Ultrasound Obstet Gynecol 2015; **45**: 530–538
Published online 8 April 2015 in Wiley Online Library (wileyonlinelibrary.com). **DOI:** 10.1002/uog.14792

Non-invasive prenatal testing for trisomies 21, 18 and 13: clinical experience from 146 958 pregnancies

H. ZHANG*†#, Y. GAO*†#, F. JIANG*†, M. FU*†, Y. YUAN*†, Y. GUO*†, Z. ZHU*†, M. LIN*†, Q. LIU*†, Z. TIAN*‡, H. ZHANG*§, F. CHEN¶**, T. K. LAU††, L. ZHAO*†, X. YI*¶, Y. YIN*¶ and W. WANG*†

**BGI-Diagnostics, Shenzhen, China; †BGI Clinical Laboratories-Shenzen, Shenzhen, China; ‡BGI Clinical Laboratories-Tianjin, Tianjin, China; §BGI Clinical Laboratories-Wuhan, Wuhan, China; ¶BGI-Shenzen, Shenzhen, China; **Section of Molecular Disease Biology, Department of Veterinary Disease Biology, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark; ††Fetal Medicine Centre, Paramount Medical Centre, Hong Kong, China*

KEYWORDS: cell-free DNA; clinical performance; CNV; false negative; false positive; low-risk population; mosaicism; NIPT; trisomy

ABSTRACT

Objectives *To report the clinical performance of massively parallel sequencing-based non-invasive prenatal testing (NIPT) in detecting trisomies 21, 18 and 13 in over 140 000 clinical samples and to compare its performance in low-risk and high-risk pregnancies.*

Methods *Between 1 January 2012 and 31 August 2013, 147 314 NIPT requests to screen for fetal trisomies 21, 18 and 13 using low-coverage whole-genome sequencing of plasma cell-free DNA were received. The results were validated by karyotyping or follow-up of clinical outcomes.*

Results *NIPT was performed and results obtained in 146 958 samples, for which outcome data were available in 112 669 (76.7%). Repeat blood sampling was required in 3213 cases and 145 had test failure. Aneuploidy was confirmed in 720/781 cases positive for trisomy 21, 167/218 cases positive for trisomy 18 and 22/67 cases positive for trisomy 13 on NIPT. Nine false negatives were identified, including six cases of trisomy 21 and three of trisomy 18. The overall sensitivity of NIPT was 99.17%, 98.24% and 100% for trisomies 21, 18 and 13, respectively, and specificity was 99.95%, 99.95% and 99.96% for trisomies 21, 18 and 13, respectively. There was no significant difference in test performance between the 72 382 high-risk and 40 287 low-risk subjects (sensitivity, 99.21%* vs *98.97%* ($P = 0.82$); *specificity, 99.95%* vs *99.95%* ($P = 0.98$)). *The major factors contributing to false-positive and false-negative NIPT results were maternal copy number variant and fetal/placental mosaicism, but fetal fraction had no effect.*

Conclusions *Using a stringent protocol, the good performance of NIPT shown by early validation studies can be maintained in large clinical samples. This technique can provide equally high sensitivity and specificity in screening for trisomy 21 in a low-risk, as compared to high-risk, population.* Copyright © 2015 ISUOG. *Published by John Wiley & Sons Ltd.*

INTRODUCTION

Conventional prenatal screening for chromosomal abnormalities, mostly trisomy 21, relies exclusively on biochemical and sonographic measurements in the first and second trimesters. With a 5% false-positive rate (FPR), conventional screening achieves a detection rate of about 60–95% for trisomy 21[1–3]. In 2008, two studies described non-invasive prenatal testing (NIPT) for trisomy 21 by sequencing cell-free DNA (cfDNA) in maternal plasma, with very low FPRs[4,5]. Both studies implied that the test could reduce the incidence of unnecessary invasive procedures and iatrogenic fetal loss. Since then, NIPT has been developed to provide early and safe detection of fetal trisomy 21 and other common aneuploidies using shotgun sequencing, targeted sequencing and single nucleotide polymorphism (SNP)-based sequencing of cfDNA[6–14]. With solid evidence of NIPT performance in small-scale populations of predominantly high-risk women, various professional societies quickly suggested that NIPT could

Correspondence to: Dr W. Wang, BGI – Shenzhen, 11th Building, Beishan Industrial Zone, Yantian District, Shenzhen City, 518083 China (e-mail: wangw@genomics.cn)

#H.Z. and Y.G. contributed equally to this study.

Accepted: 9 January 2015

be considered as a second-tier screening test for women at increased risk for aneuploidy[15–17].

Recently, several studies have suggested that NIPT in the general population can offer a performance comparable to that in a high-risk population[18–20]. One study showed a high detection rate of trisomies 21 and 18 in 11 105 singleton pregnancies, of which more than 65% were high risk[20]. In another study, NIPT considerably improved the positive predictive value (PPV) compared to that of conventional screening in a small cohort of 1914 women from the general population[21]. However, these studies were conducted in populations with a small number of low-risk pregnancies, and evaluation of a large clinical dataset on NIPT performance in the general population is urgently needed.

NIPT has been offered as a screening test in China since 2011, and may soon be considered for routine use by local Chinese health authorities after the recent approval by the China Food and Drug Administration. However, clinical experience of large-scale NIPT performance in the general population is still lacking, despite the estimation that NIPT has been offered globally to over 500 000 women to date (Bianchi DW, pers. comm.).

The present study was based on the clinical data of 146 958 NIPT tests from mainland China, the largest such cohort to our knowledge. The main objective was to report NIPT performance in detecting trisomies 21, 18 and 13 for the purpose of a large-scale clinical service for quality assurance. Possible causes for false-positive and false-negative NIPT results were investigated. In a subset of samples with known clinical outcomes, the performance of NIPT at detecting trisomy 21 in high-risk and low-risk subjects was compared.

METHODS

This was a prospective, multicenter observational study with participants enrolled from 508 medical centers in mainland China between 1 January 2012 and 31 August 2013, excluding previously reported cases[20]. NIPT was offered to the participants as either primary or secondary screening. Since the objective of the study was to evaluate its performance in detecting trisomies 21, 18 and 13, other chromosomal abnormalities were screened for but not analyzed. Eligibility for NIPT included participants of at least 18 years old with a singleton or twin pregnancy at 9 weeks' gestation or beyond. All participants underwent pretest counseling, and informed written consent was obtained prior to blood sampling. Approval was obtained from the Institutional Review Board of BGI.

