by a Health Sciences Research Grant for Research on Human Genome (H10-Genome-008) from the Ministry of Health, Labour and Welfare of Japan. After the women and their spouses in these cases had been given easy to understand, yet detailed information on this study and its purposes, all parties agreed to allow the use of parental and fetal materials for analysis. Peripheral blood from the woman and her spouse, and chorionic villi from the abortion were obtained in each case. The study was approved by the Institutional Review Board (IRB) of Institute for Developmental Research, Aichi Human Service Center, and the IRB of Nagoya City University Medical School.

Chromosomal analysis

Part of the chorionic villi from 174 cases of spontaneous abortion were cultured, and cells were harvested at 6–22 days of cultivation to analyze the chromosomes. Chromosomal analysis was performed with Q- and G-banding.

DNA preparation

Genomic DNAs for molecular studies were extracted from chorionic villi of spontaneous abortions and from peripheral blood of the parents using a DNA extraction kit (ISOTISSUE, Nippon Gene, and Dr GenTLE, Takara Bio).

Microsatellite analysis

Microsatellite markers were used to determine the parental origin of extra chromosomes and the stage at which the extra chromosomes occurred. Six to 18 microsatellite markers from the Genethon collections (Dib *et al.* 1996), which spanned the whole length of extra chromosomes in cases with double trisomy, were analyzed. Most of the primer sets used in this study were gratefully accepted gifts from Professor Y. Nakamura of the Institute of Medical Sciences, University of Tokyo, Tokyo. The remaining primers were synthesized. Genotypes of the fetus and the parents for each marker locus were determined using the DNA-sequencer-assisted method with fluorescent microsatellite marker DNAs (Fujimoto *et al.* 1998; Alf Express Fragment Manager, Amersham Pharmacia Biotech, USA).

RESULTS

Of 174 cases of spontaneous abortion that we obtained and cultured, 170 (97.7%) were successfully karyotyped. In these cases, 83 (48.8%) were euploidies and 87 (51.2%) had chromosomal abnormalities, of which 47 (27.7%) were trisomies, four (2.4%) were double trisomies, 15 (8.8%) were polyploidies, seven (4.1%) were X monosomies, six (3.5%) were translocations, and eight (4.7%) were mosaics. The karyotypes of the four cases with double trisomy were 48,XX,+16,+22 (Case 1), 48,XXY,+18 (Case 2), 48,XX,+15,+21 (Case 3) and 48,XX,+2,+5 (Case 4).

The results of polymorphic analysis of microsatellites are summarized in Table 1. More than two informative patterns were demonstrated on each extra chromosome. Examples of microsatellite polymorphic pattern of abortions and parents are given in Figs 1 and 2.

Polymorphic analysis of microsatellites on extra chromosomes 16 and 22 in Case 1 and extra chromosomes X and 18 in Case 2 were all revealed to be maternal in origin. In addition, polymorphic patterns of microsatellites on loci near

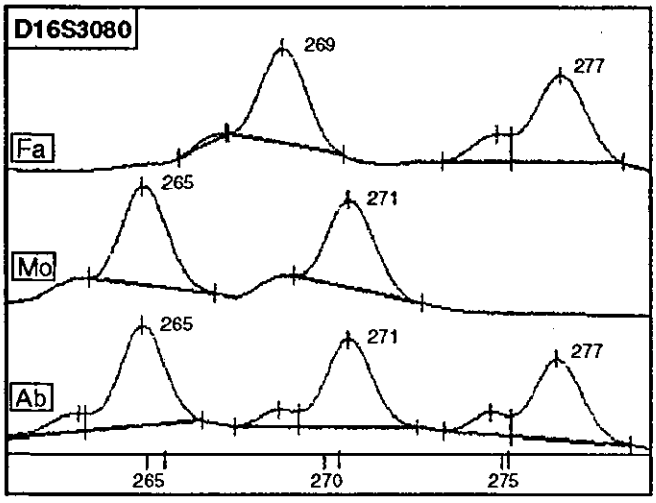

Fig. 1 Sample of microsatellite polymorphic patterns analyzed by ALF DNA sequencer (see text). The figure was obtained by polymerase chain reaction (PCR) using the primer D16S3080. Three distinct patterns are seen in the abortus (Ab) of Case 1. One pattern was derived from the father (Fa) and the other two were from the mother (Mo).

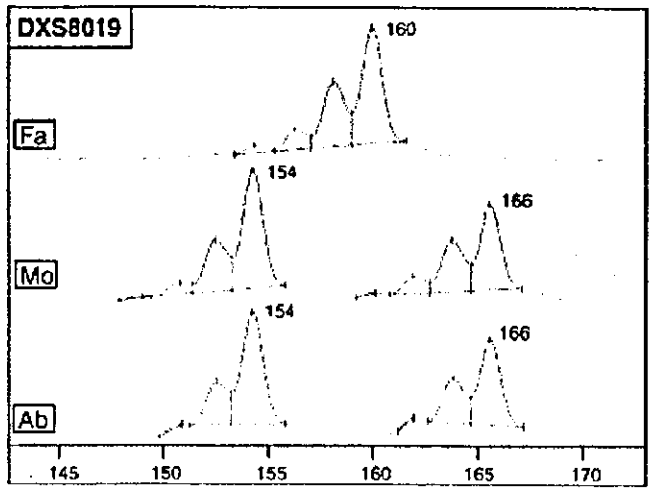

Fig. 2 Microsatellite polymorphic patterns obtained by PCR using the primer DXS8019. The figure indicates that the two X chromosomes of the abortus originated from the different X chromosomes of the mother. Ab, abortus; Fa, father; Mo, mother.

