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ORIGINAL ARTICLE

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Unexpected finding of uniparental disomy mosaicism in term placentas: Is it a common feature in trisomic placentas?

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Abstract

Objective: Non-invasive prenatal testing (NIPT) detects placental chromosome aberrations. When amniocentesis reveals a normal karyotype, confined placental mosaicism (CPM) may be assumed. In order to confirm this, placental cytogenetic studies were performed.

Method: NIPT was conducted in the course of the Dutch TRIDENT study. Placentas of 10 cases with NIPT results indicating an autosomal trisomy and showing a normal (N = 9) or low mosaic karyotype (N = 1) in amniotic fluid (AF) were investigated. The cytotrophoblast as well as the mesenchymal core of two to four placental chorionic villi biopsies were studied with single nucleotide polymorphism (SNP) array. Clinical outcome data were collected.

Results: In 10/10 cases, CPM was proven. In 3/10 cases trisomy/uniparental disomy (UPD)/biparental disomy (BPD) mosaicism was discovered. In 2/3 cases, all three cell lines were present in the placenta, whereas BPD was found in AF. In 1/3 cases trisomy 22/UPD22 was present in AF while trisomy 22/BPD22 mosaicism was found in the placenta. Five of 10 pregnancies were affected with pre-eclampsia, low birth weight, preterm delivery, and/or congenital malformations.

Conclusion: The presence of trisomy/UPD/BPD mosaicism in 3/10 cases that we investigated proves that trisomic zygote rescue may involve multiple rescue events during early embryogenesis. UPD mosaicism, when present in crucial fetal tissues, may explain the abnormal phenotype in undiagnosed cases.

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1 | INTRODUCTION

It is now recognized that confined placental mosaicism (CPM) with the chromosome aberration restricted to the placenta and absent in the fetus is the major origin of discordant results of non-invasive prenatal testing (NIPT).[1] Those who perform extended NIPT, investigating all chromosomes, already discovered that chromosome aberrations typically involved in CPM, like trisomy 16 and trisomy 7, are also commonly found with NIPT.[1-7] The trisomies involved in CPM may have a mitotic as well as meiotic origin. If meiotic, the normal fetal karyotype results from trisomic zygote rescue.[8,9] If one of the chromosomes contributed by the abnormal gamete is lost, this will result in biparental disomy (BPD) (the inheritance of one chromosome in a pair from each parent). If the chromosome contributed by the normal gamete is lost, this will result in uniparental disomy (UPD) (inheritance of both chromosomes of a pair from only one parent). BPD theoretically will occur in 2/3 and UPD in 1/3 of the cases, which actually was shown for CPM involving trisomy 16.[10] UPD may be disease causing if an imprinted chromosome (chromosome 6, 7, 11, 14, 15, or 20) is involved or through homozygosity of a gene mutation associated with a recessive disorder.[11]

In cases where amniocentesis shows normal cytogenetic results after abnormal NIPT, CPM can only be assumed. Confirmation that CPM is the origin of an abnormal NIPT result requires cytogenetic analysis of the placenta. If this confirms presence of the chromosome aberration in the placenta, another source for the abnormal NIPT result such as a maternal malignancy or a maternal constitutional chromosome aberration can be excluded. In order to prove the placental origin of the trisomy, we collected term placentas in cases in which follow-up diagnostic testing in amniotic fluid (AF) was normal or showed very low level mosaicism.

What's already known about this topic?

- Trisomic zygote rescue is the main mechanism for uniparental disomy (UPD) formation.
- Confined placental mosaicism (CPM) is the major source of discordant NIPT results.
- CPM is associated with a risk for adverse pregnancy outcome.

What does this study add?

- Trisomic zygote rescue may involve multiple rescue events based on the co-occurrence of a trisomy-, UPD- and BPD-cell line in half of the rescued cases as revealed by placental studies.

2 | MATERIALS AND METHODS

In the time period April 2014 to December 2016, 2073 NIPT were performed at our department in the Erasmus MC Rotterdam. In 15 out of 2073 cases (0.7%), extended NIPT indicated the presence of an autosomal trisomy that was assumed to be present in the placenta: five cases of trisomy 7, four of trisomy 16, one of trisomy 8, one of trisomy 12, one of trisomy 20, one of trisomy 21, and two of trisomy 22. In all these cases, follow-up amniocentesis was normal except for one case of mosaic trisomy 22 that was confirmed in AF. In all cases, the placenta was asked for in order to confirm that the chromosome aberration, as detected with NIPT, was present in the placenta. Ten placentas were received.

In all cases, NIPT was performed as part of the Dutch TRIDENT study, after first trimester screening by the combined test showed abnormal results.[4] This study was approved by the local University Medical Center Ethics Committees. Follow-up fetal diagnostic investigations in AF during pregnancy and/or in umbilical cord blood and/or buccal swab after birth were performed with SNP array (Illumina Infinium_CytoSNP_850K genotyping array) or FISH on uncultured amniotic and/or blood or buccal cells and with karyotyping of AF cell cultures (in situ method).

After birth, placental studies were performed by sampling chorionic villi (CV) from two to four different quadrants of the placenta. Both cell layers of the CV (the cytotrophoblast [CTB] and mesenchymal core [MC]) were separated according to standard techniques.[12] In some cases, an umbilical cord biopsy was taken as well. DNA was isolated, and 50 to 100 ng of DNA was hybridized to the Illumina Infinium_CytoSNP_850K genotyping array. Whole genome array profiles were analysed for presence of the trisomy involved by using Genome Studio (Illumina) and different versions of Nexus Copy Number (BioDiscovery, versions 7.0 and higher).

