Genome-wide Detection of Uniparental Disomy in a Fetus With Intrauterine Growth Restriction Using Genotyping Microarrays

Yung-Kuei Soong[1]*, Tzu-Hao Wang[1,2], Yun-Shien Lee[2,3], Chih-Ping Chen[4], Chia-Lin Chang[1], Szu-Ying Ho[1,5], An-Shine Chao[1], Po-Jen Cheng[1], Shuenn-Dyh Chang[1]

[1]Department of Obstetrics and Gynecology, Lin-Kou Medical Center, Chang Gung Memorial Hospital, Chang Gung University, [2]Genomic Medicine Research Core Laboratory, Chang Gung Memorial Hospital, [3]Department of Biotechnology, Ming-Chuan University, Tao-Yuan, [4]Department of Obstetrics and Gynecology and Department of Medical Research, Mackay Memorial Hospital, Taipei, and [5]Department of Obstetrics and Gynecology, Cathay General Hospital, Hsinchu branch, Hsinchu, Taiwan.

SUMMARY

Objective: To present the clinical and molecular features of a fetus with confined trisomy 16 mosaicism with maternal uniparental disomy (UPD), using various prenatal diagnostic techniques.

Materials and Methods: Chromosomal karyotyping was performed on samples of chorionic villi, amniotic fluid cells, amniotic membrane, umbilical cord, fetal skin, and placenta from a fetus with elevated nuchal translucency. Polymorphic short tandem repeat markers and Affymetrix single nucleotide polymorphism (SNP) mapping chips were used for molecular analyses.

Results: Karyotypes from chorionic villi and amniocytes showed 47,XX,+16 and 46,XX, respectively. Short tandem repeat markers on chromosome 16 suggested maternal UPD for chromosome 16. Affymetrix 10K SNP mapping chips were used to simultaneously confirm the difference in karyotypes between the placenta and amniocytes and to diagnose UPD for chromosome 16. Fetal ultrasonography and magnetic resonance imaging identified severe intrauterine growth restriction (IUGR). Autopsy revealed IUGR, incomplete lobulation of bilateral lungs, and malrotation of the intestines. The karyotypes of umbilical cord, fetal skin and amniotic membrane were 46,XX, and the trisomy 16 karyotype appeared to be confined to the placenta.

Conclusion: UPD should be investigated as a possible etiology in all cases of unexplained IUGR. SNP microarrays can be useful for confirming this diagnosis. [*Taiwan J Obstet Gynecol* 2009;48(2):152–158]

Key Words: microarray-based comparative genomic hybridization, single nucleotide polymorphism markers, uniparental disomy

Introduction

Uniparental disomy (UPD) occurs when both members of a particular chromosome pair derive from the same parent [1]. UPD often results from a "rescue" event; in trisomy rescue, a disomic oocyte and a monosomic sperm form a trisomic conceptus, and the loss of one homolog results in a two-thirds chance of biparental disomy and a one-third chance of maternal UPD (mUPD). In monosomy rescue, a nullisomic oocyte and a monosomic sperm form a monosomic conceptus, and this duplication of the monosomic sperm results in paternal UPD. Other mechanisms that can result in UPD include post-fertilization errors (via somatic recombination or gene conversion), gametic complementation, and somatic replacement of a derivative chromosome

*Correspondence to: Dr Yung-Kuei Soong, Department of Obstetrics and Gynecology, Lin-Kou Medical Center, Chang Gung Memorial Hospital, Chang Gung University, 5, Fu-Hsing Street, Tao-Yuan 333, Taiwan.
E-mail: yks@adm.cgmh.org.tw
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[1,2]. During trisomy rescue, trisomies that remain in the placental tissues may not have an immediately lethal consequence and are referred to as confined placental mosaicism (CPM) for trisomies [3–6].

Linkage analyses of polymorphic short tandem repeat (STR) DNA markers from the fetus and its parents have been used to detect UPD [4,6,7]. Single nucleotide polymorphism (SNP) microarrays were recently used to identify continuous or interspersed heterodisomic and isodisomic regions in six patients with Silver-Russell syndrome with mUPD for chromosome 7 [8]. In the current study, we used Affymetrix 10K mapping chips for both microarray-based comparative genomic hybridization (array CGH) analysis and detection of UPD in chromosome 16 in a fetus presented with severe intrauterine growth restriction (IUGR).

