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Confined placental mosaicism: Distribution of chromosomally abnormal cells over the term placenta

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ABSTRACT

Objective: Non-invasive prenatal testing (NIPT) investigates placental DNA and may detect confined placental mosaicism (CPM). The aim of this study was to confirm CPM in the term placenta in cases with abnormal NIPT but normal follow-up cytogenetic studies of fetus and mother. Additionally we examined the distribution of abnormal cells over the placenta.

Methods: Four chorionic villus (CV) biopsies from four placental quadrants were requested in cases where CPM was assumed. Both cell lineages of the CV, cytotrophoblast (CTB) and mesenchymal core (MC), were analyzed separately with SNP array.

Results: The chromosome aberration was confirmed in 67 % of the placentas. Three quarters of the CTB and MC biopsies from these mosaic placentas were uniformly normal (57 %) or abnormal (20 %), and a minority showed mosaicism. Among 16 cases of CPM where first trimester CV were examined as well, 11 had chromosomally normal results during pregnancy.

Discussion: Cytogenetic investigations of term placental biopsies suspected to be affected with CPM did not reveal the chromosome aberration in one third of the placentas. This is caused by the patchy pattern in which chromosomally abnormal cells are distributed over the placenta with the majority of the biopsies being uniformly normal. Further CPM research, including its clinical impact, requires the analysis of more than four biopsies to get insight into the extent of the affected part. Moreover, a subset of CPM type 1 and 3 seems to be only detectable with NIPT and not with first trimester CVS.

1. Introduction

Throughout various stages of (early) pregnancy cytogenetic testing can be conducted to identify fetal chromosomal abnormalities. In (early) embryogenesis high rates of aneuploidy are found and these are a major cause of early pregnancy loss [1,2]. Chromosomal mosaicism, the existence of cell lines with different karyotypes, is common in IVF embryos and it has been shown that the proportion of human mosaic embryos declines during early embryogenesis [3]. During early development a complex sequence of events leads to the formation of different cell lineages: the trophoblast and the inner cell mass (ICM), the latter further differentiating into epiblast, giving rise to the embryo itself and hypoblast from which the extra embryonic mesoderm (EEM) originates as shown in Fig. 1 [4]. The distribution of abnormal cells over the different compartments of the early embryo in cases of chromosomal mosaicism will determine whether it affects the entire conceptus, generalized mosaicism, or just the fetus or placenta, called confined fetal (CFM) and confined placental mosaicism (CPM), respectively [5,6].

CPM is a type of chromosomal mosaicism that was discovered in the eighties at the time of introduction of chorionic villus sampling (CVS) for prenatal diagnosis [5]. In 1–2% of the cases undergoing invasive prenatal diagnosis via CVS, a chromosome aberration was shown to be present in chorionic villi (CV) while the fetus had a normal chromosome constitution. This phenomenon of CPM regained attention after the introduction of non-invasive prenatal testing (NIPT) that investigates placental cell-free DNA in maternal blood to identify fetuses at risk for a chromosome aberration. CPM has been shown to be the major origin of discordant results of NIPT [7]. CV consists of an outer cell layer, the syncytio- and cytotrophoblast (CTB), that is derived from the trophoblast of the early embryo and an inner part, the mesenchymal core (MC)

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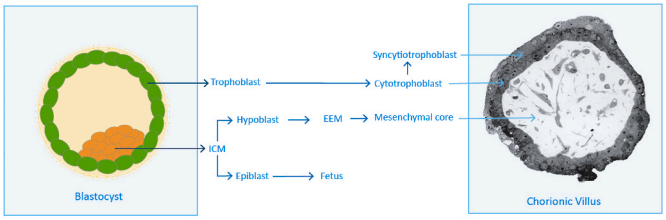

