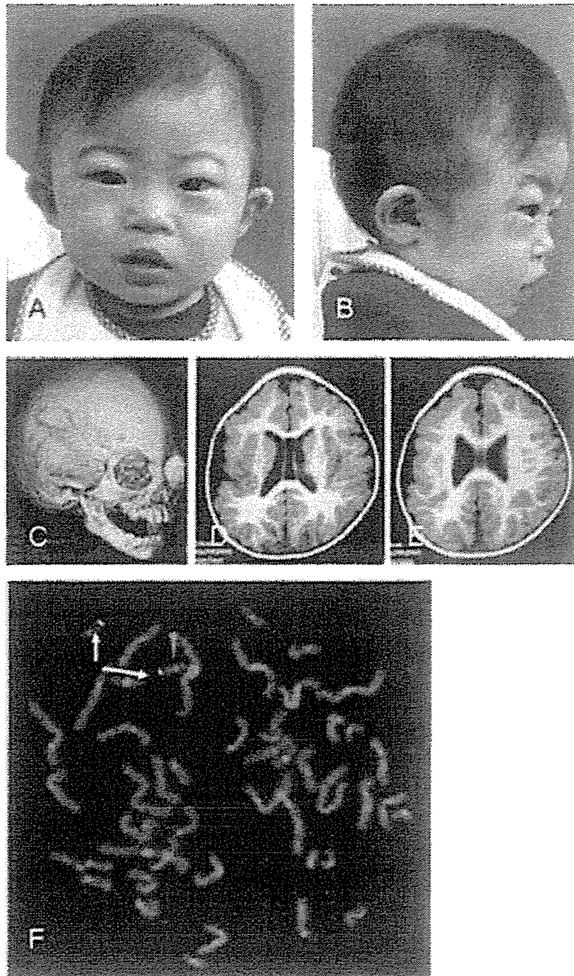

FIG. 1. The patient at age 18 months (A, B). Three-dimensional computed tomography of the skull, showing synostosis of the metopic suture (C). Brain magnetic resonance imaging, showing polymicrogyria of the right hemisphere (D, E). FISH analysis of the patient using TUPLE1 SpectrumOrange at 22q11.2 (red arrowhead) and ARSA SpectrumGreen Control Probe at 22q13.3 (green arrows) (F). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

eyelids, a low nasal bridge, hypoplastic nasal alae, low-set lop ears, a small mouth, high-arched palate, and micrognathia (Fig. 1A,B). At age 11 months, his OFC was 43 cm (−2.1 SD). When he moved his right hand, his left hand moved in a mirror fashion. A neurological examination showed left hemifacial palsy, left hemiplegia, and left ankle clonus. Radiography demonstrated complete synostosis of the metopic suture (Fig. 1C). Brain MRI showed polymicrogyria of the right hemisphere (Fig. 1D,E). Linear craniectomy of the metopic suture was performed at age 18 months. At age 2 years, he walked with support, and spoke few words. He had febrile convulsions, and his EEG showed spike activities in the right fronto-central region. Sequencing analysis of the fibroblast growth factor receptor 2 (*FGFR2*) gene detected no mutation [Yamamoto et al., 2001].

The mother was healthy and phenotypically normal. The father was of normal intelligence but had the facial gestalt characteristic of an adult with the deletion 22q11.2 syndrome, including long face, narrow palpebral fissures, square root of the nose, hypoplastic nasal alae, and small mouth. His speech was nasal.

TABLE I. FISH Analysis of the 22q11.21-11.22 Region

Probe	Band	Distance from 22pter (Mb)	Patient	Father	Mother
RP11-100K2	22q11.21	16.8	+	+	
TUPLE 1	22q11.21	17.7	−	−	+
RP11-165F18	22q11.21	19.6	−	−	
RP11-36N5	22q11.21	20.3	+	+	
RP11-757F24	22q11.22	21.1	+	+	

−, absent FISH signal; +, presence of the signal.

CYTOGENETIC STUDIES

G-banded chromosomes of the boy were 46,XY. Fluorescent in situ hybridization (FISH) analysis using LSI DiGeorge/VCFS Region Probe (Vysis, Inc., Downers Grove, IL) detected a deletion in the patient and the father, but not in the mother (Fig. 1F). FISH using probes spanning the 22q11.21-11.22 region detected a 3-Mb deletion in both the father and son (Table I).

DISCUSSION

The present patient and the father showed the typical gestalt of conotruncal anomaly face syndrome, including a narrow nose with its squared root and narrow ala nasi, and short palpebral fissures [Matsuoka et al., 1994]. These and additional clinical manifestations identified in the patient, including mental and motor developmental delay, microcephaly, seizures, polymicrogyria, and cardiac anomalies were clues for the identification of 3-Mb deletion of 22q11.2 region in this family. This clinical variability from full manifestation to extremely mild clinical anomalies in familial deletion 22q11.2 as seen in this family is a well-known phenomenon [Digilio et al., 2003]. Hemicephalic polymicrogyria revealed in the patient is also a well-known rare complication of deletion 22q11.2 syndrome [Sztriha et al., 2004], but the exception is trigonocephaly.

Trigonocephaly is seen in some chromosomal abnormalities such as monosomy 9p and partial monosomy 11q, as well as in other genetic disorders [Azimi et al., 2003; Jehee et al., 2005; Yatsenko et al., 2005]; a candidate region for monosomy 9p syndrome had recently been narrowed by our group [Kawara et al., 2006]. However, deletion 22q11.2 syndrome has never been listed as the cause of trigonocephaly. Ryan et al. [1997] summarized a

spectrum of clinical features of deletion 22q11.2 syndrome, and described five patients with craniosynostosis without descriptions of its type. McDonald-McGinn et al. [2005] reported four patients with deletion 22q11.2 syndrome associated with craniosynostosis, all of whom showed synostosis of the coronal suture but not involving the metopic suture. Therefore, the present report is the first description of a relationship between deletion 22q11.2 syndrome and trigonocephaly.

Although the exact cause of the coexistence of deletion 22q11.2 and trigonocephaly remains unknown, and coincidental occurrence might be the explanation for this. Alternatively, symptoms of trigonocephaly might be a minor complication of deletion 22q11.2. Another possibility, but less likely, is a mutated trigonocephaly gene mapped to 22q11.2 and derived from the mother was unmasked by the deletion in the patient.

ACKNOWLEDGMENTS

This study was funded by the Japan Society for the Promotion of Science to T.Y. and SORST from Japan Science and Technology Agency to N.M. and K.N.

REFERENCES

Azimi C, Kennedy SJ, Chitayat D, Chakraborty P, Clarke JT, Forrest C, Teebi AS. 2003. Clinical and genetic aspects of trigonocephaly: A study of 25 cases. Am J Med Genet Part A 117A:127–135.

Digilio MC, Angioni A, De Santis M, Lombardo A, Giannotti A, Dallapiccola B, Marino B. 2003. Spectrum of clinical variability in familial deletion 22q11.2: From full manifestation to extremely mild clinical anomalies. Clin Genet 63:308–313.

Emanuel BS, McDonald-McGinn D, Saitta SC, Zackai EH. 2001. The 22q11.2 deletion syndrome. Adv Pediatr 48:39–73.

Jehee FS, Johnson D, Alonso LG, Cavalcanti DP, de Sa Moreira E, Alberto FL, Kok F, Kim C, Wall SA, Jabs EW, Boyadjiev SA, Wilkie AO, Passos-Bueno MR. 2005. Molecular screening for microdeletions at 9p22-p24 and 11q23-q24 in a large cohort of patients with trigonocephaly. Clin Genet 67:503–510.

Kawara H, Yamamoto T, Harada N, Yoshiura K, Niikawa N, Nishimura A, Mizuguchi T, Matsumoto N. 2006. Narrowing candidate region for monosomy 9p syndrome to a 4.7-Mb segment at 9p22.2-p23. Am J Med Genet Part A 140A:373–377.

Matsuoka R, Takao A, Kimura M, Imamura S, Kondo C, Joh-o K, Ikeda K, Nishibatake M, Ando M, Momma K. 1994. Confirmation that the conotruncal anomaly face syndrome is associated with a deletion within 22q11.2. Am J Med Genet 53:285–289.

McDonald-McGinn DM, Gripp KW, Kirschner RE, Maisenbacher MK, Hustead V, Schauer GM, Keppler-Noreuil KM, Ciprero KL, Pasquariello P Jr, LaRossa D, Bartlett SP, Whitaker LA, Zackai EH. 2005. Craniosynostosis: Another feature of the 22q11.2 deletion syndrome. Am J Med Genet Part A 136A:358–362.

Ryan AK, Goodship JA, Wilson DI, Philip N, Levy A, Seidel H, Schuffenhauer S, Oechsler H, Belohradsky B, Prieur M, Aurias A, Raymond FL, Clayton-Smith J, Hatchwell E, McKeown C, Beemer FA, Dallapiccola B, Novelli G, Hurst JA, Ignatius J, Green AJ, Winter RM, Brueton L, Brondum-Nielsen K, Stewart F, Van Essen T, Patton M, Paterson J, Scambler PJ. 1997. Spectrum of clinical features associated with interstitial chromosome 22q11 deletions: A European collaborative study. J Med Genet 34:798–804.

Spiteri E, Babcock M, Kashork CD, Wakui K, Gogineni S, Lewis DA, Williams KM, Minoshima S, Sasaki T, Shimizu N, Potocki L, Pulijaal V, Shanske A, Shaffer LG, Morrow BE. 2003. Frequent translocations occur between low copy repeats on chromosome 22q11.2 (LCR22s) and telomeric bands of partner chromosomes. Hum Mol Genet 12:1823–1837.

Sztriha L, Guerrini R, Harding B, Stewart F, Chelloug N, Johansen JG. 2004. Clinical, MRI, and pathological features of polymicrogyria in chromosome 22q11 deletion syndrome. Am J Med Genet Part A 127A:313–317.

Yamamoto T, Pipo J, Ninomiya H, Ieshima A, Koeda T. 2001. Antley-Bixler syndrome and maternal virilization: A proposal of genetic heterogeneity. Clin Genet 59:451–453.

Yatsenko SA, Cheung SW, Scott DA, Nowaczyk MJ, Tarnopolsky M, Naidu S, Bibat G, Patel A, Leroy JG, Scaglia F, Stankiewicz P, Lupski JR. 2005. Deletion 9q34.3 syndrome: Genotype-phenotype correlations and an extended deletion in a patient with features of Opitz C trigonocephaly. J Med Genet 42:328–335.

© 2006 Wiley-Liss, Inc. American Journal of Medical Genetics Part A 140A:1737–1743 (2006)

Origin and Mechanisms of Formation of Fetus-in-Fetu: Two Cases With Genotype and Methylation Analyses

Shoko Miura,[1,2,8] Kiyonori Miura,[1*] Toshiyuki Yamamoto,[3] Michiko Yamanaka,[4] Keisuke Saito,[4] Tomoo Hirabuki,[4] Kenji Kurosawa,[3] Naoki Harada,[7,8] Yoko Ishizaki-Yamasaki,[1,2,8] Naomichi Matsumoto,[5,8] Fumiki Hirahara,[6] Koh-ichiro Yoshiura,[2,8] Hideaki Masuzaki,[1] and Norio Niikawa[2,8]

[1]Department of Obstetrics and Gynecology, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan
[2]Department of Human Genetics, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan
[3]Division of Medical Genetics, Kanagawa Children's Medical Center, Yokohama, Japan
[4]Division of Obstetrics, Kanagawa Children's Medical Center, Yokohama, Japan
[5]Department of Human Genetics, Yokohama City University Graduate School of Medicine, Yokohama, Japan
[6]Department of Obstetrics, Gynecology and Reproductive Science, Yokohama City University Graduate School of Medicine, Yokohama, Japan
[7]Kyushu Medical Science, Nagasaki, Japan
[8]Solution Oriented Research of Science and Technology (SORST), Japan Science and Technology Agency (JST), Kawaguchi, Japan

Received 1 May 2006; Accepted 20 May 2006

Fetus-in-fetu (FIF) is a condition in which a host infant has a fetus-like mass(es) within its body. We describe here results of molecular genetic analysis in two cases of FIF. In FIF-1, a male host had two retroperitoneal fetiform masses each with a vertebral column, and in FIF-2, a fetiform mass with vertebral column was present in the cranial cavity of a male host. Genotyping of each case using microsatellite markers showed that the host infant and its fetus(es) inherited one copy each of parental alleles and shared identical genotypes. These findings were confirmed by single nucleotide polymorphism (SNP) analysis using Affymetrix GeneChip Human Mapping 50K Array, and supported a monozygotic twin theory of FIF. Analysis of the methylation status was done in both cases at the differentially methylated region (*DMR*) within the human *IGF2-H19* locus after bisulfite treatment, methylation-specific PCR, and cloning of PCR products. Normally, only the paternal allele is methylated and the maternal allele unmethylated in *DMR*. However, in FIF-1, 7 (46.7%) of 15 clones from a fetiform mass and 6 (66.7%) of 9 clones from the other mass showed an unmethylated paternal allele, while the methylation status of a host infant and its fetiform mass in FIF-2 was the same in all clones examined with normal patterns. These data suggest that in FIF-1, two isolated blastocysts originated from one zygote, one of the two was implanted into (or included by) the other blastocyst during the process of methylation, and such abnormal implantation may have occurred in FIF-2 after the establishment of methylation. This is the first case of FIF showing different methylation patterns between a host infant and fetiform mass. © 2006 Wiley-Liss, Inc.