5 mL of peripheral blood was collected into tubes primed with ethylenediaminetetraacetic acid (EDTA) from each participant and samples were prepared for cfDNA sequencing as described previously[20]. In general, whole blood was centrifuged twice within 8 h of blood collection to extract the plasma. Plasma samples were then frozen and delivered to Ministry of Health-accredited and ISO/IEC17025-certified clinical laboratories of BGI-Health (Shenzhen, China) at which plasma was prepared for library construction, quality control and pooling. For DNA sequencing, 24 libraries were sequenced with 36-cycle single-end multiplex sequencing on Illumina HiSeq2000 platforms (Illumina, San Diego, CA, USA). A barcode tracking system was employed during sample preparation. Sequencing reads of 35 bases were trimmed and aligned to a universal unique read set, incised from the human reference genome (hg18, NCBI build 36). A binary hypothesis *t*-test and logarithmic likelihood ratio L-score between the two *t*-tests were used to classify fetal autosomal aneuploidy of trisomies 21, 18 and 13, as described previously[13,20]. The Fetal Copy-number Analysis through Maternal Plasma Sequencing (FCAPS) algorithm, which can be used to identify chromosome copy number variation (CNV) based on the binary segmentation algorithm, was implemented into the routine analytic pipeline[22].

Each participant received counseling after NIPT screening. Patients with a positive NIPT result were advised to undergo invasive testing for a prenatal diagnosis. If this was declined, the patient was monitored to record the outcome of the pregnancy. Routine antenatal care was provided to those with a negative NIPT result. Telephone interviews were performed 1 month after the expected date of delivery to obtain information on neonatal outcome, newborn physical examination or any cytogenetic testing results. To encourage reporting of false-positive and false-negative NIPT results, an insurance policy was provided for each participant as part of the test. The policy would reimburse patients for the cost of invasive tests in the case of a positive NIPT result, and would pay CNY 200 000 (approximately five times the GDP per capita in China in 2013) to each false-negative NIPT case that had aneuploidy confirmed.

Karyotyping or clinical follow-up results were used as the gold standard to calculate sensitivity and specificity of NIPT in this population, and 95% CIs were calculated on the assumption of a standard normal distribution.

Patients with NIPT outcome data were further divided into high-risk and low-risk groups. A patient with any of the following factors was classified as high risk for aneuploidy: advanced maternal age (> 35 years), a positive conventional Down syndrome screening test (cut-off 1/270 or 1/300, depending on individual hospital's criteria), abnormal sonographic markers, family history of aneuploidy or a previous pregnancy with a trisomic fetus. Patients with none of the high-risk factors were defined as low risk for aneuploidy. NIPT performance in the detection of trisomy 21 in these two groups was compared using karyotyping or follow-up results as gold standard. Owing to the small number of cases positive for trisomies 18 and 13 in the low-risk group, NIPT performance for these two trisomies was not compared between risk groups.

To identify the impact of cfDNA fetal fraction on a false-positive or negative NIPT result, cfDNA fetal fraction was estimated for samples with a false-positive or negative NIPT result, based on parent-specific homozygous SNP loci, as described previously[23]. Briefly,

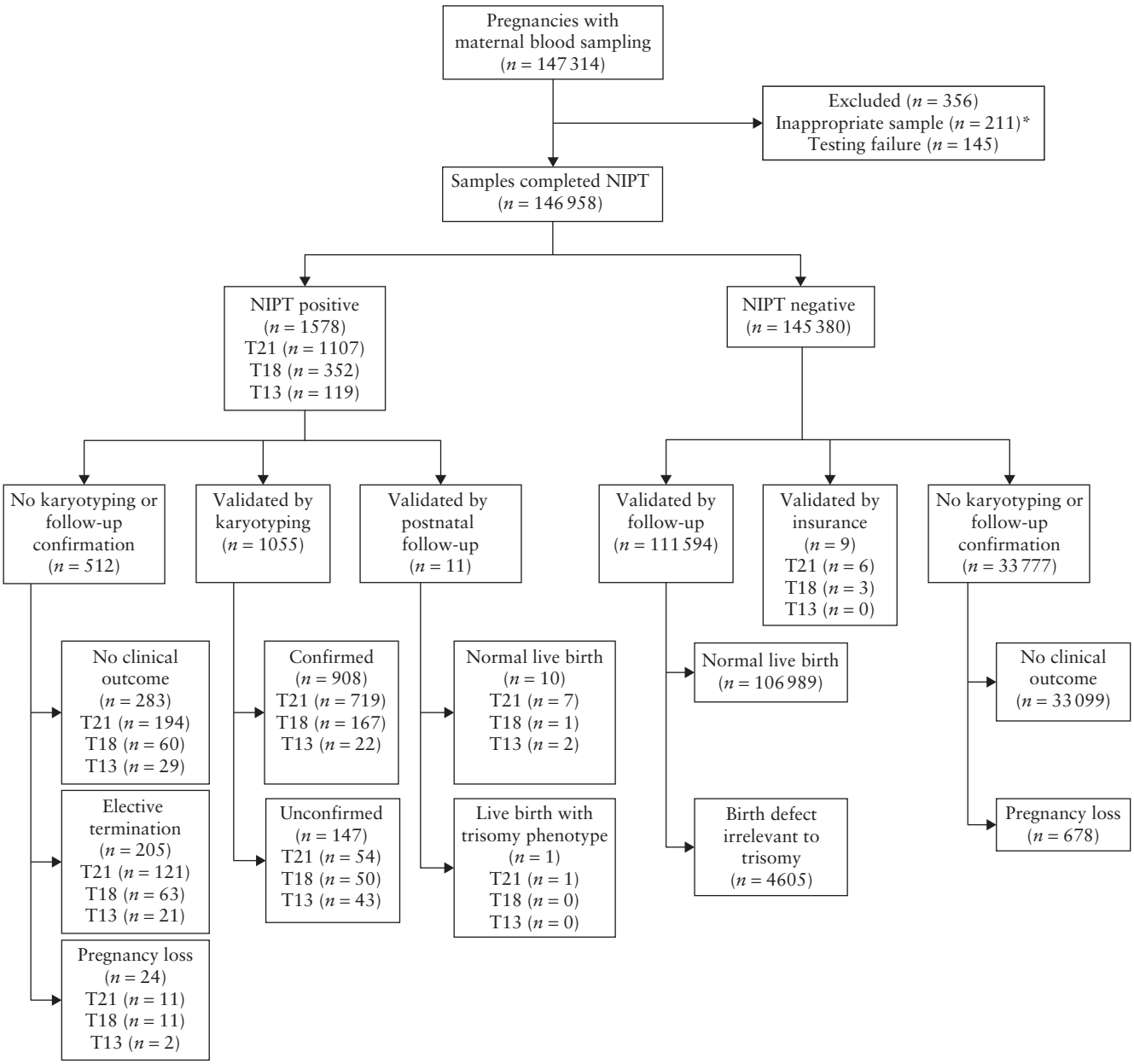

Figure 1 Flowchart of non-invasive prenatal test (NIPT) results and clinical outcome of pregnant women in mainland China undergoing screening for aneuploidies between 1 January 2012 and 31 August 2013. *Inadequate volume, contamination, sample obtained before 9 weeks' gestation or improper labeling. T, trisomy.

parent-specific homozygous loci in the form of ♀AA♂BB were selected. Then sequence reads from those loci were used to estimate the total fetal cfDNA concentration, using the formula $= \frac{2d\ (B)}{d\ (A)+d\ (B)}$, where d is the depth of the allele A or B.

