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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REFERENCES

Chen CP, Chern SR, Yeh LF, Chen WL, Chen LF, Wang W (2000) Prenatal diagnosis and genetic analysis of double trisomy 48,XXX,+18. *Prenat Diagn* **20**: 750–753.

Creasy MR, Crolla JA, Alberman ED (1976) A cytogenetic study of human spontaneous abortions using banding techniques. *Hum Genet* **31**: 177–196.

Dejmek J, Vojtassak J, Malova J (1992) Cytogenetic analysis of 1508 spontaneous abortions originating from south Slovakia. *Eur J Obstet Gynecol Reprod Biol* **46**: 129–136.

Devriendt K, Matthijs G, Meireleire J, Roelen L, van Buggenhout, Fryns JP (1998) Skin pigment anomalies and mosaicism for a double autosomal trisomy (48,XX,+18,+20). *Genet Couns* **9**: 283–286.

Dib C, Faure S, Fizames C *et al.* (1996) A comprehensive genetic map of the human genome based on 5,264 microsatellites. *Nature* **380**: 152–154.

Eiben B, Bartels I, Bahr-Porsch S *et al.* (1990) Cytogenetic analysis of 750 spontaneous abortions with the direct-preparation method of chorionic villi and its implications for studying genetic causes of pregnancy wastage. *Am J Hum Genet* **47**: 656-663.

Epstein CJ (1986) *The Consequences of Chromosome Imbalance, Principles, Mechanisms and Models.* Cambridge University Press, Cambridge.

Fujimoto M, Kantaputra PN, Ikegawa S *et al.* (1998) The gene for mesomelic dysplasia Kantaputra type is mapped to chromosome 2q24-q32. *J Hum Genet* **43**: 32–36.

Hassold T, Chen N, Funkhouser J *et al.* (1980) A cytogenetic study of 1000 spontaneous abortions. *Ann Hum Genet* **44**: 151–178.

Kajii T, Ferrier A, Niikawa N, Takahara H, Ohama K, Avirachan S (1980) Anatomic and chromosomal anomalies in 639 spontaneous abortuses. *Hum Genet* **55**: 87–98.

Lauritsen JG (1976) Aetiology of spontaneous abortion: A cytogenetic and epidemiological study of 288 abortuses and their parents. *Acta Obstet Gynecol Scand Suppl* **52**: 1–29.

Ohno M, Maeda T, Matsunobu A (1991) A cytogenetic study of spontaneous abortion with direct analysis of chorionic villi. *Obstet Gynecol* **77**: 394–398.

Park VM, Bravo RR, Shulman LP (1995) Double non-disjunction in maternal meiosis II giving rise to a fetus with 48,XXX,+21. *J Med Genet* **32**: 650–653.

Reddy KS (1997) Double trisomy in spontaneous abortions. *Hum Genet* **101**: 339–345.

Van Ravenswaaij-Arts CM, Tuerlings JH, Van Heyst AF, Nijhuis JG, Niehof J, Smeets DF (1997) Misinterpretation of trisomy 18 as a pseudomosaicism at third-trimester amniocentesis of a child with a mosaic 46,XY/47,XY,+3/48,XXY,+18 karyotype. *Prenat Diagn* **17**: 375–379.

Ya-gang X, Robinson WP, Spiegel R, Binkert F, Ruefenacht U, Schinzel AA (1993) Parental origin of the supernumerary chromosome in trisomy 18. *Clin Genet* **44**: 57–61.

Zaragoza MV, Jacobs PA, James RS, Rogan P, Sherman S, Hassold T (1994) Nondisjunction of human acrocentric chromosomes: studies of 432 trisomic fetuses and liveborns. *Hum Genet* **94**: 411–417.

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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.

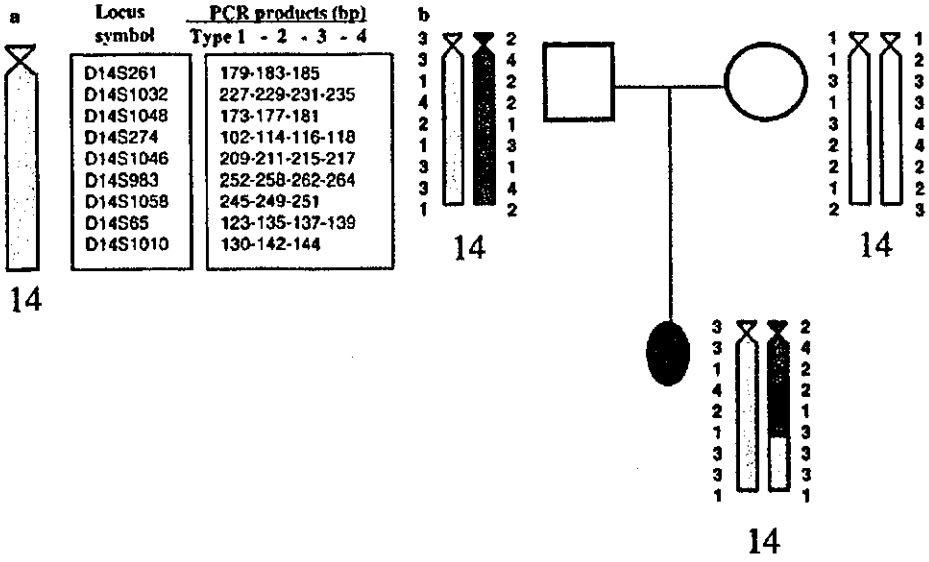

Fig. 1 Polymorphic patterns of microsatellites of chromosome 14 seen in the aborted fetus and the parents in case 35. **a** List of primers that showed informative patterns of microsatellite polymorphism, and the size of PCR products (bp). The arrangement of markers is roughly shown. **b** Polymorphic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

