Genetics in Medicine (2022) 24, 1379–1391

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Genetics in Medicine

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ACMG SYSTEMATIC EVIDENCE REVIEW

Systematic evidence-based review: The application of noninvasive prenatal screening using cell-free DNA in general-risk pregnancies

ARTICLE INFO

Article history:
Received 23 March 2022
Accepted 28 March 2022
Available online 24 May 2022

Keywords:
Cell-free DNA
Down syndrome
Noninvasive prenatal screening
Trisomy

ABSTRACT

Purpose: Noninvasive prenatal screening (NIPS) using cell-free DNA has been assimilated into prenatal care. Prior studies examined clinical validity and technical performance in high-risk populations. This systematic evidence review evaluates NIPS performance in a general-risk population.
Methods: Medline (PubMed) and Embase were used to identify studies examining detection of Down syndrome (T21), trisomy 18 (T18), trisomy 13 (T13), sex chromosome aneuploidies, rare autosomal trisomies, copy number variants, and maternal conditions, as well as studies assessing the psychological impact of NIPS and the rate of subsequent diagnostic testing. Random-effects meta-analyses were used to calculate pooled estimates of NIPS performance ($P < .05$). Heterogeneity was investigated through subgroup analyses. Risk of bias was assessed.
Results: A total of 87 studies met inclusion criteria. Diagnostic odds ratios were significant ($P < .0001$) for T21, T18, and T13 for singleton and twin pregnancies. NIPS was accurate (≥99.78%) in detecting sex chromosome aneuploidies. Performance for rare autosomal trisomies and copy number variants was variable. Use of NIPS reduced diagnostic tests by 31% to 79%. Conclusions regarding psychosocial outcomes could not be drawn owing to lack of data. Identification of maternal conditions was rare.
Conclusion: NIPS is a highly accurate screening method for T21, T18, and T13 in both singleton and twin pregnancies.

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The Board of Directors of the American College of Medical Genetics and Genomics approved this systematic evidence review on 28 February 2022.
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doi: https://doi.org/10.1016/j.gim.2022.03.019

Introduction

Since its introduction in 2011, noninvasive prenatal screening (NIPS) using cell-free DNA (cfDNA) for the detection of common fetal aneuploidies has been rapidly assimilated into prenatal care.[1] With a resolution similar to karyotyping[2] and regardless of the methodology used, cfDNA is the most sensitive and specific screening test for common chromosomal aneuploidies (chromosomes 13, 18, and 21).[3,4] Before its introduction into clinical use, no large-scale randomized control trials were performed to assess the clinical validity or clinical utility of this screening test. Subsequently, multiple studies have determined the sensitivity and specificity of this testing, focusing largely on high-risk patient populations with singleton pregnancies.[1,5-7]

Before the implementation of NIPS, screening for aneuploidy consisted mainly of multiple serum analytes with or without ultrasound to achieve a detection rate ranging from 80% to 95% for Down syndrome.[8] Although NIPS has a greater accuracy for aneuploidy detection, approximately 99% for Down syndrome at 10 weeks of gestation or greater,[4] detection rates vary slightly between laboratories owing to differences in methodologies and reporting methods.

When diagnostic testing is performed to evaluate a screen-positive high-risk result generated through NIPS, a subset of individuals will have discordant results, with varying false positive rates (FPRs) depending on the specific chromosome interrogated, the type of variant, and the prevalence of the condition. Although the intent of screening is to determine whether fetal aneuploidy is present, the specimen obtained contains predominantly maternal DNA, and the test often cannot distinguish between fetal and maternal chromosomal material. This may lead to unexpected maternal findings for which patients are unprepared, including the suggestion of maternal malignancy, a maternal submicroscopic duplication or deletion, or a maternal sex chromosome aneuploidy (SCA). Finally, all screening tests have false-positive (FP) and false-negative (FN) results but given the enhanced accuracy to detect the common trisomies, some health care providers and patients may inappropriately consider the test to be diagnostic.[9]

Current national guidelines from multiple organizations state that pregnant individuals should be made aware of both the accuracy and limitations of cfDNA screening for the detection of the common trisomies. The most recent American College of Medical Genetics and Genomics (ACMG) position statement states that "all women should be informed that NIPS is the most sensitive screening option for traditionally screened aneuploidies."[3] The American College of Obstetrics and Gynecology reinforces this statement.[8] Both organizations stress that NIPS is not equivalent to diagnostic testing.

Although initially NIPS was used to screen for the common trisomies and SCAs in singleton pregnancies, many laboratories have adapted this technology to screen twin gestations.[10] Furthermore, in some laboratories, the application has been expanded to screen for rare autosomal trisomies (RATs), as well as for both common and unique copy number variants (CNVs). However, the positive predictive values (PPVs) for these conditions are significantly lower than the PPVs for common aneuploidies and large-scale outcome studies have not been performed, nor has clinical utility of screening for these rarer conditions been established.

This systematic evidence review (SER) is designed to assess the clinical performance of NIPS in a general-risk population of both singleton and twin pregnancies. It also evaluates the use of NIPS with respect to the identification of CNVs, SCAs, RATs, and maternal conditions, its impact on the uptake of diagnostic testing, the economic implications of its use, as well as the psychological impact of this technology on the individuals undergoing prenatal screening for aneuploidy.

Materials and Methods

We performed an SER using best practices and report our methods and results in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses checklist.[11] In 2020, ACMG convened an SER workgroup to develop the evidentiary basis for a clinical guideline. The SER workgroup comprised ACMG members, including a board-certified medical geneticist and maternal fetal medicine physician (N.C.R.), clinical directors of laboratory medicine (E.S.B., M.L.L.), a laboratory genetic counselor (D.L.), and methodologists (J.M., G.P.J., M.R.M.). Working group members had no conflicts of interest according to ACMG policy. The goal of the SER was to assess the use of NIPS in a population of general-risk individuals, ie, a population reflective of a range of risks that might be encountered in general obstetrical practice, including low-risk, intermediate-risk, and high-risk patients. To address this question, a separate guideline panel external to the authors and methodologist (M.R.M.) defined the population, intervention, comparator(s), outcomes, timing, and setting and developed a set of 10 key questions (KQ) and corresponding search queries (Supplemental Material).

We initially searched Medline (PubMed) and Embase for relevant studies on July 30, 2020 and updated our search on March 26, 2021. The search strategy for Medline is presented in the Supplement. We further identified relevant studies cited by other studies or from meta-analyses. We updated our search query to account for additional synonyms used for NIPS and limited returns on the basis of publication date consistent with the original search. Results from the databases were managed in an Endnote (version 9.3.3; version 20) library that was used for deduplication. Deduplicated results were uploaded to Covidence for review and data extraction/quality assessment.

All stages of the review were performed independently by 2 reviewers. Conflicts were resolved through discussion between reviewers or adjudicated by a third reviewer. Titles and abstracts of search results were screened according to prespecified inclusion and exclusion criteria (Supplemental Material). Articles not excluded in the title/abstract screening were reviewed in their entirety for inclusion; rationale for exclusion was documented (Supplemental Material). Data extraction and risk of bias forms were created within Covidence for diagnostic accuracy and clinical utility studies; data extraction was completed in Microsoft Excel spreadsheets guided by the Consolidated Health Economic Evaluation Reporting Standards checklist.[12] Data extracted included study, population characteristics, details about NIPS and any comparators, and outcome(s). Data for true positives (TPs), true negatives (TNs), FPs, and FNs were extracted when provided or calculated by reviewers when there was sufficient confidence in the data reported. Risk of bias was assessed using the Risk of Bias in Non-Randomized Studies of Interventions (ROBINS-I)[13] framework or the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2)[14] for diagnostic accuracy studies.

Data analysis

Data exported from Covidence was cleaned in Microsoft Excel. Analysis was performed using R Studio (v.1.4.1717) (R Development Core Team), R (version 4.1.0) with the R packages "meta," "metafor," "mada," "diagmeta," and "ggplot2." An analysis plan was prespecified; random-effects meta-analyses were planned to obtain pooled point estimates and 95% CI for each of the diagnostic performance outcomes for KQ1 to KQ6. Only studies where the TPs, TNs, FPs, and/or FNs were provided or calculable with relative certainty from the data presented in the manuscript were included in meta-analyses. Studies reporting their performance without also providing the number of people in each category were not meta-analyzed and their results are reported separately. Quantitative analysis was deemed unlikely to be possible for KQ7 to KQ10 and results for those KQs were narratively synthesized. Anticipated heterogeneity was investigated through sensitivity analyses, with subgroups defined for country, year of publication, risk of bias assessment (low, moderate, high, critical), and size of population screened (<10,000, ≥10,000). Heterogeneity is reported as I^2. Publication bias was evaluated using the method described by Peters et al[15] weighted by inverse variance of average event probability and visualized with funnel plots. Results of the meta-analyses, including heterogeneity, are presented as forest plots and summarized in tables.

Results

We identified 770 articles from our literature searches and review of included studies from published meta-analyses and SERs. After deduplication, we screened 753 titles and abstracts and excluded 538 of those. We reviewed 215 studies in their entirety and determined 128 did not meet inclusion criteria (Supplemental Material). Of the 87 studies that ultimately met our inclusion criteria, 78 reported clinical outcomes and/or NIPS performance and 10 reported on economic outcomes (with 1 study reporting both). The Preferred Reporting Items for Systematic Reviews and Meta-Analyses flowchart is presented in the Supplement. A summary of all included studies is presented in the Supplement.

Trisomy 21

A total of 35 studies reported at least 1 performance characteristic (ie, sensitivity, specificity, PPV, negative predictive value (NPV), or FPR) for trisomy 21 (T21) (Supplemental Material). Of these, 28 were included in meta-analyses and the remainder were narratively synthesized. Studies reporting a statistic for >1 outcome combined are reported separately. The number of studies in the meta-analyses depended upon the specific data presented in the included studies. The pooled performance characteristics are presented in Table 1, with accompanying forest plots in the Supplement.

Two additional studies[16,17] reported sensitivity without presenting the number of TPs and/or FNs (98.9%, 95% CI = 95.90%-99.90%; 100%, 95% CI = 92%-100%, respectively). Together with the results of the meta-analysis, sensitivity ranged from 95% to 100% in 19 studies with no evidence of important heterogeneity between studies. Two additional studies reported specificity[18,19] without presenting the number of TPs and/or FNs (100%, 95% CI = 99.5%-100%; 99.95% [no CI given], respectively). Together with the results of the meta-analysis, specificity ranged from 99.89% to 100% in 17 studies. Costa et al[18] and Kypri et al[17] similarly reported PPV without presenting the number of TPs and/or FPs (100%, 95% CI = 59.0%-100%; 100%, 95% CI = 92%-100%, respectively). The pooled estimate of NPV was 100% (95% CI = 99.99%-100%) from 14 studies included in our meta-analysis. One additional study reported NPV without presenting the number of TNs and/or FNs (99.996% [no CI given]).[19] Sensitivity, specificity, PPV, and NPV of NIPS for T21 in Belgium were reported as 98.91% (95% CI = 97.24%-99.58%), 99.98% (95% CI = 99.97%-99.99%), 92.39% (95% CI = 89.34%-94.61%), and 100% (95% CI = 99.99%-100.00%), respectively.[20] Together with the results of the meta-analysis, NPV ranged from 99.99% to 100% in 16 studies and there was no important heterogeneity (I^2 = 0%) observed between the studies included in the meta-analysis. In total, 14 studies contributed to the meta-analysis for FPR; the pooled estimate was 0.04% (95% CI = 0.02%-0.08%) with considerable heterogeneity (I^2 = 76%) (Table 1). A total of 7 additional studies[18,19,21-25] reported FPR without presenting the number of TNs and/or FPs (Supplemental Material).

Table 1 Performance of NIPS in a general-risk population for trisomy 21, trisomy 18, and trisomy 13 calculated in random-effects meta-analyses

Test Statistic	No. of Studies	Result (%) (95% CI)	I^2 (%)
Trisomy 21			
Sensitivity	17	98.80 (97.81-99.34)	0.0
Specificity	14	99.96 (99.92-99.98)	75.9
PPV	28	91.78 (88.43-94.23)	68.3
NPV	14	100 (99.99-100)	0.0
FPR	14	0.04 (0.02-0.08)	75.9
Accuracy	14	99.94 (99.91-99.96)	80.2
DOR[a]	14	110,000 (44,000-260,000); $P < .0001$	55.7
Trisomy 18			
Sensitivity	6	98.83 (95.45-99.71)	0.0
Specificity	7	99.93 (99.83-99.97)	94.9
PPV	17	65.77 (45.29-81.68)	88.5
NPV	7	100 (100-100)	0.0
FPR	7	0.07 (0.03-0.17)	75.9
Accuracy	6	99.91 (99.73-99.97)	95.7
DOR[a]	6	29,000 (4800-180,000); $P < .0001$	94.9
Trisomy 13			
Sensitivity	7	100 (0-100)	0.0
Specificity	8	99.96 (99.92-99.98)	81.5
PPV	18	37.23 (26.08-49.93)	71.9
NPV	8	100 (100-100)	0.0
FPR	8	0.04 (0.02-0.08)	81.5
Accuracy	8	99.95 (99.90-99.97)	82.2
DOR[a]	7	29,000 (8900-94,000); $P < .0001$	0

Results do not include studies without adequate data to include in meta-analyses.

DOR, diagnostic odds ratio; *FPR*, false positive rate; *NIPS*, noninvasive prenatal screening; *NPV*, negative predictive value; *PPV*, positive predictive value.

[a]Data presented as odds ratio.

The diagnostic odds ratio (DOR) could be assessed in 14 studies. The estimated odds ratio of the DOR in the random-effects meta-analysis was 108,000 (95% CI 44,000-265,000). The odds for someone receiving a positive NIPS result in patients who are TP for T21 is >100,000 times higher than the odds for a positive NIPS result in patients who are TNs for T21. This highly significant ($P < .0001$) result shows that the NIPS tests are highly accurate and is consistent with an overall NIPS accuracy of 99.94% for T21 (Table 1).

In sensitivity analyses, risk of bias, country, and populations of ≥10,000 individuals were inconsistently associated with reported higher performance (Supplement). Although some subgroups were significantly different from each other, many subgroups contained only a single study and differences were not clinically meaningful. Overall, performance statistics for NIPS to detect T21 in general- or mixed-risk populations were high.

Trisomy 18

A total of 21 studies contributed to our analysis of NIPS to detect trisomy 18 (T18), whereas 2 studies reported combined results for T18 and trisomy 13 (T13) and are presented separately. Summary results and forest plots from random-effects meta-analyses for T18 are presented in Table 1 and the Supplement, respectively. In addition to the meta-analyses, Chen et al[26] reported a PPV of 54.84% (no CI given) for T18 in their mixed-risk population of 42,910 individuals with singleton pregnancies; however, PPV specifically among individuals with no clinical indications was 0%. From a cohort of 10,975 low-risk individuals in China, 166 had an adverse pregnancy outcome. Follow up with ultrasound and additional diagnostic testing identified a T18 FN from NIPS drawn at 17^{+3} weeks gestational age in a 26 year old individual.[27] In the Belgian study, sensitivity, specificity, and NPV were each reported as >95%, whereas PPV was lower, at 84.62% (95% CI = 75.82%-90.61%).[20]

We observed considerable heterogeneity in our meta-analyses. Sensitivity analyses uncovered significant between-subgroup differences on the basis of country and year of publication; however, these differences were not clinically meaningful and for country, most subgroups contained a single study (Supplemental Material). Overall, sensitivity, specificity, NPV, and accuracy of NIPS to detect T18 was high and the FPR was low (0.07%), but PPV was substantially lower than the PPV of NIPS for T21 (Table 1).

T13

A summary of the performance characteristics of NIPS for detection of T13 reported by 19 studies and meta-analysis is presented in Table 1 with corresponding forest plots and sensitivity analyses in the Supplement.

Overall, we observed high sensitivity, specificity, accuracy, and DOR for T13 with low FPRs. PPV was low at 37%, which was lower than the PPV for T18 and substantially lower than the PPV for T21. Similar to the subgroup analyses performed for T21 and T18, performance may vary, although the data are insufficient to draw conclusions about any individual subgroup. One additional study reported specificity without presenting the number of TNs and/or FPs (99.94% [no CI given]).[28] In that study of 40,265 individuals who received NIPS, diagnostic testing confirmed 4 of 33 T13 positive results.[28] Chen et al[26] reported an overall PPV of 13.79% for T13; however, in the subset of their population with no clinical indications, PPV was 25.00%. In the large study of >150,000 singleton pregnancies from Belgium, sensitivity, specificity, and NPV of NIPS for T13 was very high (each >99%), whereas PPV was considerably lower in this general-risk population: 43.90% (95% CI = 33.67%-54.68%).[20]

Combined T21, T18, T13

Most studies reported NIPS performance separately for each trisomy; however, there were some that reported overall performance for multiple outcomes. Oneda et al[29] evaluated NIPS performance for T21/T18/T13 in both prospective and retrospective populations. In their prospective cohort, sensitivity was reported as 100% (95% CI = 91.96%-100%), specificity was 99.97% (95% CI = 99.81%-100%), PPV was 97.78% (95% CI = 86.11%-99.68%), and NPV was 100% (no CI). This resulted in test accuracy of 99.97% (95% CI = 99.81%-100%). In a Chinese population of 15,626 people, Yao et al[30] reported an overall PPV of 79.07% (95% CI = 68.69%-86.80%) for T21/T18/T13 with an FPR of 0.13% (95% CI = 0.08%-0.21%).[30]

Guy et al[16] reported combined sensitivity and PPV for T18 and T13 (90.4%, 95% CI = 80.0%-96.8%; 92.2%, 95% CI = 81.5%-96.9%, respectively). Together with the results of the meta-analyses, these data present a largely positive view of NIPS as a highly accurate screening method for T21, T18, and T13, although, variability in a number of factors influenced specific test metrics.

NIPS performance in multifetal gestations

In total, 11 studies reported at least 1 performance characteristic of NIPS to detect T21, T18, or T13 in multifetal gestations, 7 of which were included in meta-analyses. A summary of results from the random-effects meta-analyses are presented in Table 2 with corresponding forest plots in the Supplement.

In the limited number of studies reporting on use of NIPS for twin gestations, diagnostic performance to detect T21, T18, and T13 was generally high, with no/little observed heterogeneity. Apart from the studies included in the meta-analysis, 4 additional studies reported outcomes pertaining to NIPS use in twin gestations.[29,31-33] NIPS screen-positive results were identified in 11 twin and 1 triplet pregnancies, accounting for 2.7% of twin pregnancies, from a prospective mixed-risk cohort of 3053 individuals.[29] Diagnostic testing confirmed the results except for 1 individual, in which it was found in the placenta of 1 twin only and reported as an FP.[29] No FP results were observed in patients with confirmatory testing for T21, T18, or T13 in either monozygotic or dizygotic pregnancies.[33] In the same study, fetal sex confirmation and zygosity calls were found to be correct in all patients.[33]

In a study of singleton and multifetal pregnancies in China, fetal sex determination was concordant in 98.6% (95% CI = 92.19%-99.96%) of twins and 97.6% (95% CI = 91.76%-99.71%) of triplets.[30] Three cases of chromosomal aneuploidy were observed in twin pregnancies. A sample from a dichorionic diamniotic pregnancy with NIPS results suggesting T21 in both fetuses resulted in termination of pregnancy that was not confirmed on the products of conception in this report. A second dichorionic diamniotic pregnancy had NIPS results of suspected T21 in only 1 twin; this finding was confirmed through karyotype and a selective feticide was performed. A live birth was reported for the other twin. Trisomy 7 (T7) was suspected in 1 twin from a monochorionic diamniotic pregnancy, with normal NIPS findings for the other. Twin-to-twin transfusion syndrome was also present and resulted in fetal demise of the receipt twin at 25 weeks and a live birth of the donor twin at 28 weeks. Importantly, the T7 finding was not confirmed through diagnostic testing; the authors hypothesized that the T7 NIPS result was likely a mosaic artifact.[30]

A report from a commercial laboratory presented the results of 30,826 mixed-risk twin samples submitted between October 2011 and December 2017.[32] Of these, 635 had positive NIPS results: T21, n = 435; T18, n = 138; T13, n = 62. Despite the large numbers of positive NIPS results, confirmation of findings was communicated by the submitting physician for only 27, 13, and 10 samples, respectively. The authors further describe an "Enhanced Sequencing" option, selected by more than half of individuals, to screen for additional aneuploidies and microdeletion syndromes. Seven samples had a positive

Table 2 Diagnostic performance statistics of NIPS in twin gestations

Test Statistic	No. of Studies	Result (%) (95% CI)	I^2 (%)
Trisomy 21			
Sensitivity	7	98.18 (88.19-99.74)	0
Specificity	7	99.93 (99.78-99.98)	0
PPV	7	94.74 (84.91-98.29)	0
NPV	7	99.98 (99.83-100)	0
FPR	7	0.07 (0.02-0.22)	0
Accuracy	7	99.82 (99.61-99.92)	0
DOR[a]	7	6586.60 (1696.39-25573.83); $P < .0001$	0
Trisomy 18			
Sensitivity	5	90.00 (67.62-97.49)	0
Specificity	6	99.95 (99.80-99.99)	0
PPV	5	90.00 (67.62-97.49)	0
NPV	6	99.95 (99.80-99.99)	0
FPR	6	0.05 (0.01-0.20)	0
Accuracy	6	99.83 (99.61-99.92)	0
DOR[a]	5	3606.40 (710.38-18308.67)	0
Trisomy 13			
Sensitivity	4	80.00 (30.90-97.28)	0
Specificity	5	99.93 (99.41-99.99)	0
PPV	4	81.75 (1.82-99.91)	0
NPV	5	99.97 (99.82-100)	0
FPR	5	0.07 (0.01-0.59)	0
Accuracy	5	99.76 (99.39-99.91)	20.7
DOR[a]	4	1350.78 (206.12-8852.31)	0

Results do not include studies without adequate data to include in meta-analyses.

DOR, diagnostic odds ratio; *FPR*, false positive rate; *NIPS*, noninvasive prenatal screening; *NPV*, negative predictive value; *PPV*, positive predictive value.

[a]Data presented as odds ratio.

NIPS result for trisomy 16 and 6 samples received positive results for microdeletions. Four of the microdeletion results were reported to have diagnostic testing; 3 were TPs and 1 was FP. The other 2 cases were not confirmed diagnostically but were reported to be consistent clinically with the suspected microdeletion syndrome. All of the samples positive for microdeletions were in higher-risk samples (ie, ultrasound finding or other high risk). Of the 7 suspected cases of T16, 6 were reported as fetal (cotwin) demise after NIPS or as spontaneous abortion. Of these, 2 were reported to be FP after karyotyping was completed from amniocentesis.[32]

Overall, few studies have comprehensively evaluated the use of NIPS for twin gestations. The results from our meta-analyses show NIPS performance in this population are generally comparable to performance in singleton pregnancies for T21, T18, and T13. Results for other aneuploidies or microdeletions were less frequently reported and no firm conclusions can be drawn about the performance of NIPS for these outcomes. Very limited data is available on triplets or higher order multiple gestations.

SCAs

In total, 33 studies reported on identification of SCAs and 28 provided sufficient data to include in random-effects meta-analyses (Supplemental Material). We analyzed studies reporting on any SCA together (overall) and separately for the specific SCA (eg, XXX).

For screening of all SCAs, our meta-analyses found sensitivity, specificity, NPV, and high accuracy of NIPS; however, the PPV for SCAs was <50%, substantially lower than the PPV of NIPS for T21. When considering individual SCAs separately, we observed similar high-performance metrics for sensitivity, specificity, accuracy, NPV, and DOR, but PPVs ranged from 30% (45, X) to 74% (47, XXY; 47, XYY). The number of studies contributing to these analyses was generally small, although most studies reported sufficient data to include in meta-analyses for PPV. FPRs were similarly variable (Supplemental Material).

In addition to the 28 studies included in meta-analyses, 5 studies reported relevant SCA outcomes for NIPS.[24,27,29,34,35] DiNonno et al[34] described NIPS performance for common trisomies and SCAs from more than 1 million test results generated from 2014 to 2017, comparing PPVs obtained in individuals of advanced maternal age to those younger than 35 years. They found combined NIPS positive result rates for T18, T13, and 45, X declined over the 4-year period, commensurate with the uptake of NIPS by younger individuals without prior risk factors. Comparing results only for those with confirmation through ultrasound, pregnancy loss, or diagnostic testing, the PPV for 45, X in individuals aged <35 years was 92.0% (95% CI = 87.5%-94.9%) vs 88.5% (95% CI = 80.1%-93.6%) in individuals aged 35 years old or older.[34]

SCAs from a mixed-risk population from Germany was reported by Tekesin et al.[24] Among the 19 individuals with a suspected SCA, only 8 had confirmatory testing through either chorionic villus sampling ($n = 2$) or amniocentesis ($n = 6$). Of the 8, 6 were reported as normal, whereas the single case of XXY and 1 of the 6 cases of XXX were confirmed. Of the 11 individuals who did not receive confirmatory diagnostic testing, 1 of the 6 suspected cases of Turner syndrome was confirmed, 4 were reported as normal, and 6 did not undergo genetic testing.[24]

Snyder et al[35] presented the results from a retrospective analysis of 113,415 NIPS tests. The authors identified 36 suspected cases of a single autosomal trisomy (T21, T18, or T13) combined with an SCA. For T21 + SCA, 11 cases had clinical outcomes: 1 was fully concordant (T21, XXX), 8 were partially concordant (T21, 45, X), and 2 cases were completely discordant. Several suspected cases of T18 and T13 were also observed in this population in conjunction with a common trisomy. Full concordance was observed in a case of T18, XXY. However, all of the positive results were obtained from individuals with a high risk.

RATs

In total, 18 studies reported data pertaining to identification of RATs. Only 3 of these adequately reported data to enable determination of full test performance characteristics[19,26,36] (Supplemental Material). At a minimum, 17 of the included studies reported the numbers of TP and FP. For each rare chromosomal trisomy, at least 1 study reported a screen-positive result. However, in those with a positive result, those with no confirmatory testing and/or missing from follow up ranged from 0% to 100%. Consequently, quantitative analysis was performed for all RATs together and results pertaining to specific trisomies are narratively described (Supplemental Material).

CNVs

In total, 17 studies reported the ability of NIPS to detect CNVs (microdeletions or microduplications). The sample sizes in each study were relatively small and the sensitivities varied greatly. Tekesin et al[24] reported 7 cases that screened positive for DiGeorge syndrome (22q11.2 deletion), yet none were confirmed via diagnostic testing. Yin et al[37] confirmed TP CNVs in 10 of the 12 cases tested through amniocentesis, whereas in the study by Zheng et al,[36] none of the 3 CNVs were confirmed.

Three additional studies reported a relatively low number of samples with CNVs detected.[21,30,38] Taken together, they detected 14 CNVs, of which 5 were TP and 9 were FP. Reported overall sensitivity to detect CNVs ranged from 69.44%[29] to 80.56%.[39] When stratified by CNV size, in general, the sensitivity to detect larger CNVs was better than for detecting smaller CNVs. The sensitivity to detect CNVs larger than 5 megabases (Mb) was >90%, whereas for those

smaller than 5 Mb, it was 68.42%.[39] In the study by Ye et al,[40] the sensitivity to detect CNVs larger than 2 Mb (81.58%, 31/38) was higher than for detecting those smaller than 2 Mb (21.43%, 3/14).

In a study by Lin et al[27] with follow up of 10,975 negative NIPS results, there were 166 cases with adverse pregnancy outcome, of which 8 had diagnostic testing. Four cases of chromosome abnormalities were confirmed, including 2 results showing microdeletions/microduplications.

Liang et al[41] was able to stratify PPV on the basis of syndromes (n = 32), 93% (DiGeorge syndrome), 68% (22q11.22 microduplication), 75% (Prader-Willi/Angelman syndrome), and 50% (cri-du-chat syndrome). For the remaining genome-wide CNVs (n = 88), combined PPVs were 32% (CNVs ≥10 Mb) and 19% (CNVs <10 Mb). Chen et al[31] showed an overall PPV of 28.99% with the best sensitivity between 5 and 10 Mb in size (20.83% for ≤5 Mb, 50.00% for 5 to 10 Mb, 27.27% for >10 Mb) for CNVs. Schwartz et al[42] had the largest sample size of screen-positive CNV cases (N = 349) with an overall PPV of 9.2%.

A large study (N = 80,449) of NIPS for a panel of microdeletion syndromes (22q11.2 deletion, 1p36 deletion, cri-du-chat, Prader-Willi, Angelman) was reported from a laboratory sample after revision of their algorithm.[43] In >42,000 individuals screened for the full panel, in those without any abnormal ultrasound findings, PPV was 18.5% for 22q11.2 deletion, 50% for 1p36 deletion, 50% for cri-du-chat, 0% for Prader-Willi, and 10% for Angelman syndromes; however, there was incomplete follow up of positive NIPS results. For individuals with abnormal ultrasound findings identified before NIPS, PPVs were significantly higher: 100% for 22q11.2, 1p36 deletion, and cri-du-chat syndromes. The authors report that the revision to their algorithm both improved PPV and reduced FPRs for these microdeletion syndromes.[43]

Psychosocial outcomes

There is limited literature regarding psychosocial outcomes after NIPS. In a study of 40 participants who received positive NIPS results, a significant portion regretted their decision to have NIPS in light of the stress and additional medical interventions they experienced. However, this was a biased sampling of individuals who posted in online forums.[44] Eight participants expressed positive opinions, 20 had mixed feelings, and 12 had negative opinions.[44] In another study that assessed the effect of genetic counseling after positive NIPS results, 76% of participants accepted confirmatory diagnostic testing, whereas 24% elected not to proceed with follow-up diagnostic testing.[45] Given the minimal evidence, no conclusions can be drawn about the impact of NIPS on psychosocial outcomes.

Maternal conditions

We identified 14 studies that included outcomes for maternal conditions (Supplemental Material). Of these, 8 were specifically directed at reporting maternal outcomes, the others were reported as part of a larger NIPS study. One study[35] included cases that were published in another study.[46] The predominant reported results were maternal neoplasms (n = 5 studies) and maternal X chromosome abnormalities (n = 3 studies). Other outcomes included actionable maternal CNVs (n = 4 studies), Duchenne muscular dystrophy gene CNV identification (n = 1), and various structural chromosomal abnormalities, such as mosaicism for an interstitial deletion and an unbalanced translocation. In a study describing the implementation of NIPS as a universal screening method in Belgium, reported maternal imbalances were found in 0.32% of NIPS results.[20] Another study similarly identified 9 clinically actionable CNVs in 3053 samples (0.29%).[29] In this study, 8 of 9 patients had symptoms of the identified disorders with 1 of 9 asymptomatic with a genetic diagnosis of Ehlers-Danlos syndrome.[29] Two confirmed maternal cases of 22q11.2 deletion were identified in a large laboratory study of NIPS from the United States for a panel of 5 microdeletion syndromes.[43] One additional maternal case was unconfirmed in the parent; however, the individual had learning disabilities and tetralogy of Fallot, which are both associated with 22q11.2 deletion syndrome.[43] Neoplasms were identified by noting unique gains and losses of multiple CNVs across chromosomes; neoplasms sometimes included uterine myomas and therefore did not consistently represent a malignancy. The Belgian population-level study reported maternal neoplasms were identified in 0.008% of NIPS results.[20] Although X chromosome anomalies were identified, including 2 interstitial X deletions,[47] 47, XXX,[46,48] and a mosaic 45, X/47, XXX complement, it is unclear if these findings had any effect on maternal health. Maternal outcomes were consistently a rare finding in NIPS and follow up with clinical outcomes was not reported.