RESULTS

Between 1 January 2012 and 31 August 2013, a total of 147 314 maternal blood samples were received for NIPT from 508 hospitals in mainland China. Of these samples, 211 (0.14%) were rejected for further processing owing to inadequate volume, contamination, having been obtained before 9 weeks' gestation or improper labeling (Figure 1). Repeat blood sampling was required in 3213 (2.18%) cases because of quality control failure, assay failure or low fetal fraction. Ultimately, 145 (0.098%) samples failed to provide informative results and were classified as a test failure.

Demographic characteristics of the remaining 146 958 cases are shown in Table 1, including 802 cases of twin pregnancy. Mean maternal age was 30.9 (range, 18–46) years and mean gestational age at NIPT was 18.7 (range, 9–36) weeks. Most (69.28%) of the samples were collected from women less than 35 years old and the vast majority (94.13%) of samples were collected during the second trimester.

Of the 146 958 samples in which an NIPT result was obtained, 1578 (1.07%) were positive, including 1107

Table 1 Demographic characteristics of pregnant women undergoing non-invasive prenatal testing (NIPT) for aneuploidies between 1 January 2012 and 31 August 2013

		Population with outcome data		
Characteristic	*Total population (n = 146 958)*	*Total (n = 112 669)*	*High risk (n = 72 382)*	*Low risk (n = 40 287)*
Maternal age (years)	30.9 (18–56)	31.0 (18–56)	32.5 (18–56)	28.3 (18–34)
18–24 years	15 668 (10.66)	11 579 (10.28)	5953 (8.22)	5626 (13.96)
25–29 years	47 228 (32.14)	37 071 (32.90)	18 264 (25.23)	18 807 (46.68)
30–34 years	38 921 (26.48)	30 146 (26.76)	14 297 (19.75)	15 849 (39.34)
35–40 years	39 784 (27.07)	30 121 (26.73)	30 121 (41.61)	—
> 40 years	5192 (3.53)	3743 (3.32)	3743 (5.17)	—
Unknown	165 (0.11)	9 (0.01)	4 (0.01)	5 (0.01)
GA at NIPT (wks)	18.7 (9–37)	18.0 (9–37)	18 (9–37)	19.4 (11–37)
First trimester (9–13 wks)	6188 (4.21)	5270 (4.68)	3085 (4.26)	2185 (5.42)
Second trimester (14–27 wks)	138 337 (94.13)	105 647 (93.77)	68 553 (94.71)	37 094 (92.07)
Third trimester (≥ 28 wks)	2165 (1.47)	1689 (1.50)	706 (0.98)	983 (2.44)
Unknown	268 (0.18)	63 (0.06)	38 (0.05)	25 (0.06)
Singleton pregnancy	146 156 (99.45)	112 265 (99.64)	NA	NA
Twin pregnancy	802 (0.55)	404 (0.36)	NA	NA
Previous DS screening result				
High risk	55 598 (37.83)	42 896 (38.07)	42 896 (59.26)	0
Low risk	31 503 (21.43)	24 536 (21.78)	5090 (7.03)	19 446 (48.27)
No test	59 857 (40.73)	45 237 (40.15)	24 396 (33.70)	20 841 (51.73)
Other high-risk factors				
Family history of aneuploidy	22 (0.01)	22 (0.02)	22 (0.03)	0
Sonographic markers of chromosomal abnormality	2366 (1.61)	2366 (2.10)	2366 (3.27)	0
Advanced maternal age	33 864 (23.04)	33 864 (30.06)	33 864 (46.79)	0

Data given as mean (range) or *n* (%). DS, Down syndrome; GA, gestational age; NA, not applicable; wks, weeks.

for trisomy 21, 352 for trisomy 18, and 119 for trisomy 13 (Figure 1). After post-test counseling, 1055 (66.86%) cases with a positive NIPT result underwent a prenatal diagnostic test, which confirmed 719 cases of trisomy 21, 167 of trisomy 18 and 22 of trisomy 13 by karyotyping, while 54, 50 and 43 cases of trisomies 21, 18 and 13, respectively, were unconfirmed (Figure 1).

Clinical outcomes of the remaining NIPT-positive cases without a prenatal diagnosis were obtained through neonatal physical examination after delivery and checking records of adverse pregnancy outcomes (Figure 1). Seven cases of trisomy 21, one of trisomy 18 and two of trisomy 13 were reported to have apparently normal phenotypes, while one case of trisomy 21 was reported to have a phenotype typical of trisomy 21 at birth. One hundred and twenty-one cases of trisomy 21, 63 of trisomy 18 and 21 of trisomy 13 underwent elective termination because of ultrasound abnormalities (increased nuchal translucency thickness, cardiac defects or malformation) and 11 cases of trisomy 21, 11 of trisomy 18 and two of trisomy 13 had a stillbirth or spontaneous miscarriage.

Data on pregnancy outcome were missing for 283 cases owing to loss of contact or because the women declined follow-up (Figure 1). Nonetheless, 232 (81.98%) of these cases had at least one high-risk indication for aneuploidy (advanced maternal age, previous high-risk Down syndrome screening result or abnormal ultrasound findings), which showed good consistency of NIPT in identifying positive cases among high-risk pregnancies (Table S1).

A total of 145 380 women had negative NIPT results, all of whom were contacted by telephone interviews 1 month after their expected date of delivery (Figure 1). Informative follow-up results were available for 111 594 cases, resulting in a successful follow-up rate of 76.76%; 106 989 cases had live births with normal neonatal examination results (Figure 1), including 405 cases who also received invasive diagnostic testing, mainly because of anxiety, and were confirmed to be normal. Another 4605 cases had neonates with birth defects confirmed as irrelevant to trisomies, including cleft palate, hearing-screening failure, metabolic disease and cardiac defects (Table S2). Pregnancy loss occurred in 678 cases, and 33 099 cases had no data on clinical outcome owing to loss of contact or because the women declined an interview.

Among all NIPT-negative cases, nine false-negative results were reported through our insurance program, all being confirmed by cytogenetic studies (Figure 1); six live births with phenotypes typical of trisomy 21 were reported shortly after birth and confirmed by the subsequent cytogenetic result. Three cases had termination of pregnancy owing to severe developmental malformations seen on ultrasound examination, and were later confirmed as trisomy 18 by cytogenetic examination of the products of conception.