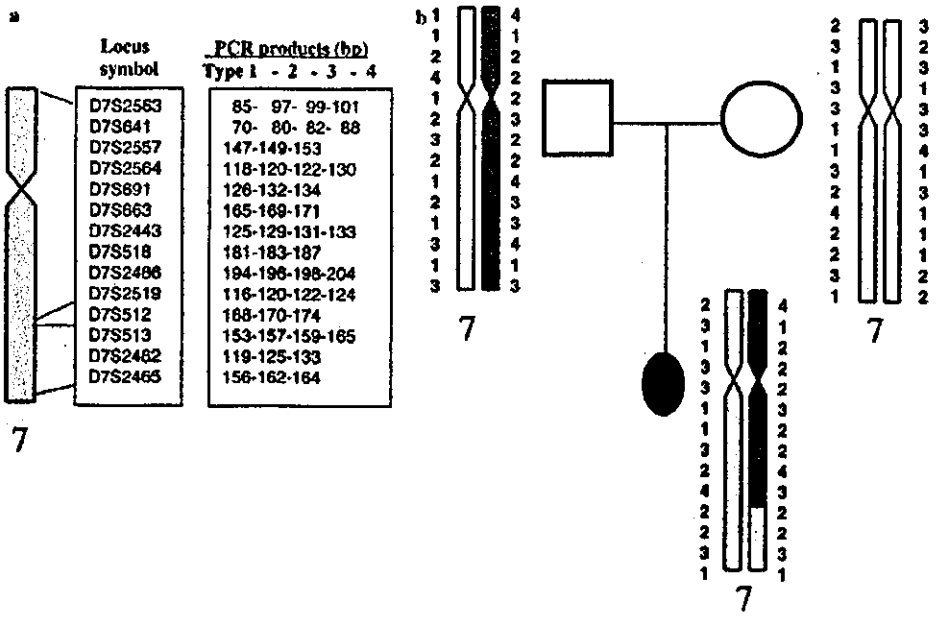

Fig. 2 Polymorphic patterns of microsatellites of chromosome 7 seen in the aborted fetus and the parents in case 107. **a** List of primers that showed informative patterns, and the size of PCR products (bp). The arrangement of markers and the locus of the centromere are roughly shown. **b** Polymorphic 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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References

Antonarakis SE, Blouin JL, Maher J, Avramopoulos D, Thomas G, Talbot CC Jr (1993) Maternal uniparental disomy for human chromosome 14, due to loss of a chromosome 14 from somatic cells with t(13;14) trisomy 14. Am J Hum Genet 52:1145–1152

Barton DE, McQuaid S, Stalling R, Griffin E, Geraghty M (1996) Further evidence for an emerging maternal uniparental disomy for chromosome 14. Analysis of a phenotypically abnormal de novo Robertsonian translocation t(13;14) carrier. Am J Hum Genet (Suppl) 59:698

Bottani A, Robinson WP, DeLozier Blanchet CD, Engel E, Morris MA, Schmitt B, Thun-Hohenstein L, Schinzel A (1994) Angelman syndrome due to paternal uniparental disomy of chromosome 15: a milder phenotype? Am J Med Genet 51:35–40

Carr DH, Gedeon M (1977) Population cytogenetics of human abortuses. In: Hook EB, Porter IH (eds) Population cytogenetics: studies in humans. Academic Press, New York San Francisco London, pp 1–9

Cotter PD, Kaffe S, McCurdy LD, Jhaveri M, Willner JP, Hirschhorn K (1997) Paternal uniparental disomy for chromosome 14: a case report and review. Am J Med Genet 70:74–79

Coveler KJ, Yang SP, Sutton R, Milstein JM, Wu YQ, Bois KD, Beischel LS, Johnson JP, Shaffer LG (2002) A case of segmental paternal isodisomy of chromosome 14. Hum Genet 110:251–256

Cox H, Bullman H, Temple IK (2004) Maternal UPD(14) in the patient with a normal karyotype: clinical report and a systematic search for cases in samples sent for testing for Prader–Willi syndrome. Am J Med Genet 127A:21–25

Dib C, Faure S, Fizames C, Samson D, Drouot N, Vignal A, Millasseau P, Marc S, Hazan J, Seboun E, Lathrop M, Gyapay G, Morissette J, Weissenbach J (1996) A comprehensive genetic map of the human genome based on 5,264 microsatellites. Nature 380:152–154

Eggermann T, Mergenthaler S, Eggermann K, Albers A, Linnemann K, Fusch C, Ranke MB, Wollmann HA (2001) Identification of interstitial maternal uniparental disomy (UPD) (14) and complete maternal UPD (20) in a cohort of growth retarded patients. J Med Genet 38:86–89

Engel E (1998) Uniparental disomies in unselected populations. Am J Hum Genet 63:962–966

Ferguson-Smith AC, Cattanach BM, Barton SC, Beechey CV, Surani MA (1991) Embryological and molecular investigations of parental imprinting on mouse chromosome 7. Nature 351:667–670

