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

W. P. Robinson,[1] I. J. Barrett,[2] L. Bernard,[1] A. Telenius,[2] F. Bernasconi,[1] R. D. Wilson,[1,3] R. G. Best,[4] P. N. Howard-Peebles,[5,6] S. Langlois,[1] and D. K. Kalousek[2]

Departments of [1]Medical Genetics, [2]Pathology, and [3]Obstetrics, University of British Columbia, Vancouver; [4]Department of Obstetrics and Gynecology, University of South Carolina, Columbia; [5]Genetics and IVF Institute, Fairfax; and [6]Medical College of Virginia, Richmond

Summary

Molecular studies were performed on 101 cases of confined placental mosaicism (CPM) involving autosomal trisomy. The origin of the trisomic cell line was determined in 54 cases (from 51 pregnancies), 47 of which were also analyzed for the presence of uniparental disomy (UPD) in the disomic cell line. An additional 47 cases were analyzed for parental origin in the disomic cell line only. A somatic (postmeiotic) origin of the trisomy was observed in 22 cases and included the majority of cases with CPM for trisomy 2, 7, 8, 10, and 12. Most cases of CPM involving trisomy 9, 16, and 22 were determined to be meiotic. Fetal maternal UPD was found in 17 of 94 informative CPM cases, involving trisomy 2 (1 case), 7 (1 case), 16 (13 cases), and 22 (2 cases). The placental trisomy was of meiotic origin in all 17 cases associated with fetal UPD ($P = .00005$). A meiotic origin also correlated with the levels of trisomy in cultured chorionic villi samples (CVS) ($P = .0002$) and trophoblast ($P = .00005$). Abnormal pregnancy outcome (usually IUGR) correlated with meiotic origin ($P = .0003$), the presence of fetal UPD ($P = 4 \times 10^{-7}$), and the level of trisomy in trophoblast ($P = 3 \times 10^{-7}$) but not with the level of trisomy in CVS or term chorion. The good fit of somatic errors with the expected results could have been observed only if few true meiotic errors were misclassified by these methods as a somatic error. These data indicate that molecular determination of origin is a useful predictor of pregnancy outcome, whereas the level of trisomy observed in cultured CVS is not. In addition, UPD for some chromosomes may affect prenatal, but not postnatal, development, possibly indicating that imprinting effects for these chromosomes are confined to placental tissues.

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Address for correspondence and reprints: Dr. Wendy P. Robinson, Department of Medical Genetics, Room 170, 950 West 28th Street, Vancouver, British Columbia V57Z 4H4, Canada. E-mail: wendyr@unixg.ubc.ca

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Introduction

Confined placental mosaicism (CPM) is detected in ~2% of viable pregnancies ascertained through chorionic villous sampling (CVS) at 10–12 wk of gestation (Kalousek 1985; Simoni 1985; Mikkelsen and Ayme 1987; Wang et al. 1993). Most commonly, this presents as a trisomic cell line observed in placental tissues and a normal diploid chromosome complement in the fetus. Although many pregnancies with CPM progress to term uneventfully, some may result in spontaneous abortion, intrauterine growth restriction (IUGR), or perinatal morbidity (Kalousek 1994). Previous data indicate that an abnormal outcome is more likely when high levels of trisomy persist to term in the placenta (Kalousek et al. 1991). However, the outcome is strongly chromosome specific, and CPMs for trisomy 2, 3, 7, and 8 are typically associated with a normal outcome.

CPM is often classified into three types: type I (aneuploidy confined to cytotrophoblast), type II (aneuploidy confined to villous stroma), and type III (aneuploid cell lines found in both cytotrophoblast and villous stroma), with specific chromosomes observed at different frequencies depending on the type of CPM observed (Kalousek and Dill 1983; Kalousek et al. 1987, 1991; Miny et al. 1991). The mosaic pattern will depend on many factors, such as the number of blastomeres at the time of the mutational event, the cell lineage affected by the mutational event, and cell viability and selection. In particular, one would expect the pattern to be strongly dependent on whether the zygote was chromosomally normal with a somatic event resulting in the abnormal (e.g., trisomic) line or whether the zygote was chromosomally abnormal with a somatic mutation resulting in "rescue" of the embryo/fetus to the diploid state.

In a study of fetal/live-born mosaic trisomy it has been previously shown that cases of generalized fetal mosaicism involving chromosomes 13, 18, 21, and X commonly arose from somatic loss of one (or more) chromosome from a trisomic fertilization (Robinson et al. 1995). These trisomies may survive to term in an apparently nonmosaic aneuploid state in the fetus,

although often supported by a mosaic placenta (Kalousek et al. 1989). In contrast, it was observed that survival of pregnancies with fetal mosaic trisomy 8 was much more likely when the trisomy had arisen somatically and was, therefore, presumed to be confined to specific tissues (Robinson et al. 1995). Placental examinations were not included in this latter study, and therefore the presence or absence of mosaicism in placenta could not be correlated with the molecular findings and clinical outcomes. However, it is clear that both somatic and meiotic origins of trisomy may occur, the frequency of each is chromosome specific, and outcome is probably influenced by the viability of trisomic cells in specific cell lineages.