Case Report

A 37-year-old, gravida 5, para 1, woman had consecutive, spontaneous abortions in her first two pregnancies. After the successful delivery of a full-term baby from her third pregnancy, her fourth pregnancy was terminated owing to trisomy 21 (47,XY,+21) diagnosed by amniocentesis. In her fifth pregnancy, an increased thickness of nuchal translucency (NT) of 5.5 mm was noted at 11 weeks' gestation. The parents were counseled regarding the increased risk of chromosomal abnormalities, structural defects, and genetic syndromes. Karyotyping of chorionic villi sampled at 12 weeks' gestation showed 47,XX,+16 (Figure 1, upper panel). Because of the possibility of trisomy rescue of the chromosome, we performed amniocentesis at 16 weeks' gestation, which revealed a 46,XX karyotype (Figure 1, lower panel). The discrepancy in karyotypes between the placenta and amniocytes was confirmed using array CGH [9] (Figure 2). Fetal ultrasonography showed severe IUGR without obvious structural defects. Magnetic resonance imaging of the fetus showed no obvious anomalous organic structures in the brain or major organs. Four significant markers out of the 22 STR markers on chromosome 16 suggested the presence of mUPD for chromosome 16 (Table 1). Affymetrix 10K SNP mapping chips were used to confirm the diagnosis of UPD for chromosome 16 (UPD16) (Table 2). The parents elected to terminate this pregnancy at 21 weeks' gestation. Autopsy of the 151-g, female abortus revealed IUGR (< fifth percentile of age-matched controls), incomplete lobulation of bilateral lungs, and malrotation of intestines. The karyotypes of the umbilical cord, fetal skin and amniotic membrane were 46,XX, and the trisomy of chromosome 16 appeared to be confined to the placenta.

Materials and Methods

After informed consent was obtained from the parents, we obtained chorionic villus tissues, amniotic fluid, amniotic membrane, skin and placental tissue samples from the fetus, and blood samples from both parents. DNA was extracted from chorionic villi and amniocytes as previously reported [10]. A panel of polymorphic STR markers was used to analyze the origin of fetal chromosome 16 [11] (Table 1). Affymetrix 10K mapping chips (Affymetrix Inc., Santa Clara, CA, USA) were used to analyze the DNA specimens isolated from placental tissues, amniocytes, and peripheral blood from the parents, according to the GeneChip mapping 10K assay protocol (http://www.affymetrix.com). Analyses of array CGH were performed using the CGcgh program as previously reported [12–14]. The CGcgh program is freely available at http://www.mcu.edu.tw/department/biotec/en%5Fpage/CGcgh/[9].

Using the tens of thousands of SNP markers on the Affymetrix 10K mapping chips, distributed among the 23 human chromosomes, we calculated all the possible combinations for each SNP marker among the mother, father and fetus (Table 2). Using the following formula, where f_o is the observed value, f_e is the expected value and the degrees of freedom are $(m-1)\times(n-1)$, we calculated the χ^2 values and derived p values for the normal inherited pattern or UPD for each chromosome:

$$\chi^2 = \sum \frac{(f_o - f_e)^2}{f_e}$$

Results

Based on the χ^2 values for all chromosomes using the tens of thousands of SNP markers in the mother, father and fetus, the p value for chromosome 16 being biparental was < 0.05 ($p = 0.0015$), whereas the p value for UPD was > 0.99999 (Table 3). These values rejected the hypothesis that the pair of chromosome 16s were inherited from both the mother and father, and supported the diagnosis of UPD16. These results were in accordance with those derived from analysis of microsatellite markers (Table 1). Severe IUGR of the fetus (150 g at 21 weeks' gestation) was compatible with the diagnosis of UPD16. Chromosomal analyses of the fetal umbilical cord, skin and amniotic membrane showed