Table 1 Polymorphic analysis of microsatellites in abotuses and their parents

Chromosome constitution	Chromosome no.	Symbol	PCR products (base pair) Father	Mother	Abortus†	Origin of an extra chromosome
48XX,+16,+22	16	D16S423	135, 139	**137, 141**	135, **137, 141**	MI
		D16S407	140, 154	**152, 156**	**152**, 154, **156**	
		D16S3080	269, 277	**265, 271**	**265, 271**, 277	
		D16S3140	135, 157	**149, 155**	135, **149, 155**	
		D16S511	186, 204	**194, 196**	186, **194, 196**	
	22	D22S446	196, 200	**194, 194**	**194, 194**, 196	MI
		D22S275	160, 166	**156, 162**	**156, 162**, 166	
		D22S1158	220, 234	**220, 238**	**220**, 234, **238**	
		D22S283	132, 134	**128, 132**	**128, 132**, 134	
		D22S1171	134, 138	**142, 144**	134, **142, 144**	
48XXY,+18	18	D18S59	142, 152	**140, 148**	**140, 148**, 152	MI
		D18S453	141, 143	**139, 141**	**139, 141**, 143	
		D18S57	88, 92	**88, 96**	**88**, 92, **96**	
		D18S1147	202, 218	**206, 216**	**206, 216**, 218	
		D18S1141	233, 235	**237, 243**	233, **237, 243**	
	X	DXS7108	252	**244, 250**	**244, 250**	MI
		DXS8019	160	**154, 166**	**154, 166**	
		DXS8020	197	**195, 199**	**195, 199**	
		DXS8094	233	**221, 221**	**221, 221**	
		DXS1073	203	**205, 207**	**205, 207**	
48XX,+15,+21	15	D15S1035	172, 178	**172, 176**	**176, 176**, 178	Mi
		D15S978	213, 217	**211, 213**	**211, 211**, 213	
		D15S155	265, 265	**253, 263**	**263, 263**, 265	
		D15S979	157, 165	**147, 157**	**147, 147**, 157	
		D15S1014	178, 180	**180, 182**	178, **182, 182**	
	21	D21S1899	161, 161	**161, 163**	161, **163, 163**	Mi
		D21S270	197, 199	**203, 207**	199, **207, 207**	
		D21S1899	268, 272	**258, 272**	**258, 258**, 268	
		D21S1890	159, 161	**153, 159**	**153, 153**, 161	
48XX,+2,+5	2	D2S281	243, 245	**245, 247**	245, **247, 247**	Mi
		D2S2153	154, 164	**146, 154**	**146, 146**, 154	
		D2S114	218, 220	**224, 228**	218, **228,228**	
		D2S163	213, 227	**221, 223**	**223, 223**, 227	
		D2S206	119, 125	**125, 133**	125, **133, 133**	
	5	D5S1987	193, 205	**201, 203**	**201, 201**, 205	Mi
		D5S646	285, 285	**277, 285**	**277, 277, 285**	
		D5S495	223, 237	**221, 223**	**221, 221**, 223	
		D5S404	186, 190	**188, 188**	186, **188, 188**	
		D5S498	177, 183	**177, 177**	**177, 177**, 183	

†PCR, products of probable maternal origin are shown in bold; MI, meiosis I; Mi, first mitotic division.

the centromere of each extra chromosome showed heterozygosity. The finding indicated that the extra chromosomes were produced by non-disjunction at the first meiotic division. However, microsatellite polymorphisms of extra chromosomes 15 and 21 in Case 3 and extra chromosomes 2 and 5 in Case 4 were also revealed to be maternal in origin, and the patterns on the loci near the centromeres all showed homozygosity. The results indicated that the extra chromosomes of two cases (Cases 3 and 4) might be produced by non-disjunction at the first mitotic division.

In the present study, the mean age of females with spontaneous abortion of double trisomy was 34.5 ± 6.6 years at the time of spontaneous abortion, and that of females with abortions of single trisomy was 32.9 ± 4.1 years. The pooled data from the present study and other studies in the literature revealed a mean age of females with abortion of double trisomy and single trisomy of 34.2 ± 5.9 years and 31.5 ± 5.6 years, respectively (Table 2) (Lauritsen 1976; Hassold *et al.* 1980; Kajii *et al.* 1980; Ohno *et al.* 1991; Dejmek *et al.* 1992; Zaragoza *et al.* 1994; Reddy 1997). The mean age of females with abortion of double trisomy is significantly higher than that of single trisomy ($P < 0.001$).

DISCUSSION

It is generally considered that most embryos with double trisomy may be spontaneously aborted (Reddy 1997). In the present study, we found four cases of double trisomy in spontaneous abortions. These abortions had double trisomies for chromosomes 16 and 22, X and 18, 15 and 21, and 2 and 5, respectively. In the literature, to our knowledge, all chromosomes except chromosomes 1 and 19 have been observed in cases of double trisomy (Reddy 1997).

The majority of investigators have also considered that increased maternal age is an important factor for the occurrence of human trisomy. The present study showed that the mean age of females with spontaneous abortion of double trisomy (34.5 ± 6.6 years) is higher than that of single trisomy (32.9 ± 4.1 years).

In reported studies, the extra chromosomes of single trisomies were predominantly maternal in origin. For instance, 85% of cases with trisomy 8, 84% of cases with trisomy 13, 100% of cases with trisomy 16, and 93% of cases with trisomy 21 were maternal (Ya-gang *et al.* 1993). In the present study, extra chromosomes in the four cases of double trisomy were all maternal in origin; this is similar to the majority of cases with single trisomy. The results also showed that the two extra chromosomes in the four cases of double trisomy were produced by non-disjunction at the same cell division. A comparison of our results with those from similar molecular studies of double trisomies in spontaneous abortions and in liveborns (Zaragoza *et al.* 1994; Park *et al.* 1995; Chen *et al.* 2000) is summarized in Table 3. Both extra chromosomes in all of 10 cases of double trisomy were of maternal origin. While some cases of mosaicism including double trisomy were reported (Van Ravenswaaij-Arts *et al.* 1997; Devriendt *et al.* 1998), these cases were excluded here. Table 3 also indicates that the cell stage, which produced the extra chromosomes by non-disjunction, is the same in each case of double trisomy.