The mitotic or meiotic origin of the trisomy was determined using the B-allele frequency (BAF) in the mosaic cases as described by Conlin et al.[13] A meiotic origin is seen when the mosaic extra chromosome contains a haplotype not present in the other two chromosomes, giving rise to two additional BAFs as compared with a mosaic trisomy of mitotic origin. If in meiotic cases the additional haplotypes were present near the centromere, this signified the presence of two different homologues, consistent with a meiosis I (MI) non-disjunction. When the additional haplotypes were absent near the centromeres and present near the telomeres, this was considered to be consistent with a MII non-disjunction. In cases without mosaic tissues (cases 4, 7, 8, and 9), but with a 100% discordancy between the karyotypes of AF or cord blood (100% normal) and placenta (100% trisomy), "digital mosaics" were made in order to elucidate the meiotic or mitotic origin. For that, the final reports (text files) produced by Genome Studio from a 100% trisomic and 100% normal sample from the same conceptus, containing LogR and BAF values for each probe on the array, were opened in an Excel file. For each probe, the average of the LogR and BAF values in both samples was calculated. When this "digital mosaic" is visualized by uploading in our analysis software (Nexus), the result will be the same as that of a "real" 50% mosaic. For validation of this method, we used two cases with a known mitotic (case 1) and meiotic (case 5) origin of the trisomy based on a mosaic placental biopsy and for which there was a 100% normal as well as a 100% abnormal placental biopsy available (see Figure S1).

The presence of UPD in the diploid cell line was assumed when the trisomic chromosome showed runs of homozygosity greater than 10 Mb in length, demonstrating results of the recombination process in meiosis,

while regions of homozygosity were absent on other chromosomes.[13,14] The presence of UPD in the mosaic trisomy cases was assumed if there was a mosaic loss of heterozygosity secondary to trisomic rescue of a meiotic non-disjunction. In cases 1, 2, 3, and 4 involving trisomy 7, which is an imprinted chromosome, UPD7 was excluded in AF by performing SNP array on DNA from AF as well as from parental blood and by comparing fetal SNPs with those from both parents. Also in case 9, SNP array was performed on maternal blood for SNP comparison between mother and fetus in order to prove the maternal origin of both the trisomy 21 and UPiD21. For comparison of fetal and parental SNPs, we used an Excel template in which the SNP data is imported for the three individuals, and a score is given for each SNP per parent. If both alleles match between child and parent, the score is IBS2 (identity by state 2). If only one allele matches with the parent, the score is IBS1, and if neither allele matches with the parent, the score is IBS0 (=discordant). Normally, for each SNP, there is contribution from each parent (no discordance). In the case of UPD, there is no contribution from one parent for that chromosome, and the score of the SNPs will be IBS0. For example, if the fetus is BB for a certain SNP, the mother AB and the father AA, then there is no paternal contribution for this SNP. If this is seen for all SNPs on a chromosome, this may be interpreted as maternal UPD.

Finally, clinical outcome data such as birth weight, gestational age at birth, and the presence of congenital malformations were collected. Small for gestational age was defined as a birthweight below[15] p10.

3 | RESULTS

3.1 | Prenatal cytogenetic follow-up studies

The results of SNP array analysis and karyotyping of AF for confirmation of an abnormal NIPT result in nine out of 10 cases are shown in Table 1. In case 7, the pregnant woman declined diagnostic testing during pregnancy. Prenatal studies revealed a normal fetal chromosome constitution in eight cases, although UPD of a nonimprinted chromosome (chromosome 16) was present in one case (case 8 in Table 1). In a case of trisomy 22 (case 10 in Table 1), AF showed low-level trisomy 22 mosaicism with UPD22 in the karyotypically normal cell line.

3.2 | Postnatal cytogenetic confirmatory studies

The results of SNP array analyses of the 10 placentas, cord blood in seven cases (including case 7 who declined invasive testing), and umbilical cord in cases 5 and 8 are shown in Table 1. In one case (case 10), also FISH was applied to a buccal swab of the newborn.

Firstly, the abnormal NIPT result was confirmed in the placenta in all cases: at least one biopsy showed the trisomy that was detected with NIPT. Moreover, in 5/10 cases extra cell lines, that were not detected prenatally, were found:

1. In 2/10 cases, additional trisomies were detected: in case 2, one placental biopsy also revealed a mosaic trisomy 13 in addition to the trisomy 7, and in case 5, an additional trisomy 14 was found besides the trisomy 12.
2. In 2/10 cases (cases 6 and 9), with a normal BPD result in AF, a UPD cell line in addition to a BPD cell line, involving the chromosomes 16 (case 6) (Figure 1) and 21 (case 9) (Figure 2) was found in the placenta.
3. In 1/10 cases (case 10) with a mosaic trisomy 22/UPhD22 in AF, a BPD22 but no UPD22 cell line was detected in the placenta (Figure 3).

This means that in three of 10 cases (cases 6, 9, and 10), mosaicism for a trisomic cell line and two different "normal" cell lines, one with a BPD and one with a UPD, was found.

Concerning the origin of the trisomy, based on the BAF profiles, all cases of trisomy 7 (case 1-4) were shown to have a mitotic origin, although a meiosis II origin without recombination formally cannot be excluded. The additional trisomy 13 in case 2 also was of mitotic origin. In cases 5, 6, 7, 8, and 10, the BAF patterns in mosaic tissues or based on digital mosaics were consistent with a meiosis I non-disjunction (case 6 in Figure 1). In case 5 with a double trisomy 12 and 14, both trisomic cell lines had a meiosis I origin (see also Figure S1). In case 9 with a 100% trisomy 21 in the CTB of biopsy 3 and a complete isodisomy 21 in the CTB of biopsy 4 (Figure 2), a meiosis II non-disjunction, without crossovers is possible, based on SNP analysis (see Figure S2). However, it is similarly plausible that the trisomy originated postzygotically through mitotic non-disjunction or isochromosome formation.

