The Journal of Molecular Diagnostics, Vol. 22, No. 12, December 2020

the Journal of Molecular Diagnostics
jmdjournal.org

Clinical Review of Noninvasive Prenatal Testing

Experience from 551 Pregnancies with Noninvasive Prenatal Testing—Positive Results in a Tertiary Referral Center

Xiaoqing Wu, Ying Li, Xiaorui Xie, Linjuan Su, Meiying Cai, Na Lin, Shengrong Du, Liangpu Xu, and Hailong Huang

From the Fujian Provincial Key Laboratory for Prenatal Diagnosis and Birth Defect, Prenatal Diagnosis Center of Fujian Provincial Maternity and Children Hospital, Affiliated Hospital of Fujian Medical University, Fuzhou City, China

Accepted for publication September 23, 2020.

Address correspondence to Liangpu Xu, Ph.D., or Hailong Huang, M.D., Fujian Provincial Key Laboratory for Prenatal Diagnosis and Birth Defect, Prenatal Diagnosis Center of Fujian Provincial Maternity and Children Hospital, Affiliated Hospital of Fujian Medical University, No. 18 Daoshan Rd., Fuzhou City, Fujian Province, 350001, China. E-mail: hl-hai@163.com or xiliangpu@fjmu.edu.cn.

A total of 551 pregnancies with positive results for noninvasive prenatal testing (NIPT) using traditional karyotyping and chromosomal microarray analysis were analyzed. Confirmatory results, positive predictive values, etiology exploration of false-positive results, and pregnancy outcomes were recorded. The study demonstrated that NIPT performed better in predicting trisomy 21 and trisomy 18 for pregnancies with advanced maternal age than for pregnancies with young maternal age; as for trisomy 13 and sex chromosomal aneuploidy (SCA) prediction, there was no significant difference between the two groups. The positive predictive values for trisomy 21, trisomy 18, trisomy 13, and SCA showed no significant upward trend when compared based on specific age categories (an interval of 5 years), which suggested that NIPT-positive result deserves equal attention from both providers and patients regardless of maternal age. In addition, the termination rates of 45,X, 47,XXY, 47,XXX, and 47,XYY were 100% (2/2), 92.9% (26/28), 33.3% (5/15), and 9.5% (2/21), respectively, which demonstrated that the decision-making regarding pregnancies varied greatly according to the types of SCAs, and further reinforce the importance of confirmatory prenatal diagnosis. The current study also supported the viewpoint that confined placental mosaicism and maternal mosaicism were the important etiology of false-positive results. *(J Mol Diagn 2020, 22: 1469—1475; https://doi.org/10.1016/j.jmoldx.2020.09.008)*

Since 2011, noninvasive prenatal testing (NIPT) based on massively parallel sequencing of cell-free fetal DNA in maternal blood has been introduced internationally for prenatal screening of trisomy 21, 18, and 13 (T21, T18, and T13, respectively). Although both are maternal peripheral blood tests, the powerful benefits of NIPT compared with traditional serologic screening are obvious.[1,2] With reduced costs of testing and growing numbers of studies demonstrating the accuracy of NIPT in the general obstetric population,[3–5] NIPT is recommended for all pregnant women.[6,7]

In China, NIPT is generally recommended for women at high risk of common chromosomal aneuploidies, such as advanced maternal age (AMA), abnormal serologic screening results, and some minor ultrasound indicators related to T21. Nowadays, as more and more low-risk pregnant women are willing to choose NIPT,[8,9] it has become the preferred method to screen for fetal chromosome abnormalities.[10] Given that NIPT is a highly reliable screening test, positive results are often associated with persistent anxiety among patients. Some of them mistakenly assume that this testing is diagnostic and proceed to pregnancy termination without confirmatory diagnostic testing.[11] Because cell-free fetal DNA is of placental origin and not of fetal origin, it is truly a screening test. The question following a positive NIPT is the likelihood of having an

Supported by the Research Fund Project of Fujian Provincial Maternity and Children's Hospital grant 2018-32 (X.W.); Youth Project of Fujian Provincial Health Commission grant 2017117 (S.D.); and Hospital Project of Fujian Maternal and Child Health Hospital grant 17-15 (S.D.).

Disclosures: None declared.

https://doi.org/10.1016/j.jmoldx.2020.09.008

affected fetus, which might be answered by the positive predictive value (PPV). All the types of chromosomal abnormalities detected by NIPT and their confirmatory results by traditional karyotyping or chromosomal microarray analysis in singleton pregnancies at high or low risk for chromosomal aneuploidy were retrospectively explored. The current research was mainly focused on assessing PPV for pregnant women with different indications and different age levels and analyzing the decision-making after a confirmed abnormality. It is hoped that the results will provide more practical evidence to aid clinicians in counseling patients regarding NIPT-positive results.

