Predicting fetoplacental chromosomal mosaicism during non-invasive prenatal testing.

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Funding: This work was made possible by grants from the University of Leuven (KU Leuven):

GOA (GOA/12/015 to J.R.V., K.D., and H.V.E.) and SymBio- Sys (PFV/10/016 to J.R.V.).

Disclosures: Prof Vermeesch's laboratory receives license fees from Agilent for the analysis pipeline used in this study. There are no other conflicts of interest.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/pd.5223

Abstract

Objective: Non-invasive prenatal detection of aneuploidies can be achieved with high accuracy through sequencing of cfDNA in the maternal blood plasma. However, false positive and negative NIPT results remain. Fetoplacental mosaicism is the main cause for false positive and false negative NIPT. We set out to develop a method to detect placental chromosomal mosaicism via genome-wide circulating cell-free maternal plasma DNA (cfDNA) screening.

Method: Aneuploidy detection was combined with fetal fraction determination to enable the detection of placental mosaicism. This pipeline was applied to whole genome sequencing data derived from 19,735 plasma samples. Following an abnormal NIPT, test results were validated by conventional invasive prenatal or postnatal genetic testing.

Results: Respectively 3.2% (5/154), 12.8% (5/39) and 13.3% (2/15) of trisomies 21, 18 and 13 were predicted and confirmed to be mosaic. The incidence of other, rare autosomal trisomies (RATs) was ~0.3% (58/19,735), 45 of which were predicted to be mosaic. Twin pregnancies with discordant fetal genotypes were predicted and confirmed.

Conclusion: This approach permits the non-invasive detection of fetal autosomal aneuploidies and identifies pregnancies with a high risk of fetoplacental mosaicism.

Knowledge about the presence of chromosomal mosaicism in the placenta influences risk estimation, genetic counseling and improves prenatal management.

What's already known about this topic?

- Non-invasive prenatal detection of fetal aneuploidies can be achieved with high accuracy through sequencing of circulating cell-free DNA (cfDNA) in maternal plasma.
- Current sequencing based bioinformatics pipelines report common trisomies (21, 18, 13) ignoring the status of other autosomal chromosomes and do not predict the presence of mosaicism

What does this study add?

- The presented aneuploidy detection pipeline, combined with fetal fraction determination, permits the non-invasive detection of aneuploidies in all chromosomes and pinpoints cases at high risk for fetoplacental mosaicism.
- Reporting about the presence of mosaicism in the placenta can influence risk estimation of a possible miscarriage, aid in the prenatal management and improve genetic counseling in future pregnancies.

INTRODUCTION

The discovery of cell-free DNA (cfDNA) of placental origin in the maternal circulation in 1997¹ and the demonstration that fetal aneuploidies can readily be identified by cell-free maternal plasma DNA analysis^{2; 3}, triggered swift clinical implementation of non-invasive prenatal testing (NIPT). NIPT allows the early detection of trisomy 13, 18 and 21 via blood sampling, avoiding the significant risk of invasive procedure-induced miscarriage⁴. Genome-wide random and targeted massively parallel sequencing^{3; 5-8} are the two main approaches applied for the detection of common fetal aneuploidies. Both approaches show high sensitivity and specificity for detection of fetal trisomy 21, 18 and 13⁹⁻¹¹. In fact, a recent bivariate meta-analysis on the performance of cfDNA screening for trisomy 21 demonstrated an overall sensitivity and specificity of 99.4% and 99.9% respectively¹². Because of this high accuracy, professional medical organizations propose that NIPT can replace conventional screening methods for common aneuploidies across the maternal age spectrum when accompanied by pre-test counselling^{13; 14}. However, a small number of false positive and false negative NIPT results remain.