3.3 | Clinical outcome

Clinical outcome data are shown in Table 1. In two of 10 cases (cases 2 and 7), the child was small for gestational age (<p10). In two cases (cases 2 and 6), the pregnancy was complicated by pre-eclampsia, and in three cases (cases 6, 7, and 10), children were born prematurely. In case 8, the child has a complete atrioventricular septum defect (AVSD).

4 | DISCUSSION

In this paper, we describe the results of cytogenetic investigations by SNP array of 10 term placentas that were primarily performed for cytogenetic confirmation of abnormal results of genome-wide NIPT as previously suggested.[16] These studies confirm that the abnormal NIPT results are caused by a placental trisomy, which makes another potential source like a maternal malignancy fairly unlikely. In almost half of the cases, at least one of the placenta biopsies was shown to be chromosomally normal, stressing the importance of analysing more than one biopsy for confirmation studies.[16-18]

As can theoretically be expected, in one-third (two of six) of meiotic trisomy cases, UPD was found in the diploid AF cells.[9] However, in one of these two cases (case 10), a BPD was found in the normal cell line in the placenta, while UPD was absent in all investigated biopsies. The reverse was found in two other cases with BPD in normal AF cells, while trisomy/UPD/BPD mosaicism was found in the placenta (cases 6 and 9). These mosaics consisting of two different diploid cell lines besides a trisomic cell line provide evidence that they originated through different trisomy rescue events during early embryogenesis in at least two out of three cases (cases 6 and 10). In one case (case 9), the mosaic trisomy21/UPiD21/BPD21 may have originated from different trisomic rescues, although a mitotic origin

TABLE 1 Results from prenatal and postnatal cytogenetic studies and clinical follow-up in 10 cases of abnormal NIPT indicating an autosomal trisomy[a]

			Postnatal Cytogenetics					
	NIPT Result	Prenatal Cytogenetics	Placenta	Cord blood	Umbilical cord	Origin Trisomy[b]	Mosaicism in Conceptus	Clinical Outcome
1	Tris 7[e]	Amniocentesis: -SNP array: normal -no UPD7 -karyo: 46,XX[12]	2 placenta biopsies: Biopsy 1: -CTB normal -MC 20% +7/BPD7 Biopsy 2: -CTB: 100% + 7 -MC normal (BPD7)	-SNP array: normal -no UPD	-	Mitotic[c]	Trisomy/BPD7	Liveborn at 39 wk, 3182 g, p20-p50 No congenital anomalies Uneventful pregnancy
2	Tris 7	Amniocentesis: -SNP array: normal -no UPD7 -karyo: 46,XX[18]	4 placenta biopsies: Biopsy 1: -CTB: 100% + 7 -MC: normal (BPD7) Biopsy 2: CTB and MC: normal (BPD7) Biopsy 3: -CTB: 90% +7/BPD7 -MC: 20% + 7/BPD7 Biopsy 4: -CTB: 20% + 13/normal (BPD7) -MC: 20% + 7/BPD7	-SNP array: normal -no UPD	-	Mitotic[c] (both tris 7 and tris 13)	Trisomy/BPD7	Liveborn at 38 1/7 wk, 2470 g, p5-p10 (SGA) No congenital anomalies Induced delivery due to pre-eclampsia
3	Tris 7	Amniocentesis: -SNP array: normal -no UPD7 -karyo: 46,XX[30]	4 placenta biopsies: Biopsy 1: -CTB: normal (BPD7) -MC: 60% + 7/BPD7 Biopsy 2: -CTB: normal (BPD7) -MC: 5% + 7/BPD7 Biopsy 3: -CTB: normal (BPD7) -MC: 90% + 7/BPD7 Biopsy 4: -CTB: normal (BPD7) -MC: normal (BPD7)	-	-	Mitotic[c]	Trisomy/BPD7	Liveborn at 38 3/7 wk, 3270 g, p50-p80 No congenital anomalies Uneventful pregnancy
4	Tris 7	Amniocentesis: -SNP array: normal -no UPD7 -karyo: 46,XX[23]	4 placentabiopsies, one analysed: Biopsy 1: CTB and MC: 100% tris 7	-	-	Mitotic[c]	Trisomy/BPD7	Liveborn at 38 3/7 wk, 2640 g, p16-p20 No congenital malformations
5	Tris 12	Amniocentesis: -SNP array: normal -no UPD12 -karyo: 46,XX[24]	4 placentabiopsies: Biopsies 1, 2, and 3: -CTB: normal -MC: normal Biopsy 4: -CTB: 100% tris 12 and tris 14 -MC: approximately 15% tris 12 and tris 14/BPD12 and 14.	-SNP array: normal -no UPD	- SNP array normal - no UPD	MI (both tris 12 and tris 14)	Trisomy/BPD12,14	Liveborn at 40 5/7 wk, 3576 g, p50 No congenital anomalies Uneventful pregnancy
6	Tris 16[f]	Amniocentesis: -SNP array: normal -no UPD16 -karyo: 46,XY[18]	3 placenta biopsies: Biopsy 1: -CTB: UPhD16(90%)/BPD16(10%) -MC: UPhD16(50%)/BPD16(50%) Biopsy 2: -CTB: 100% + 16 -MC: approximately 20% + 16/BPD16 Biopsy 3: -CTB: 80% +16/UPhD16 -MC: approximately 40% + 16/BPD16	-SNP array: normal -no UPD16	-	MI	Trisomy/UPhD/BPD	Liveborn at 36 2/7 wk, 2510 g, p20-p50 No congenital anomalies. Severe pre-eclampsia Caesarean section because of breech presentation