Uptake of diagnostic testing

We identified 10 studies that included outcomes for uptake of diagnostic testing.[18,20,29,49-55] Some studies examined the rate of uptake of diagnostic testing in those screening positive on NIPS whereas others looked at the rate of uptake of diagnostic testing over time, comparing the period before NIPS was available with the period after NIPS was available.

Screening for chromosome 7 aneuploidy as part of "supplemental NIPT" in 31,250 patients found 35 at high risk.[50] Of those, 25 patients (71%) chose diagnostic testing and 2 pregnancies had CNVs involving part of chromosome 7.[36] A general screening of 2998 patients found 278 with high-risk results. Of those, 98.5% received diagnostic

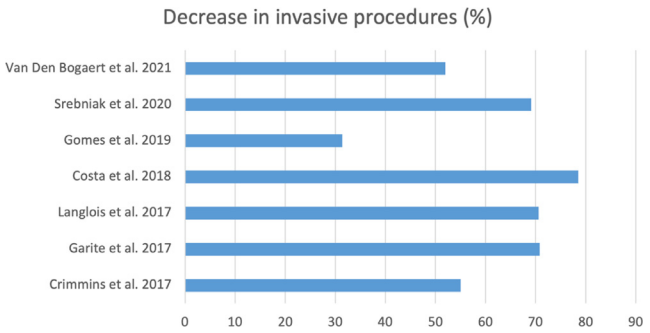

Figure 1 Percent reduction of diagnostic testing after noninvasive prenatal screening implementation.

testing, whereas only 4 patients did not.[29] Because neither of these studies looked at diagnostic testing over time, they are not included in Figure 1. In a South Korean medical center, the mean number of amniocenteses performed before NIPS was 8.8 per month that decreased to 4.1 per month after offering NIPS.[51] Because the raw data on total numbers or percentages of procedures was not provided, this study was not included in Figure 1.

One of these studies was limited to modeled data. In the model, if all participants received an amniocentesis after a "positive" result, there would be a 55% reduction in the rate of amniocentesis performed when initially screened with NIPS.[49] The total number of diagnostic procedures performed was reported to drop from 1176 in 2009 to 846 in 2015 and then 363 in 2018, likely due to the introduction and subsequent growing use of NIPS,[52] although the total number of patients screened was not provided. In another study, the rate of diagnostic testing dropped from 3.5% (before implementation of NIPS) to 2.4% (with the use of a contingent model incorporating NIPS), although, this was not statistically significant.[53] In the high-risk group, 83.3% (25/30) had a diagnostic test. In the intermediate-risk group only 12.2% (6/49) chose diagnostic testing, whereas 75.5% opted for NIPS (37/49). Costa et al[18] described that use of NIPS decreased the potential rate of diagnostic procedures from 8.2% with maternal serum screening (MSS) alone to 1.9% with a combination of NIPS and MSS. In this group of 789 patients, there were 15 diagnostic procedures performed, with potentially an additional 50 procedures in patients receiving a high-risk MSS, but a low-risk NIPS. In another study, they postulated that the rate of diagnostic testing could potentially be as high as 6.8% (79/1165) with traditional screening, whereas in their study, overall it was 2% (23/1165) with 1.2% (14/1151) of individuals with a negative NIPS result choosing diagnostic testing.[54] In the final study, Garite et al[55] found an overall 70.8% (calculated for this publication) decline in procedures (73% decrease in amniocenteses and 62% decrease in chorionic villus sampling) between the first 6 months of the control period and the last 6 months of the study period.

Although a significant majority of patients who receive a high-risk result do choose to pursue diagnostic testing, overall, it appears that the total number of patients choosing diagnostic testing has decreased over time ranging from a 31% to 79% decrease (see Figure 1) depending on the study. The findings from the Belgian population study comparing 2013, before NIPS, uptake of diagnostic testing to 2018, after universal NIPS, found a 52% reduction, which was larger than would be expected on the basis of the incidence of T21 alone.[20] This choice of whether to pursue diagnostic testing may vary based on the specific aneuploidy, availability of genetic counseling, and personal values and decision-making, however, the data were not available to assess this level of granularity.

Economic impact

Of the 10 studies that reported outcomes pertaining to the cost-effectiveness of NIPS performed in a general-risk population, only 1 was done with the societal perspective with a time horizon of the maternal lifespan, in a theoretical cohort of 4 million individuals in the United States.[56] In this study, the authors compared NIPS to detect T13/T18/T21 with NIPS for the common trisomies and 5 microdeletion syndromes. If the cost to report the microdeletions added $47 or less to the cost of NIPS for the main trisomies, NIPS plus microdeletion screening increased quality-adjusted life years by 977, decreased overall costs by $90.9 million per year, and would result in fewer neonatal deaths and second trimester miscarriages.[56] The remaining studies compared NIPS, either as a universal screening method or as a contingent method presented after some initial risk evaluation. Notably, these studies were nearly all performed from a public payer perspective and limited the time horizon to the testing duration or length of pregnancy only (Supplemental Material).

Test failure

Although not an original KQ for this SER, the guideline panel requested information regarding test failure rates, given their known association with aneuploidy. Unfortunately, this was not reported in a standard manner across studies. Some reported only the overall failure (or no-call) rate without mention of redraws, whereas others included their redraw failure (or success) rate, with some even more granular, separating out failures from the first test compared with failures from the second. Estimated failure/no-call rate of NIPS was 0.85% (95% CI = 0.58%-1.23%) in 31 studies (Supplemental Material). Although heterogeneity was considerable (I^2 = 99%), no subgroup analyses were performed owing to the inconsistency and variability of the studies. Overall, NIPS failure rate appears relatively infrequent; however, this metric may be subject to considerable publication bias.

Change in birth rates

We identified a single study that reported on a change in birth rates after implementation of universal NIPS. Belgium,

which was the first country to implement universal access and reimbursement of NIPS as a first-tier prenatal screening test, compared the rate of trisomy 21 live births from 2014 to those in 2018. The rate decreased from 0.06% of all live births to 0.04% during the time period in question, a decline that the authors could not explain through population-level changes responsible for a concurrent rise in trisomy 21 miscarriages. They posit that the reduction may result from pregnancy termination combined with the improved FPRs for NIPS, as compared with first trimester combined screening.[20]

Risk of bias assessment

We observed no evidence of publication bias across most outcomes, although there was suspicion of publication bias for test failure rate. Risk of bias for individual studies reporting the clinical or diagnostic performance outcomes uncovered serious risk of bias for confounding and missing data (ROBINS-I) and patient selection and flow and timing (QUADAS2) domains (Supplemental Material). Risk of bias was assessed across 20 domains identified in the Consolidated Health Economic Evaluation Reporting Standards checklist[12] and Drummond criteria.[57] Most compared NIPS with at least 1 option without NIPS. Except for the Avram et al[56] study, none reported a discount rate or a time horizon beyond the duration of pregnancy. An overall risk of bias was not calculated for the economic studies; however, few domains received a high risk of bias judgment for more than a single study. Unreported and under-reported data was a significant concern (Supplemental Material).

Discussion

This assessment validates that NIPS with cfDNA is the most sensitive and specific screening test for fetal Down syndrome, T13, and T18 in both singleton and twin pregnancies. In contrast to conventional serum analyte screening, it can identify maternal conditions, such as aneuploidies and malignancies. Although rare, maternal aneuploidy findings are only possible with cfDNA screening. Other outcomes, such as RATs and CNVs (predominantly deletions) in both fetus and mother can be identified. However, the clinical utility of these findings is limited, given the rarity of these events and the lack of systematic follow up of clinical outcomes.

Several recent reviews and meta-analyses have been published on NIPS.[4,58-62] Compared with traditional screening, the 2019 health technology assessment by Health Quality Ontario determined that NIPS was effective in a general or average-risk population to screen for T21, T18, and T13.[58] Our results similarly show the high performance of NIPS to screen for the common trisomies in a general population. Of the studies that used meta-analysis of NIPS to screen for SCAs, we observed that several included high-risk population studies in their analyses and their results may not be as generalizable to an average-risk population. Despite this difference, we observed relatively consistent results with our meta-analyses for SCAs to these published studies, supporting our conclusion that NIPS is also effective and accurate for SCA screening.

Our SER and meta-analysis present several strengths and limitations. Building on existing evidence, we limited our literature search for several KQs to obtain the most recent data. We considered the utility of NIPS beyond diagnostic performance by including the uptake of diagnostic tests, the impact on individuals' psychosocial status, and the identification of maternal conditions. The large number of studies included in our SER is a considerable strength.

Nevertheless, there are some limitations to our study. First, although we revised our search query to account for the variety of definitions which describes NIPS in the literature, it is possible we did not identify all relevant studies. Second, despite prespecifying an analysis plan to address expected heterogeneity, there may be other variables that we did not include in our sensitivity analyses that contribute to the variation observed between studies. Third, we included studies in our meta-analyses for which the reviewers were confident in the data reported. It is possible that this confidence was misplaced, particularly for TNs, causing us to inappropriately include studies in our quantitative analyses. Furthermore, our meta-analyses did not use the bivariate model, as detailed in Reitsma et al.[63] Although there was sparse data for many of the reported studies, we re-evaluated our analyses (data not shown) and determined that the difference between our results and the bivariate model were small (eg, T21 $\text{sensitivity}_{\text{bivariate}} = 97.6\%$ [95% CI = 96.0%-98.6%] compared with reported results [98.8%, 95% CI = 97.8%-99.3%]), although the area under the curve remained consistent regardless of the model (area under the $\text{curve}_{\text{T21}} = 99\%$). Finally, although our research questions were developed to compare NIPS with conventional serum analyte screening, we did not identify any studies reporting direct comparisons that met our inclusion criteria.

Limitations of the included studies themselves were numerous. It was often difficult to distinguish between low- and high-risk cohorts in individual studies. Information on the complete ascertainment of cases is lacking, given that there is a lack of complete follow up to identify TNs and FNs through diagnostic testing or postnatally, although these numbers are expected to be small. Studies mostly relied on local providers to evaluate fetal outcomes through physical assessment or a chart review performed to determine the newborn phenotype that may introduce error. A few studies used more objective means of obtaining this data, such as national databases. A systematic follow up of individuals with low-risk NIPS results would provide a more accurate picture of the TNs and were unavailable for review. Furthermore, the laboratory techniques used, including sequencing methods, or cutoffs for test failures or screen positives are not standardized, may differ more owing to the applications in other

countries, and the details were inconsistently reported. These failures can be due to a variety of factors. Some may have issues with the specimen itself such as inadequate sample volume or coagulation and were therefore unable to complete the sequencing process. Others may successfully complete sequencing but have no result available after an issue with analysis. This can be due to a variety of reasons, including low fetal fraction, with minimum requirements varying between laboratories and some using a method to further amplify the fetal fraction.[64] A redraw can be recommended, in which a new blood specimen is collected. In general, increased gestational age (over 20 weeks) correlates with increased fetal fraction, so collection of a specimen later in pregnancy may overcome the issue of low fetal fraction, although this would reduce the clinical utility of screening. Other issues include sample contamination, high sequence homology between maternal and fetal, or other quality control metrics.

There was limited literature available to evaluate the psychosocial outcome of individuals undergoing NIPS. Although multiple studies were identified that surveyed attitudes toward NIPS, very few were available in which NIPS was actually performed, patients received results, and then were assessed for levels of anxiety, stress, and/or regret in a systematic manner. Additional studies with a systematic evaluation approach on a large cohort is needed to better understand the psychosocial impact of NIPS, which may further elucidate the uptake (or lack thereof) of NIPS in the general population. Moreover, the psychosocial reception of NIPS may also be affected by the cost for patients and payer coverage. Economic analyses based in the United States from the patient perspective are lacking; evidence from national health care systems such as Belgium, Canada, and the Netherlands suggest most pregnant individuals find NIPS as a primary screening method for fetal chromosomal aneuploidies acceptable and have not identified significant negative impact of NIPS on psychosocial outcomes.

As described in this SER, the performance of NIPS is significantly poorer when targeting RATs and CNVs than when looking for the common trisomies. This is likely because of the rarity of RATs and the insufficient data available to properly develop a method that can distinguish between clinically relevant RATs found in the fetus vs confined placental mosaicism. In addition, the NIPS technologies were originally designed to detect the common trisomies, and not to identify small CNVs. Deletions are more difficult to identify in the background of a normal maternal karyotype than are trisomies. Large collaborative studies may be needed to generate a sufficient cohort to develop a singular method with adequate sensitivity and specificity for findings other than common trisomies. Additional outcome studies are needed to understand the unique clinical value of NIPS, specifically for SCAs, RATs, and CNVs when compared with other approaches.

Comparisons between studies are difficult, because there is no standardized testing method, fetal fraction cutoffs and calculation methods vary, and there are different initial gestational ages for testing. Further delineation of sensitivity and specificity of NIPS methodologies by independent researchers is needed to determine the best modality and to improve the diagnostic utility. Ideally, studies would include a comprehensive ascertainment of clinical outcomes to calculate the TN rate. This information would help to develop best practice guidelines and improve patient care. Despite the large number of studies included in our analysis, we identified few that considered the psychosocial impact of NIPS, particularly in light of additional information (eg, maternal conditions) that would not be captured using traditional screening techniques.

Conclusion

Worldwide, and across all laboratory platforms, NIPS using cfDNA is the most effective screening test for the autosomal T21, T18, and T13 in singleton and twin gestations, with both high detection and low FPRs. Although less accurate for SCAs, RATs, and CNVs, it is the only laboratory-based prenatal screen that can identify these at all. The incidental identification of maternal conditions is rare and makes for potentially difficult patient counseling. Finally, no conclusions can be drawn with respect to the potential psychosocial effects of this test on the screened population. Despite its accuracy, NIPS using cfDNA is a screening test for which confirmation of a screen-positive test with a diagnostic procedure remains indicated.

Conflict of Interest

N.C.R. is a consultant for The Jackson Laboratories and the ObG Project. E.S.B. and M.L.L. serve as directors in, and D.L. is employed by, clinical laboratories that perform a breadth of genetic and genomic analyses on a fee-for-service basis. All other authors declare no conflicts of interest.

Additional Information

The online version of this article (https://doi.org/10.1016/j.gim.2022.03.019) contains supplementary material, which is available to authorized users.

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Systematic evidence-based review: the application of noninvasive prenatal screening using cell-free DNA in general risk pregnancies

Supplement

Table of Contents

Search strategy for Medline (Pubmed)

Search 1:

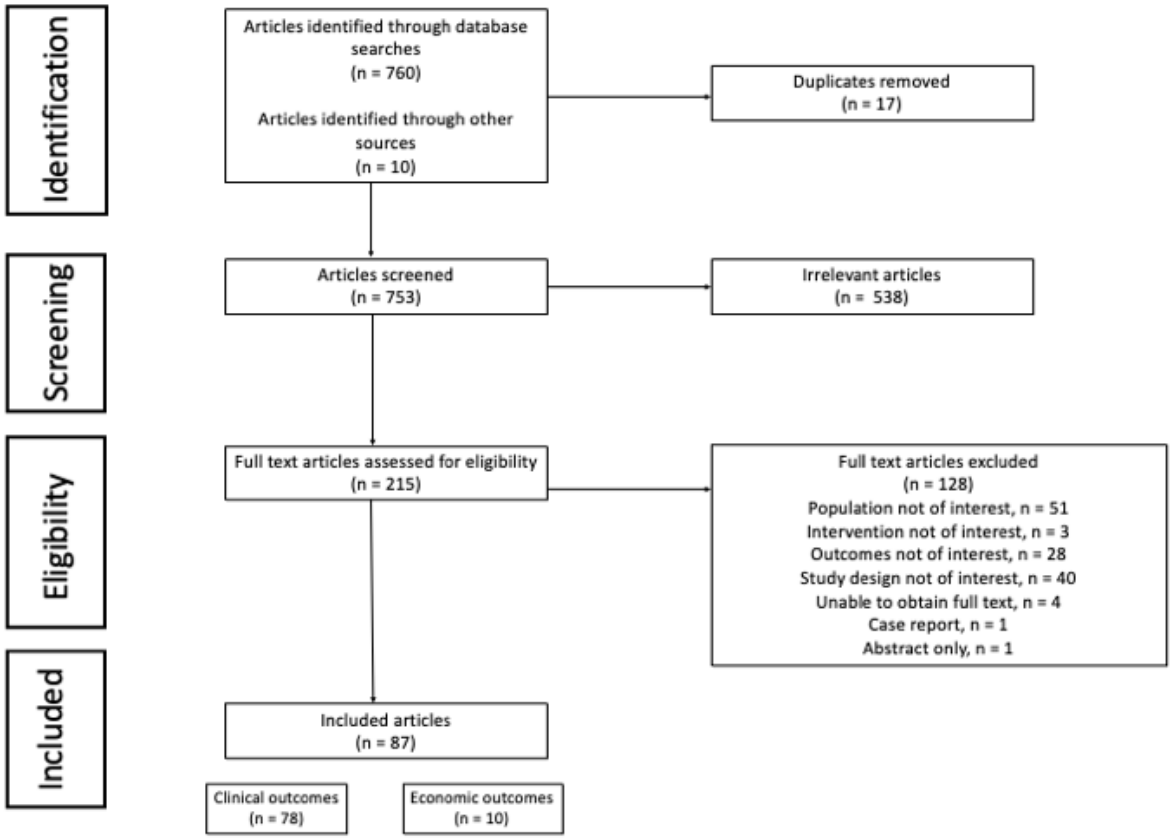
("Noninvasive prenatal testing" AND pregnancy) AND (chromosome disorders OR aneuploidy OR trisomy 13 syndrome OR trisomy 18 syndrome OR trisomy 21 syndrome OR DNA copy number variations OR DiGeorge syndrome OR Prader-Willi syndrome OR Angelman syndrome OR Williams syndrome OR Cri-du-chat syndrome) AND (prenatal diagnosis OR amniocentesis OR chorionic villi sampling OR maternal serum screening tests OR fluorescence in situ hybridization OR karyotyping OR cytogenetics OR costs and cost analysis OR quality-adjusted life years) limit to 9/2017 to present

("Noninvasive prenatal testing" AND pregnancy) AND ((trisomies NOT trisomy 13 syndrome OR trisomy 18 syndrome OR trisomy 21 syndrome) OR (prenatal diagnosis OR psychological stress OR physiological stress OR regrets OR sensitivity and specificity OR incidental findings OR uncertainty OR neoplastic pregnancy complications) OR (chromosome aberrations AND mothers))

Search 2:

((((("Noninvasive prenatal testing" OR ("cell free nucleic acids/analysis"[MeSH Terms] OR "cell-free DNA" OR "cfDNA")) AND pregnancy) AND (chromosome disorders OR aneuploidy OR trisomy 13 syndrome OR trisomy 18 syndrome OR trisomy 21 syndrome OR DNA copy number variations OR DiGeorge syndrome OR Prader-Willi syndrome OR Angelman syndrome OR Williams syndrome OR Cri-du-chat syndrome) AND (prenatal diagnosis OR amniocentesis OR chorionic villi sampling OR maternal serum screening tests OR fluorescence in situ hybridization OR karyotyping OR cytogenetics OR costs and cost analysis OR quality-adjusted life years)) NOT (("Noninvasive prenatal testing" AND pregnancy) AND (chromosome disorders OR aneuploidy OR trisomy 13 syndrome OR trisomy 18 syndrome OR trisomy 21 syndrome OR DNA copy number variations OR DiGeorge syndrome OR Prader-Willi syndrome OR Angelman syndrome OR Williams syndrome OR Cri-du-chat syndrome) AND (prenatal diagnosis OR amniocentesis OR chorionic villi sampling OR maternal serum screening tests OR fluorescence in situ hybridization OR karyotyping OR cytogenetics OR costs and cost analysis OR quality-adjusted life years))) AND (("2017/09/01"[Date - Publication] : "3000"[Date - Publication])) Sort by: Most Recent

Supplemental Figure 1. PRISMA flowchart of studies for NIPS SER.



Supplemental Table 1. PICOTS and Key Questions for NIPS SER.

PICOTS	Key Questions
Population: pregnant individuals at general risk for fetal aneuploidy (singleton and multiple gestations)	KQ1: In a general risk population, does non-invasive prenatal screening (NIPS) for T21 offer superior screening performance when compared to traditional methods of screening?
Intervention: NIPS	KQ2: In a general risk population, does NIPS for T18 or T13 offer superior screening performance compared to traditional methods of screening?
Comparators: traditional screening (e.g., serum screening, ultrasound, QUAD screen)	KQ3: In a general risk population of multifetal gestations, does NIPS for T21, T18, and T13 offer superior screening performance compared to traditional methods of aneuploidy screening?
Outcomes: detection of T21, T18, T13, RATs, SCAs, CNVs (microdeletions), maternal conditions; change in uptake of invasive diagnostic tests; cost-effectiveness,[a] cost-utility[a]	KQ4: In a general risk population, what is the evidence that supports the routine use of screening for fetal SCAs with NIPS?
Timing of NIPS: unspecified	KQ5: In a general risk population, what is the evidence for routine use of screening for fetal CNVs (e.g., microdeletions) with NIPS?
Setting: none specified (e.g., clinic, laboratory)	KQ6: In a general risk population, what is the evidence for routine use of screening for fetal RATs with NIPS?
	KQ7: In a general risk population, does the use of NIPS result in different uptake of diagnostic testing (CVS, amniocentesis) or laboratory assays (FISH, array, molecular) compared to the use of traditional screening?
	KQ8: Does the use of NIPS lead to different levels of patient anxiety/stress/regret than what occurs with traditional screening? (include inconclusive/non-reportable results here)
	KQ9: Does the use of NIPS result in identification of unknown maternal

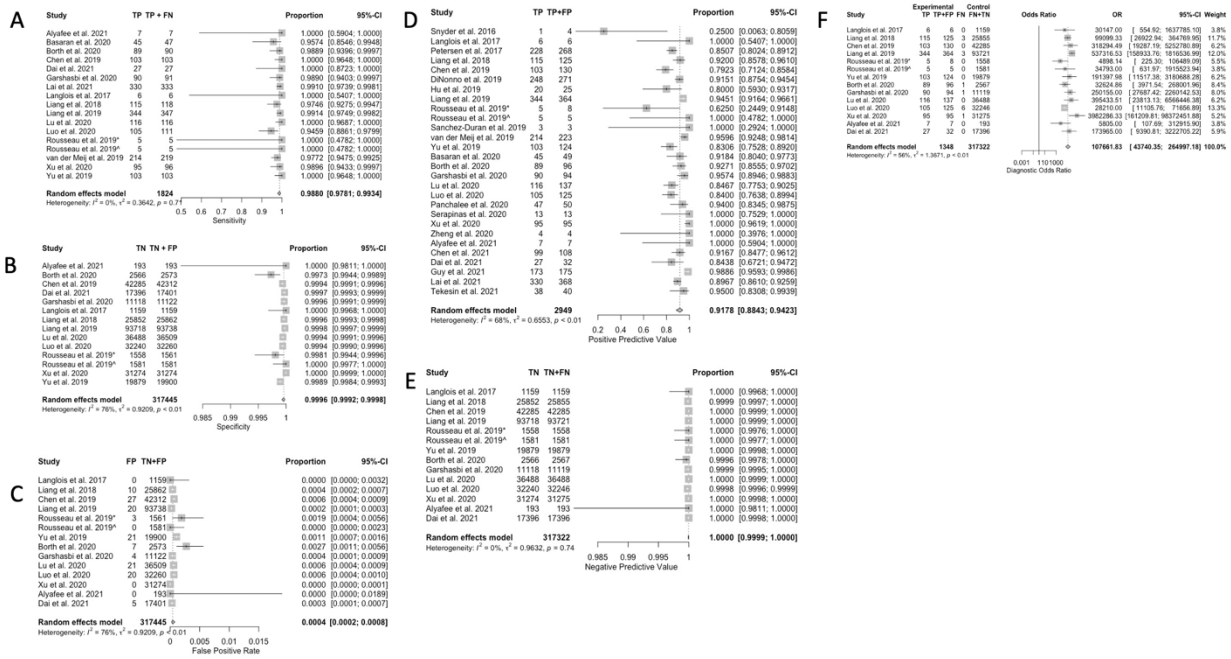
	conditions more frequently than with the use of traditional screening methods?
	KQ10: What are the economic implications of using NIPS as first-line screening for fetal aneuploidy compared to using traditional screening methods?
CNVs, copy number variants; KQ, key question; NIPS, noninvasive prenatal screening; QUAD, quad screening; RATs, rare autosomal trisomies; SCAs, sex chromosome aneuploidies; SER, systematic evidence review; T21, trisomy 21; T18, trisomy 18; T13, trisomy 13.	

Supplemental Table 2. Inclusion and exclusion criteria for NIPS SER.

Inclusion	Exclusion
General-risk pregnant individuals	High-risk population exclusively (mixed-risk patients may be included)
NIPS used as primary or secondary screening for T21, T18, T13, SCAs, RATs, CNVs, maternal conditions	Not primary literature (review articles, abstracts, editorials, guidelines, SERs or meta-analyses (used to identify relevant primary literature))
Studies reporting diagnostic performance of NIPS (i.e., sensitivity, specificity, PPV, NPV, FPR, DOR, accuracy)	NIPS method development
Studies reporting psychosocial outcomes pertaining to use of NIPS in a general-risk population	Non-English language
Studies reporting uptake of invasive diagnostic testing subsequent to NIPS	
Studies reporting economic implications of NIPS (Cost-utilities, cost-effectiveness, costs associated with NIPS, QALYs, ICERs) in a general-risk population	No economic outcomes reported
	Publication date prior to September 1, 2017 for KQ1, KQ2, KQ3, KQ4, KQ5, KQ7, KQ10

CNVs, copy number variants; DOR, diagnostic odds ratio; FPR, false positive rate; ICERs, incremental cost effectiveness ratios; KQ, key question; NIPS, noninvasive prenatal screening; NPV, negative predictive value; PPV, positive predictive value; QALY, quality-adjusted life year; RATs, rare autosomal trisomies; SCAs, sex chromosome aneuploidies; SER, systematic evidence review; T21, trisomy 21; T18, trisomy 18; T13, trisomy 13.

Supplemental Figure 2. Performance characteristics of NIPS to detect Trisomy 21 in general-risk populations from random-effects meta-analyses.



A) sensitivity; B) specificity; C) false positive rate; D) positive predictive value; E) negative predictive value; F) diagnostic odds ratio

Supplemental Table 3. Reported FPR in studies not included in NIPS SER meta-analysis.

Study	Reported False Positive Rate
Basaran et al., 2020	8.20%
Costa et al., 2018	0% (95% CI 0%-0.47%)
Kagan et al., 2018	0%
Lai et al., 2021	0.05%
Petersen et al., 2017	15%
Sanchez-Duran et al., 2019	0%
Tekesin et al., 2021	5.0% (95% CI 0.1-16.9%)

Supplemental Table 4. Subgroup analyses for specificity of NIPS for T21.

Category	N studies	Specificity (%) (95% CI)	I^2	Between-group difference
Year of publication				
2017	1	100 (0-100)	NA	
2018	1	99.96 (99.93-99.98)	NA	*Q* =1.45
2019	5	99.94 (99.89-99.99)	87.5%	
2020	5	99.96 (99.84-99.99)	74.7%	*P* = 0.84
2021	2	99.97 (99.93-99.99)	0%	
Country				
China	8	99.96 (99.93-99.98)	77.4%	
Canada	3	99.97 (99.02-100)	0%	*Q* = 19.12
Germany	1	99.73 (99.43-99.87)	NA	
Iran	1	99.96 (99.90-99.99)	NA	*P* = 0.0007
Saudi Arabia	1	100 (0-100)	NA	
Risk of bias (ROBINS-I)				
Moderate	13	99.95 (99.92-99.96)	77.7%	*Q* = 0.00
Serious	1	100 (0-100)	NA	*P* = 1.00
Population size				
<10,000	5	99.90 (99.85-99.93)	0%	*Q* = 5.56
≥10,000	9	99.96 (99.92-99.98)	80.1%	*P* = 0.0184
Table legend: NA, not applicable; NIPS, non-invasive prenatal screening				

Supplemental Table 5. Subgroup analyses for PPV of NIPS for T21.

Category	N studies	PPV (%) (95% CI)	I^2	Between-group difference
Year of publication				
2017	2	85.40 (80.71-89.11)	0%	
2018	1	92.00 (85.77-95.64)	NA	*Q* =18.20
2019	9	89.21 (82.45-93.57)	82.5%	
2020	9	94.09 (88.20-97.14)	35.3%	*P* = 0.0027
2021	6	94.02 (87.81-97.16)	59.4%	
Country				
China	12	89.51 (84.79-92.88)	68.4%	
Canada	3	93.01 (21.10-99.85)	0%	
United States	3	80.98 (49.40-94.89)	83.8%	
Germany	2	93.38 (87.77-96.52)	0%	
Saudi Arabia	1	100 (0-100)	NA	*Q* = 17.61
Spain	1	100 (0-100)	NA	
The Netherlands	1	95.96 (92.43-97.89)	NA	*P* = 0.09
Turkey	1	91.84 (80.18-96.90)	NA	
Iran	1	95.74 (89.21-98.39)	NA	
Thailand	1	94.00 (82.98-98.05)	NA	
Lithuania	1	100 (0-100)	NA	
United Kingdom	1	98.86 (95.55-99.71)	NA	
Risk of bias (ROBINS-I)				
Moderate	20	90.42 (87.22-92.88)	67.7%	*Q* = 0.73
Serious	8	94.53 (81.73-98.53)	73.5%	*P* = 0.39
Population size				
<10,000	13	92.94 (86.25-96.51)	52.1%	*Q* = 0.28
≥10,000	15	91.27 (87.18-94.14)	74.8%	*P* = 0.60

Table legend NA, not applicable; NIPS, non-invasive prenatal screening; PPV, positive predictive value

Supplemental Table 6. Subgroup analyses for FPR of NIPS for T21.