Some cases of uniparental disomy (UPD) (the inheritance of two copies of a chromosome from a single parent) have been ascertained subsequent to the finding of CPM for the involved chromosome (Cassidy et al. 1992; Purvis-Smith et al. 1992; Kalousek et al. 1993); however, trisomic zygote rescue is not the only mechanism for UPD origin (see, for example, Robinson et al. 1993). Although UPD for some chromosomes can result in clinical abnormalities, UPD for many other chromosomes results in no obvious abnormality (Ledbetter and Engel 1995). If trisomy CPM is due primarily to random loss of one chromosome from a trisomic fertilization, then UPD in the disomic cell line would be expected in one-third of cases. However, UPD would not be observed in pregnancies where the trisomic cell line arises from "duplication" of one chromosome in placental progenitors after a normal disomic fertilization.

In the present study of 101 examples of trisomy CPM, we have determined the origin of 54 trisomies (from 51 pregnancies). The origin of the fetal disomic cell line could also be determined in 47 of these cases. An additional 47 cases of CPM were examined for origin of the disomic, but not the trisomic, cell line. The objective was to determine the frequency with which CPM is the result of a somatic loss of one chromosome "rescuing" a trisomic fertilization versus somatic gain of a chromosome in a normal disomic conceptus. In addition, we evaluated whether the origin of the trisomy is a useful predictor of the level of trisomy observed at term delivery, risk of UPD, and pregnancy outcome.

Methods

Ascertainment of Cases

Most pregnancies were ascertained through mothers undergoing CVS for advanced maternal age and in which a trisomic cell line was identified. However, some cases were included in the study for which ascertainment was initially due to an abnormal triple screen result, ultrasound abnormality (usually IUGR), maternal anxiety, previous aneuploidy, mosaic amniocentesis, or IUGR noted at birth. In most cases, the abnormal cell line was absent from amniotic fluid and fetal blood. However, a few cases were also included if the level of trisomy in amniotic fluid was low (<12%) and/or no indication of trisomy mosaicism in blood was present, since it is likely that the trisomy detected in cultured amniotic fluid in such cases is due to placental contamination. However, one case was included that showed trisomy 22 mosaicism in skin and muscle despite zero trisomic cells observed in amniotic fluid or blood (case 95.66), since it fulfilled the diagnosis of CPM prenatally. It should be emphasized that this study population cannot be considered to be a random sample of mosaic cases, because inclusion of cases ascertained through IUGR may overemphasize the risks of CPM associated with poor outcome or risk of UPD. In addition, there were a higher number of CPM16 cases, since they were the initial focus of the research (e.g., Kalousek et al. 1993). Furthermore, it is likely that greater effort was made to retrieve the placenta when IUGR or another abnormality was present. Some of these cases overlap with others published previously either as case reports or chromosome-specific summaries and are identified in table 1. The reasons for ascertainment of the 51 CPM cases for which origin of trisomy could be determined is also given in table 1.

Cytogenetic studies were performed on CVS and term placenta samples as described elsewhere (Kalousek et al. 1993). The persistence to term of CPM, with the cell lineage(s) affected and the distribution within the placenta, was estimated using a combination of routine cytogenetics and FISH (Henderson et al. 1996). The number of sites taken (usually 1–2, but ≤10 sites in some cases) and number of cells analyzed varied from case to case. If multiple sites were taken from a single placental tissue, we calculated percentage figures on the basis of the combined total of all sites rather than simply average of each site. For conventional cytogenetic analysis, typically, 15 metaphases were analyzed for each tissue from each site sampled. For FISH analysis, 500 interphase nuclei were scored from each site. If FISH was used, we used those figures, because they were based on much larger numbers of cells, rather than the values obtained from conventional cytogenetics. Most of the results for trophoblast in term placenta were based on FISH analysis.

DNA Studies

Blood samples were obtained from both parents, when possible, and fetal/newborn DNA was obtained from amniotic fluid cell cultures, cord blood obtained at time of delivery, or, in cases of fetal demise, fetal tissues. Tissue from trisomic material was obtained either from CVS samples or term placenta. In the latter instance, when multiple specimens were available, DNA was extracted from the site that had been determined

to show the highest level of trisomy. In some cases, placental, but not fetal, tissue was available, and in other cases fetal samples were available but placental samples were either unavailable or the level of trisomy was too low to be insured of detecting the third allele. Therefore, the origins of both the trisomic and the disomic cell lines could be determined in only 47 of the 101 cases.

DNA was extracted following standard protocols. DNA typing of parents and child/fetus was done with highly polymorphic microsatellite markers, and PCR amplification was done as described elsewhere (Robinson et al. 1995). Markers used for origin of trisomy studies are listed in table 2. Information on primer sequence and map location is available from the Genome Database. Primers were obtained from Research Genetics, Inc. PCR amplification was performed by standard methods (usually using 55–57°C annealing temperature). A 0.5–3-μl amount of the reaction was mixed with an equal volume of loading buffer and was loaded directly onto a 0.4-mm thick 6% polyacrylamide/50% urea gel. Visualization of bands was done either by including ^{32}P-labeled cytosine in the PCR reaction and exposure of the gel to X-ray film or by silver staining.