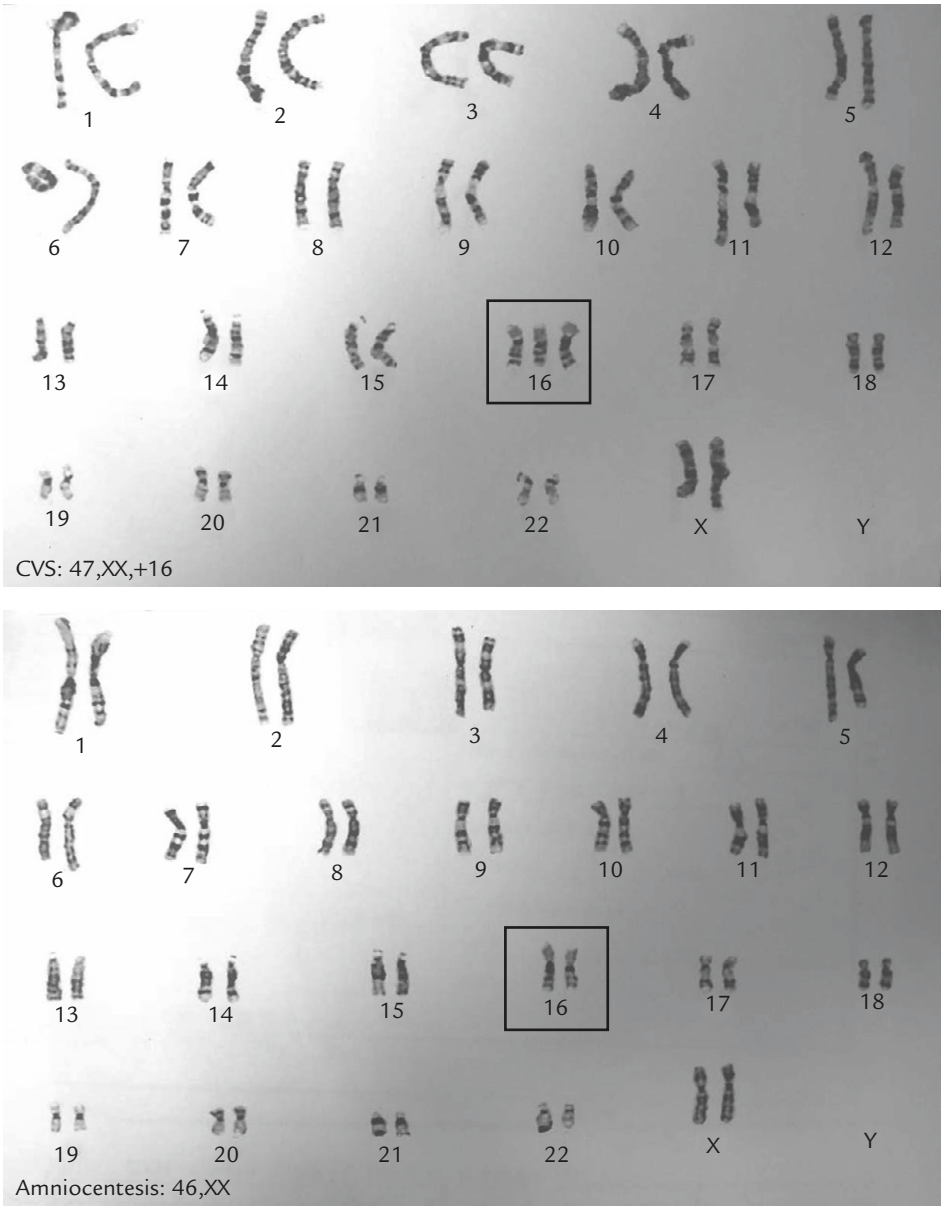

Figure 1. Chromosomal analyses of chorionic villi (upper panel, chorionic villus sampling [CVS]) and amniocytes (lower panel, amniocentesis). Trisomy 16 was noted only in chorionic villi (upper panel, boxed area), but not in amniocytes (lower panel).

46,XX, and the trisomy 16 karyotype appeared to be confined to the placenta (Figures 1 and 2). Based on these results, the diagnosis was a case of a fetus with UPD16 and CPM with trisomy 16.

Discussion

Most cases of CPM for trisomy 16 present with IUGR and various congenital abnormalities [3–6], although contradictory results have been reported [15]. Fetal growth is a complex process that is regulated by fetal, maternal and placental factors. Fetal genes are believed to determine 50% of intrauterine growth, but maternal nutritional status, maternal disease, and maternal behavior also have major impacts. The placenta is also an important factor in regulating intrauterine growth [16].

The prevalence of CPM detected in idiopathic IUGR pregnancies is >20% [17,18]. UPD may exert additional adverse effects on fetal growth associated with CPM [19]. However, a pregnancy with CPM and/or UPD may clinically present as IUGR, but show a normal karyotype after amniocentesis. CPM can often be missed, because the placental karyotype is frequently not investigated. The chance of diagnosing UPD is even more remote if there is no initial suspicion of CPM.

