Confined placental mosaicism detected with non-invasive prenatal testing: is there an association between mosaic ratio and pregnancy outcome?

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Prenatal Diagnosis

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PRENATAL DIAGNOSIS

ORIGINAL ARTICLE OPEN ACCESS

Confined Placental Mosaicism Detected With Non-Invasive Prenatal Testing: Is There an Association Between Mosaic Ratio and Pregnancy Outcome?

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Received: 3 July 2024 | **Revised:** 16 September 2024 | **Accepted:** 22 September 2024

Funding: The authors received no specific funding for this work.

ABSTRACT

Objective: Confined placental mosaicism (CPM) is associated with an increased risk for pregnancy complications, such as fetal growth restriction (FGR), preterm birth and hypertensive disorders. Pregnancies with possible CPM can be identified with non-invasive prenatal testing (NIPT). We performed a retrospective cohort study to investigate whether the mosaic ratio, as calculated with the Veriseq v2 used for NIPT, can predict adverse pregnancy outcomes in cases of CPM.

Method: A mosaic ratio for trisomies detected by NIPT and obstetric data such as fetal growth, structural fetal anomalies and birthweight were retrospectively studied in a cohort of patients with CPM diagnosed between February 2021 and October 2023. Structural and sex chromosomal aberrations were not included in this study.

Results: Of 122 CPM cases, 52 cases (42.6%) showed adverse perinatal outcomes, including FGR, low birthweight, hypertensive disorders, or preterm birth. A significantly higher mosaic ratio was found in the adverse outcome group compared to those with normal outcome, but a clear-cut threshold could not be set, except potentially for trisomy 16.

Conclusion: There is an association between the mosaic ratio and adverse pregnancy outcomes in cases of CPM. However, without a clear-cut threshold, it cannot be used for the individual patient for differentiation between CPM with and without clinical consequences.

1 | Introduction

Prenatal care routinely incorporates non-invasive prenatal testing (NIPT), which relies on the examination of placental cell-free DNA (cfDNA) circulating in the peripheral blood of a pregnant woman. This testing method was introduced in the Netherlands in 2014 [1], and has become a standard part of prenatal healthcare, first only as a second tier test and later as a first tier test. With the introduction of NIPT, confined placental mosaicism (CPM) regained attention, as it is the primary factor contributing to discordant NIPT results [2]. CPM is a type of chromosomal mosaicism characterized by the presence of one or more chromosomally abnormal cell lines in the placenta, while the chromosomal makeup of the fetus is normal [3]. CPM is associated with an increased risk for adverse pregnancy outcomes, such as fetal growth restriction (FGR) and low birthweight (LBW) [4, 5]. While pregnancies affected by CPM carry a higher risk of adverse

Summary

- What is already known about this topic?
  - CPM is associated with an increased risk of pregnancy complications
  - The clinical impact of CPM is one of the most challenging aspects of counseling of future parents
- What does this study add?
  - There is an association between the mosaic ratio and adverse pregnancy outcomes in cases of CPM
  - The mosaic ratio cannot discriminate between CPM with normal and adverse pregnancy outcomes

outcomes, about half of the cases lead to the birth of healthy babies without complications. Therefore, accurately predicting which CPM affected pregnancies are susceptible to adverse outcomes remains challenging. Numerous studies have aimed to identify predictors for adverse outcomes when CPM is diagnosed, such as level of mosaicism in first trimester chorionic villus sampling (CVS), type of CPM, origin of the involved trisomy (mitotic or meiotic) and type of chromosome aberration [6–8]. However, these CPM characteristics require the prenatal investigation of CV, whereas the majority of CPM nowadays is detected with NIPT, with diagnostic follow-up investigations mostly performed in amniotic fluid. Other studies showed the association of a low PAPP-A or high levels of free β-hCG with adverse pregnancy outcomes in cases of CPM [9–12]. To improve obstetric management in instances of suspected CPM, a noninvasive prenatal predictor, preferably derived from the NIPT data, would be considered the ultimate goal. Recently, Xiang et al. showed an association between the mosaic ratio and pregnancy outcome with the mosaic ratio being significantly higher in women who experienced adverse perinatal outcomes, such as maternal hypertensive disorders, FGR, structural fetal anomalies and LBW, than those who did not [13]. The mosaic ratio is the proportion of fetal cfDNA that is aneuploid in maternal blood [14]. The mosaic ratio has already been suggested as a predictor for fetal involvement when trisomy 13, 18, or 21 was detected with NIPT [15, 16] and also in cases with rare autosomal trisomies (RATs) [17].

