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SPECIAL REPORT

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Cytogenetic confirmation of a positive NIPT result: evidence-based choice between chorionic villus sampling and amniocentesis depending on chromosome aberration

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ABSTRACT

It has been shown that in non-invasive prenatal testing (NIPT) there is a small chance of a false-positive or false-negative result. This is partly due to the fact that the fetal cell-free DNA present in maternal plasma is derived from the cytotrophoblast of chorionic villi (CV), which is not always representative for the fetal karyotype due to chromosomal mosaicism. Therefore, a positive NIPT result should always be confirmed with invasive testing, preferably amniocentesis, in order to investigate the fetal karyotype. However, since this invasive test can only be safely performed after 15.5 weeks of gestation while NIPT can be done from the 10th week of gestation, this potentially means an unacceptable long waiting time for the prospective parents to receive a definitive result. Based on our experience with cytogenetic investigations in CV and the literature, we determined whether CV sampling may be appropriate for confirmation of an abnormal NIPT result.

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NIPT; non-invasive prenatal diagnosis; prenatal diagnosis; trisomy 13; trisomy 18; trisomy 21; autosomal trisomy; confined placental mosaicism; CPM; chorionic villi; amniocentesis

Biological origin of false positive NIPT

Non-invasive prenatal testing (NIPT), using the cell-free DNA in maternal plasma, is revolutionizing prenatal screening for the common aneuploidies (trisomy 13, 18, and 21) [1]. Moreover, there are already reports on genome-wide NIPT analysis with promising results [2,3]. For trisomy 13, 18, and 21 screening, the test performs much better than the traditional first trimester screening (combined test) [1]. Nevertheless, false positive as well as false negative results occur [4,5]. False results may have a biological as well as a technical origin. The biological origins of a positive NIPT test are diverse: confined placental mosaicism (CPM), maternal chromosomal mosaicism, maternal tumor, maternal copy number variation, and vanishing twin [6].

It is recognized that the main source of false positive results is CPM and many case reports have already been published [7,8]. CPM is a type of chromosomal mosaicism in which the chromosome abnormality is present in chorionic villi (CV)/placenta, but not in the fetus itself [9]. Since Flori et al. [10] showed that the cell-free fetal DNA in maternal plasma is derived from the outer cell layer of CV, the cytotrophoblast, detection of CPM with NIPT is to be expected at the same rate as is found with cytogenetic investigations of the cytotrophoblast of CV. This showed to be ~2% in high-risk pregnancies in our center [11]. Therefore, it is recommended to confirm a positive NIPT result by invasive prenatal testing, preferably by amniocentesis (a joint European Society of Human Genetics [ESHG]/American Society of Human Genetics [ASHG] position statement) [12]. A major drawback of confirmation in amniotic fluid is the timing of the NIPT from 10 weeks of gestation on and that of the amniocentesis, which is usually performed after the 15th week of gestation in order to prevent pregnancy complications [13]. This potentially means a very long waiting time for the pregnant woman to get a definitive result of the fetal chromosome constitution, which is highly undesirable. Moreover, the chance of fetal confirmation in cases of abnormal NIPT results involving the common aneuploidies is very high [4], so that chorionic villus sampling (CVS) may be the preferred method as it is in other pregnancies at high aneuploidy risk [14].

Long experience with cytogenetic investigations in CV for prenatal diagnosis has shown that reliable diagnosis of fetal trisomy 13, 18, and 21 is possible in CV when proper protocols are used [14]. In this paper, we will show that in the majority of cases, CVS can reliably be used for confirmation of NIPT positive for trisomy 13, 18, and 21 despite the phenomenon of CPM. For other autosomal trisomies that may be detected with genome-wide NIPT testing, the type of confirmatory test (CVS or amniocentesis) will depend on the chromosome aberration involved.