Therefore, in this population of 146 958 pregnancies, cytogenetic or phenotypic confirmation of NIPT results was available in 1066 NIPT-positive cases and 111 603 NIPT-negative cases, collectively accounting for 76.67% of the population, including twin pregnancies. Further

Table 2 Performance of non-invasive prenatal testing (NIPT) in detecting trisomies (T) 21, 18 and 13 in pregnancies with outcome data and if results with unconfirmed outcomes were considered as false negative (FN) or false positive (FP)

Trisomy	*TP* (n)	*FP* (n)	*FN* (n)	*Sensitivity* (% (95% CI))	*Specificity* (% (95% CI))	*PPV* (% (95% CI))	*NPV* (% (95% CI))	*Incidence* (%)
Overall performance ($n = 112\,669$)								
T21	720	61	6	99.17 (98.52–99.83)	99.95 (99.93–99.96)	92.19 (90.31–94.07)	99.99 (99.99–100)	0.64
T18	167	51	3	98.24 (94.93–99.63)	99.95 (99.94–99.97)	76.61 (70.99–82.23)	100 (99.99–100)	0.15
T13	22	45	0	100 (84.56–100)	99.96 (99.95–99.97)	32.84 (21.59–44.08)	100 (99.99–100)	0.02
Total	909	157	9	99.02 (98.38–99.66)	99.86 (99.84–99.88)	85.27 (83.14–87.40)	99.99 (99.99–100)	0.81
Performance in twin pregnancies ($n = 404$)								
T21	5	2	0	100 (47.82–100)	99.50 (98.20–99.94)	71.43 (29.04–96.33)	100 (99.08–100)	1.24

Theoretical PPV boundaries *Condition*	*Trisomy 21 (% (n))*	*Trisomy 18 (% (n))*	*Trisomy 13 (% (n))*	*All trisomies (% (n))*
Lower boundary				
All unconfirmed cases considered as FP	65.04 (720/1107)	47.44 (167/352)	18.49 (22/119)	57.60 (909/1578)
Upper boundary				
All unconfirmed cases considered as TP	94.49 (1046/1107)	85.51 (301/352)	62.18 (74/119)	90.05 (1421/1578)

NPV, negative predictive value; PPV, positive predictive value; TP, true positive.

calculation of NIPT sensitivity and specificity was based on this subgroup of the population with outcome data available. Among this subgroup, there were 720 true-positive cases, 61 false-positive cases and six false-negative cases of trisomy 21, resulting in a sensitivity of 99.17% and specificity of 99.95% (Table 2). The FPR and PPV for trisomy 21 were 0.05% and 92.19%, respectively. There were 167 true-positive cases, 51 false-positive cases and three false-negative cases of trisomy 18, giving a sensitivity of 98.24% and specificity of 99.95%. The FPR and PPV for trisomy 18 were 0.05% and 76.61%, respectively. There were 22 true-positive cases, 45 false-positive cases and no false-negative cases of trisomy 13, giving a sensitivity of 100% and specificity of 99.96%. The FPR and PPV for trisomy 13 were 0.04% and 32.84%, respectively. The overall sensitivity and specificity for detecting these three chromosomal anomalies combined were 99.02% and 99.86%, respectively, and the overall FPR and PPV were 0.14% and 85.27%, respectively. The incidence of trisomies 21, 18 and 13 were 0.64%, 0.15% and 0.02%, respectively. A theoretical PPV was also calculated under the two boundary conditions that all unproven NIPT-positive cases were either assumed to be a true positive or a false positive (Table 2). This provided the range of PPV for trisomies 21, 18 and 13 as 65–95%, 47–86% and 19–62%, respectively.

In this cohort of pregnancies, 802 twin pregnancies were included, of which 404 had outcome data available. Seven cases had a positive NIPT result for trisomy 21 and 795 cases had a negative NIPT result. No cases of trisomy 18 or 13 were detected. Confirmation by karyotyping revealed five true-positive cases and two false-positive cases, and follow-up results were available in 397 NIPT-negative cases, all with normal phenotypes (Table 2). Hence in twin pregnancies, detection of trisomy 21 by NIPT had a sensitivity of 100% and specificity of 99.50%.

In the samples with outcome data, 72 382 cases were classified as high risk for aneuploidy and the remaining 40 287 cases were classified as low risk (Table 1). The performance of NIPT in detecting trisomy 21 is compared in both groups in Table 3. Six hundred and twenty-four true-positive cases, 39 false-positive cases, and five false-negative cases were observed in the high-risk group, resulting in 99.21% sensitivity, 99.95% specificity and 94.12% PPV, respectively. In comparison, 96 true-positive cases, 22 false-positive cases, and one false-negative case were observed in the low-risk group, resulting in 98.97% sensitivity, 99.95% specificity and 81.36% PPV. Statistical analysis by Fisher's exact test showed no significant difference (sensitivity, 99.21% *vs* 98.97% ($P = 0.82$); specificity, 99.95% *vs* 99.95% ($P = 0.98$)) in NIPT performance between these two groups other than a decreased PPV in the low-risk group (Table 3), which was expected owing to the lower incidence of trisomy 21. Because of the low incidence of positive cases in the low-risk group, performance was not calculated and compared for trisomies 18 and 13.

A total of 157 false-positive and nine false-negative NIPT results were identified in the present study; 41 cases had an NIPT indication of biological factors, including 27 cases of maternal CNV and 12 cases of mosaicism to cause false-positive results, and two cases of confined placental mosaicism (CPM) to cause false-negative results (Tables 4 and 5). The remaining cases had no NIPT indication of

Table 3 Performance of non-invasive prenatal testing (NIPT) in detection of trisomy 21 in high-risk pregnancies and low-risk pregnancies

NIPT performance	*High-risk group (n = 72 382)*	*Low-risk group (n = 40 287)*	P*
True positive	624	96	NA
False positive	39	22	NA
False negative	5	1	NA
Sensitivity	99.21 (98.51–99.90)	98.97 (94.39–99.97)	0.82
Specificity	99.95 (99.93–99.96)	99.95 (99.92–99.97)	0.98
Positive predictive value	94.12 (92.33–95.91)	81.36 (74.33–88.38)	< 0.00001
Negative predictive value	99.99 (99.99–100)	100 (99.99–100)	0.30

Data are given as *n* or % (95% CI). *Statistical analysis by Fisher's exact test. NA, not applicable.