Fritz B, Aslan M, Kalscheuer V, Ramsing M, Saar K, Fuchs B, Rehder H (2001) Low incidence of UPD in spontaneous abortions beyond the 5th gestational week. Eur J Hum Genet 9:910–916

Fujimoto M, Kantaputra PN, Ikegawa S, Fukushima Y, Sonta S, Matsuo M, Ishida T, Matsumoto T, Kondo S, Tomita H, Deng HX, D'urso M, Rinaldi MM, Ventruto V, Takagi T, Nakamura Y, Niikawa N (1998) The gene for mesomelic dysplasia Kantaputra type is mapped to chromosome 2q24–q32. J Hum Genet 43:32–36

Georgiades P, Watkins M, Surani MA, Ferguson-Smith AC (2000) Parental origin-specific developmental defects in mice with uniparental disomy for chromosome 12. Development 127:4719–4728

Gill TJ III (1986) Immunological and genetic factors influencing pregnancy and development. Am J Reprod Immunol Microbiol 10:116–120

Ginsburg C, Fokstuen S, Schinzel A (2000) The contribution of uniparental disomy to congenital development defects in children born to mothers at advanced childbearing age. Am J Med Genet 95:454–460

Hannula K, Lipsanen-Nyman M, Kontiokari T, Kere J (2001) A narrow segment of maternal uniparental disomy of chromosome 7q31-qter in Silver–Russell syndrome delimits a candidate gene region. Am J Hum Genet 68:247–253

Harrison KJ, Allingham-Hawkins DJ, Hummel J, Meschin WS, Cox DW, Costa TM, Mak-Tam E, Teshima IF, Kamel-Reid S, Winsor EJT (1998) Risk of uniparental disomy in Robertsonian translocation carriers: identification of UPD 14 in a small cohort. Am J Hum Genet (Suppl) 63:51

Hassold T, Chen N, Funkhouser J, Jooss T, Manuel B, Matsuura J, Matsuyama A, Wilson C, Yamane JA, Jacobs PA (1980) A cytogenetic study of 1,000 spontaneous abortions. Ann Hum Genet 44:151–178

Henry I, Bonaiti-Pellie C, Chehensse V, Beldjord C, Schwartz C, Utermann G, Junien C (1991) Uniparental paternal disomy in a genetic cancer-predisposing syndrome. Nature 351:665–667

Hook EB, Hamerton JL (1977) The frequency of chromosomal abnormalities detected in consecutive newborn studies-differences between studies-results by sex and by severity of phenotypic involvement. In: Hook EB, Porter IH (eds) Population Cytogenetics: Studies in Humans. Academic Press, New York San Francisco London, pp 79–92

Hordijk R, Wierenga H, Scheffer H, Leegte B, Hofstra RM, Stolte-Dijkstra I (1999) Maternal uniparental disomy for chromosome 14 in a boy with a normal karyotype. J Med Genet 36:782–785

Johnston KM, Baker JC, Egli CA, McCaskill C, Shaffer LG (1996) Maternal uniparental isodisomy of chromosome 2 in a child with growth retardation, hypospadias and a cytogenetic abnormality. Am J Hum Genet (Suppl) 59:A95

Kaider AS, Kaider BD, Janowicz PB, Roussev RG (1999) Immunodiagnostic evaluation in women with reproductive failure. Am J Reprod Immunol 42:335–346

Kajii T, Ferrier A, Niikawa N, Takahara H, Ohama K, Avirachan S (1980) Anatomic and chromosomal anomalies in 639 spontaneous abortuses. Hum Genet 55:87–98

Kalousek DK, Langlois S, Barrett I, Yam I, Wilson DR, Howard-Peebles PN, Johnson MP, Giorgiutti E (1993) Uniparental disomy for chromosome 16 in humans. Am J Hum Genet 52:8–16

Katahira M, Kayashima T, Kishino T, Niikawa N (2002) Maternal uniparental disomy for chromosome 14 with diabetes mellitus. Intern Med 41:717–721

Kondo Y, Tsukishiro S, Tanemura M, Sugiura-Ogasawara M, Suzumori K, Sonta S (2004) Maternal uniparental disomy of chromosome 16 in a case of spontaneous abortion. J Hum Genet 49:177–181

Kotzot D, Schmitt S, Bernasconi F, Robinson WP, Lurie IW, Ilyina H, Mehes K, Hamel BC, Otten BJ, Hergersberg M, Hamel BC, Otten BJ, Hergersberg M (1995) Uniparental disomy 7 in Silver–Russell syndrome and primordial growth retardation. Hum Mol Genet 4:583–587

Kotzot D, Balmer D, Baumer A, Chrzanowska K, Hamel BC, Ilyina H, Krajewska-Walasek M, Lurie IW, Otten BJ, Schoenle E, Tariverdian G, Schinzel A (2000) Maternal uniparental disomy 7—review and further delineation of the phenotype. Eur J Pediatr 159:247–256

Kurosawa K, Sasaki H, Sato Y, Yamanaka M, Shimizu M, Ito Y, Okuyama T, Matsuo M, Imaizumi K, Kuroki Y, Nishimura G (2002) Paternal UPD14 is responsible for a distinctive malformation complex. Am J Med Genet 110:268–272