Fig. 1. Embryonic origin of the different cell lineages of chorionic villi: the cytotrophoblast (CTB) is derived from the trophoblast of the blastocyst, whereas the mesenchymal core (MC) originates from the extra-embryonic mesoderm (EEM). Both EEM and fetus are derived from the inner cell mass (ICM) of the blastocyst.

that is derived from the EEM originating from the ICM of the embryo (Fig. 1) [4,8]. We defined CTB as encompassing both syncytiotrophoblast and cytotrophoblast. Although the two layers differ functionally these are cytogenetically identical. Historically, cytogenetic classification is based on the composition of the cytotrophoblast, due to its spontaneously dividing cells, and of the mesenchymal core, whereas the syncytiotrophoblast lacks mitoses. To facilitate comparison with others studies we have opted to use the term CTB.

Depending on which cell line is affected three types of CPM can be found: type 1 when the chromosomal aberration is restricted to the CTB, type 2 when the aberration is only present in the MC and type 3 when both cell lines are affected [9,10]. Since NIPT investigates the CTB, more particularly the syncytiotrophoblast, only types 1 and 3 can be detected. In order to prove CPM after NIPT showed a chromosomal aberration, placental tissue needs to be analyzed either during pregnancy by first trimester CVS or postnatal through placental biopsies. CPM is a rare finding and detected in approximately 1–4 % of all CVS analyses for prenatal diagnosis in high genetic risk pregnancies [11,12], whereas it is less prevalent in the general obstetric population (0.17 %) [13]. CPM is associated with an increased risk of fetal growth restriction, low birthweight and premature birth as reviewed by Eggenhuizen et al. [14]. Therefore, its detection has clinical relevance.

The aim of this study was to investigate whether cytogenetic analysis of term placentas could explain the discordant abnormal NIPT results in which follow-up cytogenetic investigations of mother and fetus were normal. Moreover, since only a few, often older, studies investigated the phenomenon of CPM in term placentas [6,15,16], we examined the distribution of abnormal cells over the placenta by sampling CV of four different placental quadrants and by investigating both cell layers, CTB and MC, separately.

2. Materials and methods

2.1. Population

Pregnancies where CPM was suspected after NIPT showed a chromosome aberration (study period April 2014–december 2019) and where follow-up cytogenetic testing of fetus and mother were normal, were included in this cohort. In these cases four term placental biopsies of CV or the whole placenta were requested. In 2014, the NIPT was introduced in the Netherlands as a part of a national research program called TRIDENT. In the first phase (TRIDENT-1) the NIPT was a second-tier screening test for women with an elevated risk for trisomy 21, 13 and 18 [17]. Three years later, in 2017 the NIPT was offered as a first-tier test (TRIDENT-2) [18].

2.2. Cytogenetic analysis during pregnancy

Follow up cytogenetic testing of the fetus was performed in either amniotic fluid and/or CVS. If CVS was performed, both cell layers, CTB and MC, were analyzed with SNP array (Illumina HumanCytoSNP-12 array, Illumina Infinium-CytoSNP-850K genotyping array or Illumina Infinium GSA + MD-24 v1.0 BeadChip) as described before [19,20]. Amniotic fluid was cytogenetically investigated with the same SNP array on uncultured amniotic cells and with karyotyping or FISH of cultured AF cells (in situ method), in order to exclude mosaicism in both uncultured and cultured cells [21]. Blood of the mother was investigated for the involved chromosome aberration with SNP array as well. For parental origin and uniparental disomy (UPD) analysis blood from both parents was sampled and tested with SNP array.