Key words: fetus-in-fetu; genotype; methylation status; origin; mechanism of formation

How to cite this article: Miura S, Miura K, Yamamoto T, Yamanaka M, Saito K, Hirabuki T, Kurosawa K, Harada N, Ishizaki-Yamasaki Y, Matsumoto N, Hirahara F, Yoshiura K, Masuzaki H, Niikawa N. 2006. Origin and mechanisms of formation of fetus-in-fetu: Two cases with genotype and methylation analyses. Am J Med Genet Part A 140A:1737–1743.

INTRODUCTION

A Fetus-in-fetu (FIF) anomaly is a condition of fetiform mass(es) located within a host infant, like a Russian doll "matreshka." The incidence is estimated to be 1/500,000 births [Hoeffel et al., 2000]. Its mechanism of formation has remained unknown. Willis [1935] tried to distinguish it from teratoma by the presence of signs of axiation, metameric segmentation, delamination of germ layers, and organs

Grant sponsor: Ministry of Education, Sports, Culture, Science and Technology of Japan; Grant numbers: 16591670, 17591748, 17019055; Grant sponsor: Japan Science and Technology Agency.

*Correspondence to: Dr. Kiyonori Miura, Department of Obstetrics and Gynecology, Nagasaki University Graduate School of Biomedical Sciences, Sakamoto 1-7-1, Nagasaki 852-8501, Japan.
E-mail: kiyonori@nagasaki-u.ac.jp

DOI 10.1002/ajmg.a.31362

WILEY InterScience® DISCOVER SOMETHING GREAT

or somatic regions. These characteristics, as well as the presence of the vertebral column, segmental axis or high development of organogenesis, are widely used as definition of FIF [Hoeffel et al., 2000; Brand et al., 2004]. However, the distinction from highly differentiated teratoma maybe difficult [Hoeffel et al., 2000], for example, in the case of highly differentiated limbs or digits without the vertebral column [Eng et al., 1989; Ouimet and Russo, 1989; Naudin ten Cate et al., 1995]. There are two hypotheses on the formation of FIF. The monozygotic twin theory tries to explain FIF by inclusion of sister embryos that arise by an unequal division of the inner cell mass or anastomosis of the vitelline circulation between diamniotic monochorionic twins [Lord, 1956], whereas a highly differentiated teratoma theory is mainly based on the occasional presence of infants with both FIF and teratoma [Heifetz et al., 1988; Gilbert-Barness et al., 2003]. Alternatively, FIF and teratoma may share a causal/pathogenetic mechanism [Higgins and Coley, 2006]. Studies of genetic markers, such as a blood group, sex chromosome constitution, protein polymorphisms, and DNA markers [Hing et al., 1993; Chen et al., 1997; Kumar et al., 1999; Gilbert-Barness et al., 2003; Brand et al., 2004; Higgins and Coley, 2006], suggested that host infants and their fetiform mass are genetically identical.

We recently encountered two cases of FIF. Here we report the results of molecular genetic studies on their origin and methylation status. Possible mechanisms of formation will be discussed.

MATERIALS AND METHODS

FIF-1

A 31-year-old primigravid woman was referred to Kanagawa Children's Medical Center (KCMC) at 25 weeks of gestation for ultrasonographic diagnosis of a fetal abdominal mass. Ultrasonopgraphy (US) at 26 weeks indicated that a 43-mm retroperitoneal cystic mass contained solid tissues. Magnetic resonance imaging (MRI) at 35 weeks suggested a 90-mm irregularly shaped soft tissue mass consisting of shaft-like bones and spinal column (Fig. 1a). US at 37 weeks showed a retroperitoneal cystic mass containing a long bone-like tissue (Fig. 1b). Based on these findings, FIF was the most likely diagnosis. At 38 weeks, a cesarean section was performed, and a male newborn infant weighing 4,026 g was delivered with Apgar scores of 8 at 1 and 5 min. Retroperitoneal

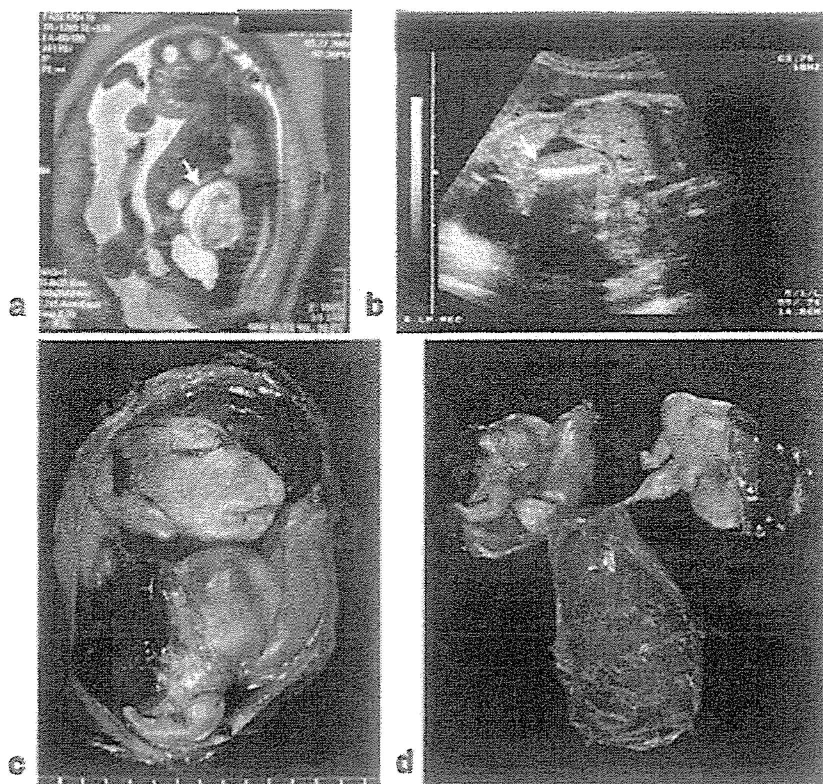

Fig. 1. FIF-1. **a**: Magnetic resonance imaging at 35 weeks showing a vertebral column (arrow). **b**: Ultrasonography (US) at 37 gestational weeks showing a retroperitoneal cystic mass containing a long bone-like structure (arrow). **c** and **d**: A cystic mass covered with a membrane containing two separate fetus-like bodies each with an umbilical cord connected to the host infant's aorta. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

tumorectomy, together with left adrenalectomy, was conducted at age 1 day. The cystic mass (9 × 9 × 5 cm) covered with an amnion-like membrane contained two separate fetiform masses, both almost totally covered with skin and having umbilical cords connected with the host aorta, and also containing oral cavity, limbs, spinal columns (one column was bifid), bowel, thymus, thyroid gland, testis, and other tissues (Fig. 1c, d). There was no placenta-like tissue. Both masses (F-1a and F-1b) were diagnosed as FIF.

FIF-2

A 31-year-old woman was referred to KCMC because of a US pattern of fetal hydrocephaly at 19 weeks of gestation (Fig. 2a). US demonstrated a large fetal head with a diameter of 82 mm (corresponding to the size for a 33-week-old fetus), multiple, high-density, intracranial calcifications, and a 60-mm intracranial tumor. Pregnancy was terminated at 21 weeks of gestation. A male fetus weighing 985 g was delivered. Autopsy showed an intracranial fetiform mass composed of limbs, vertebral column, lung, liver, adrenal, and bowel-like tissues (Fig. 2b), in addition to six irregular, fetiform tumors (Fig. 2c), thus, the largest fetiform mass (F-2) was diagnosed as FIF.

Genotyping at Microsatellite Marker Loci

Whole blood samples were obtained from Hosts 1 and 2 and from their respective parents. Fibroblast cultures were established from both host infants and three fetuses (F-1a, F-1b, and F-2). Genomic DNA was extracted from lymphocytes and fibroblasts of the host infants, and fibroblasts of the fetuses, as well as from lymphocytes of the parents. Six small pieces of fetiform tissues in FIF-2 were unavailable for the present study. A total of 38 microsatellite markers on the ABI Prism Linkage Mapping Set MD-10 (Panels 11, 12, 21, 22, 23, and 24; Applied Biosystems, Foster City, CA) were used to genotype Host 1, F-1a and F-1b, and 26 such markers (Panels 21 and 22) for Host 2 and F-2. DNA was amplified by PCR, and PCR products were analyzed on 3100 Genetic Analyzer™ (Applied Biosystems).

Microarray Analysis of Single Nucleotide Polymorphism (SNP) Loci

We used GeneChip Human Mapping 50K Array Xba™ (Affymetrix, Inc., Santa Clara, CA) for SNP genotyping. Genomic DNA (250 ng) was digested with *Xba*I (New England Biolabs, Beverly, MA), ligated by adaptor *Xba*I (Affymetrix), and subjected to PCR using primers (GeneChip Human Mapping 50K Xba Assay Kit™, Affymetrix) with conditions of 94°C for 3 min followed by initial denaturation of 94°C for 30 sec, 60°C for 45 sec, and 68°C for 60 sec for 30 cycles, and by final extension of 68°C for 7 min. PCR products were purified, fragmented using fragmentation reagents (Affymetrix), end-labeled with terminal deoxynucleotidyl transferase, and hybridized onto 50K Mapping Array™ (Affymetrix) at 48°C for 16 hr at 60 rpm. The hybridized array was washed and stained on Fluidics Station 450™ and scanned with GeneChip Scanner™

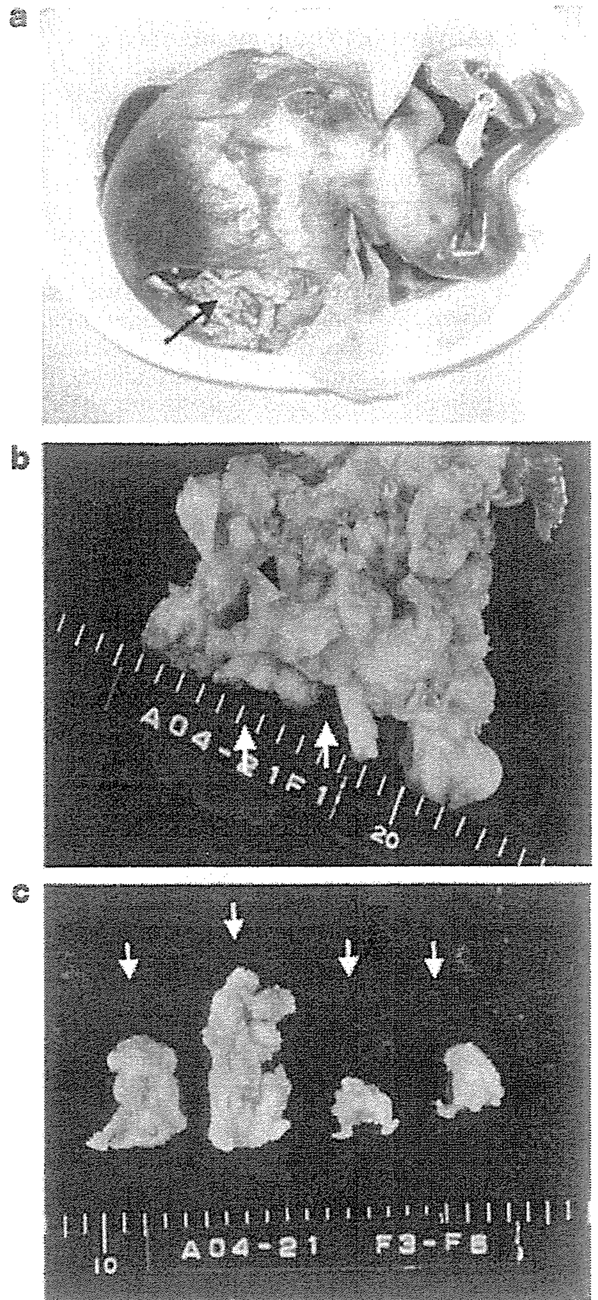

Fig. 2. FIF-2. **a**: A male infant having an intracranial fetus-like organ (arrow). **b** and **c**: Six irregular fetiform tissues (arrow) in addition to the intracranial fetus as shown in (**c**). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

(Affymetrix). The arrays were analyzed with software GDAS™ version 3.0.2 (Affimetrix) that provides rank scores for the probability of particular genotypes at SNP loci. The scores for homozygous alleles are "AA" or "BB" call and for heterozygous alleles "AB" call, and confidence scores show the accuracy of the genotype call. We chose SNPs showing discordancy (each confidence score of <0.1) between the host infants and their fetuses.