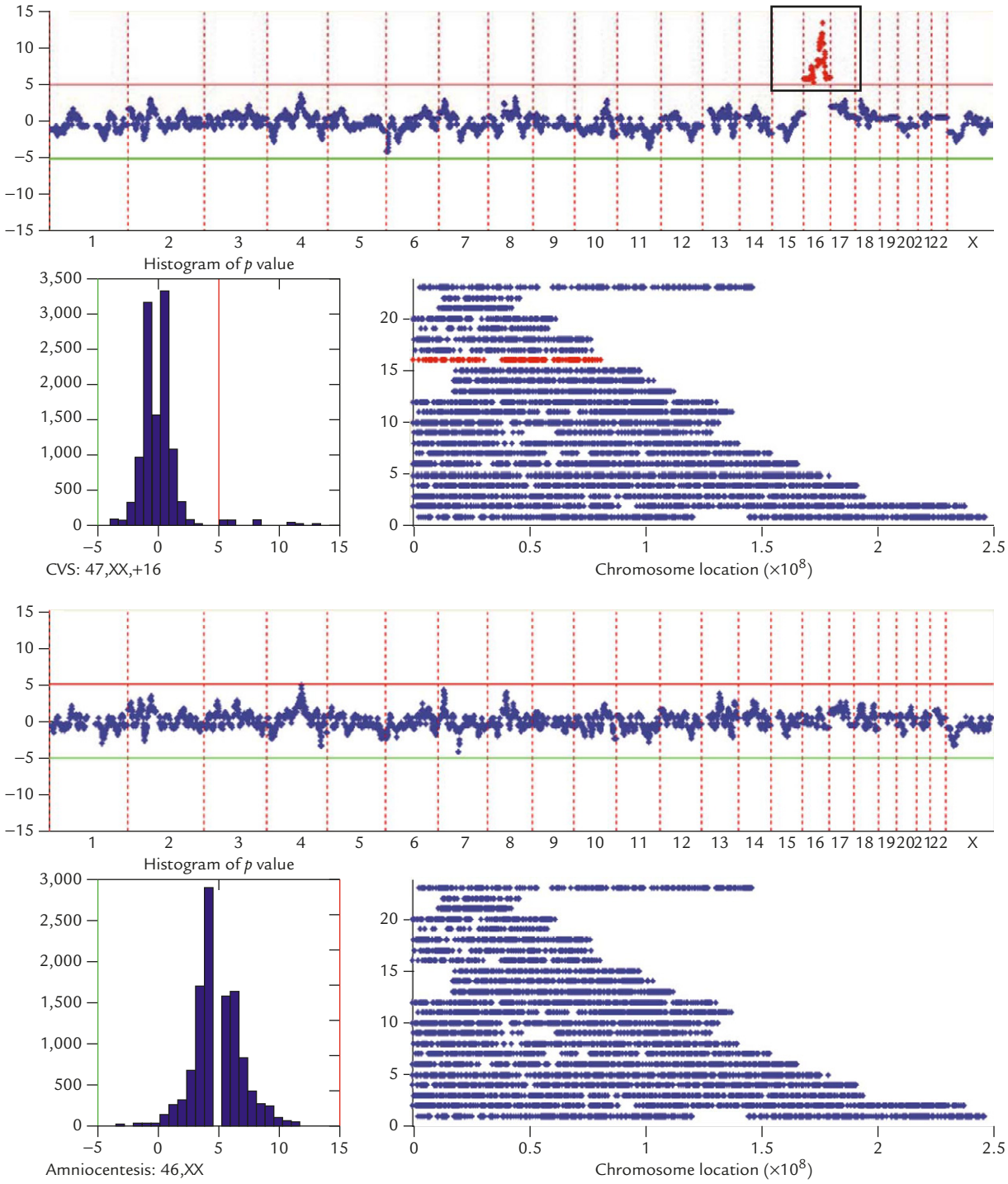

Figure 2. Microarray-based comparative genomic hybridization using the CGcgh program. Microarray-based comparative genomic hybridization confirmed the diagnosis of trisomy 16 in placental DNA (upper panel, boxed area) and the diagnosis of 46,XX in amniocyte DNA (lower panel).

CPM with trisomy 16 has been associated with elevated levels of maternal serum β-human chorionic gonadotropin and α-fetoprotein [20]. NT screening has been used to detect chromosomal abnormalities, as well as congenital heart problems [21–23]. However, no association between CPM for trisomy 16 with mUPD and increased NT has been reported according to the PubMed database (http://www.ncbi.nlm.nih.gov/sites/entrez). In our case, increased NT was noted at the first-trimester Down syndrome screening, which prompted chorionic villus sampling. This in turn revealed placental trisomy 16. Maternal UPD16 was finally confirmed by fetal karyotyping and analyses of STR markers and SNP microarrays following amniocentesis. Results of fetal

Table 1. Maternal uniparental disomy was suggested using 22 short tandem repeat (STR) markers for chromosome 16

