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High-Throughput Analysis of Chromosome Abnormality in Spontaneous Miscarriage Using an MLPA Subtelomere Assay With an Ancillary FISH Test for Polyploidy

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Chromosome analysis of spontaneous miscarriages is clinically important but is hampered by frequent tissue culture failure and relatively low-resolution analysis. We have investigated replacement of conventional karyotype analysis with a quantitative subtelomere assay performed on uncultured tissue samples, which is based on Multiplex Ligation-Dependent Probe Amplification. This assay is suitable for this purpose as approximately 98% of all observed karyotype abnormalities in spontaneous miscarriages involve copy-number change to one or more subtelomere regions. A pilot study has compared karyotyping and subtelomere analysis on 78 samples. Extensive tissue necrosis accounted for failure of both karyotyping and subtelomere testing in four (5.1%) samples. Excluding these, there were no (0/74) subtelomere test failures compared to 9.5% (7/74) karyotype failures. Twenty-two (30%) whole chromosome aneuploidies and five (6.8%) structural abnormalities were detected using the subtelomere assay. With the exception of three cases of triploidy, all karyotype abnormalities were detected by the subtelomere assay. Following on from this study, a further 100 samples were tested using the subtelomere assay in conjunction with a simple ancillary FISH test using uncultured cells to exclude polyploidy in the event of a normal subtelomere assay result. Except for three necrotic samples, tests results were obtained for all cases revealing 18 abnormalities including one case of triploidy. Taking into consideration the high success rate for the combined MLPA and FISH test results, and the very significant additional advantages of cost-effective, high-throughput batching, and automated, objective analysis, this approach greatly facilitates routine investigation of chromosome abnormalities in spontaneous miscarriage.

Key words: MLPA; prenatal diagnosis; spontaneous miscarriage; aneuploidy; subtelomere

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INTRODUCTION

Chromosome abnormalities are a well-established cause of pregnancy loss. In the first trimester, 10–15% of clinically recognized pregnancies spontaneously miscarry and approximately half of these have a detectable chromosome abnormality known to cause non-viability [Hassold et al., 1980; Lomax et al., 2000]. By far the largest abnormal category is autosomal aneuploidy (~75%), followed by polyploidy (~13%), monosomy X (~8%) and structural imbalance (~4%) [Lomax et al., 2000; Yusuf and Naeem, 2004]. Identification of the underlying cause of any individual spontaneous miscarriage is important for patient counseling and for assessment of recurrence risk and risk of viable abnormal offspring in subsequent pregnancies [Franssen et al., 2006].

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Identification of a chromosome abnormality with relevant significance not only provides an explanation but also removes the need for other investigations. Despite clinical demand, karyotyping of miscarriage material is generally not afforded as high priority as prenatal testing in many laboratories. A common problem is failure (10–40%) of samples to grow in culture [Lomax et al., 2000; Fritz et al., 2001; Benkhalifa et al., 2005]. Furthermore, metaphase quality is typically poor with this type of sample and there is a propensity for maternal rather than fetal cells to grow [Bell et al., 1999]. Recent approaches to overcome these problems have used fetal DNA for metaphase [Lomax et al., 2000; Fritz et al., 2001] or microarray [Schaeffer et al., 2004; Benkhalifa et al., 2005; Le Caignec et al., 2005] comparative genomic hybridization. We have taken a new approach to address these problems. As almost all abnormalities observed in pregnancy involve gains or losses of chromosome ends, we have used a quantitative assay that measures subtelomere copy number [Northrop et al., 2005]. Increased or decreased subtelomere copy number at both ends of any individual chromosome indicates a whole chromosome aneuploidy. Increased dosage of one chromosome end indicates a segmental aneuploidy. Increased dosage of one chromosome end with decreased dosage at the end of a different chromosome is suggestive of one abnormal chromosomes derived from a balanced parental translocation. Detection of chromosome abnormalities by karyotyping and by the subtelomere assay is compared in a pilot study of 78 unselected, spontaneous miscarriage samples, with a follow-up study of 100 samples using the subtelomere assay with an ancillary FISH test for polyploidy.