2.3. Cytogenetic analysis after pregnancy

Placental biopsies of about 1 cm^3 from the four different quadrants were received or sampled if the whole placenta was sent. From each biopsy, 20–25 mg of CV were dissected and these were cleaned with PBS. CTB and MC of the four biopsies were separated with trypsin and the MC was digested with collagenase according to standard techniques [22]. DNA was isolated from each biopsy and cytogenetically investigated with SNP array (Illumina HumanCytoSNP-12 array, Illumina Infinium-CytoSNP-850K genotyping array or Illumina Infinium GSA + MD-24 v1.0 BeadChip) [23]. In case of (partial) trisomy mosaicism in one or more biopsies, the mitotic or meiotic origin of the (partial) trisomy was determined using the B-allele frequency (BAF) as described by Conlin et al. [24]. In cases with only 100 % trisomy 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 [23]. If the chromosome aberration was not confirmed in the term placenta, it was advised to repeat the NIPT after delivery to examine the maternal cell-free DNA (cfDNA) fraction for the presence of the involved chromosome aberration. If absent, it was concluded that CPM was the (most likely) origin of the chromosome aberration despite normal placental results. If invasive testing was declined during pregnancy and no fetal component was analyzed (either MC in first trimester CV or AF), postnatally cord blood was requested to exclude fetal mosaicism.

3. Results

Between April 2014 and December 2019, NIPT was performed in our laboratory in 57,521 pregnancies (2629 TRIDENT- 1 and 54,892 TRIDENT- 2). In 150 pregnancies (0.26 %) CPM was suspected based on NIPT showing a chromosome aberration and follow-up diagnostic testing of fetus and mother during and/or after pregnancy being normal. In these cases placental biopsies were requested and received in 93 cases (62.0 %). In five cases samples were not analyzed: in two, because the chromosome aberration was already confirmed in first trimester CV, and in the other three because the tissue was not suitable for analysis (e.g. too small biopsies, tissue in formaldehyde). The number of biopsies that were analyzed in all 88 cases is shown in Supplemental Table S1. The SNP array analysis results of CTB and MC of first trimester (if available) and placental CV biopsies are shown in detail in Supplemental Table S2. The majority of the investigated term placentas were TRIDENT-2 cases (74/88, 84.1 %).

3.1. CPM type

In 59/88 (67.0 %) placentas the chromosome aberration could be confirmed in at least one of the biopsies. Comparing TRIDENT-1 (high genetic risk population, NIPT 2nd tier test) with TRIDENT-2 (general obstetric population, NIPT first tier test) we found a significant difference in confirmation rate (*p0.004)*: in all 14 TRIDENT-1 cases CPM was confirmed (100 %), compared to 45/74 (60.8 %) in TRIDENT-2. In the Trident 1 group, trisomies primarily had a meiotic origin (n = 7/12) whereas in the Trident 2 group trisomies were mainly of mitotic origin (n = 31/42) (see Supplemental Table S2).

If only one CV biopsy would have been analyzed in all cases, confirmation of CPM would have been possible in 38 cases (43.2 %). In

29 of 59 cases (49.2 %) a CPM type 1 was found (aberration restricted to CTB) and in 30 cases (50.8 %) CPM type 3 (aberration in CTB and MC) was involved. Based on the distribution of abnormal cells across CTB and MC, eight cases in the type 3 group should be considered to be CPM type 2 (abnormal cells only in MC). However, since these were detected with NIPT that essentially investigates the CTB, they were classified as CPM type 3 (highlighted with blue color in Supplemental Table S2). In three cases, the placenta was normal, but CPM was confirmed in first trimester CV (cases 35, 40 and 44 in Supplemental Table S2).

3.2. Comparison of first trimester CVS results and term placenta

Sixteen cases underwent analysis of both first trimester CV and term placenta and a comparison of both analyses could be made, as shown in Supplemental Table S2. In 7/16 cases, the findings were concordant, with two cases showing presence and five cases showing absence of the chromosomal aberration in both first trimester CV and term placenta. The remaining nine cases showed discordant results, with six having normal first trimester CV but abnormal term placenta and three cases having the opposite pattern. Among the 16 cases of CPM where first trimester CV were examined during pregnancy, eleven cases had chromosomally normal CV while six of these had an abnormal placenta.