Cytogenetic studies in CV

From many years of experience with prenatal cytogenetic diagnosis in CV, we have learned that the karyotype of the cytotrophoblast does not always represent the fetal chromosome constitution (Figure 1). The cytotrophoblast originates from the trophoblast of the embryo and traditionally is studied in direct or short-term-cultured villi (STC-villi) [15] and nowadays is tested with NIPT [10]. In contrast, the mesenchymal core of CV, which conventionally is studied in long-term-cultured villi (LTC-villi) [16], better reflects the fetal karyotype. This is due to its embryonic origin, which is the same as that of the fetus itself (inner cell mass) [17,18]. Due to their different origin, karyotypes of STC- and LTC-villi may be different leading to three types of CPM (see Table 1). Due to its trophoblastic origin, the analysis of STC-villi (cytotrophoblast) alone is not recommended for prenatal diagnosis (Association for Clinical Cytogenetics [ACC] Prenatal Diagnosis Best Practice Guidelines 2009). Proper conventional

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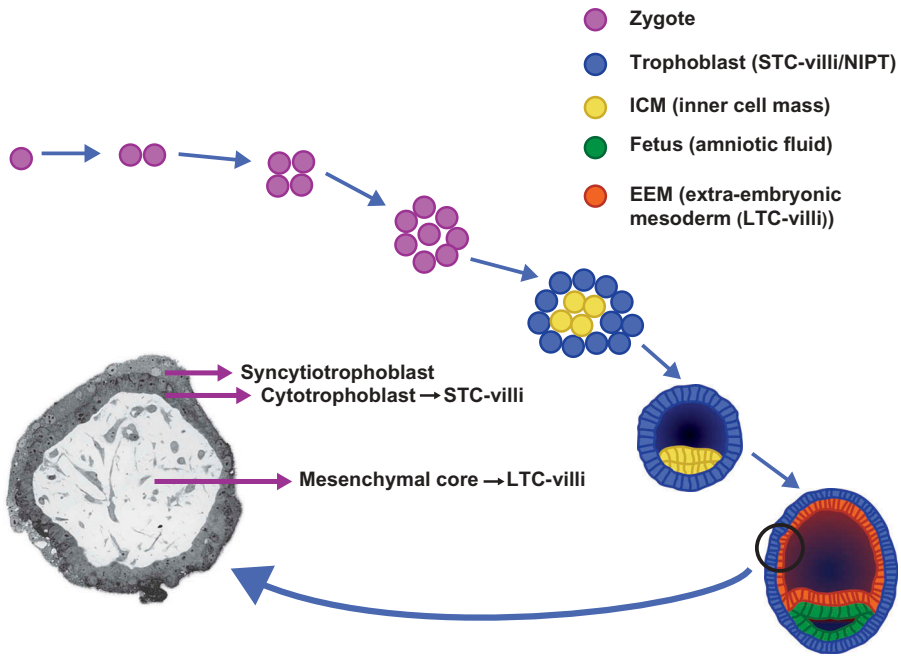

Figure 1. Early embryonic development from zygote to blastocyst. The cytotrophoblast, which is studied in short-term cultured villi (STC-villi) and with NIPT is derived from the trophoblast of the blastocyst, whereas the mesenchymal core, investigated in long-term cultured villi (LTC-villi) originates from the extra-embryonic mesoderm (EEM). Both EEM and fetus are derived from the inner cell mass (ICM) of the blastocyst [5].

Table 1. Different types of confined placental mosaicism (CPM) causing false positive NIPT results and the rare trisomies commonly involved in the different CPM types.

Type of CPM	Cytotrophoblast (STC-villi)	Mesenchymal core (LTC-villi)	Fetus	Involved trisomies*	NIPT result
CPM I	(Mosaic) abnormal	Normal	Normal	3, 7, 8, 9, 20	'False' positive
CPM II	Normal	(Mosaic) abnormal	Normal	2, 10	Normal
CPM III	(Mosaic) abnormal	(Mosaic) abnormal	Normal	15, 16, 22	'False' positive

*According to Wolstenholme [20]; Battaglia et al. [21]; Hahneman and Vejerslev [22,23].

cytogenetic investigations of CV involve the analysis of both cytotrophoblast and mesenchymal core [14,19]. This is the reason that NIPT will and can never be a diagnostic test.

Nowadays, many laboratories left karyotyping of STC- and LTC-villi and use molecular techniques like quantitative fluorescence polymerase chain reaction (QF-PCR) and/or genomic arrays on uncultured CV for cytogenetic studies. However, despite the phenomenon of CPM, some of them use DNA from the whole chorionic villus, which is a mixture of cytotrophoblast and mesenchymal core, which leads to problems with interpretation of mosaic results [24]. In order to avoid uncertain results, it is important to separate cytotrophoblast from the mesenchymal core and use the DNA from both cell lineages separately [25] or at least that of the mesenchymal core in accordance with the European cytogenetic guidelines [26].