Materials and Methods

Patients and Samples

This retrospective study reviewed 563 pregnant women with NIPT-positive results who were referred to the Fujian Provincial Prenatal Diagnosis Center (Fuzhou, China) between May 2015 and December 2019. Twelve cases were excluded as they were twin pregnancies. As a result, 551 cases were enrolled. The clinical characteristics are summarized in Table 1. The mean values of gestational age at the time of NIPT and prenatal invasive testing were 16.6 ± 2.9 and 22.0 ± 3.3 weeks, respectively. The mean age at delivery was 31.7 ± 5.3 years. Women of AMA accounted for 41% (226/551), including ages between 35 and 39 years ($N = 187$) and ages beyond 40 years ($N = 39$). The remaining 59% (325/551) cases were women at young maternal age (YMA), who were categorized into three groups: <25 years old ($N = 56$), between 25 and 29 years old ($N = 149$), and between 30 and 34 years old ($N = 123$). A total of 107 cases of younger women demonstrated multiple clinical indications, including abnormal serologic screening results (high- and critical-risk results), soft ultrasound markers, ultrasound structural abnormalities, and adverse reproductive history. Specimens for confirmation were obtained from amniotic fluid during 18 and 24 gestational weeks, and fetal cord blood beyond 24 gestational weeks.

Follow-up information was obtained through clinical records or telephone calls. The study was approved by the local Ethics Committee of Fujian Provincial Maternity and Children's Hospital. Written informed consent to participate in the study was obtained from each patient.

NIPT Data Sources

Data regarding NIPT-positive cases were obtained from different NIPT platforms, which were all certified by Chinese Food and Drug Administration for high-throughput sequencing, including NextSeq CN500 sequencer (Berry Genomics Corp., Beijing, China), NextSeq AR550 sequencer (Annoroad Gene Tech Co., Ltd., Beijing, China), BGISeq 500 sequencer (MGI Tech Co., Ltd., Shenzhen, China), and Ion Proton sequencer (Da An Gene Co., Ltd., Shenzhen, China). Most cases in the current study were investigated by NextSeq CN500 sequencer.

Table 1 Clinical Characteristic of 551 Pregnancies with NIPT-Positive Results

Variable	Value
Gestational age, mean ± SD, weeks	
At delivery	22.0 ± 3.3
At NIPT	16.6 ± 2.9
At prenatal invasive testing	31.7 ± 5.3
Specimens, *n* (%)	
Amniotic fluid	471 (85.5)
Cord blood	80 (14.5)
Clinical features, *n* (%)	
Advanced maternal age	226 (41.0)
Abnormal serologic screening	57 (10.3)
Ultrasound structural abnormalities	16 (2.9)
Soft ultrasound markers	30 (5.4)
No indications	218 (39.6)
Adverse reproductive history	4 (0.7)

NIPT, noninvasive prenatal testing.

Confirmatory Invasive Prenatal Testing and Data Analysis

All samples of amniotic fluid and fetal cord blood were analyzed using traditional karyotyping, and chromosomal microarray analysis was also offered to pregnant women who consented. Single-nucleotide polymorphism array was performed using Affymetrix CytoScan 750K array (Affymetrix Inc., Santa Clara, CA). The experimental methods and processes were similar to those described in previous reports.[12] At least five metaphases were analyzed, and 20 metaphases were counted. When mosaicism was encountered, 50 to 100 metaphases were counted. PPV, referring to the proportion of true positives, was assessed to determine the likelihood that a positive result indicates an affected fetus. For segmental imbalances, the resolution of NIPT was set as 10 Mb, and the copy number variations (CNVs) were divided into groups with CNV sizes <10 and ≥10 Mb.

Statistical Analysis

All data were entered into a Microsoft Excel 2016 (Microsoft Corp., Redmond, WA) spreadsheet, and SPSS software version 19.0 (SPSS, Inc., Chicago, IL) was used for statistical analysis. Statistical comparisons were performed using χ^2 test, and $P < 0.05$ was considered statistically significant.