3.3. Distribution of abnormal cells over the placenta

A total of 192 CTB and 157 MC biopsies were analyzed from 59 confirmed placentas (e.g. placentas in which the chromosome aberration was confirmed). The chromosome aberration was present in 50.5 % (97/192) of CTB biopsies and in 33.8 % (53/157) of MC biopsies, with the majority of the biopsies being normal. In about half of the affected CTB and MC biopsies (71/150) the chromosome aberration was present in 100 % of the cells with mosaicism in the rest as shown in Fig. 2. This means that 57 % of the samples of affected placentas were chromosomally completely normal, 20 % uniform abnormal and 23 % showed chromosomal mosaicism. In Supplemental Fig. S1 the cytogenetic results for all 88 placentas are shown with 73.4 % of biopsies being chromosomally normal.

When comparing CPM type 1 and 3, the percentage of uniformly normal samples is higher in type 1 compared to type 3 (71.7 % versus 47.0 %). For both CPM types, the proportion of mosaic biopsies was the same as the proportion of uniformly abnormal biopsies: 13.9 % mosaic and 14.4 % uniformly abnormal samples in type 1 (Fig. 3) compared to

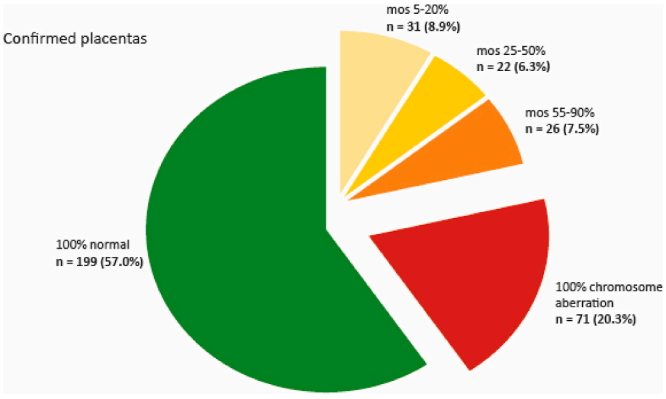

Fig. 2. Cytogenetic results in the postnatal placental biopsies of CTB (N = 192) and MC (N = 157) derived from 59 placentas in which the chromosome aberration could be confirmed (confirmed CPM). In 270/349 (77.4 %) biopsies, the result was 100 % normal (green) (57 %) or abnormal (red) (20,3 %), and in 79/349 (22.6 %) biopsies mosaicism was found, ranging from 5 to 90 % (cream 5–20 %, yellow 25–50 % and orange 55–90 %). The number of samples are shown in the chart. CTB: cytotrophoblast, MC: mesenchymal core, mos: mosaicism.

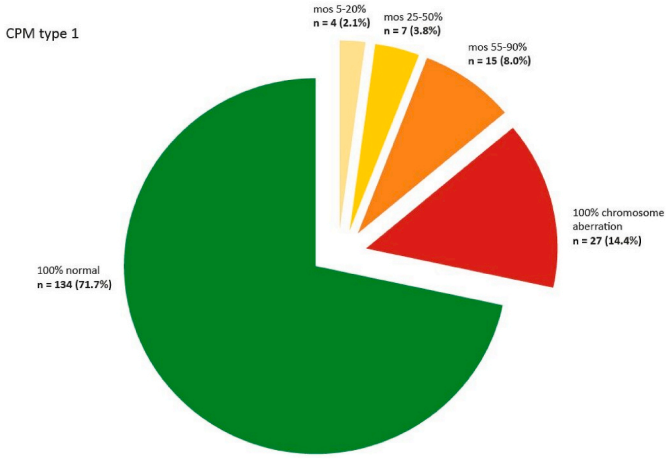

Fig. 3. Cytogenetic results in postnatal placental biopsies of the CTB (n = 108) and MC (79) derived from 30 placentas with CPM type 1 (29 with CPM proven in term placenta and one case (case 44) with normal placenta but abnormal first trimester CVS). In 161/188 (86.1 %) the result was 100 % normal (green) (71,7 %) or abnormal (red) (14,4 %). In 26/187 (13.9 %) mosaicism was found. CTB: cytotrophoblast, MC: mesenchymal core, mos: mosaicism.