The production mechanisms of double trisomy, for an instance of maternal origin, can be considered to as the following processes: (i) concurrent non-disjunctions at meiosis I, meiosis II, or first mitotic division; and (ii) occurrence of non-disjunction at different stages at meiosis I, meiosis II and first mitotic division. The present results revealed that all four cases of double trisomy may be coincident with the process (i). These findings suggest that abnormal separation of two or more chromosomes may occur simultaneously in meiotic cell division of oogonia and the first mitotic cell

Table 2 Maternal age in spontaneous abortions with double and single trisomies

	Maternal age			
	Double trisomy		Single trisomy	
Reference	No. cases	Mean age ± SD	No. cases	Mean age ± SD
Lauritsen *et al.* (1976)	2	28.5 ± 9.2	71	28.1 ± 6.3
Hassold *et al.* (1980)	12	33.2 ± 6.4	214	29.6 ± 6.1
Kajii *et al.* (1980)	7	37.9 ± 4.8	124	31.9 ± 6.5
Ohno *et al.* (1991)	4	34.2 ± 5.5	64	32.4 ± 5.7
Dejmek *et al.* (1992)	10	29.7 ± 6.0	202	27.2 ± 6.6
Zaragoza *et al.* (1994)	4	39.8 ± 3.3	56	33.7 ± 3.7
Reddy (1997)	21	35.9 ± 5.3	377	33.8 ± 5.8
Present study	4	34.5 ± 6.6	34	32.9 ± 4.1
Pooled values	64	34.2 ± 5.9	1142	31.5 ± 5.6

Table 3 Parental origin of an extra chromosome and probable stages where non-disjunction occurred in cases with double trisomy

Ascertainment	Chromosome constitution	No. extra chromosomes	Parental origin and stage	Reference
SA	48,XX,+4,+14	4	mat MII	Zaragoza *et al.* (1994)
		14	mat MII	
SA	48,XX,+10,+15	10	mat MI or MII	
		15	mat MI or MII	
SA	48,XY,+15,+16	15	mat MI or MII	
		16	mat MI or MII	
SA	48,XY,+15,+21	15	mat MI	
		21	mat MI	
LB	48,XXX,+21	X	mat MII	Park *et al.* (1995)
		21	mat MII	
LB	48,XXX,+18	X	mat MII	Chen *et al.* (2000)
		18	mat MII	
SA	48,XX,+16,+22	16	mat MI	Present study
		22	mat MI	
SA	48,XXY,+18	X	mat MI	
		18	mat MI	
SA	48,XX,+15,+21	15	mat Mi	
		21	mat Mi	
SA	48,XX,+2,+5	2	mat Mi	
		5	mat Mi	

LB, liveborn; mat, maternal; MI, meiosis I; MII, meiosis II; Mi, first mitotic division; SA, spontaneous abortion.

division of the fertilized ovum in age-advanced women, due to greatly decreased functions of structures relating to chromosomal separation such as the spindle fiber and the kinetocore, and to delayed timing of chromosomal separation.

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Original Article

Paternal uniparental disomy of chromosome 14 and unique exchange of chromosome 7 in cases of spontaneous abortion

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Abstract To investigate the involvement of uniparental disomies (UPDs) in spontaneous abortion, the polymorphic patterns of microsatellites on each chromosome were analyzed in 164 cases of abortion. Eighty-three of the 164 cases had chromosomal abnormalities. In 79 of the remaining 81 cases with normal karyotypes, the microsatellite analysis revealed that biparental patterns were present in the informative microsatellites in all chromosomes. In one of the remaining two cases, however, the polymorphic patterns of chromosome 14 appeared to be both of paternal origin. The patterns of the distal of the long arm were homozygous, and those of the remaining region were heterozygous. That is, this fetus had paternal UPD 14, originating from meiosis I nondisjunction. In the other case, the polymorphic patterns of the distal one third of the long arm of chromosome 7 were uniparental (maternal) in origin whereas those of the remaining region of this chromosome were biparental. These findings thus suggested that this chromosome might have originated from chromatid exchange between the long arms of paternal and maternal chromosome 7 at the first

mitotic division. Microsatellite analysis, however, produced no evidence of duplication or deletion of any segments. The findings also suggest the possibility that some UPDs may cause spontaneous abortion.

Keywords Uniparental disomy · Spontaneous abortion · Nondisjunction · Meiosis · Mitotic exchange

Introduction

A high percentage (around 15%) of recognized pregnancies end in spontaneous abortion. About half of these abortions have various kinds of chromosomal abnormalities such as aneuploids, polyploids, and monosomy of the X-chromosome (Hassold et al. 1980; Kajii et al. 1980; Warburton et al. 1980). The cause of the remaining cases of abortions of fetuses with a normal karyotype is mostly unknown, but immunological and other defects have been detected in some cases (Gill 1986; Kaider et al. 1999). However, the finding that typical chromosomal abnormalities account for a large portion of the causes of spontaneous abortions leads to the possibility that functional, structural, and constitutional abnormalities that are undetectable by the usual chromosomal analysis may also contribute to these abortions with a normal karyotype. These include, for example, cases with a deletion of fine chromosomal segments including a gene essential to fetal development, abnormal inactivation of the X-chromosome, or uniparental disomy (UPD) of chromosomes having an imprinting region.

Chromosomal abnormality in UPD cases cannot usually be detected by banding, except for a few cases with the phenotypically polymorphic chromosomes. To date, few UPD cases have been found among spontaneous abortions (Fritz et al. 2001; Kondo et al. 2004). To investigate the involvement of UPDs, we analyzed the chromosomal origin of spontaneous abortions in detail using microsatellite polymorphic markers and found UPD of chromosome 14 and a unique exchange of chromosome 7 in cases of spontaneous abortion.