Prior to the GeneChip analysis, we tested whether a given SNP call could be changed before and after *Xba*I digestion, because uncut *Xba*I fragments would show a seemingly aberrant allele. Two pairs of primers (a SNP and its upstream sites, and the SNP and downstream *Xba*I sites) were designed (Fig. 3a). After DNA from Host 1, F-1a and F-1b was digested with *Xba*I, it was amplified with PCR and sequenced under conditions above. Consequently, 12 *Xba*I sites randomly selected were confirmed in situ, and genotypes of Host 1 always corresponded those of F-1a and F-1b.

Regions of SNPs discordant between the host infants and their fetuses in the GeneChip analysis were sequenced in order to confirm the results. PCR was performed with primers generated for both sides of SNP sites using 2 µl of DNA, 10× PCR buffer, GeneAmp™ dNTP Mix (Applied Biosystems), and 0.5 µl of AmpliTaq Gold™ (Applied Biosystems). PCR conditions were 94°C for 10 min, and 30 cycles of 94°C for 30 sec, 60°C for 30 sec, and 72°C for 30 sec, followed by final extension at 72°C for 10 min. PCR products were analyzed by agarose gel electrophoresis and then sequenced on 3100 Genetic Analyzer (Applied Biosystems).

Methylation Assay at the *IGF2-H19* Locus

Methylation status of the differentially methylated region (*DMR*) at the human *IGF2-H19* locus at chromosome 11p15.5 was analyzed in the two cases of FIF. To differentiate parental alleles in the host infants and their fetuses, we adopted an 8097A/G SNP (Genbank Access No. AF125183, rs2107425) within *DMR* (Fig. 3b). Primer sequences (5′-3′) were as follows [Poon et al., 2002]: forward, GGACGGAATTGGTTGTAGTT; and reverse, AGG-

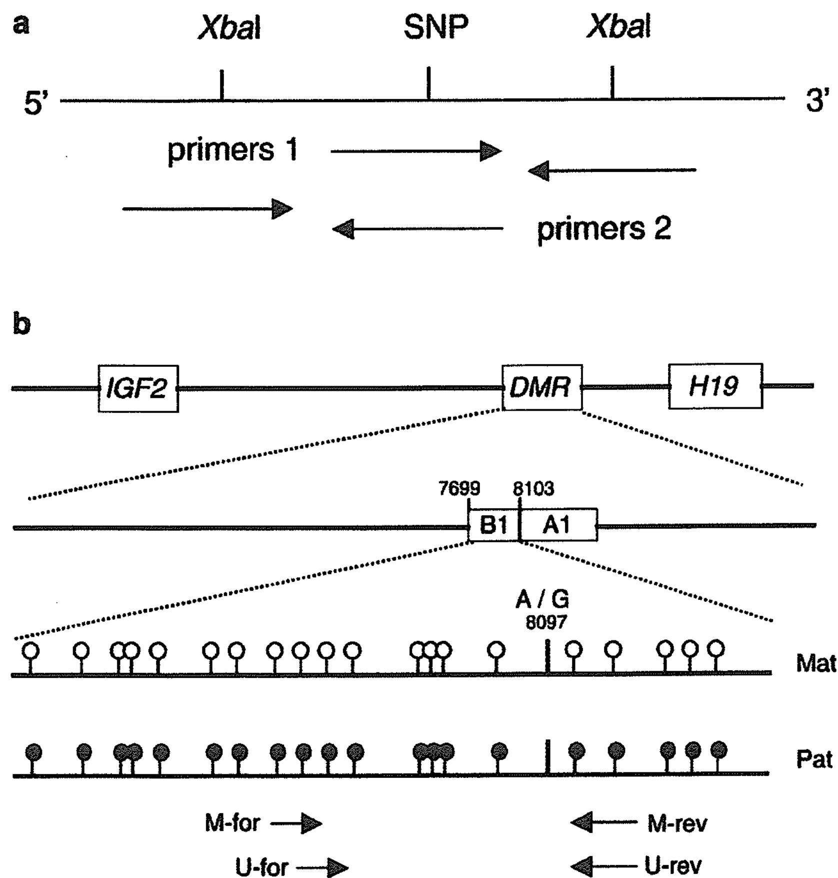

Fig. 3. **a**: *Xba*I sites around a putative SNP call for GeneChip Human Mapping 50K Array (Affymetrix) and primers designed to detect the SNP. Arrows indicate primer location and direction. **b**: Genomic structure and differentially methylated region (*DMR*) of the human *IGF2-H19* locus at 11p15.5. Numbers above bars show nucleotide numbers. Open and closed lollipops depict unmethylated and methylated regions in maternally (Mat) and paternally (Pat) derived normal chromosomes 11, respectively. Arrows indicate primer location and direction.

CAATTGTCAGTTCAGTAA. PCR was performed using Gene Amp™ PCR system 9700 (Applied Biosystems) with the conditions of 2 min at 95°C, and 35 cycles of 1 min at 95°C, 20 sec at 56°C, 20 sec at 72°C, and 10 min at 72°C. PCR products were analyzed by agarose gel electrophoresis, and sequenced by 3100 Genetic Analyzer™ (Applied Biosystems).

DNA was modified with sodium bisulfite [Kubota et al., 1997] using CpGenome™ DNA Modification Kit (Chemicon International, Temicula, CA) as instructed by the manufacturer. After conversion of DNA, the methylation status of *DMR* was examined. Methylation-specific PCR was performed in a 10 µl mixture containing 1 µl of modified DNA, 1 µl of 10× PCR buffer, 0.8 µl of GeneAmp™ dNTP Mix (Applied Biosystems), each of 1 µl forward and reverse primers, and 0.05 µl of AmpliTaq Gold™ (Applied Biosystems) under conditions of initial denaturation at 95°C for 12 min followed by 60 cycles of 95°C for 45 sec, 55°C (or 49°C) for 20 sec, and 72°C for 20 sec. Sequences (5′-3′) of primers designed by Poon et al. [2002] are as follows: M-for, TTAATTGGGGTTC-GTTCG: M-rev, CCCGACCTAAAAATCTAATACGA; U-for, GGTTTGTTTGTGGAAATGTTTT; and U-rev, CCCAACCTAAAAATCTAATACAA. PCR products were cloned using TOPO TA cloning Kit™ (Invitrogen, Carlsbad, CA) and sequenced on 3100 Genetic Analyzer.

TABLE I. Genotypes at Microsatellite Marker Loci of Two Cases of FIF

FIF-1	Markers	Mother	Father	Host 1	F-1a	F-1b
	D16S3075	2,2	1,3	1,2	1,2	1,2
	D16S3136	1,2	3,4	2,3	2,3	2,3
	D16S520	2,3	1,2	1,3	1,3	1,3
	D15S131	2,4	1,3	2,3	2,3	2,3
	D16S503	2,2	1,3	1,2	1,2	1,2
	D16S423	1,2	3,4	1,3	1,3	1,3
	D15S1007	3,3	1,2	1,3	1,3	1,3
	D7S669	1,2	1,3	2,3	2,3	2,3
	D7S502	1,3	2,4	2,3	2,3	2,3
	D8S505	2,2	1,3	1,2	1,2	1,2
	D8S285	1,3	2,3	1,2	1,2	1,2
FIF-2	Markers	Mother	Father	Host 2	F-2	
	D16S3136	1,3	2,2	2,3	2,3	
	D15S130	2,2	1,1	1,2	1,2	
	D16S515	1,2	3,3	1,3	1,3	
	D15S1002	1,2	3,4	1,3	1,3	
	D16S503	3,4	1,2	2,3	2,3	
	D15S127	1,3	2,4	2,3	2,3	
	D15S153	1,4	2,3	3,4	3,4	
	D15S117	2,3	1,1	1,3	1,3	
	D16S3046	1,2	3,4	1,4	1,4	
	D15S205	2,3	1,3	1,2	1,2	
	D15S1012	3,3	1,2	1,3	1,3	
	D15S120	2,2	1,3	2,3	2,3	
	D15S128	1,3	2,4	1,2	1,2	

RESULTS

Genotypes of Host Infants and Their Fetuses

Eleven of 38, and 13 of 26 microsatellite markers used for genotyping were informative in FIF-1 and FIF-2, respectively (Table I). Genotype analysis showed that at any informative locus, Host 1, F-1a and F-1b all inherited one copy of each parental allele, and their genotypes were all identical. The same results were obtained in Host 2 and F-2. As for the microarray analysis, the overall SNP call rate was more than 95%, indicating that all experimental steps had worked well. Host 1, and F-1a and F-1b seemed discordant (confidence < 0.1) for genotypes at 52 of some 50,000 SNP loci examined, although F-1a and F-1b were concordant at all these loci. However, sequence-based genotyping at 13 SNP loci that were randomly chosen from the 52 SNP regions showed complete concordance among the three subjects (Table II). There were no discordant alleles examined between Host 2 and F-2.

Methylation Status of *DMR* in Host Infants and Their Fetuses

Analysis of a *DMR*-SNP in FIF-1 showed that the mother was an AA homozygote, the father a GA heterozygote, and Host 1, F-1a and F-1b were all GA heterozygotes (Table III). Thus alleles A and G in the children were maternally and paternally derived, respectively. Likewise in FIF-2, the mother was a GG homozygote, the father an AA homozygote, and both Host 2 and F-2 GA heterozygotes, indicating that the children inherited one maternal and one paternal allele (Table III).

Analysis of the methylation status in *DMR* at the *IGF2-H19* locus was done by bisulfite treatment, and methylated or unmethylated sequence-specific PCR, followed by cloning of PCR products. Normally, only the paternal allele in *DMR* is methylated and the maternal allele remains unmethylated [Bartolomei and Tilghman, 1997]. This methylation pattern was altered in F-1a and F-1b. In F-1a, 8 (53.3%) of 15 clones showing unmethylated patterns had the maternally derived allele A and the remaining 7 (46.7%) had the paternally derived allele G (Table III). In F-1b, of nine such clones, three (33.3%) had the maternal and six (66.7%) had the paternal allele. On the contrary, the methylation status of Host 2 and F-2 were the same in all clones examined, showing normal patterns.

DISCUSSION

Prenatal US and MRI suggested that all three fetiform masses examined in our two cases contained vertebral columns, limb bones, and various soft tissues. The findings were confirmed by pathological analysis after birth, and ruled out teratomatous neoplasia but were consistent with FIF [Hoeffel

TABLE II. Thirteen SNP Markers, Genotypes Seemingly Discordant Between Host 1 and Two Fetuses (F-1a and 1b) by GeneChip Analysis, and Subsequent Confirmation of Concordancy by Sequencing Analysis

dbSNP ID	Chromosome	Primer	Sequence (5′-3′)	Genotype		
				Host 1	F-1a	F-1b
rs4131373	1	Forward	GCACAGTGGAGCTGCTGTTG	GG	GG	GG
		Reverse	GAGTTTGCTGAAGGCAGTGC			
rs960813	2	Forward	CGTGTTACCTTACTGTCCCT	GC	GC	GC
		Reverse	GTTCTGGTGTGTACTTTGCC			
rs4955768	3	Forward	CGATGAGGCATCTCATTACG	AA	AA	AA
		Reverse	TCTCTCTGTCCATCTGAAGG			
rs1951233	4	Forward	GCAATGATCACAGTGACTGAGG	AA	AA	AA
		Reverse	TGTGCTATTGTGGGTCTGCC			
rs60701	5	Forward	GAATCCCTTGGGTAGAACAC	TT	TT	TT
		Reverse	GGTGAATGCAGTAGTAGCCA			
rs2223995	6	Forward	CCAGCTCCACAATGAACCCA	TT	TT	TT
		Reverse	CCGTGCTTCCTAATGGTAGC			
rs1987475	7	Forward	GGAGCTCTGTGAGGAGCTGT	TT	TT	TT
		Reverse	CAAGCCCAGCTGAGCACCTT			
rs10503645	8	Forward	GCAAGAGTGTGGCTAGATCAC	CC	CC	CC
		Reverse	CCTGTTCCAGGCTGCCAGAT			
rs2077852	9	Forward	GACCTTCACCAGGACAACGT	GT	GT	GT
		Reverse	TTAGACCTTGTCAGGACGGC			
rs1159006	10	Forward	CATACCACAAGTCGTCGAGC	CT	CT	CT
		Reverse	CCTGCCAATCTAGAGGTAGG			
rs1036809	15	Forward	GGTCATCCTAGAGCAAGTCA	GC	GC	GC
		Reverse	GCACATCGTAGGGACTTAAG			
rs8587	16	Forward	CACGACCGACTGAACATTCT	AA	AA	AA
		Reverse	AAGCTGTCCTACACTTGTGC			
rs2067084	20	Forward	GCATGGTTCCTGGCATGCTG	CC	CC	CC
		Reverse	TCCACCAACCACTCTCTGTC			

et al., 2000; Brand et al., 2004]. Six separate fetiform tissues were observed in FIF-2, although we did not examine whether they had vertebral columns. Thus, in both of our cases of FIF, multiple fetuses-in-fetu were evident, as reported previously [Iyer et al., 2003]. Parthenogenetic origin or ovarian teratoma [Miura et al., 1999] was also totally ruled out in the two cases, because host infants were both males.