29 % mosaic and 24 % uniformly abnormal biopsies in type 3 (Fig. 4).

We also compared the distributions of abnormal cells in CPM involving a trisomy of meiotic origin versus those with a mitotic origin. In 55/75 (73 %) trisomic cases the mitotic or meiotic origin could be determined with SNP array: 18/55 (32.7 %) trisomies were of meiotic origin, while 37/55 (67.3 %) had a mitotic origin. The percentage of uniformly normal biopsies was much higher in the mitotic as compared to the meiotic group (67.3 % vs 38.9 %). Also the percentage with a uniform abnormal result was much higher in the meiotic vs mitotic group (44.2 % vs 9.8 %). In the mitotic group, there were more mosaic abnormal (22.9 %) than uniformly abnormal (9.8 %) samples. (Figs. 5 and 6).

3.4. CPM cases with the chromosomal aberrations unconfirmed in placenta

There were 29 cases in which placental studies showed completely

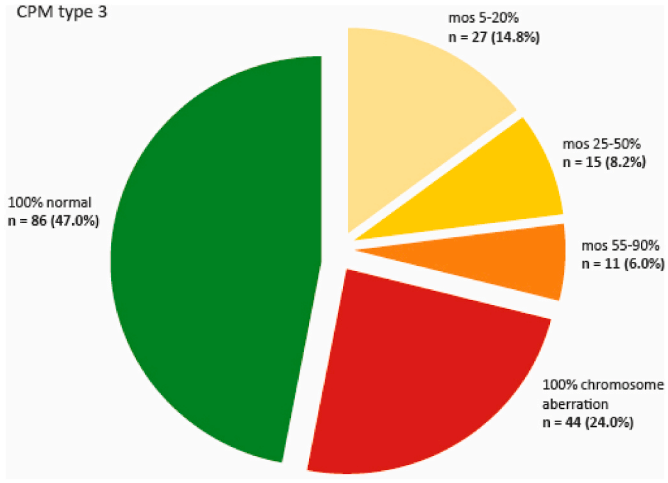

Fig. 4. Cytogenetic results in postnatal placental biopsies of the CTB (n = 96) and MC (n = 87) derived from 32 placentas with CPM type 3 (30 with CPM proven in the term placenta and 2 (cases 35 and 40) with CPM in first trimester CVS but normal placenta). In 130/183 (71.0 %) the result was 100 % normal (green) (47 %) or abnormal (red) (24 %). In 53/183 (29.0 %) mosaicism was found. CTB: cytotrophoblast, MC: mesenchymal core, mos: mosaicism.

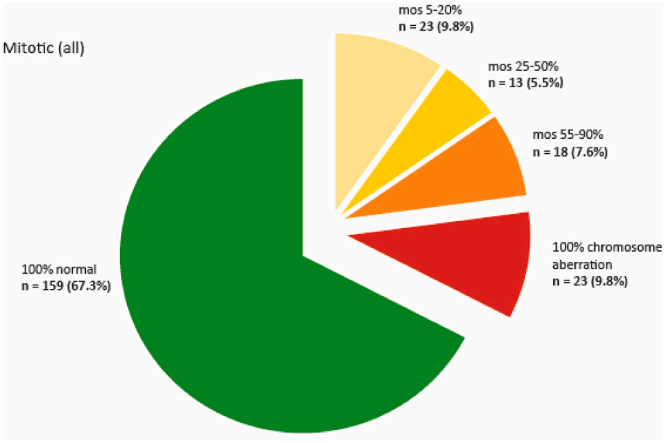

Fig. 5. Cytogenetic results in the postnatal placental biopsies of CTB (n = 130) and MC (n = 106) derived from 37 placentas with a mitotic origin. In 182/236 (77.1 %) the result was 100 % normal (green) (67,3 %) or abnormal (red) (9,8 %). In 54/236 (22.9 %) mosaicism was found. CTB: cytotrophoblast, MC: mesenchymal core, mos: mosaicism.

