Outcomes in pregnancies with a confined placental mosaicism and implications for prenatal screening using cell-free DNA

Francesca Romana Grati, MSc, PhD¹, Jose Ferreira, MD, PhD^{2,3,4}, Peter Benn, DSc, PhD⁵, Claudia Izzi, MD⁶, Federica Verdi, MD⁷, Elena Vercellotti, MD⁸, Cristina Dalpiaz, MD⁹, Patrizia D'Ajello, MD⁸, Elisa Filippi, MD, PhD¹⁰, Nicola Volpe, MD, PhD¹¹, Francesca Malvestiti, MSc¹, Federico Maggi, MSc, PhD¹, Giuseppe Simoni, MSc, PhD¹, Tiziana Frusca, MD, PhD¹¹, Gaetana Cirelli, MD¹², Gabriella Bracalente, MD¹⁰, Antonino Lo Re, MD⁹, Daniela Surico, MD⁸, Tullio Ghi, MD, PhD¹¹ and Federico Prefumo, MD, PhD⁶

Purpose: To assess the association between confined placental mosaicism (CPM) and adverse pregnancy outcome.

Methods: A retrospective cohort study was carried out evaluating the outcome of pregnancies with and without CPM involving a rare autosomal trisomy (RAT) or tetraploidy. Birthweight, gestational age at delivery, fetal growth restriction (FGR), Apgar score, neonatal intensive care admission, preterm delivery, and hypertensive disorders of pregnancy were considered.

Results: Overall 181 pregnancies with CPM and 757 controls were recruited. Outcome information was available for 69% of cases ($n = 124$) and 62% of controls ($n = 468$). CPM involving trisomy 16 (T16) was associated with increased incidence of birthweight <3rd centile ($P = 0.007$, odds ratio [OR] = 11.2, 95% confidence interval [CI] = 2.7–47.1) and preterm delivery ($P = 0.029$, OR = 10.2, 95% CI = 1.9–54.7). For the other RATs, an association with

prenatally diagnosed FGR was not supported by birthweight data and there were no other strong associations with adverse outcomes.

Conclusion: Excluding T16, the incidence of adverse pregnancy outcomes for pregnancies carrying a CPM is low. RATs can also be identified through genome-wide cell-free DNA screening. Because most of these will be attributable to CPMs, we conclude that this screening is of minimal benefit.

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INTRODUCTION

Confined placental mosaicism (CPM) is defined as the presence of a chromosomal abnormality in the placenta but not in the fetus.^{1,2} This condition is detected when cytogenetic analysis of a chorionic villus sample (CVS) shows the presence of cell lines with normal and abnormal karyotypes but follow-up analysis on amniotic fluid cells or fetal tissue is normal. Depending on the distribution of the abnormal cell line in the two placental layers, three different types of CPM can be identified: type I, with the abnormal cell line identified in the cytotrophoblast only; type II, with the abnormality in

the mesenchyme only; and type III, with both placental layers containing the abnormal cell line.^{3,4}

The most common type of CPM involves a trisomy/disomy. The mechanisms generating such fetoplacental discrepancies are generally either a mitotic chromosome segregation error occurring in an initially chromosomally normal conceptus, or a meiotic error resulting in trisomy with subsequent postzygotic “trisomic rescue.”⁵ Meiotic origin is generally associated with high levels of trisomy in trophoblasts. A model for this association is mosaic trisomy 16 (T16) that is usually detected as CPM type III and is associated with fetal