(Continues)

TABLE 1 (Continued)

	NIPT Result	Prenatal Cytogenetics	Postnatal Cytogenetics			Origin Trisomy[b]	Mosaicism in Conceptus	Clinical Outcome
			Placenta	Cord blood	Umbilical cord			
7	Tris 16[g]	-	4 placenta biopsies: -CTB of 4 biopsies: 100% + 16 -MC of 1 biopsy: 100% + 16	-SNP array: normal -no UPD16	-	MI	Trisomy/BPD	Liveborn at 34 1/7 wk, 1435 g, <p5 (IUGR) No congenital anomalies. Emergency caesarean section IUGR from 20 wk of gestation Good catch-up growth after birth Normal psychomotor development at 1 y
8	Tris 16	Amniocentesis: -SNP array: normal -UPhD16 -karyo 46,XX[21]	4 placentabiopsies: -CTB and MC of all 4 biopsies: 100% trisomy 16	-SNP array: normal -UPhD16	-SNP array: normal -UPhD16	MI	Trisomy/UPhD	Liveborn, at 38 1/7 wk, 2500 g, <p16 Pregnancy complicated by oligohydramnios Induction of labour Emergency caesarean section (nonreassuring CTG and failure to progress) Complete AVSD (not detected by ultrasound)
9	Tris 21	Amniocentesis: -SNP array: normal -no UPD21 Maternal blood: SNP array normal	4 placentabiopsies: Biopsies 1 and 2: CTB and MC normal (BPD21) Biopsy 3: -CTB: 100% trisomy 21 -MC: normal (BPD21) Biopsy 4 -CTB: UPiD21 -MC: normal (BPD21)	-	-	MII or mitotic[d]	Trisomy/UPiD/BPD	Liveborn, 40 4/7 wk, 3370 g, p20-p50 No congenital anomalies Uneventful pregnancy
10	Tris 22	Amniocentesis: -SNP array: 10% +22 or 10% BPD22 -UPhD22 in normal cell line -karyo: 46,XY[33]	4 placentabiopsies: Biopsy 1: -CTB: approximately 30% +22/BPD22 -MC: approximately 50% +22/BPD22 Biopsies 2, 3, and 4: CTB and MC 100% tris 22	-SNP array: normal -UPhD22 -FISH buccal swab and cord blood (probe: BCR(22q11)/ABL(9q34) dual fusion, Vysis): normal in 200 nuclei	-	MI	Trisomy/UPhD/BPD	Liveborn at 31 3/7 wk, caesarean section, 1160 g, p10-p16 No congenital anomalies Pregnancy complicated by recurrent vaginal bleeding and premature labour Emergency caesarean section due to premature labour, breech position, and previous caesarean section Hospitalized for 9 wk due to prematurity

Note. "-" means: not available.

Abbreviations: AVSD, atrioventricular septum defect; BAF, B-allele frequency; BPD, biparental disomy; CTB, cytotrophoblast; Karyo, karyotyping; MI, meiosis I; MII, meiosis II; MC, mesenchymal core; UPD, uniparental disomy; UPiD, uniparental isodisomy; UPhD, uniparental heterodisomy.

[a]Underlined cell lines represent cell lines in the placenta that were not detected prenatally.

[b]The origin of the trisomy was determined based on the BAF profiles of the true mosaics in the placenta (cases 1, 2, 3, 5, 6, and 10) or digital mosaics (cases 4, 7, 8, and 9) (see section 2)

[c]Based on the BAF profiles of cases 1, 2, 3, and 4, a mitotic origin was assumed in the trisomy 7 cases based on the absence of a third haplotype across the whole chromosome, although a MII origin cannot be excluded. This fits earlier studies.[34-36]

[d]Although a MII origin is possible, it is similarly plausible that the trisomy arose postzygotically through isochromosome formation or non-disjunction (see Figure S2).

[e]Published previously: case 2.5 in table S2 of Van Opstal et al.[1]

[f]Published previously: case 2.12 in table S2 of Van Opstal et al.[1]

[g]Published previously: case 2.13 in table S2 of Van Opstal et al.[1]

of the trisomy 21 and UPiD21 is as plausible for explaining the results. To the best of our knowledge, mosaic trisomy/UPD/BPD as the consequence of different trisomic rescues has only been suggested once in a patient with Prader-Willi syndrome.[19] In that case, besides a cell line with maternal UPhD15, there was a normal cell line with a maternal and paternal chromosome 15. As the authors suggested, this mosaic could only have originated from two independent trisomy rescue events, although trisomic cells were not detected. Trisomic rescue has been generally considered to be a single event during the early cleavage divisions leading to fetal UPD in 1/3 of the cases, which was actually shown in the present study in AF/cord blood.[20] However, our follow-up cytogenetic investigations of the placentas revealed that trisomic zygote rescue may involve more than one rescue event giving rise to trisomy/UPD/BPD mosaicism.