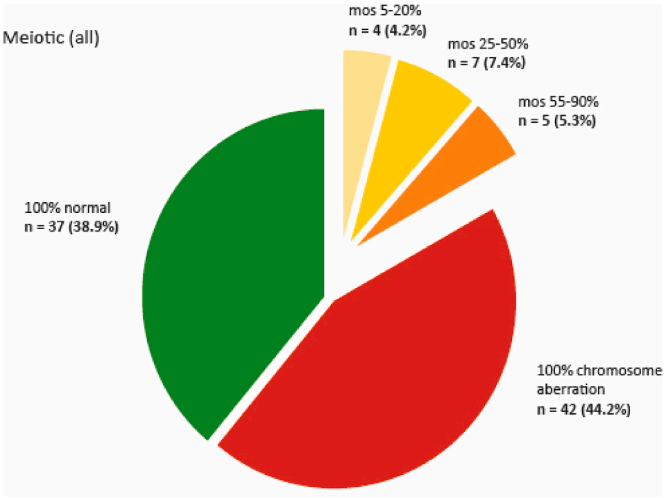

Fig. 6. Cytogenetic results in the postnatal placental biopsies of CTB (n = 52) and MC (n = 43) derived from 18 placentas with a meiotic origin. In 79/95 (83.2 %) the result was 100 % normal (green) (38,9 %) or abnormal (red) (44,2 %). In 16/95 (16.8 %) mosaicism was found. CTB: cytotrophoblast, MC: mesenchymal core, mos: mosaicism.

normal results of which three had the chromosome aberration confirmed in first trimester CV leaving 26 cases in the undefined group (Supplemental Table S2). Eighteen involved a rare or common autosomal trisomy and eight a structural chromosomal aberration (Supplemental Table S2). In all these negative cases a fetal origin was excluded with investigation of CV, AF and/or cord blood. A maternal origin was also excluded in 21 of 26 cases: in both genomic DNA from blood and maternal cfDNA (N = 10), in genomic DNA only (N = 8) and in cfDNA only (N = 3). In 10 cases the cfDNA was investigated shortly after delivery and in three during a previous or later pregnancy. In three cases a leiomyoma was seen, in one a vanishing twin (VT) was assumed (case 55 in Supplemental Table S2) and in one (case 47) both were encountered (Supplemental Table S2).

4. Discussion

In this paper we describe the results of confirmatory studies in 88 term placentas of patients where NIPT showed a chromosome aberration that was suspected to be present in the placenta, based on the type of chromosome aberration and on follow-up cytogenetic investigations of fetus and mother. Placental cytogenetic studies are rarely done and little is known about the distribution of cells with the chromosome aberration over the placenta in cases of CPM. This study is rather unique due to the analysis of multiple placental CV biopsies, and for the separate analysis of both cell lineages, CTB and MC, that have a different embryonic origin (trophectoderm and inner cell mass, respectively). The main findings of this study are that it turned out to be rather difficult to prove CPM in the term placenta despite very strong suspicion, with normal cytogenetic results in one third of the cases notwithstanding the analysis of multiple biopsies. And that this has to do with the "patchy" distribution of chromosomally abnormal cells over the placenta, which means that in the "confirmed" cases (e.g. placentas in which the chromosome aberration could be identified in at least one of the biopsies) the majority of the samples showed a uniform normal (57 %) cytogenetic constitution, with the rest of the biopsies being uniformly abnormal (20 %) or mosaic (23 %). Considering all placentas, almost three quarters of the biopsies were normal. The finding that more than half of the placental biopsies is normal in "confirmed" CPM cases is in line with a recent study where 54 % of the placental biopsies were normal, without making a distinction between CTB and MC [25]. Therefore, for confirmatory studies of CPM, it is strongly recommended to retrieve more than 4 biopsies from distinct placental sites. Additionally, it is important to analyze CTB and MC separately, so that low level mosaicism in the CTB will not go undetected when "diluted" with a normal MC in case of CPM type 1. We show that even the sampling of four placental sites can still miss the affected placental part in about one third of the cases and if one biopsy would have been analyzed in all cases, the diagnosis of CPM would have been missed in more than half of the cases.