The diagnosis of uniparental disomy was based on uniparental inheritance for at least two loci along the chromosome in question. In four cases, only the mother was available. In two of these cases, 10 markers were typed with one allele always matching the mother, and, for several loci, one allele could not have come from the mother. This was taken as sufficient evidence of biparental inheritance. In another two cases involving chromosome 16, the offspring did not carry any nonmaternal alleles at any of 15 loci, and it could be inferred that these were cases of maternal UPD. A summary of the number of cases tested versus number of UPD by chromosome is given in table 3. Three cases showed double trisomy (one for 2 and 18; one for 8 and 16; and one for 10 and 12) and have been scored in table 3 for each chromosome separately.

Origin of the Trisomy

The observation of a marker amplifying three distinct alleles from trisomic tissue confirms a meiotic origin of the extra chromosome. In a few cases of UPD16, placental material was not available, but a maternal meiotic origin could be inferred by the presence of two different maternal alleles in the diploid UPD cell line. Meiosis I (MI) errors are distinguished from meiosis II (MII) errors by use of markers that map near the centromere: nonreduction (heterozygosity present in both parent and child) at the centromere indicates an MI error, and reduction (heterozygosity present in parent and reduced to homozygosity in child) indicates an MII error. Multiple markers are, however, needed to indicate a high probability of a somatic origin of the extra nondisjunction in the trisomic or UPD cell line (Robinson et al. 1993). Since 50 cM corresponds to one chiasma, for most chromosomes, multiple chiasmata will take place during meiosis. At least one chiasma is expected to occur for any chromosome pair, including male meiosis for chromosome 21 and X-Y, and therefore at least some loci would retain parent-of-origin heterozygosity if the nondisjunction event originated as a meiotic error. It is therefore assumed that, when all markers spanning the chromosome pair show reduction to homozygosity, the extra chromosome has arisen by a postzygotic duplication mechanism (Antonarakis et al. 1993; Robinson et al. 1993, 1994). We cannot however exclude that occasionally a case designated as "somatic" is actually an MII error that has followed absent MI recombination.

Fisher's exact test of a 2 × 2 table was used in all pairwise comparisons. A one-tailed test was used because a meiotic error would be expected to be associated with high, not low, levels of trisomy , UPD, or abnormal outcome. Since estimates of the level of trisomy are based on different sample sizes in each case and are not normally distributed, statistical comparison is difficult. We therefore chose to use a simple and conservative test for these comparisons. First, the median value of percent trisomy was determined for each category (e.g., CVS samples), and then the number of cases in each subgroup (e.g., CVS-meiotic and CVS-somatic), which were above or below the group median value, were determined and compared by the Fisher's test.

Results

Table 1 summarizes results for all cases for which placental material was available and origin of the trisomic cell line could be determined (N = 54). Samples with <15% trisomy were excluded from the analysis of origin of trisomy. Somatic errors were observed for most chromosomes examined. However, meiotic errors predominated for chromosomes 16 and 22, as has been observed in studies of fetal trisomy in pregnancy loss (Zaragoza et al. 1994; Hassold et al. 1995). In addition, both cases of CPM9 showed a meiotic origin of trisomy.

Table 3 summarizes the number of cases for each chromosome for which UPD testing was performed. Overall UPD was observed in 17 of 94 cases, including maternal UPD for chromosomes 2 (1 case), 7 (1 case), 16 (13 cases), and 22 (2 cases). UPD was observed in 13 (46%) of 28 pregnancies with CPM for trisomy 16, and 2 (50%) of 4 cases with trisomy 22 mosaicism, but in only 2 (3%) of 62 cases for the remaining chromosomes.

An increased level of trisomic cells was found for trisomies of meiotic origin relative to those of somatic origin in cultured prenatal CVS (P = .0002) and term trophoblast (P = .00005) (table 4). A lower level of trisomy was also observed in cultured term chorionic stroma and plate; however, these differences were not

Table 1

Summary of 54 Cases (51 Pregnancies) for Which Origin of Trisomy Could Be Determined