¹Unit of Research and Development, Cytogenetics and Medical Genetics TOMA, Advanced Biomedical Assays, Impact Lab Group, Busto Arsizio, Varese, Italy; ²Faculty of Medicine, Eduardo Mondlane University, Maputo, Mozambique; ³Department of Obstetrics and Gynecology, Maputo Central Hospital, Maputo, Mozambique; ⁴Genomed S.A., Warsaw, Poland; ⁵Department of Genetics and Genome Sciences, University of Connecticut Health Center, Farmington, CT, USA; ⁶UO Diagnosi Prenatale, Azienda Ospedaliera Spedali Civili, Brescia, Italy; ⁷Ostetricia e Ginecologia, Ospedale di Bolzano, Bolzano, Italy; ⁸Università del Piemonte Orientale, Novara, Italy; ⁹UO Ostetricia e Ginecologia, Clinica Pederzoli, Peschiera del Garda, Italy; ¹⁰UOC Ginecologia e Ostetricia, Osp. Cà Foncello Treviso, Treviso, Italy; ¹¹Department of Medicine and Surgery, Unit of Surgical Sciences, Obstetrics and Gynecology, University of Parma, Parma, Italy; ¹²Ostetricia e Ginecologia, Ospedale Santorso AULSS7 Pedemontana (VI), Santorso, Italy. Correspondence: Francesca Romana Grati (fgrati@tomalab.com)

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abnormality, fetal growth restriction (FGR), and other pregnancy complications.⁵ Meiotic CPM may also be associated with the presence of fetal uniparental disomy (UPD), which is defined as a pair of homologous chromosomes derived from only one parent. UPD is of particular clinical interest when it involves chromosomes 6, 7, 11, 14, 15, or 20 because these chromosomes carry imprinted regions that are associated with defined syndromes. Phenotypes can include fetal growth restriction (FGR) and reduced postnatal growth.⁶

The prevalence of mosaicism in CVS for viable pregnancies at 10–12 weeks of gestation is approximately 2%.^{3,7–9} When a rare autosomal trisomy (RAT, defined as any autosomal trisomy other than T21, T18, and T13) is detected in CVS, in 97% of the cases it is a CPM.³ Although this is a common finding, the possible association between CPM and adverse pregnancy outcome is unclear and counseling is therefore challenging. Some authors reported a higher risk of adverse pregnancy outcome, mainly FGR,^{10–15} while others did not.^{16–22} Many studies had a small number of cases, did not consistently combine the analyses of the direct preparation with the long-term cultures, or lacked a confirmatory amniocentesis to discriminate between CPM and true fetal mosaicism (TFM) in all cases.

Because of the limitations of past studies and the controversy around the significance of CPM, we undertook a retrospective cohort study based on review of pregnancy outcomes in affected pregnancies and in controls to assess the association between CPM and adverse pregnancy outcome.

MATERIALS AND METHODS

This retrospective cohort study was based on medical record review of women who underwent CVS between May 2000 and January 2018 at seven Italian referral centers for prenatal diagnosis. The villus samples of each patient were submitted to a single laboratory, which conducted the cytogenetic analysis by means of both cytotrophoblast and mesenchymal karyotyping.^{23,24} Informed consent for the analyses was obtained from all subjects. The most common indications for CVS in the entire data set were maternal anxiety/elective decision (women <35 years) or advanced maternal age (approximately 80% of the cases). All cases of RATs or with tetraploidy greater than 20% observed during the study period were included. A mosaicism was defined as the presence of a mosaic abnormality (MA) or nonmosaic abnormality (NMA) in one of the two placental tissues, or MA in both tissues or NMA in one tissue and MA in the other. Rare cases with both abnormal nonmosaic layers were also included when associated with normal ultrasound findings. TFM was defined as the presence of at least two colonies from two AF cultures showing the same abnormality as that previously observed at CVS.

All patients with a diagnosis of mosaic of CVS involving a RAT or a tetraploidy had a genetic consultation and were offered a follow-up amniocentesis with UPD analysis (for cases with trisomy 6, 7, 11, 14, 15, 20) and a detailed ultrasound anomaly scan. Methods for cytogenetic and UPD

analyses have been described in detail in elsewhere³ (Supplementary Materials and Methods).

Inclusion criteria were (1) singleton pregnancy; (2) absence of ultrasound abnormalities (except increased nuchal translucency or soft markers); (3) an abnormal cell line showing a RAT, tetraploidy, or a whole autosomal arm imbalance detected in the two placental layers (type III), or in the long-term culture (type II) or in direct preparation only (type I); (4) a follow-up amniocentesis that showed a normal karyotype. Exclusion criteria were (1) twin pregnancy, (2) confirmed TFM on amniocytes, (3) incomplete CVS analysis (direct or long-term culture, only), (4) CPM for common trisomies (T21,18,13) or sex chromosome aneuploidies.