MATERIALS AND METHODS

Sample Collection, DNA Extraction and Cytogenetic Analysis

This prospective study tested spontaneous miscarriage samples referred to the Cytogenetics Laboratory of Victorian Clinical Genetics Services for karyotype analysis. In the pilot study, MLPA analysis was performed on a consecutive set of spontaneous miscarriage samples (34 chorionic villus (PV) and 44 fetal tissue (FT) samples) referred for karyotyping. The mean gestational age was 19 weeks. The follow-up study used samples (16 PV and 84 FT) referred from a single specialist obstetrics unit, the higher proportion of FT samples reflecting its tertiary level practice. The mean gestational age for these was 22 weeks. Karyotyping was not performed on the follow-up study samples.

For PV samples, maternal decidua, mucus, and blood clots were dissected using a stereo microscope and removed; strands of chorionic villi were sampled from at least two sites. FT (1–5 mg, pilot study; 5–10 mg, follow-up study) was taken for DNA extraction and the remainder used for cell culture and cytogenetic analysis. Samples for DNA extraction were digested in a 200 μl mix, consisting of 10 μl of proteinase K and 190 μl of G2 Buffer (Qiagen, Victoria, Australia; www.qiagen.com), for 16 hr at 56°C. Genomic DNA was extracted using the Progenome™ II Extraction Kit (Progen, QLD, Australia; www.progen.com.au). DNA was diluted in distilled water to a concentration of 50 ng/μl and stored at −20°C until use. Cell culture, G-banding and karyotype analysis was performed using standard protocols.

Polyploidy Testing Using FISH

An uncultured cell suspension was prepared from each sample in the follow-up study on receipt. One to five milligrams of tissue was chopped finely with a scalpel blade, suspended in culture media, dropped onto a Polysine™ slide (Menzel-Glaser; www.menzel.de) and immediately fixed. All slides were stored at room temperature in the dark until required. If the subtelomere assay showed no abnormality, that is, excluded any aneuploidy, the slide was processed using dual color interphase FISH with locus-specific DNA probes for chromosomes 13 (*RB1* gene) and 21 (D21S59, D21S341, and D21S342) (Vysis, NSW, Australia; www.vysis.com) to test for polyploidy. For each sample, 20 interphase cells were analyzed. The analysis was performed using a Zeiss Axioscop microscope equipped with a Cytovision Image Analysis System (Applied Imaging Ltd, Newcastle, UK; www.aicorp.com).

MLPA Subtelomere Test

The subtelomere test was performed as previously described [Northrop et al., 2005] using DNA samples (250 ng) with appropriate positive and normal controls. Two independent sets of subtelomeric probes (P036B and P070) were used which target two loci at each chromosome subtelomere region (MRC-Holland, www.mrc-holland.com). The P036B and P070 probe sets each target one unique locus per subtelomeric region for all chromosomes except the acrocentric chromosomes where pericentric, long arm loci are used. Probes for the pseudoautosomal regions in the X and Y chromosomes and Y-specific probes are included. The two probe sets have no subtelomeric probes in common. The median distance of target sequences from the telomere is 0.54 and 0.37 Mb for the P036B and P070 sets, respectively. PCR products were separated using capillary electrophoresis (MegaBace, General Electric, Victoria, Australia; www.ge.com/en).

Data Analysis

Data analysis used the relative peak height (RPH) method as previously described [Slater et al., 2003, 2004; Northrop et al., 2005]. Briefly, each individual subtelomeric region's peak height is divided by the sum of all subtelomere peak heights. Each normalized peak height is then divided by the corresponding average, normalized peak height from five normal control samples to derive the RPH. Theoretically, heterozygous deletions and duplications show an RPH of 0.5 and 1.5, respectively. Thresholds were determined as the mean of P036B and P070 data from the total sample data [Northrop et al., 2005] and are RPH values of $\leq$0.65 and $\geq$1.35 for deletions and duplications, respectively. The mean RPH $\pm$ 1.96 SD (95% CI) was used to define the normal range for all subtelomeric probes (P036B and P070) as 0.84–1.16.