Results

Overall NIPT and Verification Results

A total of 442 samples of amniotic fluid and 109 samples of fetal cord blood were collected from the 551 fetuses

Table 2 The Details of 256 Abnormal Results Confirmed by Karyotype Analysis and/or CMA

		TP			Outcome		
Type	NIPT positive, *n*	*n* (%)	Type	*n*	TOP, *n* (%)*	Live birth, *n* (%)*	Lost to follow-up, *n* (%)
T21	150	122 (81.9)	Standard T21	108	107 (99.1)	1 (0.9)	0
			Translocation T21	3	3 (100)	0	0
			Mosaic T21	11	9 (90.0)	1 (10.0)	1 (9.1)
T18	52	18 (34.6)	Standard T18	17	17 (100)	0	0
			Rearrangement T18	1	1 (100)	0	0
T13	36	9 (25.0)	Standard T13	7	7 (100)	0	0
			Translocation T13	2	2 (100)	0	0
SCAs	258	97 (37.6)	45,X	2	2 (100)	0	0
			47,XXX	16	5 (33.3)	10 (66.7)	1 (0.6)
			47,XXY	31	26 (92.9)	2 (7.1)	3 (9.7)
			47,XYY	22	19 (90.5)	2 (9.5)	1 (4.5)
			48,XXYY	1	1 (100)	0	0
			Mosaicism	23	13 (56.5)	10 (43.5)	0
			46,del(X)	2	2 (100)	0	0
Multiple aneuploidy	10	1 (10.0)	48,XXY,inv(9) (p12q13),+18	1	1 (100)	0	0
RAA	19	1 (5.3)	Mosaic trisomy 9	1	1 (100)	0	0
Structural abnormality	26	8 (30.7)	CNVs $\geq$ 10 Mb	6	4 (80.0)	1 (20.0)	1 (16.7)
			CNVs $<$ 10 Mb	2	0	2 (100)	0

*Cases lost to follow-up were not included.

CMA, chromosomal microarray analysis; CNV, copy number variation; NIPT, noninvasive prenatal testing; RAA, rare autosomal aneuploidy; SCA, sex chromosomal aneuploidy; T13, trisomy 13; T18, trisomy 18; T21, trisomy 21; TP, true positive; TOP, termination of pregnancy.

enrolled. Of these, traditional karyotyping alone was performed on 200 samples, whereas on the remaining 351 samples, chromosomal microarray analysis was performed concurrently. A total of 256 fetuses were confirmed to possess chromosomal abnormalities concordant or partially concordant with NIPT results (common inversion anomalies and polymorphism changes were not considered), composed of 122 (47.8%) cases of T21, 18 (7.1%) cases of T18, 9 (3.5%) cases of T13, 97 (37.6%) cases of sex chromosomal abnormalities, 8 (2.7%) cases of structural abnormalities, and 1 (0.4%) cases of rare autosomal aneuploidy. The details are summarized in Table 2. The PPVs for T21, T18, T13, and sex chromosomal aneuploidies (SCAs) were 81.3% (122/150), 34.6% (18/52), 25.0% (9/36), and 37.6% (97/258), respectively. Among the 26 cases with chromosomal structural abnormalities, 6 fetuses with CNVs $\geq$10 Mb and 2 cases with CNVs $<$10 Mb were confirmed.

To explore the etiology of false-positive results, maternal blood tests were performed for 10 cases with positive results of SCAs, and maternal mosaicism was confirmed in 4 cases. In addition, placental biopsies were obtained after delivery or pregnancy termination for 10 of 295 fetuses with normal karyotype, and confined placental mosaicism (CPM) was confirmed in 6 cases. Among them, four CPMs involving chromosomes 9, 13, 21, and 22 manifested as fetal growth restriction with or without other ultrasound findings; the remaining two cases had normal development during the 15 months of follow-up.

PPVs for T21, T18, T13, and SCAs, according to Maternal Age at Delivery

The verification results of T21, T18, T13, and SCA positive cases for pregnant women aged $<$25, 25 to 29, 30 to 34, 35 to 39, and $>$40 years are shown in Table 3; those aged $>$40 years had the highest PPV for T21, T18, and SCAs, but there was no significant positive association between PPV and specific age categories, especially for T21 and SCAs (Figure 1). However, the PPVs for T21 and T18 in YMA pregnancies was significantly lower than those in AMA pregnancies [T21: 73.5% versus 87.8% ($P < 0.05$); T18: 8.0% versus 59.3% ($P < 0.05$)]; the PPVs for T13 and SCAs in these two groups showed no statistical differences [T13: 15.0% versus 37.5% ($P > 0.05$); SCAs: 39.2% versus 34.1% ($P > 0.05$)].