Case No.	Abnormal Cell Line	Ascertainment	Origin		Percent Trisomy					Birthweight or Outcome	Previous Publication
					Prenatal-CVS		Term Chorion				
			Fetus	Placenta	Culture	Direct	Plate	Villi	Term Trophoblast		
95.22	47,+2	US-Ab, IUGR	UPDm	MI, maternal	43	100	13	50	85	IUGR, neonatal death	Hansen et al. (in press)
94.25	47,+2	IUGR	N	MI, maternal	...	...	75	65	20	IUGR	Case 6, Shaffer et al. (1996)
92.29	47,+2	AMA	N	Somatic	100	...	...	...	0	Normal	Case 4, Shaffer et al. (1996)
93.1	47,+2	AMA	N	Somatic	12	0	18	3	7	Normal	...
95.21	47,+3	AMA	N	Somatic	100	0	13	0	19	Normal	...
91.53	47,+7	AMA	UPDm	MI, maternal	100	...	100	...	93	IUGR	Case 4, Kalousek et al. (1996)
91.33	47,+7	AMA	N	Somatic	75	0	60	84	0	Normal	Case 2, Kalousek et al. (1996)
91.40	47,+7	AMA	N	Somatic	88	...	47	66	...	Normal	Case 3, Kalousek et al. (1996)
91.80	47,+7	AMA	N	Somatic	35	...	66	46	0	Normal	Case 5, Kalousek et al. (1996)
93.150	47,+7	AMA	N	Somatic	27	...	0	25	18	Normal	Case 7, Kalousek et al. (1996)
94.85	47,+7	AMA	N	Somatic	100	0	20	40	14	Normal	Case 8, Kalousek et al. (1996)
95.23	47,+7	AMA	N	Somatic	66	...	...	18	11	Normal	Case 9, Kalousek et al. (1996)
96.7	47,+7	?	N	Somatic	100	0	...	40	...	?	...
96.64	47,+8	AMA	...	Somatic	0	100	...	0	60	IUGR, maternal preeclampsia	...
94.79	47,+8	AMA	N	Somatic	93	10	66	16	18	TA	...
89.20	47,+8	AMA	N	Somatic	60	...	...	20	...	Normal	...
93.84	47,+9	AMA	N	M, maternal	20	...	33	0	0	Normal	...
91.96	47,+9	AMA	N	M, maternal	90	...	36	25	0	Normal	...
91.89	47,+10	?	...	Somatic	25	...	26	35	0	Normal, NSVD	...
91.69	47,+10	Previous 45X	N	Somatic	10	...	0	55	0	Normal	...
93.53	47,+12	AMA	N	Somatic	10	...	100	50	0	Normal	...
95.67	47,+12	AMA	N	Somatic	96	...	...	...	...	?	...
96.34	47,+12	AMA	N	MI, maternal	71	...	...	...	...	Normal	...
95.47	46,+i(15q)	?	...	Somatic	21	...	20	0	...	Normal	...
94.115[c]	47,+15	Mosaic AF	...	MI	...	...	100	100	...	TA/Normal US	...
93.103	47,+16	AMA	UPDm	MI, maternal	100	100	...	...	...	Normal	...

CPM16-8	47,+16	AMA	UPDm	MI, maternal	100	100	100	20	100	IUGR, IUD	Case 1, Kalousek et al. (1993)
91.14	47,+16	US-Ab	UPDm	MI, maternal	100	100	100	100	100	IUGR, IUD	Case 2, Kalousek et al. (1993)
91.71	47,+16	AMA	N	MI, maternal	100	100	34	100	100	IUGR	Case 3, Kalousek et al. (1993)
92.25	47,+16	Maternal anxiety	UPDm	MI, maternal	ND	100	100	...	100	IUGR	Case 4, Kalousek et al. (1993)
90.90	47,+16	Maternal anxiety	UPDm	MI, maternal	100	...	26	28	0	Normal	Case 5, Kalousek et al. (1993)
91.55	47,+16	AMA	N	MI, maternal	100	...	0	0	0	Normal	Case 7, Kalousek et al. (1993)
92.48	47,+16	AMA	N	MI, maternal	100	...	...	100	88	Normal	Case 8, Kalousek et al. (1993)
92.49	47,+16	AMA	UPDm	MI, maternal	100	100	100	100	80	IUGR	Case 9, Kalousek et al. (1993)
92.95[c]	47,+16	AMA	UPDm	MI, maternal	...	...	94	4	96	IUGR	...
93.43	47,+16	US-Ab	UPDm	MI, maternal	...	...	0	16	44	TA, IUGR	...
93.94[c]	47,+16	AMA	UPDm	MI, maternal	100	...	...	100	...	IUGR	...
94.50[c]	47,+16	TT-Ab	UPDm	MI, maternal	...	...	100	100	54	TA	...
93.134	47,+16	AMA	N	Somatic, paternal	69	...	13	11	0	Normal	...
94.116	47,+16	AMA	N	MI, maternal	100	...	100	70	60	Normal	...
95.28	47,+16	AMA	UPDm	MI, maternal	100	...	...	...	...	IUGR, neonatal death	...
94.21	47,+16	TT-Ab	N	MI, maternal	100	...	...	...	...	Normal	...
96.32	47,+16	Mosaic AF	UPDm	MI, maternal	...	...	...	...	...	Anomalies	...
95.64[c]	47,+22	AMA	UPDm	MII, maternal	100	...	13	100	25	IUGR[a]	...
95.41	47,+22	AMA	N	M	100	0	...	20	18	Normal	...
91.56	47,+22	AMA	...	M	70	66	73	31	22	IUGR	...
95.66	47,+22	IUGR	UPDm	MI, maternal	...	...	...	100	46	IUGR[d]	...
93.7	47,+22	AMA	N	M, maternal	100	...	...	100	19	Normal	...
91.78	47,+8; 48,+8,+21	AMA	...	Somatic (8); Somatic (21)	10; 60	...	0; 58	3; 88	16[b]	Normal	...
92.21	48,+10,+12	AMA	N (10); N (12)	MII, maternal (10); MI, maternal (12)	50; 50	...	100; 100	83; 83	0; 0	Normal	... ...
93.48	47,+16; 48,+8,+16	AMA	UPD (16); N (8)	MI, maternal (16); Somatic? (8)	81; 19	...	93; 7	100; 0	85; 0	TA, IUGR by US	... ...