Category	N studies	FPR (%) (95% CI)	I^2	Between-group difference
Year of publication				
2017	1	0.00 (0-1.00)	NA	
2018	1	0.04 (0.02-0.07)	NA	*Q* =1.45
2019	5	0.06 (0.03-0.11)	87.5%	
2020	5	0.04 (0.01-0.16)	74.7%	*P* = 0.84
2021	2	0.03 (0.01-0.07)	0%	
Country				
China	8	0.04 (0.02-0.07)	77.4%	
Canada	3	0.03 (0-0.98)	0%	*Q* = 19.12
Germany	1	0.27 (0.13-0.57)	NA	
Saudi Arabia	1	0 (0-1.00)	NA	*P* = 0.0007
Iran	1	0.04 (0.01-0.10)	NA	
Risk of bias (ROBINS-I)				
Moderate	13	0.05 (0.04-0.08)	77.7%	*Q* = 0.00
Serious	1	0 (0-1.00)	NA	*P* = 1.00
Population size				
<10,000	4	0.03 (0-0.98)	0%	*Q* = 0.04
≥10,000	10	0.04 (0.02-0.08)	81.8%	*P* = 0.83
Table legend FPR, false positive rate; NA, not applicable				

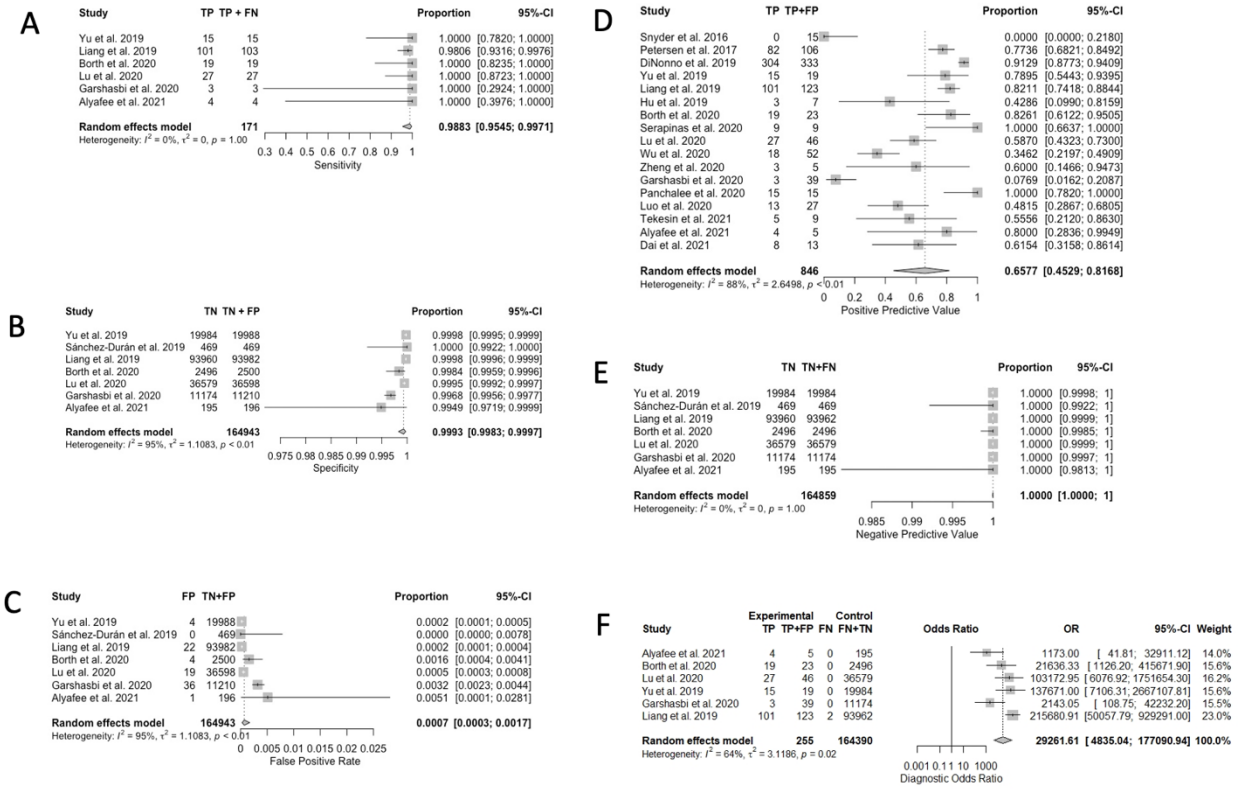
Supplemental Table 7. Subgroup analyses for DOR of NIPS for T21.

Category	N studies	DOR (95% CI)	I^2	Between-group difference
Year of publication				
2017	1	30,000 (550; 1,600,000)	NA	
2018	1	99,000 (27,000-367,000)	NA	*Q* = 0.85
2019	5	130,000 (23,000-700,000)	54.4%	
2020	5	140,000 (27,000-740,000)	68.6%	*P* = 0.93
2021	2	42,000 (1,600-110,000)	45.0%	
Country				
China	8	203,000 (64,000-650,000)	66.8%	
Canada	3	14,000 (1,700-110,000)	0%	*Q* = 8.58
Germany	1	33,000 (4,000-270,000)	NA	
Saudi Arabia	1	5,800 (100-313,000)	NA	*P* = 0.07
Iran	1	250,000 (28,000-230,000)	NA	
Risk of bias (ROBINS-I)				
Moderate	13	89,000 (38,000-210,000)	50.0%	*Q* = 5.02
Serious	1	4,000,000 (160,000-98,000,000)	NA	*P* = 0.025
Population size				
<10,000	4	11,000 (1,800-72,000)	0%	*Q* = 6.39
≥10,000	10	170,000 (63,000-450,000)	74.8%	*P* = 0.0115
Table legend DOR, diagnostic odds ratio; NA, not applicable				

Supplemental Table 8. Subgroup analyses for accuracy of NIPS for T21.

Category	N studies	Accuracy (95% CI)	I^2	Between-group difference
Year of publication				
2017	1	99.96 (99.32-100)	NA	
2018	1	99.95 (99.91-99.97)	NA	*Q* = 0.49
2019	5	99.93 (99.86-99.97)	86.3%	
2020	5	99.94 (99.86-99.97)	85.9%	*P* = 0.97
2021	2	99.94 (99.61-99.99)	52.8%	
Country				
China	8	99.95 (99.93-99.97)	81.8%	
Canada	3	99.88 (99.66-99.96)	3.4%	*Q* = 22.30
Germany	1	99.70 (99.40-99.85)	NA	
Saudi Arabia	1	99.75 (96.15-99.98)	NA	*P* = 0.0002
Iran	1	99.96 (99.89-99.98)	NA	
Risk of bias (ROBINS-I)				
Moderate	13	99.93 (99.90-99.95)	78.8%	*Q* = 8.98
Serious	1	100 (99.98-100)	NA	*P* = 0.0027
Population size				
<10,000	4	99.86 (99.66-99.95)	0%	*Q* = 2.86
≥10,000	10	99.94 (99.91-99.96)	85.1%	*P* = 0.09
Table legend NA, not applicable				

Supplemental Figure 3. Performance characteristics of NIPS to detect Trisomy 18 in general-risk populations from random-effects meta-analyses.



A) sensitivity; B) specificity; C) false positive rate; D) positive predictive value; E) negative predictive value; F) diagnostic odds ratio

Supplemental Table 9. Subgroup analyses for specificity of NIPS for T18.

Category	N studies	Specificity (%) (95% CI)	I^2	Between-group difference
Year of publication				
2019	3	99.98 (99.97-99.98)	0%	Q = 19.70
2020	3	99.87 (99.66-99.95)	95.2%	
2021	1	99.49 (96.47-99.93)	NA	P < 0.0001
Country				
China	3	99.97 (99.95-99.98)	73.3%	
Spain	1	100 (0-100)	NA	Q = 64.64
Germany	1	99.84 (99.57-99.94)	NA	
Iran	1	99.68 (99.56-99.77)	NA	P < 0.0001
Saudi Arabia	1	99.49 (96.47-99.93)	NA	
Risk of bias (ROBINS-I)				
Moderate	6	99.93 (99.81-99.97)	95.7%	Q = 0.00
Serious	1	100 (0-100)	NA	P = 1.00
Population size				
<10,000	2	99.85 (98.94-99.98)	0%	Q = 0.60
≥10,000	5	99.94 (99.83-99.98)	96.5%	P = 0.44
Table legend: NA, not applicable; NIPS, non-invasive prenatal screening				

Supplemental Table 10. Subgroup analyses for PPV of NIPS for T18.

Category	N studies	PPV (%) (95% CI)	I^2	Between-group difference
Year of publication				
2016	1	0 (0-100)	NA	
2017	1	77.36 (68.43-84.34)	NA	*Q* = 3.45
2019	4	81.93 (66.09-91.34)	82.5%	
2020	8	68.14 (32.20-90.59)	77.2%	*P* = 0.49
2021	3	62.96 (43.77-78.78)	0%	
Country				
China	8	60.30 (46.14-72.92)	82.4%	
United States	3	42.45 (1.39-97.47)	85.4%	*Q* = 25.03
Lithuania	1	100 (0-100)	NA	
Germany	1	74.84 (53.34-88.56)	NA	*P* = 0.0003
Iran	1	7.69 (2.50-21.30)	NA	
Saudi Arabia	1	80.00 (30.90-97.28)	NA	
Thailand	1	100 (0-100)	NA	
Risk of bias (ROBINS-I)				
Moderate	13	67.74 (46.91-83.31)	82.5%	*Q* = 0.27
Serious	4	50.82 (7.56-92.88)	84.7%	*P* = 0.60
Population size				
<10,000	7	77.07 (47.02-92.72)	77.7%	*Q* = 1.14
≥10,000	10	56.75 (30.39-79.77)	90.6%	*P* = 0.29
Full reporting of data				
Yes	5	60.37 (27.58-85.91)	90.6%	*Q* = 0.14
No	1	68.12 (42.43-86.10)	88.3%	*P* = 0.70

Table legend: NA, not applicable; NIPS, non-invasive prenatal screening; PPV, positive predictive value

Supplemental Table 11. Subgroup analyses for FPR of NIPS for Trisomy 18.

Category	N studies	FPR (%) (95% CI)	I^2	Between-group difference
Year of publication				
2019	3	0.02 (0.02-0.03)	0%	*Q* = 19.70 *P* < 0.0001
2020	3	0.13 (0.05-0.34)	95.2%	
2021	1	0.51 (0.07-3.53)	NA	
Country				
China	3	0.03 (0.02-0.05)	73.3%	*Q* = 64.64 *P* < 0.0001
Germany	1	0.16 (0.06-0.43)	NA	
Iran	1	0.32 (0.23-0.44)	NA	
Saudi Arabia	1	0.51 (0.07-3.53)	NA	
Risk of bias (ROBINS-I)				
Moderate	6	0.07 (0.03-0.19)	95.7%	*Q* = 0.00 *P* = 1.00
Serious	1	0 (0-100)	NA	
Population size				
<10,000	2	0.15 (0.02-01.06)	96.5%	*Q* = 0.60 *P* = 0.44
≥10,000	5	0.06 (0.02-0.17)	96.5%	
Full reporting of data				
Yes	6	0.06 (0.02-0.17)	95.7%	*Q* = 1.85 *P* = 0.17
No	1	68.12 (42.43-86.10)	88.3%	

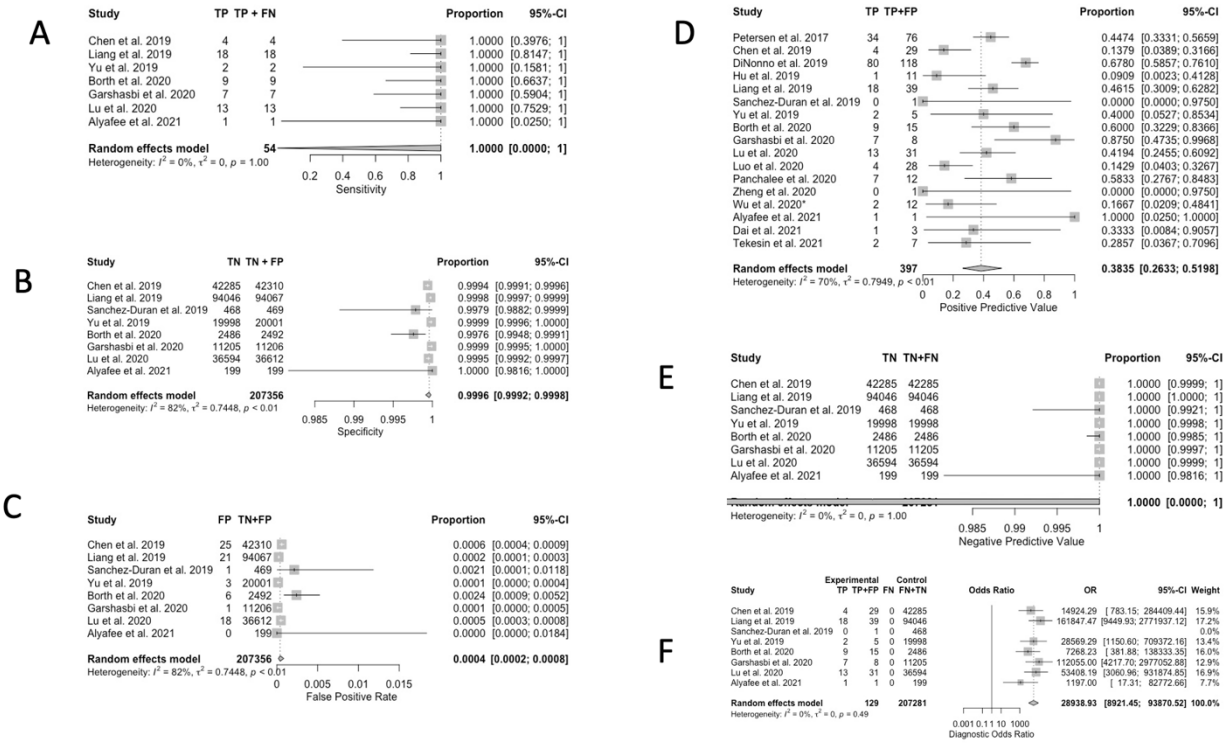
Table legend: FPR, false positive rate; NA, not applicable; NIPS, non-invasive prenatal screening

Supplemental Table 12. Subgroup analyses for DOR of NIPS for T18.

Category	N studies	DOR (95% CI)	I^2	Between-group difference
Year of publication				
2019	2	200,000 (53,000-730,000)	0%	*Q* = 9.69 *P* = 0.0079
2020	3	17,000 (1,900-160,000)	41.7%	
2021	1	1,200 (42-33,000)	NA	
Country				
China	3	180,000 (54,000-580,000)	0%	*Q* = 13.73 *P* = 0.0033
Germany	1	22,000 (1100-420,000)	NA	
Iran	1	2100 (110-42,000)	NA	
Saudi Arabia	1	1,200 (42-33,000)	NA	
Population size				
<10,000	1	1,200 (42-33,000)	NA	*Q* = 4.05 *P* = 0.04
≥10,000	5	53,000 (10,000-270,000)	52.1%	
Full reporting of data				
Yes	5	30,000 (3,500-250,000)	70.4%	*Q* = 0.03 *P* = 0.87
No	1	22,000 (1,100-420,000)	NA	

Table legend: DOR, diagnostic odds ratio; NA, not applicable; NIPS, non-invasive prenatal screening

Supplemental Figure 4. Performance characteristics of NIPS to detect T13 in general-risk populations from random-effects meta-analyses.



A) sensitivity; B) specificity; C) false positive rate; D) positive predictive value; E) negative predictive value; F) diagnostic odds ratio

Supplemental Table 13. Subgroup analyses for specificity of NIPS for T13.

Category	N studies	Specificity (%) (95% CI)	I^2	Between-group difference
Year of publication				
2019	4	99.97 (99.94-99.98)	81.7%	*Q* = 0.33 *P* = 0.85
2020	3	99.95 (99.79-99.99)	87.2%	
2021	1	100 (0-100)	NA	
Country				
China	4	99.97 (99.94-99.98)	79.4%	*Q* = 21.74 *P* = 0.0002
Germany	1	99.76 (99.47-99.89)	NA	
Iran	1	99.99 (99.94-100)	NA	
Spain	1	99.79 (98.50-99.97)	NA	
Saudi Arabia	1	100 (0-100)	NA	
Risk of bias (ROBINS-I)				
Moderate	6	99.97 (99.92-99.99)	84.5%	*Q* = 1.46 *P* = 0.23
Serious	2	99.94 (99.91-99.96)	36.9%	
Population size				
<10,000	2	99.85 (98.95-99.98)	0%	*Q* = 1.68 *P* = 0.20
≥10,000	6	99.96 (99.92-99.98)	85.9%	

Table legend: NA, not applicable; NIPS, non-invasive prenatal screening

Supplemental Table 14. Subgroup analyses for PPV of NIPS for T13.

Category	N studies	PPV (%) (95% CI)	I^2	Between-group difference
Year of publication				
2017	1	44.74 (34.00-55.99)	NA	
2019	6	31.83 (13.90-57.46)	82.9%	*Q* = 1.03
2020	7	41.49 (21.99-64.07)	66.3%	*P* = 0.79
2021	3	36.36 (14.33-66.12)	0%	
Country				
China	9	24.53 (14.80-37.80)	50.4%	
Germany	2	50.00 (30.24-69.76)	44.0%	
United States	2	57.05 (40.54-72.12)	89.9%	*Q* = 15.48
Iran	1	87.50 (46.27-98.27)	NA	
Spain	1	0 (0-100)	NA	*P* = 0.0169
Thailand	1	58.33 (30.76-81.52)	NA	
Saudi Arabia	1	100 (0-100)	NA	
Risk of bias (ROBINS-I)				
Moderate	12	39.06 (24.79-55.47)	49.4%	*Q* = 0.03
Serious	5	36.71 (17.28-61.69)	85.0%	*P* = 0.87
Population size				
<10,000	8	32.15 (17.69-51.09)	15.7%	*Q* = 0.76
≥10,000	9	43.32 (26.90-61.36)	80.9%	*P* = 0.38

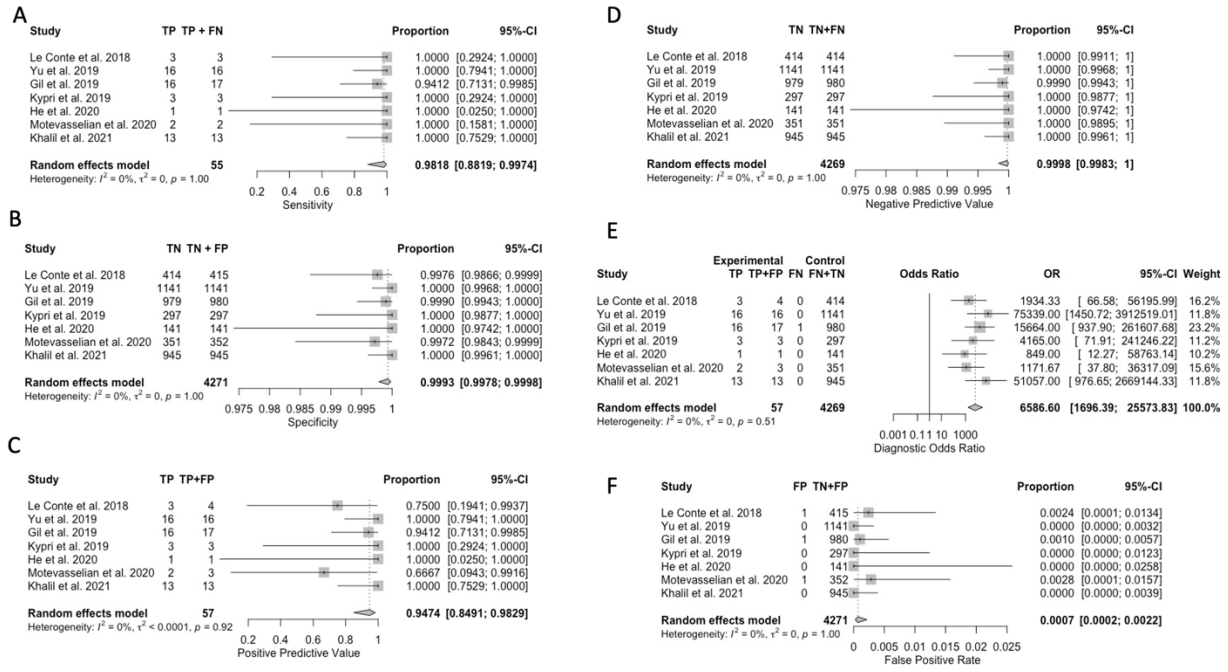
Table legend: NA, not applicable; NIPS, non-invasive prenatal screening; PPV, positive predictive value

Supplemental Table 15. Subgroup analyses for FPR of NIPS for T13.

Category	N studies	FPR (%) (95% CI)	I^2	Between-group difference
Year of publication				
2019	4	0.03 (0.02-0.06)	81.7%	*Q* =0.33 *P* = 0.85
2020	3	0.05 (0.01-0.21)	87.2%	
2021	1	0 (0-100)	NA	
Country				
China	4	0.03 (0.02-0.06)	79.4%	*Q* = 21.73 *P* = 0.0002
Germany	1	0.21 (0.03-1.50)	NA	
Iran	1	0.01 (0-0.06)	NA	
Spain	1	0.21 (0.03-1.50)	NA	
Saudi Arabia	1	0 (0-100)	NA	
Risk of bias (ROBINS-I)				
Moderate	6	0.03 (0.01-0.08)	84.5%	*Q* = 1.46 *P* = 0.23
Serious	2	0.06 (0.04-0.09)	36.9%	
Population size				
<10,000	2	0.15 (0.02-1.05)	0%	*Q* = 1.68 *P* = 0.20
≥10,000	6	0.04 (0.02-0.08)	85.9%	

Table legend: FPR, false positive rate; NA, not applicable; NIPS, non-invasive prenatal screening

Supplemental Figure 5. Diagnostic performance of NIPS for multifetal pregnancies.



A) sensitivity; B) specificity; C) positive predictive value; D) negative predictive value; E) diagnostic odds ratio; F) false positive rate

Supplemental Table 16. Diagnostic performance of NIPS for SCAs.

Test Statistic	# of Studies	Result (%) (95% CI)	I^2
Overall SCAs			
Sensitivity	11	99.63 (94.83-99.98)	0%
Specificity	9	99.80 (99.69-99.88)	87.6%
PPV	29[†]	43.13 (37.92-48.50)	71.0%
NPV	9	100 (99.99-100)	0%
FPR	9	0.20 (0.12-0.31)	87.6%
Accuracy	9	99.78 (99.71-99.83)	89.3%
DOR*	9	12688.01 (3059.76-52613.82)	75.2%
Monosomy X/Turner syndrome			
Sensitivity	7	97.68 (84.25-99.70)	0%
Specificity	6	99.84 (99.67-99.92)	88.7%
PPV	23[†]	29.52 (22.72-37.36)	70.1%
NPV	6	100 (99.98-100)	0%
FPR	6	0.16 (0.08-0.33)	88.7%
Accuracy	6	99.82 (99.71-99.89)	88.4%
DOR*	6	8451.3850 (1809.46-39473.51)	42.7%
Trisomy X (XXX)			
Sensitivity	5	100 (0-100)	0%
Specificity	4	99.97 (99.96-99.98)	0%
PPV	16[†]	53.95 (40.58-66.77)	68.4%
NPV	4	100 (0-100)	0%
FPR	4	0.03 (0.02-0.04)	0%
Accuracy	4	99.97 (99.96-99.98)	0%
DOR*	4	122075.54 (27498.37-541938.91)	0%
Klinefelter syndrome (XXY)			
Sensitivity	4	99.25 (78.13-99.98)	0%
Specificity	4	99.99 (99.98-99.99)	0%
PPV	17[†]	74.05 (59.47-84.73)	75.5%
NPV	4	100 (99.98-100)	0%
FPR	4	0.01 (0.02-0.02)	0%
Accuracy	4	99.98 (99.98-99.99)	0%
DOR*	4	131772.21 (32519.67-533951.24)	0%
Jacob's syndrome (XYY)			
Sensitivity	4	100 (0-100)	0%

Test Statistic	# of Studies	Result (%) (95% CI)	I^2
Specificity	4	99.99 (99.99-100)	0%
PPV	14[†]	74.45 (58.40-85.81)	59.6%
NPV	4	100 (0-100)	0%
FPR	4	0.01 (0-0.01)	0%
Accuracy	4	99.99 (99.99-100)	0%
DOR*	4	202461.83 (38930.01-1052935.65)	0%

*Data presented as odds ratio

†Rousseau et al., 2019 data reported separately for Illumina and Thermo-Fisher.

[@]Results do not include studies without adequate data to include in meta-analyses.

Table legend: DOR, diagnostic odds ratio; FPR, false positive rate; NIPS, non-invasive prenatal screening; NPV, negative predictive value; NR, not reported; PPV, positive predictive value; SCA, sex chromosome aneuploidies

Supplemental Table 17. Subgroup analyses for specificity of NIPS for SCAs.

Category	N studies	Specificity (%) (95% CI)	I^2	Between-group difference
Year of publication				
2019*	5	99.80 (99.57-99.91)	86.6%	*Q* =0.78 *P* = 0.68
2020	2	99.85 (99.61-99.94)	92.6%	
2021	2	99.77 (99.66-99.84)	94.1%	
Country				
China	5	99.78 (99.72-99.83)	87.2%	*Q* = 30.50 *P* < 0.0001
Canada*	2	99.46 (99.13-99.66)	33.1%	
Iran	1	99.93 (99.86-99.96)	NA	
Cyprus	1	99.95 (99.85-99.98)	NA	
Population size				
<10,000*	2	99.46 (99.13-99.66)	33.1%	*Q* = 13.73 *P* = 0.0002
≥10,000	7	99.84 (99.75-99.89)	88.2%	

*****Rousseau listed twice for the different platforms.

Table legend: NA, not applicable; NIPS, non-invasive prenatal screening; SCA, sex chromosome aneuploidies

Supplemental Table 18. Subgroup analyses for PPV of NIPS for SCAs.

Category	N studies	PPV (%) (95% CI)	I^2	Between-group difference
Year of publication				
2015	1	21.31 (12.80-33.33)	NA	
2017	1	40.32 (32.06-49.17)	NA	
2018	2	41.14 (34.09-48.58)	54.7%	*Q* =10.84
2019	7	42.16 (26.86-59.12)	74.3%	*P* = 0.05
2020	13	47.67 (37.42-58.12)	74.9%	
2021	5	41.78 (37.46-46.24)	46.3%	
Country				
China	16	41.21 (37.83-44.68)	79.4%	
Canada	2*	10.53 (2.65-33.74)	NA	
United States	2	31.16 (19.30-46.15)	84.3%	
Turkey	1	50.00 (28.42-71.58)	NA	
Belgium	1	37.29 (29.05-46.34)	NA	*Q* = 50.04
Iran	1	72.41 (53.76-85.56)	NA	
Cyprus	1	78.57 (50.57-92.93)	NA	*P* < 0.0001
Italy	1	77.27 (55.64-90.21)	NA	
Thailand	1	68.18 (46.63-84.01)	NA	
Australia	1	26.04 (18.25-35.71)	NA	
Lithuania	1	100 (0-100)	NA	
Risk of bias (ROBINS-I)				
Moderate	21	45.02 (37.98-52.27)	74.7%	*Q* = 1.87
Serious	8	38.80 (33.66-44.21)	56.8%	*P* = 0.17
Population size				
<10,000	10	47.95 (34.46-61.76)	66.4%	*Q* = 0.78
≥10,000	19	41.26 (36.17-46.54)	74.0%	*P* = 0.38

Table legend: NA, not applicable; NIPS, non-invasive prenatal screening; PPV, positive predictive value; SCA, sex chromosome aneuploidies

Supplemental Table 19. Subgroup analyses for FPR of NIPS for SCAs.

Category	N studies	FPR (%) (95% CI)	I^2	Between-group difference
Year of publication				
2019	5	0.20 (0.09-0.43)	86.6%	*Q* =0.78 *P* = 0.68
2020	2	0.15 (0.06-0.39)	92.6%	
2021	2	0.23 (0.16-0.34)	94.1%	
Country				
China	5	0.22 (0.17-0.28)	87.2%	*Q* = 30.50 *P* <= 0.0001
Canada	2	0.54 (0.34-0.87)	33.1%	
Iran	1	0.07 (0.04-0.14)	NA	
Cyprus	1	0.05 (0.02-0.15)	NA	
Population size				
<10,000	2	0.54 (0.34-0.87)	33.1%	*Q* = 13.73 *P* = 0.0002
≥10,000	7	0.16 (0.11-0.25)	88.2%	

Table legend: FPR, false positive rate; NA, not applicable; NIPS, non-invasive prenatal screening; SCA, sex chromosome aneuploidies

Supplemental Table 20. Subgroup analyses for DOR of NIPS for SCAs.

Category	N studies	DOR (95% CI)	I^2	Between-group difference
Year of publication				
2019	5	9,900 (1,200-83,000)	64.6%	*Q* = 0.80 *P* = 0.67
2020	2	32,000 (5,800-170,000)	0%	
2021	2	11,000 (270-440,000)	85.7%	
Country				
China	5	24,000 (3,100-190,000)	82.6%	*Q* = 8.59 *P* = 0.0352
Canada	2*	540 (54-5,500)	0%	
Iran	1	29,000 (3,500-240,000)	NA	
Cyprus	1	41,000 (2,000-830,000)	NA	
Population size				
<10,000	2	540 (54-5,500)	66.4%	*Q* = 7.10 *P* = 0.0077
≥10,000	7	26,000 (4,900-140,000)	79.9%	

Table legend: DOR, diagnostic odds ratio; NA, not applicable; NIPS, non-invasive prenatal screening; SCA, sex chromosome aneuploidies

Supplemental Table 21. Diagnostic performance of NIPS for RATs.

Test Statistic	# of Studies	Result (%) (95% CI)	I^2
Sensitivity	2	92.31 (60.94-98.93)	0%
Specificity	3	99.95 (99.93-99.96)	46.9%
PPV	17	13.42 (8.07-21.48)	70.3%
NPV	3	100 (99.99-100)	0%
FPR	3	0.05 (0.04-0.07)	46.9%
Accuracy	3	99.95 (99.93-99.96)	42.1%
DOR*	2	16,000 (2,900-90,000); $P < 0.0001$	0%

*Data presented as odds ratio
Results do not include studies without adequate data to include in meta-analyses.

Narrative Summary of NIPS for Individual Trisomies

Trisomy 1

A single suspected case of T1 was reported from a very large (N=86,193) study from China. Following SNP-microarray testing which revealed arr(1-22)x2,(XN)x1, the case was categorized as a false positive and the follow-up from the patient was reported as a normal live birth[1].

Trisomy 2

Five cases of suspected T2 were identified in a large (N=89,817) study from the United States. Three of these were found to be mosaic and two of these were cases of UPD. Both UPD/TFM pregnancies were terminated, while the third TFM pregnancy resulted in a live birth with intrauterine growth restriction (IUGR), but no dysmorphology at birth. Two cases were found to be confined placental mosaicism (CPM). One of these resulted in a live normal birth while the other ended in fetal demise at 17 weeks with severe IUGR, anhydramnios, and multiple congenital anomalies[2]. Liang et al. report a NIPS+ for T2 in a fetus found to have oligohydramnios and a single umbilical artery; the pregnancy was terminated[3]. Confined placental mosaicism was identified in the single case of T2 NIPS+ identified in a large prospectively reported study from a single laboratory. The outcome was a live birth with fetal growth restriction[4]. Two cases, both high-risk, received NIPS+ results for T2 in a large mixed-risk study reported by Wan et al. (2018). One patient reported normal follow-up while the other identified arr2p25.3p11.2x2 hmz (87 Mb) and arr2q11.1q37.3x2 hmz (147.22 Mb), both categorized as uncertain significance, by CMA[5]. Two suspected T2 cases, one of which was considered high-risk from serum screening, were identified in a study of 18,016 mixed-risk patients from a single center in China. Confirmatory testing revealed arr 2p25.1p22.3x2 hmz (24.36 Mb) in one case and arr 15q14q23x2 hmz (31.20 Mb), both of uncertain pathogenicity. Pregnancy outcomes were fetal loss and vaginal bleeding in the first case and a normal liveborn in the second[6]. A large (N=34,620) general population study from China identified a single suspected case of T2 using NIPS which was confirmed through additional testing[7].