We have shown that in both cases analyzed host infants and their fetuses shared the same genotypes at all polymorphic loci examined. In other words, they originated from the same zygote. Our data support the monozygotic twin theory [Gilbert-Barness et al., 2003; Beaudoin et al., 2004; Brand et al., 2004]. However, a simple monozygotic (monochorionic, diamniotic) twin theory may not explain all cases of FIF. There have been some reports on the simultaneous presence of a fetus and teratoma [Magnus et al., 1999; Gilbert-Barness et al., 2003], multiple fetuses were involved in FIF [Iyer et al., 2003] as in our cases, fetiform mass was composed of underdeveloped organs or tissues [de Lagausie et al., 1997; Hopkins et al., 1997], and such mass grew in any body part of a fetus [Afshar et al., 1982; Magnus et al., 1999; Beaudoin et al., 2004; Borges et al., 2005]. A monozygotic, dichorionic, diamniotic twin theory [Beaudoin et al., 2004] may answer the questions. Normally, 30 hr after fertilization, a zygote consists of two cells, but if a zygote divides into two separate embryos, they may become monozygotic, dichorionic, diamniotic twins. Subsequently, around the 5th day of development, if one blastocyst that is derived from one conceptus is implanted into the other blastocyst instead of normal implantation into the uterine endometrium, or the former blastocyst is enclosed by the latter if they are implanted closely, and such an implant or inclusion body may not develop normally, leading to FIF. From an embryological point of view, the existence of the vertebral column in fetiform mass may reflect its derivation from a primitive streak. The formation of the primitive streak normally starts during the 3rd week, together with gastrulation that will lead to the notochord formation and subsequently to the

TABLE III. Genotypes of a SNP in the DMR at the *IGF2-H19* Locus and Methylated or Unmethylated Alleles After Bisulfite Conversion

Cases	Family members	Genotypes	Alleles	
			Methylated	Unmethylated (% of clones)
FIF-1	Mother	AA	A	A
	Father	GA	A	G (100)
	Host 1	GA	G	A (100)
	F-1a	GA	G	A (53.3), G (46.7)
	F-1b	GA	G	A (33.3), G (66.7)
FIF-2	Mother	GG	G	G
	Father	AA	A	A
	Host 2	GA	A	G (100)
	F-2	GA	A	G (100)

vertebral column and segmental axis. Thus, FIF likely arises from a zygote at a primitive-streak stage and fetiform mass develops to a certain degree in a manner similar to normal fetal development.

In our FIF-1, methylated patterns of F-1a and F-1b at the *IGF2-H19* locus were different from their host infant. Although the detailed methylation status at this locus during early human development has remained obscure, parental imprints in mice are erased once during gametogenesis [Reik et al., 2001; Li, 2002] and an early embryo is unmethylated and gradually begins to be methylated during blastocyst stage [Reik et al., 2001; Li, 2002]. In FIF-1, two isolated blastocysts originating from one zygote may have been implanted into (or included by) the other host blastocyst during a process of establishing methylation. The reprogramming of methylation in the two abnormally implanted embryos may have not proceeded thereafter by certain unknown mechanisms such as tumorization. Ovarian tumors from various stages of oogenesis do maintain their methylation status at their original stage [Miura et al., 1999]. On the other hand, such abnormal implantation may have occurred in FIF-2 after the establishment of methylation, since methylation patterns in Host 2 and F-2 were identical.

Thus, we may have provided another line of evidence that the FIF anomaly is of monozygotic origin. This is the first case of FIF showing different methylation patterns between a host infant and fetiform mass. Our data of the methylation analysis will contribute to future studies on pathogenesis of FIF.

ACKNOWLEDGMENTS

This study was supported by Grants-in-Aid for Scientific Research (Category C, 16591670 for K. M. and Category C, No. 17591748 for H. M.), Grant-in-Aid on Priority Areas "Applied Genomics" (No. 17019055 for N. N.) from the Ministry of Education, Sports, Culture, Science and Technology of Japan, and by SORST from Japan Science and Technology Agency (JST) (for N. N.)

REFERENCES

Afshar F, King TT, Berry CL. 1982. Intraventricular fetus-in-fetu. J Neurosurg 56:845–849.

Bartolomei MS, Tilghman SM. 1997. Genomic imprinting in mammals. Annu Rev Genet 31:493–525.

Beaudoin S, Gouizi G, Mezzine S, Wann AR, Barbet P. 2004. Mediastinal Fetus in fetu case report and embryological discussion. Fetal Diagn Ther 19:453–455.

Borges E, Lim-Dunham JE, Vade A. 2005. Fetus in fetu appearing as a prenatal neck mass. J Ultrasound Med 24:1313–1316.

Brand A, Alves MC, Saraiva C, Loio P, Goulao J, Malta J, Palminha LM, Martins M. 2004. Fetus in fetu-Diagnostic criteria and differential diagnosis—A case report and literature review. J Pediatr Surg 39:616–618.

Chen CP, Chern SR, Liu FF, Jan SW, Lee HC, Sheu JC, Lee WT, Wang TY. 1997. Prenatal diagnosis, pathology, and genetic study of fetus in fetu. Prenat Diagn 17:13–21.

de Lagausie P, de Napoli Cocci S, Stempfle N, Truong QD, Vuillard E, Ferkadji L, Aigrain Y. 1997. Highly differentiated teratoma and fetus-in-fetu: A single pathology? J Pediatr Surg 32:115–116.

Eng HL, Chuang JH, Lee TY, Chen WY. 1989. Fetus in fetu: A case report and review of the literature. J Periatr Surg 24:296–299.

Gilbert-Barness E, Opitz JM, Debich-Spicer D, Muller T, Arnold SR, Quintero R. 2003. Fetus-in-fetu form of monozygotic twinning with retroperitoneal teratoma. Am J Med Genet Part A 120A:406–412.

Heifetz SA, Alrabeeah A, Brown BS, Lau H. 1988. Fetus in fetu: A fetiform teratoma. Pediatr Pathol 8:215–226.

Higgins KR, Coley BD. 2006. Fetus in fetu and fetiform teratoma in 2 neonates: An embryologic spectrum? J Ultrasound Med 25: 259–263.

Hing A, Corteville J, Foglia RP, Bliss DP Jr, Donis-Keller H, Dowton SB. 1993. Fetus in fetu: Molecular analysis of a fetiform mass. Am J Med Genet 47:333–341.

Hoeffel CC, Khoag QN, Tran TT, Fornes P. 2000. Fetus in fetu: A case report and literature review. Pediatrics 105:1335–1344.

Hopkins KL, Dickson PK, Ball TI, Ricketts RR, O'Shea PA, Abramowsky CR. 1997. Fetus-in-fetu with malignant recurrence. J Pediatr Surg 32:1476–1479.

Iyer KV, Vinaya K, Haller JO, Maximin S, Barrerras J, Velchek F. 2003. Multiple fetuses in fetu: Imaging findings. Pediatr Radiol 33:53–55.

Kubota T, Das S, Christian SL, Baylin SB, Herman JG, Ledbetter DH. 1997. Methylation-specific PCR simplifies imprinting analysis. Nat Genet 16:16–17.

Kumar AN, Chandak GR, Rajasekhar A, Reddy NCK, Singh L. 1999. Fetus-in-fetu: A case report with molecular analysis. J Pediatr Surg 34:641–644.

Li E. 2002. Chromatin modification and epigenetic reprogramming in mammalian development. Nat Rev Genet 3:662–673.

Lord JM. 1956. Intra-abdominal foetus in foetu. J Pathol Bacteriol 72:627–641.

Magnus KG, Millar AJ, Sinclair-Smith CC, Rode H. 1999. Intrahepatic fetus-in-fetu: A case report and review of the literature. J Pediatr Surg 34:1861–1864.

Miura K, Obama M, Yun K, Masuzaki H, Ikeda Y, Yoshimura S, Akashi T, Niikawa N, Ishimaru T, Jinnno Y. 1999. Methylation imprinting of H19 and SNRPN gene in human benign ovarian teratomas. Am J Hum Genet 65:1359–1367.

Naudin ten Cate L, Vermeij-Keers C, Smit DA, Cohen-Overbeek TE, Gerssen-Schoorl KBJ, Dijkhuizen T. 1995. Intracranial teratoma with multiple fetuses: Pre- and post-natal appearance. Hum Pathol 26:804–807.

Ouimet A, Russo P. 1989. Fetus in fetu or not? J Pediatr Surg 24:926–927.

Poon LLM, Leung TN, Lau TK, Chow KCK, Lo YMD. 2002. Differential DNA methylation between fetus and mother as a strategy for detection fetal DNA in maternal plasma. Clin Chem 48:135–141.

Reik W, Dean W, Walter J. 2001. Epigenetic reprogramming in mammalian development. Science 293:1089–1093.

Willis RA. 1935. The structure of teratoma. J Pathol Bacteriol 40: 1–36.

Congenital Neuroblastoma in a Patient With Partial Trisomy of 2p

Yuri Dowa, MD,[*] Toshiyuki Yamamoto, MD,[*] Yasuko Abe, MD,[†] Masaki Kobayashi, MD,[‡] Rikuo Hoshino, MD,[‡] Kazumi Tanaka, MD,[§] Noriko Aida, MD,[||] Hiroshi Take, MD,[¶] Keisuke Kato, MD,[#] Yukichi Tanaka, MD,[#] Jyunpei Ariyama,[**] Naoki Harada,[**] Naomichi Matsumoto, MD,[††] and Kenji Kurosawa, MD[*]

Summary: We report the fourth example of a patient with germline partial trisomy of 2p21-pter and congenital neuroblastoma. The male infant had a dysmorphic facial expression and presented with congenital heart disease, supernumerary nipples, hypospadias, shawl scrotum, hemilateral persistent hyperplastic primary vitreous, and neuroblastoma. His germline karyotype of 46,XY,der(8)t(2;8)(p21;p23.2) was inherited from a maternal-balanced translocation, which indicates that the proto-oncogene *MYCN* region of 2p24.3 is tripicated in germline cells. A cytogenetic study of the biopsied tumor cells did not show *MYCN* amplification, but the DNA index was 2.4 and histologic fluorescent in situ hybridization analysis indicated somatic mutation with near-pentaploidy of the tumor cells. This could be an alternative mechanism of *MYCN* activation in the process of the tumorigenesis of neuroblastoma.

Key Words: neuroblastoma, *MYCN*, 2p trisomy, translocation

(*J Pediatr Hematol Oncol* 2006;28:379–382)

In Japan, neuroblastoma is the most common malignant solid tumor in childhood and accounts for 10% of all cases of childhood cancer.[1] Eighty percent of cases are diagnosed before the age of 5 years, with a mean age at diagnosis of 2 years. The tumor arises from primitive neural crest cells that form the adrenal medulla and sympathetic nervous system. Cytogenetic and molecular analysis of neuroblastoma has identified several structural chromosomal aberrations, including amplification of *MYCN* (a member of the MYC gene family), loss of 1p36, and gain of 17q.[1] It is now estimated that approximately 25% of neuroblastomas carry amplified *MYCN*, making this gene one of the most reliable prognostic factors for neuroblastoma. Normally, only a single copy of *MYCN* resides on chromosome 2.

Partial trisomy of 2p was first reported over 30 years ago.[2] Lurie et al[3] recently reviewed over 50 cases, including 3 new reports. Characteristic clinical features include anomalies of the face, trunk, limbs, and genitalia, and psychomotor delay. Other congenital defects that seem to be associated with this trisomy involve the neural tube, lung, heart, and diaphragm. Thus far, there have been 3 reports of partial trisomy of 2p associated with congenital neuroblastoma.[4–6] Recently, we encountered an additional patient having the partial 2p trisomy syndrome associated with neuroblastoma.