NOTE.—Ellipses (. . .) = not done or not available; TT-Ab = triple test in abnormal range; AFP US-Ab = abnormality or IUGR detected by ultrasound; UPDm = maternal uniparental disomy; N = biparental inheritance of chromosomes; TA = therapeutic abortion; IUD = intrauterine death; AF = amniotic fluid.

[a] A low level of trisomy was detected in blood.

[b] Sixteen percent trisomy 8 detected by FISH, no analysis for chromosome 21.

[c] A low level (4%–13%) of trisomy was detected in AF.

[d] A low level of trisomy was detected in muscle and skin but not in blood or AF.

Table 2

List of Markers Used for Origin of Trisomy Studies

Chromosome	Markers Tested
2	D2S131, D2S171, D2S177, D2S337, D2S139, D2S114, D2S156, D2S389, D2S172, D2S155
3	D3S1284, D3S1312, D3S1271, D3S1308, D3S1261, D3S1285, D3S1591, D3S1274, D3S1581
7	D7S531, D7S664, D7S691, D7S645, D7S1870, D7S669, D7S649, D7S800, D7S495, D7S505, D7S550, D7S637
8	D8S264, D8S265, D8S261, D8S255, D8S166, D8S260, D8S279, D8S286, D8S270, D8S256, D8S528, D8S558, D8S537, D8S284, D8S373
9	D9S291, D9S162, D9S165, D9S152, D9S176
10	D10S249, D10S141, D10S199, D10S205, D10S190, D10S217, D10S197, D10S201, D10S558, D10S211
12	D12S352, D12S241, D12S94, D12S77, D12S87, D12S95, D12S83, D12S88, D12S78, D12S343
15	D15S541, D15S542, D15S543, D15S11, D15S128, D15S117, D15S123, D15S100, D15S642
16	D16S291, D16S423, D16S407, D16S543, D16S410, D16S403, D16S401, D16S409, D16S261, D16S308, D16S310, D16S398, D16S305, D16S422, D16S413, D16S402, D15S543
21	D21S1257, D21S1899, D21S369, D21S11, D21S120, D21S1262, D21S212, D21S265, D21S259, D21S268, D21S1253, D21S258
22	F8SVWFP, D22S420, D22S427, D22S944, D22S311, D22S306, D22S425, D22S421, D22S277, D22S283, D22S270, D22S274

statistically significant with the test used (which compared numbers of cells in each group above or below the median value). The weaker correlation with term chorion (plate and stroma) may be due in part to the bias that trisomy origin could be determined only when tissue with high levels of trisomy were available, which was most often obtained from chorionic villus. A meiotic origin also strongly correlated with the presence of UPD (P = .00005) (table 5), since all cases of UPD were derived from meiotic trisomies.

Pregnancy outcome was compared with origin of trisomy and the presence/absence of UPD (table 6). Cases resulting in therapeutic abortion with no other clinical details were excluded from these tables but were counted as abnormal if abnormalities or IUGR were diagnosed by ultrasound. Case 93.48 with double trisomy 8 and 16 resulting from a probable somatic trisomy 8 and meiotic trisomy 16 was counted as meiotic. IUGR or other abnormalities were seen only in cases for which the trisomy was of meiotic origin (tables 2 and 6) (P = .0003), with the exception of one case of "somatic" CPM8. In this case (96.64), labor was induced at 21–22 wk, because of preeclampsia in the mother; the baby, who was small for gestational age, did not survive. Although CPM8 is rarely associated with an abnormal outcome, this was also one of the few cases ascertained in this study where the trisomy was confined to the trophoblast, and it is impossible to determine whether the CPM8 could have contributed to preeclampsia. Of 12 cases with CPM2 or CPM7 for which the origin of trisomy was determined, only the 3 cases determined to have a meiotic origin were associated with IUGR or any other abnormal findings. Two of these three cases were also associated with UPD, and clinical details have been published elsewhere (Langlois et al. 1995; Shaffer et al. 1996; Hansen et al., in press). For chromosome 16 (N = 18), all but one case was determined to be associated with a maternal MI error. In the one exception, three alleles were never observed; however, the paternal allele appeared darker than the maternal allele when informative, indicating a somatic duplication of the paternal chromosome had occurred. The trisomy in this case was present only at low levels in chorion of the term placenta and was not present in trophoblast. Birth weight and phenotype of the newborn baby were normal.