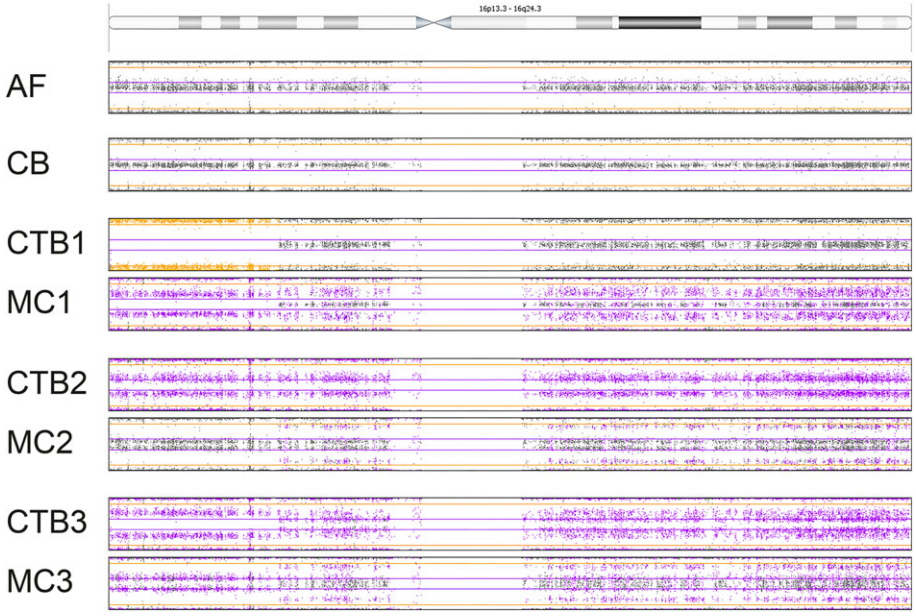

FIGURE 1 B-allele frequency (BAF) plots of chromosome 16 in different tissues of case 6: AF, amniotic fluid; CB, umbilical cord blood; CTB1, 2, and 3, cytotrophoblast of placental biopsies 1, 2, and 3; MC1, 2, and 3, mesenchymal core of placental biopsies 1, 2, and 3. These BAF profiles show a meiotic origin of trisomy 16 with additional BAF lines representing genotypes present in the trisomic cell line that are not present in the diploid cell line. Mosaicism of a trisomy 16 cell line with two different diploid cell lines, one with BPD, and one with UPD was found. AF and CB both show a normal BAF profile fitting a 100% BPD16. CTB1 and MC1 of placental biopsy 1, both with a normal LogR profile (data not shown), show UPD16/BPD16 mosaicism of different levels (approximately 90%/10% in CTB and approximately 50%/50% in MC) as shown by a region of mosaic loss of heterozygosity at the p-arm telomere. It should be noted that the BAF profile in CTB1 may also fit a low mosaic trisomy 16 of approximately 10% (with UPD16 in 90%). CTB2 of biopsy 2 shows a 100% trisomy 16. MC2 shows approximately 20% trisomy 16 with BPD 16 in the diploid cell line based on absence of a mosaic region of homozygosity near the p-arm telomere. CTB3 of biopsy 3 shows approximately 80% trisomy 16 with UPD16 in the diploid cell line shown by the altered pattern near the telomere of the p-arm. MC3 shows approximately 40% trisomy 16 with BPD16 in the diploid cell line based on absence of a region of homozygosity in the p-arm. Based on the BAF profile, and as compared with the BAF profile of MC2, especially at the p-arm, it cannot be excluded that besides a trisomy 16 and BPD16, also a UPD16 cell line is present in MC3 [Colour figure can be viewed at wileyonlinelibrary.com]

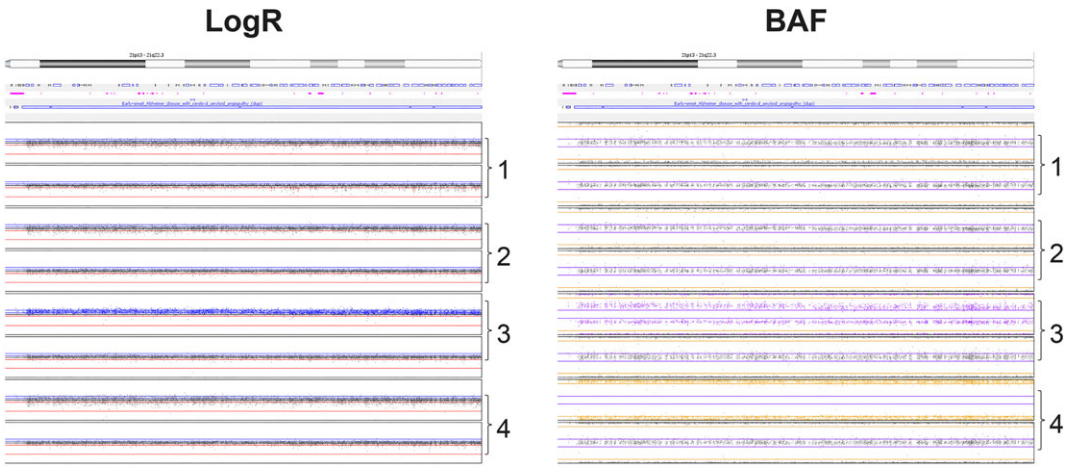

FIGURE 2 LogR (left) and B-allele frequency (BAF) profiles of part of the long arm of chromosome 21 in four placental biopsies (1-4) of case 9. For each biopsy, cytotrophoblast (CTB) and mesenchymal core (MC) were investigated separately, with the upper plot within biopsies 1 to 4 showing the result of CTB and the lower one of the MC. The LogR plots (left) show a normal diploid result in CTB and MC of biopsies 1, 2, and 4 and of the MC of biopsy 3. In the CTB of biopsy 3, a 100% trisomy 21 was found. The BAF profiles (right) show a normal BPD 21 in CTB and MC of biopsies 1 and 2 and in the MC of biopsies 3 and 4. A 100% trisomy 21 was found in the CTB of biopsy 3 whereas a UPiD21 was present in the CTB of biopsy 4, shown by a complete loss of heterozygosity [Colour figure can be viewed at wileyonlinelibrary.com]