Materials and methods

Cases of spontaneous abortion

Of the 164 cases of spontaneous abortion analyzed, 133 patients that aborted a fetus were admitted to the Department of Obstetrics and Gynecology, Nagoya City University School of Medicine,

Nagoya, Japan. The remaining 31 cases were obtained from the Cell Bank constructed with a Health Science Research Grant for Research on Human Genome (H10-Genome-008) from the Ministry of Health, Labor and Welfare of Japan. These cases were aborted at 6–9 weeks of gestation. All of the patients and their spouses in these cases agreed to allow the use of parental and fetal materials for analysis, after being given understandable and detailed information on this study and its purposes. Peripheral blood of the patient with the spontaneous abortion and her spouse, and chorionic villi from the abortion, were obtained for each case. This study was approved by the IRB of the Institute for Developmental Research, Aichi Human Service Center, and the IRB of Nagoya City University Medical School.

Chromosomal analysis

The tissue of chorionic villi was separated under a stereomicroscope from three different parts of the samples from each abortion and was cultured separately using AmnioMAX C-100 medium (Invitrogen). All specimens were cultured within 18 h following abortion sampling. Cells were harvested for the chromosomal preparation at 6–19 days of cultivation. Peripheral blood lymphocytes from the patient with the abortion and from her spouse were also cultured and harvested conventionally for chromosomal analysis. Chromosomes were analyzed by G and Q banding.

Polymorphic analysis of microsatellites

Genomic DNA was extracted from the chorionic villi of the abortions and the blood of the patients with abortion and their spouses by the standard method. Two hundred polymorphic microsatellite markers on about every 20 Mb in all autosomes and the X-chromosome were selected from the Genethon collections (Dib et al. 1996). Some of the primers used in this study were provided by Prof. Y. Nakamura, Institute of Medical Sciences, University of Tokyo, Tokyo, Japan, and the others were synthesized. The microsatellite polymorphic patterns of the fetus and the parents for each marker locus were determined using a DNA-sequencer-assisted method with fluorescent microsatellite marker DNAs (Mansfield et al. 1994) with slight modifications (Fujimoto et al. 1998). When a polymorphic pattern suggesting disomy was obtained, further analysis using other synthesized primers that can detect many microsatellites in the same chromosome region was performed to clearly identify the parental origin of the chromosome.

Results

Of the 164 cases of spontaneous abortions investigated, 83 had chromosomal abnormalities, which were various but similar to those seen frequently in spontaneous abortions. In the remaining 81 cases with a normal karyotype—46,XX in 40 cases and 46,XY in 41 cases—polymorphic analysis of microsatellites was performed. The polymorphic analysis of villi from 79 of the 81 cases revealed that the informative microsatellite patterns of all of the autosomes, and of the X-chromosome of the 46,XX cases, were biparental patterns: one paternal (pat) and the other maternal (mat). These results indicate that every paired chromosome originated from one pat chromosome and one mat chromosome.

In one (case 35) of the remaining two 46,XX cases, the microsatellite polymorphic patterns of all paired autosomes except chromosome 14, and the X-chromosome were one pat and one mat each; however, the informative patterns of the three microsatellites of chromosome 14 were dual pat. Further polymorphic analysis of this case was performed using other synthesized primer sets for chromosome 14. Thirteen microsatellites showed informative patterns, clearly indicating both pat origin (Fig. 1), and all patterns of the other 17 microsatellites were consistent with pat origin. The results also showed that the microsatellite patterns in the region from the centromere to about two thirds of the chromosomal length away from the centromere of chromosome 14 (D14S261–D14S983) were heterozygous while those in the remaining region from that point to the distal end (D14S1058–D14S1010) were homozygous. That is, the results indicated that this fetus had pat iso- and heterodisomy of chromosome 14. The constitution of chromosome 14 in this case also suggests that both chromosomes 14 originated from pat meiosis I nondisjunction of dyad 14 that accompanied a crossover at a point about two thirds of the long arm away from the centromere.

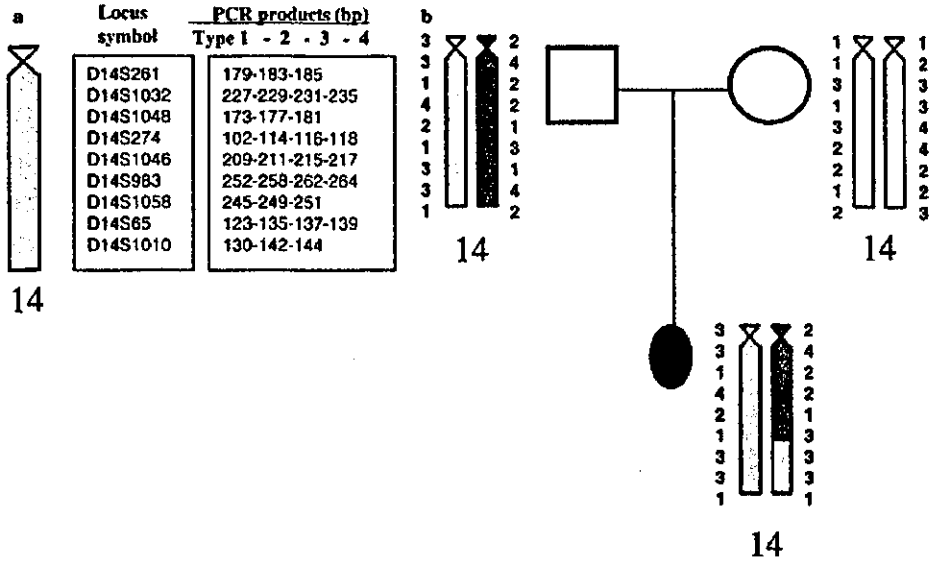

Fig. 1 Polymorphic patterns of microsatellites of chromosome 14 seen in the aborted fetus and the parents in case 35. **a** List of primers that showed informative patterns of microsatellite polymorphism, and the size of PCR products (bp). The arrangement of markers is roughly shown. **b** Polymorphic patterns of microsatellites in the fetus (case 35) and the parents. The polymorphic analysis indicates that this is a case of paternal iso-/heterodisomy of chromosome 14