Ledbetter DH, Engel E (1995) Uniparental disomy in humans: development of an imprinting map and its implications for prenatal diagnosis. Hum Mol Genet 4:1757–1764

Mansfield DC, Brown AF, Green DK, Carothers AD, Morris SW, Evans HJ, Wright AF (1994) Automation of genetic linkage analysis using flourescent microsatellite markers. Genomics 24:225–233

Manzoni MF, Pramparo T, Stroppolo A, Chiaino F, Bosi E, Zuffardi O, Carrozzo R (2000) A patient with maternal chromosome 14 UPD presenting with a mild phenotype and MODY. Clin Genet 57:406–408

Martin RA, Sabol DW, Rogan PK (1999) Maternal uniparental disomy of chromosome 14 confined to an interstitial segment (14q23–14q24.2). J Med Genet 36:633–636

McGowan KD, Weiser JJ, Horwitz J, Berend SA, McCaskill C, Sutton VR, Shaffer LG (2002) The importance of investigating for uniparental disomy in prenatally identified balanced acrocentric rearrangements. Prenat Diagn 22:141–143

Miyoshi O, Hayashi S, Fujimoto M, Tomita H, Sohda M, Niikawa N (1998) Maternal uniparental disomy for chromosome 14 in a boy with intrauterine growth retardation. J Hum Genet. 43:138–142

Morison IM, Reeve AE (1998) A catalogue of imprinted genes and parent-of-origin effects in humans and animals. Hum Mol Genet 7:1599–1609

Nicholls RD, Knepper JL (2001) Genome organization, function, and imprinting in Prader–Willi and Angelman syndromes. Annu Rev Genomics Hum Genet 2:153–175

Nicholls RD, Knoll JH, Butler MG, Karam S, Lalande M (1989) Genetic imprinting suggested by maternal heterodisomy in non-deletion Prader–Willi syndrome. Nature 342:281–285

Offiah AC, Cornette L, Hall CM (2003) Paternal uniparental disomy 14: introducing the "coat-hanger" sign. Pediatr Radiol 33:509–512

Papenhausen PR, Mueller OT, Johnson VP, Sutcliffe M, Diamond TM, Kousseff BG (1995) Uniparental isodisomy of chromosome 14 in two cases: an abnormal child and a normal adult. Am J Med Genet 59:271–275

Ralph A, Scott F, Tieman C, Caubere M, Kollegger S, Junio J, Roberts C, Ewen K, Slater HR (1999) Maternal uniparental isodisomy for chromosome 14 detected prenatally. Prenat Diagn 19:681–684

Robinson WP, Dutly F, Nicholls RD, Bernasconi F, Penaherrera M, Michaelis RC, Abeliovich D, Schinzel AA (1998) The mechanisms involved in formation of deletions and duplications of 15q11–q13. J Med Genet. 35:130–136

Sanlaville D, Aubry MC, Dumez Y, Nolen MC, Amiel J, Pinson MP, Lyonnet S, Munnich A, Vekemans M, Morichon-Delvallez N (2000) Maternal uniparental heterodisomy of chromosome 14: chromosomal mechanism and clinical follow up. J Med Genet 37:525–528

Shaffer LG, McCaskill C, Egli CA, Baker JC, Johnston KM (1997) Is there an abnormal phenotype associated with maternal isodisomy for chromosome 2 in the presence of two isochromosomes? Am J Hum Genet 61:461–462

Shaffer LG, McCaskill C, Adkins K, Hassold TJ (1998) Systematic search for uniparental disomy in early fetal losses: the results and a review of the literature. Am J Med Genet 79:366–372

Smith MJ, Creasy MR, Clarke A, Upadhyaya M (1998) Sex ratio and absence of uniparental disomy in spontaneous abortions with a normal karyotype. Clin Genet 53:258–261

Splitt MP, Goodship JA (1997) Another case of maternal uniparental disomy chromosome 14 syndrome. Am J Med Genet 72:239–240

Temple IK, James RS, Crolla JA, Sitch FL, Jacobs PA, Howell WM, Betts P, Baum JD, Shield JP (1995) An imprinted gene(s) for diabetes? Nat Genet 9:110–112

Tomkins DJ, Roux AF, Waye J, Freeman VC, Cox DW, Whelan DT (1996) Maternal uniparental isodisomy of human chromosome 14 associated with a paternal t(13q14q) and precocious puberty. Eur J Hum Genet 4:153–159

Walter CA, Shaffer LG, Kaye CI, Huff RW, Ghidoni PD, McCaskill C, McFarland MB, Moore CM (1996) Short-limb dwarfism and hypertrophic cardiomyopathy in a patient with paternal isodisomy 14: 45,XY,idic(14)(p11). Am J Med Genet 65:259–265

Wang JC, Passage MB, Yen PH, Shapiro LJ, Mohandas TK (1991) Uniparental heterodisomy for chromosome 14 in a phenotypically abnormal familial balanced 13/14 Robertsonian translocation carrier. Am J Hum Genet 48:1069–1074

Warburton D, Stein Z, Kline S, Susser M (1980) Chromosome abnormalities in spontaneous abortions: data from the New York City study. In: Porter IH, Hook EB (eds) Human embryonic and fatal death. Academic Press, New York, pp 261–268

Webb AL, Sturgiss S, Warwicker P, Robson SC, Goodship JA, Wolstenholme J (1996) Maternal uniparental disomy for chromosome 2 in association with confined placental mosaicism for trisomy 2 and severe intrauterine growth retardation. Prenat Diagn 16:958–962