PATIENT REPORT

The patient was the second child of nonconsanguineous parents. The first son was healthy and the mother had a history of ovarian cancer. Prenatal examination by ultrasonography revealed a fetal double-outlet right ventricle (DORV) and a ventricle septum defect (VSD). Intrauterus growth retardation was detected in the third trimester. The male child was delivered by forceps at 37-week gestation after induction for nonreassuring fetal heart rate. At birth, the child showed severe intrauterus growth retardation, with a weight of 1776 g (−1.7 SD), height of 41 cm (−3.4 SD), and head circumference of 31 cm (−1.0 SD). He was intubated immediately because of respiratory distress that developed after birth. Significant physical findings consisted of unusual facial features with a prominent forehead, hypertelorism, a depressed nasal bridge, and posteriorly angulated ears (Fig. 1A). Supernumerary nipples, hypospadias, and a shawl scrotum were also noted (Fig. 1B). The prenatally detected congenital heart defect with DORV and VSD was confirmed by ultrasonographic examination, as was pulmonary stenosis.

The child was extubated at postnatal day 7. From 13 days of age, his abdomen began slowly distending, and the liver was palpable 2 cm under the costal arch. When the patient was 1 month old, abdominal ultrasonography and an abdominal computed tomography (CT) scan revealed a retroperitoneal mass and intrahepatic tumor (Fig. 1C). A biopsy was obtained from the metastatic tumor in the liver, and the findings of the pathologic examination were consistent with stage 4S neuroblastoma, poorly differentiated type, and favorable histology, according to the Shimada System (Figs. 1D, E).[1]

At 8 months, the patient had opacification of the left lens and aspiration was performed via the corneal limbus. We also

Received for publication September 6, 2005; accepted April 7, 2006.
From the Departments of *Medical Genetics, †Oncology, ‡Neonatology, §Ophthalmology, ||Radiology, ¶Surgery, and #Pathology, Kanagawa Children's Medical Center; ††Department of Human Genetics, Yokohama City University Graduate School of Medicine, Yokohama; and **Kyushu Medical Science Nagasaki Laboratory Inc, Nagasaki, Japan.
Reprints: Toshiyuki Yamamoto, MD, International Research and Educational Institute for Integrated Medical Sciences (IREIIMS), Tokyo Women's Medical University, 8-1 Kawada-cho, Shinjuku-ku, Tokyo, 162-8666, Japan (e-mail: yamamoto@imcir.twmu.ac.jp).
Copyright © 2006 by Lippincott Williams & Wilkins

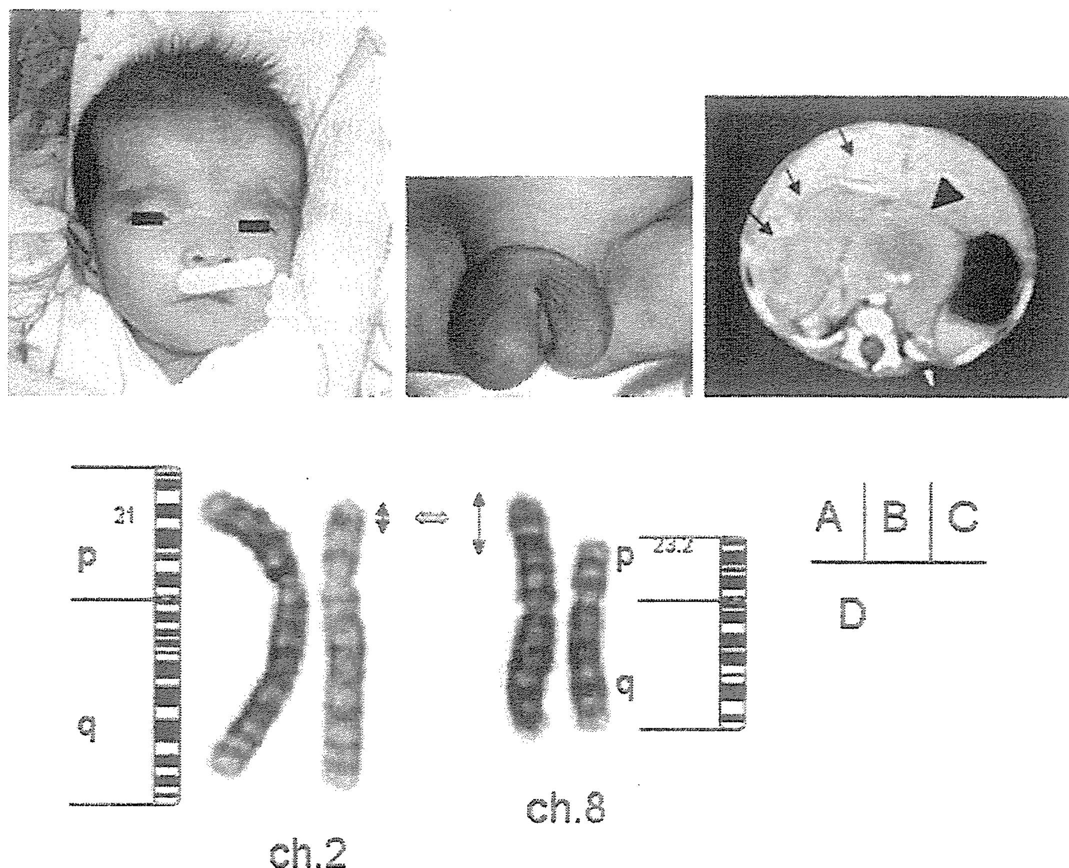

FIGURE 1. Photographs of dysmorphic facial appearance (A) and shawl scrotum (B) at 2 months of age. A large retroperitoneal mass (arrowhead) surrounding the abdominal aorta with punctual calcifications is shown by contrast-enhanced computed tomography (C). The enlarged right lobe of the liver including metastatic masses with irregular margin (arrows) can be seen. G-banded partial karyotype of chromosomes 2 and 8 of the mother (D). Derivative chromosomes are inside, which indicate the karyotype of the patient as 46,XY,der(8)t(2;8)(p21;p23.2)mat.

detected abnormal vascularization from the posterior element of the left lens, indicating that the secondary cataract was induced by persistent hyperplastic primary vitreous (PHPV).[7]

He has been receiving primary chemotherapy with low-dose cyclophosphamide and vincristine, and now he is 1.5 years old without any progression of the neuroblastoma.[8] However, he showed severe psychomotor developmental delay as he had never sat up alone, and had been fed by tube because of feeding difficulty.

CYTOGENETIC STUDIES

The clinical features were suggestive of a partial 2p trisomy, and a cytogenetic study was performed using his peripheral leukocytes. Routine G-banded chromosome analysis showed a male karyotype with a derivative chromosome 8 with unknown origin on the short arm. To confirm the origin of this additional region, parental karyotypes were analyzed. His father's karyotype was normal with 46,XY, and his mother showed reciprocal translocation between 2p and 8p with a karyotyping 46,XX,t(2;8)(p21;p23.2) (Fig. 2A). Detailed fluorescent in situ hybridization (FISH) analyses using BAC/PAC clones were performed to detect the breakpoint of each chromosome, according to a method described elsewhere.[9] BAC/PAC clones of RP11-24K2, RP11-128C5, RP11-72C11, RP11-633O21, RP11-819H19, RP11-907L18, RP11-130P22, and RP11-91C18 on the short arm of chromosome 2, and RP11-412I11, RP11-121F7, RP11-590N22, RP11-243D4, RP11-89I12, RP11-296E5, RP11-3N20, RP11-143D15, and RP11-118D21 on the short arm of chromosome 8 were selected by the UCSC genome browser (http://genome.ucsc.edu/). The patient's final karyotype was determined to be 46,XY,der(8) t(2;8)(p21;p23.2)mat. ish der(8)t(2;8)(p21;p23.2)(RP11-24K2+,RP11-128C5+,RP11-72C11+,RP11-633O21+, RP11-819H19+,RP11-907L18+,RP11-130P22−,RP11-91C18−,RP11-412I11−,RP11-121F7−,RP11-590N22+, RP11-243D4+,RP11-89I12+,RP11-296E5+,RP11-3N20+, RP11-143D15+,RP11-118D21+). This result indicates partial trisomy of 2p21-pter, in which *MYCN* is included, and partial monosomy of 8p23.2-pter, not including the locus of *GATA4*.

 © 2006 Lippincott Williams & Wilkins

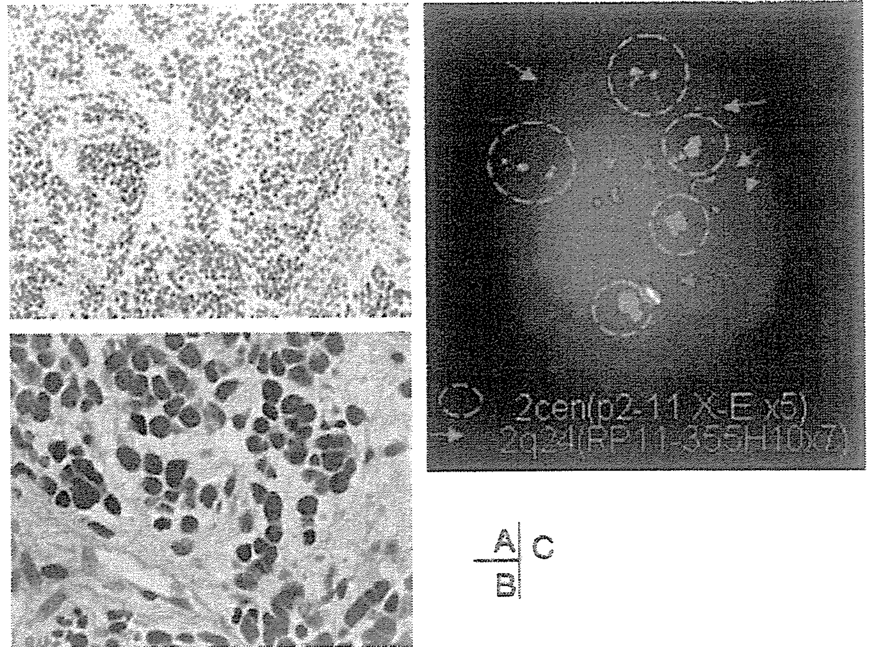

FIGURE 2. Pathologic examination with hematoxylin/eosin staining, ×10 (A) and ×40 (B). The wedge-resected tissue showed nests of neuroblastic cells with neurofibrillary matrix delineated by edematous stroma with hepatic cell cords. The stroma was composed of a fine eosinophilic matrix, but the Schwannian stroma was less developed. Mitoses and karyorrhexis were observed at a rate less than 2% of the entire neoplastic cells. Histologic FISH analysis of touch imprint of the biopsy specimens (C). Signals from probes for the chromosome 2 centromere (D2Z1) and *MYCN* are visible, with 5 and 7 signals, respectively, per cell.

Slot-blot hybridization analysis of biopsy specimens to detect gene amplification of *MYCN* did not show amplification (SRL, Tokyo). However, a *MYCN* proto-oncogene DNA index of 2.4 was obtained (SRL, Tokyo). Further histologic FISH analysis of a touch imprint of the tumor was performed using a chromosome 2 centromere probe prepared from p2-11 *X-E* (a generous gift from Dr H.F. Willard) and a *MYCN* probe prepared from RP11-355H10 (BAC/PAC Resources Center, Children's Hospital Oakland Research Institute, CA). The result showed that most of the neoplastic cells displayed 3 to 7 signals of the chromosome 2 centromere and 5 to 9 signals of *MYCN*. The median observations were 5 signals of the chromosome 2 centromere and 7 signals of *MYCN*, indicating somatic mutation with near-pentaploidy of the tumor cells (Fig. 2B).

DISCUSSION

The patient's characteristic clinical features of a prominent forehead, hypertelorism, a depressed nasal bridge, posteriorly angulated ears, hypospadias, and a shawl scrotum are compatible with partial duplication of chromosome 2p. This patient developed neuroblastoma within a month after birth and a secondary cataract induced by PHPV at the age of 8 months. This is the fourth report of partial 2p trisomy syndrome associated with neuroblastoma. Previous studies have found that associated chromosomal deletions in these patients were variable and included 13q34, 16p11, and 8p23.[4–6] However, triplication of *MYCN* in the germline cells (in the triplicated region of 2p24.3) was common to all 4 patients (Table 1).