It has been demonstrated that the presence of (i) a (vanishing) twin, (ii) maternal copy number variants (CNVs) as small as 500kb, or (iii) the occurrence of undiagnosed cancer in pregnant women influences the genomic representation profiles (GR-profiles) and can skew the overall Z-scores which in turn may lead to erroneous fetal aneuploidy estimates¹⁵⁻¹⁹. Independent lines of evidence derived from confirmatory amniocentesis or follow-up studies analyzing the cytotrophoblast suggest however that the main cause for biological false positive NIPT results is the occurrence of fetoplacental mosaicism^{20; 21}. Confined placental mosaicism (CPM), in which the abnormal cell line occurs almost solely in the placenta and not in amniotic cells or other fetal tissue, has been reported to occur in 1-2% of pregnancies during the first trimester^{22; 23}. The presence of aneuploid placental cell lineages can affect the placental function. In particular, 16% to 21% of pregnancies with CPM show prenatal complications^{22; 24; 25}. Recently, Pertile et al. show the association of rare autosomal trisomies (RATs) detected by NIPT with miscarriages, true fetal mosaicism, intrauterine growth retardation (IUGR), intrauterine fetal death and confirmed or suspected uniparental disomy²⁶.

In the current study, we implement a novel analytical approach which detects the presence and estimates the degree of (C)PM. The novel analysis pipeline was applied retrospectively to

almost 20,000 sequenced cfDNA samples. We demonstrate that the presence of fetoplacental mosaicism is accurately predicted in 90% of the cases. In addition, the method can identify discordant chromosomal constitution in twin pregnancies. This additional information has important clinical implications which can guide genetic counselling, invasive testing, pregnancy monitoring and ultrasound examinations resulting in a better overall pregnancy management.

MATERIALS AND METHODS

Sample processing and sequencing

A consecutive non-selected series of 19,735 pregnant women were tested for common fetal aneuploidies by cfDNA profiling. The pregnant women chose NIPT analysis because of maternal comfort (61.7 %), advanced maternal age (over 36 years of age) (24.2%), increased risk for fetal aneuploidy as indicated by the combined test (12.2%), familial history of congenital or hereditary diseases (1.8%) or NIPT following pre-implantation genetic diagnosis (PGD) and in vitro fertilization (IVF) (0.1%). Peripheral blood samples were collected in Cell-Free DNA BCT tubes (Streck, Omaha, NE, USA) or Cell-Free DNA collection tubes (Roche Diagnostics, Germany) from 10 weeks of gestation onwards (mean gestational age: 13.2 ± 2.32 SD). Standard centrifugation methods were used for plasma isolation. CfDNA extraction was either performed manually with the QIAamp Circulating Nucleic Acid Kit (Qiagen Benelux B.V., Venlo, Netherlands) or automatically, using the Maxwell HT cfDNA kit (Promega) on the Hamilton Liquid Handler according to the manufacturer's recommendations. Library preparation, whole genome sequencing as well as genome-wide genomic representation profiling was performed as previously described^{16; 17}.

Mosaicism detection

A retrospective analysis of the NIPT sequencing profiles of the study cohort was performed as follows. Read counts were collected per 50kb bin. These counts were normalised, GC-corrected, and aggregated per chromosome, as described in Bayindir et al. (2015) to produce a Chromosomal Read Count (ChromRC). The decision rules taken from the same paper were applied to generate (i) a genotype call (trisomy, monosomy, borderline-but-no-trisomy, borderline-but-no-monosomy, or normal) for all autosomes, (ii) a prediction of fetal sex (female, male, or undetermined), and (iii) an overall sample quality assessment fail or pass. Maternal constitutional CNVs were identified by CNV analysis as previously described in Brison *et al* (2016) to exclude false positive results of sub-chromosomal fetal aneuploidy or

false mosaicism. Fetal fraction was estimated using the SeqFF method as described in Kim et al. (2015)²⁷. From these data, samples with fetal sex undetermined or sample quality fail were removed and the following values were calculated per chromosome:

1. Mean.ChromRC.normal: the chromosome's mean ChromRC as observed in the subset of samples with genotype call *normal*
2. SD.ChromRC.normal: the chromosome's standard deviation of ChromRC as observed in the subset of samples with genotype call *normal*

Given Mean.ChromRC.normal and a particular fetal fraction (FF), the expected read count Mean.ChromRC.trisomy.FF for a full trisomy (i.e. 100% of the placenta) can be calculated as

$$\text{Mean.ChromRC.trisomy.FF} = \text{Mean.ChromRC.normal} * (1 + 0.5 * \text{FF})$$

to account for the fact that the fetus contributes 50% extra alleles (3 instead of 2) of the chromosome of interest to the circulating cfDNA. In Fig. 1 and Suppl. Fig. 1 and Fig.2 the full diagonal line corresponds to the expected read count Mean.ChromRC.trisomy.FF.