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Clinical Report

Inv dup del(4)(:p14 → p16.3::p16.3 → qter) With Manifestations of Partial Duplication 4p and Wolf-Hirschhorn Syndrome

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An 8-year-old girl with a combination of clinical manifestations of partial duplication 4p and the Wolf–Hirschhorn syndrome was studied. Chromosomal G-banding and FISH analyses showed a 33.2-Mb segment of inverted duplication at 4p14-p16.3 and a 2.8-Mb segment of deletion at 4p16.3-pter (including the Wolf–Hirschhorn syndrome critical region). The chromosomes of the parents were normal. Her karyotype was thus 46,XX, inv dup del(4)(:p14 → p16.3:: p16.3 → qter) de novo. The inverted duplication deletion was assumed to have arisen through chromatid breakage at 4p16.3, U-type reunion at the breakpoints to produce a dicentric intermediate, breakage of the dicentric to result in a monocentric, and telomere capture/healing of the broken end. Olfactory receptor gene clusters at 4p16.3 were ruled out as an intermediary of the duplication deletion process.

KEY WORDS: inv dup del(4p); FISH; Wolf–Hirschhorn syndrome; 4p duplication

INTRODUCTION

Inverted duplications with terminal deletions have been reported in an increasing number of chromosomes, but the mechanisms leading to most of such rearrangements remain unknown. Best characterized among them is inv dup del 8p. Maternal heterozygous inversion polymorphism involving two olfactory receptor gene clusters at 8p23.1 has been implicated to mediate the rearrangements [Giglio et al., 2001; Matsumoto et al., 2001]. Similar olfactory receptor gene clusters and their inversion polymorphism have been found at 4p16.3 and proposed to mediate an unbalanced der(4)t(4;8)(p16;p23) translocation with clinical manifestations of Wolf–Hirschhorn syndrome (OMIM #194190) [Giglio et al., 2002]. Inv dup del(4p) has been reported only once [Cotter et al., 2001].

Here we report a girl with inv dup del(4p) with clinical manifestations of both partial duplication 4p and the Wolf–Hirschhorn syndrome. Possible participation of the olfactory receptor gene clusters at 4p16.3 in the rearrangement was studied.

CLINICAL REPORT

The patient is an 8-year-old girl, the second child born to a 33-year-old G2P2 mother and a non-consanguineous 39-year-old father. Family history was unremarkable. The pregnancy was uncomplicated, with spontaneous vaginal delivery at 40 weeks of gestation. Birth weight was 2,580 g (−1.3 SD), length 46 cm (−1.5 SD), and OFC 31 cm (−1.6 SD). Developmental milestones were delayed: she lifted her head at age 6 months, sat alone at 2 years, and walked unsupported at 6 years. At age 7 months, she developed tonic-clonic seizures controllable with medication. When referred to us at age 6 years, she weighed 14.4 kg (−2.0 SD), measured 104 cm (−2.4 SD), and had an OFC of 45 cm (−4.4 SD). She

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had microcephaly, prominent grabella, inverted eyelashes, large and malformed ears, a nose with bulbous tip, hypoplastic alae nasi, and high and wide nasal root, a short philtrum, a small mouth with downturned corners, high-arched palate, and micrognathia (Fig. 1a,b; Table I). She had exudative otitis media, chronic sinusitis, and moderate sensorineural hearing loss. Scoliosis and clubfoot were noted. Bone age was delayed (2 years at age 4 years). She spoke no meaningful words. The serum growth hormone level was within the normal range. Magnetic resonance imaging of the brain showed mild delay of myelination.

CYTOGENETIC AND FISH STUDIES

G-banded chromosomes of cultured peripheral blood lymphocytes from the patient included a chromosome 4 with an inverted duplication of distal 2/3 of its short arms and an apparent loss of the 4p16.3 band (Fig. 2a). Both parents had normal chromosomes.

Microdissection of the short arms of the abnormal chromosome 4 and PCR amplification of the dissected DNA were performed as described previously [Hirota et al., 1992; Ohta et al., 1993]. The probe generated was fluorescence-labeled, and, together with Cot-1 DNA, was hybridized to metaphase chromosomes from a normal individual. It gave a homogeneous staining pattern on the short arms of normal chromosomes 4, with no signals on other chromosomes, an indication that the excess 4p segment was derived from 4p.

FISH analysis was carried out using the following 15 BAC/PAC clones mapped to 4pter-4p14 (Map Viewer database, http://www.ncbi.nlm.nih.gov/cgi-bin/Entrez/hum_srch?chr=hum_chr.inf&query). BAC/PAC clone DNA was labeled with SpectrumOrange™ or SpectrumGreen™-dUTP (Vysis, Downers Grove, IL) using nick translation reagent kit (Vysis). The probes were hybridized to denatured chromosomal DNA at 37°C for 16 hr. FISH on the patient's chromosomes using the 14 clones gave three different patterns of signals (Figs. 2b and 3). Two clones—GSHP-36P21 (4p-subtelomeric probe) [Knight et al., 2000] and RP11-262P20—showed no signal on the abnormal chromosome 4, but each gave a signal on the normal chromosome 4 (Fig. 2b).