In 1983, Schwab et al[10] reported the amplification of *MYCN* in neuroblastoma cell lines and in 1 tumor. In 1984, Brodeur and colleagues[11] reported the correlation of *MYCN* amplification with advanced-stage disease. Clinical studies have confirmed that amplification of the *MYCN* proto-oncogene is one of the best prognostic indicators of poor outcome.[12] Although approximately 30% of neuroblastoma tumors present *MYCN* amplification at diagnosis, FISH studies have recently shown that *MYCN* is occasionally duplicated at its resident site in neuroblastoma cell lines without further amplification, suggesting that it could be an alternative mechanism of *MYCN* activation.[13,14] However, only 4 patients with trisomy 2p showing congenital neuroblastoma have thus far been reported.[4–6]

The present patient also had a partial monosomy of 8p23.2-pter. There are many reported patients with small

TABLE 1. Summary of Germline 2p Trisomy Associated With Neuroblastoma

	Duplicated Region	Deleted Region	Origin	Neuroblastoma	The Other Combined Symptoms
Patel et al[6]	2p23-pter	13q34-qter	Unknown	+	Postaxial polydactyly, seizures
Our patient	2p21-pter	8p23.2-pter	Maternal	+	DORV, VSD, PS, hypospadias, shawl scrotum, cataract, severe mental retardation
Say et al[4]	2p21-p25	—	Unknown	+	Lung agenesis, dead at 35 d
Nagano et al[5]	2p13-pter	16p11-pter	Paternal	+	Microphthalmos, congenital cataract, micropenis

© 2006 Lippincott Williams & Wilkins

distal 8p deletions.[15,16] The findings in these patients suggest that major congenital anomalies, especially congenital heart defects, are frequent, but facial dysmorphism may be subtle and mental retardation less severe than in those with deletions associated with more proximal breakpoints.[15,16] Mutations and haploinsufficiency of *GATA4* in this region with 8p23.1 have been shown to be responsible for congenital heart defects.[17,18] According to our detailed FISH study, the locus of *GATA4* is at least 8 Mb proximal from the breakpoint of 8p23.2. Thus, congenital heart defects associated with DORV, VSD, and pulmonary stenosis of this patient, cannot be explained by haploinsufficiency of *GATA4*.

In patients with PHPV, the primary vitreous and embryonic vitreous vasculature remains and grows even after birth, although it is generally isolated and unilateral. The incidence of this condition is unknown, but it is not rare. Although PHPV has been associated with numerous genetic syndromes, the mechanism contributing to these conditions remains unclear.[19]

REFERENCES

1. Brodeur G, Sawada T, Tsuchida Y, et al. *Neuroblastoma.* Amsterdam: Elsevier; 2000.
2. Bender K, FAU-Reinwein H, Gorman LZ, et al. Familial 2-C translocation: 46,XYt(2p-;Cp+) and 46,XXCp+. *Humangenetik.* 1969;8:94–104.
3. Lurie IW, Ilyina HG, Gurevich DB, et al. Trisomy 2p: analysis of unusual phenotypic findings. *Am J Med Genet.* 1995;55:229–236.
4. Say B, Carpenter NJ, Giacoia G, et al. Agenesis of the lung associated with a chromosome abnormality (46,XX,2p+). *J Med Genet.* 1980;17:477–478.
5. Nagano H, Kano Y, Kobuchi S, et al. A case of partial 2p trisomy with neuroblastoma. *Jinrui Idengaku Zasshi.* 1980;25:39–45.
6. Patel JS, Pearson J, Willatt L, et al. Germline duplication of chromosome 2p and neuroblastoma. *J Med Genet.* 1997;34:949–951.
7. Dass AB, Trese MT. Surgical results of persistent hyperplastic primary vitreous. *Ophthalmology.* 1999;106:280–284.
8. Rubie H, Coze C, Plantaz D, et al. Localised and unresectable neuroblastoma in infants: excellent outcome with low-dose primary chemotherapy. *Br J Cancer.* 2003;89:1605–1609.
9. Shimokawa O, Kurosawa K, Ida T, et al. Molecular characterization of inv dup del(8p): analysis of five cases. *Am J Med Genet A.* 2004;128:133–137.
10. Schwab M, Alitalo K, Klempnauer KH, et al. Amplified DNA with limited homology to myc cellular oncogene is shared by human neuroblastoma cell lines and a neuroblastoma tumour. *Nature.* 1983;305:245–248.
11. Brodeur GM, Seeger RC, Schwab M, et al. Amplification of N-myc in untreated human neuroblastomas correlates with advanced disease stage. *Science.* 1984;224:1121–1124.
12. Bown N. Neuroblastoma tumour genetics: clinical and biological aspects. *J Clin Pathol.* 2001;54:897–910.
13. Corvi R, Savelyeva L, Schwab M. Duplication of N-MYC at its resident site 2p24 may be a mechanism of activation alternative to amplification in human neuroblastoma cells. *Cancer Res.* 1995; 55:3471–3474.
14. Valent A, Le Roux G, Barrois M, et al. MYCN gene overrepresentation detected in primary neuroblastoma tumour cells without amplification. *J Pathol.* 2002;198:495–501.
15. Hutchinson R, Wilson M, Voullaire L. Distal 8p deletion (8p23. 1—8pter): a common deletion? *J Med Genet.* 1992;29:407–411.
16. Wu BL, Schneider GH, Sabatino DE, et al. Distal 8p deletion (8)(p23.1): an easily missed chromosomal abnormality that may be associated with congenital heart defect and mental retardation. *Am J Med Genet.* 1996;62:77–83.
17. Garg V, Kathiriya IS, Barnes R, et al. GATA4 mutations cause human congenital heart defects and reveal an interaction with TBX5. *Nature.* 2003;424:443–447.
18. Hirayama-Yamada K, Kamisago M, Akimoto K, et al. Phenotypes with GATA4 or NKX2.5 mutations in familial atrial septal defect. *Am J Med Genet A.* 2005;135:47–52.
19. Pellegrino JE, Engel JM, Chavez D, et al. Oculo-palatal-cerebral syndrome: a second case. *Am J Med Genet.* 2001;99:200–203.

© 2006 Lippincott Williams & Wilkins

© 2006 Wiley-Liss, Inc.
American Journal of Medical Genetics Part A 140A:1827–1833 (2006)

Clinical Outcome of Infants With Confined Placental Mosaicism and Intrauterine Growth Restriction of Unknown Cause

Kiyonori Miura,[1]* Koh-ichiro Yoshiura,[2,7] Shoko Miura,[1,7] Tatsuro Kondoh,[3] Naoki Harada,[4,7] Kentaro Yamasaki,[1] Yoko Fujimoto,[1] Yoko Yamasaki,[1] Terumi Tanigawa,[1] Yuriko Kitajima,[1] Takako Shimada,[1] Atsushi Yoshida,[1] Daisuke Nakayama,[1] Masato Tagawa,[3] Shuichiro Yoshimura,[1] Joseph Wagstaff,[5] Yoshihiro Jinno,[6] Tadayuki Ishimaru,[1,8] Norio Niikawa,[2,7] and Hideaki Masuzaki[1]

[1]Department of Obstetrics and Gynecology, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan
[2]Department of Human Genetics, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan
[3]Department of Pediatrics, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan
[4]Kyushu Medical Science, Nagasaki, Japan
[5]Department of Pediatrics, Clinical Genetics Program, Carolinas Medical Center, Charlotte, North Carolina
[6]Department of Molecular Genetics, University of the Ryukyus School of Medicine, Okinawa, Japan
[7]SORST, Japan Science and Technology Agency (JST), Kawaguchi, Japan
[8]Sasebo Chuo Hospital, Sasebo, Japan

Received 2 December 2005; Accepted 8 June 2006

The purpose of this study was to know a role of confined placental mosaicism (CPM) in perinatal outcome and postnatal growth and development of infants with intrauterine growth restriction (IUGR). We selected 50 infants with IUGR (<−2.0 SD) from 3,257 deliveries in a regional medical center during the past 10-year period, and carried out cytogenetic and molecular analyses in their placenta and cord blood. Of the 50 infants, 8 had CPM (CPM group) and were composed of five single (CPM2, 7, 13, 22, and 22), one double (CPM7/13), and one quadruple trisomy (CPM2/7/15/20), and one partial monosomy [del(2)(p16)]. The origin of an extra chromosome of trisomy was maternal in six cases of CPM, paternal in one, and undetermined in one. Uniparental disomy in disomic cell lines was ruled out in all these mosaics. We also compared clinical parameters for perinatal outcome between CPM group and infants without evidence of CPM (non-CPM group), such as maternal and gestational age, birth weight, *Apgar* score, cord blood pH, gender, and uterine artery patterns by Doppler ultrasonography, as well as weight, height, and developmental quotient (DQ) by Denver Developmental Screening Test at age 12 months. Phenotypic abnormalities were noted in two infants with CPM and three infants of non-CPM group: One with CPM22 had ASD and hypospadias, one with CPM7/13 had Russell–Silver syndrome (RSS), and one without CPM had polydactyly, and two without CPM had RSS. All but one infant with CPM are alive at age 12 months. Among the clinical parameters, the detection rate of a notch waveform pattern of the uterine artery was significantly higher in the CPM group ($P < 0.05$). However, no significant difference was noted in perinatal outcome of pregnancy and in DQ at age 12 months between the two groups. Interestingly, short stature (<−2 SD) at age 12 months was more frequently seen in CPM group (7/8 infants with CPM vs. 8/15 infants without CPM), although no statistically significant difference was obtained. The information obtained will be useful for perinatal care and genetic counseling for infants with IUGR and CPM. © 2006 Wiley-Liss, Inc.

Key words: confined placental mosaicism; intrauterine growth restriction; uniparental disomy; Doppler ultrasonography

How to cite this article: Miura K, Yoshiura K, Miura S, Kondoh T, Harada N, Yamasaki K, Fujimoto Y, Yamasaki Y, Tanigawa T, Kitajima Y, Shimada T, Yoshida A, Nakayama D, Tagawa M, Yoshimura S, Wagstaff J, Jinno Y, Ishimaru T, Niikawa N, Masuzaki H. 2006. Clinical outcome of infants with confined placental mosaicism and intrauterine growth restriction of unknown cause. Am J Med Genet Part A 140A:1827–1833.

Grant sponsor: Ministry of Education, Sports, Culture, Science and Technology of Japan; Grant numbers: 16591670, 17591748, 17019055; Grant sponsor: Japan Science and Technology Agency (JST).

*Correspondence to: Kiyonori Miura, Department of Obstetrics and Gynecology, Nagasaki University Graduated School of Biomedical Sciences, 1-7-1 Sakamoto, Nagasaki 852-8501, Japan.
E-mail: kiyonori@nagasaki-u.ac.jp

DOI 10.1002/ajmg.a.31389

WILEY InterScience® DISCOVER SOMETHING GREAT

INTRODUCTION

Confined placental mosaicism (CPM) may be defined as the presence of chromosomal mosaicism confined to tissues of extraembryonic origin. The mosaicism may be confined to the cytotrophoblast (type 1 CPM), the villous stroma (type 2 CPM), or both tissues (type 3 CPM) [Kalousek and Vekemans, 1996]. Chromosome mosaicism is detected in 1%–2% of chorionic villus samples (CVS) [Ledbetter et al., 1992; Wolstenholme et al., 1994; Stetten et al., 2004]. The correlation between CPM and intrauterine growth restriction (IUGR) of infants has remained controversial. Some studies showed higher frequency (6.5%–25%) of IUGR in pregnancies with CPM than those (5.0%–8.3%) in those without CPM [Kalousek et al., 1991; Ledbetter et al., 1992; Kalousek, 1994; Wolstenholme et al., 1994; Artan et al., 1995; Wilkins-Haug et al., 1995; Krishnamoorthy et al., 1995; Stipoljev et al., 2001], whereas others gave negative association between IUGR and CPM [Schwinger et al., 1989; Kennerknecht et al., 1993]. When trisomy rescue occurs (Fig. 1), one-third of fetuses may have uniparental disomy (UPD). IUGR is sometimes associated with UPD for chromosome 7, 11, 14, 15 or 16 [Ledbetter and Engel, 1995; European Collaborative Research on Mosaicism in CVS, 1999; Monk and Moore, 2004]. Maternal UPD for chromosome 7 is known to be one of the causes of Russell–Silver syndrome (RSS) that exhibits severe IUGR [Eggerding et al., 1994; Kotzot et al., 1995]. In addition, there have been two cases of IUGR who had CPM for chromosome 2 or 22 and maternal UPD for the respective chromosome [Ariel et al., 1997; Balmer et al., 1999], as well as cases of IUGR associated with CPM and maternal UPD for chromosome 16 [Eggermann et al., 2004]. In CPM-derived UPD, it is generally difficult to ascertain whether IUGR is caused by UPD in fetus or CPM in its placenta.