Deviations from this linear prediction can be attributed partly to variations in the measurement of both ChromRC and FF, partly to the possibility that the fetus does not contribute exactly 50% extra alleles, for instance due to CPM. Ideally the measurement variation should be estimated from cases with known degrees of mosaicism ranging between 0% and 100%. In practice however, such precise data are only available for normal cases, where the degree of mosaicism is assumed to be exactly 0%. In Fig. 1, the standard deviation SD.ChromRC.normal calculated from these normal cases is used to mark a $\pm 3 * \text{SD.ChromRC.normal}$ zone not only around Mean.ChromRC.normal (the X-axis and horizontal dashed line), but also around Mean.ChromRC.trisomy.FF (the diagonal dashed lines). The underlying assumption is that the variation in ChromRC is independent of the degree of mosaicism and can be generalized from normals to (non) mosaic trisomies. The X-axis and dashed lines then mark zones where normal (horizontal) and non-mosaic trisomies (diagonal) are likely to appear. Moreover, next to the more common Z-score, which quantifies to what extent an observed ChromRC deviates from normality, a TriZ-score can be defined, which quantifies to what extent a ChromRC value deviates from the value expected in a non-mosaic trisomy at a particular FF:

$$\text{TriZ} = (\text{ChromRC} - \text{Mean.ChromRC.trisomy.FF}) / \text{SD.ChromRC.normal}$$

Cases with a $Z > 3$ and $\text{TriZ} < -3$ are labelled as TriZ-low (Table 2A,B). These represent cases where the observed ChromRC is higher than expected in normals, but lower than expected in non-mosaic trisomies. Placental mosaicism is one potential source of TriZ-low scores. For instance, with a 50% mosaic trisomy, the ChromRC at a particular fetal fraction can be expected to appear halfway between the lines marking Mean.ChromRC.normal and Mean.ChromRC.trisomy.FF. More generally, given the fetal fraction and the expected ChromRC for normals, the expected percentage placental mosaicism can be calculated as:

$$\% \text{ placental mosaicism} = \frac{\text{ChromRC} - \text{Mean. ChromRC. normal}}{\text{Mean. ChromRC. trisomy. FF} - \text{Mean. ChromRC. normal}}$$

Notice this percentage ranges between 100%, for $\text{ChromRC} = \text{Mean.ChromRC.trisomy.FF}$ and 0% for $\text{ChromRC} = \text{Mean.ChromRC.normal}$. Again, in absence of a “golden standard” of known degrees of mosaicism, it is currently not possible to either validate the percentage placental mosaicism calculation or provide a confidence interval for it.

Confirmatory invasive testing

When cfDNA profiling indicated the presence of a chromosomal abnormality, the women were offered follow-up by standard invasive prenatal diagnosis based on DNA extracted from chorionic villus sampling (CVS) or amniotic fluid (AF). Subsequently, when a discrepancy between the NIPT and the invasive genetic test result was detected, women were proposed to donate the placenta upon delivery. To this end, upon their informed consent, the women were provided with a sampling vial which they presented to the midwife upon delivery.

Chromosome microarray analysis was performed on DNA extracted from CVS or AF as described in Brady et al. (2015)²⁸, using the 8x 60 K CytoSure ISCA v2 or v3 microarray (Oxford Gene Technology Oxford, UK). Fluorescence in situ hybridization (FISH) was performed following standard procedures. For chromosomes 7, 11, 14, 15 and 16, the presence of a uniparental disomy (UPD) was tested by polymorphic short tandem repeat (STR) analysis using a PCR master mix (Promega (<https://www.promega.com/products/pcr/endpoint-pcr/pcr-master-mix/?catNum=M7501>)) following a standard protocol ²⁹.

Ethical approval

This study was approved by the institutional ethics review board (S60268).

