachromosomal rearrangements or the translocation breakpoints were far distal, and WCP probes failed to demonstrate the translocations.

Although very interesting, a detailed analysis of the chromosomal composition of the embryos studied in these 18 cases and, in particular, a discussion of the distribution of balanced and unbalanced gametes would go beyond the scope of this more technically oriented paper. While recent publications listed some of these results (23,25,26), we plan to compile the extensive data in a future communication.

In summary, many affected couples can now receive help from centers offering assisted reproduction and PGD. If the woman carries the translocation, preselection of eggs prior to IVF should increase the chances of fertilization and nidation. The analysis can be performed on first polar bodies biopsied from eggs retrieved for IVF (6,7,9,16). If the male partner carries the translocation, eggs are fertilized first and allowed to develop for three days. At that point, blastomeres can be biopsied for PGD (8,18). As demonstrated above, reverse cloning of translocation breakpoints in YAC clones allows unambiguous differentiation between normal and derivative chromosomes in single interphase cells. Without modification, this procedure can detect intrachromosomal rearrangements such as deletions or inversions (23). This will greatly facilitate

the selection of oocytes for IVF and embryos for transfer following blastomere analysis.

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