In the other 46,XX case (case 107), on the other hand, polymorphic analysis of microsatellites revealed that the distal one third of the long arm of chromosome 7 appeared to be mat uniparental in origin whereas the segments from the distal part of the short arm to about two thirds distal from the centromere of the long arm of chromosome 7 were biparental in origin (Fig. 2). The microsatellite polymorphic patterns of the X-chromosome and all autosomes except for chromosome 7 were one pat and one mat. The segments of the distal part of the long arm of chromosome 7 that appeared to be both mat showed isodisomy. This would seem to suggest that one of the chromosomes 7 might have originated from an exchange between chromatids of the long arms of pat and mat chromosome 7 at the first mitotic division. Polymorphic analysis of microsatellites also revealed no evidence of the presence of cells with other chromosome constitutions derived from such chromatid exchange at the first mitotic division. Detailed investigation of the area around the breakpoint of the exchange revealed that, although all of the informative patterns obtained by analysis using primers from D7S2519 to those existing in more centromeric regions of the long arm of chromosome 7 were biparental, all of the informative patterns in all primers from D7S512 to those in the distal side of the long arm showed isodisomy. Further analysis using other primers between D7S2519 and D7S512 revealed no informative patterns. In addition, as a result of

microsatellite analysis, there was no evidence of duplication or deletion of any segments around the breakpoint of the exchange.

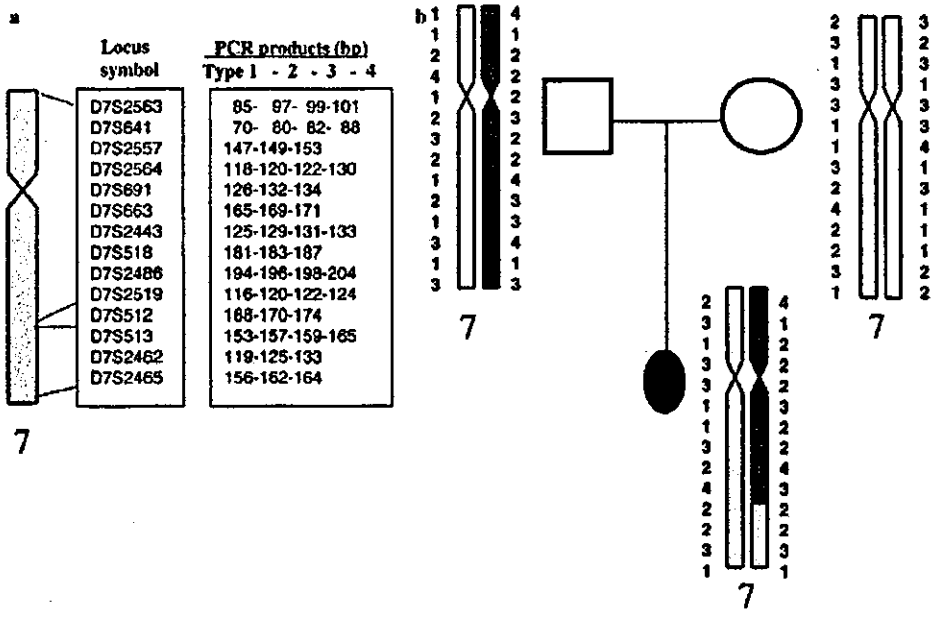

Fig. 2 Polymorphic patterns of microsatellites of chromosome 7 seen in the aborted fetus and the parents in case 107. **a** List of primers that showed informative patterns, and the size of PCR products (bp). The arrangement of markers and the locus of the centromere are roughly shown. **b** Polymorphic patterns of microsatellites in the fetus (case 107) and the parents. The polymorphic analysis indicates that this is a case of partial mat isodisomy of chromosome 7. There was no evidence of deletion or duplication of any segments around the breakpoint of exchange

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Case 35 was the first pregnancy of a 32-year-old woman. Her spouse was 34 years old. The fetus was diagnosed as having a stopped heartbeat in the eighth week of pregnancy and aborted during the following week (8w5d) in a typical spontaneous abortion in which the fetus could not be found. There was nothing remarkable either before or during the pregnancy. The karyotypes of the woman and her spouse were normal. Case 107 was from the first pregnancy of a 36-year-old woman. Her husband was 37 years old. The fetal heartbeat stopped at the seventh week of pregnancy, and the fetus was aborted the following week (7w5d) in a typical spontaneous abortion in which the fetus could not be found. There was nothing remarkable either before or during the pregnancy. The chromosomes of the woman and her husband were normal.