However, notwithstanding the same embryonic origin for fetus and mesenchymal core, the latter does not always show the fetal karyotype due to chromosomal mosaicism like CPM types II and III (Table 1). Because abnormal NIPT results require confirmation and the recommended amniocentesis may cause long waiting times for the patient, we investigated whether CVS may be advisable after NIPT shows an abnormal result. Therefore, we reviewed large data sets and the literature in order to investigate whether the chromosomal constitution of the mesenchymal core after the detection of a (mosaic) trisomy in the cytotrophoblast (e.g. NIPT) may be considered to correctly reflect the fetal karyotype. If so, this would indicate that a CVS is appropriate after a positive NIPT. We did this investigation not only for trisomy 13, 18, and 21, but also for other autosomal trisomies that are frequently encountered in STC-villi, and therefore potentially also with genome-wide NIPT: trisomy 2, 3, 7, 8, 9, 15, 16, 20, and 22 [19,21–23].

The chromosomal constitution of mesenchymal core and its relation to the fetal karyotype

In order to examine whether the mesenchymal core is representative for the fetal karyotype, we gratefully made use of the ACC CVS database 2005 [27] in which the karyotyping results, presented in 171 papers on CV cytogenetics as well as the cytogenetic data from different UK laboratories, are collected and organized according to results in STC-and LTC-villi with information on fetal follow-up investigations [28]. Abnormal cases with a (mosaic) trisomy in cytotrophoblast that would lead to a positive NIPT were reviewed. Only cases with clinical and/or cytogenetic follow-up investigations were included.

1. Trisomy 13, 18, and 21

The results for trisomy 13, 18, and 21 are shown in Table 2. It can be concluded that if the mesenchymal core reveals a 100% trisomy or a 100% normal result, the fetus will,

Table 2. Predictive value of LTC-villi in cases of (mosaic) trisomy 13, 18, and 21 in STC-villi (based on the ACC CVS database 2005 [27]). Only cases with follow-up investigations in the fetus were considered.

		Karyotype in LTC-villi		
Karyotype in STC-villi	Total *N*	100% trisomy (trisomy confirmed in the fetus)*	Mosaic trisomy (trisomy confirmed in the fetus)*	Normal (trisomy confirmed in the fetus)*
(mos) +13	120	87 (87)	10 (5)	23 (0)
(mos) +18	219	202 (202[1])	7 (1)	10 (0[2])
(mos) +21	365	347 (347[3])	6 (5)	12 (0[4])
Total	704	636 (636)	23 (11)	45 (0)

*Confirmed in the fetus means 'clinically or cytogenetically confirmed in the fetus' according to the ACC CVS database 2005 [27]. STC-villi: short-term-cultured villi in which the cytotrophoblast is studied; LTC-villi: long-term-cultured villi in which the mesenchymal core is studied.

[1]There was one exceptional case (C98-2363) in which not a trisomy 18, but another chromosome aberration (mosaic trisomy 8) was found in postnatal tissue. This case is not included in the table.

[2]One exceptional case (C920883) of fetal trisomy 18 had a false negative result (46,XX) in LTC-villi. This case was not included in the table since only one 'cell colony' in the LTC-villi was investigated due to small sample size, which does not meet good practice criteria and maternal cell contamination (MCC) has not been excluded.

[3]Trisomy 21 was not confirmed in one rare case in which the fetus showed another unbalanced chromosome aberration (partial trisomy 9) (case ID 95/870C). This case is not included in the table.