This study explores the association between the mosaic ratio as calculated from the NIPT data and adverse pregnancy outcomes in cases of CPM. Although CPM is associated with an increased risk of adverse pregnancy outcome, we previously showed that when CPM involves a trisomy of meiotic origin, FGR and LBW are significantly more often observed [8]. Since CPM involving a meiotic trisomy is more likely to have high levels of mosaicism in the cytotrophoblast (CTB), which will lead to an increased amount of trisomic cfDNA in maternal blood, a higher mosaic ratio is expected.

Therefore, we hypothesize that a higher mosaic ratio is positively correlated with FGR and LBW. If the mosaic ratio proves to be a reliable predictor of adverse pregnancy outcomes, we aim to propose a specific threshold to identify high risk CPM pregnancies.

2 | Methods

2.1 | NIPT and Cytogenetic Investigations

Between February 2021 and October 2023, all cases of (potential) CPM involving a trisomy that were detected with NIPT performed at the Erasmus MC and with follow-up cytogenetic studies in the Erasmus MC (Rotterdam, the Netherlands) and LUMC (Leiden, the Netherlands) were collected. These NIPTs were performed as part of the TRIDENT-2 study, which ended on April the first 2023. Since then, NIPT has been offered as part of the national prenatal screening program as a first-tier screening test to all pregnant women [18]. TRIDENT-2 was licensed by the Minister of Health, Welfare, and Sport (1017420-153371-PG) and approved by the Medical Center Ethics Committee of Erasmus MC, Rotterdam (MEC-2018-1685).

NIPT was performed using Veriseq v2 software and WISECONDOR [19]. The Veriseq v2 also calculates the mosaic ratio, which is the proportion of the fetal material that is aneuploid. This metric is based on the ratio of the fetal fraction inferred from the coverage of the region to the fetal fraction for the sample [14]. For CPM cases involving two trisomies and therefore two mosaic ratio's, the higher mosaic ratio was adopted.

If the NIPT result indicated a chromosome aberration, patients were counseled for invasive prenatal diagnosis. The study included all cases with singleton pregnancies diagnosed or suspected to have CPM based on follow-up investigations. Structural chromosomal aberrations were not included in this study. CPM was confirmed when a chromosomal aberration was present in first trimester chorionic villi or postnatal placenta tissue but absent in amniotic fluid (AF), cord blood, or fetal tissue and maternal blood. In these cases, the type of CPM and origin of the trisomy (mitotic or meiotic) could be determined. CPM was suspected when a rare or common trisomy typically associated with CPM was detected, with normal findings in AF, cord blood, fetal tissue and maternal blood, but without sufficient placental tissue for cytogenetic confirmation.

Cytogenetic analysis of chorionic villi (CV), AF, other fetal tissue and maternal blood was performed using the SNP array (Illumina Infinium GSA + MD-24 v1.0 BeadChip) as previously described. In cases of confirmed CPM, the type of CPM and origin of the trisomy were determined as previously outlined [8]. Specifically, both cell layers of the CV, CTB and mesenchymal core (MC) were analyzed with an SNP array (Illumina Infinium GSA + MD-24 v1.0 BeadChip) as described in prior studies [20, 21].

Based on affected cell lineages CPM can be divided into three different types (type 1, 2, and 3) [22, 23]. If the chromosome aberration is only present in the cytotrophoblast (CTB), this is called CPM type 1. When the chromosomal abnormality is restricted to the mesenchymal core (MC) of the chorionic villi, it is categorized as type 2. Type 3 is defined as the presence of the abnormality in both cell-layers, MC and CTB. Only types 1 and 3 can be detected with NIPT.