Clinical outcome of pregnancy and manifestations of infants with CPM varied from normal to abnormal delivery and phenotypes [Wolstenholme et al., 1994; Saks et al., 1998; Bryan et al., 2002; Roberts et al., 2003]. Abnormal outcome may depend on meiotic or mitotic origin of trisomy and on chromosomes involved in CPM [James and Jacobs, 1996]. It is also the consequence of abnormal placental functions due to abnormal karyotype, UPD or abnormal gene expression [Robinson et al., 1997]. However,

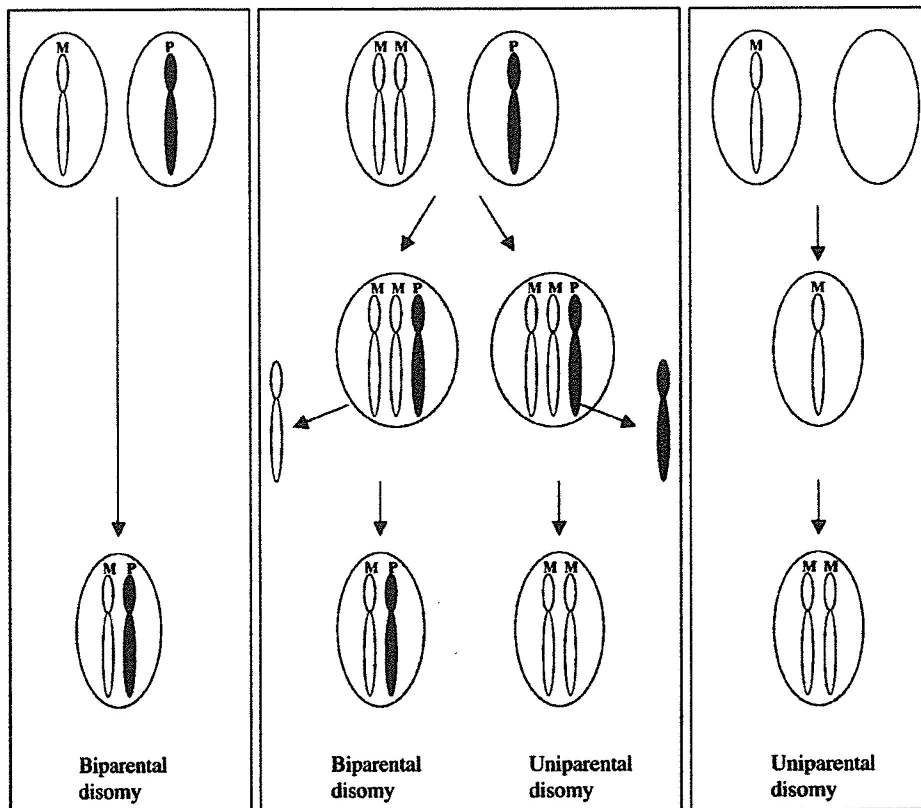

Fig. 1. Diagram illustrating the difference between biparental and uniparental disomy. **Right panel:** biparental disomy from normal fertilization. **Middle panel:** biparental and uniparental disomy from trisomic rescue. **Left panel:** monosomy duplication in a monosomic conceptus. M and P denote maternal allele and paternal allele, respectively.

American Journal of Medical Genetics Part A: DOI 10.1002/ajmg.a

it remains obscure whether CPM affects on postnatal growth and development. Therefore, molecular determination of origin of CPM may be useful to predict such outcome.

We have been following up a large series of pregnancies with IUGR of unknown cause and CPM. The purpose of this study was to know a role of CPM in perinatal outcome and postnatal growth and development of infants with IUGR.

MATERIALS AND METHODS

Patients

During a period from April 1995 to July 2004, there were 3,257 deliveries in Nagasaki University Hospital. Of these, 124 were with IUGR, judged prenatally on the basis of low estimated weight (<−2 SD) for gestational age defined by ultrasonography, and confirmed at delivery. Those with the following risk factors for IUGR were: multiple pregnancy; fetal chromosomal abnormality, infections and TORCH syndrome; maternal hypertension; maternal diabetes; maternal smoking (>20 cigarettes per day); or cord factor. Fifty fetuses without these risk factors, whose weight and length at birth were less than −2 SD, were included in the study. Biopsied chorionic villus specimens from two or more placental sites and cord blood were collected at delivery as were parental peripheral blood samples. All study protocols were approved by the Committee for the Ethical Issue on Human Genome and Gene Analysis in Nagasaki University.

Clinical Evaluation of Infants With IUGR

Clinical parameters for perinatal outcome included maternal age and gestational age at birth, birth weight, *Apgar* score, blood pH in the umbilical artery, sex, and Doppler ultrasonography of uterine artery (Fig. 2). At age 12 months after birth, the weight and length were measured of the infants, and their development was assessed using Denver Developmental Screening test (DDST) [Frankenburg and Dodds, 1967].

Cytogenetic Analysis

Tissue specimens from two or more sites of the term placenta were cultured and harvested. In each sample, 50–100 G-banded metaphases were analyzed in two flasks. Mosaicism was defined when a same chromosome abnormality was found in both two separate culture flasks.

Molecular Genetic Analysis

DNA was extracted from cord blood, placenta and parental blood using standard method. DNA samples from cases with CPM were genotyped using a microsatellite marker panel, ABI PRISM linkage-mapping set-MD10 (Applied Biosystems,

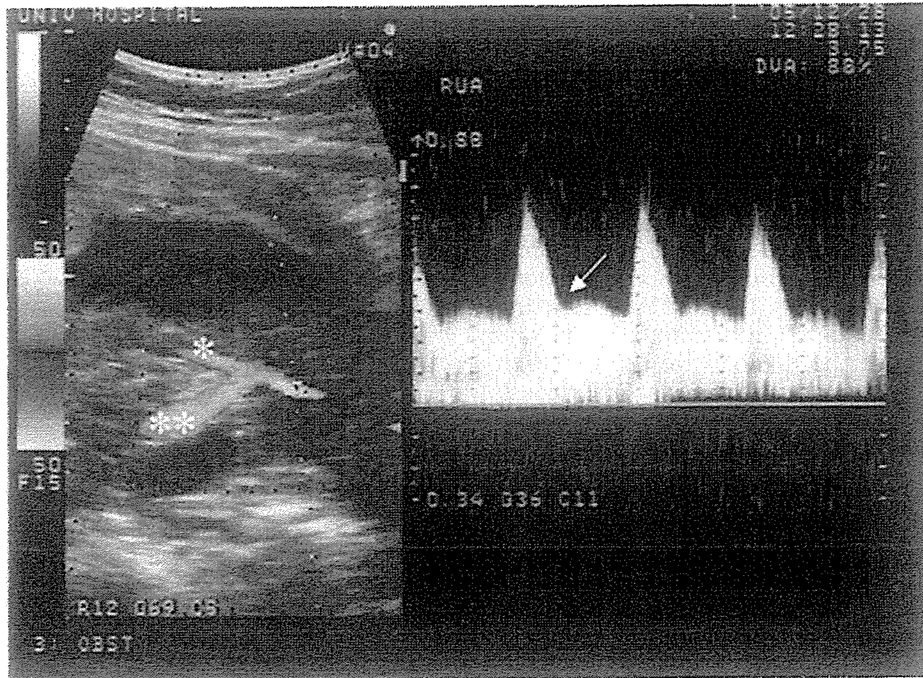

Fig. 2. Photograph of a notch-waveform ultrasonographic color-Doppler pattern of the uterine artery. White arrow points to a notch-waveform. * and ** denote uterine artery and external iliac artery, respectively.

Foster City, CA) and GeneAmp PCR System 9700 (Applied Biosystems). Each genotyping was repeated at least three times to avoid erroneous results. The PCR condition and the procedure of genotyping were described in our previous manuscript [Masuzaki et al., 2004].

Infants whose placental villi were disomy/trisomy mosaics were studied for possible UPD of the chromosome pairs involved in the trisomies. The analysis was carried out in DNA samples from the infants and their parents, using microsatellite marker panels corresponding to the chromosomes involved in the trisomies. Placental DNA samples with disomy/trisomy mosaicism were analyzed in the same manner, in order to learn about the origin of the trisomies involved.

RESULTS

Confined Placental Mosaicism

Of the 50 infants with IUGR and their placentas studied, 8 (16%) had a chromosomally abnormal cell line restricted to the placenta (Table I). Patient 1 had non-mosaic trisomy 22; Patients 2 to 5 were trisomy mosaics; Patient 6 was a double trisomy mosaic; Patient 7 a quadruple trisomy mosaics; and Patient 8 was a normal/del(2)(p16)mosaic. Cultured cord blood lymphocytes from these infants were chromosomally normal with either a 46,XX (three cases) or 46,XY (five cases) karyotype.

Origin of Placental Trisomic Cell Lines

Microsatellite markers close to the centromeres were analyzed in the six placentas with trisomic cell lines and peripheral blood samples from their parents (Table I). Analysis in Patients 1 to 4 (trisomies 22, 2, 7, and 13, respectively) indicated that their trisomic cell lines arose from maternal first meiotic non-disjunction (mat MI). Trisomy 7 in Patient 5 resulted from paternal first meiotic non-disjunction (pat MI). Trisomies 7 and 13 in Patient 6 both originated from either maternal second meiotic or mitotic non-disjunction (mat MII or mitotic), although to distinguish MII from mitotic events was impossible because the markers we used did not detect the presence of recombinations. Trisomies 2, 7, and 15 in Patient 7 likewise arose from either maternal second meiotic or mitotic events. The origin of trisomy 20 in the same infant, however, was not determined. Analysis of cord blood samples from the six infants with placental trisomic cell lines showed no instance of UPD for the chromosome pairs involved in the trisomies.

Phenotypic Abnormalities in the Infants With CPM

Patient 1, a boy, had an atrial septal defect (ASD) and hypospadias, both of which were surgically

TABLE I. Results of Cytogenetic and Molecular Analysis, and Clinical Outcome of Pregnancies With CPM

				Karyotype							
Case no.	Age (yrs)	Sex	Maternal age (yrs)	Placenta	Cord blood	Origin of trisomy	Gestatational age (weeks)	Birth weight (SD)	Cord blood pH	Notch-waveform pattern of uterine artery	Phenotypic abnormality
1	6	M	29	47,XY,+22[50]	46,XY	Mat MI	37	−4.6	7.31	+	ASD, hypospadias
2	6	M	28	46,XY[97]/47,XY,+2[3]	46,XY	Mat MI	34	−2.5	7.27	−	—
3	3	F	25	46,XX[62]/47,XX,+7[8]	46,XX	Mat MI	37	−3.2	7.14	+	—
4	9	M	26	46,XY[45]/47,XY,+13[5]	46,XY	Mat MI	35	−2.2	7.29	−	—
5	3	F	31	46,XX[10]/47,XX,+22[40]	46,XX	Pat MI	36	−2.9	7.34	+	—
6	1[a]	M	29	46,XY[98]/48,XY,+7,+13[2]	46,XY	Mat MII or mitotic	38	−5.0	7.19	+	Russell–Silver syndrome
7	10	M	22	46,XY[48]/50,XY,+2,+7,+15,+20[2]	46,XY	Mat MII or mitotic[b]	38	−3.2	7.30	+	—
8	7	F	29	46,XX[97]/46,XX,del(2)(p16)[3]	46,XX		36	−2.9	7.25	−	—

Mat MI, maternal first meiotic non-disjunction; Mat MII, maternal second meiotic non-disjunction; Pat MI, paternal first meiotic non-disjunction.
[a]Died at age 12 months.
[b]Origin of trisomy 20 unknown.

repaired. Patient 6, a boy, had RSS with triangular face, cleft lip, and hemihypertrophy. At age 9 months, he weighed 2,680 g (−6.8 SD), measured 49.5 cm (−8.8 SD), and had developmental retardation. At age 12 months, he died of unknown cause. Analysis with a microsatellite marker panel spanning the long arm of chromosome 7 ruled out UPD for all or a part of chromosome 7.

Growth and Development of Infants With or Without CPM

Growth and development were evaluated with DDST in 7 infants with CPM and another 13 infants without CPM (Table II). In both groups, growth retardation as judged by weight was less pronounced at age 12 months than at birth, although at age 12 months 6 of the 7 infants with CPM and 8 of the 13 infants without CPM were still less than −2 SD.

There was no appreciable difference between the two groups in the frequency of delayed development. Patient 2, a boy with placental mosaicism of 46,XY/47,XY,+2, showed delayed gross and fine motor performance and delayed social ability, although his speech development was normal. Patient 17, a boy without CPM, were delayed in all categories. Patient 29, a girl without CPM, likewise were delayed in all categories tested, and had polydactyly of right preaxial finger, which was non-syndromic.

DISCUSSION

By a retrospective screening of 124 pregnancies with IUGR, we have collected 50 infants without any evidence of having risk factors for IUGR. Among them, eight infants (16%) had CPM that was detected by biopsy on two or more placental sites, followed by karyotyping on cultured preparations. The value is comparable to 8.7% obtained by Kalousek and Dill 1983 as well as to data by others [Wilkins-Haug et al., 1995; Lestou and Kalousek, 1998]. However, as our

TABLE II. Physical and Developmental Evaluation at Age 12 Months

	With CPM	Without CPM
No. infants studied	7[a]	13
Weight (SD)	−1.7 to −3.5	−0.7 to −3.5
Length (SD)	−0.4 to −3.3	−0.7 to −3.6
Delayed development (No. delayed/total)		
Gross motor performance	1/7	2/13
Fine motor performance	1/7	2/13
Speech development	0/7	1/13
Social ability	2/7	2/13
Phenotypic abnormalities	ASD and hypospadias	Polydactyly

[a]Patient 6 was not available from study, having died of Russell–Silver syndrome.