The parameters and performance of the quantitative MLPA subtelomere assay have been described in detail [Northrop et al., 2005]. By using two independent probes per subtelomere region, that is, one from the P036B and one from the P070 probe sets, and calculating the mean RPH, the number of subtelomere copies for all chromosomes can be readily determined. The distributions of two-copy measurements (normal karyotypes), one-copy abnormalities (whole chromosome or segmental monosomies) and three-copy abnormalities (whole chromosome or segmental trisomies) were found to be almost nonoverlapping and fit well with the threshold values determined previously. The P036B and P070 copy number measurements for any one subtelomere region are almost always concordant. Rare discordant results (none observed in this study) are almost certainly due to probe binding site or copy number polymorphism, even though careful selection of loci has minimized this possibility. Any discordance between the copy number indicated by a pair of subtelomere probes should be resolved using FISH or quantitative PCR analysis. Interpretation of results should take into account any inference using copy number at the probe loci to regions beyond these loci, especially where only one subtelomere region shows an abnormality. Again, further investigation using FISH, quantitative PCR or parental karyotyping might be appropriate.

RESULTS

A blinded pilot study was conducted to compare the results of MLPA subtelomere analysis and karyotyping in 78 spontaneous miscarriage samples with respect to detection of chromosome abnormalities. There were 34 placental villi and 44 fetal tissue samples collected. The parameters and performance of the quantitative MLPA subtelomere assay have been described in detail [Northrop et al., 2005]. The appropriateness of these thresholds for this application is valid. The mean RPH values calculated for the pilot study (Table I) for the one, two, and three subtelomere copy measurements are 0.50 ± 0.10 ($n = 8$), 1.03 ± 0.16 ($n = 46$), 1.58 ± 0.23 ($n = 23$)

TABLE I. Pilot Study-Comparison of Karyotype and Subtelomere Test Results on 78 Spontaneous Miscarriages

Case	Karyotype	Subtelomere assay	Cases	PV	FT	RPH (mean $\pm$ SD, n)
1–18	46,XX	No abnormality detected (F)	18	4	14	1.02 ± 0.13, n = 92
19–38	46,XY	No abnormality detected (M)	20	6	14	1.02 ± 0.12, n = 92
39–40	69,XXY	No abnormality detected (M)	2	2	0	1.04 ± 0.20, n = 92
41	68,XXX,−22	del22p(subtel);del22q(subtel) (F)	1	1	0	0.66, n = 2
42	47,XY, +5	dup5p(subtel);dup5q(subtel) (M)	1	1	0	$1.56 + 0.40$, n = 4
43	47,XX, +9	dup9p(subtel);dup9q(subtel) (F)	1	1	0	1.42 ± 0.25, n = 4
44	47,XX, +10	dup10p(subtel);dup10q(subtel) (F)	1	1	0	1.53 ± 0.43, n = 4
45	47,XY, +10	dup10p(subtel);dup10q(subtel) (M)	1	1	0	1.53 ± 0.21, n = 4
46	47,XX, +14	dup14p(subtel);dup14q(subtel) (F)	1	1	0	1.41 ± 0.26, n = 4
47–48	47,XX, +16	dup16p(subtel);dup16q(subtel) (F)	2	2	0	1.84 ± 0.51, n = 4
49–50	47,XY, +16	dup16p(subtel);dup16q(subtel) (M)	2	2	0	1.62 ± 0.18, n = 4
51	47,XY, +18	dup18p(subtel);dup18q(subtel) (M)	1	0	1	1.59 ± 0.14, n = 4
52–54	47,XY, +21	dup21p(subtel);dup21q(subtel) (M)	3	1	2	1.45 ± 0.25, n = 4
55	47,XX, +21	dup21p(subtel);dup21q(subtel) (F)	1	0	1	1.40 ± 0.08, n = 4
56–58	47,XX, +22	dup22p(subtel);dup22q(subtel) (F)	3	3	0	1.64 ± 0.25, n = 4
59–60	45,X	delX/Yp(subtel);delX/Yq(subtel) (F)	2	2	0	0.51 ± 0.05, n = 4
61	47,XY, +16[6]/46,XY[39]	dup16p(subtel);dup16q(subtel) (M)	1	1	0	1.64 ± 0.09, n = 4
62	47,XX,t(8;16)(q11.2;q13)mat, +16	dup16p(subtel);dup16q(subtel) (F)	1	1	0	1.74 ± 0.11, n = 4
63	46,XY,der(4)t(4;13)(p15.32;q32)pat	del4p(subtel);dup13q(subtel) (M)	1	1	0	0.33, n = 2; 1.5, n = 2
64	46,XY,der(6)t(6;8)(q25.3;q24.11)mat	del6q(subtel);dup8q(subtel) (M)	1	0	1	0.47, n = 2; 1.65, n = 2
65	46,XX,add(18)(p11.2)de novo	del18p(subtel);dup20p(subtel) (F)	1	0	1	0.47, n = 2; 1.8, n = 2
66	46,XX,add(22)(q13) de novo	del22q(subtel) (F)	1	0	1	0.58, n = 2
67	46,XY,del(4)(q33)de novo	del4q(subtel) (M)	1	0	1	0.47, n = 2
68–69	Culture failure	No abnormality detected (F)	2	0	2	1.03 ± 0.20, n = 92
70–73	Culture failure	No abnormality detected (M)	4	1	3	1.05 ± 0.18, n = 92
74–77	Culture failure	No DNA available	4	1	3	—
78	Culture failure	dup16p(subtel);dup16q(subtel) (M)	1	1	0	1.62 ± 0.04, n = 4
		Total	78	34	44	