Although levels of trisomy in CVS and chorionic samples were lower in the normals than in those with IUGR or other abnormal outcome, these differences were barely or not statistically significant (table 7). Abnormal

Table 3

Frequency of Fetal UPD in 94 Cases of Trisomy CPM

Chromosome	UPD	BPD	Total
2	1	11	12
3	0	2	2
4	0	1	1
7	1	11	12
8	0	6	6
9	0	5	5
10	0	3	3
12	0	5	5
13	0	2	2
14	0	2	2
15	0	3	3
16	13	15	28
17	0	2	2
18	0	4	4
20	0	1	1
21	0	2	2
22	2	2	4
Total	17	77	94

Table 4

Origin versus Mean Level of Trisomy

Tissue Sampled	Origin of Trisomy (%)		P[a]
	Meiotic	Somatic	
Cultured CVS (stroma)	89.3	56.1	.0002
Term trophoblast	50.1	9.6	.00005
Term chorionic stroma	58.1	36.2	n.s.
Term chorionic plate	66.5	39.0	n.s.

[a] Comparing no. in each group above and below the median; n.s. = not significant.

outcome was, however, very highly correlated with a high level of trisomy in term trophoblast ($P = 3 \times 10^{-7}$).

Discussion

Origin of Trisomy

The present data confirm that CPM can arise from either loss of a chromosome from a trisomic conceptus or somatic duplication of one chromosome in a normal disomic zygote. The frequency with which CPM for various trisomies arise as a postzygotic, somatic error cannot be accurately assessed. However, it is likely that the somatic cases are more common than table 1 indicates, for two reasons: (1) Somatic errors are associated with lower levels of trisomy in the placenta and are expected usually to involve only one cell lineage (CPM type I or II). Somatic errors are thus less likely than meiotic errors to be associated with either ultrasound abnormalities/IUGR or detectable levels of trisomy in small single cultured samples of prenatal CVS (resulting in lower rate of ascertainment). (2) Somatic errors are less likely to show sufficient levels of trisomic cells (>20%) in term placenta to determine origin of trisomy. (CVS material was often not available for DNA extraction.) These problems are illustrated by the CPM2 cases: the two cases ascertained by IUGR were meiotic in origin, whereas the two cases ascertained by advanced maternal age and trisomy 2 in CVS were both consistent with a postzygotic somatic origin; placental material was available for three other cases of CPM2 with normal pregnancy outcome, but the level of trisomy in the available tissue was too low to establish the origin of the extra chromosome 2.

Despite ascertainment bias, it is clear that there are chromosome-specific differences in the relative rates of meiotic versus somatic origin. Conceptions with nonmosaic trisomy 16 are almost exclusively due to a maternal MI error (Hassold et al. 1995), as is also true for cases of CPM16 examined in this study. Only one case of paternal somatic origin was observed. Recently, another case of a paternal origin of fetal trisomy 16 was reported (Meck et al. 1995). Somatic errors for chromosome 16 may not be rare when compared with other chromosomes; however, maternal meiotic errors are quite frequent for this chromosome and account for the majority of cases. Similarly, all five cases of CPM22 were meiotic in origin, as was previously found for the majority of fetal trisomy 22 cases (Zaragoza et al. 1994).

All four samples of CPM8 examined in the present study were of somatic origin, as is also typical of mosaic trisomy 8 found in live borns (Robinson et al. 1995). In the only case with high levels of trisomy 8 in trophoblast,

Table 5

Origin versus Presence of Fetal UPD

Origin of Fetal Disomy	Origin of Trisomy		P
	Meiotic	Somatic	
UPD	17	0	.00005
BPD	13	17	

Table 6

Outcome Compared with Origin and UPD (Cases with Origin of Trisomy Data)

Origin	Outcome		P
	IUGR, IUD, Abnormality	Normal	
Meiotic trisomy	17	12	.0003
Somatic trisomy	1	16	
Fetal UPD	14	2	.0000004
Fetal BPD	2	23	
Fetal BPD—meiotic trisomy	2	10	n.s.
Fetal BPD—somatic trisomy	1	13	

Note.—n.s. = not significant.

Table 7

Outcome versus Mean Level of Trisomy

Tissue	Outcome (%)		P[a]
	IUGR, IUD, Abnormality	Normal	
Cultured CVS (stroma)	84.4	64.7	n.s.
Term trophoblast	70.3	11.4	.0000003
Term chorionic stroma	69.4	43.0	n.s.
Term chorionic plate	60.4	45.2	n.s.

[a] Comparing no. in each group above and below the median; n.s. = not significant.

pregnancy outcome was abnormal (IUGR and maternal preeclampsia). Because all cases of nonmosaic trisomy 8 ascertained from spontaneous abortions have been due to a meiotic origin (Bernasconi et al. 1996*a*; James and Jacobs 1996), the lack of meiotic errors in viable pregnancies appears to be due to selection against this class. Either trisomy 8 pregnancies are unlikely to undergo "trisomic zygote rescue" by the loss of a chromosome in primitive embryonic progenitors or trisomy 8 in trophoblast is not well tolerated. Trophoblast is the first tissue to differentiate and is responsible for exchange of nutrients and wastes between mother and fetus, expression of placental hormones important in maintaining pregnancy, and anchoring of the placenta into the maternal decidua. It is therefore expected that the genetic constitution of this tissue may be particularly critical to continuation of pregnancy.