TABLE I. Clinical Manifestations in dup(4)(p14→pter) and Wolf–Hirschhorn Syndrome

Dup(4)(p14→pter)[a]	Wolf–Hirschhorn Syndrome[b,c]
Growth retardation	**Growth retardation**
Obesity	
Mental retardation	**Mental retardation**
Seizures	**Seizures**
Hypotonia	Hypotonia
Microcephaly	**Microcephaly**
	Prominent glabella
Synophrys	
	Large, protruding eyes
	Hypertelorism
Downslanting palpaebral fissure	
Strabismus	**Strabismus**
Coloboma	Coloboma
	Broad/beaked nose
Short pug nose	
Wide-nasal bridge	**High-nasal bridge**
Dysplastic ears	**Dysplastic ears**
	Preauricular tag/pit
	Deafness
	Short philtrum
	Downturned corners of the mouth
High palate	
	Cleft lip/palate
Pointed-chin/short chin	
	Micrognathia
Short neck	
Scoliosis	**Scoliosis**
Retarded bone age	
Congenital heart disease	Congenital heart disease
Hypoplastic genitalia/ hypospadias	Hypospadias
	Renal anomaly
Clinodactyly of fifth fingers	
	Club foot

Boldface letters indicate manifestations in the patient.
[a]Schinzel [2001].
[b]Zollino et al. [2000].
[c]Wieczorek et al. [2000].

These findings indicate that a 2.8-Mb segment at 4p16.3-pter was deleted in the abnormal chromosome. Five clones—CITB-2008A15, RP11-141P6, RP11-79E3, RP11-143G24, and RP11-136D4—each gave single signals of equal intensity on both the normal chromosome 4 and the inv dup(4p) chromosome. The other

Fig. 1. The patient at the age of 7 10/12 years (a and b).

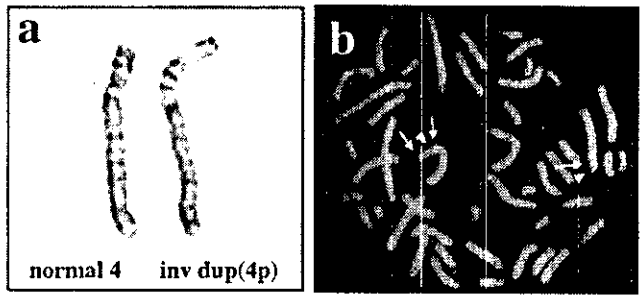

Fig. 2. The patient's chromosomes 4 (a), and two-color FISH using probes, RP11-262P20 (red, arrowhead) and RP11-20M7 (green, arrow) (b). RP11-262P20 signal is absent on the inv dup del(4p) but present on normal chromosome 4. RP11-20M7 shows two separate signals on the inv dup del(4p) chromosome and a single signal on normal chromosome 4.

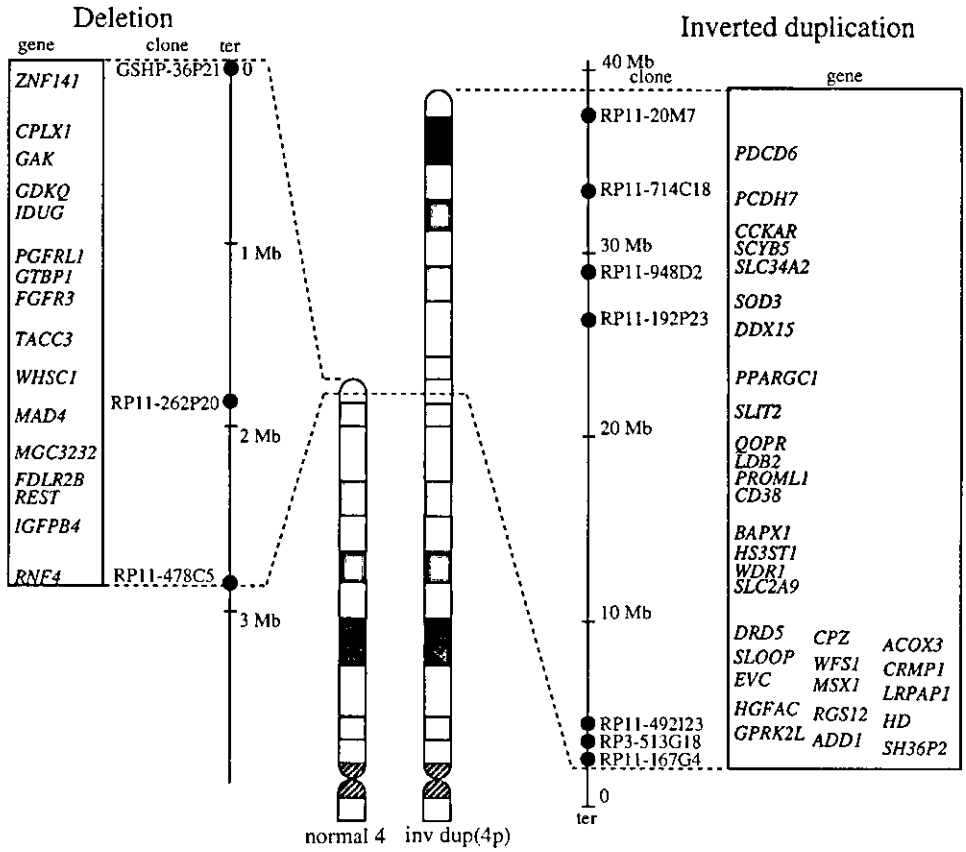

Fig. 3. Schematic presentation of a 2.8-Mb deleted segment and a 33.2-Mb duplicated segment on the inv dup del(4p) chromosome of the patient. Filled circles indicate clones used for FISH, and locus names in boxes the genes mapped to the segments.