which fit the threshold values of >0.65 and <1.35 for normal samples.

Results are summarized in Table I. Karyotyping was successful in 67 (86%) cases and the subtelomere assay was successful in 74 (95%) cases. There were four necrotic samples where both failed. Of the other 74 samples, 38 showed a normal karyotype and subtelomere assay result (cases 1–38). There were 22 whole chromosome aneuploidies in total (cases 42–62 and 78). The assay detected the trisomy in case 78 where culture failure precluded a karyotype. The trisomies found were of chromosomes 5, 9, 10, 14, 16, 18, 21, and 22 and there were two cases of monosomy X (cases 59 and 60) (Table I and Fig. 1). The karyotype in case 61 showed a mosaic trisomy 16 result in a male conceptus. Six samples (cases 62–67) showed one or more structural abnormalities, which were detected by both karyotyping and subtelomere testing. Three of these were unbalanced segregants, shown by follow-up testing to be inherited from a carrier parent (cases 62–64). The subtelomere test provided a more detailed description of the karyotype abnormality in cases 65 and 66, where an add(18) and an add(22) described in the karyotypes, was shown to be a der(18)t(18p;20p) (Figs. 1f and 2a) and a simple del(22q) (Fig. 1g), respectively.

Non-concordant results were obtained in cases 39 and 40 where the subtelomere assay did not detect an abnormality but karyotyping showed triploidy. Polyploidy is not detectable using this subtelomere assay as RPHs are adjusted to the same extent by the normalization process. Although essentially triploid, case 41 contained disomy 22, which was detected by the subtelomere assay.

The ratio of female to male karyotypes was 34–33 (1–1.03). A subtelomere abnormality was found in 20 out of 33 (61%) and 8 out of 41 (19.5%) of the PV and FT samples tested, respectively.

To address the non-specificity of the subtelomere assay for polyploidy, a simple FISH assay using probes for chromosomes 13 (*RB1* gene) and 21 (D21S59, D21S341 and D21S342) (Vysis Aneu-Vysion) was introduced using a slide prepared from an uncultured cell suspension at the time of sample receipt. These slides were tested if no whole or segmental aneuploidy was detected using the subtelomere assay result.

A further 100 samples (16 PV and 84 FT) were tested in a follow-up study using the subtelomere assay with this ancillary FISH test and 18 abnormalities were detected (Table II) including one case of triploidy (Fig. 2b). For these tests, DNA was extracted from 5 to 10 mg rather than 1 to 5 mg of tissue and this reduced the failure rate for the subtelomere assay from 5 to 3%.