The mitotic or meiotic origin of the trisomy was determined using the B-allele frequency (BAF) as described by Conlin et al. [24]. In cases with only 100% abnormal and normal results in different biopsies, digital mosaics were made in order to elucidate the meiotic or mitotic origin according to a method described before [25].

2.2 | Clinical Follow-Up

Pre- and postnatal data were collected at the Erasmus MC (Rotterdam, the Netherlands) and LUMC (Leiden, the Netherlands), midwife practices and referral hospitals. All participants provided written informed consent as a component of the TRIDENT study. Ultrasound measurements were collected from the first trimester through the third trimester, which included an (expert) ultrasound screening for congenital anomalies between 18 and 24 weeks of gestation.

Adverse perinatal outcomes:

- *FGR*: Defined as (1) estimated fetal weight (EFW) < 10th percentile, (2) fetal AC < 10th percentile, or (3) declining fetal growth (decline of minimal 20 percentiles of AC and/or EFW). Estimated fetal weight was calculated with the use of Hadlock 3 [26].
- *Maternal hypertensive disease*: Including pregnancy-induced hypertension, pre-eclampsia, or HELLP syndrome (*hemolysis, elevated liver enzymes, and low platelet count syndrome*).
- Termination of pregnancy (TOP) due to markers of adverse outcome.
- Intra uterine fetal demise (IUFD).
- *Preterm birth*: Defined as birth before 37 weeks of gestation.
- *LBW*: Defined as a birthweight below the 10th percentile according to the Hoftiezer curve [27].
- *Structural fetal anomalies*: Either prenatally detected through ultrasound analysis or postnatally.

2.3 | Statistical Analysis

All statistical analyses were performed with IBM SPSS Statistics (version 26; IBM Corporation, Chicago, IL). A p value of < 0.05 in two-tailed tests was considered statistically significant. The following tests were used: chi-square tests for categorical variables and Mann–Whitney U tests for continuous variables.

3 | Results

During the study period, 124 CPM involving a trisomy were detected through NIPT of which 2 were lost to follow up, as shown in Figure 1. Of the 122 cases, 25 involved a common aneuploidy and 97 involved a rare autosomal trisomy (RAT). Trisomy 7 and 13 were most common ($n = 40$, 32.8% and $n = 20$, 16.4%, respectively) as shown in Figure 2. In 2/122 (1.6%) cases, the pregnancy ended in IUFD, one pregnancy at 17 weeks of gestation (without structural fetal anomalies or FGR) and the other at 37 weeks of gestation with extreme FGR. In 1/122 (0.8%) cases, parents opted for TOP. This pregnancy was complicated by an anhydramnion with no signs of rupture of membranes. In 52 cases (42.6%), one or multiple adverse perinatal outcomes were present. All cases are shown in Table S1.