[4]A case of 46,XX of our own group (1364_LC) was not included since MCC most likely explained the false negative result.

respectively, be trisomic or normal as well. Not only the older literature, but also recent papers that are not included in the database confirm this finding as well [21,29,30]. However, in 3.3% (23/704) of cases, LTC-villi revealed a mosaic trisomy, which requires follow-up investigations in amniotic fluid, since in about half of the cases (12/23) the trisomy will be confined to the placenta (Table 2). CPM is more likely for trisomy 18 mosaicism (86% of mosaic cases are CPM) than for trisomy 13 (50% CPM) or trisomy 21 mosaicism (only 17% CPM) (Table 2). So in only 3.3% of the cases with positive NIPT results, CVS will not give definitive results and further testing in amniotic fluid will be necessary. This figure does not appear to be very different from the situation where CVS is done due to high aneuploidy risk (women with abnormal first trimester screening results or advanced maternal age), where circa 2% of the cases require follow up karyotyping in amniotic fluid if only STC-villi are investigated [11]. Therefore, it seems to be appropriate to offer CVS as a confirmatory test if NIPT is positive for trisomy 13, 18, or 21 (Figure 2). In that way, most of the pregnant women at high risk for an abnormal fetal test result will benefit from a rapid definitive result, while just a few will have to undergo an amniocentesis as well. In NIPT positive cases, first trimester ultrasound investigations may be of value in the decision-making process on the type of confirmatory invasive test. Moreover, if a vanishing twin is suspected, a CVS may probably be contra-indicated in order to prevent a misdiagnosis.

Although the data in the ACC CVS database are biased, this figure of 3.3% requiring further investigations in amniotic fluid correspondents well with the figure of 2–4%, at least for trisomies 18 and 21, as recently published by