Mosaicism of a cell line with UPD and one with BPD is only rarely diagnosed in humans. Apart from the case of Horsthemke et al[19] mentioned above, five other cases of Prader-Willi syndrome caused by mosaic maternal UPD15/BPD15 can be found in the literature,[21-24] one of Silver-Russell syndrome caused by mosaic maternal UPD7/BPD7 (case 52 in that publication)[25] and two cases of Silver-Russell

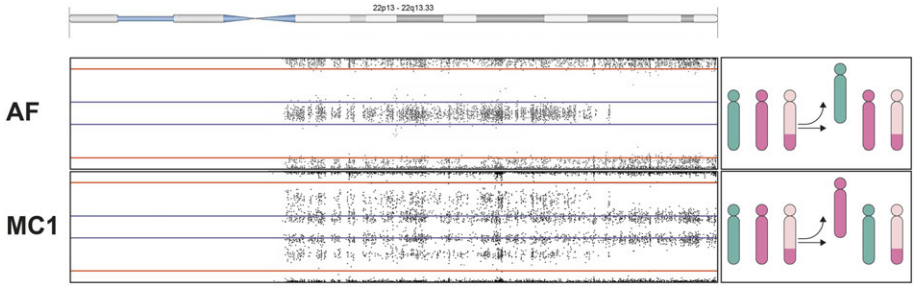

FIGURE 3 The B-allele frequency (BAF) profiles of chromosome 22 in amniotic fluid (AF) and the mesenchymal core of biopsy 1 (MC1) of case 10. In AF, a low mosaic trisomy 22 of about 10% was found with UPhD in the diploid cell line, as shown by loss of heterozygosity at the telomeric end of the q-arm. The UPhD originated from loss of the "green chromosome" as illustrated in the figure on the right; however, it should be noted that the BAF profile, with a normal LogR, could also fit a low mosaic BPD22 of about 10% with UPD22 in the remaining 90%. In MC1, a mosaic trisomy 22 was found of about 50% with a BPD22 in the diploid cell line based on absence of a region of homozygosity. This BPD originated through loss of another trisomic chromosome during early embryogenesis, namely, the pink one, as illustrated in the figure on the right [Colour figure can be viewed at wileyonlinelibrary.com]

syndrome caused by mosaic maternal UPD11/BPD11.[26,27] However, in all these cases, the UPD cell line involved a complete isodisomy (UPiD) suggesting another origin than trisomic rescue. A postfertilization error with loss of a chromosome followed by endoduplication was suggested to be the most likely mechanism in these cases.[9]

As suggested by Izumi et al, the reason for the rarity of detection of UPD/BPD mosaicism in humans might be the fact that low-level mosaicism may not result in an apparent phenotype or because of the technical difficulty associated with its assessment (ie, methylation-sensitive PCR may not be sufficiently sensitive for detecting very low-level mosaicism).[24] Therefore, they proposed the use of SNP array as an adjunct to the standard methylation analysis in the evaluation of Prader-Willi syndrome, given its ability of detecting low-level mosaicism as well as its capability of identifying regions of homozygosity. By investigating term placentas, we show evidence that the reason for its rarity may also be restriction of UPD mosaicism to certain tissues that are usually not investigated. Perhaps like aneuploid cells, UPD is also involved in CPM with preferential allocation of UPD cells to the compartment of the placenta in order to "rescue" the fetus. The fact that trisomic rescue probably occurs during very early embryogenesis,[28] and since the majority of cells of the early embryo are destined to become the placenta, may explain preferential allocation of abnormal cell lines to the placenta. On the other hand, presence in the placenta and absence in AF/blood does not exclude that other tissues are affected. Detection of mosaicism is difficult since routinely only one tissue (blood) is investigated.[27] Therefore, hidden UPD/BPD mosaicism may be the cause of unexplained disease in cases in which an imprinted chromosome is involved or through homozygosity of a mutation in a recessive disease gene. In the present study, only nonimprinted chromosomes 16, 21, and 22 were involved in the trisomy/UPD/BPD mosaic cases, and therefore, no abnormal phenotypes were to be expected apart from the risk for a recessive disease. However, in five of 10 cases, the pregnancy was complicated by the birth of a small for gestational age child, pre-eclampsia, and/or prematurity. Moreover, in one case, a child with an atrioventricular septum defect (AVSD) was born. This confirms the association of CPM with fetal growth problems, pre-eclampsia, and/or MCA, at least in high risk pregnancies,[1,29,30] although adverse obstetric outcome seems to depend on the chromosome involved, level of mosaicism, and distribution of abnormal cells.[31] Moreover, it cannot be excluded that prematurity in case 10, with low-level mosaicism trisomy 22 in AF, may also be the consequence of cryptic fetal trisomy mosaicism despite normal cytogenetic results in cord blood and buccal swab or fetal UPD22. Nevertheless, given the increased chance for an unfavourable obstetric outcome for both fetus and mother, pregnancies with discordant NIPT results should be considered as "high risk" for adverse obstetric outcome events. Expert fetal ultrasound, fetal growth surveillance, and high-level obstetric maternal care should be offered.