Control subjects from singleton pregnancies with no evidence of CPM were selected from the laboratory database by identifying four contemporaneous cases (two male and two female fetuses) with similar maternal age, gestational age at the time of the procedure, and indication for prenatal diagnosis. Cases and controls were coded and blinded prior to medical record review. Each clinical center had an assigned analyst for the extraction of follow-up data from the medical records.

The following pregnancy outcome information was considered:

1. Birthweight percentile and birthweight below the 3rd percentile (frequently used as a classifier for small for gestational age).
2. Prenatal diagnosis of FGR, based on medical chart documentation.
3. Apgar score at 5 minutes.
4. Admission to a neonatal intensive care unit (NICU), excluding the subjects with prenatal assignment of FGR (outcome 2).
5. Hypertensive disorders (HD) of pregnancy (HELLP syndrome, preeclampsia, pregnancy induced hypertension).
6. Spontaneous preterm delivery (PTD) including cases with or without prior premature rupture of membranes.
7. Fetal anomaly, stillbirth, miscarriage, or termination of pregnancy.
8. Other, less common, pregnancy complications (e.g., acute chorioamnionitis, cholestasis, gestational diabetes, abruptio placentae, placenta previa).

We used INTERGROWTH-21st project charts to calculate birthweight percentile from gestational age at birth, birthweight, and newborn sex, because they are independent of ethnicity, or any other maternal factors that have been previously suggested to influence fetal growth.²⁵

Institutional review board (IRB) approval was obtained from TOMA laboratory IRB (#0000023/2018).

Statistical data analysis

The associations between the diagnosis of CPM and each outcome variable were assessed by an independent analyst, after de-coding and classification into cases and controls. Mann–Whitney exact test was used for numeric and ordinal

outcome variables and exact Chi-square test for categorical binary outcome variables. Linear regression was used for associations between the degree of abnormal cell lines in placenta and quantitative outcome variables.

Associations between CPM and outcomes were assessed for CPMs involving a RAT (excluding T16) or a whole autosomal arm imbalance, for CPMs with tetraploidy only, and CPM with T16 only.

Birthweights (outcome 1 above) were treated as quantitative (interval) variables and categorical binary variables, respectively. Apgar scores (outcome 3) were treated as ordinal variables; all the other outcomes were treated as categorical binary variables.

Statistical testing was carried out using PASW Statistics (version 18, 30 July 2019, New York, NY, USA). A *P* value <0.05 was considered significant. Correction for multiple testing was not applied (see Supplementary Materials and Methods).

RESULTS

In this series of 76,104 CVS, 1603 cases (2.1%) displayed a chromosomal mosaicism. Of these, 1212 cases were subsequently investigated by a midtrimester amniocentesis. In 1051 of them (86.7%) the amniotic fluid karyotype showed no evidence of chromosome abnormality; they were therefore classified as CPMs. Of these, 443 cases (42.2%) involved a RAT (176 CPM type I, 200 CPM type II, and 54 CPM type III), 72 cases (5.9%) involved a tetraploidy (32 CPM type I, 15 CPM type II, and 25 CPM type III), and 23 (1.9%) a whole arm autosomal imbalance, mainly isochromosome (6 CPM type I, 10 CPM type II, and 3 CPM type III).

The seven participating centers accounted for 181 cases or 41% of the total CPM (*n* = 443) in the full data set. The remaining 262 cases were from multiple smaller referral locations where it was impractical to gather follow-up. There were 757 matched controls. One or more of the clinical outcomes were obtained for 124/181 (68.5%) CPM cases and 468/757 (62%) controls. The missing outcomes were due to outpatient document disposal according to Italian regulations (after 10 years), or because the patient delivered in a different institution. Figure 1 summarizes the study design. There was no difference in maternal age distribution and in indications for testing between cases and controls (Table S1 and S2).

Figure 2 shows the number of the specific chromosome abnormalities present as CPM in the cases. We included the three cases with isochromosomes of 11q, 7p, and 20p as RATs. Trisomies 2, 3, 7, and 16 were the most frequently encountered CPMs.