Table 4 Analysis of biological factors causing 157 false-positive results in pregnant women undergoing non-invasive prenatal testing (NIPT) for trisomies 21, 18 and 13

Contributing factor	*Value*
Maternal age (years)*	31.65 (21–24)
Gestational age (weeks)*	18.5 (13–27)
Fetal fraction (%)*	9.74 (3.54–21.94)
Suspected maternal CNV†	
Trisomy 21	15 (13) (0.59–6.72 Mb)
Trisomy 18	9 (6) (0.5–3.35 Mb)
Trisomy 13	3 (2) (4.89 – 14 Mb)
Suspected mosaicism/CPM‡	
Trisomy 21	3 (—)§
Trisomy 18	2 (—)¶
Trisomy 13	9 (2 CPM)**
Live birth normal phenotype with no prenatal diagnosis (*n*)	
Trisomy 21	7
Trisomy 18	1
Trisomy 13	2
NIPT-suspected multiple chromosomal aneuploidy	1 trisomy 13 FP (chr 13, chr 14, chr 20)
Twin pregnancy	2 trisomy 21 FP

*Data given as mean (range). †Data given as no. suspected cases (no. confirmed cases) (CNV size range). ‡Data given as no. suspected cases (no. confirmed cases). §Three cases suspected to be mosaic. Placental tissues were not available for further investigation, thus none was confirmed. ¶Two cases suspected to be mosaic. Placental tissues were available for further sequencing and results showed no detected mosaicism. **Seven cases suspected to be mosaic. Placental tissues were not available, thus none of the seven cases was confirmed. Placental tissues were available in two other cases, and karyotyping showed 70% CPM in one case and complete CPM in another case. chr, chromosome; CNV, copy number variant; CPM, confined placental mosaicism; FP, false positive.

biological factors and could not be confirmed, mainly because of the lack of samples for confirmation.

Among the false-positive cases, 27 had chromosome breakpoints and very large regional T-score identified by FCAPS analysis, which indicated the existence of a maternal background of CNV (Table 4). Confirmation using maternal white blood cell (WBC) sequencing showed that 21/27 cases had maternal chromosome duplication ranging from 0.5 Mb to 14 Mb on the relevant chromosomes, including 13 cases of trisomy 21, six cases of trisomy 18 and two cases of trisomy 13. Twelve cases were suspected of having fetal or placental mosaicism based on the finding that the fetal fraction estimated by the trisomic chromosome was dramatically lower than the fetal fraction estimated by the Y chromosome (Table 4). For confirmation, placental tissues were available in four cases for further sequencing or karyotyping, showing CPM of trisomy 13 in two cases (one with 70% placental mosaicism and one with complete placental trisomy 13), and no mosaicism was detected in the other two (Table 4).

In addition, 10 false-positive results were based on the phenotypic description of the neonate with no cytogenetic confirmation, and thus we cannot rule out the possibility of fetal mosaicism, CPM or maternal CNV. One false-positive trisomy 13 case had an NIPT result of multiple chromosome aneuploidies, which could have been caused by sequencing data deviation or other biological factors. Unfortunately consent was not available for testing maternal WBC in this case, so the maternal genetic background was unknown. Two false-positive trisomy 21 results were from twin pregnancies, both of which underwent amniocentesis and karyotyping to reveal normal karyotypes of both fetuses.

Among the NIPT false-negative cases (Table 5), maternal WBC sequencing found no maternal CNV background or mosaicism. Placental samples were obtained in two false-negative cases of trisomy 18 and both showed low-level CPM of trisomy 18. In particular, one of the false-negative trisomy 18 cases contained 30% trisomy 18 mosaicism and 60% XO mosaicism in multiple placental samples. The NIPT result in this case showed a high risk for monosomy X but did not detect trisomy 18.

To verify the role of cfDNA fetal fraction in false-positive and negative NIPT results, 120 false-positive cases and eight false-negative cases with extra plasma sample aliquots were tested for cfDNA fetal fraction. The false-positive cases had a mean fetal fraction of 9.74% (range, 3.54–21.94%) (Table 4), with all above the 3.5% minimum requirement of an NIPT test. In the false-negative cases, the mean fetal fraction was 10.2% (range, 5.18–13.39%) (Table 5), with all greater than the minimum requirement of the test.

DISCUSSION

NIPT has been widely used to screen for trisomies 21, 18 and 13 in the past few years, yet large-scale clinical studies on its efficacy in the general population are lacking,

Table 5 Summary of clinical outcome and results of further investigation of nine cases with false-negative non-invasive prenatal test (NIPT) results, including six trisomy (T) 21 and three T18 cases

NIPT result	*MA (yrs)*	*GA (wks)*	*Ultrasound result*	*Down syndrome screening result*	*FF (%)**	*Pregnancy outcome*	*Cytogenetic confirmation*	*Further sequencing*
Aneu ND	26	19	Norm singleton	T21 high risk (1/285)	9.9	LB, tris pheno	NB: 46,XY + 21	WBC norm
Aneu ND	28	22	Echogenic bowel, VM 0.8 cm	Low risk	12.9	LB, tris pheno	NB: 46,XY + 21	WBC norm
Aneu ND	26	25	Norm singleton	T21 med risk (1/590)	13.4	LB, tris pheno	NB: 46,XY der(21:21) (q10:q10) + 21	WBC norm
Aneu ND	22	14 + 4	Singleton, NT 3.2 mm	No test	NA	LB, tris pheno	NB: 46,XX + 21	WBC norm
Aneu ND	28	25 + 4	NT 6.8 mm	No test	10.9	LB, tris pheno	NB: 46,XX + 21	WBC norm; NB: T21
Aneu ND	37	13	Norm singleton	No test	13.1	LB, tris pheno	NB: 46,XX + 21	WBC norm
Aneu ND	27	17 + 3	Norm singleton	T13 high risk (1/59)	5.2	Elective TOP (dev malf on US at 22 wks)	POC: 47,XY + 18	WBC norm
Aneu ND	29	19 + 4	Norm singleton	T21 med risk (1/360)	5.3	Elective TOP (dev malf on US at 22 wks)	POC: 47,XY + 18; PT: 20–40% mosaicism T18 and tetraploidy in 6 placental places	WBC norm
XO	24	20 + 4	Norm singleton	T21 high risk (1/45)	9.5	Elective TOP (dev malf on US)	AF: 47,XX + 18; POC: 47,XX + 18; PT: 30% mosaicism T18, 60% mosaicism XO	WBC norm

*Fetal fraction (FF) measured in eight cases with extra sample aliquots. AF, amniotic fluid; Aneu ND, aneuploidy not detected; dev malf, developmental malformation; GA, gestational age; LB, live birth; MA, maternal age; med risk, medium risk; NA, not applicable; NB, neonatal blood; norm, normal; NT, nuchal translucency; POC, products of conception; PT, placental tissue; TOP, termination of pregnancy; tris pheno, typical trisomy phenotype; US, ultrasound examination; VM, ventriculomegaly; WBC, maternal white blood cells; wks, weeks; XO, monosomy X; yrs, years.

and concerns have been raised about its performance in routine clinical practice[24]. To provide a large clinical dataset as an NIPT audit assessment, this multicenter prospective study collected over 140 000 clinical samples. The present study was performed in mainland China, where prenatal screening for Down syndrome occurs mainly from the second trimester onwards and NIPT is used predominantly as a secondary screening test. Thus most pregnant women had NIPT after 13 weeks' gestation and more than half of the population was found to be at high risk by previous biochemical screening.