DISCUSSION

A wide variety of trisomies was detected by the subtelomere assay in the two studies by increased dosage at four subtelomere loci, two at each chromosome end (Fig. 1a). All (trisomies 5,9,10,12,13,14, 15,16,18,21,22) were readily detected. This wide range of trisomies is typical of that observed in the karyotypes of spontaneous miscarriage material, especially from the first trimester. Unlike trisomy, whole-chromosome monosomy is rarely observed, probably due to conceptus demise in the very early stages of pregnancy. The exception is monosomy X, which is relatively common and is detected by single copy dosage of the loci located in the short (PAR 1) and long arm (PAR 2) pseudoautosomal regions common to both the X and Y chromosomes. Cases 59 and 60 showed these changes (Table I and Fig. 1e). Although, whole chromosome aneuploidy is inferred from the copy-number changes of loci at both ends of the chromosome in question, case 62 (Table I) demonstrates the need for careful interpretation. Here an interchange trisomy, derived from a reciprocal translocation of chromosomes 8 and 16, is the underlying origin of trisomy 16 but this is not revealed by the subtelomere assay. Although not observed in this series, this etiology is much more likely with Robertsonian translocations such as the common t(14;21)(q10;q10). It is therefore important to consider the possibility of a balanced translocation in a carrier parent, especially in cases of recurrent spontaneous miscarriage.

The karyotype of case 61 (Table I) showed mosaic trisomy 16; the mosaic component of this abnormality was not revealed by the subtelomere test. This trisomy in a male karyotype must be of post-zygotic origin and therefore has no significance for further pregnancies. It is of note that the mosaicism detected in the karyotype is relatively low level (13%) and the subtelomere test would not have the sensitivity to detect copy-number changes at this level. What has almost certainly happened here is that the normal cells have had growth advantage over the aneuploid cells. This exemplifies a well-recognized problem in the culture of miscarriage material, especially placental biopsies.

Six of the seventy-four cases tested showed structural abnormalities. Cases 63, 64, and 65 (Table I) showed concomitant subtelomere duplication and deletion of two different chromosomes. These findings suggest malsegregation of a parental reciprocal translocation and this was shown to be the case in cases 63 and 64 (Table I). The significant imbalances inferred from the parental karyotypes are the likely cause of miscarriage. Detection of this type of abnormality is of obvious importance for the management of future pregnancies, especially if there is a possibility of an alternative, abnormal segregant, which might be viable at term. The reproductive implications for other potential carrier relatives also need to be considered as illustrated for cases 63 (Table I) and 95 (Table II) where two brothers carrying the same reciprocal translocation