seven clones—RP11-167G4, RP3-513G18, RP11-492I23, RP11-192P23, RP11-948D2, RP11-714C18, and RP11-20M7—showed a single signal on the normal chromosome 4, and duplicated signals on the abnormal chromosome 4 (Fig. 2b). The more distal the clones used as probes, the more separated were the duplicated signals. RP11-20M7, for instance, gave two widely separated signals on the abnormal chromosome 4 (Fig. 2b), whereas RP11-167G4 produced closely situated but separate signals (data not shown). These findings indicated that the duplicated segment was inverted. RP11-478C5 gave a signal on the abnormal chromosome weaker than that on the normal chromosome 4, an indication that the clone spanned the junction of the deletion. The size of the duplicated segment was estimated to be 33.2 Mb (Fig. 3). The patient's karyotype was thus interpreted as 46,XX, der(4)(:p14→p16.3::p16.3→qter) de novo.

DISCUSSION

The girl we described had an inverted duplication for 4p14-p16.3 and deletion for 4p16.3-pter. The interpretation was supported by chromosomal FISH analyses using a number of probes spanning the duplication and deletion regions. It was further supported by the presence in the girl of the clinical manifestations of both duplication 4p16.3-14 and deletion 4p16.3-pter (Wolf–Hirschhorn syndrome) (Table I).

We are aware of only one previous report of inv dup del(4p) [Cotter et al., 2001]. The report dealt with a 4-year-old girl with inverted duplication of 4p15.3-p16.3 and deletion of 4p16.3-pter. Inverted duplication deletions with one breakpoint at the terminal band have been described in 1q, 2q, 3p, 4p, 5p, 7q, 8p, 9p, and 11p [see Cotter et al., 2001, for references; Bonaglia et al., 2000; de Brasi et al., 2001; Fisher et al., 2002]. The simplest mechanism leading to inverted duplication deletion is two chromatid break, U-type reunion of the broken ends to generate a dicentric intermediate, and a rescue by subsequent random breakage and telomere healing/capture. This is likely to be the case in the inv dup del 4p we described.

The most frequent and best characterized among the inverted duplication deletions reported is that which involves 8p. Inv dup del 8p has been proposed to arise through illegitimate recombination during maternal meiosis between inverted repeats mediated by two olfactory receptor gene clusters, situated 6-Mb apart with each other at 8p23.1 [Giglio et al., 2001; Matsumoto et al., 2001]. Similar olfactory receptor gene clusters and inversion polymorphism exist in 4p16, and, in combination with those at 8p23, they leads to recurrent unbalanced translocation, der(4)t(4;8)(p16;p23), with

clinical manifestations of 4p16.3 deletion (Wolf–Hirschhorn syndrome) [Giglio et al., 2002]. Involvement of the olfactory receptor gene clusters at 4p16 in the inv dup del(4p) we described was ruled out for the following two reasons. First, RP3-513G18, mapped close but distal to the olfactory gene clusters, gave duplicated signals on the inv dup 4p chromosome, an indication that the distal breakpoint of the inverted duplication was situated distal to the gene clusters. Second, the short arms of the abnormal chromosome 4 in the patient we described had inverted duplication and deletion of the terminal band. This was at variance with inv dup del 8p which was composed of—distal to proximal—a deleted segment, a single-copy segment, and an inverted duplication segment [Giglio et al., 2001; Matsumoto et al., 2001]. The absence in the inv dup del 4p chromosome of a single-copy segment distal to the duplicated segment suggests that it was formed through a mechanism different from that for inv dup del 8p.

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REFERENCES

Bonaglia MC, Giorda R, Poggi G, Raggi ME, Rossi E, Baroncini A, Giglio S, Borgatti R, Zuffardi O. 2000. Inverted duplications are recurrent rearrangements always associated with a distal deletion: Description of a new case involving 2q. Eur J Hum Genet 8:597–603.

Cotter PD, Kaffe S, Li L, Gershin IF, Hirschhorn K. 2001. Loss of subtelomeric sequence associated with a terminal inversion duplication of the short arm of chromosome 4. Am J Med Genet 102:76–80.

de Brasi D, Rossi E, Giglio S, D'Agostino A, Titomanlio I, Farnia V, Andria G, Sebastio G. 2001. Inv dup del (1)(pter → q44::q44 → q42:) with the classical phenotype of trisomy 1q42-qter. Am J Med Genet 104:127–130.

Fisher AM, Thomas NS, Cockwell A, Stecko O, Kerr B, Temple IK, Clayton P. 2002. Duplications of chromosome 11p15 of maternal origin result in a phenotype that includes growth retardation. Hum Genet 111:290–296.

Giglio S, Broman KW, Matsumoto N, Calvari V, Gimelli G, Neumann T, Ohashi H, Voullaire L, Larizza D, Giorda R, Weber JL, Ledbetter DH, Zuffardi O. 2001. Olfactory receptor-gene clusters, genomic-inversion polymorphisms, and common chromosome rearrangements. Am J Hum Genet 68:874–883.