Table 1 summarizes the classification of the 124 CPMs (types I, II, and III) and the average percentage of the abnormal cell line in the affected placental layers. The relative proportions of the three CPM types in the study were similar to those seen in the full set of CVS cases. Most CPMs were types I and II. RATs that were present in both placental layers (type III) generally showed a higher percentage of abnormal

cells. This agrees with the expectation that type III abnormalities are mostly of meiotic origin.⁵

UPD was investigated for CPMs involving imprinted chromosomes (7, 11, 14, 15, or 20) (Table 2). The only UPD identified in this cohort was in one case of T14. That pregnancy was terminated due to the UPD and abnormalities identified by ultrasound.

Table 3 and S3 summarize the outcomes for the various types of CPM. For CPMs involving RATs (excluding trisomy 16), a statistically significant association was found with FGR (odds ratio [OR] 3.4; 95% confidence interval [CI] 1.3–9.3). There was also a weak association with NICU admission excluding FGR (OR 3.3; 95% CI 1.0–10.2) (Table S3). For NICU admission, the proportion of abnormal cells in cytotrophoblasts and mesenchyme was not statistically significant. There was no association with HD, PTD, or birthweight percentile (below third or when all birthweights were considered).

Because of literature suggestive of poor outcomes with CPM involving T16, this subgroup (11 cases) was analyzed separately. There was a statistically significant excess of cases with birthweight <3rd percentile (OR 11.2; 95% CI 2.7–47.1). However, when all birthweights were considered as quantitative outcomes, a significant correlation could not be demonstrated. CPM T16 cases also appeared to show an association with FGR (OR 8.4; 95% CI 1.6–43.2). In addition, there was a statistically significant association with spontaneous PTD (OR 10.2; 95% CI 1.9–54.7) and low Apgar score (*P* = 0.006).

CPMs involving T16 only were also analyzed to determine whether the proportion of abnormal cells in cytotrophoblast and mesenchyme was associated with birthweight. There were three T16 CPM cases that had a birthweight less than the 3rd percentile and all had a mosaicism level of 100% in both cytotrophoblasts and mesenchyme. There was only one other T16 case with this level of mosaicism that had a birthweight percentile >3rd (24th percentile). The average percentage of T16 cells for the cases with a birthweight percentile >3rd was 46% (min 0%, max 100%) in the mesenchyme and 40% (min 0%, max 100%) in the cytotrophoblast. There were three T16 cases whose birthweight percentile was above 90% and the levels of abnormal cells in cytotrophoblast and mesenchyme were 76% and 29%, 0% and 9%, and 0% and 10%, respectively. A linear regression analysis of abnormal cell percentage for all T16 cases with birthweight percentile showed an association in both mesenchyme (adjusted r^2 = 0.044; *P* = 0.03) and cytotrophoblast (adjusted r^2 = 0.07 *P* = 0.008) when analyzed independently. When analyzed in a multiple variable regression model, the *P* value was 0.028 and the adjusted r^2 was 0.044. These data suggest that the increased degree of trisomy in mesenchyme and cytotrophoblast explains only 4.4% of the variation in birthweight percentile and that the degree of mosaicism in cytotrophoblast has a bigger effect than the degree of mosaicism in mesenchyme.

Fig. 1 Study design. CPM confined placental mosaicism, CTs common trisomies, CV chorionic villus, RATs rare autosomal trisomies, SCAs sex chromosome aneuploidies, TETRA tetraploidy, TFM true fetal mosaicism, WAI whole autosomal arm imbalance, *whole arm Xp or Xq trisomies, unbalanced structural rearrangements, monosomies, triploids.

There were only three miscarriages (all controls) and two stillbirths (one T16 caused by tearing of the umbilical cord, and one control). There were six elective pregnancy terminations, two of them in CPM cases (one in a CPM for T22 with 100% abnormal cells in both placental layers, and the other in a CPM for T14, in which talipes and UPD were present), and four in controls (one for increased nuchal translucency and subsequent detection of a cardiac abnormality, one with a cardiac anomaly, one with a dup 22q11, and one because of very early onset FGR). No other fetal abnormalities were recorded. These numbers of fetal anomalies, miscarriage, stillbirth, and terminations of pregnancy were insufficient for a meaningful statistical analysis. Similarly, there appeared to be only a small number of other pregnancy complications (data not shown) with insufficient numbers for formal statistical comparisons.