Discussion

Mat and pat UPDs for various chromosomes in humans have been identified in individuals by ascertaining medical problems. Findings of imprinting disturbances, non-Mendelian inheritance of recessive genes, and chromosomally abnormal patterns indicated UPD (Ledbetter and Engel 1995; Engel 1998). Among these, abnormal clinical features have been distinctly shown in both pat and mat UPDs of chromosomes 14 and 15 (Nicholls et al. 1989; Bottani et al. 1994; Cotter et al. 1997; Sanlaville et al. 2000). Abnormal clinical features have also been shown in UPDs of chromosomes 2, 7, and 16 of only mat origin (Kalousek et al. 1993; Kotzot et al. 1995; Johnston et al. 1996) and in UPDs of chromosomes 6 and 11 of only pat origin (Henry et al. 1991; Temple et al. 1995). In particular, serious clinical features have been described for some UPDs, such as mat UPD 2 associated with severe growth retardation, pulmonary dysplasia, and renal failure (Webb et al. 1996; Shaffer et al. 1997); and pat UPD 14, which shows the phenotypes of thoracic narrowing and skeletal dysplasia (Cotter et al. 1997; Kurosawa et al. 2002). In contrast, some such as UPD 1, 13, 21, and 22 have almost no clinical features with either pat or mat UPDs (Ledbetter and Engel 1995; Engel 1998; Morison and Reeve 1998). On the other hand, UPDs such as chromosomes 3, 12, or 17–19 have not been found in any case to date. These facts suggest the possibility that some UPD cases may exhibit abnormalities before birth. Whereas mouse studies have clearly indicated that some UPDs affect the development of embryos and the placenta (Ferguson-Smith et al. 1991), it has not been ascertained in humans whether UPDs affect embryogenesis and fetal development.

In the present study, we found the first case of pat UPD 14 in human spontaneous abortion. Human chromosome 14q shares synthetic homology with the distal half of mouse chromosome 12, in which there is the imprinting region. Georgiades et al. (2000) demonstrated that mice with UPD 12 resulted in parent-origin-specific developmental defects, and the placentomegaly and abnormality of maternal artery supply were likely to contribute to the progressive loss of pat UPD 12 fetus after E15.5. To determine the effects of human UPD 14, further data such as ultrasonographic findings of the placenta and fetus are important, but we failed to obtain detailed findings in the present study (case 35).

To the best of our knowledge, of the UPD 14 cases in liveborns reported to date in the literature, 37 were mat (Antonarakis et al. 1993; Papenhausen et al. 1995; Barton et al. 1996; Tomkins et al. 1996; Splitt and Goodship 1997; Harrison et al. 1998; Miyoshi et al. 1998; Hordijk et al. 1999; Martin et al. 1999; Ralph et al. 1999; Ginsburg et al. 2000; Manzoni et al. 2000; Sanlaville et al.

2000; Eggermann et al. 2001; Katahira et al. 2002; Cox et al. 2004) and 8 pat (Wang et al. 1991; Papenhausen et al. 1995; Walter et al. 1996; Cotter et al. 1997; McGowan et al. 2002; Coveler et al. 2002; Kurosawa et al. 2002; Offiah et al. 2003). As mentioned above, most cases of pat UPD 14 have characteristic and often serious clinical features, including blepharophimosis, small thorax, and joint contractures, while the main features of mat UPD 14 are low birth weight, poor postnatal growth, fleshy nasal tip, and scoliosis. The number of reports of UPD 14 suggests that the frequency of pat UPD 14 cases in liveborns is actually fewer than that of mat UPD cases. The difference in the frequencies between pat and mat UPD 14 cases might have resulted from a difference in the actual rate of occurrence or in the rate of selective elimination during embryogenesis and fetal development. On the assumption that UPDs are formed by fertilization between gametes nullisomic and disomic for the same chromosome, the frequency of pat and mat UPDs for the same chromosome might be equal. If, in cases of selective elimination, developmental defects of UPD fetuses appear also to be abortion, pat UPD 14 cases may be seen more frequently in spontaneous abortions than mat UPD 14 cases. Generally, chromosomal abnormalities actually seen in liveborns, such as trisomies 18 and 21, are also seen in spontaneous abortions at several times the rate in liveborns (Carr and Gedeon 1977; Hook and Hamerton 1977). These facts suggest that UPDs with congenital abnormalities seen in liveborns may become a cause of spontaneous abortions in the same way as trisomies do.

Until now, however, the relationship of UPDs to abortion has not been well understood. In the literature to date, only three cases of UPDs 9, 16, and 21 have been found among spontaneous abortions (Fritz et al. 2001; Kondo et al. 2004), and the present case is the first report of UPD 14 in a spontaneous abortion. In other reports, the association of UPDs with spontaneous abortions has not been found (Shaffer et al. 1998; Smith et al. 1998). The combined frequency of UPD cases from these four studies and the present study is 1.69% (4/236), which indicates a low incidence of UPD in spontaneous abortion. However, there is a methodological limitation to the polymorphic analysis of microsatellites. Even using this kind of analysis, for instance, the mosaic cases of UPDs due to the trisomy rescue mechanism could not be detected in the ascertainment of UPD cases in spontaneous abortions.

Another case found in the present study (case 107) is a very rare one that was produced by an exchange between the chromatids of the long arm of pat and mat chromosome 7. This case had partial mat UPD of 7q. It is well known that abnormal clinical features are shown in mat UPD 7, including this region (Kotzot et al. 2000; Hannula et al. 2001). Other than this case, there is no reported case of spontaneous abortion in which there was demonstrated to be an exchange between

pat and mat chromosome. In liveborns, on the other hand, many cases with an exchange between pat and mat chromosomes have been reported. For example, an exchange between pat and mat chromosomes is one of the mechanisms in contiguous gene syndromes, such as Prader–Willi and Angelman syndromes (Robinson et al. 1998; Nicholls and Knepper 2001), in which partial UPD and deletion of segments resulted from unequal exchange between chromatids of pat and mat homologous chromosomes. Although in the present case we could not find any deletion or duplication near the breakpoint of the exchange of chromosome 7 by polymorphic analysis of microsatellites, there remains a possibility that further detailed analyses may in fact show deletion and duplication resulting from the exchange. Generally, an exchange between chromatids of two homologous chromosomes produces two kinds of cells, each with different chromosome constitutions. Whereas this case had only one of the two kinds of karyotypes expected, it is possible that the cell with the other karyotype could not increase because of disadvantage due to microdeletions, duplication, or UPD resulting from an exchange.