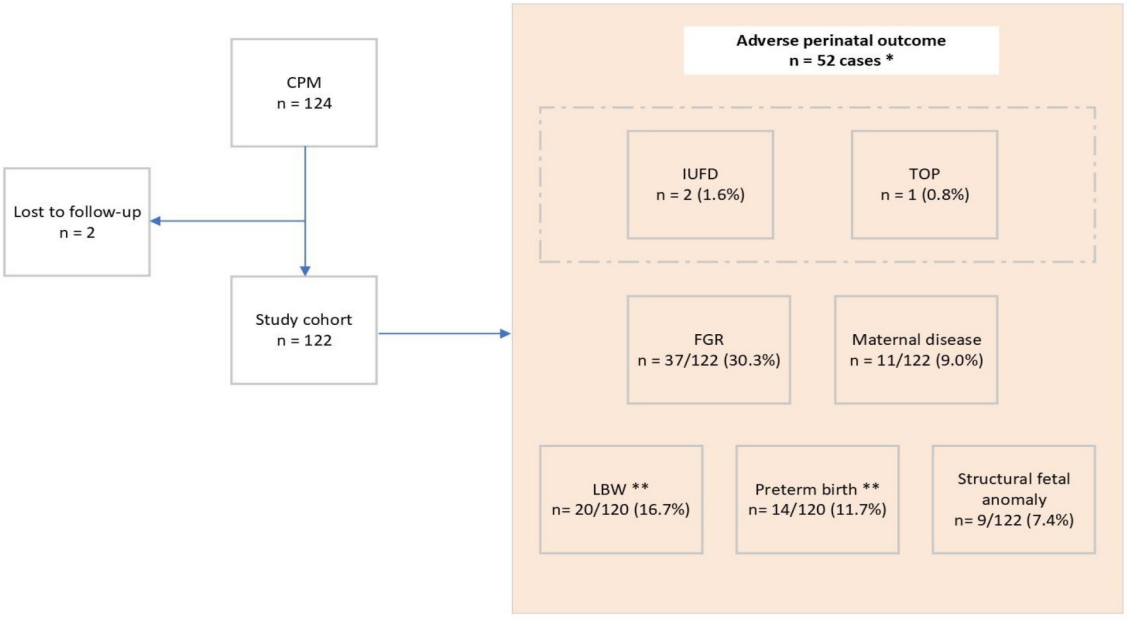

FIGURE 1 | Flowchart of this study, with in the orange frame, numbers and nature of adverse perinatal outcomes. CPM, confined placental mosaicism; FGR, fetal growth restriction; IUFD, intra uterine fetal demise; LBW, low birthweight; NIPT, non-invasive prenatal testing; TOP, termination of pregnancy. *Total number of cases with an adverse perinatal outcome is 52, with some cases having multiple adverse outcomes. **In two cases (one IUFD at 17 weeks and a TOP at 19 weeks) no birthweight was available and these were not included in the preterm birth group, therefore in both LBW and preterm birth the total number of cases is 120. [Colour figure can be viewed at wileyonlinelibrary.com]