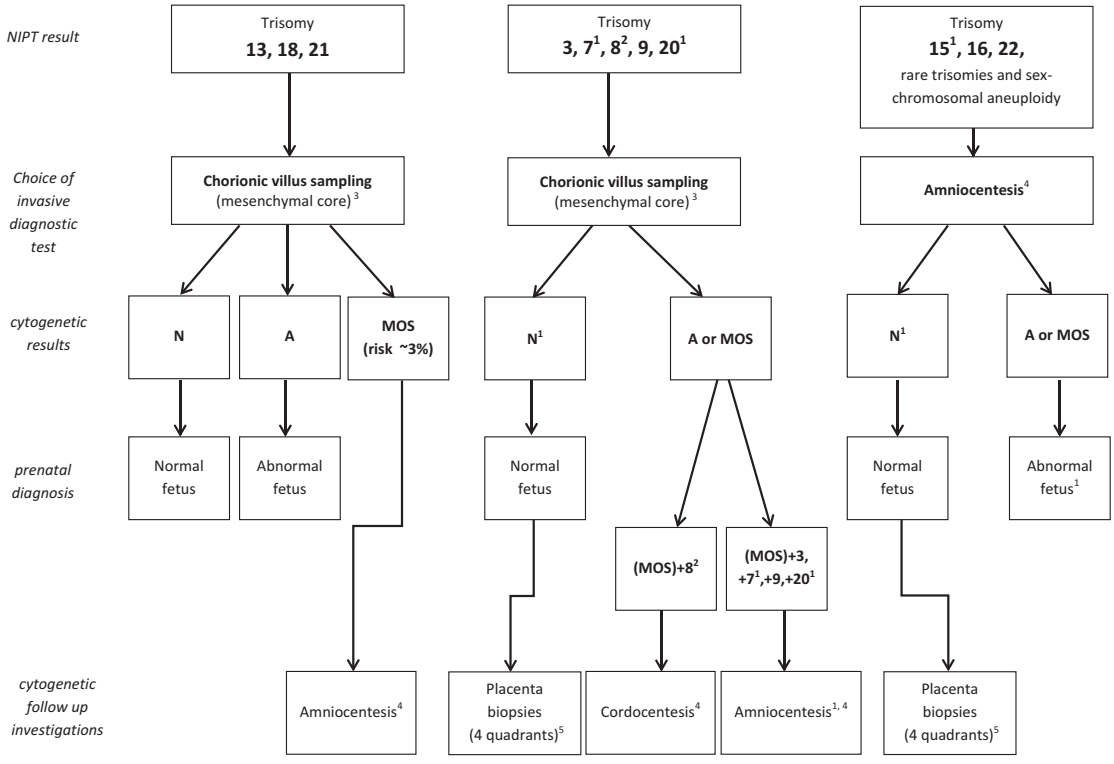

Figure 2. The choice of invasive test for confirmatory studies depends on the chromosome aberration detected with NIPT. A: abnormal; N: normal; MOS: mosaic. [1] If imprinted chromosomes like 6, 7, 11, 14, 15 or 20 are involved, UPD in the normal cell line should be excluded. [2] For trisomy 8, an amniocentesis may not be the appropriate follow-up test due to tissue–specific chromosomal mosaicism, although present in the fetus, sometimes being absent in amniotic fluid [22] [3]investigate uncultured mesenchymal core, preferentially with SNP array, to exclude mosaicism. Also investigate uncultured cytotrophoblast (to confirm NIPT). [4] Investigate both uncultured cells with SNP array and cell cultures (AF cell colonies) [31]. [5] Investigate both uncultured cytotrophoblast and mesenchymal core with SNP array of at least 4 placenta biopsies.

Table 3. Risk for a confirmatory amniocentesis after CVS for the different common aneuploidies based on the present study (of ACC CVS database [27]) and the single center study of Grati et al. [32].

Chromosome aberration	Present study $N = 704$	Grati et al. (2015) $N = 1512$
Trisomy 13	8.3%	22%
Trisomy 18	3.2%	4%
Trisomy 21	1.6%	2%

Grati et al. [32], based on their single center experience with >50,000 CV samples. The risks for a confirmatory amniocentesis after CVS per chromosome aberration based on the present study and the large cohort presented by Grati et al. [32] are shown in Table 3. In both studies, this risk is the smallest for trisomy 21 and the biggest for trisomy 13. For trisomy 18 and 21, the figures are comparable, whereas for trisomy 13 their figure is higher (22% vs. 8.3%) due to the fact that Grati and her colleagues consider a trisomy that is restricted to the cytotrophoblast (mesenchymal core normal) to be an uncertain result requiring an amniocentesis as well. This accounts for most cases of trisomy 13 mosaicism (74%) that they found and less for trisomy 18 or 21. However, as reviewed by van den Berg et al. [33], false negative LTC-villi (normal mesenchymal core while the fetus has a (mosaic) trisomy) are extremely rare and the exceptional cases in the literature are probably mostly attributable to maternal cell contamination (MCC), which in the conventional cytogenetic era was not routinely excluded in female cases, at least at the DNA level. MCC is a well-known problem of culturing CV that are often contaminated with maternal tissue requiring proper separation of maternal deciduas [26]. Moreover, it is a well-known phenomenon that long-term culturing in cases of mosaicism may lead to loss of the abnormal cell line through culture induced selection mechanisms [31]. Therefore, in order to prevent misdiagnoses, it should be recommended to avoid CV culturing, so that the risk of MCC and cell line selection is minimized. This is especially important since the detection of chromosomal mosaicism is crucial for recognizing those cases that require confirmatory amniocentesis. Therefore, like Grati et al. [32], we encourage the investigation of both cytotrophoblast and mesenchymal core, but not with karyotyping of STC- and LTC-villi as these authors suggest, but by using molecular techniques on DNA isolated from uncultured cytotrophoblast and uncultured mesenchymal core. If QF-PCR is used, which is only able to exclude mosaicism of about >15% [34], and the results in the mesenchymal core are normal, this testing should be complemented with SNP array analysis which will enable the detection of >5% mosaicism of meiotic origin [35].

2. Other autosomal trisomies and sex-chromosomal aneuploidies

For the other trisomies that often are found in CV cytotrophoblast (3, 7, 8, 9, 15, 16, 20, 22), the numbers in the ACC CVS database are very small and only individual case reports are published. Therefore, no reliable risk figure as for trisomy 13, 18, or 21 can be drawn from the database or literature.

Table 4. The karyotype in LTC-villi in cases of (mosaic) autosomal trisomy in STC-villi and its relation to the fetal karyotype (based on the ACC CVS database 2005 [27]). Only cases with follow-up investigations were considered.