A very interesting question is how many abnormalities undetectable by the usual banding method, such as UPDs and somatic exchanges that were detected in the present study, are concerned with miscarriage. Although polymorphic analysis of DNAs including microsatellite polymorphic analysis is one effective means for such elucidation, the data based on these methods remain insufficient. To clarify the relationship between constitutional abnormalities including UPDs and spontaneous abortion, or the effects during the developmental stages in humans, further investigations of abortions using DNA polymorphic markers and other means are needed.

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Rapid Publication

On the Reported 8p22-p23.1 Duplication in Kabuki Make-Up Syndrome (KMS) and its Absence in Patients With Typical KMS

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To the Editor:

Kabuki make-up syndrome (KMS) was described originally and independently by Niikawa et al. [1981] and Kuroki et al. [1981]. KMS was characterized by a distinctive facial appearance resembling the Kabuki actor's make-up, mild to moderate mental retardation, skeletal abnormality, postnatal growth retardation, and dermatoglyphic abnormality. (See a review by Matsumoto and Niikawa [2003].) Multiple organ involvement implies that KMS is a contiguous syndrome, but its cause remains unknown. Milunsky and Huang [2003] recently reported that all of six KMS patients they examined had an approximate 3.5-Mb duplication at 8p22-p23.1 demonstrated by comparative genomic hybridization (CGH) and fluorescence in situ hybridization (FISH) using four BAC clones (RP11-112G9, RP11-252K12, RP11-31B7, and RP11-92C1) as probes. They also suggested that a paracentric inversion, detected by RP11-122N11, separated from the duplicated region may contribute to the occurrence of the condition.

We analyzed a total of 26 Japanese and 2 Thai patients with KMS and 52 phenotypically normal controls regarding such duplication and inversion by FISH using 15 BAC clones covering 8p22-8p23.1 after obtaining written informed consent and with the approval by IRB of Nagasaki University. All of these patients were referred to us after making a definitive diagnosis of KMS. Their metaphase chromosomes were prepared for FISH from immortalized lymphoblastoid cell lines or peripheral blood lymphocytes according to standard protocols. Eight BAC clones (GS-77L23, RP11-245H16, RP11-5E15, RP11-399J23, RP11-403C10, RP11-589N15, RP11-252C15, RP11-45O16) selected from the UCSC Genome Browser version July 2003 (http://genome.ucsc.edu/cgi-bin/hgGateway?org=human) in addition to 7 clones (RP11-122N11, RP11-235F10, RP11-112G9, RP11-252K12, RP11-31B7, RP11-92C1, RP11-23H1) used by Milunsky and Huang [2003] were labeled with SpectrumGreen™-11-dUTP or Spectrum Orange™-11-dUTP (Vysis, Downers Grove, IL) and used as probes for FISH. FISH signals of all clones were carefully examined on both of metaphase chromosomes and interphase nuclei. Duplication was determined if signal intensity of probe A was much stronger than that of probe B in metaphases and two dots were surely observed in interphases using two-color FISH. At least 10 metaphases and 10 interphases were scored in each experiment.

None of the four clones reported to be duplicated in patients by Milunsky and Huang [2003] revealed any duplication in our 28 KMS patients examined (Fig. 1). All other clones, but RP11-122N11, also showed a single-copy signal, not duplicated (Fig. 1). The RP11-122N11 locus was reported to be inverted in six KMS patients and two of their mothers [Milunsky and Huang, 2003]. However, using RP11–122N11 as a probe we observed "duplicated" rather than "inverted" signals (Fig. 1). As the signal of two green signals for this probe looked similar in size and intensity (Fig. 1a), duplication is more likely. Of 22 KMS patients analyzed with this probe, 15 had a homozygous duplication and 7 a heterozygous one, and thus the allele frequency for the duplication is 88.5%. Similarly, of 52 normal persons, 40 and 12 had a homozygous and a heterozygous duplication, respectively, the frequency for the duplication being 84.1%, which is not statistically different in the patients by χ^2 analysis ($P = 0.47$). Thus, findings of RP11-122N11 signal were likely to be a polymorphism (Fig. 2A–D).