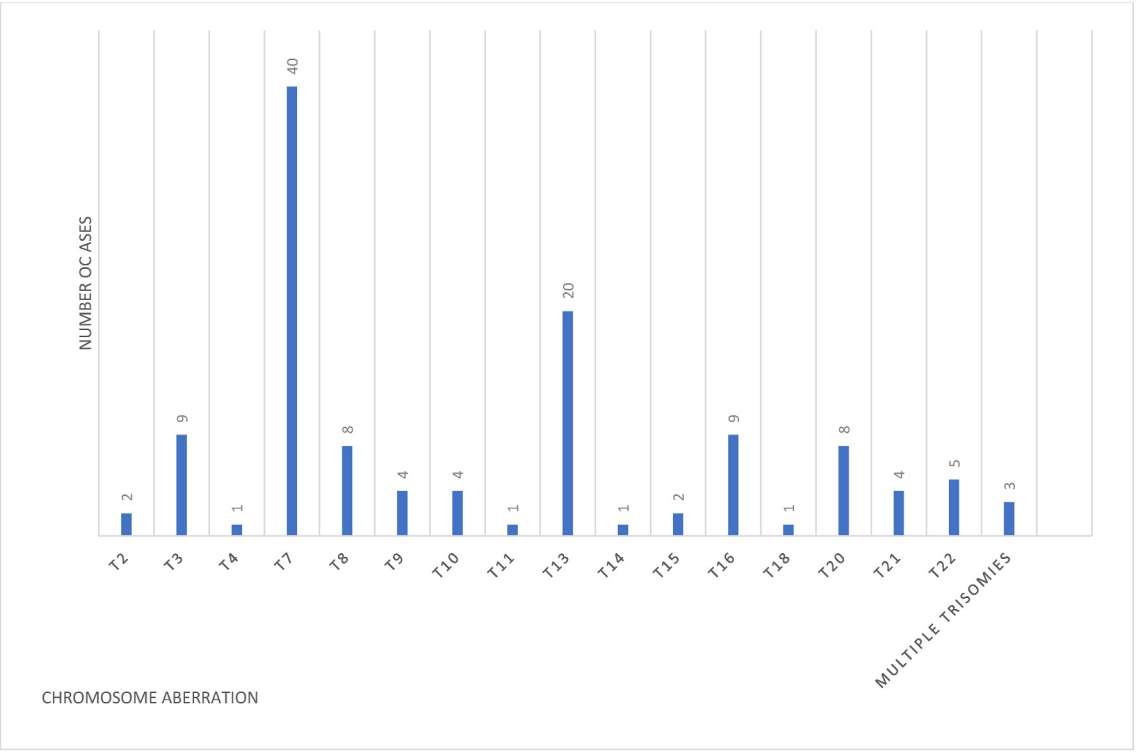

FIGURE 2 | Number of cases per chromosome aberration: different chromosome aberrations on *X*-axis and number of cases on *Y*-axis. T, trisomy. [Colour figure can be viewed at wileyonlinelibrary.com]

3.1 | Association of Mosaic Ratio and Pregnancy Outcome

The median mosaic ratio in the total cohort was 0.39 (IQR 0.28–0.62). In the group with adverse perinatal outcomes, the median mosaic ratio was 0.46 (IQR 0.29–0.79) compared to 0.37 (0.27–0.48) in the group without. The mosaic ratio was significantly higher ($p = 0.021$) in the group with adverse perinatal outcomes ($n = 52$) compared to the group without ($n = 70$). The distributions of the mosaic ratio for the cases with and without adverse perinatal outcomes are shown in Figure 3. Comparing the mosaic ratio between cases with and without a LBW, a significant difference was found ($p = 0.006$), as shown in Figure 4. However, if only FGR was considered, no significant difference was found ($p = 0.654$), as shown in Figure 5.

We divided the mosaic ratio into six subcategories, as shown in Table 1. When the mosaic ratio was < 0.61, the risk of adverse outcome was ~33% in the three categories. When the mosaic ratio exceeded 0.61, the risk of adverse outcome increased in both categories. We were not able to set a clear cut-off, as adverse perinatal outcomes were present across all six categories.

Due to the number of cases (≥ 9) of CPM trisomy 3, 7, 13, and 16, a subgroup analysis was possible and is presented in Supporting Information S1: Figure S1A–D. No significant associations were found between the height of the mosaic ratio and the presence of adverse pregnancy outcomes considering the subgroups CPM trisomy 3 ($p = 0.730$), trisomy 7 ($p = 0.861$), and trisomy 13 ($p = 0.710$). Only in the subgroup CPM trisomy 16 a significant association was present ($p = 0.024$, as shown in Supporting Information S1: Figure S1D).

3.2 | Association Between Mosaic Ratio and Cytogenetic Characteristics of the CPM

In 46 cases, the origin of the trisomy could be determined: 38/46 cases (82.6%) were of mitotic origin and 8/46 cases (17.4%) were of meiotic origin. A statistically significant difference in mosaic ratios was found between those groups, with the meiotic group having a higher median mosaic ratio (0.97 [IQR 0.79–1.18] versus 0.42 [IQR 0.33–0.66]) (as shown in Supporting Information S1: Figure S2). All eight meiotic cases had a mosaic ratio above 0.70, with seven of these cases experiencing adverse perinatal outcomes.

In 47 cases CPM type could be determined, 29/47 cases (61.7%) involved CPM type 1 and 18/47 cases (38.3%) type 3. Although type 1 CPM had a lower median mosaic ratio (0.42 [IQR 0.35–0.68]) compared to type 3 (0.73 [IQR 0.44–0.97]), the difference was not statistically significant (as shown in Supporting Information S1: Figure S3).