STRs	Father	Mother	Amniocytes
D16S676	NA	NA	NA
D16S2640	286,290	286,290	290
D16S672	271	NA	NA
D16S753*	256	254	254
D16S477	NA	NA	NA
D16S771	242,250	242	242
D16S310	158	158	158
D16S751	NA	226,230	226,230
D16S539	NA	NA	NA
D16S2622	128,140	128	128
D16S541	155	151,155	151,155
D16S750	108	108	108
D16S2618	134,137	134,137	NA
D16S687	218,226	218,222	218,222
D16S490*	338	342	342
D16S770	126	126,138	126,138
D16S526	202	202	202
D16S752	106	106,114	106,114
D16S2619	145	145,149	145,149
D16S2623	235,239	235,239	235,239
D16S3398*†	196	194,202	194,202
D16S767*†	164	160,168	160,168

**Informative for maternal or paternal origins; †fully informative.*

Table 3. Statistical chances of the fetus being normal or having uniparental disomy (UPD)

Chromosome	*p* value to be normal*	*p* value to be UPD*
1	0.64531	0.00044
2	0.94154	<0.00001
3	0.43687	0.0004
4	0.73531	<0.00001
5	0.44359	0.0003
6	0.15776	0.03565
7	0.60605	0.00015
8	0.55629	0.00006
9	0.53161	0.00319
10	0.57129	0.00043
11	0.91605	0.00104
12	0.66252	0.00337
13	0.85513	0.00018
14	0.67137	0.02781
15	0.76302	0.03301
16	0.00156	>0.99999
17	0.86383	0.02905
18	0.88278	0.02181
19	0.78926	0.01305
20	0.85513	0.03887
21	0.87113	0.02178
22	0.75182	0.03710

**A p value < 0.05 was considered to be statistically significant.*

Table 2. Possible fetal combinations of all single nucleotide polymorphism markers inherited from the mother and father

Possible combinations	a	b	c	d	e	f	g	h	i
Mother	AA	AA	AA	AB	AB	AB	BB	BB	BB
Father	AA	AB	BB	AA	AB	BB	AA	AB	BB
Fetus	AA	AA,AB	AB,AA	AA,AB	AA,AB,BB	AB,BB	AB,AB	AB,BB	BB
Expected in a normal fetus	a	b/2, b/2	c, 0	d/2, d/2	e/4, e/2, e/4	f/2, f/2	g, 0	h/2, h/2	i
Expected in a UPD fetus	a	b, 0	0, c	0, d	0, e, 0	f, 0	0, g	0, h	i
Observed	a	b, b1	c, c1	d, d1	e, e1, e2	f, f1	g, g1	h, h1	i

UPD = uniparental disomy.

magnetic resonance imaging and autopsy excluded congenital heart diseases as the cause of increased NT in this case, suggesting that increased NT during the first trimester could be a primary sign of CPM for trisomy 16 with mUPD.

The American College of Medical Genetics recommends that multiple STR markers be used to identify UPD and that they should be tested for each chromosome of interest [1]. Two fully informative loci showing either uniparental or biparental inheritance are the minimal requirements to make a diagnosis. If a chromosome of interest has at least two loci supportive of the diagnosis of UPD, biparental inheritance should be proven on all other chromosomes and multiple loci should be used to distinguish UPD from deletion. In this case, we analyzed 22 STR markers and only four were informative for maternal or paternal origins (D16S753, D16S490, D16S3398, D16S767; Table 1). Among those four loci, two could not exclude the possibility of maternal monosomy, thus only two out of a panel of 22 markers (D16S3398 and D16S767; Table 1) were fully informative. If the karyotypes of both the

placenta (47,XX,+16) and the fetus (46,XX) had not been available before the STR markers were analyzed, we would have needed an exhaustive panel of markers for other chromosomes to confirm or exclude the diagnosis of UPD.

Affymetrix SNP microarrays allowed us to perform array CGH and UPD detection simultaneously. Using the intensity of each SNP probe set on Affymetrix SNP microarrays, we modified the freely available CGcgh software to perform array CGH on complementary DNA microarrays and Affymetrix 10K, 100K, 250K and 500K microarrays [9]. Other platforms, such as Agilent oligonucleotide microarrays (http://www.agilent.com/chem), CMDX BAC, and oligonucleotide microarrays (http://www.cmdiagnostics.com), which only analyze probe intensity, can also be used to perform array CGH. However, only the microarrays with SNP information could be used to perform linkage analyses among the triad of mother, father and fetus (Table 2). This genome-wide linkage analysis using SNP information is instrumental in simultaneously detecting UPD for every chromosome (Table 3).

IUGR has been associated with UPD of many chromosomes, in addition to chromosome 16 [5,24–28]. Several commercial SNP microarray platforms with increasing numbers of probes on each new microarray are now available, and we, therefore, suggest that UPD should be investigated as a possible etiology in all cases of IUGR that cannot be explained by chromosomal aneuploidy or adverse maternal medical conditions.

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