Study	Trisomy 2 TP	Trisomy 2 FP	Unverified/Missing	TOP
Pertile 2017	3 (TFM x3)^	2 (CPM)		2^
Liang 2018				1
Scott 2018	1-mosaic			
Wan 2018	2-VUS			
Gou 2020		2		
Chen 2021	1			
Van Den Bogaert 2021	1			

Trisomy 3

A patient who received a T3 NIPS+ had no confirmatory genetic testing but had a live birth[4]. Four patients (one low-risk) received T3 NIPS+ results. Two patients reported normal follow-up without additional testing, one patient underwent karyotyping that did not confirm the T3 NIPS result, and one patient underwent CMA which identified two benign chromosome 4 and chromosome 14 anomalies but did not confirm the T3 finding[5]. Both T3 NIPS+ results were confirmed to be false positives in the study by Chen et al. (2019)[8]. The Dutch TRIDENT-2 study evaluating the national implementation of NIPS as a first-tier screening test identified 3 suspected T3 cases, none of which were confirmed[9]. A single suspected case of T3 was found to be false positive with a pregnancy outcome of a normal liveborn in a patient with intermediate risk from serum screening[6]. Among the five suspected T3 cases identified by Chen et al. (2021), three were confirmed to be false positives by additional testing and two were unverified[7]. The single T3 NIPS+ result was similarly found to be a false positive in the study by Lai et al. (2021) and follow-up was reported as a live birth and normal[1]. Neither T3 NIPS finding was verified in the study by Pertile et al. (2017)[2].

Study	Trisomy 3 TP	Trisomy 3 FP	Unverified/Missing	TOP
Pertile 2017			2	
Scott 2018			1	
Wan 2018		2	2	
Chen 2019		2		
Van der Meij 2019		3		
Gou 2020		1		
Chen 2021		3	2	
Lai 2021		1		

Trisomy 4

In a large study (N=89,817) from the United States, three cases of T4 were confirmed. Two cases were uniparental disomy and the third had intrauterine fetal demise[2]. Three patients from a single laboratory were reported to have T4 NIPS+. No abnormality was detected in confirmatory genetic testing and all three were live births; however, two of the three had fetal growth restriction[4]. Chen et al. (2019) reported one T4 NIPS+ result which was unverified in their study[8]. The single suspected case of T4 in the TRIDENT-2 study was found to be a false positive[9]. Two suspected cases of T4 were identified from 34,620 general risk pregnant individuals. One of these was confirmed to be a false positive after additional testing, while one remained unverified[7].

Study	Trisomy 4 TP	Trisomy 4 FP	Unverified/Missing	TOP
Pertile 2017	3 (2 UPD, 1 IUFD)			
Scott 2018			3	

Chen 2019			1	
Van der Meij 2019		1		
Chen 2021		1	1	

Trisomy 5

Fetal growth restriction, ultrasound-identified fetal structural abnormality and postnatal anomalies were observed in the single patient who received a T5 NIPS+, despite genetic testing which did not detect any abnormalities[4]. Two high-risk patients from a mixed-risk population reported normal clinical outcomes after receiving a T5 NIPS+ result but did not undergo additional testing[5]. The single T5 NIPS+ result reported by Chen and colleagues was confirmed to be a true positive[8]. Two cases of suspected T5 were observed in the TRIDENT-2 study; however, both were later confirmed to be false positives[9]. In a large study of 34,620 women in China, Chen et al. (2021) identified 3 cases of suspected T5. Two of these were found to be false positives and one remained unverified[7].

Study	Trisomy 5 TP	Trisomy 5 FP	Unverified/Missing	TOP
Pertile 2017			1	
Scott 2018		1		
Wan 2018			2	
Chen 2019	1			
Van der Meij 2019		2		
Chen 2021		2	1	

Trisomy 6

A single suspected case of T6 was found to be false positive with a pregnancy outcome of a normal liveborn in a patient with intermediate risk from serum screening[6].

Trisomy 7

Thirteen studies reported positive NIPS for Trisomy 7 (T7). Of these, 2 were focused exclusively on T7 results[10, 11]. In nine T7 NIPS+ cases, Pertile et al. confirmed one case as TP, 5 cases as FP, while 3 patients were missing follow-up. The positive case was determined to be fetal mosaicism and had intrauterine growth restriction[2]. A single case of T7 was found to be a false-positive in a follow-up study of women from the United States with positive NIPS[12]. Six positive NIPS results were obtained in a prospective study by Scott et al. Of the six, one patient did not have additional testing, 1 case was found to be CPM, and three patients had genetic testing with no abnormalities detected. Clinical outcomes for the six NIPS+ cases were termination of pregnancy in one case with ultrasound-detected fetal structural abnormality and five live births, two of which had fetal growth restriction[4]. Eighteen patients from a mixed-risk study received T7 NIPS+ results. Neither of the two low-risk patients underwent additional testing but reported normal follow-up. Two patients with unknown risk from serum screening underwent additional testing: in one, the karyotyping was normal and in the other, a benign chromosome 14 anomaly was identified. Among the 14 high-risk patients, only one patient received a pathogenic finding (arr7p22.3q36.3x2~3 (159.08 Mb)) and one patient received an

uncertain finding (arr7q11.23q21.11x2 hmz (5.08 Mb)) from CMA. All others received findings of benign/likely benign chromosomal anomalies by CMA or reported normal follow-up in patients which did not undergo additional testing[5]. T7 NIPS+ results were the most numerous RAT reported by Chen et al. (2019). However, none of the 14 were true positives; nine were confirmed false positives while five were unverified[8]. Qi and colleagues reported findings of T7 NIPS+ cases in a large study from China. Of the thirty-five cases with suspected T7, 25 underwent additional testing for confirmation. In the patients with suspected T7 alone, only one was found to have 7q11.23x3 via CMA despite a normal karyotype; this patient terminated the pregnancy. The other cases with suspected T7 reported healthy children with normal development postnatally and without congenital anomalies requiring surgical intervention[10]. None of the 32 suspected T7 NIPS+ results were confirmed in the TRIDENT-2 study [van der Meij 2019]. Eleven suspected cases of T7 were identified in a large, mixed-risk population from China. Confirmatory CMA/karyotyping revealed benign chromosomal anomalies in two patients; however, all cases resulted in a normal live birth. Of 15 NIPS+ T7 results among 34,620 general risk pregnancies, only one was confirmed to be a true positive and 10 were confirmed to be false positives. Four results remained unverified[7]. Lai et al. (2021) reported 4 suspected cases of T7 among 86,193 general risk pregnancies. Three were confirmed to be false positives and one pregnancy was terminated[1]. From a combined 70,411 NIPS tests from two cohorts in China between 2015-2019, 39 were suspected T7 cases. Of these, a single case was confirmed as a true positive and 27 were confirmed to be false positives; however, 11 were unverified[11].

Study	Trisomy 7 TP	Trisomy 7 FP	Unverified/Missing	TOP
Pertile 2017	1	5	3	
Petersen 2017		1		
Scott 2018	1 mosaic	3	1	1
Wan 2018	1; 1-VUS	16		
Chen 2019		9	5	
Qi 2019	2*	33		2
Van der Meij 2019		32		
Gou 2020		11		
Chen 2021	1	10	4	
Lai 2021		3		1
Zhu 2021	1	27	11	
*includes case w/multiple aneuploidies				

Trisomy 8

Pertile and colleagues (2017) reported three cases of suspected T8 from a cohort of 89,817 in the United States. Two of these were unverified, but one resulted in a normal live birth. The third case was found to be a case of maternal mosaicism (10% T8) following microarray, while placental biopsy did not confirm the T8 NIPS finding. This pregnancy was terminated[2]. Two cases of Trisomy 8 were detected with NIPS amongst more than 23,000 samples submitted to a

single laboratory. Both cases resulted in live births and with no abnormalities detected with subsequent genetic testing[4]. Five patients received a T8 NIPS+ result in a large mixed-risk study. Karyotyping was normal in one patient and in another patient without additional testing, the outcome was reported as normal at follow-up. In three patients who underwent subsequent CMA, the chromosomal anomalies identified were benign except for an arr1q44x1 (242 kb) which was categorized as uncertain[5]. Three of the five T8 NIPS+ results were confirmed false positives, while the remaining two were unverified in a study of more than 40,000 Chinese women[8]. Thirteen suspected cases of T8 were identified in the TRIDENT-2 study; however, none were confirmed[9]. In three cases of suspected T8, confirmatory testing failed to detect the trisomy and all pregnancy outcomes were for a normal live birth[6]. Seven suspected cases of T8 were found to be false positives in the study reported by Chen and colleagues (2021), while two suspected cases were unverified[7]. Three cases of suspected T8 were all found to be false positives with follow-up reported as live births and normal. Two of these had confirmatory SNP-microarray with arr(1-22)x2,(XN)x1 results while the third was unverified[1].

Study	Trisomy 8 TP	Trisomy 8 FP	Unverified/Missing	TOP
Pertile 2017		1^ (maternal mosaic, 10% T8)	2	1^
Scott 2018			2	
Wan 2018		4	1	
Chen 2019		3	2	
Van der Meij 2019		13		
Gou 2020		3		
Chen 2021		7	2	
Lai 2021		3^	1^	
Van Den Bogaert 2021	3			
^Patient listed in multiple categories				

Trisomy 9

Four cases of suspected T9 were reported by Pertile and colleagues (2017). One of these was in a twin pregnancy with a normal live birth and co-twin demise at 9 weeks. Karyotyping of newborn blood failed to confirm the T9 NIPS result. A second case ended in miscarriage at 11 weeks and the NIPS result was unverified. Microarray in a third case confirmed the T9 NIPS result and there were multiple anomalies present on ultrasound. This pregnancy was terminated. The fourth case reported by Pertile et al. resulted in a live birth with IUGR and cleft palate; however, neither microarray nor karyotyping confirmed the T9 NIPS result [2]. An unconfirmed T9 NIPS result in a patient who received NIPS at 19 weeks was followed up by ultrasound which confirmed ventricular septal defect, cleft lip and palate, and pulmonary stenosis. This pregnancy was terminated [3]. Both cases of T9 were observed to be false

positives in the Petersen et al. study[12]. Genetic testing on the products of conception following a miscarriage at 11 weeks confirmed a T9 NIPS+ finding[4]. A T9 NIPS+ result was unconfirmed by CMA in a patient of unknown risk from serum screening in a large, mixed-risk population[5]. Of the two T9 NIPS+ reported in Chen et al. (2019), one was unverified, and one was confirmed false positive[8]. Of the four suspected T9 cases identified in the study of NIPS implementation in The Netherlands, only one was found to be a true positive[9]. Two cases of suspected T9 were identified by NIPS in a study reported by Gou et al. (2020)[6]. In one case, follow-up CMA/karyotyping was normal; in the other case, an uncertain finding of arr 20p12.1x1 (420 kb) was revealed. In both cases, the pregnancy outcome was of a normal live birth. The single suspected case of T9 from a large study of Chinese women (N=34,620) was confirmed to be a false positive[7], as was the single case among 86,193 pregnancies in China reported by Lai et al. (2021)[1].

Study	Trisomy 9 TP	Trisomy 9 FP	Unverified/Missing	TOP
Pertile 2017	1^	2	1	1^
Liang 2018				1
Petersen 2017		2		
Scott 2018	1			
Wan 2018		1		
Chen 2019		1	1	
Van der Meij 2019	1	3		
Gou 2020		2		
Chen 2021		1		
Lai 2021		1		
Van Den Bogaert 2021	1			

Trisomy 10

Three cases of suspected T10 were reported by Pertile et al. (2017). Amniocentesis in two cases failed to confirm the NIPS result, although one case was determined to be CPM (65% T10). The third case was unverified and ended in miscarriage [2]. One case of T10 NIPS+ was determined to be likely fetal mosaicism after confirmatory CVS aCGH identified 50%-60% T10 in the fetus and ultrasound abnormalities including posterior cranial defect and diaphragmatic hernia. The pregnancy was terminated [4]. A patient with low *a priori* risk determined by serum screening reported normal outcome for the fetus, but without confirmatory testing. A high-risk patient from the same study underwent CMA which identified arr14q32.33x3 and arr1p21.1x1, both of which were benign [5]. The single case of T10 NIPS+ was unconfirmed in the study by Chen and colleagues (2019)[8]. The suspected T10 case in the Dutch TRIDENT-2 study was determined to be a false positive[9], as was the suspected T10 case reported by Gou et al. (2020)[6] and all three cases identified by Chen et al. (2021)[7].

Study	Trisomy 10 TP	Trisomy 10 FP	Unverified/Missing	TOP

Pertile 2017		2 (CPM x1)	1	
Scott 2018	1 (mosaic)			1
Wan 2018		1		
Chen 2019			1	
van der Meij 2019		1		
Gou 2020		1		
Chen 2021		3		

Trisomy 11

One false positive was reported by Chen et al. (2019)[8], by van der Meij and colleagues (2019)[9], by Oneda et al. (2020)[13], and Lai et al. (2021)[1]. In the Oneda (2020) study, the suspected T11 case was a situation of a vanishing twin; results of amniocentesis on the twin brother were normal. Of the three T11 NIPS+ results reported by Chen et al. (2021), two were confirmed to be false positives and one remained unverified[7].

Study	Trisomy 11 TP	Trisomy 11 FP	Unverified/Missing	TOP
Chen 2019		1		
Van der Meij 2019		1		
Oneda 2020		1		
Chen 2021		2	1	
Lai 2021		1		

Trisomy 12

One study reported on findings of T12 (Pallister-Killian syndrome, OMIM# 601803) and identified three patients who were confirmed to have T12 (no false positive findings) [14]. A single high-risk patient received a T12 NIPS result which was not confirmed by karyotyping [5]. The single T12 NIPS+ result was verified as a false positive by Chen et al. (2019)[8]. Of the four suspected T12 cases in the Dutch TRIDENT-2 study, only one was confirmed to be true positive [9].

Study	Trisomy 12 TP	Trisomy 12 FP	Unverified/Missing	TOP
Chau 2020	3			
Wan 2018		1		
Chen 2019		1		
Van der Meij 2019	1	3		

Trisomy 14

The patient receiving a NIPS+ for T14 was found to be a false positive in Petersen et al.'s study[12]. Two patients from a large laboratory received T14 NIPS+ results. Amniocentesis found no abnormalities detected with aCGH and the pregnancy resulted in a live birth with fetal growth restriction, fetal structural abnormalities, and postnatal anomalies (diaphragmatic hernia). The other case resulted in miscarriage and the T14 was confirmed through genetic testing of the products of conception [4]. In a large mixed-risk population, two high-risk individuals received T14 NIPS results. One had CMA which identified arr14q32.33x3 (603kb) and arr22q11.22x3 (134 kb), both of which were categorized as benign. Follow-up for the other patient was reported as normal [5]. Chen et al. reported three T14 NIPS+ results; two of the three were verified false positive and one was unconfirmed [8]. All three of the suspected T14 cases reported by van der Meij et al. were false positives [9], as were both cases identified in the study by Chen et al. (2021)[7]. The patient receiving T14 NIPS+ results was unverified through additional testing; however, follow-up was reported as a live birth and normal [1]. A single case of suspected T14 was reported in a large study from the United States; however, the result was unverified and the pregnancy ended in miscarriage [2].

Study	Trisomy 14 TP	Trisomy 14 FP	Unverified/Missing	TOP
Pertile 2017			1 (miscarriage)	
Petersen 2017		1		
Scott 2018	1		1	
Wan 2018		2	1	
Chen 2019		2	1	
Van der Meij 2019		3		
Chen 2021		2		
Lai 2021		1^	1^	
^Patient listed in multiple categories				

Trisomy 15

Pertile et al. reported 14 cases of suspected T15. Of the verified cases, all three true positives ended in miscarriage, while a false positive was found to be CPM. Ten cases, all unverified, ended in miscarriage [2]. Two cases of T15 were reported by Scott et al. (2018). One resulted in a miscarriage at 11 weeks and genetic testing confirmed the T15 result. The other pregnancy was terminated after confirmatory genetic testing found fetal mosaicism and uniparental disomy [4]. A patient with high-risk serum screening results received a T15 NIPS result but considered a false positive as follow-up for the patient was reported as normal [5]. Verification of the two T15 NIPS+ results identified only one as a true positive [8]. Only one of the four suspected T15 cases identified in the TRIDENT-2 study was confirmed as a true positive [9]. A patient of intermediate risk after serum screening received a suspected T15 result from NIPS. CMA and karyotyping revealed a 46,XN(53)/47,XN,+15(47) result and the pregnancy was terminated [6]. A confirmed case of T15 was identified in a prospective study of 3,169 patients

from China [13]. All three suspected cases of T15 were confirmed as false positives in study of 34,620 pregnant individuals from China [7].

Study	Trisomy 15 TP	Trisomy 15 FP	Unverified/Missing	TOP
Pertile 2017	3 (miscarriage x3)	1 (UPD, CPM)	10 (miscarriage x10)	
Scott 2018	1; 1 (mosaic, UPD)^			1^
Wan 2018		1		
Chen 2019	1	1		
van der Meij 2019	1	3		
Gou 2020	1^			1^
Oneda 2020	1			
Chen 2021		3		
^Patient listed in multiple categories				

Trisomy 16

Seven suspected cases of T16 were reported in a large study from the United States [2]. Of these, three were unverified (no diagnostic testing resulting in a live normal birth, one ectopic pregnancy, one miscarriage). Among the four cases that underwent amniocentesis, TFM was identified in one (termination of pregnancy), CPM/UPD/IUGR in one (fetal demise at 23 weeks), and UPD with fetal demise at 17 weeks in the third. The fourth case resulted in a normal live birth [2]. Of the three patients receiving a NIPS+ for T16, only one was confirmed to be a true positive [12]. Four patients received a T16 NIPS+ reported by Scott et al. (2018). Of these, three were found to have no abnormalities detected through additional genetic testing. All three resulted in a live birth; however, one was affected with fetal growth restriction and a second affected by fetal growth restriction, ultrasound-identified fetal structural abnormalities, and postnatal anomalies. The fourth case was found to have fetal mosaicism and structural abnormalities on ultrasound; the pregnancy was terminated [4]. Five patients received a NIPS+ result for T16 in a large mixed-risk study. Four of these were in patients deemed at high-risk based on serum screening. Clinical outcomes for the pregnancies was mixed: two high risk patients reported normal follow-up, one high-risk patient underwent CMA which found several variants which were categorized as benign, and two patients (one low-risk) both reported fetal loss but without confirmatory genetic testing [5]. All nine T16 NIPS+ were verified to be false positives [7], as were the 4 cases reported by Chen et al. (2019) and the single case from Gou et al. (2020)[6, 8]. Fourteen suspected T16 cases were identified in the TRIDENT-2 study; two of them were confirmed to be true positive and the remainder were not confirmed in the fetus [9].

Study	Trisomy 16 TP	Trisomy 16 FP	Unverified/Missing	TOP
Pertile 2017	2 (TFM x1, UPD x1)	2^ (CPM x1)	3^ (ectopic x1, miscarriage x1)	1 (TFM)

Petersen 2017	1	2		
Scott 2018	1 (mosaic)^	3		1^
Wan 2018		1	4	
Chen 2019		4		
Van der Meij 2019	2	12		
Gou 2020		1		
Chen 2021		9		
Van Den Bogaert 2021	4			
^Patient listed in multiple categories				

Trisomy 17

T17 NIPS+ result was not confirmed by CMA in a high-risk patient from a mixed-risk study reported by Wan and colleagues (2018). CMA findings were for arr14q32.33x3 (458 kb) and arr16p13.11x1 (204 kb), both benign [5]. A single suspected T17 result from NIPS was not confirmed in the large Dutch TRIDENT-2 study [9]. The suspected T17 NIPS+ results were determined to be false positives in two large studies of general risk patients from China [1, 7].

Study	Trisomy 17 TP	Trisomy 17 FP	Unverified/Missing	TOP
Wan 2018		1		
Van der Meij 2019		1		
Chen 2021		1		
Lai 2021		1^	1^	
^Patient listed in multiple categories				

Trisomy 19

CMA findings of arr7q11.21x1 (619 kb) and arr14q32.33x3 (702 kb), both benign, did not confirm T19 NIPS+ result in a high-risk patient [5].

Trisomy 20

Three cases of suspected T20 were reported by Pertile et al. in a large study from the United States. None of the cases were verified, one pregnancy ended in miscarriage, one resulted in a diagnosis of IUGR and delivery at 35+2 weeks, while there was no outcome data for the third [2]. One case of T20 was reported in a large prospective study of pregnant patients in Australia. The case resulted in a live birth and no abnormalities were detected through amniocentesis [4]. Two patients (one low-risk from serum screening) received T20 NIPS+ results, neither of which was confirmed by karyotyping, in a large, mixed-risk study [5]. In a large study of more than 40,000 individuals, 5 T20 NIPS+ were identified. Of the four which were verified, only one was a true positive [8]. All eleven of the suspected T20 cases from the Netherlands were confirmed to be false positives [9], as was the single suspected case from a large, single study center from

China [6], and the two cases identified in another large (N=34,620) study from China [7]. Another case of suspected T20 was unverified, but also categorized as a false positive and follow-up was reported as a live birth and normal [1].

Study	Trisomy 20 TP	Trisomy 20 FP	Unverified/Missing	TOP
Pertile 2017			3	
Scott 2018		1		
Wan 2018		2		
Chen 2019	1	3	1	
van der Meij 2019		11		
Gou 2020		1		
Chen 2021		2		
Lai 2021		1^	1^	
^Patient listed in multiple categories				

Trisomy 22

A large study from the United States confirmed a single case of fetal mosaicism (50% T22) after microarray and one false positive. The mosaic case ended in miscarriage at 12 weeks. Three others remained unverified, two of these ended in miscarriage [2]. A patient with a positive NIPS for T22 that was not confirmed by diagnostic testing was found to have a fetus with ventricular septal defect and persistent left superior vena cava by ultrasound and the pregnancy was terminated [3]. Three cases of T22 were reported by Scott et al. (2018). All three were confirmed to be true positives and all three resulted in miscarriage [4]. Five high-risk patients and one patient of unreported risk status received T22+ NIPS results. Three of these underwent karyotyping which failed to confirm the T22 NIPS result and one patient reported normal clinical follow-up without testing. Two patients underwent CMA analysis that identified benign chromosomal anomalies, but not T22 [5]. Two of the four T22 NIPS+ results were confirmed false-positive while the other two were unverified [8]. Among the five suspected cases of T22 from the TRIDENT-2 study, additional testing confirmed one true positive and four false positives[9]. Four suspected cases of T22 were reported by Gou et al. (2019); three of the four had a pregnancy outcome of a normal live birth following normal or benign CMA/karyotyping results, while the fourth case had a miscarriage at 15 weeks[6]. A mixed-risk prospective study in China identified one suspected case of T22 which was not confirmed after amniocentesis [13], nor was the single case reported by Lai et al. following SNP-microarray [1]. Of the eight T22 NIPS+ results obtained in a study of general risk patients from China, only one was confirmed to be a true positive. Three of the remaining cases were confirmed as false positives and four were unverified [7].

Study	Trisomy 22 TP	Trisomy 22 FP	Unverified/Missing	TOP
Pertile 2017	1 (TFM, miscarriage)	1	3 (miscarriage x2)	
Liang 2018		1^		1^
Scott 2018	3 (miscarriage x3)			

Wan 2019		4	1	
Chen 2019		2	2	
Van der Meij 2019	1	4		
Gou 2020		4 (miscarriage x1)		
Oneda 2020		1		
Chen 2021	1	3	4	
Lai 2021		1		
Van Den Bogaert 2021	2			

Other Rare Suspected Aneuploidies

Six studies reported other rare suspected aneuploidies among their NIPS results. Six cases of suspected monosomies (M14 x4, M16 x2) were reported in a study of 15,362 mixed-risk pregnancies; CMA was performed in 2 high-risk (determined by serum screening) cases with arr14q32.33x3 (690 kb and 748 kb), both benign. In the other 4 cases without additional testing (1 high risk, 1 low risk, 2 risk NR), follow-up contact was reported as normal [5].

Four cases of suspected dual aneuploidy (T7/T2; T7/T3; T7/T11; T7/X0) and two cases of suspected multiple aneuploidy (T7/T8/T20/M13/M22/T3; T7/T8/T2) were identified among the 35 singleton pregnancy patients out of 31,250 who received NIPS in a study from China [10]. All six of the cases underwent karyotyping of amniotic fluid cells; the patient with a T7/X0 NIPS result was confirmed for monosomy X, while CMA testing revealed 7q21.13q36.3x3/Xp22.33p11.22x1. The fetus was delivered at 37+4 weeks and exhibited macrocephaly and discordance of limbs with body size at birth. Karyotyping in the patient with the T7/T8/T20/M13/M22/T3 NIPS+ result was normal (46,XN); however, the patient elected to terminate the pregnancy. The patient with suspected T7/T8/T2 experienced a miscarriage at 16 weeks; karyotyping did not confirm the suspected aneuploidies. The remaining cases resulted in live births with normal physical and psychomotor development postnatally and no congenital anomalies that required surgical intervention.

The Dutch TRIDENT-2 study reported 3 cases of dual aneuploidy (T5/T7; T7/T13; T13/T20) in a cohort of 56,818 individuals with expanded findings (non-common aneuploidies, CNVs). None of the three dual aneuploidies was confirmed in the fetus [9].

In addition to RATs, five rare autosomal monosomies (Chr 14, Chr 16 (x3), Chr 22) were identified among more than 18,000 individuals tested from a single center in China [6]. Clinical outcomes for the suspected M14 and M22 cases were normal live births and no chromosomal anomalies detected using CMA/karyotyping. In two of the suspected M16 cases, CMA/karyotyping detected no anomalies; however, one case identified fetal structural abnormalities and the pregnancy was terminated. The other M16 case with normal CMA/karyotyping resulted in a normal live birth. CMA analysis identified an arr16p11.2x1 (1.18

Mb) in the third suspected M16 case; this anomaly was categorized as benign, and the clinical outcome was a normal live birth.

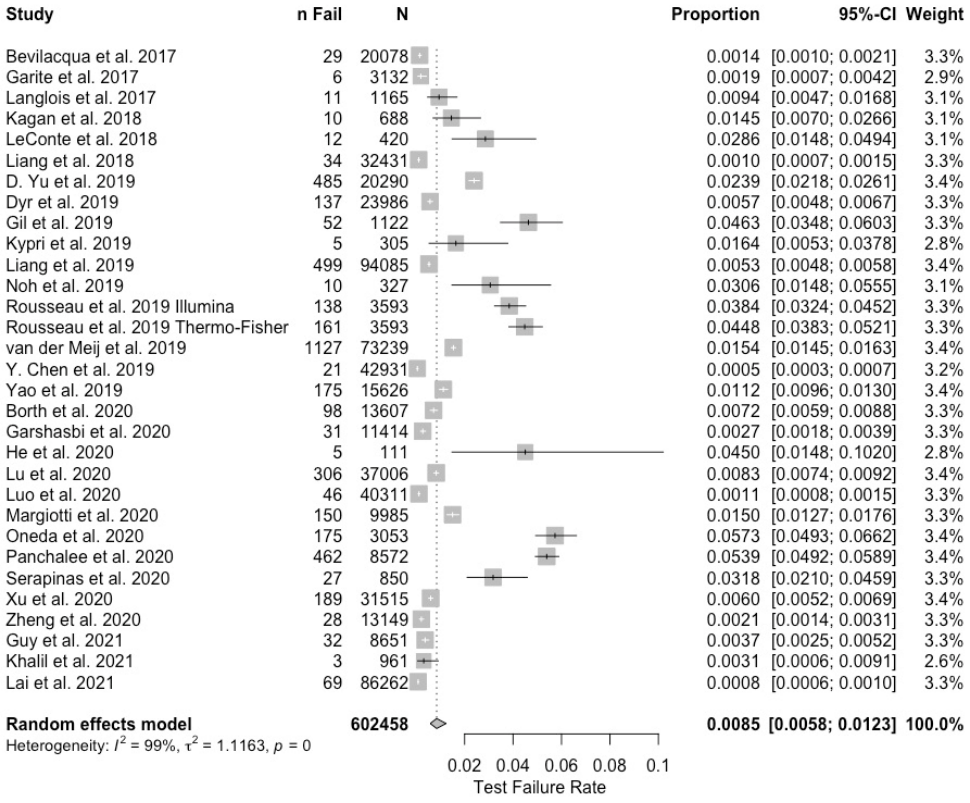
Four suspected cases of monosomy 14 (M14) were reported from a large population of general risk pregnancies in China. Three of the four were confirmed to be false positives and the last case remained unverified [7].

Lai et al. (2021) reported two cases of suspected rare monosomy (M14, M16). Both were categorized as false positives and reported follow-up of live births and normal offspring; however, only the M14 case was confirmed false positive with SNP-microarray. Additionally, they reported 24 cases of suspected multiple RATs, many of which also included suspected common (T13/T18/T21) trisomies. Four of these were categorized as true positives [1].

Supplemental Table 22. Maternal conditions identified through NIPS.

Study	N	Results
Malignancies		
Amant et al. 2015	3	Ovarian 1; Lymphoma 2
Bianchi et al. 2015	10	8 were known
Dharajiya et al. 2018	18/55	55 nonreportable NIPT cases with altered genomic profiles were cataloged. Of these, 43 had additional information available to enable follow- up. A maternal neoplasm was confirmed in 40 of these cases: 18 malignant, 20 benign uterine fibroids, and 2 with radiological confirmation but without pathological classification.
Ji et al. 2019	41	Breast cancer, n=10; lymphoma, n=9; liver cancer, n=9; gastric cancer, n=4; colorectal cancer, n=3; teratoma, n=1; nasopharyngeal carcinoma, n=1; lung cancer, n=1; leiomyosarcoma, n=1; dysgerminoma, n=1; cervical cancer, n=1; majority of cancers were stage IV at diagnosis
Snyder et al. 2016	5	Cases reported in Bianchi et al. 2015
SCAs		
Bianchi et al. 2015	2	47, XXX (2); significant no follow-up (204 SCA; no f/up for 143)
Martin et al. 2020	100	Suspected maternal X chromosome abnormality confirmed in 100/106 cases
Yang et al. 2021	1	mos 45,X[85]/47,XXX[15]
CNVs		
Brison et al. 2017	5	Clinically actionable CNVs
Brison et al. 2019	16	DMD CNVs: 10 pathogenic, 4 benign, 2 unclassified. 3 were known DMD families.
Martin et al. 2018	2/1	6 cases suspected based on fetal risk score of 50% for 22q11.2 deletion; follow-up available for 3 individuals; 2 with confirmed maternal 22q11.2 deletion, 1 with confirmed fetal deletion and unconfirmed maternal copy number for 22q11.2 region but with tetralogy of Fallot and learning disabilities (associated with 22q11.2 deletion syndrome)
Oneda et al. 2020	9/3053	8/9 had symptoms of identified disorders; 1/9 asymptomatic Ehlers-Danlos genetic diagnosis
Zhou et al. 2019	6	Reported as ways to demonstrate a higher FPR in fetal results; these were not pathogenic.
Other results		
Snyder et al. 2016	1	Mosaic trisomy 8

Supplemental Figure 6. NIPS test failure/no call rates in random-effects meta-analysis.