PPVs for T21, T18, T13, and SCAs, according to Indication

The enrolled cases included pregnancies with no indications and pregnancies with AMA, abnormal serologic screening (including high and critical risk of serologic screening), ultrasound structural abnormalities, and soft ultrasound markers. Numbers of NIPT-positive case in these groups and PPVs for T21, T18, T13, and SCAs are shown in Table 4. The PPV in the ultrasound structural abnormality group was the highest in predicting T21 fetuses, which was 100%, followed by 87.8% in AMA group. The AMA group

Table 3 Performance of NIPT for T21, T18, T13, and SCAs in Pregnancies with Different Age Groups

Age category, years	T21		T18		T13		SCAs	
	NIPT positive, *n*	TP, *n* (PPV, %)	NIPT positive, *n*	TP, *n* (PPV, %)	NIPT positive, *n*	TP, *n* (PPV, %)	NIPT positive, *n*	TP, *n* (PPV, %)
YMA								
<25	16	14 (87.5)	9	1 (11.1)	2	0 (0)	21	9 (42.9)
25–29	30	24 (80.0)	11	1 (9.1)	9	1 (11.1)	76	23 (30.3)
30–34	22	12 (54.5)	5	0 (0)	9	2 (22.2)	73	35 (47.9)
Total	68	50 (73.5)	25	2 (8.0)	20	3 (15.0)	170	67 (39.4)
AMA								
35–39	63	53 (84.1)	22	12 (54.5)	15	6 (40.0)	78	24 (30.8)
≥40	19	19 (100)	5	4 (80.0)	1	0 (0)	10	6 (60.0)
Total	82	72 (87.8)*	27	16 (59.3)*	16	6 (37.5)	88	30 (34.1)*

*$P < 0.05$ compared with same group YMA via χ^2 test.

AMA, advanced maternal age; NIPT, noninvasive prenatal testing; PPV, positive predictive value; SCA, sex chromosomal aneuploidy; T13, trisomy 13; T18, trisomy 18; T21, trisomy 21; TP, true positive; YMA, young maternal age.

had the highest PPV for T18 (57.7%) and T13 (37.5%). Even though cases with various high-risk indications were excluded, no statistical difference in PPVs for T13 and SCAs between YMA and AMA pregnancies were observed; the PPVs for T21 and T18 in YMA pregnancies remained significantly lower than those in AMA group.

Pregnancy Outcomes

Follow-up was obtained in 95.5% (527/551) of the patients. The outcomes of fetuses with confirmed abnormalities are presented in Table 2. Of the 149 pregnancies with confirmed T13, T18, and T21, one case of T21 was lost to follow-up; the remaining cases ended in termination except for one case of standard T21 and one case of low-level mosaic T21. Of the patients with a positive prenatal diagnosis for SCAs (mosaicism included), 55.4% (51/92) elected to terminate. The termination rates for pregnancies with confirmed 45,X, 47,XXY, 47,XXX, and 47,XYY were 100% (2/2), 92.9% (26/28), 33.3% (5/15), and 9.5% (2/21), respectively. No significantly abnormal phenotype was observed for the surviving infants during the short time follow-up, except for the cases of standard T21 who showed typical features of Down syndrome.

Discussion

Almost all the publications regarding the performance of NIPT involved only one or two NIPT platforms. Although the accuracy of NIPT varies somewhat with the platform used,[13] in actual clinical practice, as long as the results come from qualified and approved institutions, counseling should be conducted on the basis of their combined risk, and further diagnostic testing should be offered according to the gestational age, irrespective of the platform used. The study center is a tertiary referral prenatal diagnostic center; thus, many pregnant women with NIPT-positive results seek appropriate genetic counseling for pregnancy management. The information provided in present study may be more helpful for clinical consultants.

Overall, the PPVs for T21, T13, and SCAs in the present study were close to those reported in recent studies with large sample sizes.[9,14,15] But for T18, the PPV of 34.6% was considerably lower than those documented in recent studies, which ranged from 54.5% to 77.8%. This may be due to the age composition of the enrolled cases. In the current study, women of YMA with no clinical indication accounted for 39.6%, which is almost equal to that in women with AMA. This situation reflected the increase in uptake of NIPT in the low-risk population to some extent, as suggested by Chen et al.[16] On comparing the detection rates between women of YMA and AMA, the total PPV for T21 and T18 for women with AMA was found to be significantly higher than that in women with YMA, regardless of whether other indications were considered or not; however, for T13 and SCAs, there was no significant difference, which emphasized the age-specific risk for T18 and T21.[17] However, no significant upward trend of PPVs was observed when pregnant women were subgrouped with an age interval of 5 years. This further suggests that NIPT-positive results should receive

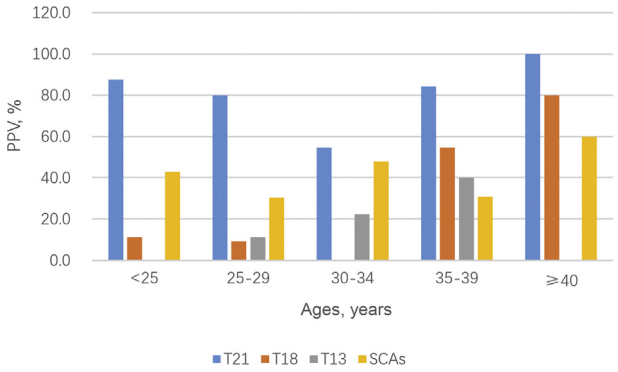

Figure 1 Comparison of the positive predictive values (PPVs) for trisomy 21 (T21), trisomy 18 (T18), trisomy 13 (T13), and sex chromosomal aneuploidies (SCAs) among different age categories.