4 | Discussion

The aim of this study was to investigate whether the mosaic ratio of a trisomy involved in CPM and detected with NIPT could be used as a non-invasive marker for the identification of CPM cases with a higher likelihood of having adverse perinatal outcomes. Counseling and informed decision making in cases of (additional) findings from genome-wide NIPT is considered challenging, particularly when the clinical relevance is unclear [28]. When CPM is diagnosed prenatally, this may challenge

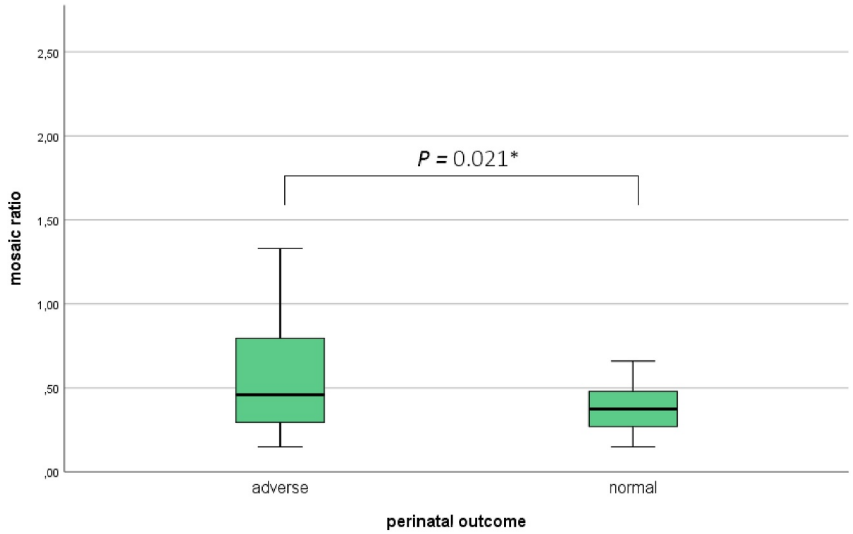

FIGURE 3 | Association between the mosaic ratio and perinatal outcome in 122 cases of CPM, with adverse perinatal outcome ($N = 52$) on the left and normal outcome ($N = 70$) on the right. *p*-value is given inside the boxplot. *Statistical significant. [Colour figure can be viewed at wileyonlinelibrary.com]

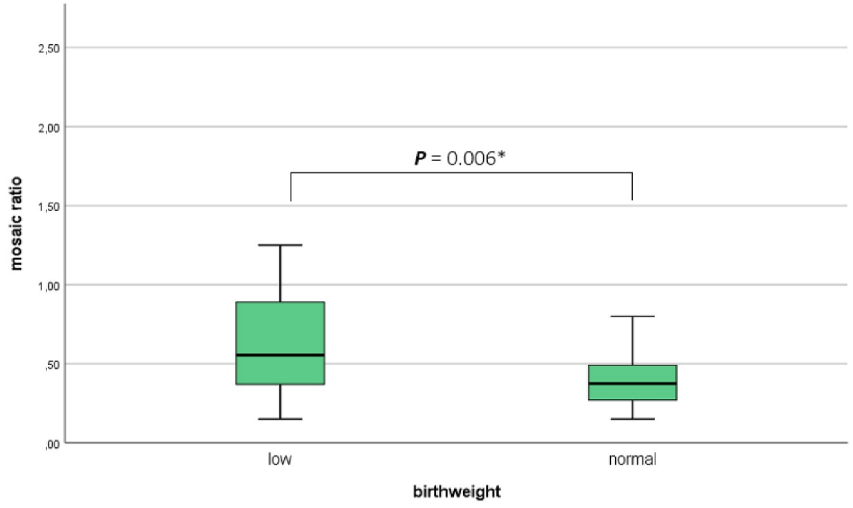

FIGURE 4 | Association between mosaic ratio and birthweight in 120 cases of CPM, with low birthweight (below the 10th percentile according to Hoftiezer) on the left ($N = 20$) and normal birthweight on the right ($N = 100$) *p*-value is given in the boxplot. *Statistically significant. [Colour figure can be viewed at wileyonlinelibrary.com]