Giglio S, Calvari V, Gregato G, Gimelli G, Camanini S, Giorda R, Ragusa A, Guerneri S, Selicorni A, Stumm M, Tonnies H, Ventura M, Zollino M, Neri G, Barber J, Weiczorek D, Rocchi M, Zuffardi O. 2002. Heterozygous submicroscopic inversions involving olfactory receptor-gene clusters mediate the recurrent t(4;8)(p16;p23) translocation. Am J Hum Genet 71:276–285.

Hirota T, Tsukamoto K, Deng HX, Yoshiura K, Ohta T, Tohma T, Kibe T, Harada N, Jinno Y, Niikawa N. 1992. Microdissection of human chromosomal regions 8q23.3-q24.11 and 2q33-qter: Construction of DNA libraries and isolation of their clones. Genomics 13:349–354.

Knight SJ, Lese CM, Precht KS, Kuc J, Ning Y, Lucas S, Regan R, Brenan M, Nicod A, Lawrie NM, Cardy DL, Nguyen H, Hudson TJ, Riethman HC, Ledbetter DH, Flint J. 2000. An optimized set of human telomere clones for studying telomere integrity and architecture. Am J Hum Genet 67: 320–332.

Matsumoto N, Harada N, Giglio S, Kurosawa K, Ledbetter DH, Niikawa N. 2001. Inverted low copy repeats and a common 8p23 inversion polymorphism. Am J Hum Genet 69:318.

Ohta T, Tohma T, Soejima H, Fukushima Y, Nagai T, Yoshiura K, Jinno Y, Niikawa N. 1993. The origin of cytologically unidentifiable chromosome abnormalities: Six cases ascertained by targeted chromosome-band painting. Hum Genet 92:1–5.

Schinzel A. 2001. Catalogue of unbalanced chromosome aberrations in man. 2nd Edn. Berlin, New York: Walter de Gruyter, p 195–198.

Wieczorek D, Krause M, Majewski F, Albrecht B, Horn D, Riess O, Gillessen-Kaesbach G. 2000. Effect of the size of the deletion and clinical manifestation in Worl–Hirschhorn syndrome: Analysis of 13 patients with a de novo deletion. Eur J Hum Genet 8:519–526.

Zollino M, Stefano CD, Zampino G, Mastroiacovo P, Wright TJ, Sorge G, Selicorni A, Tenconi R, Zappala A, Battaglia A. Di Rocco M, Palka G, Pallotta R, Altherr M. 2000. Genotype–phenotype correlation and clinical diagnostic criteria in Wolf–Hirschhorn syndrome. Am J Med Genet 94:254–261.

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Clinical Report

Mosaic Supernumerary inv dup(15) Chromosome With Four Copies of the *P* Gene in a Boy With Pigmentary Dysplasia

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Association of the pink-eye-dilution gene (*P*) with hypopigmentation is seen in patients who have oculocutaneous albinism type 2 (OCA2) and Prader–Willi syndrome (PWS) or Angelman syndrome (AS). However, it remains unknown whether duplication or amplification of the *P* gene causes hyperpigmentation. We previously reported a woman who had hyperpigmentation with a duplication of the proximal part of 15q, including the *P* gene. Here, we describe an additional patient with mosaicism of inv dup(15) and clinical manifestations of severe psychmoter retardation, epilepsy, and pigmentary dysplasia showing mottled and linear patterns of hyperpigmentation. His karyotype was 47,XY,+idic(15)(pter→q14::q14→pter)[38]/46, XY[12] de novo. Chromosomal fluorescence in situ hybridization (FISH) showed six copies of the *P* gene. Therefore, his cutaneous mosaicism might be caused by the presence of both normal and hyperpigmented skin due to multicopies of the *P* gene.

KEY WORDS: chromosome 15q; chromosomal mosaicism; *P* gene; pigmentary dysplasia; hyperpigmentation; gene dosage hypothesis

INTRODUCTION

About one-fourth of patients with either Prader–Willi syndrome (PWS) or Angelman syndromes (AS) have chromosomal deletions of 15q11-q13 and exhibit hypopigmentation of the skin, hair, and eyes [Butler, 1989; King et al., 1994]. Patients with oculocutaneous albinism type 2 (OCA2) due to homozygous loss-of-function mutations of the pink-eye-dilution gene (*P*) located at 15q11.2-q12 also manifest generalized hypopigmentation, frequently severe [Rinchik et al., 1993; Lee et al., 1994]. We recently reported a woman with duplication of 15q11.2-q14, including the *P* gene, who had generalized skin hyperpigmentation, and proposed that a duplication of the gene might account for her hyperpigmentation [Akahoshi et al., 2001].

Here, we describe another patient with linear skin pigmentation (pigmentary dysplasia) associated with mosaicism for a marker chromosome [mos inv dup(15)].

CLINICAL REPORT

The patient, a 10-year-old Japanese boy, has been followed-up for severe psychomotor retardation and epilepsy since age 4 years. His non-consanguineous parents and elder sister were all healthy and had no pigmentary dysplasia. He was born at 38 weeks of gestation with a birth weight of 3,480 g, following a normal pregnancy and delivery. Left inguinal hernia and left knee dislocation were surgically repaired in early infancy. At age 8 months, he developed head-nodding seizures (West syndrome) and was placed on ACTH. He attained head control at age 8 months, rolled over at 12 months, started to make sounds at 21 months,

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