The finding that 77 % of all biopsies of "confirmed" cases were uniformly normal or abnormal also is in agreement with recent similar observations in first trimester miscarriages in which three biopsies were taken from the products of conception (POC) and in which 68 % of all mosaic cases showed heterogeneously distributed mosaicism (e.g. patches with a uniform normal or abnormal chromosome constitution next to mosaic patches). The authors conclude that this may explain the underestimation of chromosomal mosaicism in POC samples when only one biopsy is investigated [26].

In the present study the four placental biopsies were taken randomly from 4 different quadrants, without taking into account the cotyledon structure of the human placenta. Our observations seem to fit recent findings that every bulk placental sample taken randomly is in fact derived from a single parental branch that is genetically distinct [27]. We speculate that the chromosomal constitution of each cotyledon is uniformly normal or abnormal and that mosaicism that we observed only occurred if the sampling was performed at the borders of different cotyledons. However, this should be further investigated since no studies have been performed on the cytogenetic constitution of different cotyledons of a human placenta when the pregnancy is complicated with CPM; in other placental studies, biopsies were also taken randomly [6, 15]. These results indicate that the analysis of multiple biopsies of a term placenta, perhaps one per cotyledon, is necessary to improve our understanding of the embryonic origin and further evolution of CPM during pregnancy and also its effect on fetal development. We may conclude that the examination of just four placental biopsies does not give any insight into the chromosomal constitution of the rest of the placenta that is not investigated. Research on an association between the level of mosaicism in the term placenta and the impact on fetal growth will require another approach in which more than four biopsies from the different cotyledons are analyzed.

In 16 cases both first trimester CV and term placenta were investigated and the diagnosis of CPM could be made in both tissues, in only 12.5 % of the cases. Moreover, CPM was not detected in 68.8 % of first trimester CV, suggesting that CVS is a less sensitive technique than NIPT if the prenatal diagnosis of CPM has to be established [28]. This may be

considered for instance if ultrasound investigations show fetal growth problems and invasive cytogenetic diagnosis is normal. It may also suggest that the prenatal diagnosis of CPM, made in 1–2% of pregnancies at increased genetic risk, based on CVS studies, is underestimated. However, it is also possible that NIPT and CVS may detect different subsets of CPM since the manner in which samples are collected is different: NIPT probably gathers CTB from throughout the placenta whereas CVS collects samples locally. This may explain why recently Lund et al. came to an opposite conclusion that NIPT showed a low detection rate for mosaicism since not all cases of CPM that were detected with CVS were found with NIPT [29]. Both studies may have contrary conclusions since they look from a different angle: the study of Lund starts with an abnormal CVS and then investigates whether NIPT is able to detect the chromosome aberration, whereas the present study starts with an abnormal NIPT that we try to confirm in first trimester and term placental CV.