Although UPD/BPD mosaicism is rare in humans, it has frequently been seen in in vitro fertilization (IVF) embryos.[32] It is well-known that the first cell cycles following IVF are prone to chromosome instability, which is characterized by an elevated rate of gains and losses of complete chromosomes or segments of chromosomes per cell cycle resulting in cell-to-cell variability.[33] Vanneste et al[32] showed, apart from mosaic whole chromosome and segmental aneuploidy, mosaic whole chromosome UPD in 9% (2/23) of the embryos, which supports the findings in the present study. Probably, the placenta reflects the cytogenetic embryonic chaos and functions as a litter basket for chromosomally abnormal cells.

In all four cases of CPM trisomy 7, the trisomy was shown to have originated postzygotically, which confirms previous studies.[34-36] Although figures on the origin of trisomy 7 are still small, this might indicate that the risk for UPD7 as the consequence of trisomic rescue in cases of placental trisomy 7 is rather small. However, more studies are needed to confirm this assumption. The mitotic origin of the trisomy 7 may also explain the normal array results in the CTB of four different biopsies in case 3. These normal results were not expected since the NIPT, which investigates cfDNA from the CTB, showed trisomy 7. Possible explanations are that low-level mosaicism was present but under the detection level of the SNP array that we used. Indeed, SNP arrays are very sensitive for detection of low-level trisomy mosaicism but not if of mitotic origin. Another explanation may be placental variation with CTB at other placental sites that were not investigated, being affected with trisomy 7.[17,18] The reason why the trisomy 12 but not the trisomy 14 was detected with NIPT in case 5 is unclear. Both had a meiotic

origin, but unfortunately, we could not determine the parental origin since parental blood was not available. Assuming the same parental origin, both chromosome aberrations should have been present in the same cell line, which is supported by the array results of the abnormal biopsy. However, probably overall higher levels of trisomy 12 than trisomy 14 were present in the placenta as the result of rescues at a different time point: an earlier rescue of trisomy 14 may have led to lower levels of trisomy 14, especially in that part of the placenta that we did not investigate. This again stresses the importance of investigating multiple placental sites as shown in the past.[17]

Since trisomy/UPD/BPD mosaicism seems to be common in rescued trisomic conceptuses of meiotic origin, caution should be taken when interpreting SNP arrays. As shown by Conlin et al,[13] SNP arrays are very sensitive for detection of low-level trisomy mosaicism, especially if the trisomy is of meiotic origin. However, low-level trisomy mosaicism (<10%, not showing clear LogR elevation) and low mosaic UPD may show the same BAF profile. Therefore, both should be considered when interpreting SNP array results. This means that the BAF profile of the AF in case 10, that was interpreted as being a case of low-level trisomy 22 mosaicism, in fact may also represent a case of low-level BPD/UPD mosaicism (10% BPD and 90% UPD).

In conclusion, by investigating term placentas in the context of confirmatory studies of abnormal NIPT results, we found evidence that trisomy/UPD/BPD mosaicism as a consequence of more than one trisomy rescue event during early embryogenesis may be more common than generally thought. Perhaps it may be the cause of unexplained disease if an imprinted chromosome is involved or through homozygosity of a recessive disease in some unresolved cases. This argues for storage of placental DNA in cases of newborns presenting with congenital malformations after NIPT revealed a chromosome aberration that was not confirmed in AF, so that in case there is no genetic diagnosis in DNA obtained from blood cells of the newborn, further studies can be performed. In such cases, genetic studies of the placenta may reveal chromosomal mosaicism that might also be present in the child, which may explain the child's phenotype.