One strength of this study is the estimation of NIPT sensitivity and specificity in a large prospective population with cytogenetic or phenotypic outcomes. Similar estimation has not been reported in several recent studies owing to small sample sizes or insufficient follow-up[19,21,25,26]. Our data show that NIPT in large-scale clinical practice maintained high sensitivity (99.17%, 98.24% and 100%) and specificity (99.95%, 99.95% and 99.96%) for trisomies 21, 18 and 13, respectively. When compared to studies in high-risk populations[6,8–13,27,28], our data showed comparable, if not better, performance (Table S3). Thus with a strict protocol and quality management, the clinical efficacy of NIPT did not deteriorate in large-scale practice.

Another strength of this study is the comparison of NIPT performance in 72 382 high-risk and 40 287 low-risk pregnancies, which is the largest comparison so far. Previous comparisons were conducted with small sample sizes[21,29], which meant that conclusive evidence was still required based on large-scale clinical experience. Here, we defined the low-risk group according to stringent criteria, containing none of the known high-risk factors. Our data showed comparable sensitivity ($P = 0.82$) and specificity ($P = 0.98$) in the detection of trisomy 21 in the high-risk and low-risk groups, adding to the recent data that only assessed PPV[21]. Although NIPT has been recommended only in the high-risk population, similar fetal cfDNA distributions were found recently in the low-risk population, which provides the basis for uniform NIPT performance in the general population[30,31]. Importantly, our comparison data corroborate the effective performance of NIPT in the general population. Nonetheless, the reduced PPV in the low-risk group as a consequence of lower disease prevalence reaffirmed that NIPT should not be used as a diagnostic test and that confirmation by invasive testing is still necessary.

Several conditions have been known to contribute to false-positive and negative NIPT results: (1) low fetal fraction[32]; (2) maternal chromosome abnormality[33]; (3) genetic discordance between the fetus and placenta i.e. CPM[34,35]; (4) fetal mosaicism[12]; (5) vanishing twin[36,37]. In this study, low fetal fraction may not have been a

major factor in routine practice, since all the false-positive and negative cases that were tested in our data had fetal fractions above the NIPT requirement. In contrast, biological factors such as maternal background of CNV and CPM had important roles in causing the false-positive and negative results. In addition, fetal pathogenic CNV and fetal mosaicism have also been reported to cause false results[12,32,38,39]. These factors must therefore be taken into account when interpreting NIPT results, and post-test genetic counseling should be provided to pregnant women following recommendations such as those of the National Society of Genetic Counselors' statement[15]. The NIPT algorithm in this study could not identify the maternal or fetal contribution to abnormality (i.e. CNV or mosaicism). Thus we recommend that each case with CNV or mosaicism identified by NIPT should be confirmed as to the source of the abnormality using maternal WBC, amniotic fluid or multiple placental samples.

Most cases with suspected mosaicism or CPM could not be confirmed, mainly because of the difficulty of obtaining confirmatory samples, and the insufficient quantity of cells (~20 cells) for examination during routine cytogenetic analysis. We could not verify vanishing twins in this study, owing to a lack of sonographic information on the cases. Also, owing to the patient's declining further testing, we could not confirm the reason for one false-positive case with multiple chromosome aneuploidies, which may relate to maternal malignancy[40]. About two-thirds of false NIPT results had no obvious biological factors as an explanation. This probably reflects the inadequate resolution of conventional karyotyping in identifying mosaicism and submicroscopic CNV compared to NIPT[38]. Given improved confirmation methods such as microarray, more biological factors may be identified in these NIPT false-positive and negative cases.

The limitation of this study was the incomplete follow-up of NIPT results, which could introduce bias into the performance evaluation. In NIPT-positive cases, only 66.8% of women provided the result of a confirmatory diagnosis, mainly because they declined to provide clinical outcomes (17.9%) or they underwent pregnancy termination (13.0%). This raised the importance of reinforcing genetic counseling in future clinical utilization of NIPT and avoiding potential misuse of the test as a diagnostic method. Nonetheless, outcome data were available for nearly 77% of the population, and the demographic characteristics of this subgroup were similar to those of the total population. Thus performance based on this subgroup represented that of the total population well. Lastly, with a well-operated insurance program to encourage confirmation of a positive result and reporting of false-negative results, the possibility of NIPT false results going undetected remained low.

In conclusion, our study represents the largest clinical experience of NIPT to date. We have shown that the performance of NIPT in detecting trisomies 21, 18 and 13 was maintained at a high level, comparable to that of previous small-scale validation studies. Maternal genetic background and fetal/placental mosaicism played important roles in false-positive and negative NIPT results, whereas low fetal fraction is unlikely to be a significant contributor. NIPT performance in detecting trisomy 21 showed no statistical difference between the low-risk and high-risk groups. Our findings suggest that it is appropriate to offer NIPT as a routine screening test for fetal trisomies 21, 18 and 13 in the general population.

ACKNOWLEDGMENT

This study was funded by Shenzhen Birth Defect Screening Project Lab, BGI-Shenzhen, Shenzhen, China [JZF No. [2011]861]. The authors thank all collaborating medical centers, patients and their families.

Disclosure

Hongyun Zhang, Ya Gao, Fuman Jiang, Meili Fu, Yuying Yuan, Yulai Guo, Zhongyi Zhu, Mengmeng Lin, Qiufang Liu, Zhongming Tian, Haiquan Zhang, Lijian Zhao, Xin Yi, Ye Yin, and Wei Wang are employees of BGI Diagnostics. Fang Chen is an employee of BGI – Shenzhen. Tze Kin Lau has no financial relationship with BGI Diagnostics or BGI – Shenzhen.