		Karyotype in LTC-villi		
Karyotype in STC-villi	Total N	100% trisomy (confirmed in the fetus)*	Mosaic trisomy (confirmed in the fetus)*	Normal (confirmed in the fetus)*
(mos) +3	22	1 (0)	0 (0)	21 (0)
(mos) +7	32	2 (0)	9 (0)	21 (0)
(mos) +8	11	0 (0)	5 (1)	6 (0)
(mos) +9	11	2 (1)	5 (2)	4 (0)
(mos) +15	16	2 (1)	10 (1)	4 (0)
(mos) +16	45	34 (1)	9 (0)	2 (0)
(mos) +20	23	2 (0)	9 (2)	12 (0)
(mos) +22	6	4 (3)	1 (1)	1 (0)

*Confirmed means 'clinically or cytogenetically confirmed in the fetus' according to the ACC CVS database 2005 [27]. STC-villi: short-term-cultured villi in which the cytotrophoblast is studied; LTC-villi: long-term-cultured villi in which the mesenchymal core is studied.

Nevertheless, it may be concluded that a normal result in LTC-villi means a normal karyotype in the fetus as well (Table 4). If a 100% or mosaic trisomy is present in LTC-villi, most will be confined to the placenta, but depending on the chromosome aberration, some cases may be confirmed in the fetus. As already shown by Wolstenholme [20], the distribution of a chromosomal abnormality between the cytotrophoblast and extra-embryonic mesoderm cell lineages, shows a highly specific pattern for each chromosome aberration (Table 1). As a rule, CVS may be an appropriate confirmatory test if the trisomy mostly is involved in CPM type 1. For trisomies that are mostly involved in CPM type 3, an amniocentesis will always be the confirmatory procedure of choice. These confirmatory studies not only need to include chromosomal investigations, but also molecular testing for uniparental disomy if an imprinted chromosome (6, 7, 11, 14, 15, 20) is involved. Moreover, cytogenetic studies should include the investigation of both uncultured as well as cultured cells, the latter for detection of an exceptional case of tissue specific mosaicism that may be missed in uncultured cells, as was shown before [31]. Figure 2 shows the choice of invasive test for confirmatory studies depending on the chromosome aberration involved.

For all other rare autosomal (1, 2, 4, 5, 6, 10, 11, 12, 14, 17, and 19) and sex-chromosomal numerical aberrations, an amniocentesis should be the preferred confirmatory invasive test (Figure 2). Detection of trisomy 2 or 10 with NIPT most probably will be extremely rare since these trisomies mainly are involved in CPM type 2 [20,21] (Table 1). If detected, an amniocentesis should be advised. Experience with the other rare trisomies in CV is very small and therefore, we would recommend amniocentesis when detected with NIPT. If sex-chromosomes are included in the NIPT test, and if the test reveals an increased risk for sex-chromosomal aneuploidy, an amniocentesis should be recommended as well due to the relatively low predictive value of sex-chromosomal abnormalities in CV for the fetal chromosomal status. Especially, the detection of 45,X in CV has shown to be an unhelpfully erratic indicator of the fetal karyotype [8,36]. Moreover, it was shown that a relatively high frequency of sex-chromosomal aneuploidies (8.6%) as detected with NIPT is due to maternal mosaicism [37]. Therefore, as suggested, maternal karyotyping in all cases of NIPT positive

for sex-chromosomal aneuploidies should be considered. It is however important to realize that confirmation of a maternal mosaic does not exclude that the fetus is affected as well. Therefore, an amniocentesis will still be necessary to elucidate the fetal chromosome constitution.

If NIPT involves another trisomy than trisomy 13, 18, or 21, CPM is by far the most likely origin of the positive result in such cases. Although one could consider CPM to be irrelevant, it is associated with an increased risk of intrauterine growth retardation [38], fetal loss [39] or poor perinatal outcome [40]. Therefore, close follow-up investigations with ultrasound to monitor fetal growth in cases of CPM are indicated. Brady et al. [6] suggested that in the case of normal development upon ultrasound examination, further invasive actions may not be warranted. However, in such cases it is important to realize that although fetal ultrasound abnormalities are to be expected in case of a fetal trisomy, their absence does not exclude that the fetus is affected with trisomy mosaicism. Moreover, if an imprinted chromosome (6, 7, 11, 14, 15, 20) is involved, there is a risk for uniparental disomy, which causes disease that may not be detectable with ultrasound investigations and which may be a reason for invasive testing [41].