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CONFLICT OF INTEREST

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REFERENCES

1. Van Opstal D, van Maarle MC, Lichtenbelt K, et al. Origin and clinical relevance of chromosomal aberrations other than the common trisomies detected by genome-wide NIPS: results of the TRIDENT study. *Genet Med*. 2017. https://doi.org/10.1038/gim.2017.132
2. Pescia G, Guex N, Iseli C, et al. Cell-free DNA testing of an extended range of chromosomal anomalies: clinical experience with 6,388 consecutive cases. *Genet Med*. 2017;19(2):169-175.
3. Hahnemann JM, Vejerslev LO. European collaborative research on mosaicism in CVS (EUCROMIC)—fetal and extrafetal cell lineages in 192 gestations with CVS mosaicism involving single autosomal trisomy. *Am J Med Genet*. 1997;70(2):179-187.
4. Oepkes D, Page-Christiaens LC, Bax CJ, et al. Trial by Dutch laboratories for evaluation of non-invasive prenatal testing. Part I—clinical impact. *Prenat Diagn*. 2016;36(12):1083-1090.
5. Bayindir B, Dehaspe L, Brison N, et al. Noninvasive prenatal testing using a novel analysis pipeline to screen for all autosomal fetal aneuploidies improves pregnancy management. *Eur J Hum Genet*. 2015;23(10):1286-1293.
6. Fiorentino F, Bono S, Pizzuti F, et al. The clinical utility of genome-wide non invasive prenatal screening. *Prenat Diagn*. 2017;37(6):593-601.
7. Pertile MD, Halks-Miller M, Flowers N, et al. Rare autosomal trisomies, revealed by maternal plasma DNA sequencing, suggest increased risk of feto-placental disease. *Sci Transl Med*. 2017;9(405):eaan1240.
8. Kotzot D. Complex and segmental uniparental disomy updated. *J Med Genet*. 2008;45(9):545-556.
9. Eggermann T, Soellner L, Buiting K, Kotzot D. Mosaicism and uniparental disomy in prenatal diagnosis. *Trends Mol Med*. 2015;21(2):77-87.
10. Wolstenholme J. An audit of trisomy 16 in man. *Prenat Diagn*. 1995;15(2):109-121.
11. Kotzot D. Prenatal testing for uniparental disomy: indications and clinical relevance. *Ultrasound Obstet Gynecol*. 2008;31(1):100-105.
12. Smidt-Jensen S, Christensen B, Lind AM. Chorionic villus culture for prenatal diagnosis of chromosome defects: reduction of the long-term cultivation time. *Prenat Diagn*. 1989;9(5):309-319.
13. Conlin LK, Thiel BD, Bonnemann CG, et al. Mechanisms of mosaicism, chimerism and uniparental disomy identified by single nucleotide polymorphism array analysis. *Hum Mol Genet*. 2010;19(7):1263-1275.
14. Papenhausen P, Schwartz S, Risheg H, et al. UPD detection using homozygosity profiling with a SNP genotyping microarray. *Am J Med Genet A*. 2011;155A(4):757-768.
15. Visser GH, Eilers PH, Elferink-Stinkens PM, Merkus HM, Wit JM. New Dutch reference curves for birthweight by gestational age. *Early Hum Dev*. 2009;85(12):737-744.
16. Van Opstal D, Srebniak MI. Cytogenetic confirmation of a positive NIPT result: evidence-based choice between chorionic villus sampling and amniocentesis depending on chromosome aberration. *Expert Rev Mol Diagn*. 2016;16(5):513-520.
17. Henderson KG, Shaw TE, Barrett IJ, Telenius AH, Wilson RD, Kalousek DK. Distribution of mosaicism in human placentae. *Hum Genet*. 1996;97(5):650-654.
18. Schuring-Blom GH, Keijzer M, Jakobs ME, et al. Molecular cytogenetic analysis of term placentae suspected of mosaicism using fluorescence in situ hybridization. *Prenat Diagn*. 1993;13(8):671-679.
19. Horsthemke B, Nazlican H, Husing J, et al. Somatic mosaicism for maternal uniparental disomy 15 in a girl with Prader-Willi syndrome: confirmation by cell cloning and identification of candidate downstream genes. *Hum Mol Genet*. 2003;12(20):2723-2732.
20. Kotzot D. Complex and segmental uniparental disomy (UPD): review and lessons from rare chromosomal complements. *J Med Genet*. 2001;38(8):497-507.
21. Morandi A, Bonnefond A, Lobbens S, et al. A girl with incomplete Prader-Willi syndrome and negative MS-PCR, found to have mosaic maternal UPD-15 at SNP array. *Am J Med Genet A*. 2015;167A(11):2720-2726.

22. Zilina O, Kahre T, Talvik I, Oiglane-Shlik E, Tillmann V, Ounap K. Mosaicism for maternal uniparental disomy 15 in a boy with some clinical features of Prader-Willi syndrome. *Eur J Med Genet*. 2014;57(6):279-283.
23. Wang JC, Vaccarello-Cruz M, Ross L, et al. Mosaic isochromosome 15q and maternal uniparental isodisomy for chromosome 15 in a patient with morbid obesity and variant PWS-like phenotype. *Am J Med Genet A*. 2013;161A(7):1695-1701.
24. Izumi K, Santani AB, Deardorff MA, et al. Mosaic maternal uniparental disomy of chromosome 15 in Prader-Willi syndrome: utility of genome-wide SNP array. *Am J Med Genet A*. 2013;161A(1):166-171.
25. Fuke T, Mizuno S, Nagai T, et al. Molecular and clinical studies in 138 Japanese patients with Silver-Russell syndrome. *PLoS One*. 2013;8(3):e60105.
26. Bullman H, Lever M, Robinson DO, Mackay DJ, Holder SE, Wakeling EL. Mosaic maternal uniparental disomy of chromosome 11 in a patient with Silver-Russell syndrome. *J Med Genet*. 2008;45(6):396-399.
27. Luk HM, Ivan Lo FM, Sano S, et al. Silver-Russell syndrome in a patient with somatic mosaicism for upd(11) mat identified by buccal cell analysis. *Am J Med Genet A*. 2016;170(7):1938-1941.
28. Los FJ, Van Opstal D, van den Berg C. The development of cytogenetically normal, abnormal and mosaic embryos: a theoretical model. *Hum Reprod Update*. 2004;10(1):79-94.
29. Robinson WP, Penaherrera MS, Jiang R, et al. Assessing the role of placental trisomy in preeclampsia and intrauterine growth restriction. *Prenat Diagn*. 2010;30(1):1-8.
30. Kalousek DK. Current topic: confined placental mosaicism and intrauterine fetal development. *Placenta*. 1994;15(3):219-230.
31. Wolstenholme J, Rooney DE, Davison EV. Confined placental mosaicism, IUGR, and adverse pregnancy outcome: a controlled retrospective U.K. collaborative survey. *Prenat Diagn*. 1994;14(5):345-361.
32. Vanneste E, Voet T, Le Caignec C, et al. Chromosome instability is common in human cleavage-stage embryos. *Nat Med*. 2009;15(5):577-583.
33. Geigl JB, Obenauf AC, Schwarzbraun T, Speicher MR. Defining 'chromosomal instability'. *Trends Genet*. 2008;24(2):64-69.
34. Kalousek DK, Langlois S, Robinson WP, et al. Trisomy 7 CVS mosaicism: pregnancy outcome, placental and DNA analysis in 14 cases. *Am J Med Genet*. 1996;65(4):348-352.
35. Robinson WP, Barrett IJ, Bernard L, et al. 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*. 1997;60(4):917-927.
36. Mergenthaler S, Wollmann HA, Burger B, et al. Formation of uniparental disomy 7 delineated from new cases and a UPD7 case after trisomy 7 rescue. Presentation of own results and review of the literature. *Ann Genet*. 2000;43(1):15-21.

SUPPORTING INFORMATION

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