REFERENCES

1. Malone FD, Canick JA, Ball RH, Nyberg DA, Comstock CH, Bukowski R, Berkowitz RL, Gross S J, Dugoff L, Craigo SD, Timor-Tritsch IE, Carr SR, Wolfe HM, Dukes K, Bianchi DW, Rudnicka AR, Hackshaw AK, Lambert-Messerlian G, Wald NJ, D'Alton ME. First-trimester or second-trimester screening, or both, for Down's syndrome. *N Engl J Med* 2005; **353**: 2001–2011.
2. Wald NJ, Rodeck C, Hackshaw AK, Walters J, Chitty L, Mackinson AM; SURUSS Research Group. First and second trimester antenatal screening for Down's syndrome: the results of the Serum, Urine and Ultrasound Screening Study (SURUSS). *Health Technol Assess* 2003; **7**: 1–77.
3. Nicolaides KH. Screening for fetal aneuploidies at 11 to 13 weeks. *Prenat Diagn* 2011; **31**: 7–15.
4. Fan HC, Blumenfeld YJ, Chitkara U, Hudgins L, Quake SR. Noninvasive diagnosis of fetal aneuploidy by shotgun sequencing DNA from maternal blood. *Proc Natl Acad Sci USA* 2008; **105**: 16 266–16 271.
5. Chiu RW, Chan KC, Gao Y, Lau VY, Zheng W, Leung TY, Foo CH, Xie B, Tsui NB, Lun FM, Zee BC, Lau TK, Cantor CR, Lo YM. Noninvasive prenatal diagnosis of fetal chromosomal aneuploidy by massively parallel genomic sequencing of DNA in maternal plasma. *Proc Natl Acad Sci USA* 2008; **105**: 20 458–20 463.
6. Chiu RW, Akolekar R, Zheng YW, Leung TY, Sun H, Chan KC, Lun FM, Go AT, Lau ET, To WW, Leung WC, Tang RY, Au-Yeung SK, Lam H, Kung YY, Zhang X, van Vugt JM, Minekawa R, Tang MH, Wang J, Oudejans CB, Lau TK, Nicolaides KH, Lo YM. Non-invasive prenatal assessment of trisomy 21 by multiplexed maternal plasma DNA sequencing: large scale validity study. *BMJ* 2011; **342**: c7401.
7. Chen EZ, Chiu RW, Sun H, Akolekar R, Chan KC, Leung TY, Jiang P, Zheng YW, Lun FM, Chan LY, Jin Y, Go AT, Lau ET, To WW, Leung WC, Tang RY, Au-Yeung SK, Lam H, Kung YY, Zhang X, van Vugt JM, Minekawa R, Tang MH, Wang J, Oudejans CB, Lau TK, Nicolaides KH, Lo YM. Noninvasive prenatal diagnosis of fetal trisomy 18 and trisomy 13 by maternal plasma DNA sequencing. *PLoS One* 2011; **6**: e21791.
8. Ehrich M, Deciu C, Zwiefelhofer T, Tynan JA, Cagasan L, Tim R, Lu V, McCullough R, McCarthy E, Nygren AO, Dean J, Tang L, Hutchison D, Lu T, Wang H, Angkachatchai V, Oeth P, Cantor CR, Bombard A, van den Boom D. Noninvasive detection of fetal trisomy 21 by sequencing of DNA in maternal blood: a study in a clinical setting. *Am J Obstet Gynecol* 2011; **204**: 205.e1–11.
9. Palomaki GE, Kloza EM, Lambert-Messerlian GM, Haddow JE, Neveux LM, Ehrich M, van den Boom D, Bombard AT, Deciu C, Grody WW, Nelson SF, Canick JA. DNA sequencing of maternal plasma to detect Down syndrome: an international clinical validation study. *Genet Med* 2011; **13**: 913–920.
10. Palomaki GE, Deciu C, Kloza EM, Lambert-Messerlian GM, Haddow JE, Neveux LM, Ehrich M, van den Boom D, Bombard AT, Grody WW, Nelson SF, Canick JA. DNA sequencing of maternal plasma reliably identifies trisomy 18 and trisomy 13 as well as Down syndrome: an international collaborative study. *Genet Med* 2012; **14**: 296–305.
11. Ashoor G, Syngelaki A, Wagner M, Birdir C, Nicolaides KH. Chromosome-selective sequencing of maternal plasma cell-free DNA for first-trimester detection of trisomy 21 and trisomy 18. *Am J Obstet Gynecol* 2012; **206**: 322.e1–5.