The data set included 18 cases with tetraploidy CPM. There was no evidence for a statistically significant association between this finding and any of the adverse outcomes analyzed.

DISCUSSION

In this study we have evaluated the clinical significance of CPM involving aneuploidies other than the common ones in CVS. Only T16 was found to be strongly associated with a risk for an adverse outcome ($OR > 8$). CPMs involving RATs appeared to be significantly associated with FGR. However, except for those involving T16, there was no association with low birthweight less than the 3rd percentile or when considering all birthweight percentiles, suggesting that the FGR association for RATs (excluding T16) may have been spurious. For example, in this group, there were 21 pregnancies assigned as having FGR, of which only 13 had a birthweight <10th percentile. Conversely, of 19 newborns with a birthweight <3rd, only 8 were assigned as FGR. Heterogeneous criteria were used for defining FGR with differences between centers; some estimated fetal weight percentiles were calculated using Hadlock's charts²⁶ whereas others used Yudkin's curves.²⁷ Actual birthweight centiles are based on appropriate population standards and should constitute a more reliable measure of fetal growth.²⁵ It is also

Fig. 2 Rare autosomal trisomies and tetraploid abnormalities included in the study. Arranged with most common abnormalities listed first. The red line denotes the cumulative number (%). CPM confined placental mosaicism.

possible that the reporting CPM itself resulted in an ascertainment bias in the assignment of FGR, attributable to prior literature suggesting an association. The diagnosis of FGR, with possible bias, could also have been the indication for admission to the NICU. Therefore, we excluded subjects assigned as having FGR when considering the association between RATs and NICU admission. After this exclusion, NICU admission was found to be of borderline significance ($P = 0.049$) and this weak association could still be attributable to biased referral of cases to NICUs.

There is a substantial body of evidence to believe that CPM involving trisomy 16 is associated with FGR, prematurity, preeclampsia, and fetal abnormalities.^{28–31} In this study, which only included 12 cases, a significant association was found between CPM T16 and birthweight <3rd percentile. Interestingly, the association between CPM T16 and the quantitative variable birthweight percentile failed to reach statistical significance. It is possible that CPM T16 causes a severe growth restriction in a small number of cases but in the full set of cases CPM has only a minor effect. Consistent with this, we observed that cases with birthweight percentile <3rd were atypical in having 100% abnormal cells in both placental layers. No association was found with HD although preeclampsia is a known complication of these pregnancies.^{28,29} An association with PTD and low Apgar score was also identified for T16 CPM.

No association was found between CPM involving tetraploidy and any adverse outcome. Tetraploidies are commonly detected in cytotrophoblasts and counseling is challenging because this result might be an in vitro cultural artifact or a true clonal abnormality.³ This study shows that, in absence of

fetal ultrasound abnormalities, these women can be reassured that pregnancy outcomes are like those in a control population.

Previous studies assessing the significance of CPM have been controversial with conflicting results, possibly due to differences in study design. The study methodology may be, at least in part, a contributing factor; there have been case-control studies that assessed whether CPM is more frequent in FGR pregnancies.^{11–13,20} Other studies assessed differences in clinical presentation, perinatal outcome, and postnatal growth and development between infants for FGR pregnancies with or without CPM.³² Finally, there have been cohort studies that assessed whether there is an increased risk of FGR and pregnancy complications in CPM compared with non-CPM matched pregnancies.^{16,17,21–23} The present study is of this latter type. Differences in sample size, with smaller samples being more prone to biases, may also explain the differences in conclusions.

Overall, the results of this study are reassuring: only CPM for T16 showed an increased risk for birthweight percentile <3rd and spontaneous PTD while for most CPMs for rare autosomal trisomies (excluding T16) detected prenatally by CVS analysis, the general health of the mother and fetus can be expected to be close to that seen for pregnancies with a normal CV karyotype. These results agree with those of Amor and collaborators²¹ who found no increase in prenatal complications or adverse neonatal outcomes.