RESULTS

Detection of chromosomal mosaicism

Full, non-mosaic chromosomal trisomies are predicted when $Z > 3$ and $\text{TriZ} \geq -3$ are labelled as TriZ-high/normal (Table 1A,B). Cases with a $Z > 3$ and $\text{TriZ} < -3$ are labelled as TriZ-low and are predicted to be mosaic chromosomal trisomies. TriZ-low was observed and reported for a total of 45 samples (Table 2A and red circles in Fig. 1 and Suppl. Fig 1). Invasive follow-up information was available for 34 samples. For almost two thirds of these samples ($n=20$), invasive follow-up indeed showed the presence of mosaicism, twelve with common aneuploidies (trisomy 13, 18 or 21) and eight with rare autosomal trisomies (RATs) (Table 2A,B). All but two of the cases with predicted common aneuploidies were shown to be present in the fetus (i.e. detected in amniotic fluid; Table 2B). All but one of the chromosome 2, 7, 8, 16 and 20 RATs were confined to the placenta, with a different degree of placental mosaicism depending of the region sampled (Table 2B). For instance, analysis of five different placental biopsies in a case with a predicted trisomy 2 and normal fetal genotype confirmed the presence of trisomy 2 in 3%, 26%, 20%, 14% and 25% of the cells in the respective placental biopsies. The exception was one case of trisomy 16 where FISH analysis revealed low grade fetal mosaicism (7% of amniocytes) in combination with high grade placental mosaicism upon arrayCGH analysis (>75% of placental cells).

In 12 cases, only AF was sampled and no mosaicism was detected. These results may indicate false positives. More likely, they may represent CPM, since placenta was not investigated. In 2 cases, both AF and CVS samples did not show evidence of the predicted mosaicism. Whereas these might indeed indicate false positives, we cannot exclude the possibility that the tested regions of the placenta do not represent the whole placenta. For the remaining cases, no placental material could be obtained to further test for the presence of confined placental mosaicism (Table 2A). In conclusion, in 20/22 or 90% of cases where a placental mosaicism was detected, the mosaicism was confirmed upon sampling the placenta. UPD testing was performed in 12 cases (7, 2, 1 and 2 cases of trisomy 7, 11, 15 and 16 respectively). Only in one pregnancy, the fetus showed a mosaic trisomy with UPD for chromosome 15. This case was previously reported in Bayindir et al., 2015¹⁶.

In twin pregnancies, one twin can have a trisomy whereas the other twin can be normal. Hence, the algorithm presented should allow to predict the presence of a chromosomal

aneuploidy in one of the placentas of either twin. Indeed, this was observed in two pregnancies, one trisomy 13 and another with a trisomy 21 (arrows in Fig. 1).

Predicted non-mosaic trisomies can be mosaic

The power of the algorithm to detect placental mosaicism increases with increasing fetal fraction. In effect, if the fetal fraction is below the intersect of the standard deviation of normal read counts versus the predicted trisomic read count, a potential mosaicism would remain undetected in any possible case. This intersect varies amongst chromosomes because the standard deviation differs as a consequence of differences in variation amongst chromosomes. For trisomy 21, this intersect is at about 5% fetal fraction. A fetal fraction of less than 5% was observed in 7.3% of all samples. Except for trisomy 16 and 22, the intersect is lower. With increasing fetal fraction, the potential to detect and determine the degree of placental mosaicism increases.

In addition, due to the presence of differences in the degree of mosaicism in placenta and fetus, a fetal mosaicism may not be identified if the placenta is not mosaic. For the common trisomies (13, 18 and 21), the prediction of non-mosaic trisomies was confirmed in all fetuses and/or placentas (Table 1A) except for 3 cases that proved to be fetal mosaic aneuploidies. For the RATs, 4 out of 20 predicted predicted non-mosaic trisomies were proven mosaic upon invasive testing (Table 1B). Among these, one pregnancy with a predicted trisomy 15 was terminated at 23 weeks of gestation due to anhydramnion. For this case, arrayCGH, FISH and molecular analysis on fetal blood showed a normal result with the absence of UPD15. Another case with a trisomy 22 was normal upon amniotic fluid analysis (arrayCGH and FISH analysis) whereas follow-up FISH of 3 placental biopsies confirmed the presence of trisomy 22 in 75-80% and ~37% of the cells respectively.