12. Bianchi DW, Platt LD, Goldberg JD, Abuhamad AZ, Sehnert AJ, Rava RP. Genome-wide fetal aneuploidy detection by maternal plasma DNA sequencing. *Obstet Gynecol* 2012; **119**: 890–901.
13. Jiang F, Ren J, Chen F, Zhou Y, Xie J, Dan S, Su Y, Yin B, Su W, Zhang H, Wang W, Chai X, Lin L, Guo H, Li Q, Li P, Yuan Y, Pan X, Li Y, Liu L, Chen H, Xuan Z, Chen S, Zhang C, Zhang H, Tian Z, Zhang Z, Jiang H, Zhao L, Zheng W, Li S, Li Y, Wang J, Wang J, Zhang X. Noninvasive Fetal Trisomy (NIFTY) test: an advanced noninvasive prenatal diagnosis methodology for fetal autosomal and sex chromosomal aneuploidies. *BMC Med Genomics* 2012; **5**: 57.
14. Zimmermann B, Hill M, Gemelos G, Demko Z, Banjevic M, Baner J, Ryan A, Sigurjonsson S, Chopra N, Dodd M, Levy B, Rabinowitz M. Noninvasive prenatal aneuploidy testing of chromosomes 13, 18, 21, X, and Y, using targeted sequencing of polymorphic loci. *Prenat Diagn* 2012; **32**: 1233–1241.
15. Devers PL, Cronister A, Ormond KE, Facio F, Brasington CK, Flodman P. Noninvasive prenatal testing/noninvasive prenatal diagnosis: the position of the National Society of Genetic Counselors. *J Genet Couns* 2013; **22**: 291–295.
16. American College of Obstetricians and Gynecologists Committee on Genetics. Committee Opinion No. 545: Noninvasive prenatal testing for fetal aneuploidy. *Obstet Gynecol* 2012; **120**: 1532–1534.
17. Benn P, Borell A, Chiu R, Cuckle H, Dugoff L, Faas B, Gross S, Johnson J, Maymon R, Norton M, Odibo A, Schielen P, Spencer K, Huang T, Wright D, Yaron Y. Position statement from the Aneuploidy Screening Committee on behalf of the Board of the International Society for Prenatal Diagnosis. *Prenat Diagn* 2013; **33**: 622–629.
18. Song Y, Liu C, Qi H, Zhang Y, Bian X, Liu J. Noninvasive prenatal testing of fetal aneuploidies by massively parallel sequencing in a prospective Chinese population. *Prenat Diagn* 2013; **33**: 700–706.
19. Gil MM, Quezada MS, Bregant B, Ferraro M, Nicolaides KH. Implementation of maternal blood cell-free DNA testing in early screening for aneuploidies. *Ultrasound Obstet Gynecol* 2013; **42**: 34–40.
20. Dan S, Wang W, Ren J, Li Y, Hu H, Xu Z, Lau TK, Xie J, Zhao W, Huang H, Xie J, Sun L, Zhang X, Wang W, Liao S, Qiang R, Cao J, Zhang Q, Zhou Y, Zhu H, Zhong M, Guo Y, Lin L, Gao Z, Yao H, Zhang H, Zhao L, Jiang F, Chen F, Jiang H, Li S, Li Y, Wang J, Wang J, Duan T, Su Y, Zhang X. Clinical application of massively parallel sequencing-based prenatal noninvasive fetal trisomy test for trisomies 21 and 18 in 11,105 pregnancies with mixed risk factors. *Prenat Diagn* 2012; **32**: 1225–1232.
21. Bianchi DW, Parker RL, Wentworth J, Madankumar R, Saffer C, Das AF, Craig JA, Chudova DI, Devers PL, Jones KW, Oliver K, Rava RP, Sehnert AJ. DNA sequencing versus standard prenatal aneuploidy screening. *N Engl J Med* 2014; **370**: 799–808.
22. Chen S, Lau TK, Zhang C, Xu C, Xu Z, Hu P, Xu J, Huang H, Pan L, Jiang F, Chen F, Pan X, Xie W, Liu P, Li X, Zhang L, Li S, Li Y, Xu X, Wang W, Wang J, Jiang H, Zhang X. A method for noninvasive detection of fetal large deletions/duplications by low coverage massively parallel sequencing. *Prenat Diagn* 2013; **33**: 584–590.
23. Zheng J, Xu C, Guo J, Wei Y, Ge H, Li X, Zhang C, Jiang H, Pan L, Tang W, Xie W, Zhang H, Zhao Y, Jiang F, Chen S, Wang W, Xu X, Chen F, Huang H. Effective noninvasive zygosity determination by maternal plasma target region sequencing. *PLoS One* 2013; **8**: e65050.
24. Lutgendorf MA, Stoll KA, Knutzen DM, Foglia LM. Noninvasive prenatal testing: limitations and unanswered questions. *Genet Med* 2014; **16**: 281–285.
25. 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.
26. McCullough RM, Almasri EA, Guan X, Geis JA, Hicks SC, Mazloom AR, Deciu C, Oeth P, Bombard AT, Paxton B, Dharajiya N, Saldivar JS. Non-invasive prenatal chromosomal aneuploidy testing – clinical experience: 100,000 clinical samples. *PLoS One* 2014; **9**: e109173.
27. Sparks AB, Struble CA, Wang ET, Song K, Oliphant A. Noninvasive prenatal detection and selective analysis of cell-free DNA obtained from maternal blood: evaluation for trisomy 21 and trisomy 18. *Am J Obstet Gynecol* 2012; **206**: 319.e1–9.
28. Norton ME, Brar H, Weiss J, Karimi A, Laurent LC, Caughey AB, Rodriguez MH, Williams J 3rd, Mitchell ME, Adair CD, Lee H, Jacobsson B, Tomlinson MW, Oepkes D, Hollemon D, Sparks AB, Oliphant A, Song K. Non-Invasive Chromosomal Evaluation (NICE) Study: results of a multicenter prospective cohort study for detection of fetal trisomy 21 and trisomy 18. *Am J Obstet Gynecol* 2012; **207**: 137.e1–8.
29. Pergament E, Cuckle H, Zimmermann B, Banjevic M, Sigurjonsson S, Ryan A, Hall MP, Dodd M, Lacroute P, Stosic M, Chopra N, Hunkapiller N, Prosen DE, McAdoo S, Demko Z, Siddiqui A, Hill M, Rabinowitz M. Single-nucleotide polymorphism-based noninvasive prenatal screening in a high-risk and low-risk cohort. *Obstet Gynecol* 2014; **124**: 210–218.
30. Hudecova I, Sahota D, Heung MM, Jin Y, Lee WS, Leung TY, Lo YM, Chiu RW. Maternal plasma fetal DNA fractions in pregnancies with low and high risks for fetal chromosomal aneuploidies. *PLoS One* 2014; **9**: e88484.
31. Brar H, Wang E, Struble C, Musci TJ, Norton ME. The fetal fraction of cell-free DNA in maternal plasma is not affected by a priori risk of fetal trisomy. *J Matern Fetal Neonatal Med* 2013; **26**: 143–145.
32. Canick JA, Palomaki GE, Kloza EM, Lambert-Messerlian GM, Haddow JE. The impact of maternal plasma DNA fetal fraction on next generation sequencing tests for common fetal aneuploidies. *Prenat Diagn* 2013; **33**: 667–674.
33. Yao H, Zhang L, Zhang H, Jiang F, Hu H, Chen F, Jiang H, Mu F, Zhao L, Liang Z, Wang W. Noninvasive prenatal genetic testing for fetal aneuploidy detects maternal trisomy X. *Prenat Diagn* 2012; **32**: 1114–1116.
34. Hall AL, Drendel HM, Verbrugge JL, Reese AM, Schumacher KL, Griffith CB, Weaver DD, Abernathy MP, Litton CG, Vance GH. Positive cell-free fetal DNA testing for trisomy 13 reveals confined placental mosaicism. *Genet Med* 2013; **15**: 729–732.
35. Faas BH, de Ligt J, Janssen I, Eggink AJ, Wijnberger LD, van Vugt JM, Vissers L, Geurts van Kessel A. Non-invasive prenatal diagnosis of fetal aneuploidies using massively parallel sequencing-by-ligation and evidence that cell-free fetal DNA in the maternal plasma originates from cytotrophoblastic cells. *Expert Opin Biol Ther* 2012; **12** (Suppl 1): S19–S26.
36. Lau TK, Cheung SW, Lo PS, Pursley AN, Chan MK, Jiang F, Zhang H, Wang W, Jong LF, Yuen OK, Chan HY, Chan WS, Choy KW. Non-invasive prenatal testing for fetal chromosomal abnormalities by low-coverage whole-genome sequencing of maternal plasma DNA: review of 1982 consecutive cases in a single center. *Ultrasound Obstet Gynecol* 2014; **43**: 254–264.
37. Futch T, Spinosa J, Bhatt S, de Feo E, Rava RP, Sehnert AJ. Initial clinical laboratory experience in noninvasive prenatal testing for fetal aneuploidy from maternal plasma DNA samples. *Prenat Diagn* 2013; **33**: 569–574.
38. Srinivasan A, Bianchi DW, Huang H, Sehnert AJ, Rava RP. Noninvasive detection of fetal subchromosome abnormalities via deep sequencing of maternal plasma. *Am J Hum Genet* 2013; **92**: 167–176.
39. Lau TK, Jiang FM, Stevenson RJ, Lo TK, Chan LW, Chan MK, Lo PS, Wang W, Zhang HY, Chen F, Choy KW. Secondary findings from non-invasive prenatal testing for common fetal aneuploidies by whole genome sequencing as a clinical service. *Prenat Diagn* 2013; **33**: 602–608.
40. Osborne CM, Hardisty E, Devers P, Kaiser-Rogers K, Hayden MA, Goodnight W, Vora NL. Discordant noninvasive prenatal testing results in a patient subsequently diagnosed with metastatic disease. *Prenat Diagn* 2013; **33**: 609–611.

SUPPORTING INFORMATION ON THE INTERNET

The following supporting information may be found in the online version of this article:

Table S1 Details of 283 NIPT positive cases with no pregnancy outcome or diagnostic results from the collaborative medical units. 232 (82%) cases had advanced maternal age, previous high-risk Down syndrome screening result or abnormal ultrasound findings, and therefore were high-risk pregnancies. Although with no definitive diagnostic confirmation, based on the information, the likelihood of false-positive results was small

Table S2 Information on 4605 NIPT negative cases that had live birth with birth defects was collected from collaborative medical units

Table S3 Comparison of non-invasive prenatal testing (NIPT) performance in detecting trisomies (T) 21, 18, and 13 in the present study and previous validation studies in a high-risk population