Strengths of the study

This represents the most extensive cohort to date for CPM pregnancy outcomes. All cytogenetic analyses were carried

Table 1 The average percentage of the abnormal cells in the placental layers for CPM types I, II, and III

CPM types	Overall				RAT (excluding T16)				T16 only				Tetraploidy			
	Cases		% Abnormal cells mean (min-max)		Cases		% Abnormal cells mean (min-max)		Cases		% Abnormal cells mean (min-max)		Cases		% Abnormal cells mean (min-max)	
	#	%	Dir	LTC	#	%	Dir	LTC	#	%	Dir	LTC	#	%	Dir	LTC
I	55	44.4	45	47.9	30.9 (5.5–100)	1	8.3	40.0 ^a	9	50.0	83.7 (50–100)		9	50.0	83.7 (50–100)	
II	49	39.5	40	42.6	33.5 (5.4–100)	5	41.7		4	22.2	38.4 (9.1–75)		4	22.2		89.5 (75–100)
III	20	16.1	9	9.6	64.1 (3.9–100)	6	50.0	95.0 (100–100)	5	27.8	71.8 (40–100)		5	27.8	71.8 (40–100)	24.5 (7–50)
Total	124		94			12			18				18			

CPM confined placental mosaicism, Dir direct preparation, LTC long-term culture, RAT rare autosomal trisomy, T16 trisomy 16.

^aOne case.

Table 2 Evaluation for uniparental disomy (UPD) in cases with CPM for trisomy or partial trisomy for chromosomes 6, 7, 11, 14, 15, or 20

Imprinted chromosome	Number present	Tested cases (n)	UPD detected (n)
Trisomy 6	0	0	0
Trisomy 7	21	15	0
Trisomy 11	3	3	0
Trisomy 14	1	1	1
Trisomy 15	10	10	0
Trisomy 20	5	3	0
Total	39	32	1

CPM confined placental mosaicism.

out at a single laboratory with uniform policies for systematic direct preparation, long-term culture, and follow-up amniocenteses. The study included a large number of matched controls ($n = 468$). Follow-up was gathered in a blinded fashion with a high completion rate (63%). Most patients did not receive prior ultrasound or maternal serum screening that could preselect cases with particular types of CPM.

Limitations of the study

Due to the retrospective nature of this study, there were no standard diagnostic criteria for FGR during the study period. Furthermore, except for CPM involving trisomy 16 and tetraploidies, we could not investigate associations for each individual chromosome abnormality involved due to insufficient power. Therefore, additional studies are needed to determine if T16 CPM is the only specific CPM that is associated with growth restriction. We cannot exclude that the possibility that cases with follow-up were biased toward those with either abnormal or normal pregnancy outcomes. For example, pregnancies terminated following the ultrasound detection of abnormality prior to confirmatory amniocentesis would not be included. Finally, as shown in Supplementary Table S3, our sample size was only sufficient to identify strong associations between the CPM RATs and the assessed outcomes. We could not exclude small associations, such as OR less than 3 to 10.

Implications for cfDNA screening

The results of the present study have implications on the clinical utility of RAT detection through genome-wide cell-free DNA (cfDNA) testing. Direct CVS analysis and cfDNA are both based on the trophoblast cell lineage and both will identify RATs, most of which will be CPMs.³³ The main advantage claimed for detecting RATs through cfDNA is because of the association with pregnancy complications.^{34–36} RATs can be readily detected by genome-wide technologies in which counting data are already available. Therefore, the relevant question is whether there is additional useful information that could be extracted by looking for RATs when the cfDNA test is being done anyway.