Supplemental Table 23. Summary of all included studies reporting clinical outcomes and diagnostic performance of NIPS in a general-risk population.

Study Information	Population	NIPS	Results
Alyafee et al., 2021 **Country** Saudi Arabia **Time frame** October 2019 to August 2020 **Risk of Bias** ROBINS-I: moderate **Funding/potential COI** None	**N** = 200 low risk: 187 (93.5%) high risk: 13 (6.5%) **Inclusion criteria** singleton pregnancy, natural conception, gestational age ≥10 wks (confirmed by ultrasound) **Exclusion criteria** NR **Participant characteristics** Mean (range) age: 35.69 (21-48) yrs	**NIPS Platform** IONA NIPT (commercially marketed by YourGene Health) **NIPS description** NSG of the multiplexed DNA libraries were performed according to the protocol provided by the manufacturer (Ion Chef and IonS5 XL, Life Technologies, SD, United States), and 12 samples per chip (Ion 540TM Chip-Life Technologies) were analyzed Mean (range) FF: 13.38% (4-31%)	**T21**: TP 7; TN 193; FP 0; FN 0 All low-risk cases were confirmed to be TN; 7/7 (100%) high-risk cases were TP **T18**: TP 4; TN 195; FP 1; FN 0 All low-risk cases were confirmed to be TN, 4/5 (80%) high-risk cases were TP **T13**: TP 1; TN 199; FP 0; FN 0 All low-risk cases were confirmed to be TN; 1/1 (100%) high-risk case was TP **SCA**: NR **CNV**: NR **RAT:** NR **Diagnostic Procedures**: NR **Identification of maternal conditions**: NR

Study Information	Population	NIPS	Results
	Mean (range) gestational age: 19.14 (10-32) wks Mean (range) BMI: 30.84 (15-48)		**Psychosocial outcomes**: NR
Amant et al., 2015 **Country** Belgium **Timeframe** NR **Risk of Bias** ROBINS-I: Serious **Funding/potential COI** One author reports being the founder of and stockholder in Cartagenia, which provides software for clinical analysis of genomics data. The analysis used in this study has been licensed to Cartagenia, for which the author's laboratory receives license fees	**N** = 4000 screened, 3 with abnormal profiles **Inclusion criteria** 3 profiles with an aberrant quality score and reproducible genome-wide representation (GR) profiles reminiscent of cancer-related CNV. **Exclusion criteria** NR **Participant characteristics** NR	**NIPS Platform** NR **NIPS description** Samples with QS >2 labeled as poor quality which prompted repeat sampling. 25/4000 had elevated QS, 4/23 repeat samples had QS >2. 3/4 GR profiles were reproducible Individuals with repeatedly high QS values and reproducible aberrant GR profile involving aberrations of >2	**T21**: NR **T18**: NR **T13**: NR **SCA**: NR **CNV**: NR **RAT**: NR **Diagnostic Procedures**: NR **Identification of maternal conditions**: all 3 tumor-like NIPS-derived GR profiles were confirmed by FISH or CMA (3/4000); diffusion-weighted magnetic resonance imaging (WB-DWI), which revealed a tumorous mass in all 3 cases **Psychosocial outcomes**: NR

Study Information	Population	NIPS	Results
		chromosomes were referred to the oncology unit.	
Basaran et al., 2020 **Country** Turkey **Timeframe** November 2013 and October 2016 **Risk of Bias** ROBINS-I: moderate **Funding/potential COI** None	**N** = 101 **Inclusion criteria** NR **Exclusion criteria** NR **Participant characteristics** Mean (range) maternal age: 37.5 (29-44) yrs Gestational age (range): 12.0-24.5 wks	**NIPS Platform** variety of commercial NIPS: Nifty, Materni21, Panorama, Harmony, Prena, Clarigo, b-sure (Verify), Tranquility **NIPS description** NR; vary by manufacturer Cases were classified into five groups according to the ultrasound findings. Karyotyping, interphase FISH and micro-array techniques were used for follow-up studies.	**T21**: TP 45; FP 4; FN 2 **T18**: TP 6; FP 4 **T13**: TP 2; FP 5 **SCA**: TP MX, 5; XXX, 2; XXY, 2; XYY, 0 TN FP MX,5; XXX, 0; XXY, 3; XYY, 1 FN **CNV**: TP 1; FP 3 **RAT**: NR **Diagnostic Procedures**: NR **Identification of maternal conditions**: NR **Psychosocial outcomes**: NR
Bevilacqua et al., 2018	**N** = 14115	**NIPS Platform**	**T21**: NR

Study Information	Population	NIPS	Results
Country Belgium and Spain **Timeframe** April 2013 to December 2016 **Risk of Bias** ROBINS-I: Serious **Funding/potential COI** None	**Inclusion criteria** patients undergoing NIPS in 2 centers who opted for SCA analysis **Exclusion criteria** NR **Participant characteristics** Cohort 1: patients had NIPS for 1) high risk for common fetal trisomies (assessed by 1st-trimester combined testing, 2nd-trimester triple/quadruple biochemistry testing, or ultrasound findings) (n=552, 17.5%) or 2) because NIPS was chosen as the primary method	Harmony Prenatal Test (Ariosa Diagnostics, Inc., San Jose, CA, USA) **NIPS description** NR Follow-up with prenatal or postnatal karyotyping was available for 118/161 NIPS+ cases (73.3%); calculated PPV and range of FPR for each SCA	**T18**: NR **T13**: NR **SCA**: (overall) TP 44 FP 74 Other: Overall FPR min-max %: 0.52%-0.83% 45,X+ n=80; f/u in n=61; PPV: n=16 (24.6%); FPR min-max %: 0.33%-0.46% 47,XXX+ n=35; f/u in n=22; PPV: n=5 (22.7%); FPR min-max %: 0.12%-0.21% 47,XXY+ n=36; f/u in n=30; PPV: n=19 (63.3%); FPR min-max %: 0.08%-0.12% 47,XYY+ n=10; f/u in n=5; PPV: n=5 (100%); FPR min-max %: 0.0%-004% **CNV**: NR **RAT**: NR **Diagnostic Procedures**: NR **Identification of maternal conditions**: NR **Psychosocial outcomes**: NR

Study Information	Population	NIPS	Results
	of screening (n=2610, 82.5%) Cohort 2: NR Median (range) age at testing: 36.5 (18.6-49.8) yrs Median (range) gestational age: 13.3 (10.0-34.7) wks		
Bianchi, Parsa, et al., 2015 **Country** United States **Timeframe** NR **Risk of Bias** ROBINS-I: Serious **Funding/potential COI** All authors are employees of Illumina or receive	**N** = 18,161 **Inclusion criteria** Individuals undergoing NIPS for autosomal aneuploidy who also selected the fetal sex test option and had a result for SCA status reported in the laboratory information management	**NIPS Platform** Illumina **NIPS description** Genome-wide massively parallel sequencing of cfDNA isolated from maternal plasma was performed as per previously validated laboratory procedures using methods for sample preparation,	**T21**: NR **T18**: NR **T13**: NR **SCA**: Other: 2 FP cases of XXX were documented to be maternal in origin (out of 18,161 samples with sex chromosome results) No sex aneuploidy detected: XX (n=8721) concordant 52/8721; discordant karyotype 8/8721; discordant ultrasound 10/8721; no follow-up 8651/8721

Study Information	Population	NIPS	Results
honorarium/research funding from Illumina	system database used for this query **Exclusion criteria** NR **Participant characteristics** Mean (SD) age: 35.7 (4.9) yrs Mean (SD) gestational age: 14.0 (4.0) wks	sequencing, and analysis that were similar to those reported by Futch et al. SCA were classified into one of six discrete categories: XX, XY, monosomy X, XXX, XXY, and XYY based on the normalized chromosome values obtained for both X and Y.	XY n=9236: concordant 49/9236; discordant karyotype 4/9236; discordant ultrasound 10/9236; no follow-up 9173/9236 Sex aneuploidy detected n=204: MX (n=148) concordant 9/148; discordant (karyotype 35/148; no follow-up 104/148 XXX (n=38) concordant 1/38; discordant 12/38; no follow up 25/38 XXY (n=12) concordant 2/12 Discordant 0/12; no follow up 10/12 XYY (n=6) concordant 1/6; discordant 1/6; no follow-up 4/6 **CNV**: NR **RAT**: NR **Diagnostic Procedures:** NR **Identification of maternal conditions**: NR **Psychosocial outcomes**: NR
Bianchi, Chudova, et al., 2015 **Country** United States	**N** = 125,426; 3757 positive for 1+ aneuploidies **Inclusion criteria** NIPS performed within specified	**NIPS Platform** verifi Prenatal Test (Illumina) **NIPS description** screens for the presence of whole	**T21**: NR **T18**: NR **T13**: NR **SCA:** NR

Study Information	Population	NIPS	Results
Timeframe February 15, 2012, to September 30, 2014 **Risk of Bias** ROBINS-I: Serious **Funding/potential COI** Authors are employees of/receive research funding/honoraria from multiple commercial laboratories (Illumina, Myriad Genetics, Novartis, Pfizer, Sequenom, Ariosa)	time frame. Additional info from those with abnormal results by NIPS whose clinician voluntarily informed the laboratory at any time prior to November 15, 2014, that maternal cancer had been diagnosed after NIPS **Exclusion criteria** NR **Participant characteristics** Mean (range) age: 35 (23-39) yrs Mean (range) gestational age: 13.9 (10-20) wks	chromosome aneuploidy for chromosomes 13, 18, and 21. Testing for sex chromosome aneuploidy by analyzing sequencing counts for chromosomes X and Y is optional. The method uses massively parallel sequencing of cfDNA isolated from maternal plasma To evaluate the frequency of reported maternal malignancies in relation to the overall frequency of aneuploidy positive results, all clinical laboratory reports, as well as all tests that were cancelled due to abnormal underlying chromosomal	**CNV**: NR **RAT**: NR **Diagnostic Procedures**: NR **Identification of maternal conditions**: Seven of the 10 cases of maternal cancer reported to the clinical laboratory had multiple aneuploidies (Table 2). Of the 39 cases of multiple aneuploidy, 7 cases (18% [95% CI,7.5%-33.5%]) were in pts with an occult cancer. 3757 (3.0%) were positive for 1 or more aneuploidies involving chromosome 13, 18,21, X, or Y. In 10 of these aneuploidy-detected cases, the referring clinician voluntarily reported to the clinical laboratory within weeks to months after the initial discussion regarding the clinical significance of the positive NIPS results that the patient had been diagnosed with a malignancy. In 2 cases (leiomyosarcoma and unspecified adenocarcinoma), the referring physicians reported that the women were critically ill, and they declined to approach them for consent to participate in this study, so 8 patients in study. The expected cancer rate in pregnant women is about 0.1%. This series of cancer cases, reported voluntarily, represents 0.008% (10/125 426) of the laboratory case volume, a cancer frequency that is 10-fold lower than what might be expected. However, this patient series is inherently incomplete. **Psychosocial outcomes**: NR

Study Information	Population	NIPS	Results
		patterns generated within the study time frame, were reviewed and the findings were grouped into 1 of 5 categories: single trisomy, single monosomy, single sex chromosome aneuploidy, single sex chromosome aneuploidy plus single trisomy, or multiple aneuploidies. Statistical analysis of the reported proportions was performed using Clopper-Pearson exact binomial 2-sided confidence intervals at the 95% level (using R version 3.1.2)	
Borth et al., 2020 **Country** Germany	**N** = 13,607 consecutive pts **Inclusion criteria**	**NIPS Platform** bioinformatic re-analysis of existing sequencing data	**T21**: TP 89; TN 2566; FP 7; FN 1; PPV = 89/96 **T18**:

Study Information	Population	NIPS	Results
Timeframe December 2017 to April 2019 **Risk of Bias** ROBINS-I: Moderate **Funding/potential COI** None	Individuals who previously underwent NIPS; Both singleton and twin pregnancy samples of ≥10 weeks gestation were included in the study **Exclusion criteria** known vanishing twin or a higher-grade multiple pregnancy **Participant characteristics** Mean (SEM) age: 33.68 (0.04) yrs Mean (SEM) gestational age: 12.48 (0.02) wks Mean (SEM) BMI: 24.87 (0.05)	using VeriSeq NIPS Solution v2 pipeline **NIPS description**: NIPS results positive for fetal aneuploidy were considered confirmed when validated by either invasive prenatal diagnostics or an anomaly observed on ultrasound that matched the high-risk NIPS call.	TP 19; TN 2496; FP 4; FN 0; PPV = 19/23 **T13**: TP 9; TN 2486 FP 6; FN 0; PPV = 9/15 **SCA**: MX TP 5; TN 2482; FP 5; FN 0; PPV = 5/10 **CNV**: NR **RAT**: NR **Diagnostic Procedures**: NR **Identification of maternal conditions**: NR **Psychosocial outcomes**: NR

Study Information	Population	NIPS	Results
	Reason for screening (%): AMA, 42.3; positive US/other screen, 6.0; other medical, 5.5; patient wish, 46.2		
Brison et al., 2017 **Country** Belgium **Risk of Bias** ROBINS-I: Serious **Funding/potential COI** One author reports being the founder of and stockholder in Cartagenia, which provides software for clinical analysis of genomics data. The analysis used in this study has been licensed to Cartagenia, for which the author's laboratory receives license fees	**N** = 9289 **Inclusion criteria** ≥11 wks gestation age **Exclusion criteria** NR **Participant characteristics** NR	**NIPS Platform** Illumina **NIPS description** CNV analysis: Appears to be "in-house" but not explicitly stated, and unclear if "KU Leuven, Belgium" or "University Hospital, Antwerp, Belgium." Blood sampling cfDNA extraction and library preparation were performed as described in previous study. Massively parallel sequencing was performed on the HiSeq2500 or	**T21**: NR **T18**: NR **T13**: NR **SCA**: NR **CNV**: NR **RAT**: NR **Diagnostic Procedures**: NR **Identification of maternal conditions**: Consistent with population estimates, 10% nonrecurrent and 0.4% susceptibility CNVs for low-penetrant genomic imbalances were identified. 5 clinically actionable variants were reported. **Psychosocial outcomes**: NR

Study Information	Population	NIPS	Results
		NextSeq500 sequencer (Illumina) in fast mode, producing 50-bp or 75-bp single end reads, respectively. The results are part of the routine clinical work-up and paid-for-service. Routine diagnostic analysis of chromosomal Z, ZZ, bin median (BM), and other median (OM) scores in combination with a visual inspection of the genomic representation profiles was performed as described. In this way, clinically relevant maternal aberrations were identified. Microarray confirmation	

Study Information	Population	NIPS	Results
		performed for each of the 5 cases	
Brison et al., 2019 **Country** Belgium **Timeframe** July 2017 through June 2018 **Risk of Bias** ROBINS-I: Moderate **Funding/potential COI** None	**N** = 26,123 NIPS analysis; 16 maternal CNVs in the DMD gene were detected **Inclusion criteria** Those who consented to receive secondary findings **Exclusion criteria** NR **Participant characteristics** NR	**NIPS Platform** NR **NIPS description** NIPS and CNV detection were carried out as described in other studies. Briefly, low-pass genome sequencing generated ~10 million single-end reads of 36 bp per sample	**T21**: NR **T18**: NR **T13**: NR **SCA**: NR **CNV**: NR **RAT:** NR **Diagnostic Procedures**: NR **Identification of maternal conditions**: CNVs in DMD are thus present in 1/1632 women. 9 DMD/BMD or variable, 7 LB or VUS **Psychosocial outcomes**: NR
Chau et al., 2020 **Country** China **Timeframe** 2016-2017 **Risk of Bias** ROBINS-I: Moderate	**N** = 29,007; 3 w/12p **Inclusion criteria** cases with abnormal amount of DNA originating from	**NIPS Platform** NR **NIPS description** genome wide NIPS methodology for screening	**T21**: NR **T18**: NR **T13**: NR **SCA**: NR **CNV**:

Study Information	Population	NIPS	Results
Funding/potential COI 2 authors are employees of a company that provides NIPS in Hong Kong and Macau; other authors with no COI to declare	the entire p-arm of chromosome 12 by NIPS **Exclusion criteria** NR **Participant characteristics** Mean age: 33.1 yrs Mean gestational age: 12^{+5} wks Mean FF: 12.66%		Other 3 cases with abnormal amount of DNA originating from the entire p-arm of chromosome 12 were detected, yielding an incidence of 3/27800 (0.011% or 1 in ~9266) in singleton pregnancies. Clinical details, diagnostic testing results, and pregnancy outcome were available and reviewed which disclosed PKS in these fetuses **RAT**: NR **Diagnostic Procedures**: NR **Identification of maternal conditions**: NR **Psychosocial outcomes**: NR
Y. Chen et al., 2019 **Country** China **Timeframe** April 2015 to December 2018 **Risk of Bias** ROBINS-I: Serious **Funding/potential COI** None	**N** = 42910 (42,931 originally sampled, however, 21 cases failed) **Inclusion criteria** (1) gestational age between 12^{+0} wks and 26^{+6} wks, (2) single pregnancy, and (3) BMI < 100	**NIPS Platform** JingXin BioelectronSeq 4000 System **NIPS description** Semiconductor sequencing	**T21**: TP 103 FP 27 PPV 79.23% **T18**: TP 17 FP 14 PPV 54.84% **T13**: TP 4 FP 25 PPV 13.79%

Study Information	Population	NIPS	Results
	Exclusion criteria (1) Individuals with chromosomal abnormalities, (2) multifetal pregnancy, (3) those who have received stem cell therapy and transplant surgery, (4) those who received allogeneic blood products within 1 year, and (5) received immunotherapy within 4 weeks **Participant characteristics** Maternal blood samples were collected from Ningbo Women and Children Hospital in China		**SCA**: Overall TP 37 FP 75 PPV (overall) 33.04% **CNV**: TP 20 FP 49 PPV (overall) 28.99% **RAT**: TP 3 FP 29 PPV (overall) 9.38 **Diagnostic Procedures**: NR **Identification of maternal conditions**: NR **Psychosocial outcomes**: NR

Study Information	Population	NIPS	Results
	Gestational age group, %: 12-15^{+6}: 12.9% 16-19^{+6}: 57.7% 20-23^{+6}: 24.5% 24-26^{+6}: 4.9% Age, range: 18-49 yrs		
M. Chen et al., 2019 **Country** China **Timeframe** December 2016 to April 2018 **Risk of Bias** ROBINS-I: Moderate **Funding/potential COI** Government and foundation funding; no COI noted	**N** = 362 multifetal & singleton mixed **Inclusion criteria** Multifetal gestations opting for NIPS; Random selection of singleton gestations opting for NIPS **Exclusion criteria** NR **Participant characteristics** 203 randomly selected	**NIPS Platform** Illumina **NIPS description** Extraction of cfDNA was performed with QIAamp Circulating Nucleic Acid Kit (Qiagen, Hilden, Germany) according to the manufacturer protocol. Libraries were built using TruSeq Nano DNA Library Prep Kit from Illumina. DNA libraries were subjected to 50bp long paired-end sequencing on	**T21**: Other: detected 2 cases trisomy 21. (1) from DCDA twin - TOP, no karyotype (2) other DCDA, NIPT Normal/T21, Karyotype Normal/T21 when the FF was above 3%, the Z-score value was higher than 3 (above the cut-off value) and the fetal aneuploidy could be detectable with a theoretical sensitivity of 51.9%. When the FF increased to 4%, the theoretical sensitivity was 85.7% **T18**: NR **T13**: NR **SCA**: NR **CNV**: NR **RAT**: NR **Diagnostic Procedures**: NR

Study Information	Population	NIPS	Results
	singleton pregnancies, 69 twins, and 90 higher-order multifetal pregnancies (85 triplets, 2 quadruplets, 1 quintuplet, 1 sextuplet, and 1 octuplet) Mean (SD) age: 31.2 (5.7) Mean (SD) gestational age: Singleton, 17 (3) wks Twin, 20 (5) wks Triplet, 13 (2) wks Quadruplet, 12, 13 Quintuplet, 12 Sextuplet, 11 Octuplet, NA	NextSeq CN500 platform.	**Identification of maternal conditions:** NR **Psychosocial outcomes**: NR
Chen et al., 2021 **Country** China	**N** = 34620 **Inclusion criteria**	**NIPS Platform** NIFTY (BGI, China) **NIPS description**	**T21**: n=121; f/u in 108; TP=99; PPV 91.67% **T18**:

Study Information	Population	NIPS	Results
Timeframe October 2017 to March 2019 **Risk of Bias** ROBINS-I: Moderate **Funding/potential COI** None	consecutive recruitment; opted or referred for basic NIPS **Exclusion criteria** NR **Participant characteristics** Mean age: 31.5 yrs AMA, 32.81% BMI: normal, 72.31% FF mean: 9.94% Test failure 1st time: n=270 (0.78%)	NR	n=44; f/u in 31; TP=16 **T13**: n=44; f/u in 35; TP=9; PPV 23.68% **SCA**: (n=124 (45,X: n=54; 47,XXX: n=24; 47,XXY: n=24; 47,XYY: n=5; unclassified other SCA n=17); 45,X TP=9, PPV=22.50%; 47,XXX TP=8; PPV=53.33%; 47,XXY TP=14, PPV=87.50%; 47,XYY TP=5, PPV=100%; other SCA PPV=6.25% **CNV**: n=57, f/u in 41; TP=21, PPV (CNVs >= 5Mb)=51%; PPV Chr5=25.00%; PPV Chr4=66.67%; PPV Chr7=100%; PPV Chr2=100% **RAT**: n=71, f/u in 55; TP=3, PPV=5.66% **Diagnostic Procedures**: NR **Identification of maternal conditions**: NR **Psychosocial outcomes**: NR
Costa et al., 2018 **Country** France **Timeframe** May 2015 to February 2016 **Risk of Bias**	**N** = 924 **Inclusion criteria** NR **Exclusion criteria** Individuals exhibiting fetal	**NIPS Platform** NR **NIPS description** massive parallel sequencing using a whole-genome approach, as	**T21**: The FPR and PPVs were 6.6% (95% CI, 5%-8.6%) and 8.8% (95% CI, 2.9%-19.3%) for MSS versus 0% (95% CI, 0%-0.47%) and 100% (95% CI, 59.0%-100%) for NIPS. Specificity MSS 93.4% (91.4%-95.0%) vs NIPS 100% (99.5%-100%)

Study Information	Population	NIPS	Results
ROBINS-I: Moderate **Funding/potential COI** 3 authors employees/shareholders of CERBA; other authors report no COI	anomalies on the 1st-trimester scan (including nuchal translucency ≥3.5 mm) **Participant characteristics** Individuals undergoing aneuploidy screening w/NIPS in 9 centers; 546 with spontaneous pregnancies; 378 with ART-induced pregnancies) Median (IQR) age: 33.3 (30.0-37.5) yrs AMA: 36.6% Median (IQR) BMI: 22.5 (20.6-25.7) Median (IQR) gestational age: 12^{+4} (12^{+2} – 13^{+1})	described by Jensen et al. w/some slight modifications. Z-scores were calculated for the targeted chromosomes 13, 18, and 21, as previously described; and the FF was evaluated using the coverage method, as described by Kim et al. The results were expressed as positive or negative according to the following metric criteria: total count 9 million and estimated fetal DNA fraction 4%. compared the FPR and PPV of standard MSS w/those of NIPS for T21. when ultrasounds were normal, blood	FN MSS 28.6% (3.7%-71.0%) vs NIPS 0% (0-41.0%) **T18**: No cases **T13**: one patient was positive for trisomy 13 but did not choose to undergo invasive testing. Placental biopsies were performed at birth, abnormal profiles for markers located on chromosome 13, thus suggesting confined placenta mosaicism. The baby suffered from IUGR yet presented a normal karyotype at birth **SCA**: NR **CNV**: NR **RAT**: NR **Diagnostic Procedures**: MSS hi NIPS+ n=5, all got invasive; MSS low NIPS+ n=2 both got invasive, MSS low NIPS— n=730, 8 got invasive, MSS hi NIPS— n=52 invasive=0 **Identification of maternal conditions**: NR **Psychosocial outcomes**: NR

Study Information	Population	NIPS	Results
		samples for both conventional MSS and NIPS	
Dai et al., 2021 **Country** China **Timeframe** July 13, 2017 to January 22, 2020 **Risk of Bias** ROBINS-I: Moderate **Funding/potential COI** None	**N** = 17,428 **Inclusion criteria** NR **Exclusion criteria** 1. Either parent had chromosome abnormalities; 2. Either parent had family history of genetic diseases; 3. Ultrasound-identified fetal structural abnormalities; 4. Pregnant individual had malignant tumors during pregnancy. **Participant characteristics** NR	**NIPS Platform** NR **NIPS description** In-house	**T21**: 37/17428 NIPS+ 32/37 had confirmatory amnio TP: 27, FP: 5 **T18**: 16/17428 NIPT+ 13/16 w/amnio TP: 8; FP: 5 **T13**: NR **SCA** (overall) 91/17428 NIPS+ 78/91 had confirmatory amnio TP: 30, FP: 48 **CNV**: NR **RAT**: NR **Diagnostic Procedures**: NR **Identification of maternal conditions**: NR **Psychosocial outcomes**: NR

Study Information	Population	NIPS	Results
Dharajiya et al., 2018 **Country** United States **Timeframe** NR; >3 yrs of NIPS **Risk of Bias** ROBINS-I: Serious **Funding/potential COI** Employment, leadership, consultant, stock ownership in commercial laboratories (Sequenom, Pathway Genomics)	**N** = 450,000 pregnant patients. Additional analysis performed for >79,000 research-consented samples. In total, 55 nonreportable NIPS cases with altered genomic profiles were cataloged. Of these, 43 had additional information available to enable follow-up **Inclusion criteria** NR **Exclusion criteria** NR **Participant characteristics** NR	**NIPS Platform** NR NIPS description Maternal blood samples (approximately 10 mL) were collected in Streck BCT tubes. Anticoagulated blood samples were subjected to plasma fractionation, DNA extraction, library preparation, and next-generation sequencing as previously described developed a novel algorithm to identify additional neoplasms with CNAs located elsewhere in the genome	**T21**: NR **T18**: NR **T13**: NR **SCA**: NR **CNV**: NR **RAT**: NR **Diagnostic Procedures**: NR **Identification of maternal conditions**: 20 benign neoplasms, 12 lost to follow-up, 5 unknown/none, 18 malignant. In total, 55 nonreportable NIPS cases with altered genomic profiles were cataloged (out of 450000). Of these, 43 had additional information available to enable follow-up. A maternal neoplasm was confirmed in 40 of these cases: 18 malignant, 20 benign uterine fibroids, and 2 with radiological confirmation but without pathological classification. In a population of pregnant women who submitted a blood sample for NIPS, an abnormal genomic profile not consistent with fetal abnormalities was detected in about 10 out of 100000 cases. A subset of these observations (18 of 43; 41.9%) was attributed to maternal malignant neoplasms **Psychosocial outcomes**: NR

Study Information	Population	NIPS	Results
DiNonno et al., 2019 **Country** United States **Timeframe** 2014-2017 w/quarterly assessments **Risk of Bias** ROBINS-I: Serious **Funding/potential COI** Authors are employees/hold stock/paid consultants for Natera, Inc.	**N** = 1,035,844 **Inclusion criteria** NR **Exclusion criteria** NR **Participant characteristics** NR	**NIPS Platform** Natera **NIPS description** Testing was subject to revisions in the protocols in April 2015 (version 2), February 2016, and January 2018 (version 3). An algorithm to screen for a select group of microdeletions was introduced in March 2014 with procedural and algorithm changes in April 2015, February 2016, and January 2018	**T21**: 7802 T21 positives, 884 were followed up. 837 confirmed by genetics. PPV - 94.7% **T18**: 2205 T18 positives, 333 were followed up. 304 confirmed by genetics. PPV - 91.3%. **T13**: 1207 T13 positives, 118 were followed up. 80confirmed by genetics. PPV - 67.8% **SCA** (MX): 2017 MX positives, 120 were followed up. 93 confirmed by genetics. PPV - 77.5% **CNV**: NR **RAT**: NR **Diagnostic Procedures**: NR **Identification of maternal conditions**: NR **Psychosocial outcomes:** NR
Dyr et al., 2019 **Country** United States	**N** = 30,826 multifetal samples; average risk: n=1562	**NIPS Platform** NR; implied Sequenom **NIPS description**	Average risk: positive 5/1562 (0.32%) All other stats for overall group: **T21**: reported negative n=28,561; reported positive n=435; communicated TP n=16; communicated FP n=4;

Study Information	Population	NIPS	Results
Timeframe October 2011-December 2017 **Risk of Bias** ROBINS-I: Serious **Funding/potential COI** multiple authors employed by commercial lab (Sequenom)	**Inclusion criteria** Multifetal **Exclusion criteria** NR **Participant characteristics** Samples submitted as average risk screening, no high-risk indication reported, comprised 5.1% of the total sample cohort and only 0.8% of all positives reported	All samples were tested for T21 as well as presence or absence of chromosome Y. Beginning in February 2012 all samples were also tested for T18 and T13. Select samples were opted in for "Enhanced Sequencing" by their ordering healthcare provider for seven common microdeletions associated with eight syndromes: 22q deletion (DiGeorge syndrome), 5p deletion (Cri-du-chat syndrome), 15q deletion (Prader-Willi syndrome/ Angelman syndrome), 1p36 deletion syndrome, 11q deletion	communicated FN n=7; relative observed sensitivity 98.40%; relative observed specificity 99.99%; relative observed PPV 99.08% **T18**: reported negative n=28,814; reported positive n=138; communicated TP n=8; communicated FP n-1; communicated FN n=4; relative observed sensitivity 97.16%; relative observed specificity >99.99%; relative observed PPV 99.28% **T13**: reported negative n=28,887; reported positive n=62; communicated TP n=3; communicated FP n=7; communicated FN n=0; relative observed sensitivity >99.99%; relative observed specificity 99.98%; relative observed PPV 88.71% **SCA**: NR **CNV**: NR **RAT**: NR **Diagnostic Procedures**: NR **Identification of maternal conditions**: NR **Psychosocial outcomes**: NR

Study Information	Population	NIPS	Results
		(Jacobsen syndrome), 8q deletion (Langer-Giedion syndrome), and 4p deletion (Wolf-Hirschhorn syndrome). T16 and T22 were also analyzed for additional chromosomal events as part of the Enhanced Sequencing Series	
Garite et al., 2017 **Country** United States **Timeframe** January 2012 through June 2014; historical cohort: January through June 2010 **Risk of Bias** ROBINS-I: Moderate **Funding/potential COI**	**N** = 3074; control: 1414 **Inclusion criteria** Individuals undergoing either amniocentesis or CVS for genetic testing **Exclusion criteria** NR **Participant characteristics**	**NIPS Platform** Varied by practice **NIPS description** NR	The frequency of positive aneuploid test results (autosomal trisomy and sex chromosome aneuploidy) per procedure was more than double, increasing from 6.9% during the control period to 14.8% during the last 6 months of the study period. However, while the number of total abnormal and aneuploidy results per procedure increased, the overall number of abnormal results dropped from 21.8/month to 13.7/month, and aneuploidy decreased from 16.7/month to 10.5/month in the last 6 months, a decrease of 37% for each. **SCA**: NR **CNV:** NR **RAT:** NR