Molecular studies on trisomy for chromosomes 10 and 12 have not been reported before, and so it is of interest to note that both somatic and meiotic errors were observed. However, for these and the remaining CPMs examined, too few cases for each chromosome were observed to make any general conclusions.

Origin versus Level of Trisomy

The results presented here show, as expected, that a meiotic origin is correlated with high levels of trisomy in placental tissues. The strongest association was seen with the level of trisomy in term trophoblast. However, this finding may simply be a consequence of ascertainment on the presence of the trisomy in cultured CVS (villous stroma). Only type II or type III CPM would be ascertained by this scheme, the difference between the two being the presence or absence of the trisomy from trophoblast. One would expect type II CPM to be more often associated with somatic errors and type III with meiotic errors. Recently, Wolstenholme (1996) tried to predict the frequency with which somatic versus meiotic errors occur for each chromosome, by use of type III CPM as an indicator of meiotic origin. Although this correlation exists, a meiotic origin does not always imply a high level of trisomy in term placenta. For example, case 91.55, with a meiotic trisomy 16, showed no detectable trisomy in term placenta (despite 100% detected in CVS). Similarly, case 92.21 showed no evidence of the 48,+10,+12 cell line in term trophoblast but had high levels in villous stroma, and case 95.41 showed 100% trisomy 22 in cultured CVS but 0% in direct CVS analysis; yet the cases were also clearly of meiotic origin as determined by the presence of three alleles for multiple markers. A great discrepancy between the level of trisomy can occur with either early loss of the extra chromosome from trophoblast progenitor cells or selection against the trisomic cell line in one or the other placental tissue.

Likewise, trisomy can be present in both tissues, even when the trisomy is of somatic origin, if the duplication occurs prior to the blastocyst stage and differentiation of the trophoblast. Although a number of the somatic errors analyzed here did show the presence of trisomy in both stroma and trophoblast, it did not exceed 18% trisomy in trophoblast in these cases (in contrast to a mean of 51% trisomy in trophoblast of meiotic cases).

A major assumption of the present analysis was that complete homozygosity along the chromosome arm was considered to be a somatic error rather than an MII error associated with no meiotic recombination. There is no direct evidence to confirm this assumption, but lack of high levels of trisomy in both placental tissues, absence of fetal UPD, and lack of abnormal outcome attributable to the trisomic placenta, in any case classified as somatic origin, could be observed only if MII errors were not being misclassified at a significant rate.

Double Trisomy

Double trisomy was present in three cases of CPM for which origins of the trisomy were determined. In one case, only one abnormal cell line was observed with constitution 48,+10,+12 (92.21). Both nondisjunction events were of maternal meiotic origin, but the trisomy 10 was most likely derived from a maternal MII error, as judged by reduction of maternal alleles to homozygosity for marker D10S144 (10q11), whereas the trisomy 12 was the result of an MI error, as judged by the presence of maternal heterozygosity at D12S87 (12p11) and D12S83 (12q13-q14). Thus, both chromosomes were disomic in the oocyte, but the errors originated at different stages of meiosis. A copy of each was lost in either the same cell division or progressive cell divisions with loss of the intervening 47 chromosome cell line.

In a second case (91.7), two abnormal cell lines were observed: 47,+8 and 48,+8,+21. Three alleles were never observed for either chromosome 8 or chromosome 21 loci, despite a high level (70%) of both trisomies in the sample used for DNA analysis. A somatic duplication of chromosome 8 presumably occurred first, with subsequent duplication of chromosome 21.

In a third case (93.48), two abnormal cell lines were also present, a 47,+16 and a 48,+8,+16 cell line. In this case, the zygote most likely originated with the 47,+16 constitution, with gain of a chromosome 8 in one derivative cell line and loss of the 16 in an independent cell line contributing to the diploid fetal precursor cells. Ten chromosome 8 markers were typed in this case, and a faint third allele was not observed, consistent with somatic duplication. However, the level of trisomy (7%) may well have been too low to enable detection of a third allele, and thus a meiotic origin could not be completely excluded.

Origin of UPD

All cases of UPD observed in this study were associated with a meiotic origin of the extra chromosome.