Table 3 Summary of all association studies between CPMs and outcomes

Outcome	CPM (RATs excluding T16) N = 94	CPM (tetraploidy only) N = 18	CPM (T16 only) N = 12	Controls N = 468	Significant associations
Binary outcome variables (X ²)	# Abnormal outcome/total (%) P value ^a			# Abnormal outcome/ total (%)	
Birthweight percentile <3rd	3/87 (3.4%) P 1.0	0/17 P 0.672	3/11 (27.3%) P 0.007	13/401 (3.2%)	T16 only
FGR	7/92 (7.6%) P 0.018	2/17 (11.8%) P 0.072	2/12 (16.7%) P 0.039	10/428 (2.3%)	RATs (excluding t16) T16 only
NICU admission (excluding FGR)	5/84 (6%) P 0.049	1/18 (5.6%) P 0.317	1/10 (10%) P 0.192	8/420 (1.9%)	RATs (excluding T16)
HD	6/92 (6.5%) P 0.60	1/18 (5.6%) P 1.0	1/12 (8.3%) P 1.0	20/428 (4.7%)	None
Spontaneous PTD	4/81 (4.9%) P 0.48	0/16 (0%) P 1.0	2/9 (22.2%) P 0.029	11/403 (2.7%)	T16 only
Quantitative outcome variables (Mann–Whitney rank-sum test)	N (median, IQR, 25th–75th percentile) P value ^a			N (median, IQR, 25th–75th percentile)	
Birthweight percentile	N = 87 (49, 52, 26–78) P 0.257	N = 17 (44, 37, 25–63) P 0.186	N = 11 (35, 89, 2–91) P 0.181	N = 401 (55, 50, 31–81)	None
Apgar score at 5 minutes	N = 87 (10, 1, 9–10) P 0.477	N = 17 (9, 1, 9–10) P 0.120	N = 11 (9, 0, 9–9) P 0.006	N = 408 (10, 1, 9–10)	T16 only

^aP values compare the CPM group versus controls.

CPM confined placental mosaicism, FGR fetal growth restriction, HD hypertensive disorders of pregnancy, IQR interquartile range, NICU neonatal intensive care unit, PTD preterm birth, T16 trisomy 16.

Statistically significant *p*-values are in bold underlined *p* < 0.05.

Initial studies with genome-wide cfDNA screening have reported high rates of FGR but these need to be interpreted cautiously because they may be subject to ascertainment biases similar to those we encountered in this study.^{37,38} Our data indicate that 0.41% of cases show a CPM (type I or III) involving a RAT in cytotrophoblasts and, of these,³ 6/98 (6.1%) will be associated with a birthweight percentile <3rd meaning that routine detection of CPM in a general population might result in identification of $0.41\% \times 6.1\% = 0.025\%$ of cases with birthweight percentile <3rd. This represents only $0.025\% / 3\% = 0.8\%$ of all such cases present in the population. Under the assumption that CVS and cfDNA have comparable efficacy, the yield of low birthweight babies identifiable through a RAT would therefore seem to be minimal. We therefore conclude that cfDNA screening is unlikely to be an effective screening test for low birthweight.

This study shows that T16 is the only individual RAT that may be worth looking for and reporting through cfDNA screening. However, even for mosaic T16, pregnancy outcomes are highly variable and optimal prenatal management has not been defined. Genome-wide cfDNA screening will also potentially lead to the identification of some cases with UPD, but this yield is expected to be very low.³ Cell-free DNA screening will also identify nonmosaic RATs, which are associated with early fetal losses, but these are neither preventable nor actionable. Screening for RATs is not endorsed by the American College of Medical Genetics and Genomics.³⁹

SUPPLEMENTARY INFORMATION

The online version of this article (<https://doi.org/10.1038/s41436-019-0630-y>) contains supplementary material, which is available to authorized users.

DISCLOSURE

P.B. is a consultant for and holds stock options in Natera, Inc. F.R. G. and F. Malvestiti are full-time employees of TOMA laboratory, Impact Lab Group without ownership shares. F.R.G. is an expert panel member for Roche and consultant for Menarini Biomarkers. G.S. is a consultant for TOMA laboratory, Impact Lab Group. F. Maggi holds stock options in TOMA laboratory, Impact Lab Group. F.P. received consulting fees from Tamas srl for an economic evaluation study of cell-free fetal DNA screening in the Italian national health service. The other authors declare no conflicts of interest.

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