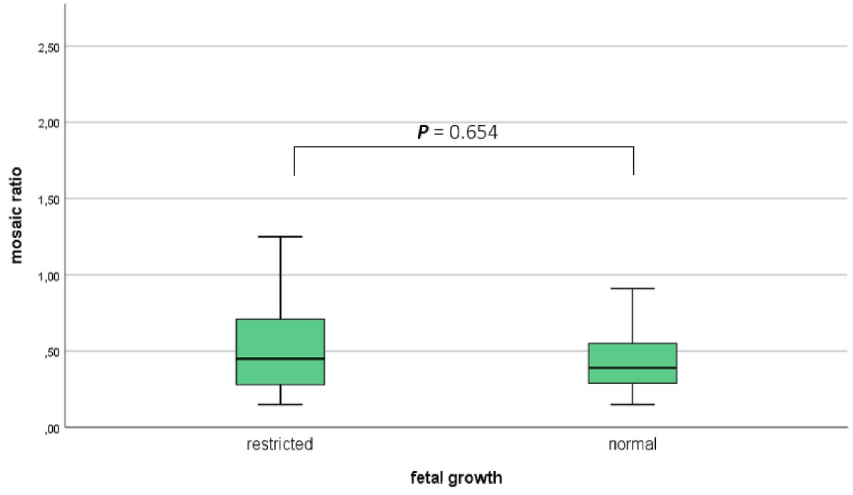

FIGURE 5 | Association between mosaic ratio and fetal growth in 122 cases of CPM, with restricted growth ($N = 37$) on the left and normal growth ($N = 85$) on the right. *p*-value is given in the boxplot. [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 1 | Mosaic ratio divided in six categories. The number of cases and percentages with normal and adverse perinatal outcome(s) is shown per category.

	Perinatal outcome	
Mosaic ratio category	**Normal**	**Adverse**
0–0.2	8/12 (66.7%)	4/12 (33.3%)
0.21–0.4	33/52 (63.5%)	19/52 (36.5%)
0.41–0.6	18/27 (66.7%)	9/27 (33.3%)
0.61–0.8	6/14 (42.9%)	8/14 (57.1%)
0.81 to ≥ 1.0	5/17 (29.4)	12/17 (70.6%)
Total	70/122 (57.4%)	52/122 (42.6%)

pregnancy management since such pregnancies are at increased risk for complications such as FGR, LBW and maternal hypertensive disorders, although about half will be uneventful. It would be clinically very helpful to be able to differentiate between those with and without a risk of adverse pregnancy outcomes so that proper clinical follow-up can be offered efficiently only to those at risk. This follow-up may include intensified monitoring by serial blood pressure measurements or ultrasound evolution of the fetal growth and Doppler velocity measurements.

Our data show that the mosaic ratio identified through NIPT was significantly higher in women who experienced adverse perinatal outcomes compared with those who did not. This confirms the findings of Xiang et al. [13]. However, our data also indicate that it is not possible to define a clear cut-off value for CPM cases with and without adverse outcomes. Only for trisomy 16 such a threshold could be set with cases having a mosaic ratio < 0.5 having a normal outcome and if the mosaic ratio was > 0.7 pregnancy complications occurred. However, this observation must be made with some reservations, as it is based on a limited subset of only nine cases. In the total cohort, only a trend was seen that higher mosaic ratios were associated with a greater chance of having adverse outcomes. For instance, if the mosaic ratio was < 0.6 the chance of adverse outcomes was about 30%, whereas this increased to 80% when the mosaic ratio was > 0.81. However, this also means that in the latter case, outcome was normal in still 20% of the cases. Moreover, if the mosaic ratio was really low (< 0.2), the risk of adverse outcome was still substantial (~30%). Therefore, our study demonstrates that the mosaic ratio cannot be used for differentiating between CPM with and without pregnancy complications, with the (reservedly) exception of trisomy 16. However, it can give an indication on the chances of adverse outcome, being in our cohort ~30% when the mosaic ratio is < 0.6 and which increases to 80% with higher mosaic ratios. Further studies on larger numbers will be necessary to further nuance and confirm these figures.