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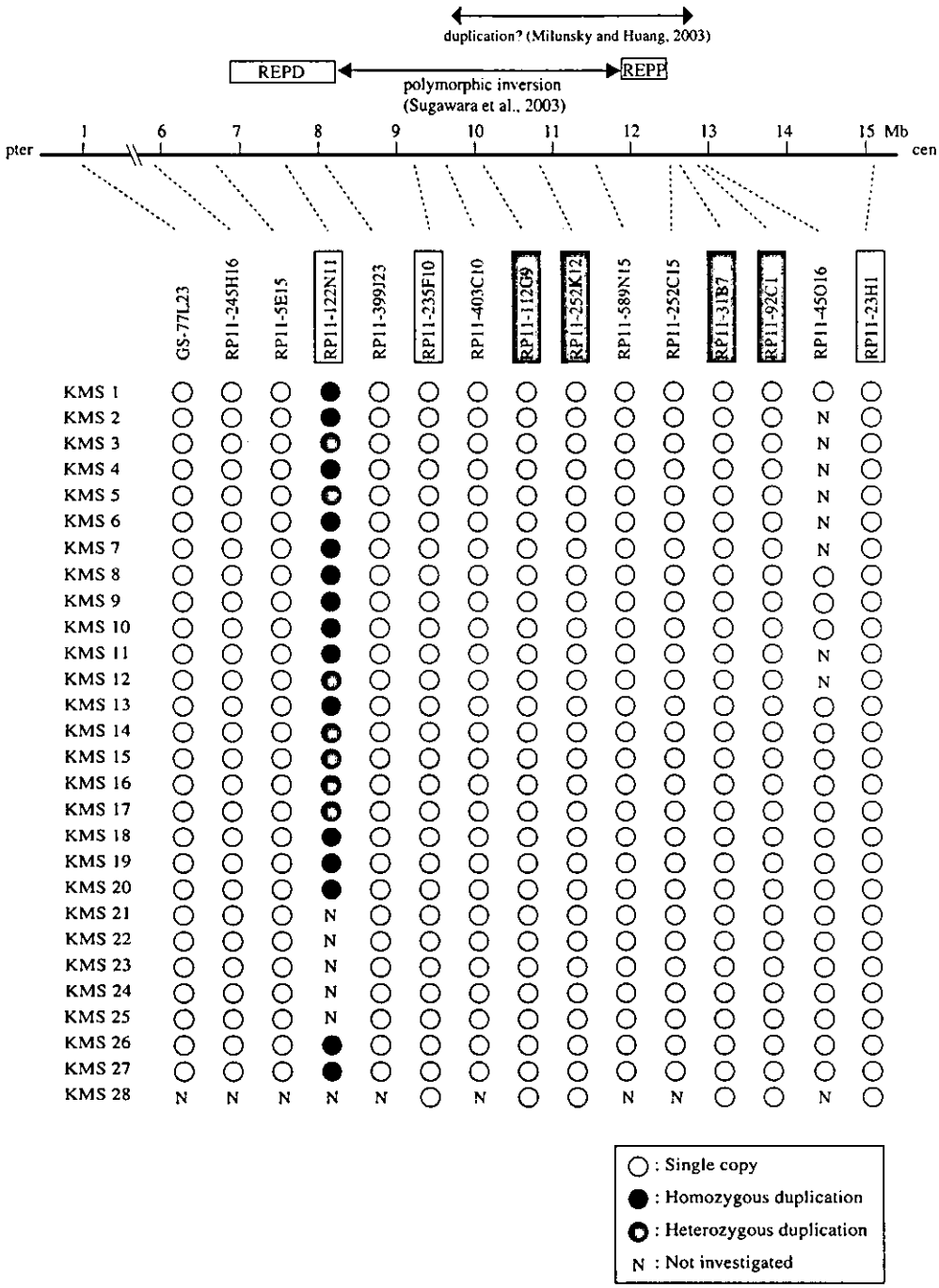

Fig. 1. Results of FISH analysis on 28 patients with Kabuki make-up syndrome (KMS). From top to bottom, a duplicated region reported by Milunsky and Huang [2003], polymorphic inversion reported by Sugawara et al. [2003], a scale from the 8p telomere to the centromere, BAC/PAC clones used for FISH study and their locations, and results of FISH studies in all patients. REPD, repeat distal; REPP, repeat proximal. BAC clones in square and grey square were those used for the study and reported to be duplicated, respectively, by Milunsky and Huang [2003]. Open, black and gray circles indicate a single-copy FISH signal, homozygous duplication, and heterozygous duplication, respectively. N, not investigated.

Unlike the data by Milunsky and Huang [2003], we were not able to detect any interstitial duplication at 8p22-8p23.1 in our series of 28 KMS patients. There must be some reasons for these discrepant results. The patient populations studied in two investigations may be different clinically. From our examination of the facial photographs of cases 1 and 2 in the report by Milunsky and Huang [2003], they may not have typical KMS and could be "8p23.1-p22 duplication syndrome." Alternatively, the discrepancy may be due to the complexity of the 8p23 region. We have constructed a comprehensive physical map covering low copy repeats (LCRs), and a common inverted region at 8p23. Although we did not incorporate the clone, RP11-122N11, to our previous map or evaluate it [Sugawara et al., 2003], we now assign it within one of LCRs

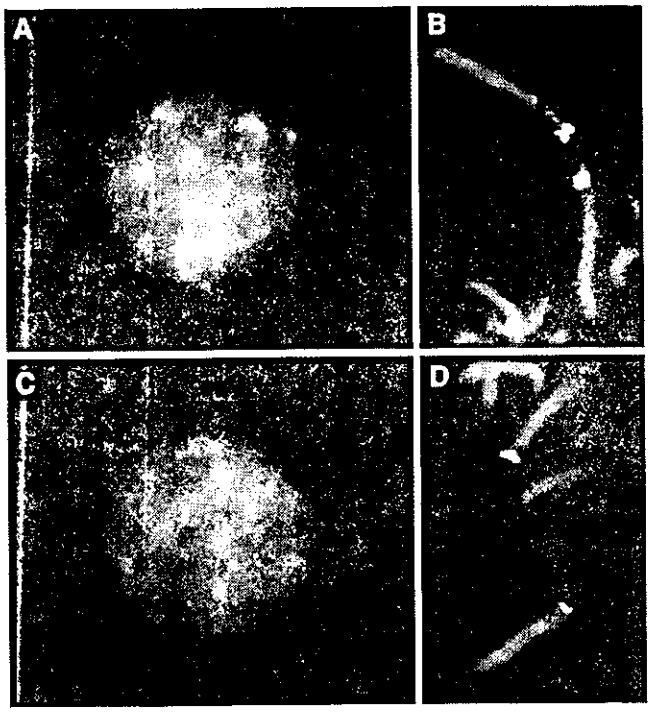

Fig. 2. FISH analysis using RP11–122N11 labeled with Spectrum-Green™ and RP11–235F10 labeled with Spectrum-Orange™ in normal controls, showing a homozygous duplication (**A**: An interphase nucleus and (**B**) metaphase chromosomes) and a heterozygous duplication (**C**: An interphase nucleus and (**D**) metaphase chromosomes).

according to both the UCSC database and our map. The clone would have shown seeming duplicated signals on both of homologous chromosomes, but the allele frequency of the RP11-122N11 duplication in the normal Japanese is 84.1%, not 100%. This implicates that this region has more complicated structure than expected, and should well be characterized.

In conclusion, our data suggest that the cause of KMS in most patients is still unknown, and further studies will be necessary absolutely.

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