Limitations of using CVS data for NIPT interpretation

The main limitation of using the ACC CVS database is that all data are the results from conventional chromosome studies in STC- and LTC-villi, whereas many laboratories nowadays use molecular techniques such as QF-PCR and/or genomic arrays. Since these techniques, and especially SNP array, are generally more sensitive for mosaicism detection [35], the prevalence of chromosomal mosaicism in CV and therefore the need for confirmatory studies in amniotic fluid may be underestimated. However, the ACC data set contains the best available data set (confirmed by recent literature in large cohorts) and until large studies showing the separate analysis of cytotrophoblast and mesenchymal core with molecular cytogenetic techniques such as microarray become available, it will remain the reference database for mosaicism in CVS.

Another limitation that should be taken into account is cohort selection. In contrast to the population that nowadays has access to NIPT, all patients traditionally studied with CVS were at high genetic risk for (segmental) aneuploidy. Moreover, the CVS data may be representative for only a part of the placenta, especially when villi were sampled transcervically, whereas probably the whole placental trophoblast will shed DNA into the maternal circulation. It may therefore be relevant to take into account that placental variation, which has been shown in term placenta [42], may potentially explain rare cases of false positive NIPT results that are not confirmed with CVS or a single placenta biopsy. Since with transabdominal CVS a larger area of the placenta is biopsied than with the transcervical approach, which may be restricted to a small placental part, cytogenetic results of the former may potentially better represent the cell-free DNA results.

However, although these limitations may have an effect on currently known incidences of chromosomal mosaicism in prenatal diagnosis, in our opinion they probably will not change existing protocols on the interpretation of chromosomal mosaicism in CV. However, only the investigation of both CV and term placentas for confirmation of positive NIPT results will show how the chromosomal constitution of the cell-free DNA relates to that of first trimester CV and term placenta.

Postnatal confirmatory studies

When an amniocentesis is performed instead of CVS and the trisomy is not confirmed in uncultured as well as cultured cells [31] and when other biological sources of a positive NIPT such as a vanishing twin, maternal CNV, or maternal mosaicism are excluded, CPM may still only be assumed. Therefore, in order to confirm that the abnormal NIPT result is the consequence of CPM, we would like to advise investigation of the term placenta. It is important to investigate at least four biopsies of the different quadrants due to placental site variation [42] and similar to first trimester CV, the separate investigation of both cytotrophoblast as well as mesenchymal core is indicated. If the results are normal, a maternal tumor may be considered to be the origin of the abnormal NIPT result and further investigations may be warranted [43].

Expert commentary

Due to the placental origin of the cell-free 'fetal' DNA, NIPT never will and can be a diagnostic test on its own. Therefore, a confirmatory invasive test is recommended by a joint ESHG/ASHG position statement, with amniocentesis being the preferred test. However, since NIPT may be performed from 10 weeks of gestation on, this potentially means a very long waiting-time for the pregnant woman to get a definitive result about the fetal chromosome constitution. This may cause distress which should be avoided. Therefore, since CVS allows fetal cytogenetic diagnosis in the first trimester of pregnancy, this potentially could be a more suitable confirmatory test. However, because of the phenomenon of CPM, this is often discouraged. Indeed, in some cases the result in CV will be inconclusive requiring a secondary invasive test to get a definitive diagnosis. In this paper, we show that CVS will allow a rapid definitive result at an early gestational age in the vast majority (~97%) of pregnant women with an abnormal NIPT showing one of the common trisomies (trisomy 13, 18, and 21). Only a small percentage (~3%) will have to undergo a secondary invasive test for a definitive diagnosis (1.6% for trisomy 21, 3.2% for trisomy 18, and 8.3% for trisomy 13). An early diagnosis facilitates the reproductive autonomy as it allows early termination of pregnancy (TOP) on the parents request in affected cases. It has been shown that this is associated with less psychological distress as compared to TOP after 14 weeks of gestation [44]. Another advantage of CVS is that it allows the investigation of the cytotrophoblast, which in cases of a normal mesenchymal core enables to confirm the NIPT, if CPM is the cause. Moreover, if the cytotrophoblast is normal, and when other biological sources are excluded, rapid follow-up investigations on maternal cancer may be indicated allowing early interventions that may be lifesaving [43]. If amniocentesis is performed, confirmation studies in term placenta are important in order to learn about the clinical performance of NIPT and the frequency of CPM in high as well as in low risk populations.