Study Information	Population	NIPS	Results
All but one author are employees of MedNax, Inc. which owns/ manages Obstetrix/ Pediatrix Medical Group (funded study)	patients undergoing NIPS within large multi-state MFM practices consortium		**Diagnostic Procedures**: During the control period, there were a total of 1,440 procedures in 1,414 mothers (2 procedures in 28 twins and 3 procedures in 1 triplet) of which there were 1,169 amniocenteses (193 per month) and 280 CVS (47 per month). During the 30 months of the study period, there were 3,132 procedures in 3,074 mothers. In the last 6 months of the study period, amniocenteses dropped to 52/month and CVS to 18/month, for an overall decline from the control period of 73% and 62%, respectively. There were significant decreases in the percentage of procedures performed because of advanced maternal age and abnormal serum screening, while there were significant increases in the percentage performed because of abnormal ultrasound findings and because of NIPS results. **Identification of maternal conditions**: NR **Psychosocial outcomes**: NR
Garshasbi et al., 2020 **Country** Iran **Timeframe** July 1, 2015, to December 31, 2016 **Risk of Bias**	**N** = 11414 samples obtained. 11223 w/completed NIPS **Inclusion criteria**	**NIPS Platform** BGI Hong Kong **NIPS description** identify T21, T18, T13 and SCA, all other chromosomes	**T21**: TP 90 TN 11118 FP 4 FN 1 Other Twin pregnancy (n=443): T21 3 true pos, 0 FP, 0 FN, 440 TN, PPV 100% (29.42-100.00), NPV 100.00 (99.17-100.00);

Study Information	Population	NIPS	Results
ROBINS-I: Moderate **Funding/potential COI** None	pregnant women from over 150 medical centers, located in 27 cities of Iran. NIPS was provided as a secondary screen test for T21/T18/T13 and SCA in high-risk pregnancies, including pregnant individuals >16 years old, w/a singleton pregnancy, >10 wks of gestation **Exclusion criteria** NR **Participant characteristics** Mixed-risk Median (range) age: 35 (14-49) yrs	were screened for abnormalities	**T18:** TP 3 TN 11174 FP 36 FN 0 Other Twin pregnancy (n=443): T18 1 true pos, 0 FP, 0 FN, 442 TN, PPV 100% (2.50-100.0), NPV 100.00 (99.17-100.00) **T13**: TP 7 TN 11205 FP 1 FN 0 Other **SCA** (Overall): TP 21; TN 11042; FP 8; FN 1 MX: TP 10; FP 5; TN 11197; FN 1 XXX: TP 4; FP 2; TN 11207; FN 0 XXY: TP 4; FP 1; TN 11208; FN 0 XYY: TP 3; FP 0; TN 11210; FN 0 **CNV**: NR **RAT**: NR **Diagnostic Procedures**: NR

Study Information	Population	NIPS	Results
	AMA, 55.34% Median (range) gestational age: 15 (10-37) No risk factors: 31%		**Identification of maternal conditions**: NR **Psychosocial outcomes**: NR
Gil et al., 2020 **Country** England, Belgium **Timeframe** October 2012 to January 2018 **Risk of Bias** ROBINS-I: Moderate **Funding/potential COI** Funded by non-profit; some costs covered by Ariosa Diagnostics which had no role in any aspect of data analysis or the manuscript	**N** = 997 twin pregnancies **Inclusion criteria** 1st-trimester gestational age **Exclusion criteria** NR **Participant characteristics** twin pregnancies self-referred for NIPS testing; individuals referred for NIPS testing after routine combined testing	**NIPS Platform** Harmony (Ariosa Diagnostics, CA) **NIPS description** DANSR assays targeting sequences on chrs 13, 18 and 21 for chr quantitation and SNPs on chrs 1-12 for fetal-fraction measurement. Products of the DANSR assays can be quantified using either NGS or a custom CMA; both were used during the course of this study. The data were analyzed using the	**T21**: n=17 TP 16/17 (94.1%) FP: 1 FN: 1 **T18**: n=10 TP: 9/10 (90%) 1 case was MC w/both affected FP: 1 FN: 1 **T13:** n=2 TP: 1/2 (50%) FN: 1 All the other trisomic cases were a DC pregnancy in which only one fetus was trisomic and the cotwin was non-trisomic. NIPS correctly called 962/968 TN (99.4%) In the non-trisomic group, four cases were classified as trisomy 13, one

Study Information	Population	NIPS	Results
	Median (IQR) age: 38.0 (34.5-41.0) Median (IQR) gestational age: 12.1 (10.7-12.9)	FORTE algorithm, which calculates probability scores for fetal trisomy, with >1% considered to be high probability.	as trisomy 18 and one as trisomy 21 and, therefore, the combined FPR was 0.62% (6/968) **SCA**: NR **CNV**: NR **RAT:** NR **Diagnostic Procedures**: NR **Identification of maternal conditions**: NR **Psychosocial outcomes**: NR
Gomes et al., 2019 **Country** Portugal **Timeframe** March 2017-Feb 2018 **Risk of Bias** ROBINS-I: Serious **Funding/potential COI** None	**N** = 1272 (1193 low risk; 49 intermediate risk, 30 high risk) **Inclusion criteria** NR **Exclusion criteria** multifetal pregnancy or a major fetal abnormality were excluded **Participant characteristics**	**NIPS Platform** NR **NIPS description** NR	**T21**: NR **T18**: NR **T13**: NR **SCA**: NR **CNV**: NR **RAT**: NR **Diagnostic Procedures**: Study group: high risk 83.3% had invasive tests; intermediate risk 12.2% had invasive test **Identification of maternal conditions**: NR **Psychosocial outcomes**: NR

Study Information	Population	NIPS	Results
	Individuals attending 1st semester combined screening at a single institution Mean (SD) age: 30.05 (5.9) Mean (SD) BMI: 25.06 (5.31)		
Gou et al., 2020 **Country** China **Timeframe** March 2017 to February 2020 **Risk of Bias** ROBINS-I: Moderate **Funding/potential COI** None	**N** = 18016 **Inclusion criteria** Gestational age 12-23 wks undergoing NIPS **Exclusion criteria** NR **Participant characteristics** Individuals undertaking self-pay NIPS through NHS or private	**NIPS Platform** NR **NIPS description** Whole-genome sequencing of cffDNA from maternal blood was performed on an ion proton platform; retrospective analysis of de-identified patient information.	**T21**: NR **T18**: NR **T13**: NR **SCA**: NR **CNV**: NR **RAT**: 33 RATs were detected by NIPT from 18,016 samples, with a screening rate of 0.18%. 20/33 had normal pregnancy outcome; 4/33 adverse pregnancy outcomes (TOP, miscarriage, fetal loss); 3/33 lost to follow-up **Diagnostic Procedures**: NR

Study Information	Population	NIPS	Results
	healthcare providers after either a low chance (<1:150) combined test result or no prior screening Patient choice of confirmatory test (amnio, karyotyping, CMA); 14/33 were NA for risk from serum screening; rest were either high or intermediate risk Mean maternal age: 30.0 yrs Mean gestational age: 21.1 wks		**Identification of maternal conditions**: NR **Psychosocial outcomes**: NR
Guy et al., 2021 **Country** United Kingdom **Timeframe**	**N** = 8655 samples, (8651 w/ outcomes) **Inclusion criteria**	**NIPS Platform** IONA **NIPS description** NR	**T21**: sens 173/175 (98.9%, 95% CI 95.9-99.9) PPV: 96.7% (95% CI 92.8-98.4%) **Combined T18/T13**: Sens 47/52 (90.4%; 95% CI 80.0%-96.8%)

Study Information	Population	NIPS	Results
January 2016 to March 2019 **Risk of Bias** ROBINS-I: Serious **Funding/potential COI** Yourgene is contracted to supply the IONA system to St. Georges University Hospitals NHS Foundation Trust as the basis of the SAFE test. None of the authors have pecuniary interests in Yourgene or the SAFE test service	electing to undertake self-pay NIPT through NHS or private healthcare providers after either a low chance (<1:150) combined test result or no prior screening **Exclusion criteria** Pregnancies lost to follow up or w/incomplete reporting of outcomes **Participant characteristics** Samples originated from secondary screening for a high-chance (>1:150) combined screening result or a screen-	pregnancy outcomes were divided into non-trisomic, T21, T18 or T13 by either confirmed karyotype (n=219) or phenotypical normality of the neonate. All abnormal karyotype results were cross checked with regional cytogenetic registers to ensure accuracy.	PPV 92.2% (95% CI 81.5%-96.9) **SCA**: NR **CNV**: NR **RAT**: NR **Diagnostic Procedures**: NR **Identification of maternal conditions**: NR **Psychosocial outcomes**: NR

Study Information	Population	NIPS	Results
	positive QUAD test in the SAFE test collaborative network (a total of 14 NHS trusts) and from individuals electing to undertake self-pay NIPT through NHS or private healthcare providers after either a low chance (<1:150) combined test result or no prior screening (a total of 13 providers). Median (IQR) age: 34.6 (31.1-38.1) Median (IQR) gestational age: 12.0 (11.0-14.0)		
He et al., 2020 **Country** China	**N** = 146 twin pregnancies	**NIPS Platform** In-house	**T21**: Of the 141 cases included in the study, only one DCDA case had a high-risk NIPS result for T21 (Z scores=10.46) and no cases of T18 or T13 were detected. Confirmation by

Study Information	Population	NIPS	Results
Timeframe March 2016 to January 2018 **Risk of Bias** ROBINS-I: Moderate **Funding/potential COI** None	**Inclusion criteria** participants ≥18years old with twin pregnancies ≥10 wks gestation **Exclusion criteria** Individuals who had a definite chromosomal abnormality or a family history of genetic disorders or who suffered from tumors or received allogeneic blood transfusion and transplantation recently **Participant characteristics** Median (range) age: 33 (22-45) Median (range) gestational age: 16.1 (10-23)	**NIPS description** sequencing performed using a Fetal aneuploidies Trisomy Detection Kit (semi-conductor sequencing; Daan Gene Corp.). Sequencing data analyzed by a standard pipeline according to the manufacturer's protocol. The results were from the chromosome-wide aneuploidy test for whole chromosomes (Stouffers Z-scores). Z-scores ≥3 marked as high risk. Karyotyping or clinical follow up were used as the gold standard to evaluate sensitivity and specificity of NIPS in this population	karyotyping revealed one true-positive case T21. According to the follow-up results, the 140 cases with negative NIPS results were euploid, and the sens and spec for T21 by NIPS were both 100% **T18**: no cases **T13**: no cases **SCA**: NR **CNV**: NR **RAT**: NR **Diagnostic Procedures**: NR **Identification of maternal conditions**: NR **Psychosocial outcomes**: NR

Study Information	Population	NIPS	Results
	DCDA, n=107 (73.2%) MCDA, n=39 (26.7%)		
Hu et al., 2019 **Country** China **Timeframe** March 2016 to May 2017 **Risk of Bias** ROBINS-I: Moderate **Funding/potential COI** None	**N** = 8152 undergoing NIPT. 11 failed QC. 8141 remaining. **Inclusion criteria** NR **Exclusion criteria** NR; samples failing QC **Participant characteristics** Mixed-risk from a single hospital Age by groups, AMA=13.79% Gestational age range, 9-34 wks	**NIPS Platform** NR **NIPS description** NR	**T21**: TP 20 FP 5 PPV: 80% **T18**: NR TP 3 FP 4 PPV: 60% **T13**: TP 1 FP 10 PPV: 14.28% **SCA**: Overall TP 11 FP 13 PPV: 45.83% **CNV**: TP 13 FP 18 **RAT**: NR

Study Information	Population	NIPS	Results
			Diagnostic Procedures: NR **Identification of maternal conditions**: NR **Psychosocial outcomes**: NR
Ji et al., 2019 **Country** China **Timeframe** NR **Risk of Bias** ROBINS-I: Moderate **Funding/potential COI** None	**N** = 639 NIPS; 542 w/follow-up **Inclusion criteria** Positive for multiple chr aneuploidies on initial NIPS **Exclusion criteria** NR Participant characteristics Mean (SD) age: 32.1 (5.6) yrs Mean (SD) gestational age: 17 (3.3) wks	**NIPS Platform** BGI **NIPS description** NR Participants were classified as having maternal malignancies based on the confirmed medical record within 1-yr of NIPS	**T21**: NR **T18**: NR **T13**: NR **SCA**: NR **CNV**: NR **RAT**: NR **Diagnostic Procedures**: NR **Identification of maternal conditions**: 41 cancer cases; 501 non-cancer multiple chromosomal aneuploidies findings of any type (reproducible, non-reproducible, or uncertain) were associated with a PPV of 7.6% (41/542) for diagnosing maternal malignancies **Psychosocial outcomes**: NR
Kagan et al., 2018 **Country** Germany	**N** = 1400 (699 randomized to FTCS; 701	**NIPS Platform** Harmony Prenatal Test (Roche)	**T21**: NR In the US+NIPS group, there were no FP cases, while the age-adjusted FPR in the FTCS group was 2.5%.

Study Information	Population	NIPS	Results
Timeframe October 2015- December 2016 **Risk of Bias** ROBINS-I: **Funding/potential COI** One author is employed by Roche; Roche/Ariosa provided kits for Harmony Prenatal Test	randomized to US+NIPS) **Inclusion criteria** Individuals undergoing 1st-trimester screening at a single institution **Exclusion criteria** maternal age <18 yrs, CRL measurement >84 mm or <45 mm, and multiple pregnancy, including vanishing twins **Participant characteristics** US+NIPS group, median risk for T21 was 1:10000; FTCS median risk for T21 was 1:3787.	**NIPS description** DANSR and simultaneous microarray-based assay of non-polymorphic (chro-mosomes 13, 18, 21, X and Y) and polymorphic loci to estimate chromosome proportion and FF	**T18**: NR **T13**: NR **SCA**: NR **CNV**: NR **RAT**: NR **Diagnostic Procedures**: 6/17 high risk for T21 opted for diagnostic testing **Identification of maternal conditions**: **Psychosocial outcomes**: NR

Study Information	Population	NIPS	Results
	Median age: FTCS 33.9 yrs; NIPS 33.9 yrs Median gestational age: FTCS 12.7; NIPS 12.7		
Kagan et al., 2019 **Country** Germany **Timeframe** October 2015 to December 2016 **Risk of Bias** ROBINS-I: **Funding/potential COI** One author is employed by Roche; Roche/Ariosa provided kits for Harmony Prenatal Test	**N** = 1400 (699 randomized to FTCS; 701 randomized to US+NIPS) **Inclusion criteria** Unselected individuals undergoing 1st-trimester screening **Exclusion criteria** maternal age <18 yrs, CRL measurement >84 mm or <45 mm, and multiple pregnancy, including vanishing twins;	**NIPS Platform** Harmony Prenatal Test (Roche) **NIPS description** DANSR and simultaneous microarray-based assay of non-polymorphic (chro-mosomes 13, 18, 21, X and Y) and polymorphic loci to estimate chromosome proportion and FF	**T21**: 24 cases (1.7%) no follow-up Median risk of T21 w/FTCS 1:3,787. Adding 3 new markers median risk was 1:6,418. If the risks of T21 were calculated without MSS, they ranged between 1:2,787 and 1:6,219 depending on the combination of markers used. In the US+NIPS group, median risk was 1:10,000 irrespective of the mode of risk calculation in those with uninformative NIPS test results. Only 0-0.6% of cases had a risk between 1:100-1:999 While there were no FP in the US+NIPS group, with eFTCS, the FPR were between 0.9 and 3.2%. **T18**: NR **T13**: NR **SCA**: NR **CNV**: NR

Study Information	Population	NIPS	Results
	miscarriage w/out further autopsy or genetic analysis; no results for either screening or newborn exam/genetic testing avail **Participant characteristics** Median age: FTCS 33.9 yrs; NIPS 33.9 yrs Median gestational age: FTCS 12.7; NIPS 12.7		**RAT**: NR **Diagnostic Procedures**: NR **Identification of maternal conditions**: NR **Psychosocial outcomes**: NR
Kagan et al., 2020 **Country** Germany **Timeframe** January to December 2018 **Risk of Bias** ROBINS-I: Moderate	**N** = 1127 (1062 low-risk; 65 high-risk) **Inclusion criteria** NR **Exclusion criteria** maternal age <18 years, CRL	**NIPS Platform** Harmony Prenatal Test (Roche) performed by TOMA Advanced Biomedical Assays NIPS description NR	**T21**: NR **T18**: NR **T13**: NR **SCA**: NR **CNV**: NIPS 22q11.2DS+ n=3 (all in low-risk group); FP=3, TP=0

Study Information	Population	NIPS	Results
Funding/potential COI One author employed by Roche; 3 authors employed by TOMA Laboratory w/out ownership shares; one author expert panel member for Roche and Menarini Biomarkers; Ariosa sponsored the investigator-initiated study	measurement of >84 or <45 mm, and multiple pregnancy, including vanishing twins; miscarriage w/o further autopsy or genetic analysis **Participant characteristics** Median (IQR) age: low risk, 33.9 (31.0-36.7); high risk, 35.8 (30.4-38.3) Median (IQR) gestational age: low risk, 12.9 (12.5-13.3); high risk, 12.9 (12.4-13.2)	FISH analysis was performed when CMA provided a normal result to exclude the presence of rare confined placental mosaicism for 22q11.2DS in cytotrophoblasts. Parental testing was carried out by FISH analysis. After delivery, a detailed neonatal clinical examination was performed, including further genetic testing on cord blood or placenta by FISH and/or CMA. For those w/negative NIPS: all children are examined directly after birth and ≥6 times by a pediatrician w/in the 1st yr of life (all were	**RAT**: NR **Diagnostic Procedures**: NR **Identification of maternal conditions**: NR **Psychosocial outcomes**: NR

Study Information	Population	NIPS	Results
		>6 mos of age at time of manuscript)	
Khalil et al., 2021 **Country** United Kingdom **Timeframe** February 2015 to June 2018 **Risk of Bias** ROBINS-I: Moderate **Funding/potential COI** 3 authors current/former employees of Yourgene (formerly Premaitha Health)	**N** = 1003 twin pregnancies **Inclusion criteria** ≥16 yrs old; US documentation of twin pregnancy at ≥10 wks gestational age **Exclusion criteria** Participants who have Down syndrome or other chr abnormality themselves, children <16 yrs old, adults w/ learning disabilities or mental illness or who are unable to give informed consent for themselves, adults who are unconscious or	**NIPS Platform** IONA **NIPS description** NGS and a proprietary algorithm. Screening was for trisomies 21,13, and 18. Primary outcome DR and specificity for twin gestations for the three trisomies and test failure rate.	**T21**: MC: n=276; T21+ n=1, normal n=275 DC: n=685; T21+ n=13, T18+ n=1, T13+ n=1, normal n=670 TP: T21 n=13; T13 n=1 FP: T18 n=1 TN: n=942 FN: T18 n=1 Sample failure rate 3/961 (0.31%) **SCA**: NR **CNV**: NR **RAT**: NR **Diagnostic Procedures**: NR **Identification of maternal conditions**: NR **Psychosocial outcomes**: NR

Study Information	Population	NIPS	Results
	very severely ill; adults who have a terminal illness or current malignancy, adults in emergency situations, prisoners and young offenders, or any person considered to have a particularly dependent relationship with investigators. The exclusion criteria included higher order multiple pregnancies, fetal demise or vanishing twin, known mosaicism, partial trisomy or translocations, or known aneuploidy or malignancy in the		

Study Information	Population	NIPS	Results
	pregnant individual **Participant characteristics** 2 groups of pregnant individuals w/twin pregnancies: Group 1 those with a low chance of carrying a fetus with a chr abnormality, on the basis of the conventional prenatal screening tests Group 2 included women w/a high chance, on the basis of conventional prenatal screening tests (>1:150 at term), and who attended the fetal medicine		

Study Information	Population	NIPS	Results
	clinics at the study sites for prenatal counseling and possible diagnostic testing (CVS or amnio).		
Kypri et al., 2019 **Country** Cyprus **Timeframe** NR; large prospective samples from lab until February 2018 **Risk of Bias** ROBINS-I: Moderate **Funding/potential COI** Majority authors current/former employees of NIPD Genetics Public Company	**N** = 10564; SCA n=305; twin T21/T18/T13 n=306 **Inclusion criteria** NR **Exclusion criteria** samples w/insufficient fetal fraction **Participant characteristics** general population pregnant individuals (singletons and twins) from multiple referral	**NIPS Platform** Veracity; Illumina **NIPS description** NR	Twins: (blinded mixed retrospective and prospective validation of n=306 samples) T21: NIPS+ n=3; TP=3 T18: NIPS+ n=1; TP=1 T13: NIPS+ n=1; TP=1 Singletons: (mixed-risk) NIPS: n=10280; follow-up n=10280; TN n=10280; spec=99.98% (95% CI 99.93%-99.998%); NPV=100% (95% CI 99.96%-100%) T21: NIPS+ n=126; follow-up n=44; TP=44; sens=100% (92-100%); PPV=100% (92-100%) T18: NIPS+ n=24; follow-up n=10; TP=10; sens=100% (69-100%); PPV=100% (69-100%) T13: NIPS+ n=16; follow-up n=7; TP=5; sens=100% (48%-100%); PPV=71% (29-96%) Twins: SCAs (blinded retrospective validation of n=305 plasma samples)

Study Information	Population	NIPS	Results
	centers in 21 countries		45,X: NIPS+=7; TP=7 47,XXY: NIPS+ n=4; TP=4 47,XXX: NIPT+ n=2; TP=2 47,XYY: NIPT+ n=1; TP=1 Singletons: NIPS: n=6200; follow-up n=6200; TN=6200; spec=99.95% (99.86-99.99%); NPV = 100% (99.94-100%) 45,X+ n=16; follow-up n=7; TP=4; sens=100% (40-100%); PPV=57% (18-90%) 47,XXX+ n=6; follow-up n=2; TP=2 47,XXY+ n=10; follow-up n=4; TP=4 47,XYY+ n=3; follow-up n=0 48,XXYY+ n=1; follow-up n=1; TP=1 **CNV**: NR **RAT**: NR **Diagnostic Procedures**: NR **Identification of maternal conditions**: NR **Psychosocial outcomes**: NR
Lai et al., 2021 **Country** China **Timeframe** May 2015 to December 2018	**N** = 86193 **Inclusion criteria** Age ≥16 yrs, singleton pregnancy 12 wks	**NIPS Platform** NR **NIPS description** Libraries of 96 samples with	**T21+** n=368; TP=330; FP=38; FN=3, refused=51; sens=99.1%; spec=99.95%; PPV=89.67%; NPV=99.996%; FPR=0.05%; FNR=0.90%; screen positive rate=0.5%

Study Information	Population	NIPS	Results
Risk of Bias ROBINS-I: Moderate **Funding/potential COI** NR	gestational age, and no history of transfusion or transplantation during past years **Exclusion criteria** Individuals w/intermediate/ high-risk pregnancy from 1st or 2nd trimester screening; structural abnormalities reported by US include cardiac malformations, cleft lip and palate, fetal hydrops, limb malformations, cystic hygroma, renal dysplasia, lung cystadenomas **Participant characteristics**	barcodes were then pooled together with equimolar and subjected for single-end sequencing (37 base-pairs with another 8 base-pairs as index) on a Nextseq-500 platform; required sequencing quality value (Q30) was >85%, and GC content ranged from 38 to 42; After GC correction, FF was estimated by using elastic net (ENET) algorithm Chr aneuploidy was reported using the criteria of Z-score $\geq$3 (trisomy) or $\leq$ -3 (monosomy). When different fetal fractions were reported by two algorithms (ENET and chromosome Y-	**T18+** n=100; TP=84, FP=16, refused=8; FN=1; sens=98.82%; spec-99.98%; PPV=84%; NPV=99.999%; FPR=0.02%; FNR=1.18%; SPR=0.13% **T13+** n=57; TP=30, FP=27, refused=2; FN=0; sens=100%; spec-99.97%; PPV=52.63%; NPV=100%; FPR=0.03%; FNR=0%; SPR=0.07% **SCA**: 45,X+ n=191; TP=23, FP=168, refused=48; FN=3; sens=88.46%; spec=99.8%; PPV=12.04%; NPV=99.996%; FPR=0.2%; FNR=11.54%; SPR=0.29% 47,XXX+ n=53; TP=36, FP=17, refused=18; FN=0; sens=100%; spec=99.98%; PPV=67.92%; NPV=100%; FPR=0.02%; FNR=0%; SPR=0.08% 47,XXY+ n=113; TP=78, FP=35, refused=29; FN=0; sens=100%; spec=99.96%; PPV=69.03%; NPV=100%; FPR=0.04%; FNR=0%; SPR=0.17% 47,XYY+ n=18; TP=14, FP=4, refused=5; FN=0; sens=100%; spec=100%; PPV=77.78%; NPV=100%; FPR=0%; FNR=0%; SPR=0.04% 46,XY(delX)+ n=25; TP=23, FP=168, refused=48; FN=3; sens=88.46%; spec-99.8%; PPV=12.04%; NPV=99.996%; FPR=0.2%; FNR=11.54%; SPR=0.29% **CNVs+** (<= 5Mb) n=12; TP=4, FP=8, refused=1; FN=16; sens=20%; spec-99.99%; PPV=33.33%; NPV=99.981%; FPR=0.01%; FNR=80%; SPR=0.02%

Study Information	Population	NIPS	Results
	General population	based), mosaic chromosome aneuploidy was considered. The analytical algorithm for CNVs was reported in previous studies, with a resolution of 5 Mb. ~2500 lost to follow-up	**RATS+** n=44; TP=9, FP=35, refused=12; FN=1; sens=90%; spec-99.96%; PPV=20.45%; NPV=99.999%; FPR=0.04%; FNR=10%; SPR=0.07% **Diagnostic Procedures**: NR **Identification of maternal conditions**: NR **Psychosocial outcomes**: NR
Langlois et al., 2017 **Country** Canada **Timeframe** November 2013 to June 2017 **Risk of Bias** ROBINS-I: Moderate **Funding/potential COI** The authors are investigators in a Research Project funded under the auspices of Genome Canada and the Canadian Institutes for Health Research (both	**N** = 1198 **Inclusion criteria** ≥19 yrs old, singleton gestation, recruited <14 wks gestation, have decided to undertake the provincially funded screening test and agreed to have the NIPS screening result provided to them at the same time as the result of	**NIPS Platform** HARMONY Prenatal test; T21, T13, T18 only **NIPS description** NR 33 lost to follow-up	T21+ traditional screening n=68 TP=5; FP=63; TN=1096; Detection rate=83% (36%-99%); FPR=5.4% (4.2%-6.9%) T21+ NIPS: n=6; TP=6; FP=0; TN=1159; DR=100% (54%-100%); FPR=0% (0-0.3%) 0 cases of T18 or T13; NIPS+ T18 FP=1; NIPS+ T13 FP=1 FPR for both T18 & T13 0.09% (0-0.48%) **SCA**: NR TP TN FP FN Other **CNV**: NR TP TN

Study Information	Population	NIPS	Results
non for-profit organizations funded by the Canadian government) but that call for some mandatory in-kind contributions from other partners. This funding is at arm's length from the scientific component of the Research Project. The funders had no role in the design of the study, interpretation of the results, or approval of the manuscript	their standard screen. Participants also consented to a review of their and their newborn's medical records and/ or phone call at 6 wks postpartum for details of invasive testing, if done, course of their pregnancy and outcome, as well as information about the health of their newborn and results of any postnatal genetic testing **Exclusion criteria** NR **Participant characteristics** low-risk pregnant individuals		FP FN Other **RAT**: NR TP TN FP FN Other **Diagnostic Procedures**: total invasive diagnostic procedure rate was 2% (23/1165; 95% CI, 1.3%-3%) but could have been as high as 6.8% (79/1165; 95% CI, 5.4%-8.4%) based on traditional screening and ultrasound examination without NIPS analysis. The rate of invasive diagnostic testing in the NIPS negative women was 1.2% (14/1151;95% CI, 0.7%-2%) **Identification of maternal conditions**: NR **Psychosocial outcomes**: NR

Study Information	Population	NIPS	Results
	seeking publicly-funded screening		
LeConte et al., 2018 **Country** France & Belgium **Timeframe** 1 November 2013 to 31 August 2015 **Risk of Bias** ROBINS-I: Moderate **Funding/potential COI** 3 authors are employees of CERBA, in which they are also shareholders	**N** = 492 Inclusion criteria twin pregnancies with no abnormal fetal ultrasound finding and with nuchal translucency <3.5 mm **Exclusion criteria** NR **Participant characteristics** Individuals w/ twin pregnancy undergoing routine screening	**NIPS Platform** **NIPS description** massively parallel sequencing using a whole-genome approach; Z-scores were calculated for the targeted chromosomes (13, 18 and 21) and classification was based upon a standard normal transformed cut-off value of Z=3 for chromosome 21 and Z=3.95 for chromosomes 18 and 13. Results are expressed as positive or negative when the metric criteria (total count of reads should be 9 million and the estimated fetal DNA fraction 8%) are	Overall: (n=420) NIPT+ n=6; TP=4; FP=2; TN=414 **T21**: TP=3, FP=1; sens=100% (29.2-100%); spec=99.8% (98.7-100%) **T18**: TP=1, FP=0 **T13**: TP=0, FP=1 59 pts (12%) lost to follow-up & no karyotype for 13 SCA: NR **CNV**: NR **RAT**: NR **Diagnostic Procedures**: NR **Identification of maternal conditions**: NR **Psychosocial outcomes**: NR

Study Information	Population	NIPS	Results
		fulfilled and no-result if they are not. A theoretical value of 8% was used whatever the chorionicity, assuming that each fetus contributes adequate amounts of DNA to the maternal plasma to ensure accurate results, compared with the 4% value validated previously for use in singleton pregnancies	
Liang et al., 2018 **Country** China **Timeframe** August 2011 to December 2016 **Risk of Bias** ROBINS-I: Moderate **Funding/potential COI** None	**N** = 32431 **Inclusion criteria** ≥12 weeks of gestation with a singleton pregnancy **Exclusion criteria** NR **Participant characteristics**	**NIPS Platform** NR **NIPS description** Whole-genome massively parallel shotgun sequencing was performed in all cases; Starting from 2012, screening for other genome-wide RATs and CNVs was added to the aspect	**T21**: TP n=115; FP n=10; TN n=25852; FN n=3; sens=97.45% (92.79%-99.13%); spec=99.96% (99.93%-99.98%); PPV=92% (85.90%-95.60%); NPV=99.99% (99.97%-100%) **T18**: TP n=23; FP n=16; TN n=25941; FN n=0; sens=100% (85.69%-100%); spec=99.94% (99.90-99.96%); PPV=58.97% (43.42%-72.92%); NPV=100% (99.99%-100%) **T13**: TP n=3; FP n=10; TN n=25967; FN n=0; sens=100% (43.85%-100%); spec=99.96% (99.93-99.98%); PPV=23.08% (8.18%-50.26%); NPV=100% (99.99%-100%) SCA: (Overall):