The rationale for using the mosaic ratio for the prediction of pregnancy outcome is based on previous observations that pregnancy outcomes in cases of CPM are dependent on the origin of the trisomy, with meiotic trisomies being significantly associated with adverse pregnancy outcomes [8, 29]. Due to its pre-zygotic origin, higher levels of trisomic cells are expected in the CTB of CV as compared to those of post zygotic origin, which may lead to a higher mosaic ratio. Also, the type of CPM affects the clinical outcomes with type 3 being more often associated with FGR and LBW than CPM type 1 [7, 8]. Based on a study of 88 placentas, it was recently shown that 72% of term placenta CTB biopsies were uniformly normal in type 1, whereas this was only 47.0% in type 3 [30]. This may contribute to lower levels of abnormal cells in the CTB in type 1 versus type 3 and also here lower mosaic ratios are expected. In the present study, we indeed show that the mosaic ratio in CPM type 1 is lower compared to CPM type 3. Moreover, CPM involving a meiotic trisomy has a significantly higher mosaic ratio than CPM involving a mitotic trisomy. However, despite these differences, there was an overlap of mosaic ratio distributions in meiotic versus mitotic trisomy cases as well as in CPM type 1 versus type 3, and therefore, no clear-cut distinctions could be made. This may explain the inability to differentiate between CPM with and without high risk of adverse pregnancy outcomes based solely on the mosaic ratio.

There are various explanations for this. Firstly, the mosaic ratio is calculated using the fetal fraction as measured by Veriseq v2. It was recently shown that current methods for fetal fraction determination, including the Veriseq v2 method that we use, do not allow for a reliable and consistent fetal fraction (FF) estimation and that estimated FF should be regarded as a laboratory-specific range rather than a precise number [31]. Therefore, if the FF measurement is not precise, the same concerns apply to the mosaic ratio that uses the FF in its calculation. Secondly, it is unknown whether the whole placenta evenly sheds cfDNA from the CTB into the maternal bloodstream through apoptosis. We previously showed that CPM is characterized by placental patches showing a uniformly normal and abnormal cytogenetic constitution [30]. If apoptosis primarily takes place at an abnormal patch, the mosaic ratio may increase, whereas the rest of the placenta may be cytogenetically normal. Depending on where the apoptosis takes place, the mosaic ratio may not reflect the cytogenetic constitution of the whole placental CTB. This may explain the large overlap of the mosaic ratio ranges in meiotic versus mitotic trisomy cases as well as type 1 versus type 3 CPM. Moreover, based on our experience, the NIPT result, including the FF measurement and *Z*-scores of the chromosome aberration, is a snapshot that may differ at different time points and most probably will depend on the apoptotic activity of the CTB at the specific moment in pregnancy under the given circumstances. Although the FF gradually increases during pregnancy, it may sometimes be observed that the FF is smaller and *Z*-score lower when measured in blood taken a few weeks after the original result. Therefore, the mosaic ratio may be a dynamic metric that probably not always represents the actual load of trisomic cells in the placenta CTB.

5 | Conclusion

In conclusion, we showed an association between the mosaic ratio and pregnancy outcome in cases of CPM with higher mosaic ratios correlating with increased risks for pregnancy complications such as FGR, LBW and maternal hypertensive

disorders. However, at an individual level, the mosaic ratio cannot distinguish clinically relevant from clinically irrelevant CPM. Based on our findings, we recommend closely monitoring all pregnancies where CPM is suspected, regardless of the mosaic ratio, since even low mosaic ratios < 0.2 are associated with a substantial risk (~30%) of pregnancy complications.

Acknowledgments

The authors would like to thank the Dutch NIPT Consortium for organizing the Trident studies. They also wish to acknowledge the dedicated work of their laboratory colleagues from the reception, cell culture, karyotyping/FISH, array, NGS and DNA/RNA isolation units as well as the prenatal genetic counselors from the Department of Clinical Genetics. We would also like to thank all obstetric care providers for providing follow-up.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data available on request from the corresponding author.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.