In one third of placentas the chromosome aberration that was detected with NIPT could not be confirmed in the term placenta, nor in first trimester CV that were also analyzed in five cases. CPM is still the most likely explanation for the abnormal NIPT in these cases since the fetus showed a normal karyotype and maternal cytogenetic investigations of genomic DNA and cfDNA were normal as well in most cases. Moreover, in the majority a trisomy was involved which is typically found in CPM [7]. However, in some cases, it cannot be excluded that the chromosome aberration detected with NIPT had a different origin. For instance an early VT could shed abnormal cfDNA in the maternal circulation during the first trimester. Although it was an exclusion criterium during the study period, in one and perhaps two cases it was discovered after follow up. Another possible explanation could be a leiomyoma, seen in four cases. Leiomyomas may be chromosomally abnormal and may contribute to the cfDNA fraction in maternal plasma [13]. However, it is worth noting that one patient exhibited normal cfDNA during a subsequent pregnancy and one had normal cfDNA results the day after delivery, which makes a myoma origin less likely in two of four cases. At last, a technical false positive cannot be excluded in an exceptional case with a low z-score in the NIPT.

The separate analysis of both cell lineages, CTB and MC, allowed investigation of the CPM type and the use of SNP array for cytogenetic analysis allowed the determination of the mitotic or meiotic origin of the trisomy that was found in most CPM cases. Especially CPM type 1 and CPM involving a mitotic trisomy are difficult to confirm since ~70 % of all biopsies were uniformly normal. But also CPM type 3, with both CTB and MC being affected, exhibited a normal cytogenetic result in almost half of the biopsies. And if the chromosome aberration was present, in half of the samples it was found in mosaic form, which may go undetected if CTB and MC are not investigated separately. A CPM of meiotic origin has the highest chance of being diagnosed since almost 60 % of the biopsies were uniformly or mosaic abnormal, probably due to the pre-zygotic origin of the trisomy. This explains the higher confirmation rate in Trident 1 cases as compared to Trident 2 cases, the former primarily involving trisomies of meiotic origin and the latter of mitotic origin. It probably also explains the higher risk of pregnancy complications that is seen in CPM involving a meiotic trisomy [30,31]. Determination of the pre- or post-zygotic origin of the chromosome aberration involved in CPM may therefore be helpful to identify pregnancies at higher risk for pregnancy complications. Since NIPT seems to be the most sensitive method for diagnosing CPM, the development of techniques that can differentiate between a meiotic or mitotic origin of the CPM with NIPT, would be of great clinical relevance [32].

4.1. Conclusions

In conclusion, to our knowledge, this is the first study describing in detail the distribution of chromosomally abnormal cells in term placentas affected by CPM that was initially detected with NIPT. This study illustrates the challenge of diagnosing CPM in term placentas even when four biopsies are taken and CTB and MC are investigated separately. We show that this is caused by the patchy distribution of the chromosome aberration over the term placenta with most placental patches being uniformly normal and half of the affected biopsies being mosaic. As a result, for further placental studies on CPM we suggest taking multiple (more than 4) biopsies from different cotyledons and analyzing the CTB and MC separately. This study also illustrates the challenge of diagnosing CPM in first trimester CV with almost 70 % of first trimester CVS being chromosomally normal in cases of CPM, illustrating its potential underdiagnosis prenatally. However, it is also possible that with NIPT a subset of CPM may be detected that cannot be revealed with CVS and vice versa due to the different manners in which the CTB is sampled. Nevertheless, if CPM is suspected during pregnancy and invasive testing with amniocentesis or CVS is normal, NIPT may be considered for diagnosing CPM.

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CRediT authorship contribution statement

G.M. Eggenhuizen: Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Data curation. **S. van Veen:** Writing – review & editing, Formal analysis. **N. van Koetsveld:** Writing – review & editing, Formal analysis. **A.T.J.I. Go:** Writing – review & editing, Investigation, Formal analysis. **K.E.M. Diderich:** Writing – review & editing, Formal analysis. **M. Joosten:** Writing – review & editing, Formal analysis. **M. van den Born:** Writing – review & editing, Formal analysis. **M.I. Srebniak:** Writing – review & editing, Formal analysis. **D. Van Opstal:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.placenta.2024.06.008.

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