Study Information	Population	NIPS	Results
	mixed-risk population of pregnant individuals from Eastern China	of screening as an additional service, and women need to consent to this separately to the common aneuploidies.	TP=28, FP=29; TN=NR; FN=NR, Sens=NR; Spec=NR; PPV=49.12% (36.62%-61.74%); NPV=NR **CNV**+ n=37, validated n=21; TP=6; FP=15 **RATs**+ n=53, validated n=24; TP=3; FP=21 **Diagnostic Procedures:** NR **Identification of maternal conditions:** NR **Psychosocial outcomes:** NR
Liang et al., 2019 **Country** China **Timeframe** November 2015 to December 2017 **Risk of Bias** ROBINS-I: Moderate **Funding/potential COI** 7 authors are employees of Berry Genomics Corporation; 1 author holds stocks in the company	**N** = 94085 **Inclusion criteria** singleton pregnancy **Exclusion criteria** NR **Participant characteristics** the general population who had naturally conceived a singleton pregnancy Median (range) age (low-risk):	**NIPS Platform** In house using Berry Genomics kit; sequencing on Illumina NextSeq **NIPS description** NIPS-PLUS Median FF: 10.8%	965 NIPS-Plus positive results, there were 526 fetuses at high risk for T21, T18, or T13 **T21** (n=364) was the most common, followed by **T18** (n=123) and **T13** (n=39). Of these, there were 20 pregnancies incorrectly scored as high risk (FPs) for T21, 22 for T18, and 21 for T13, yielding positive predictive values (PPVs) of 95%, 82%, and 46%, respectively **SCA**: 390 NIPS+ 45,X: NIPS+ n=190; FP=141; PPV=26% 47,XXY: NIPS+ n=76; FP=13; PPV=83% 47,XXX: NIPS+ n=81; FP=31; PPV=62% 47,XYY: NIPS+ n=24; FP=6; PPV=75% 46,XY(Xdel): n=19; FP=17; PPV=11% **CNV**: 120 P/LP fetal CNVs were followed up in validation studies: 32 cases of MMS associated with classical chr diseases. This comprised 14 cases at high risk of DGS, 6 cases of 22q microduplication syndrome, 4 cases of PWS, 6

Study Information	Population	NIPS	Results
	29 (15-34) yrs Median (range) GA: 17^{+3} (11-39) wks		cases of CDC, and 2 cases of 1p36 del syndrome. DGC, 14 suspected cases, 13TP, 1FP, PPV=93%; 6 cases 22q11.2 microduplications 4TP 2FP PPV=67%; PWS 4 suspected cases, 3TP, 1FP PPV 75%; CDC 6 suspected cases 3TP 3FP PPV 50% The remaining 88 of 120 fetal CNVs comprised genome-wide segmental CNVs that were classified as nonsyndromic MMS because no specific syndromes could be identified in any current databases as associated with these changes. Of these, there were 23 TPs and 49 FPs forCNVs, ≥10 Mb (PPV 32%) and 3 TPs and 13 FPs for CNVs <10 Mb (PPV 19%) (nonsyndromic). **RAT:** NR **Diagnostic Procedures:** NR **Identification of maternal conditions:** NR **Psychosocial outcomes:** NR
Lin et al., 2020 **Country** China **Timeframe** January 2017 to December 2017 **Risk of Bias** ROBINS-I: Serious **Funding/potential COI** None	**N** = 11175 **Inclusion criteria** have low risk NIPS results to assess False negative rate **Exclusion criteria** NR	**NIPS Platform** In-house; BGI sequencer **NIPS description** Analysis was performed for all samples on aneuploidies of chr 13, 18, 21, X, and Y, as well as other	**T21**: NR **T18**: FN, 1 **T13**: NR **SCA**: NR **CNV & RATs**: 3/10,975 FN for T18, RAT (12p) & microdeletions **Diagnostic Procedures**: NR **Identification of maternal conditions**: NR

Study Information	Population	NIPS	Results
	Participant characteristics NIPS performed at a single hospital; low risk: n=10975; benign pregnancy outcomes: n=10310; loss to follow-up: n=499; adverse pregnancy outcome: n=166	genome-wide RAT and sub-chr CNV. Pregnancies with low-risk NIPS results were recommended for routine prenatal care and interviewed by telephone at 3 months after delivery	**Psychosocial outcomes**: NR
Lu et al., 2020 **Country** China **Timeframe** January 2017 to December 2019 **Risk of Bias** ROBINS-I: Moderate **Funding/potential COI** None	**N** = 37006 recruited. 93 (0.25%) excluded. 36913 remained. AMA n=9516 (high risk, 1118; low risk, 8398) Normal age n=27397 (high risk, 12575; low risk, 14822) **Inclusion criteria** Singleton gestations, had	**NIPS Platform** NR **NIPS description** NR	**T21**: TP, n=116; FP, n=21 [15 from further dx testing]; TN, n=36488; FN; n=0 sens=100%; spec=99.94%; FPR=0.06%; FNR=0%; PPV=84.67%; NPV=100% **T18**: TP, n=27; FP, n=19 [16 from further dx testing]; TN, n=36579; FN; n=0 sens=100%; spec=99.95%; FPR=0.05%; FNR=0%; PPV=58.70%; NPV=100% **T13**: TP, n=13; FP, n=18 [15 from further dx testing]; TN, n=36594; FN; n=0 sens=100%; spec=99.95%; FPR=0.05%; FNR=0%; PPV=41.94%; NPV=100% **SCA** (Overall): TP, n=51; FP, n=102 [53 from further dx testing]; TN, n=36472; FN; n=0

Study Information	Population	NIPS	Results
	pretest counseling **Exclusion criteria** NR **Participant characteristics** Individuals from single center in China. Median (range) maternal age: 29 (18-54) yrs Median (range) gestational age was 17^{+4} (12-32) wks Repeat sample: n=306 (0.83%) 213 of them obtained effective NIPS results		sens=100%; spec=99.72%; FPR=0.28%; FNR=0%; PPV=33.33%; NPV=100% **CNV**: NR **RAT**: NR **Diagnostic Procedures**: NR **Identification of maternal conditions**: NR **Psychosocial outcomes**: NR
Lu et al., 2021	**N** = 45773	**NIPS Platform** In-house	**T21**: NR

Study Information	Population	NIPS	Results
Country China **Timeframe** June 1, 2015, to June 30, 2019 **Risk of Bias** ROBINS-I: Serious **Funding/potential COI** None	**Inclusion criteria** [1] gestational week between 12^{+0}-26^{+6} wks [2] singleton pregnancy **Exclusion criteria** [1] gestational age <12 wks; [2] multifetal pregnancies; [3] definite chr abnormalities; [4] individuals who underwent an allogeneic blood transfusion, stem cell therapy, transplant surgery, or other procedure; [5] a family history of genetic disease or an indication for a high risk of genetic disease in the fetus; [6] individuals w/ malignant	**NIPS description** NR	**T18**: NR **T13**: NR **SCA** (Overall): TP, n=58; FP, n=85; PPV, 40.56% range of PPV 30.23% in age <30 yrs to 71.43% in age >39 yrs **CNV**: NR **RAT**: NR **Diagnostic Procedures**: NR **Identification of maternal conditions**: NR **Psychosocial outcomes**: NR

Study Information	Population	NIPS	Results
	tumors; and [7] other conditions that might affect the accuracy of the results. **Participant characteristics** Mixed-risk population ages 16 to 45 yrs		
Luo et al., 2020 **Country** China **Timeframe** January 2011 to December 2018 **Risk of Bias** ROBINS-I: Moderate **Funding/potential COI** None	**N** = 40311 **Inclusion criteria** ≥12 weeks of gestation with a singleton pregnancy **Exclusion criteria** multifetal pregnancies, one of the parents w/ chr abnormalities, and individuals who had received allogeneic blood transfusion, transplantation,	**NIPS Platform** In-house; sequenced at BGI **NIPS description** NR	**T21**: NIPS+ n=145; TP=105; PPV=84% **T18**: NIPS+ n=21; TP=13; PPV=48.15% **T13**: NIPS+ n=21; TP=4; PPV=14.29% **SCA**: PPV 35.32% **CNV**: NR **RAT**: NIPS+ n=69 54 w/invasive testing. TP=5 **Diagnostic Procedures**: NR **Identification of maternal conditions**: NR **Psychosocial outcomes**: NR

Study Information	Population	NIPS	Results
	stem cell therapy and immunotherapy w/in a year Participant characteristics		
Margiotti et al., 2020 **Country** Italy **Timeframe** January 2018 to January 2020 **Risk of Bias** ROBINS-I: Moderate **Funding/potential COI** Multiple authors employed by laboratory	N = 9985 **Inclusion criteria** NR **Exclusion criteria** NR **Participant characteristics** mixed-risk population	**NIPS Platform** Ion S5 NGS (ThermoFisher Scientific, Waltham, MA, USA) **NIPS description** NR	**T21**: NR **T18**: NR **T13**: NR **SCA**: Total SCA+ n=31; validated n=22; TP=17, FP=5; PPV=77.3% (54.2%-91.3%); unconfirmed n=9 45,X+ n=19; validated n=13, TP=9, FP=4, PPV=69.2% (38.9%-89.6%); unconfirmed n=6 47,XXX+ n=4; validated n=3, TP=3, FP=0; PPV=100% (31%-100%), unconfirmed n=1 47,XXY+ n=6; validated n=5, TP=4, FP=1, PPV=80% (29.9%-98.9%), unconfirmed n=1 47,XYY+ n=2; validated n=1; TP=1; FP=0; PPV=100% (5.5%-100%); unconfirmed n=1 **CNV**: NR **RAT**: NR **Diagnostic Procedures**: NR

Study Information	Population	NIPS	Results
			Identification of maternal conditions: NR **Psychosocial outcomes**: NR
Martin et al., 2018 **Country** United States **Timeframe** February 2014-February 2015 **Risk of Bias** ROBINS-I: moderate/serious **Funding/potential COI** Multiple authors are employees/paid consultants of Natera; study was funded by Natera, Inc.	**N** = 80449 **Inclusion criteria** NR **Exclusion criteria** test canceled, draw <9 weeks GA, insufficient blood volume, contamination, multiple gestations, and low fetal fraction **Participant characteristics** Maternal age (yr), mean (SD): 32.0 (5.8) Gestational age (wks), mean (SD): 13.7 (4.1) Maternal weight (lbs), mean (SD): 157.3 (38.3)	**NIPS platform** Natera **NIPS description** Screened for panel of microdeletion syndromes (22q11.2 deletion, 1p36, cri-du-chat, Prader-Willi, Angelman microdeletions), N=42,326; screened for 22q11.2 deletion only, N=21,948	**T21:** NR **T18:** NR **T13:** NR **SCA:** NR **CNV: (revised algorithm)** 22q11.2: w/ abnormal findings (known prior to NIPS): TP, n=18; FP, n=0; PPV=100%; missing confirmation, n=6; w/o abnormal findings or detected after NIPS: TP, n=5; FP, n=22; PPV=18.5%; missing confirmation: n=8 1p36: w/ abnormal findings (known prior to NIPS): TP, n=1; FP, n=0; PPV=100%; missing confirmation, n=0; w/o abnormal findings or detected after NIPS: TP, n=1; FP, n=1; PPV=50%; missing confirmation: n=0 Cri-du-chat: w/ abnormal findings (known prior to NIPS): TP, n=2; FP, n=0; PPV=100%; missing confirmation, n=0; w/o abnormal findings or detected after NIPS: TP, n=2; FP, n=2; PPV=50%; missing confirmation: n=0 Prader-Willi: w/ abnormal findings (known prior to NIPS), none identified w/o abnormal findings or detected after NIPS: TP, n=0; FP, n=1; PPV=0%; missing confirmation: n=3

Study Information	Population	NIPS	Results
	Fetal fraction (%), mean (SD): 10.5 (4.3)		Angelman: w/ abnormal findings (known prior to NIPS): none identified w/o abnormal findings or detected after NIPS: TP, n=1; FP, n=9; PPV=10%; missing confirmation: n=7 **RAT:** NR **Diagnostic procedures:** NR **Identification of maternal conditions:** 6 cases suspected based on fetal risk score of 50% for 22q11.2 deletion; follow-up available for 3 individuals; 2 with confirmed maternal 22q11.2 deletion, 1 with confirmed fetal deletion and unconfirmed maternal copy number for 22q11.2 region but with tetralogy of Fallot and learning disabilities (associated with 22q11.2 deletion syndrome) **Psychosocial outcomes:** NR
Martin et al., 2020 **Country** NR; assumed United States **Timeframe** May 12 to December12, 2018 **Risk of Bias** ROBINS-I: Moderate **Funding/potential COI**	**N** = 194385 Group A: Suspected Maternal ChrX n=149; sequentially enrolled to n=106 Group B: Suspected fetal Chr: n=613;	**NIPS Platform** Natera **NIPS description** NR	**T21**: NR **T18**: NR **T13**: NR **SCA**: 149 suspected maternal x chr abnormalities. Group A (suspected group 100/106 had anomalies 94.3% PPV). 45,X: NIPS+ n=58, TP mosaic or non-mosaic 38/58 (65.5%) 47,XXX: NIPS+ n=40; TP=38 In Group B (n=107), no maternal CMA abnormalities reported, NPV= 100% (1-sided 97.5% CI, 96.6%-100.0%)

Study Information	Population	NIPS	Results
Multiple authors are employees/paid consultants of Natera; Natera, Inc contributed to the design; participated in the collection, analysis, and interpretation of data; and collaborated on writing, reviewing, and approving the final version. This study was funded by Natera, Inc.	sequentially enrolled to n=107 **Inclusion criteria** All tests unable to evaluate fetal risk for aneuploidy because of uninformative algorithm results were eligible for inclusion. Group A (n=106) where a maternal X chr abnormality was suspected and Group B (control group, n=107) where a fetal chr abnormality involving chr 13, 18, 21, or X was suspected but did not meet criteria for reporting; ≥9 wks gestation and the FF ≥ 2.8%. **Exclusion criteria**		**CNV**: NR **RAT**: NR Other **Diagnostic Procedures**: NR **Identification of maternal conditions**: NR **Psychosocial outcomes**: NR

Study Information	Population	NIPS	Results
	Multifetal pregnancies and pregnancies involving egg donors or surrogates; Risk assessment was not performed if the pregnancy was known to have been complicated by a vanishing twin or a known maternal history of chr abnormality or malignancy **Participant characteristics**		
Motevasselian et al., 2020 **Country** Iran **Timeframe** March 2016 and December 2018	**N** = 500 twin pregnancies; 144 pregnancies (28.8%) were excluded **Inclusion criteria**	**NIPS Platform** ion Torrent (Life Technology) **NIPS description** NR	**combined T21/T18/T13**: NIPT+ n=7; TP=6; FP=1; combined FPR=0.28%; combined sens=100%; combined spec=99.7% **SCA**: NR **CNV**: NR **RAT**: NR **Diagnostic Procedures**: NR

Study Information	Population	NIPS	Results
Risk of Bias ROBINS-I: Serious **Funding/potential COI** None	referred to Nilou Clinical Laboratory **Exclusion criteria** No follow-up (n=94, 18.8%); no karyotype (n=22, 4.4%), IUFD of both fetuses (n=7, 1.4%); selective embryonic reduction (n= 2, 0.4%); TOP due to preterm labor (n=11), PROM (n=7), severe pre-eclampsia (n=1) **Participant characteristics** mixed risk population of twin pregnancies		**Identification of maternal conditions**: NR **Psychosocial outcomes**: NR
Noh et al., 2019 **Country** South Korea	**N** = 817; 490 (60.0%) chose the integrated test as their primary serum screening	**NIPS Platform** Green Cross Genome NIPStest **NIPS description**	**T21**: NR **T18**: NR **T13**: NR

Study Information	Population	NIPS	Results
Timeframe July 2016 to April 2018 **Risk of Bias** ROBINS-I: Moderate **Funding/potential COI** None	method, 327 (40.0%) chose NIPS **Inclusion criteria** singleton and twin pregnancies undergoing prenatal screening for fetal trisomy **Exclusion criteria** NR **Participant characteristics** tertiary urban academic medical center in Seoul, South Korea (Samsung Medical Center).	shotgun massively parallel sequencing (s-MPS) by Sequenom MaterniT21 PLUS, (Sequenom, Inc., San Diego, CA, USA)	**SCA**: NR **CNV:** NR **RAT**: NR **Diagnostic Procedures:** The mean number of amnio performed at our institution prior to NIPS was 8.8/mo (8.8¬±4.8,r ange: 4-14). Post-NIPS: decreased to 4.1/mo (4.1¬±2.3, range: 2-8); *P* < 0.01 **Identification of maternal conditions**: NR **Psychosocial outcomes**: NR
Norwitz et al., 2019 **Country** United States with samples from multiple countries	**N** = 126 (overlapping cohorts for analysis) **Inclusion criteria**	**NIPS platform** Natera **NIPS description** SNP-based NIPS, using an algorithm previously validated	**T21**: (samples w/confirmation) MZ, n=1; DZ, n=4; no FP **T18**: (samples w/confirmation) DZ, n=5; no FP **T13**: (samples w/confirmation) DZ, n=1; no FP **SCA** NR **CNV** NR **RAT** NR **Diagnostic procedures** NR

Study Information	Population	NIPS	Results
Timeframe April 2013 – February 2017 **Risk of Bias** ROBINS-I: Moderate **Funding/Potential COI** multiple authors were/are employed by, are on the advisory board of, and/or own stock/stock options of Natera	Individuals ≥18 yrs old) with sonographically confirmed twin pregnancies. Patients had to be 9 weeks gestation or greater. Subset (n=56) with at least 1 additional criterion: Confirmed affected with aneuploidy by invasive testing, non-invasive prenatal test (NIPT) "high-risk" result, serum screening risk of greater than 1:100, or observed ultrasound abnormalities suggestive of aneuploidy **Exclusion criteria**	for singleton pregnancies, with modifications for twin gestations	**Identification of maternal conditions** NR **Psychosocial outcomes** NR **Other** Zygosity: samples w/confirmation, MZ, correct calls 29/29; DZ, correct calls 64/64 Fetal sex confirmation: MZ, correct calls 40/40 (20 males, 20 females); DZ, correct calls 62/62 (2 males, n=20; 1 male, n=34, 0 males, n=8)

Study Information	Population	NIPS	Results
	singleton pregnancies or the use of a surrogate or egg donor; samples with multiple aneuploidy conditions **Participant characteristics** Reported separately for each analysis: Zygosity, n=95 MZ:DZ: 30:65 Maternal age, mean (SD): 32.8 (5.2) yrs Gestational age, mean (SD): 15.4 (4.7) weeks Fetal sex, n=103 MZ:DZ 40:63 Maternal age, 32.8 (5.3) Gestational age, 15.4 (4.6)		

Study Information	Population	NIPS	Results
	Aneuploidy, n=117 MZ:DZ 40:77 Maternal age, 33.0 (5.5) Gestational age, 15.6 (4.8)		
Oneda et al., 2020 **Country** Switzerland **Timeframe** NR **Risk of Bias** ROBINS-I: Moderate **Funding/potential COI** NR	**N** = 3053 for prospective; 2998 with result (98.2%). 91 cases for retrospective **Inclusion criteria** Prospective: pregnant women after 9 weeks gestation, who opted to have NIPT **Retrospective**: pts w/results from invasive prenatal testing who agreed to participate **Exclusion criteria** NR	**NIPS Platform** In-house; sequencing on NextSeq550 or HiSeq2500 (Illumina) **NIPS description** NR confirmed fetal trisomy ratio in twins, the percentage was 1.3% (4 in 301 fetuses)	**T21**: TP=28; FP=0; T21 + XXX, TP=1, FP=0 **T18**: TP=26; FP=0 **T13**: TP=8; FP=0 **Combined T21/T18/T13 prospective only**: Sens 100.00% (95% CI 91.96-100) Spec 99.97% (95% CI 99.81-100) PPV 97.78 (95% CI 86.11-99.68) NPV: 100% Accuracy: 99.97% (95% CI 99.81-100) **SCA**: MX, TP=9; FP=0; XXX, TP=3, FP=0; XYY, TP=1, FP=0; XXY, TP=1, FP=0; **Combined SCA prospective only:** Sens 100.00% (95% CI 2.5-100) Spec 99.93% (95% CI 99.76-99.99) PPV 33.33 (95% CI 11.12-66.65) NPV: 100% Accuracy: 99.93% (95% CI 99.76-99.99) **CNV**: prospective only:

Study Information	Population	NIPS	Results
	Participant characteristics Prospective: Median (range) gestational age, 12 (9-28) wks AMA, 35.2% Retrospective: Median (range) gestational age, 14 (11-35) wks		Sens 75% (95% CI 19.41-99.37) Spec 99.74% (95% CI 99.46-99.89) PPV 30% (95% CI 14.45-52.10) NPV: 99.96% (95% CI 99.80-99.99) Accuracy: 99.7% (95% CI 99.41-99.87) **RAT**: combined prospective only: Sens 100.00% (95% CI 2.5-100) Spec 99.93% (95% CI 99.76-99.99) PPV 33.33 (95% CI 11.12-66.65) NPV: 100% Accuracy: 99.97% (95% CI 99.73-99.99) **Diagnostic Procedures**: NR **Identification of maternal conditions**: 9 pts w/clinically relevant CNVs **Psychosocial outcomes**: NR
Panchalee et al., 2020 **Country** Thailand **Timeframe** October 1, 2013 to May 31, 2018 **Risk of Bias** ROBINS-I: Moderate **Funding/potential COI**	**N** = 8,659 enrolled; 8572 w/ confirmed singleton pregnancy. 8434 w/conclusive results **Inclusion criteria** singleton pregnancies, all	**NIPS Platform** Natera **NIPS description** SNP-based	**T21**: Out of 63 calls for T21, confirmatory testing was done for 50 samples (79.4%). TP=47. FP = 3. PPV = 94% **overall (T21+T18+T13)** calls are 96. Confirmatory testing was done for 77samples (80.2%). TP=69. FP = 8. PPV = 89.6% **T18**: 20 calls for T18, confirmatory testing was done for 15samples (75%). TP=15. FP = 0. PPV = 100%

Study Information	Population	NIPS	Results
Some authors w/tech transfer agreements w/ Natera Inc., USA and Bangkok Cytogenetics Center Co. Ltd., Thailand. Neither of them was involved with analysis of data and preparation of the manuscript. Some authors received travel bursary from Bangkok Cytogenetics Ltd. And Natera Inc. to actively participate in their sponsored lecture events. The other authors declare no conflicts of interest	self-pay, gestational age >9 wks **Exclusion criteria** gestational age <9 wks, multifetal gestation, donor egg pregnancy, surrogate carrier, missing patient information or incomplete con-sent documents, sample received >6 days after collection, insufficient blood volume (<13 ml), wrong collection tube used, or if the sample was apparently damaged, non-Thai ethnicity **Participant characteristics**		**T13**: 13 calls for T13, confirmatory testing was done for 12samples (92.3%). TP=7. FP = 5. PPV = 58.3% **SCA**: 45,X: 18 calls; testing in 12 (67%); TP=8; FP=4; PPV=66.7% (42.9-84.2); FN=0 10 calls for non-45,X SCA; 100% w/testing. TP=7. FP = 3. PPV = 70% **CNV**: NR **RAT**: NR **Diagnostic Procedures**: NR **Identification of maternal conditions**: NR **Psychosocial outcomes**: NR

Study Information	Population	NIPS	Results
	Mean (SD) gestational age: 13.2 (2.1) wks Mean (SD) age: 35.0 (3.5)		
Pertile et al., 2017 **Country** United States; Australia **Timeframe** Cohort 1: October 2013 to September 2014 Cohort 2: April 2015 to August 2016 **Risk of Bias** ROBINS-I: Serious **Funding/potential COI** NR	**N** = Cohort 1: 72,932 subjects; Cohort 2: 16,885 **Inclusion criteria** gestational age at time of sampling was greater than or equal to 10 weeks; (ii) a value for the NCDQ parameter was available; (iii) blood samples had been drawn into nonexpired Streck DNA Blood Collection Tubes (BCT) and had arrived at the laboratory within the time frame required for analysis and with	**NIPS Platform** Illumina **NIPS description** WGS cfDNA w/bioinformatics algorithms to detect anomalies from all chromosomes; cohort 1 data from Illumina, cohort 2 data from Victorian Clinical Genetics Services; flagged samples if normalized chromosome denominator quality <50	**T21**: NR **T18**: NR **T13**: NR **SCA**: NR **CNV**: NR **RAT**: NIPS+ 246 of 518 flagged samples (Cohort 1: 47.5%) 60/109 flagged samples (Cohort 2: 55.0%). Of 52 single RATs with outcome data, 22 samples (42%) were associated with an early or missed miscarriage (<11 to 12 weeks of gestation). Miscarriage was reported in 13 of 14 samples (93%) with trisomy15 and in 3 of 5 samples (60%) with trisomy 22. Single cases of trisomies 9, 10, 14, and 20 and two cases of trisomy 16 were also recorded as miscarriages. Another case of trisomy 9 was associated with a co-twin demise at 9 weeks of gestation. Cytogenetic investigation on products of conception (POC) was carried out in five miscarriage samples. In each case, the RAT was confirmed by using SNP microarrays: three cases of trisomy 15 (placental villi), one case of TFM for trisomy 22 (fetal skin), and one case of non-mosaic trisomy 9 (fetal skin) in a

Study Information	Population	NIPS	Results
	sufficient volume for testing; and (iv) if multiple test samples at different gestational ages were received from the same pregnancy, only one blood sample was selected for study **Exclusion criteria** (i) a gestational age of less than 10 weeks, (ii) inadequate blood volume, and (iii) blood collected into tubes other than Streck DNA BCT **Participant characteristics** Mean (SD) age: Cohort 1, no flag 34.6 (5.4) vs flag 35.1 (5.8); Cohort		pregnancy that was terminated after multiple fetal anomalies were observed on ultrasound examination. There were 17 pregnancies involving single RATs that proceeded to amniocentesis. Normal amniocentesis results were obtained in seven pregnancies (13%) for single samples associated with trisomies 2, 7, 9, 16, and 22and for two cases of trisomy 10. These pregnancies proceeded to phenotypically normal live births, except for the case with trisomy 9, which was associated with IUGR and cleft palate at birth. **Diagnostic Procedures**: NR **Identification of maternal conditions**: NR **Psychosocial outcomes**: NR

Study Information	Population	NIPS	Results
	2, no flag 34.4 (4.3) vs flag 35.6 (4.9); ages significant w/in & between groups Mean (SD) GA: Cohort 1, no flag 13.8 (4.2) wks vs flag 13.9 (4.4) wks; Cohort 2, no flag 11.0 (1.9) wks vs flag 11.2 (2.6) wks; GA significant between groups		
Pescia et al., 2017 **Country** Switzerland **Timeframe** March 2013-May 2015 **Risk of Bias** ROBINS-I: Moderate **Funding/potential COI** Some authors are minority shareholders of Sonic Healthcare, which	**N** = 6388 **Inclusion criteria** NR **Exclusion criteria** transportation time >48h, total DNA concentrations ≤4ng/ul, and visible hemolysis (degree defined	**NIPS Platform** Illumina HighSeq 2000 **NIPS description** Shotgun sequencing	**T21**: NR **T18**: NR **T13**: NR **SCA**: NR **CNV**: NR **RAT**: 50/6388 samples RATs (0.78%); T7, n=16; associated w/UPD. The group with a high or very high risk for an unfavorable outcome if the fetus were affected comprised seven cases (14%).

Study Information	Population	NIPS	Results
owns Aurigen, Fasteris, and Genesupport	by photographic references). **Participant characteristics** Mean (SD) GA: 13.19 (2.36) wks Low-risk: 28%		T6, T7, T14, T15, and T16, were considered abnormal or likely abnormal because UPD can be symptomatic even in diploid fetuses after trisomy or monosomy rescue. All other trisomies were rated abnormal or likely abnormal based on the relative evidence for further workup. Follow-up with amnio in 19/50 (38%): 100% (3/3) for T22, 50% (2/4) for T16, and 37.5% (6/16) for T7, which included routine molecular UPD analysis in addition to karyotyping. Four fetal aneuploidies were confirmed; all three T22 mosaicism cases were fetal, as was one case of T12 mosaicism. For all remaining cases, amnio revealed normal diploid results; in the cases with potential UPD, no single fetal UPD was identified. This resulted in a nominal FPR of 0.71% and a low PPV of 8% **Diagnostic Procedures**: NR **Identification of maternal conditions**: NR **Psychosocial outcomes**: NR
Petersen et al., 2017 **Country** United States **Timeframe** April 2012 to June 2017 **Risk of Bias** ROBINS-I: Serious **Funding/potential COI**	**N** = 712 **Inclusion criteria** previous positive from Initial NIPS **Exclusion criteria** NR **Participant characteristics**	**NIPS Platform** Multiple (Ariosa Diagnostics, BGI, Natera, Sequenom, and Illumina) **NIPS description** Follow-up at Baylor Genetics	**T21** NIPS+ n=268 TP= 228., PPV =85%. FPR =15% **T18** NIPS+ n=106. TP= 82., PPV =77%. FPR =23% **T13** NIPS+ n=76. TP= 34., PPV =45%. FPR =55% **SCA**: XXY NIPS+ n=20. TP= 17, PPV =85%, FPR =15% XXX NIPS+ n=11. TP= 5., PPV =45%. FPR = 55% XYY NIPS+ n=4. TP= 4.