Table 4 Performance of NIPT for T21, T18, T13, and SCAs in Pregnancies with Different Clinical Indications

	T21		T18		T13		SCAs	
Indications	NIPT positive, *n*	TP, *n* (PPV, %)	NIPT positive, *n*	TP, *n* (PPV, %)	NIPT positive, *n*	TP, *n* (PPV, %)	NIPT positive, *n*	TP, *n* (PPV, %)
None (YMA)	32	23 (71.9)	17	0 (0)	12	2 (16.7)	122	49 (40.2)
AMA	82	72 (87.8)*	27	16 (59.3)*	16	6 (37.5)	88	30 (34.1)
Abnormal serologic screening	16	12 (75.0)	4	1 (25.0)	5	1 (20)	30	11 (36.7)
Ultrasound structural abnormalities	5	5 (100)	0		1	0 (0)	7	3 (42.9)
Soft ultrasound markers	13	10 (76.9)	4	1 (25.0)	2	0 (0)	9	3 (33.3)
Reproductive history of children with chromosomal aneuploidy	2	1 (50.0)	0		0		2	1 (50.0)
Total	150	122 (81.3)	52	18 (34.6)	36	9 (25)	258	97 (37.6)

*$P < 0.05$ versus same group YMA via χ^2 test.

AMA, advanced maternal age; NIPT, noninvasive prenatal testing; PPV, positive predictive value; SCA, sex chromosomal aneuploidy; T13, trisomy 13; T18, trisomy 18; T21, trisomy 21; TP, true positive; YMA, young maternal age.

the same attention regardless of maternal age. Although NIPT was not frequently recommended for fetuses with abnormal ultrasound findings, some pregnant women considered NIPT to be an acceptable alternative to invasive diagnostic testing. In the present study, the ultrasound structural abnormality group and soft ultrasound markers group demonstrated the highest PPVs of 100% and 76.9%, respectively, in predicting T21 fetuses, which indicated that a positive NIPT result for T21 combined with abnormal ultrasound findings increases the possibility of an affected fetus.

Rare autosomal aneuploidies were the most common findings using genome-wide NIPT analysis in addition to targeted chromosomal aneuploidies,[18,19] and they were always confirmed to be false positives. He et al[20] suggested that the PPV and pregnancy outcomes might be influenced by the duration of gestation at NIPT as well as the indications; the later the gestation stage during NIPT testing, the less likely the fetus was affected. In the study province, NIPT is usually performed at a relatively late gestation stage at around 14 weeks, compared with 10 weeks[19] or 12 weeks[20] in other reports. Among 19 cases with rare autosomal aneuploidy–positive results, only one case of true fetal mosaicism was identified; one fetus developing fetal growth restriction was finally confirmed to be CPM. In addition, the efficiency for CNV detection, which has recently gained much attention, was also analyzed. The PPV for CNVs (30.8%) was similar to those reported in previous publications.[14,21–26] The PPVs for CNVs <10 Mb and CNVs ≥10 Mb were 28.6% and 31.6%, respectively; these values were close to those of T13, T18, and SCAs, which potentially demonstrated the accuracy of the test for fetal CNVs. Xu and Li[27] suggested that for most women with no indications for an invasive procedure, no routine methods are available for pathogenic CNV screening, but NIPT can be considered in these cases. However, the utilization of NIPT for CNVs remains controversial, and its routine implementation is not recommended to date.[28,29]

False-positive NIPT results can be explained by CPM,[19,30] death of a twin *in utero*,[31] or maternal incidental findings.[32] In the present study, 295 of 442 cases were confirmed to be false positive; however, only 10 cases of placental biopsies were available, and 6 of them were confirmed to have CPM. Notably, four cases of CPM involved trisomy 9, 13, 21, and 22, manifested as fetal growth restriction, which supports the view that CPM involving some specific chromosomes may be related to poor perinatal outcomes.[33–36]