DISCUSSION

We developed and implemented a method to identify samples with a high likelihood of placental chromosomal mosaicism. The sensitivity of the method to predict placental mosaicism increases with increasing fetal fraction. When a mosaicism was predicted by the algorithm, the placental mosaicism was confirmed in about 90% (20/22) cases where the placenta was sampled. In the remaining cases, no aneuploidy was detected in the analyzed placental biopsies, which does not exclude the presence of an aneuploidy cell line elsewhere in the placenta. Overall, this analysis provides a unique view on the incidence of mosaic chromosomal aneuploidies in trisomic fetuses and placentas. The proportion of mosaicism for

the common aneuploidies trisomy 21, 18 and 13 was 3.2% (5/154), 12.8% (5/39) and 13.3% (2/15) respectively (Table 2A). These numbers are slightly higher than previously reported by Konialis and Pangalos (2015) ³⁰. In their large study of 73,268 fetal conventional karyotypes, trisomy 21, 18 and 13 mosaicism was detected in 2%, 4.8% and 9.6% respectively. The slightly higher numbers obtained with NIPT likely indicates that NIPT is more sensitive to detect placental mosaicism. The overall frequency of RATs in our study was 0.3%, very similar to 0.34% observed by Pertile et al. (2017). The large majority of RATs were mosaic (Fig. 3). For example, all trisomy 7 cases have are confined to and mosaic in the placenta. It seems likely that RATs originated postzygotic and/or that full placental trisomies for those RATs are not viable ³¹. It is interesting to note that also for chromosome 18 and 13, there is a large proportion of mosaic samples. This is in accordance with some older observations made by conventional karyotyping ^{24; 31-33}, suggesting that placentas with trisomy 18 or trisomy 13 are always mosaic in viable pregnancies²². Intriguingly, a small but significant number (21/154) of trisomy 21 TriZ values are higher than expected. Since such higher values are not observed for any of the other aneuploidies, we could assume that these placentas contain a subset of cells carrying four chromosomes 21. Interestingly, a case with tetrasomy/trisomy mosaicism has been reported ³⁴.

The ability to identify pregnancies with chromosomal fetoplacental mosaicisms and the ability to estimate the degree of placental mosaicism can improve clinical guidance of the pregnancy. CPM was first described by Kalousek and Dill ²² in term placentas of infants born with unexplained IUGR. It has been estimated that 16 to 21% of pregnancies with CPM show prenatal or perinatal complications²². The detection of RATs, which are often CPMs, have been associated with a spectrum of fetal manifestations ranging from normal pregnancy outcome to IUGR and even intrauterine death of a chromosomally normal fetus ^{22; 26}. The degree of mosaicism and its effect in fetoplacental outcome is chromosome dependent. Genetic testing of amniotic fluid cells can confirm the presence of fetal mosaicism. When mosaicism is detected, there is a risk that the baby will develop birth defects. The severity depends on the fraction and the tissue distribution of the aneuploid cells. Although fetuses with mosaic aneuploidies are mainly observed for chromosomes 21, 18 and 13, mosaic trisomies involving most other autosomes have been reported in patients with birth defects ³⁵. However, for RATs, the mosaicism is most often confined to the placenta. If a chromosomal aneuploidy is restricted to the placenta, NIPT analysis would suggest the presence of an

aneuploidy whereas the genetic analysis of the fetus is normal. To test this hypothesis, we sampled placentas from 14 pregnant women where the NIPT and invasive genetic testing results were conflicting. In 9 of these we demonstrated that the positive NIPT result was indeed the consequence of placental mosaicism. CPM was especially pronounced in the RATs (Fig. 3).

Another value of detecting chromosomal mosaicism is that they can be indicators for increased risk for fetal uniparental disomy³⁶. UPD in humans is caused primarily by meiotic nondisjunction events, followed by trisomy or monosomy 'rescue'. Not surprisingly, the incidence of UPD increases with maternal age. UPDs were initially detected as mosaic trisomies during routine prenatal diagnosis by chorionic villus sampling or amniocentesis³⁷. Now, we are capable of detecting some mosaic trisomies non-invasively. The risk for (fetal) developmental disorders is the highest for chromosomes known to carry imprinted genes. The most severe developmental disorders are UPD7 (Silver-Russel Syndrome), UPD14, and UPD15 (Prader-Willi / Angelman Syndrome). When NIPT indicated the presence of (mosaic) trisomy but invasive testing was normal, UPD testing was performed. Only in one pregnancy, the fetus proved to be a mosaic trisomy and UPD for chromosome 15. This case was earlier reported in Bayindir et al., 2015¹⁶. In a published analysis of 16,885 pregnancies, seven rare autosomal trisomies were shown to have resulted in a uniparental disomy cell line in the fetus²⁶ which involved chromosomes 2, 4, 15 and 16.