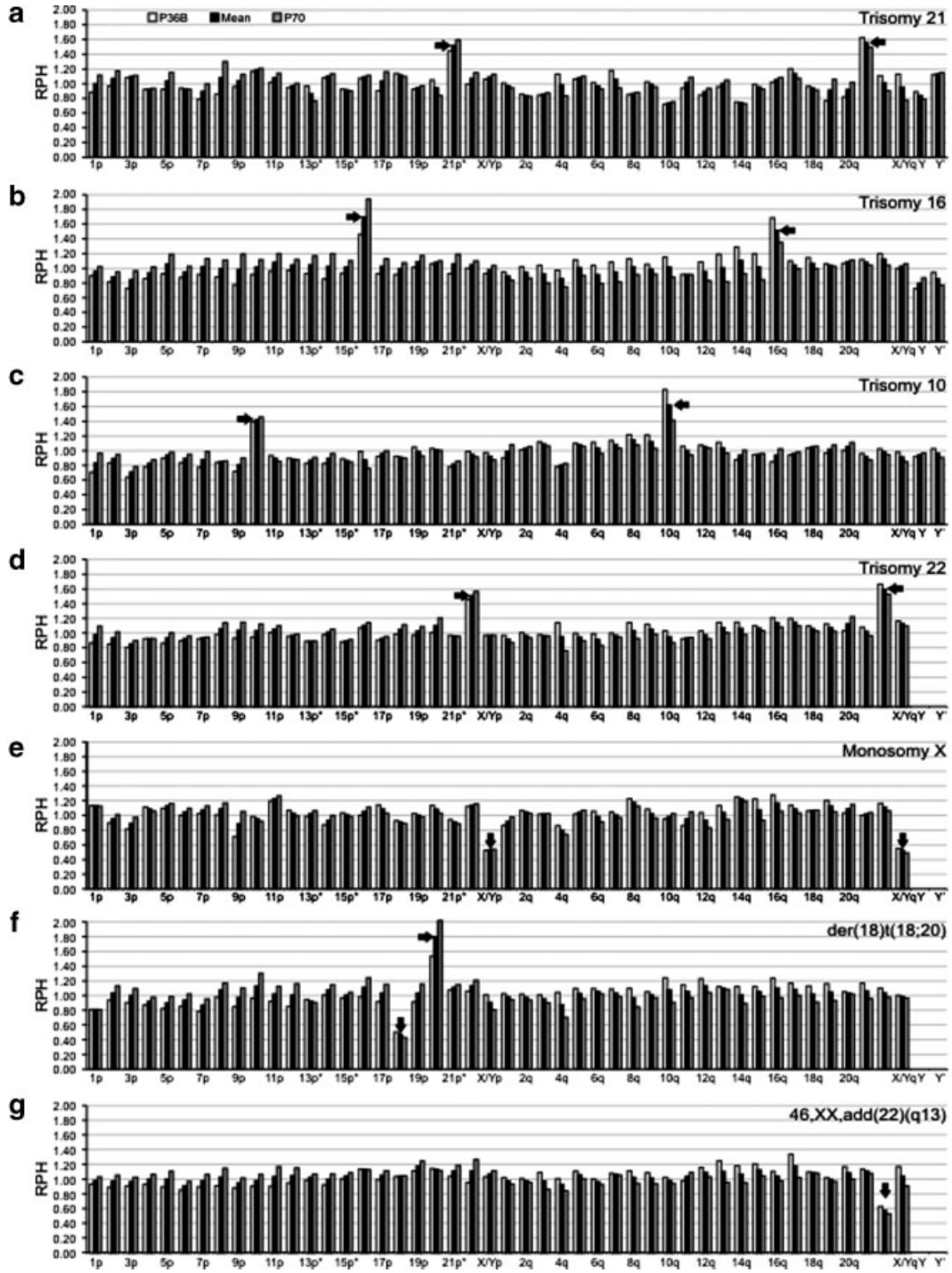

FIG. 1. Detection of whole chromosome and segmental aneuploidy using the subtelomere assay. Copy number (RPH) is indicated for each subtelomere from each probe set as well as the average value (see key). *Indicates a pericentric long arm probe location for acrocentric chromosomes. RPH values of 0.5, 1.0, and 1.5 indicate one, two and three subtelomere copies respectively except for the Y where 1.0 indicates one copy. **a**–**d**: Selected trisomies showing increased copy number at both ends of the chromosome in question. **e**: Monosomy X shown by a copy number of one for the X/Yp and X/Yq subtelomeres. **f**: Copy number of one for the subtelomere of 18p and three for 20p consistent with a derivative of a translocation between these chromosomes. **g**: Copy number of one for the subtelomere of 22q and two for all other subtelomeres indicating a simple terminal deletion.

have transmitted exactly the same derivative chromosome to miscarried pregnancies.

Cases 66 and 67 (Table I) showed subtelomere deletion in one chromosome only. This finding is more likely to be de novo and this was demonstrated for cases 66 and 67 through parental testing. Without the karyotype, the extent of the deletions would be unknown and even here the accuracy of breakpoint