It is recommended that patients with a positive NIPT for autosomal aneuploidies elect prenatal diagnosis, and almost all of the confirmed positive cases choose to terminate the pregnancy.[37] As for the SCAs, there may be some differences. Ramdaney et al[38] reported that nearly 65.7% of patients with a positive NIPT for SCAs declined prenatal diagnosis. In the present study, all the NIPT-positive cases that received post-test counseling by a genetic counselor were willing to perform invasive prenatal testing, consistent with some other studies.[39,40] It is known that individuals with SCAs are phenotypically normal in the neonatal stage without physical or intellectual disability; as a result, pregnancies with confirmed SCAs were less likely to be terminated. The termination rate of 55.4% for cases with confirmed SCAs in this study was relatively lower than the 61.1% to 81% reported in previous studies.[38,41,42] The leading factors for the variation can be attributed to the type of SCAs, maternal age, and opinion of the genetic consultant. Thus, confirmatory invasive prenatal testing is of great importance to determine the type of SCAs. On one hand,

pregnancy decisions should be made on the basis of a definite result for the fetus. On the other hand, prenatal diagnosis of SCAs may provide an opportunity for early intervention and comprehensive postnatal management, improving the quality of life of the affected child.

There are a few limitations to the present study. Studies on placentas and maternal karyotypes were not routinely conducted to access the etiology of discordance between positive cell-free fetal DNA results and normal karyotype. In addition, postnatal blood testing was not routinely performed for infants who were prenatally identified as mosaicism to further assess their prognosis.

Conclusion

The present study demonstrated that NIPT performed better in predicting T21 and T18 for pregnancies with AMA than for pregnancies with YMA; however, PPVs of T21, T18, T13, and SCAs showed no significant upward trends when compared on the basis of more specific age grouping. The decision-making regarding pregnancies varied greatly according to the types of SCAs, which reinforces the importance of confirmatory test after an NIPT-positive result for SCAs.

Acknowledgments

We thank Min Zhang, Yan Wang, and Lingji Chen for technical support on single-nucleotide polymorphism array analysis.