The ability to predict mosaicism is limited by technical and biological factors. Technically, for trisomy 21 the method does not allow the detection of mosaicism if the fetal fraction is below 5%. Biologically, the distribution of the chromosomal mosaicism in placenta and fetus appears to be random. The analysis of placenta further pinpoints CPM as the main cause for discrepancies between NIPT and invasive testing results. The sampling of a single region of the placenta does not reflect the actual constitution of the whole placenta as different regions can contain different degrees of trisomic cells. For instance, sampling five regions of the placenta of a predicted trisomy 2 with normal fetal genotype revealed CPM for trisomy 2 ranging from 3% to 25%. The consequence of the apparent random distribution of chromosomal changes is that the method can never exclude the presence of an aneuploidy restricted to the fetus or, conversely, a non-mosaic trisomy in the placenta with a normal diploid fetus. In addition, cfDNA is derived from the cytotrophoblast whereas placental biopsies can contain both cytotrophoblasts as well as the mesenchymal core cells³⁸.

Discrepancies between the genetic constitution of the different cell layers of the placenta, trisomy or monosomy confined to the cytotrophoblast or mesenchyme, have been well described in the literature ³⁹. Despite all potential advantages, it remains controversial whether genome-wide NIPT analysis should be implemented in the routine clinical setting. The main concern is a potential increase in the number of invasive procedures to follow-up (false) positive results which can be either biological (a consequence of CPM) or technical in origin. As shown here, many of the RATs are indeed confined to the placenta. However, we believe this information can improve pregnancy management. It should be noted that aneuploidy detection does not necessarily need to result in invasive testing but may rather lead to expert ultrasound follow-up. Subsequently, results of both the genetic testing and fetal ultrasound can be disclosed to the pregnant women. In our experience, after discussing the low risk for developmental anomalies associated with the rare aneuploidies, some women opt against invasive testing. For other trisomies, including the aneuploidies for chromosomes with imprinted genes, invasive testing could be offered with the possibility of associated UPD testing. In 44/58 rare autosomal trisomies, a follow-up invasive test was performed. The others are lost to follow-up. It should be noted that this is only 0.2% of the study cohort and this does not constitute a significant increase in invasive testing compared to exclusive testing of the common aneuploidies. In 9 out of 44 (20%) follow-up invasive diagnostic test confirmed aneuploidy in the fetus. In 35 cases the fetus was normal. Interestingly, for 25 pregnancies our method predicted mosaicism. Hence, identification of potential mosaicism may further improve future counselling and further reduce the number of unnecessary invasive tests. Indeed, here we show that pregnant women undergoing genetic counselling following an abnormal NIPT result should be informed that the tissue being sampled is not of fetal origin and that mosaic conditions can also occur.

Acknowledgments

We sincerely thank Kristien Philippe and Sofie Verbeke for contributing to the genetic counseling of the patients. This work was made possible by grants from the University of Leuven (KU Leuven): GOA (GOA/12/015 to J.R.V., K.D., and H.V.E.) and SymBio- Sys (PFV/10/016 to J.R.V.).

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Figure 1: Normalized chromosomal read count in relation to the fetal fraction (SeqFF) for chromosome 13 (A), 18 (B), 21 (C) and 7 (D). The X axis depicts the fetal fraction and the Y axis the normalized chromosomal read count. Horizontal and diagonal lines respectively mark the predicted normal and trisomic normalised read counts (full lines) and $\pm 3 \times \text{SD}$ as measured in the normal cases (X-axis and dashed lines). Values within $3 \times \text{SD}$ from expected trisomy counts are plotted as blue triangles. Values outside the area delineated by the X-axis and dashed lines represent cases with $|Z| > 3$ and $|\text{TriZ}| > 3$. These are plotted as red circles and indicate pregnancies at risk for fetoplacental mosaicism. The arrows indicate discordant twins.

Figure 2: Incidence of rare autosomal trisomies.

Table 1: NIPT results and invasive follow-up of common (A) or uncommon (B) aneuploidies.