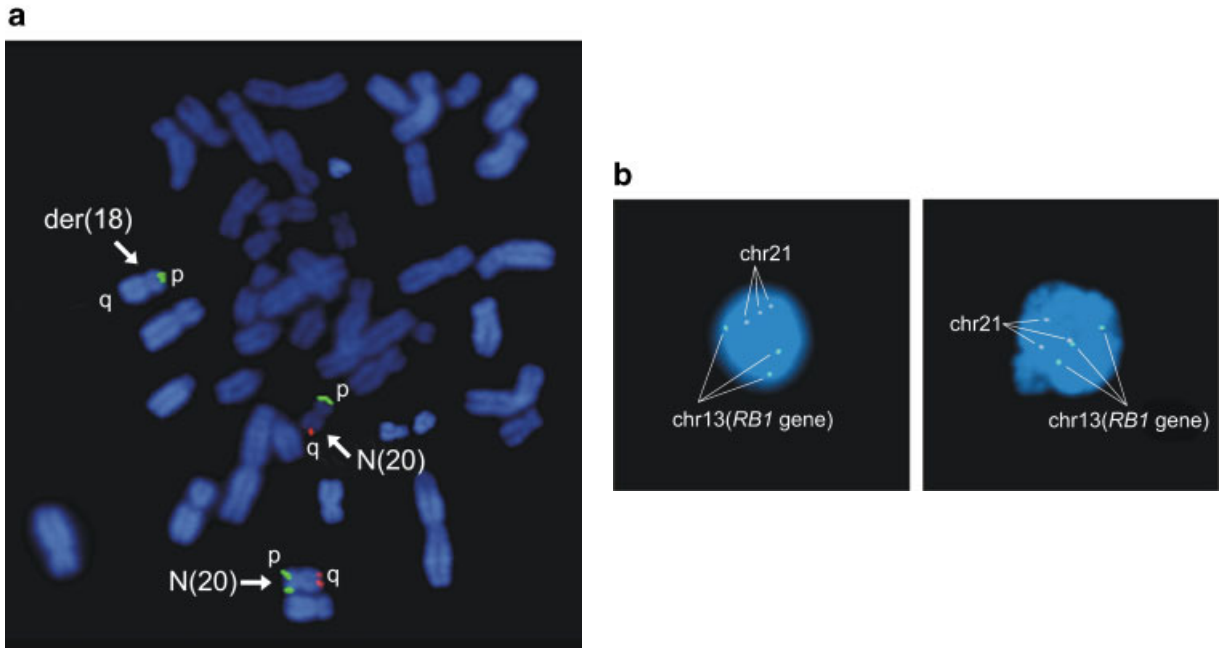

FIG. 2. Detection using FISH of (**a**) segmental duplication of the 20psubtel region in case 65 (Table I). The chromosome 20psubtel and 20qsubtel specific probes (Vysis) are indicated. **b**: Detection of triploidy in case 80 (Table II) by FISH performed on a slide prepared from an uncultured cell suspension. The probes for chromosomes 13 (*RB1* gene) and 21 (D21S59, D21S341, and D21S342) (Vysis) are indicated. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

determination is poor. If this information is required, mapping using quantitative PCR can be performed or FISH using an uncultured cell preparation as discussed further below.

The proportion of samples in the pilot study showing no abnormality is notably lower than that in the follow-up study (29% vs. 43% for PV and 64% vs. 86% for FT samples). As the incidence of karyotype abnormality is well established to be inversely proportional to gestation, this probably reflects the difference in the average gestation of the two groups (19 weeks vs. 22 weeks).

The main limitation of the assay is its inability to detect polyploidy, which is a relatively common cause of spontaneous miscarriage (~13%). Cases 39 and 40 (Table I), therefore, showed a normal subtelomere result discordant with the karyotypes. Case 41 (Table I) is essentially triploid but the disomy

TABLE II. Follow-Up Study-Results of Tests on 100 Spontaneous Miscarriages Using the Subtelomere Assay and FISH Test for Polyploidy*

Case	Subtelomere assay	Cases	PV	FT	RPH (mean ± SD, n)
1–40	No abnormality detected (F)	40	5	35	1.00 ± 0.10, n = 96
41–79	No abnormality detected (M)	39	2	37	1.01 ± 0.11, n = 96
80	No abnormality detected (F)[a]	1	1	0	1.01 ± 0.10, n = 96
81	dup12p(subtel);dup12q(subtel) (F)	1	1	0	1.50 ± 0.09, n = 4
82	dup13p(subtel);dup13q(subtel) (F)	1	1	0	1.60 ± 0.13, n = 4
83	dup15p(subtel);dup15q(subtel) (M)	1	0	1	1.60 ± 0.14, n = 4
84	dup16p(subtel);dup16q(subtel) (M)	1	1	0	1.40 ± 0.16, n = 4
85–86	dup18p(subtel);dup18q(subtel) (M)	2	0	2	1.50 ± 0.17, n = 4
87–89	dup21p(subtel);dup21q(subtel) (M)	3	0	3	1.40 ± 0.15, n = 4
90–91	dup21p(subtel);dup21q(subtel) (F)	2	0	2	1.40 ± 0.08, n = 4
92	dup22p(subtel);dup22q(subtel) (F)	1	1	0	1.63 ± 0.18, n = 4
93–94	delX/Yp(subtel);delX/Yq(subtel) (F)	2	1	1	0.52 ± 0.06, n = 4
95	del4p(subtel);dup13q(subtel) (F)[b]	1	0	1	0.48, n = 2; 1.51, n = 2
96	del5p(subtel) (M)	1	0	1	0.51, n = 2
97	dup3p(subtle) (F)	1	0	1	1.42, n = 2
98–100	No DNA available	3	3	0	—
	Total	100	16	84	

*FISH for polyploidy was performed in all cases where the subtelomere assay showed a normal result. This used a slide prepared using an uncultured cell suspension prepared at the time of sample receipt.