TABLE III. Outcome of Infants With or Without Confined Placental Mosaicism

	With CPM	Without CPM
No. infants studied	8	42
Age (years)	1–10	1–10
Male/female	5/3	27/15
Maternal age (years)		
Range	22–31	23–40
Mean	28.6	29.1
Gestational age (weeks)	34–38	34–39
Birth weight (SD)	−2.2 to −5	−2 to −4.7
Cord blood pH	7.14–7.34	7.11–7.39
Notch-waveform pattern in uterine artery (present/total)	4/8	5/42
Phenotypic abnormalities		
Russell–Silver syndrome	Patient 6	Two infants
Other abnormalities	ASD, hypospadias	Polydactyly

karyotyping was not done on non-cultured, direct preparation of the placenta, we did not screen for type 1 CPM or distinct between types 2 and 3. This might lead to underestimate the frequency of CPM in our series of pregnancy. Since CPM in four of the eight infants of CPM group were composed of a few trisomic cells (2–3/50 cells), the existence of trisomic cells with much lower frequency or type 1 CPM was not totally ruled out in some infants of non-CPM group. Genotyping at polymorphic allele sites examined revealed that chromosomal aberrations in five of seven infants with CPM arose at the maternal meiosis. This may reflect more frequent occurrence of meiotic non-disjunction during oogenesis than spermatogenesis [Hassold and Hunt, 2001], and meets with previously reported data that CPM of meiotic origin correlates with an increased risk of IUGR [Robinson et al., 1997]. The mean of maternal age in infants of both CPM and non-CPM groups was not high (28.6 vs. 29.1 years). It is often difficult to ascertain whether the pathological findings of pregnancy with CPM are caused by UPD in the fetus or CPM itself in the placenta. As UPD was excluded in all our infants with CPM, UPD does not explain the etiology of the majority of adverse outcome of pregnancies with CPM.

Comparisons of various parameters as indicators of perinatal outcome showed no significant difference between IUGR infants with and without CPM, except for the higher rate of notch waveform patterns in the uterine artery in the CPM group (Table III). Non-catching-up stature (<−2 SD) at age 12 months was seen in 7 (87.5%) of 8 infants with CPM and 8 (53.3%) of 15 infants without CPM, although the frequencies were not statistically different. There was no difference in motor, speech, and social development between the two groups, too. Clinical manifestations in some infants merit comments. There were three infants with RSS (one of CPM group and two of non-CPM group). Patient 2, who had CPM for del(2)(p16), showed normal developmental performance and

was catching up on her growth at age 12 months. There have been two reports of infants with IUGR and CPM for partial monosomy: a liveborn with CPM for del(13)(q13) [Wolstenholme et al., 1994] and a stillbirth with CPM for del(16)(p16) [Stipoljev et al., 2001]. Our infant with CPM for del(2)(p16) is added to the list of CPM for partial monosomy that may cause IUGR. Patient 6 had RSS without UPD died at age 12 months, as did four reported cases of RSS in infancy [Imaizumi et al., 1983; Donnai et al., 1989]. The clinical severity of RSS might depend on the presence of a cell line of UPD7, though previous study suggested indistinguishable phenotypes between RSS patients with and without UPD7 [Bernard et al., 1999].

A notch-waveform ultrasonographic pattern of the uterine artery was frequently seen in the CPM group of our series of IUGR infants. This reflects high flow-resistance of the artery caused by inadequate physiological changes of spiral arteries that are important for the uteroplacental circulation [Coleman et al., 2000], and the flow-resistance is related to a reduced trophoblast migration into the myometrium. Such patterns may also be related to an association of decidual vasculopathy with CPM. Sagol et al. 1999 showed a positive association between pathological changes of the placental bed and high flow-resistance of the uterine artery in women with pre-eclampsia and IUGR. Wilkins-Haug et al. 1995 demonstrated that placental changes consistent with decidual vasculopathy were found in two cases of CPM-associated IUGR, but in none of the remaining cases of IUGR or control pregnancies. Wilkins-Haug et al. 2006 recently showed that decidual vasculopathy, placental infarcts, and intervillous thrombus were more frequently observed in infants with CPM compared to chromosomally normal placenta of infants with IUGR. On the other hand, morphologically normal placenta in many cases of either CPM or IUGR without CPM was also reported [Krishnamoorthy et al., 1995].

In conclusion, we have demonstrated associations between CPM and IUGR and between CPM and notch waveform patterns of the uterine artery. We also confirmed that perinatal outcome of pregnancy was not markedly different between IUGR infants with and without CPM. Short stature tended to be more frequently observed at age 12 months in IUGR infants with CPM than in those without CPM. The information obtained in our study will be useful for perinatal care and genetic counseling for infants with IUGR and CPM.

ACKNOWLEDGMENTS

This study was supported in part by Grants-in-Aid for Scientific Research (Category C, No. 16591670 for K.M. and Category C, No. 17591748 for H.M.) and Grant-in-Aid on Priority Areas "Applied Genomics" (No. 17019055 for N.N.) from Ministry of Education, Sports, Culture, Science and Technology of Japan, and SORST from Japan Science and Technology Agency (JST) (for N.N.).

REFERENCES

Ariel I, Lerer I, Yagel S, Cohen R, Ben-Neriah Z, Abeliovich D. 1997. Trisomy 2: Confined placental mosaicism in a fetus with intrauterine growth retardation. Prenat Diagn 17:180–183.

Artan S, Basaran N, Hassa H, Ozalp S, Sener T, Sayli BS, Cengiz C, Ozdemir M, Durak T, Dolen I, Ozgunen T, Tuna M. 1995. Confined placental mosaicism in term placentae: Analysis of 125 cases. Prenat Diagn 15:1135–1142.

Balmer D, Baumer A, Rothlisberger B, Schinzel A. 1999. Severe intra-uterine growth retardation in a patient with maternal uniparental disomy 22 and a 22-trisomic placenta. Prenat Diagn 19:1061–1064.

Bernard LE, Penaherrera MS, Van Allen MI, Wang MS, Yong SL, Gareis F, Langlois S, Robinson WP. 1999. Clinical and molecular findings in two patients with Russell–Silver syndrome and UPD7: Comparison with non-UPD7 cases. Am J Med Genet 87:230–236.

Bryan J, Peters M, Pritchard G, Healey S, Payton D. 2002. A second case of intrauterine growth retardation and primary hypospadias associated with a trisomy 22 placenta but with biparental inheritance of chromosome 22 in the fetus. Prenat Diagn 22:137–140.

Coleman MA, McCowan LM, North RA. 2000. Mid-trimester uterine artery Doppler screening as a predictor of adverse pregnancy outcome in high-risk women. Ultrasound Obstet Gynecol 15:7–12.

Donnai D, Thompson E, Allanson J, Baraitser M. 1989. Severe Silver–Russell syndrome. J Med Genet 26:447–451.

Eggerding FA, Schonberg SA, Chehab FF, Norton ME, Cox VA, Epstein CJ. 1994. Uniparental isodisomy for paternal 7p and maternal 7q in a child with growth retardation. Am J Hum Genet 55:253–265.

Eggermann T, Curtis M, Zerres K, Hughes HE. 2004. Maternal uniparental disomy 16 and genetic counseling: New case and survey of published cases. Genet Counsel 15:183–190.

European Collaborative Research on Mosaicism in CVS. 1999. Trisomy 15 CPM: Probable origins, pregnancy outcome and risk of fetal UPD: European Collaborative Research on Mosaicism in CVS (EUCROMIC). Prenat Diagn 19:29–35.

Frankenburg WK, Dodds JB. 1967. The Denver developmental screening test. J Pediatr 71:181–191.

Hassold T, Hunt P. 2001. To err (meiotically) is human: The genesis of human aneuploidy. Nat Rev Genet 2:280–91.

Imaizumi K, Ogino T, Kajii T. 1983. Silver syndrome: An infant with necropsy findings. Jpn J Hum Genet 28:231–234.

James RS, Jacobs PA. 1996. Molecular studies of the aetiology of trisomy 8 in spontaneous abortions and the liveborn population. Hum Genet 97:283–286.

Kalousek DK. 1994. Current topic: Confined placental mosaicism and intrauterine fetal development. Placenta 15:219–230.

Kalousek DK, Dill FJ. 1983. Chromosomal mosaicism confined to the placenta in human conceptions. Science 221:665–667.

Kalousek DK, Vekemans M. 1996. Confined placental mosaicism. J Med Genet. 33:529–533.

Kalousek DK, Howard-Peebles PN, Olson SB, Barrett IJ, Dorfmann A, Black SH, Schulman JD, Wilson RD. 1991. Confirmation of CVS mosaicism in term placentae and high frequency of intrauterine growth retardation association with confined placental mosaicism. Prenat Diagn 11:743–750.

Kennerknecht I, Kramer S, Grab D, Terinde R, Vogel W. 1993. A prospective cytogenetic study of third-trimester placentae in small-for-date but otherwise normal newborns. Prenat Diagn 13:257–269.

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

Krishnamoorthy A, Gowen LC, Boll KE, Knuppel RA, Sciorra LJ. 1995. Chromosome and interphase analysis of placental mosaicism in intrauterine growth retardation. J Perinatol 15: 47–50.

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.

Ledbetter DH, Zachary JM, Simpson JL, Golbus MS, Pergament E, Jackson L, Mahoney MJ, Desnick RJ, Schulman J, Copeland KL, Verlinsky Y, Yang-Feng T, Schonberg SA, Babu A, Tharapel A, Dorfmann A, Lubs HA, Rhoads GG, Fowler SE, De La Cruz F. 1992. Cytogenetic results from the U.S. Collaborative Study on CVS. Prenat Diagn 12:317–345.

Lestou VS, Kalousek DK. 1998. Confined placental mosaicism and intrauterine fetal growth. Arch Dis Child Fetal Neonatal Ed 79: 223–226.

Masuzaki H, Miura K, Yoshiura KI, Yoshimura S, Niikawa N, Ishimaru T. 2004. Detection of cell free placental DNA in maternal plasma: Direct evidence from three cases of confined placental mosaicism. J Med Genet 41:289–92.

Monk D, Moore GE. 2004. Intrauterine growth restriction—genetic causes and consequences. Semin Fetal Neonat Med 9:371–378.

Roberts E, Dunlop J, Davis GS, Churchill D, Davison EV. 2003. A further case of confined placental mosaicism for trisomy 2 associated with adverse pregnancy outcome. Prenat Diagn 23: 564–565.

Robinson WP, Barrett IJ, Bernard L, Telenius A, Bernasconi F, Wilson RD, Best RG, Howard-Peebles PN, Langlois S, Kalousek DK. 1997. Meiotic origin of trisomy in confined placental mosaicism is correlated with presence of fetal uniparental disomy, high levels of trisomy in trophoblast, and increased risk of fetal intrauterine growth restriction. Am J Hum Genet 60:917–927.

Sagol S, Ozkinay E, Oztekin K, Ozdemir N. 1999. The comparison of uterine artery Doppler velocimetry with the histopathology of the placental bed. Aust N Z J Obstet Gynaecol 39:324–329.

Saks E, Mccoy MC, Damron J, Kelly TE. 1998. Confined placental mosaicism for trisomy 8 and intra-uterine growth retardation. Prenat Diagn 18:1202–1204.

Schwinger E, Seidl E, Klink F, Rehder H. 1989. Chromosome mosaicism of the placenta—a cause of developmental failure of the fetus? Prenat Diagn 9:639–647.

Stetten G, Escahleeon CS, South ST, McMichael JL, Saul DO, Blakemore KJ. 2004. Reevaluating confined placental mosaicism. Am J Med Genet Part A 131A:232–239.

Stipoljev F, Latin V, Kos M, Miskovic B, Kurjak A. 2001. Correlation of confined placental mosaicism with fetal intrauterine growth retardation. A case control study of placentas at delivery. Fetal Diagn Ther 16:4–9.

Wilkins-Haug L, Roberts DJ, Morton CC. 1995. Confined placental mosaicism and intrauterine growth retardation: A case-control analysis of placentas at delivery. Am J Obstet Gynecol 172: 44–50.

Wilkins-Haug L, Quade B, Morton CC. 2006. Confined placental mosaicism as a risk factor among newborns with fetal growth restriction. Prenat Diagn 26:428–432.

Wolstenholme J, Rooney DE, Davison EV. 1994. Confined placental mosaicism, IUGR, and adverse pregnancy outcome: A controlled retrospective U.K. collaborative survey. Prenat Diagn 14:345–361.