The high rate of somatic errors for many chromosomes, especially those for which generalized trisomy is rarely observed, therefore appears to explain why the observed frequency of UPD in CPM cases is much less than the one-third of cases expected with rescue of a trisomic zygote. In contrast, for chromosomes 16 and 22, where all but one case was determined to be of maternal meiotic origin, UPD was found in 47% of the evaluated cases. This is not significantly more than the expected 33% given random loss of one chromosome. It is possible that a bias toward IUGR and abnormal cases results in a slightly greater than expected frequency of UPD. It is somewhat surprising that the correlation between UPD and IUGR or abnormal outcome is so strong, given that maternal UPDs for chromosomes 2, 16, and 22 have all been found in individuals with a normal phenotype arguing against imprinting effects in fetal/adult tissues (Kalousek and Barrett 1994; Schinzel et al. 1994; Bernasconi et al. 1996*b*). However, the possibility exists that the UPD cell line may show imprinting effects confined to the placental tissues, thereby resulting in greater pregnancy complication (discussed further below).

The frequency of UPD15 is of particular interest, since it is one of the few UPDs associated with a clear phenotype: Prader-Willi syndrome for maternal UPD15 and Angelman syndrome for paternal UPD15. Including the present cases, there are a total of eight cases of CPM15 investigated (excluding cases reported solely because UPD15 was present) (Simoni and Sirchia 1994; Christian et al. 1996). Of these eight cases, only one was associated with UPD15, although all informative cases were meiotic in origin. An additional case of UPD15 was identified by Christian et al. (1996) from three cases analyzed prospectively where trisomy 15 mosaicism was seen at amniocentesis. Although 2/11 is not significantly less than the expected 1/3, the possibility remains that the risk is slightly lower because of occasional cases of somatic origin.

Origin of Trisomy and Pregnancy Outcome

Because a meiotic origin correlates with both high levels of trisomy in both placental cell lineages (trophoblast and chorionic stroma) and UPD, it is difficult to distinguish whether an abnormal outcome associated with UPD is due to the UPD itself (i.e., imprinting effects or homozygosity for recessive traits) or to the presence of excessive trisomic cells in placenta and/or undetected trisomy in the fetal cells. The observation of two cases of fetal UPD16 with normal outcome, and IUGR associated with high levels of placental trisomy 16 in the absence of fetal UPD, suggests that IUGR associated with CPM16 correlates primarily with a high percentage of trisomic cells in placenta (Kalousek and Barrett 1994). In addition, cases with UPD16 show some postnatal catch-up growth, further supporting a placental cause of the IUGR. Because most CPM16 cases were meiotic in origin, we decided to test outcome versus presence of UPD for all CPM16 cases, even when the origin of the trisomy had not been determined (table 8). A significant correlation of UPD16 with abnormal pregnancy outcome was observed ($P = .02$), suggesting that imprinting may exist for chromosome 16 but that these effects are limited to the placental tissues and in utero growth. It is possible that the UPD16 cell line simply does not outcompete the trisomy cell line as well as a normal biparental disomic 16 cell line does.

Table 8

Correlation of Outcome with UPD in All CPM16 Cases

	Outcome		
CPM16	IUGR, IUD, Abnormality	Normal	*P*
Fetal UPD	11	2	.02
Fetal BPD	5	8	

NOTE.—Includes cases for which origin of trisomy was not determined.

Only two cases of CPM2-associated UPD2 have been reported (including the present case), both with poor pregnancy outcome. One showed evidence of generalized trisomy 2 mosaicism (Harrison et al. 1995), and in the other case the abnormal outcome could be explained by either poor placental function (because of trisomy 2 mosaicism) or by the presence of UPD in both placenta and the fetus (Hansen et al., in press). Since a case of UPD2 of somatic origin has recently been identified in an adult with no obvious abnormality (Bernasconi et al. 1996*b*), an imprinting effect of UPD2 would likely be confined to placental tissues.

UPD for chromosome 7 is now well established to be associated with severe growth restriction and is found in ~10% of cases with the Silver-Russell syndrome phenotype (Kotzot et al. 1995). It is interesting that some of these cases have been associated with prenatal growth restriction, whereas in other cases only postnatal growth retardation was noted. In the present case (clinical details published by Langlois et al. 1995), fetal growth retardation was detected at birth, and 90%–100% trisomic cells were observed in chorionic plate and trophoblast. It could be hypothesized that the difference between these cases is the level of trisomy in the placenta, with higher levels more likely to be associated with IUGR. As mentioned above for chromosomes 16 and 2, another possibility is that the UPD7 cell in placenta has an effect on growth, and cases of UPD7 arising from a somatic event (e.g., Eggerding et al. 1994) may not show as severe prenatal effects.

The most interesting result of the present study is that only one case determined to have a somatic origin of

trisomy (96.64) was associated with adverse outcome (IUGR and maternal preeclampsia), and it is possible that the cause in this exception may have been unrelated to the CPM. Although many of the CPM cases of meiotic origin were also associated with UPD, 2 of 12 cases with biparental inheritance in the diploid cell line also showed IUGR. It is impossible to ever prove a "somatic" origin, and suggestive evidence (as used here) requires the typing of many markers along the chromosome, which may not be practical in terms of prenatal diagnosis. In contrast, the majority of cases of meiotic origin will show the presence of three alleles if 5–10 highly polymorphic markers spanning the chromosome are used. This can be done without parental samples and may in the future provide a useful first screen for predicting pregnancy outcome, if enough outcome data for each chromosome can be compiled.

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