[a]Triploidy was detected in this case using the ancillary FISH test.

[b]The same abnormality as in case 63 of Table I but from another carrier.

22 was detected by the subtelomere assay which identified the low RPHs for all four chromosome 22-specific loci relative to those for all other chromosomes (Table I). Consequently, to identify polyploidy in a follow-up study (Table II), we initiated FISH testing of an uncultured cell preparation, made on receipt of each sample, for all cases showing a normal subtelomere result. Polyploidy is readily detected (Fig. 2b) using probes for chromosomes 13 (*RB1* gene) and 21 (D21S59, D21S341, and D21S342).

Other discordances were found where karyotyping (cases 65 and 66; Table I) and subtelomere testing (cases 41, 61, 62; Table I) provided incomplete descriptions of the abnormalities present. These arise out of the limitations of both tests.

A major aim of using subtelomere testing is to reduce the karyotype failure rate which is a very significant problem and disincentive for routine spontaneous miscarriage testing [Lomax et al., 2000; Schaeffer et al., 2004]. Four cases (cases 74–77; Table I) showing extensive tissue necrosis failed both tests. Other than these, there were 7/78 (9.0%) karyotype failures and 0/74 (0%) subtelomere test failures. In a follow-up series of 100 samples (Table II: 16 PV and 84 FT) tested using the subtelomere assay with the ancillary FISH test for polyploidy, the amount of tissue used for DNA extraction was increased from 1–5 to 5–10 mg. This eliminated the problem of inability to perform the subtelomere assay due to inadequate DNA amounts in all except necrotic samples.

Array analysis using Comparative Genomic Hybridization (array CGH) has also been used for analysis of spontaneous miscarriage material [Schaeffer et al., 2004; Le Caignec et al., 2005]. The first of these studied only eight spontaneous miscarriage samples with no aneuploidies detected but the second analyzed 41 chorionic villus samples and reported a total of 15 aneuploidies and five structural abnormalities. Two of the structural abnormalities were interstitial deletions coincidental with an autosomal trisomy: a duplication of 15q11-q13 unlikely to have significance in pregnancy loss and a putative unconfirmed deletion of 9p21. A duplication of 10qtel was found but this has been reported and interpreted in three cases elsewhere as a probable polymorphism [Le Caignec et al., 2005]. With the exception of the interstitial deletions, all the abnormalities found are detectable using the subtelomere assay. As for subtelomere testing, detection of polyploidy is not possible using array CGH and neither of the above studies addressed this issue. However, an earlier study [Lomax et al., 2000] using CGH with metaphase chromosomes used flow cytometry as an adjunct test for this purpose. Similarly, balanced translocations are not detected by either technique although these are a rare cause of spontaneous miscarriage.

Both array CGH and the MLPA subtelomeric assay detect the currently observed range of relatively large-scale chromosome abnormality found in spontaneous miscarriages. However, rare cases of interstitial abnormality are found by karyotyping and it cannot be assumed that cryptic interstitial abnormalities might not also account for some spontaneous miscarriages. The MLPA subtelomeric assay will not detect any such interstitial abnormalities. On the other hand, a suitable array CGH platform would, but this is not suitable for high-throughput and has the current caveat of cost of testing. For both approaches, an ancillary test for polyploidy would still be necessary.

In summary, the MLPA subtelomere assay in conjunction with the ancillary FISH test offers significant advantages over conventional karyotyping. In particular, it overcomes the problem of culture failure for all except necrotic samples. MLPA assays are widely used in many applications [Sellner and Taylor, 2004; White et al., 2004] where copy number measurement is required and have proved to be extremely robust. Batch processing using standard 96-multiwell format and data analysis automation provides very significant gains in efficiency and cost-effectiveness to facilitate testing of spontaneous miscarriage samples.

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