Overview of samples with a chromosomal trisomy call of chromosome 13, 18 and 21 (A) or RAT (B) respectively in combination with a TriZ-score that is not low. AF; Amniotic fluid. CVS; Chorionic Villus Sampling. CPM; Confined Placental Mosaicism. DCDA twin; dichorionic-diamniotic twin.

Table 1a

Trisomy	Trisomy call & triZ high/normal	Trisomies (Trisomy call & TriZ high/normal) confirmed upon invasive testing					No follow-up	False negative
		Total confirmed	AF	CVS/placenta	Fetal biopsy	Postnatally		
13	7	4	2	1	1		3	
18	24	20	18	2			4	2*
21	134	107	90	9	2	6	26 + 1 (miscarriage)	

*1 false negative trisomy 18 in singleton pregnancy and 1 in early DCDA twin pregnancy

° 1 trisomy 18 confirmed in CVS but not in amniotic fluid

Table 1b

NIPT result	Follow-up invasive testing							
	Confirmed (True positive)				Not Confirmed (false positive)		No follow-up	
Trisomy call & triZ high/normal	AF	Predicted % of mosaicism by NIPT	CVS/placenta	Fetal biopsy	Postnatally	AF	Fetal biopsy	
2	1 (5-13% mosaic)	0.53						
9						1	1	1 (miscarriage)
10								1
15	1 (mosaic T15 + UPD15)	0.35					1 (TOP)	
16	1 (25% mosaic)	0.69				4		1 + 1 (miscarriage)
17						1		
20		0.68	1 (CPM)					
22						2	2	

Table 2: NIPT results and invasive follow-up of mosaic aneuploidies. (A) Overview of samples with a chromosomal trisomy call in combination with a TriZ-low-score. **(B)** Detailed overview per case when the detected aneuploidy was confirmed in the fetus. FU; Follow-up. AF; Amniotic fluid. CVS; Chorionic Villus Sampling. CPM; Confined Placental Mosaicism. FA; Fetal Aneuploidy. FM; Fetal Mosaicism.

Table 2a

Trisomy	Trisomy call & triZ low	Trisomies (Trisomy call & TriZ low) confirmed upon invasive testing			No follow-up
		Confirmed [°]	Not Confirmed (AF)	Not Confirmed (AF + placenta)	
13	3	2 (miscarriages)			1
18	9	5 (FA)	1		3
21	8	5 (FA)		1	2
2	1	1 (CPM)			
7	8	3 (CPM)	4*		1
8	4	1 (CPM)	1		2
11	1			1	
15	1				1
16	6	1 (CPM) + 1 (CPM + FM)	3		1
20	3	1 (CPM)	2		
22	1		1		
Total number	45	20	12	2	11

*among which 1 partial T7

[°] see Table 2B for more details per case

Table 2b

Type of mosaic aneuploidy	Chromosome	Sample ID	Follow-up invasive testing			
			AF	CVS	Placenta	Miscarriage
<i>Confined placental Mosaicism</i>	2	61242702	Not confirmed		Confirmed (3-25%)	
	7	60873268	Not confirmed	Confirmed		
	7	60992731	Not confirmed		Confirmed (<10%)	
	7	61240708	Not confirmed		Confirmed (~40%)	
	8	60933711	Not confirmed		Confirmed (5-14%)	
	16	61551017	Not confirmed		Confirmed (53-65%)	
	20	61353006	Not confirmed		Confirmed (5-10%)	
<i>Fetal (high grade mosaic) Aneuploidies</i>	13	61052199				Confirmed
	13	60668954				Confirmed (40%)
	18	60582876	Confirmed (<85%)			
	18	60615688	Confirmed (<85%)			
	18	60841330	Confirmed (<85%)			
	18	60878828	Confirmed (<85%)			
	18	61129105	Confirmed (<85%)			

	21	60609668	Confirmed (<85%)			
	21	60791239	Confirmed (<85%)			
	21	60977435	Confirmed (<85%)			
	21	61321214	Confirmed (<85%)			
	21	61355675	Confirmed (<85%)			
<i>Fetal + Placental Mosaicism</i>	16	60585849	Confirmed (7%)		Confirmed (>75%)	
<i>Unexplained so far</i>	21	60977275	Not confirmed		Not confirmed (3 biopsies)	