References

1. Costa JM, Letourneau A, Favre R, Bidat L, Belaisch-Allart J, Jouannic JM, Quarello E, Senat MV, Broussin B, Tsatsaris V, Demain A, Kleinfinger P, Lohmann L, Agostini H, Bouyer J, Benachi A: Cell-free fetal DNA versus maternal serum screening for trisomy 21 in pregnant women with and without assisted reproduction technology: a prospective interventional study. Genet Med 2018, 20: 1346–1353
2. Nshimyumukiza L, Menon S, Hina H, Rousseau F, Reinharz D: Cell-free DNA noninvasive prenatal screening for aneuploidy versus conventional screening: a systematic review of economic evaluations. Clin Genet 2018, 94:3–21
3. Gregg AR, Gross SJ, Best RG, Monaghan KG, Bajaj K, Skotko BG, Thompson BH, Watson MS: ACMG statement on noninvasive prenatal screening for fetal aneuploidy. Genet Med 2013, 15:395–398
4. Liao C, Zhengfeng X, Zhang K: DNA sequencing versus standard prenatal aneuploidy screening. N Engl J Med 2014, 371:577–578
5. Fairbrother G, Johnson S, Musci TJ, Song K: Clinical experience of noninvasive prenatal testing with cell-free DNA for fetal trisomies 21, 18, and 13, in a general screening population. Prenat Diagn 2013, 33: 580–583
6. Benn P, Borrell A, Chiu RW, Cuckle H, Dugoff L, Faas B, Gross S, Huang T, Johnson J, Maymon R, Norton M, Odibo A, Schielen P, Spencer K, Wright D, Yaron Y: Position statement from the Chromosome Abnormality Screening Committee on behalf of the Board of the International Society for Prenatal Diagnosis. Prenat Diagn 2015, 35:725–734
7. Gregg AR, Skotko BG, Benkendorf JL, Monaghan KG, Bajaj K, Best RG, Klugman S, Watson MS: Noninvasive prenatal screening for fetal aneuploidy, 2016 update: a position statement of the American College of Medical Genetics and Genomics. Genet Med 2016, 18:1056–1065
8. Hu H, Liu H, Peng C, Deng T, Fu X, Chung C, Zhang E, Lu C, Zhang K, Liang Z, Yang Y: Clinical experience of non-invasive prenatal chromosomal aneuploidy testing in 190,277 patient samples. Curr Mol Med 2016, 16:759–766
9. Xu L, Huang H, Lin N, Wang Y, He D, Zhang M, Chen M, Chen L, Lin Y: Non-invasive cell-free fetal DNA testing for aneuploidy: multicenter study of 31 515 singleton pregnancies in southeastern China. Ultrasound Obstet Gynecol 2020, 55:242–247
10. Zhang H, Gao Y, Jiang F, Fu M, Yuan Y, Guo Y, Zhu Z, Lin M, Liu Q, Tian Z, Zhang H, Chen F, Lau TK, Zhao L, Yi X, Yin Y, Wang W: Non-invasive prenatal testing for trisomies 21, 18 and 13: clinical experience from 146,958 pregnancies. Ultrasound Obstet Gynecol 2015, 45:530–538
11. Dar P, Curnow KJ, Gross SJ, Hall MP, Stosic M, Demko Z, Zimmermann B, Hill M, Sigurjonsson S, Ryan A, Banjevic M, Kolacki PL, Koch SW, Strom CM, Rabinowitz M, Benn P: Clinical experience and follow-up with large scale single-nucleotide polymorphism-based noninvasive prenatal aneuploidy testing. Am J Obstet Gynecol 2014, 211:527.e1–527.e17
12. Wu X, An G, Xie X, Su L, Cai M, Chen X, Li Y, Lin N, He D, Wang M, Huang H, Xu L: Chromosomal microarray analysis for pregnancies with or without ultrasound abnormalities in women of advanced maternal age. J Clin Lab Anal 2020, 34:e23117
13. Society for Maternal-Fetal Medicine Publications Committee: Electronic address PSO, prenatal aneuploidy screening using cell-free DNA. Am J Obstet Gynecol 2015, 212:711–716
14. Chen Y, Yu Q, Mao X, Lei W, He M, Lu W: Noninvasive prenatal testing for chromosome aneuploidies and subchromosomal microdeletions/microduplications in a cohort of 42,910 single pregnancies with different clinical features. Hum Genomics 2019, 13:60
15. Xue Y, Zhao G, Li H, Zhang Q, Lu J, Yu B, Wang T: Non-invasive prenatal testing to detect chromosome aneuploidies in 57,204 pregnancies. Mol Cytogenet 2019, 12:29
16. Chen KM, White K, Shabbeer J, Schmid M: Maternal age trends support uptake of non-invasive prenatal testing (NIPT) in the low-risk population. J Matern Fetal Neonatal Med 2019, 32:4039–4042
17. Yamada T, Sekizawa A, Fujii Y, Hirose T, Samura O, Suzumori N, Miura K, Sawai H, Hirahara F, Murotsuki J, Kamei Y, Sago H; Japan NIPT consortium: Maternal age-specific risk for trisomy 21 based on the clinical performance of NIPT and empirically derived NIPT age-specific positive and negative predictive values in Japan. J Hum Genet 2018, 63:1035–1040
18. Bianchi DW, Chiu RWK: Sequencing of circulating cell-free DNA during pregnancy. N Engl J Med 2018, 379:464–473
19. Pertile MD, Halks-Miller M, Flowers N, Barbacioru C, Kinnings SL, Vavrek D, Seltzer WK, Bianchi DW: Rare autosomal trisomies, revealed by maternal plasma DNA sequencing, suggest increased risk of feto-placental disease. Sci Transl Med 2017, 9: eaan1240
20. He Y, Liu YH, Xie RG, Liu SA, Li DZ: Rare autosomal trisomies on non-invasive prenatal testing: not as adverse as expected. Ultrasound Obstet Gynecol 2019, 54:838–839
21. Liang D, Cram DS, Tan H, Linpeng S, Liu Y, Sun H, Zhang Y, Tian F, Zhu H, Xu M, Wang H, Yu F, Wu L: Clinical utility of noninvasive prenatal screening for expanded chromosome disease syndromes. Genet Med 2019, 21:1998–2006
22. Gross SJ, Stosic M, Mcdonald-Mcginn DM, Bassett AS, Norvez A, Dhamankar R, Kobara K, Kirkizlar E, Zimmermann B, Wayham N, Babiarz JE, Ryan A, Jinnett KN, Demko Z, Benn P: Clinical experience with single-nucleotide polymorphism-based non-invasive prenatal screening for 22q11.2 deletion syndrome. Ultrasound Obstet Gynecol 2016, 47:177–183