Five-year view

Together with the technical improvement of the NIPT technique and after a period of intensive follow-up investigations as recommended in this paper, it will probably be shown that most cases of false positive NIPT do have a biological instead of technical origin. If so, perhaps in the future it will no longer be necessary to do invasive testing in all positive cases, meeting one of the major goals of introducing NIPT, namely reducing the number of invasive tests [12]. Since trisomy 21, in all cases, and trisomy 13 and 18, in the presence of ultrasound abnormalities, are generally considered to be 'certain fetal' abnormalities when encountered in STC-villi (cytotrophoblast) [45], perhaps confirmation in CV or amniotic fluid in the future may be skipped when fetal ultrasound abnormalities are present. However, in order to avoid the abortion of an unaffected fetus it then will be necessary not only to rely on *z*-scoring, but to use an analysis pipeline that allows whole chromosome analysis. This enables differentiation of high *z*-scores due to fetal trisomy from those caused by maternal CNVs [2,46,47]. Moreover, also other causes of false positive NIPT results, such as maternal mosaicism or a vanishing twin, should be excluded in such cases before TOP may be considered. As always, it is of great importance that patients with an abnormal NIPT result are informed about all possible options during post-test counseling, which will enable prospective parents to make informed choices about pregnancy management. And finally as NIPT opens the possibility for noninvasive screening of many more genetic disorders, one could expect that the scope of prenatal screening will be broadened from the common aneuploidies to all unbalanced chromosome aberrations. This will then allow more first trimester diagnoses that will require confirmation studies. In order to avoid maternal anxiety due to long waiting times after a positive NIPT result at 10–11 weeks of gestation, early protocols for confirmatory follow-up studies preferably at as early as 11–12 weeks of gestation should be developed as an alternative for second-trimester amniocentesis and fetal anomaly scan.

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Key issues

- Due to the origin of the cell-free 'fetal' DNA in maternal blood, namely the cytotrophoblast of CV, false-positive and false-negative NIPT results are to be expected (Table 1 and Figure 1).
- According to international guidelines, a positive NIPT should be confirmed with invasive testing in order to investigate the fetal karyotype.
- Confirmation of positive NIPT results by amniocentesis potentially leads to a very long waiting time for the pregnant woman to get a definitive result of the fetal chromosome constitution.
- Whether CVS may be appropriate for confirmation studies of an abnormal NIPT result will depend on the involved chromosome aberration.
- If NIPT is positive for trisomy 13, 18, or 21, chromosome analysis of the mesenchymal core of CV will be representative for the fetus in ca. 97% of cases (Table 2 and Figure 1).
- If NIPT indicates a trisomy 3, 7, 8, 9, or 20, CVS may be performed for confirmatory testing instead of amniocentesis since these trisomies are mostly involved in CPM type I (Tables 1 and 4 and Figure 2).
- If NIPT is positive for trisomy 15, 16, or 22, amniocentesis is the technique of choice for confirmatory testing as these trisomies are mostly involved in CPM type III (Tables 1 and 4 and Figure 2).
- If NIPT is positive for other rare trisomies (1, 2, 4, 5, 6, 10, 11, 12, 14, 17, 19) or sex-chromosomal aneuploidy amniocentesis is recommended (Figure 2).
- If CVS is performed for confirmation of an abnormal NIPT result, we encourage the separate analysis of the cytotrophoblast (for confirmation of the NIPT result) and of mesenchymal core (for investigation of the fetal chromosomal constitution) (Figure 2).
- If CVS is performed for confirmatory studies, molecular techniques should be used on DNA from uncultured mesenchymal core in order to prevent misdiagnoses due to MCC or cell line selection in cell cultures (Figure 2).
- If molecular techniques are used for cytogenetic studies of CV, we encourage the use of SNP array after normal QF-PCR results which is better able to detect low-level mosaicism, which is crucial for recognition of those cases that will require a confirmatory amniocentesis as well (Tables 2 and 3 and Figure 2).
- If an abnormal NIPT result is not confirmed in amniotic fluid cells, we advise post-partum cytogenetic investigation of at least four placenta biopsies. If negative, and when other biological sources (maternal CNV or mosaicism, vanishing twin) are excluded, further patient examination on the presence of a tumor potentially may be warranted (Figure 2).

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