23. Helgeson J, Wardrop J, Boomer T, Almasri E, Paxton WB, Saldivar JS, Dharajiya N, Monroe TJ, Farkas DH, Grosu DS, Mccullough RM: Clinical outcome of subchromosomal events detected by whole-genome noninvasive prenatal testing. Prenat Diagn 2015, 35:999−1004
24. Sahoo T, Hovanes K, Strecker MN, Dzidic N, Commander S, Travis MK: Expanding noninvasive prenatal testing to include microdeletions and segmental aneuploidy: cause for concern? Genet Med 2016, 18:275−276
25. Petersen AK, Cheung SW, Smith JL, Bi W, Ward PA, Peacock S, Braxton A, Van Den Veyver IB, Breman AM: Positive predictive value estimates for cell-free noninvasive prenatal screening from data of a large referral genetic diagnostic laboratory. Am J Obstet Gynecol 2017, 217:691.e1−691.e6
26. Martin K, Iyengar S, Kalyan A, Lan C, Simon AL, Stosic M, Kobara K, Ravi H, Truong T, Ryan A, Demko ZP, Benn P: Clinical experience with a single-nucleotide polymorphism-based non-invasive prenatal test for five clinically significant microdeletions. Clin Genet 2018, 93:293−300
27. Xu LL, Li DZ: Clinical utility of noninvasive prenatal screening for pathogenic copy number variants. Am J Obstet Gynecol 2019, 221: 660
28. Di Renzo GC, Bartha JL, Bilardo CM: Expanding the indications for cell-free DNA in the maternal circulation: clinical considerations and implications. Am J Obstet Gynecol 2019, 220:537−542
29. Committee opinion No. 640: cell-free DNA screening for fetal aneuploidy. Obstet Gynecol 2015, 126:e31−e37
30. Xu Y, Chen L, Liu Y, Hao Y, Xu Z, Deng L, Xie J: Screening, prenatal diagnosis, and prenatal decision for sex chromosome aneuploidy. Expert Rev Mol Diagn 2019, 19:537−542
31. Curnow KJ, Wilkins-Haug L, Ryan A, Kirkizlar E, Stosic M, Hall MP, Sigurjonsson S, Demko Z, Rabinowitz M, Gross SJ: Detection of triploid, molar, and vanishing twin pregnancies by a single-nucleotide polymorphism-based noninvasive prenatal test. Am J Obstet Gynecol 2015, 212:79.e1−79.e9
32. Bianchi DW: Cherchez la femme: maternal incidental findings can explain discordant prenatal cell-free DNA sequencing results. Genet Med 2018, 20:910−917
33. Chen CP, Tsai C, Lin MH, Chern SR, Chen SW, Lai ST, Chen WL, Pan CW, Wang W: Application of non-invasive prenatal testing in late gestation in a pregnancy associated with intrauterine growth restriction and trisomy 22 confined placental mosaicism. Taiwan J Obstet Gynecol 2017, 56:691−693
34. Nagamatsu T, Kamei Y, Yamashita T, Fujii T, Kozuma S: Placental abnormalities detected by ultrasonography in a case of confined placental mosaicism for trisomy 2 with severe fetal growth restriction. J Obstet Gynaecol Res 2014, 40:279−283
35. Stipoljev F, Latin V, Kos M, Miskovic B, Kurjak A: Correlation of confined placental mosaicism with fetal intrauterine growth retardation: a case control study of placentas at delivery. Fetal Diagn Ther 2001, 16:4−9
36. Amiel A, Bouaron N, Kidron D, Sharony R, Gaber E, Fejgin MD: CGH in the detection of confined placental mosaicism (CPM) in placentas of abnormal pregnancies. Prenat Diagn 2002, 22:752−758
37. Dobson LJ, Reiff ES, Little SE, Wilkins-Haug L, Bromley B: Patient choice and clinical outcomes following positive noninvasive prenatal screening for aneuploidy with cell-free DNA (cfDNA). Prenat Diagn 2016, 36:456−462
38. Ramdaney A, Hoskovec J, Harkenrider J, Soto E, Murphy L: Clinical experience with sex chromosome aneuploidies detected by noninvasive prenatal testing (NIPT): accuracy and patient decision-making. Prenat Diagn 2018, 38:841−848
39. Scibetta EW, Gaw SL, Rao RR, Silverman NS, Han CS, Platt LD: Clinical accuracy of abnormal cell-free fetal DNA results for the sex chromosomes. Prenat Diagn 2017, 37:1291−1297
40. Reiss RE, Discenza M, Foster J, Dobson L, Wilkins-Haug L: Sex chromosome aneuploidy detection by noninvasive prenatal testing: helpful or hazardous? Prenat Diagn 2017, 37:515−520
41. Shaffer BL, Caughey AB, Norton ME: Variation in the decision to terminate pregnancy in the setting of fetal aneuploidy. Prenat Diagn 2006, 26:667−671
42. Christian SM, Koehn D, Pillay R, Macdougall A, Wilson RD: Parental decisions following prenatal diagnosis of sex chromosome aneuploidy: a trend over time. Prenat Diagn 2000, 20:37−40