Study Information	Population	NIPS	Results
Six authors affiliated w/Baylor Genetics	NR		**CNV**: NR **RAT**: NIPS+ n=12 monosomies 13 and 18 and T7, T9, T14, and T16. None of the 5 NIPS screen-positive monosomy (13 and 18) cases were confirmed, and only T16 was confirmed **Diagnostic Procedures**: NR **Identification of maternal conditions**: NR **Psychosocial outcomes**: NR
Qi et al., 2019 **Country** China **Timeframe** April 2015 to November 2017 **Risk of Bias** ROBINS-I: Serious **Funding/potential COI** None	**N** = 35/31,250 **Inclusion criteria** consecutive pts w/abnormal NIPS results **Exclusion criteria** **Participant characteristics** Mean GA: 20^{+4} wks Mean age: 30.8 yrs	**NIPS Platform** JingXin **NIPS description** Reported in previous paper	**T21**: NR **T18**: NR **T13**: NR **SCA**: NR **CNV**: NR **RAT**: chr 7 aneuploidies were suspected from NIPS results in 0.11% of patients (35/31,250). In 20/20 amnios, normal result (suggests CPM) however 2 of these had a CNV involving chromosome 7. 9/10 CVS showed placental chimerism. (some patients had both amnio and CVS) **Diagnostic Procedures**: 25/35 chose invasive testing following suspected chr 7 abnormality **Identification of maternal conditions**: NR **Psychosocial outcomes:** NR
Rousseau et al., 2019 **Country** Canada	**N** = 1660 baseline risk **Inclusion criteria**	**NIPS Platform** Illumina and ThermoFisher	**T21**: ThermoFisher: TP=5, FP=3, TN=1558, FN=0; FPR=3/1561 (0.19% (0.04%-0.56%)); spec: 1558/1561 (99.8% (99%-100%))

Study Information	Population	NIPS	Results
Timeframe November 2013 – April 2016 **Risk of Bias** ROBINS-I: Moderate **Funding/potential COI** None	Age ≥19 yrs; GA 10-13^{+6} wks undergoing screening for Down syndrome **Exclusion criteria** multifetal pregnancy, twin demise (spontaneous or elective), or history of malignancy **Participant characteristics** (Baseline risk only): Mean (SD) age: 32.9 (4.5) yrs Mean (SD) GA: 12.2 (1.0) wks	**NIPS description** Illumina: Optical-based ThermoFisher: semiconductor randomly removed 329 euploid samples before testing and an additional 61 were lost to follow-up; 27 had insufficient samples or did not meet inclusion criteria	Illumina: TP=5, FP=0, TN=1581, FN=0; FPR=0/1581 (0% (0%-0.23%)); spec=1581/1581 (100% (99%-100%)) **T18**: ThermoFisher: TP=0, FP=3, TN=1563, FN=0; FPR=3/1566 (0.19% (0.04%-0.56%)); spec: 1563/1566 (99.8% (99%-100%)) Illumina: TP=0, FP=3, TN=1583, FN=0; FPR=3/1586 (0.19% (0.04%-0.55%)); spec=1583/1586 (99.8% (99%-100%)) **T13**: ThermoFisher: TP=0, FP=4, TN=1562, FN=0; FPR=4/1566 (0.26% (0.07%-0.65%)); spec: 1562/1566 (99.7% (99%-100%)) Illumina: TP=0, FP=4, TN=1582, FN=0; FPR=4/1586 (0.25% (0.07%-0.64%)); spec=1582/1586 (99.7% (99%-100%)) **SCA**: 45,X ThermoFisher: TP=1, FP=11, TN=1554, FN=0; FPR=11/1565 (0.70% (0.35%-2%)); spec: 1554/1565 (99.2% (98%-100%)) 45,X Illumina: TP=1, FP=6, TN=1579, FN=0; FPR=6/1585 (0.38% (0.14%-0.82%)); spec=1579/1585 (99.6% (99%-100%)) **CNV**: NR **RAT**: NR **Diagnostic Procedures**: NR **Identification of maternal conditions**: NR **Psychosocial outcomes**: NR

Study Information	Population	NIPS	Results
Sanchez-Duran et al., 2019 **Country** Spain **Timeframe** February 2016-March 2017 **Risk of Bias** ROBINS-I: Serious **Funding/potential COI** None	**N** = 2639 **Inclusion criteria** Singleton gestations w/a known outcome. Intermediate risk defined as 1:11 to 1:1500 by FTS or QUAD **Exclusion criteria** vanishing twin pregnancy and unknown karyotype or unknown neonatal phenotype **Participant characteristics** Median (IQR) age: 32.1 (28.1-36.0) yrs Median (IQR) GA: 13.2 (12-5-14.1) wks	**NIPS Platform** Ariosa Diagnostics **NIPS description** NR	**T21**: TP=3; FP=0 **T18**: TP=0; FP=0 **T13**: TP=0; FP=0 **SCA**: NR **CNV**: NR **RAT**: NR **Diagnostic Procedures**: NR **Identification of maternal conditions:** NR **Psychosocial outcomes**: NR Results of survey about testing preferences: results showed that 374 (81.8%) women would have preferred cfDNA testing as the second line contingent test, 80 (17.5%) would have preferred an invasive procedure, and 3 (0.7%) women not doing anything
Sandow et al., 2020	**N** = 47219	**NIPS Platform**	**T21:** NR

Study Information	Population	NIPS	Results
Country Australia **Timeframe** March 2013 to December 2018 **Risk of Bias** ROBINS-I: Moderate **Funding/potential COI** None	**Inclusion criteria** NIPS as 1st or 2nd tier screening; confirmed GA ≥10 wks **Exclusion criteria** Multifetal gestations **Participant characteristics** Mean (SD) age: 36.4 (4.6) yrs Mean (SD) BMI: 24.2 (3.5) Median (IQR) GA: 11.0 (10.4-11.7)	Multiple **NIPS description** Mix of whole-genome, targeted, and CMA-based platforms; FF assessed using two different methods Mean (SD) FF: 9.4 (4.2)%	**T18:** NR **T13:** NR **SCA**: NIPS+ n=107; 9 declined testing, 2 lost to follow-up TP=25; FP=71; PPV<30% **CNV:** NR **RAT:** NR **Diagnostic Procedures:** NR **Identification of maternal conditions:** NR **Psychosocial outcomes:** NR
Schwartz et al., 2018 **Country** United States **Timeframe** 2014-2016 **Risk of Bias** ROBINS-I: Serious	**N** = 349 **Inclusion criteria** screened positive by NIPS for a CNV involving 1p, 4p,5p, 15q, or 22q; underwent	**NIPS Platform** Multiple **NIPS description** NR	**T21:** NR **T18:** NR **T13:** NR **SCA:** NR **CNV**: PPV=9.2%; when a CNV was confirmed, 39.3% of samples had additional abnormal CMA findings; unrelated abnormal

Study Information	Population	NIPS	Results
Funding/potential COI Multiple authors work for testing companies or are members of advisory boards/speakers/receive honorarium and/or research support	diagnostic studies by CVS or amnio **Exclusion criteria** NR **Participant characteristics** NR		CMA findings in 11.8% of pts w/an unconfirmed CNV; stretches of homozygosity associated w/FP NIPS result **RAT:** NR **Diagnostic Procedures:** NR **Identification of maternal conditions:** NR **Psychosocial outcomes:** NR
Scott et al., 2018 **Country** Australia **Timeframe** March 2015 to August 2017 **Risk of Bias** ROBINS-I: Moderate **Funding/potential COI** NR	**N** = 23,388 **Inclusion criteria** Singleton gestation, no obvious anomalies, ≥10 wks GA **Exclusion criteria** GA <10 wks, insufficient sample volume, >5 days between sample collection & lab receipt, detection of fetal abnormality **Participant characteristics**	**NIPS Platform** In-house; sequencing on Illumina NextSeq **NIPS description** SAFeR algorithms calculating normalized chromosome values (NCVs) for chromosomes 13, 18, 21, X and Y. Chromosome coverage value (CCV) analysis is also calculated for the 22 autosomes	**T21:** NR **T18:** NR **T13:** NR **SCA:** NR **CNV:** NR **RAT**: 28 RAT cases identified: T2, n=1; T3, n=1; T4, n=3; T5, n=1; T7, n=6; T8, n=2; T9, n=1; T10, n=1; T14, n=2; T15, n=2; T16, n=4; T20, n=1; T22, n=3. Of the 28RAT cases, six miscarried, half due to anomalies in chromosome 22 (all three trisomy, 22 cases). Two cases had true fetal mosaicism (TFM) confirmed on amniocentesis, of which one also had structural anomalies and the other had both trisomy and UPD 15 on amniocentesis but no structural anomalies seen on ultrasound. One case with mosaic trisomy 10 on CVS and structural abnormalities seen on ultrasound had a likely, but unproven, fetal mosaicism. Termination of pregnancy occurred in four cases (the two TFM cases, the trisomy 10 case, and one trisomy 7 case, which had structural abnormalities despite a normal amniocentesis).

Study Information	Population	NIPS	Results
	Mean (range) age: 35.5 (27-43) yrs 93% samples collected in 1st-trimester		**Diagnostic Procedures:** NR **Identification of maternal conditions:** NR **Psychosocial outcomes:** NR
Serapinas et al., 2020 **Country** Lithuania **Timeframe** 2014-2019 **Risk of Bias** ROBINS-I: Moderate **Funding/potential COI** None	**N** = 862 cases; after excluding, n=850 [low risk n=808, high risk n=15, no call n=27] **Inclusion criteria** singleton pregnancy and in three groups: (1) aged ≥35 yrs; (2) with a high risk identified after the FTS (3) no increased risk **Exclusion criteria** multifetal gestation; GA ≥21 wks	**NIPS Platform** Natera Panorama **NIPS description** SNP-based, NATUS algorithm analysis	**T21**: n=15; confirmatory test, n=13; FP=0, PPV=100% **T18**: n=10; confirmatory test n=9; FP=0, PPV=100% **T13**: NR **SCA**: 45,X+ n=1 (confirmed); FP=0, PPV=100% XYY+ n=1 (confirmed); FP=0, PPV=100% **CNV**: NR **RAT**: NR **Diagnostic Procedures:** NR **Identification of maternal conditions:** NR **Psychosocial outcomes:** NR

Study Information	Population	NIPS	Results
	Participant characteristics Group 1: mean (range) age 37.7 (35-49) yrs Group 2: mean (range) age 34.1 (23-42) yrs Group 3: mean (range) age 28.5 (19-32) yrs Median (range) GA: all: 11 (9-21) wks		
Snyder et al., 2016 **Country** United States **Timeframe** February 2012-August 2014 **Risk of Bias** ROBINS-I: Serious **Funding/potential COI** Authors are employees of commercial lab	**N** = 138; 67 had neonatal karyotypes available **Inclusion criteria** subset of singleton samples w/NIPS results of single autosomal monosomy or multiple aneuploidies. Included six previously	**NIPS Platform** Illumina 124erify **NIPS description** Whole-genome	**Combined results**: single autosomal monosomy, n=65; single autosomal trisomy w/SCA, n=36; multiple aneuploidies, n=37 79 cases w/clinical outcomes: M13/M18/M21: 1 partially concordant result, 3 discordant results Single trisomy + SCA: T13+SCA/T18+SCA/T21+SCA: 1 fully concordant, 8 partially concordant, 2 discordant Multiple aneuploidies: 3 fully concordant, 13 partially concordant, 42 discordant **Diagnostic Procedures**: NR

Study Information	Population	NIPS	Results
	published cases originating from pregnancies w/an occult maternal malignancy **Exclusion criteria** NR **Participant characteristics** NR		**Identification of maternal conditions**: 6/79 cases malignancy **Psychosocial outcomes**: NR
Srebniak et al., 2020 **Country** The Netherlands **Timeframe** 2009-2018 **Risk of Bias** ROBINS-I: Serious **Funding/potential COI** None	**N** = 8608 **Inclusion criteria** pregnancies w/out fetal ultrasound anomalies at the time of sampling, that were referred for diagnostic CMA due to AMA, abnormal ftCT (with NT <3.5 mm), recurrence risk for chromosome aberrations or	**NIPS Platform** NR **NIPS description** NR	**T21/T18/T13:** substantial increase in diagnostic yield over time **SCA**: NR **CNV**: NR **RAT**: NR **Diagnostic Procedures**: substantial decrease of the number of diagnostic tests in pts w/out fetal US anomalies: 2009: n=1176 (AMA or abnormal ftCT), 2015: n=846 (no AMA needed, NIPS as 2nd tier) 2018: n=363 (NIPS as 1st tier) **Identification of maternal conditions:** NR **Psychosocial outcomes:** NR

Study Information	Population	NIPS	Results
	abnormal NIPS results **Exclusion criteria** Fetuses tested due to the presence of a chromosome aberration in one of the parents were excluded from the analysis, as the results were dependent on chromosome segregation and type of aberration and not on a selection based on screening or US; samples detected elsewhere but confirmed by lab **Participant characteristics** NR		
Tekesin et al., 2021	**N** = 81 w/NIPS+; 73	**NIPS Platform**	**T21:** NIPT+ n=40; confirmed + n= 38. Confirmed neg. n= 2. PPV = 95% (83.1-99.4%); FPR=5.0% (0.1-16.9%)

Study Information	Population	NIPS	Results
Country Germany **Timeframe** 09/2013 to 12/2019 **Risk of Bias** ROBINS-I: Serious **Funding/potential COI** None	w/confirmatory testing **Inclusion criteria** NIPS+ results for autosomal aneuploidies (T21, T18, T13), SCAs (X0, XXX, XXY, XYY) or a 22q11.2 microdeletion (DiGeorge syndrome) **Exclusion criteria** No confirmatory testing **Participant characteristics** Median (range) age: 37 (22-44) yrs Median (range) GA: 13.6 (11.6-26.6) wks	Harmony (Roche); PrenaTest (Eurofins Lifecodexx AG, Germany); PreviaTest (Eluthia GmbH, Germany) **NIPS description** NR	**T18:** NIPT+ n=9 confirmed + n= 5. Confirmed neg. n= 4. PPV = 55.6% (21.2-86.3%); FPR = 44.4% (13.7-78.8%) **T13:** NIPT+ n= 7 confirmed + n= 2. Confirmed neg. n= 5. PPV = 28.6% (3.7-71.0%); FPR=76.9 % (46.2-95.0%) **SCA:** Overall: NIPT+ n=13; confirmed + n=3, confirmed neg. n=10; PPV=23.1% (5.5-57.2); FPR=76.9% (46.2-95.0%) X0: NIPT+ n=5; confirmed + n=1; dx neg n=4; PPV=20%; FPR=80% XXX: NIPT+ n=5; confirmed + n=1; dx neg n=4; PPV=20%; FPR=80% XXY: NIPT+ n=1; confirmed + n=1; PPV=100% **CNV:** DiGeorge syndrome: NIPT+ n=5; dx neg n=5; FPR=100% (7.8-100%) **RAT:** NR **Diagnostic Procedures:** NR **Identification of maternal conditions:** NR **Psychosocial outcomes:** NR
Van Den Bogaert et al., 2021	**N** = 183621	**NIPS platform** VeriSeq NIPT v2 or	**T21** (n=494): unconfirmed, n=100; sens 98.91% (95% CI 97.24-99.58); spec, 99.98% (95% CI 99.97-99.99); PPV,

Study Information	Population	NIPS	Results
Country Belgium **Timeframe** July 2017-June 2019 **Risk of Bias** ROBINS-I: Moderate **Funding/Potential COI**: none	**Inclusion criteria** NR **Exclusion criteria** higher-order (e.g., triplets) pregnancies **Participant characteristics** NR	other Illumina sequencer; Ion Proton system **NIPS description** Next-generation sequencing was performed with either the Ion Proton system (ThermoFisher scientific) or the VeriSeq NIPT v2 solution, HiSeq1500, HiSeq2500, HiSeq3000, HiSeq4000, Novaseq6000, NextSeq500 or NextSeq550 sequencer (Illumina). Genome-wide genomic representation profiling and interpretation was performed using the VeriSeq NIPT Assay Control Software	92.39% (95% CI 89.34-94.61); NPV, 100% (95% CI 99.99-100.00); 3/5 FPs were confirmed CPM; FN, n=4 **T18** (n=115): unconfirmed, n=24; sens 97.47% (95% CI 91.23-99.30); spec, 99.99% (95% CI 99.98-99.99); PPV, 84.62% (95% CI 75.82-90.61); NPV, 100% (95% CI 100.00-100.00); of 1/3 FPs were confirmed CPM; FN, n=2 **T13** (n=91): unconfirmed, n=9; sens 100.00% (95% CI 90.36-100.00); spec, 99.97% (95% CI 99.96-99.98); PPV, 43.90% (95% CI 33.67-54.68); NPV, 100% (95% CI 100.00-100.00); 8/16 FPs were CPM **SCA** NR **CNV** (n=109) NIPS suggested possible fetal segmental imbalance; unconfirmed, n=17 **RAT** (n=339; rare autosomal monosomy, n=11): unconfirmed RAT, n=73; confirmed: n=11 (Trisomy 2, n=1; Trisomy 8, n=3; Trisomy 9, n=1; Trisomy 16, n=4; Trisomy 22, n=2); 28/51 FPs were confirmed CPM Unconfirmed rare autosomal monosomy, n=11 UPD testing (n=64 pregnancies): confirmed, n=3 (trisomy 7, n=1; trisomy 15, n=2) **Diagnostic procedures:** 2013, n=6,279; 2018, n=3,047; normalized to number of live births represents a 52% reduction in invasive tests; reduction in number of diagnostic tests is larger than the incidence of trisomy 21 **Identification of maternal conditions** reported maternal imbalances: 0.32%; maternal cancers: 0.008%

Study Information	Population	NIPS	Results
		v2.0.0 (Illumina) or as previously described.	**Psychosocial outcomes** NR **Other**: incidence of trisomy 21 live births: 2014, 77 births; 2018, 52 births
Van der Meij et al., 2019 **Country** The Netherlands **Timeframe** April 1, 2017 to April 1, 2018 **Risk of Bias** ROBINS-I: Moderate **Funding/potential COI** None	**N** = 73239 **Inclusion criteria** Pts who elected to have NIPS performed as a 1st-tier test; have a Dutch social security number and Dutch health insurance and needed to be able to provide informed consent **Exclusion criteria** pregnancies w/a vanishing or dichorionic twin, fetal US anomalies incl. a NT of ≥3.5 mm, or GA <11^{+0} wks. Pts < 18 yrs or couples known to	**NIPS Platform** Multiple sites; NR **NIPS description** performed with either the Illumina HiSeq4000 or the NextSeq500 sequencer (Illumina); test failure: n=1127; 1020 were repeated, 86% resulted in conclusive result	**T21**+ (n=239): IUFD n=14; TOP n=2; cases w/confirmatory dx testing TP=214 FP=9; FN=5; sens=98% (95-99%); PPV=96% (93-98%) **T18**+ (n=49): IUFD n=0; TOP n=0; cases w/confirmatory dx testing TP=48 FP=1 FN=5; sens=91% (79-97%); PPV=98% (87-100%) **T13**+ (n=55): IUFD n=3; TOP n=1; cases w/confirmatory dx testing TP=27 FP=24; FN=0; sens=100% (87-100%); PPV=53% (43-63%) **SCA**: NR **CNV**: NR **RAT**s+ (n=101): IUFD n=0; TOP n=1; cases w/confirmatory dx testing TP=6; FP=91; PPV=6% **Diagnostic Procedures:** NR **Identification of maternal conditions:** NR **Psychosocial outcomes: NR**

Study Information	Population	NIPS	Results
	carry a(balanced) chromosomal abnormality; pts w/a current malignancy; who, in the past three months, had received blood transfusions, stem cell therapy, or immunotherapy to treat a malignancy; or who had an organ trans-plantation; at high risk for the common trisomies, based on FCTR 1/200 or medical history, but not on AMA alone, were enrolled in the TRIDENT-1 study and excluded from this paper		

Study Information	Population	NIPS	Results
	Participant characteristics Mean (range) age: 31.7 (18-52) yrs Mean (range) GA: 11.9 (11-41) wks		
Wan et al., 2018 **Country** China **Timeframe** February 2015 to January 2018 **Risk of Bias** ROBINS-I: Moderate **Funding/potential COI** None	**N** = 15362 **Inclusion criteria** NR **Exclusion criteria** NR; pretest US to determine fetal number, GA, and to exclude major structural abnormalities **Participant characteristics** Mean (range) age: 33 (19-45) yrs Mean (range) GA: 15 (12-24) wks	**NIPS Platform** In-house **NIPS description** Whole genome sequencing by Ion Proton semiconductor (Life Technologies)	**T21:** NR **T18:** NR **T13:** NR **SCA:** NR **CNV:** NR **RAT:** screening positive rate for RAT is 0.38% (59/15362). Invasive prenatal diagnosis was performed in 61% (36/59) of the cases. A majority of the RATs detected by NIPS (94.9%, 56/59) were false positive, probably resulting from CPM **Diagnostic Procedures:** NR **Identification of maternal conditions:** NR **Psychosocial outcomes:** NR
Wu et al., 2020	**N** = 551	**NIPS Platform**	**T21:** NIPT+ n=150, TP=122, PPV=81.3%

Study Information	Population	NIPS	Results
Country China **Timeframe** May 2015 to December 2019 **Risk of Bias** ROBINS-I: Moderate **Funding/potential COI** None	**Inclusion criteria** Pts w/NIPS+ results **Exclusion criteria** Twin gestations **Participant characteristics** Mean (SD) GA: 16.6 (2.9) wks AMA, 41.0% No indications, 39.6%	Multiple (NextSeq CN500; NextSeq AR550; BGI Seq500; Ion Proton) **NIPS description** NR; most cases used NextSeq CN500	T21 YMA/no indication: NIPT+ n=32, TP=23, PPV=71.9% **T18:** NIPT+ n=52, TP=18, PPV=34.6% T18 YMA/no indication: NIPT+ n=17, TP=0, PPV=0% **T13:** NIPT+ n=36, TP=9, PPV=25% T13 YMA/no indication: NIPT+ n=12, TP=2, PPV=16.7% **SCA:** NIPT+ n=258, TP=97, PPV=37.6% SCA YMA/no indication: NIPT+ n=122, TP=49, PPV=40.2% **CNV:** NR **RAT:** NR **Diagnostic Procedures:** NR **Identification of maternal conditions:** NR **Psychosocial outcomes:** NR
Xu et al., 2020 **Country** China **Timeframe** June 2012 to May 2017 **Risk of Bias** ROBINS-I: Serious **Funding/potential COI** None	**N** = 31515 **Inclusion criteria** Pts undergoing NIPS **Exclusion criteria** Twin gestations **Participant characteristics**	**NIPS Platform** NextSeq CN500 sequencer (Berry Genomics Corporation, Beijing, China); **NIPS description** Whole-genome; analysis w/Bambni system	**T21:** Detection rate (low-risk only): 17/6093 (0.28%) Overall: TP=95; FP=18; FPR=0.06%; TN=31274; FN=1; FNR=1.04%; sens=98.96%; spec=99.94%; PPV-84.07%; NPV=99.997% **T18:** Detection rate (low-risk only): 8/6093 (0.13%) Overall: TP=25; FP=11; FPR=0.03%; TN=31352; FN=0; FNR=0%; sens=100%; spec=99.96%; PPV=69.44%; NPV=100% **T13:** Detection rate (low-risk only): 3/6093 (0.05%) Overall: TP=7; FP=8; FPR=0.03%; TN=31373; FN=0; FNR=0; sens=100%; spec=99.97%; PPV=46.67%; NPV=100%

Study Information	Population	NIPS	Results
			SCA: Detection rate (low-risk only): 44/6093 (0.72%) Overall: TP=61; FP=82; FPR=0.26%; TN=31245; FN=0; FNR=0; sens=100%; spec=99.74%; PPV=42.66%; NPV=100% 47,XXX: TP=15; FP=8; FPR=0.03%; TN=31365; FN=0; FNR=0; sens=100%; spec=99.97%; PPV=65.22%; NPV=100% 47,XXY: TP=15; FP=5; FPR=0.02%; TN=31368; FN=0; FNR=0; sens=100%; spec=99.98%; PPV=75%; NPV=100% 45,X: TP=20; FP=57; FPR=0.18%; TN=31311; FN=0; FNR=0; sens=100%; spec=99.82%; PPV=25.97%; NPV=100% 47,XYY: TP=10; FP=2; FPR=0.01%; TN=31376; FN=0; FNR=0; sens=100%; spec=99.99%; PPV=83.33%; NPV=100% 46,XY(delX): TP=1; FP=10; FPR=0.03%; TN=31377; FN=0; FNR=0; sens=100%; spec=99.97%; PPV=9.09%; NPV=100% **CNV:** NR **RAT:** NR **Diagnostic Procedures:** NR **Identification of maternal conditions:** NR **Psychosocial outcomes:** NR
Yang et al., 2021 **Country** China	**N** = 47800 **Inclusion criteria**	**NIPS Platform** In-house **NIPS description**	**T21:** NR **T18:** NR **T13:** NR

Study Information	Population	NIPS	Results
Timeframe January 2015 to September2019 **Risk of Bias** ROBINS-I: Moderate **Funding/potential COI** None	aged 18-45 yrs and (ii) GA >12 wks. GA determined by US. Twins were included. **Exclusion criteria** NR **Participant characteristics** GA at (groups), 12-24 wks, 80.8% Age (groups), <35 yrs, 88.10%	JingXin BioelectronSeq 4000 System semi-conductor FF: 13.11% (CI: 5.53-17.70)	**SCA:** 238 cases + for SCA, 170 underwent PNDx, 64 declined. 85 true positives. no false negatives in the 47, 562 delivered by newborn screening 9 cases identified in 1530 twins, and 6 had PNDx **CNV:** NR **RAT:** NR **Diagnostic Procedures:** NR **Identification of maternal conditions:** identified a mos 45,X[85]/47,XXX **Psychosocial outcomes:** NR
Yao et al., 2019 **Country** China **Timeframe** May 2011 to December 2014 **Risk of Bias** ROBINS-I: Moderate **Funding/potential COI** None	**N** = 15626 **Inclusion criteria** ≥18 yrs old; GA >10 wks; willing to undergo NIPS for 1st- or 2nd-tier screening **Exclusion criteria** unclear clinical information or	**NIPS Platform** Illumina **NIPS description** Analyzed by BGI-Shenzhen 175 (1.12%) blood samples had to be re-sampled, and 10 (0.06%) samples failed to generate informative results	**Combined T21/T18/T13:** TP=68; FP=18; TN=13651; FN=0; FPR=0.13% (0.08%-0.21%); PPV=79.07% (68.69%-86.80%); incidence=0.50% PPV dropped from 79.07% reporting just T21/T18/T13 with each additional category reported (SCA, CNV, other) **SCA:** (overall): TP=26; FP=16; TN=13651; FN=1; FPR=0.12% (0.07%-0.19%); PPV=61.90% (45.65%-76.01%); incidence=0.20% 45,X: TP=4; FP=10; TN=13651; FN=0; FPR=0.07% (0.04%-0.14%); PPV=28.57% (9.58%-58.00%); incidence=0.03%

Study Information	Population	NIPS	Results
	known maternal aneuploidy **Participant characteristics** Mean (range): 29.99 (18-50) Mean (range) GA: 18.65 (10-36) wks		47,XXX: TP=11; FP=2; TN=13651; FN=0; FPR=0.01% (0.00%-0.06%); PPV=84.62% (53.66%-97.29%); incidence=0.08% 47,XXY:TP=9; FP=3; TN=13651; FN=1; FPR=0.02% (0.01%-0.07%); PPV=75.00% (42.84%-93.31%); incidence=0.07% 47,XYY: TP=2; FP=1; TN=13651; FN=0; FPR=0.01% (0.00%-0.05%); PPV=66.67% (12.53%-98.23%); incidence=0.01% **CNV:** TP=4; FP=3; TN=13651; FN=0; FPR=0.03% (0.01%-0.07%); PPV=57.14% (20.24%-88.19%); incidence=0.03% **RAT:** NR **Diagnostic Procedures:** NR **Identification of maternal conditions:** NR **Psychosocial outcomes:** NR
Ye et al., 2021 **Country** China **Timeframe** NR **Risk of Bias** ROBINS-I: Moderate **Funding/potential COI** None	**N** = 873 **Inclusion criteria** NR **Exclusion criteria** NR **Participant characteristics** Median (range) age: 28.16 (21-45) yrs	**NIPS Platform** In-house; BGISEQ-500 (MGI, China) **NIPS description** Previously reported	**T21:** NR **T18:** NR **T13:** NR **SCA:** NR **CNV:** Total abnormal = 52. 34 TP. 18 FP. Sensitivity 65.38%. Specificity 97.45% CNV >=2Mb = 38. 31 TP. 7 FP. Sensitivity 81.58%. Specificity 98.18% CNV >=2Mb = 14. 3 TP. 11 FP. Sensitivity 21.43%. Specificity 99.27%

Study Information	Population	NIPS	Results
	Median (range) GA: 17.29 (11-24) wks		**RAT:** NR **Diagnostic Procedures:** NR **Identification of maternal conditions:** NR **Psychosocial outcomes:** NR
Yin et al., 2020 **Country** China **Timeframe** December 2017 to June 2019 **Risk of Bias** ROBINS-I: Serious **Funding/potential COI** None	**N** = 6239 **Inclusion criteria** singleton gestations, natural conceptions, consents were signed, and US performed prior to the blood draw for GA and NT **Exclusion criteria** NR **Participant characteristics** Groups: Age, % (yrs) 18-25, 15.6% 26-35, 64.0% 36-44, 20.0% >44, 0.4%	**NIPS Platform** FlexiGene **NIPS description** Ion Proton Sequencing System (Life Technologies)	**T21:** NR **T18:** NR **T13:** NR **SCA:** NIPT+ n=17; confirmed by amnio, n=11; TP=64.7% Among the 6 cases with inconsistent results, 5 were 45,X and 1 was an 47,XYY. In the SCA cases, 1 case of serological screening showed a high risk of T21; 3 cases of serological screening showed abnormal MoM; 1 case of serological screening showed a low risk; and 4 cases were AMA. **CNV:** NIPT+ n=16; confirmed by amnio, n=9; TP=56% 2 cases were T18 NIPT dup + but n=1 confirmed All abnormal microdeletion/microduplications were de novo. **RAT:** NR **Diagnostic Procedures:** NR **Identification of maternal conditions:** NR **Psychosocial outcomes:** NR

Study Information	Population	NIPS	Results
	GA, % (wks) 12-13^{+6}, 32.9% 14-15^{+6}, 50.6% 16-20^{+6}, 14.9% >21, 1.6% High risk, 10.6% Low risk, 20% NIPS 1st tier, 63.5%		
D. Yu et al., 2019 **Country** China **Timeframe** 30 July 2015 and 30 June 2016 **Risk of Bias** ROBINS-I: Moderate **Funding/potential COI** None	**N** = 20,232 **Inclusion criteria** NR **Exclusion criteria** No confirmatory amnio; loss of contact **Participant characteristics** Mean (SD) age: 32.2 (5.3) yrs Mean (SD) GA: 18.2 (2.8) wks	**NIPS Platform** In-house; sequenced on NextSeq 550AR (Annoroad Gene Technology, China) **NIPS description** MagMAX cfDNA isolation kit; 229 samples removed from analysis (positive for RATS, monosomies other than 21/18/13; CNVs w/unknown clinical significance	**T21:** T21: TP=103; FP=21; FN=0; TN=19879; sens=100%; spec=99.89% **T18:** T18: TP=15; FP = 4; FN = 0; TN = 19984; sens=100%; spec=99.98% **T13:** T13: TP=2; FP=3; FN=0; TN=19998; sens=100%; spec=99.99% **SCA:** NR **CNV:** w/confirmed results by invasive testing: TP = 29; FP = 7; FN = 7; sensitivity = 80.56%; PPV= 80.56%; FNR = 19.44% **RAT:** NR **Diagnostic Procedures:** NR **Identification of maternal conditions:** NR **Psychosocial outcomes:** NR

Study Information	Population	NIPS	Results
W. Yu et al., 2019 **Country** China **Timeframe** 1 October 2015 to 1 August 2017. **Risk of Bias** ROBINS-I: Moderate **Funding/potential COI** NR	**N** = 1160 twin pregnancies **Inclusion criteria** (1) twin pregnancies between 1 October 2015, and 1 August 2017; (2) age >18 years old; (3) US-confirmed; (4) voluntary NIPS for fetal T21, T18, T13, and SCAs, w/ or w/out prior serum screening result; (5) GA ≥8 wks; (6) absence of chr abnormalities phenotypically in either parent (7) no receiving of foreign blood transfusion, transplant surgery, cell therapy, or immunotherapy	**NIPS Platform** In-house; Ion torrent sequencing **NIPS description** NR	**T21:** NR **T18:** NR **Overall:** Aneuploidy was detected in 26 fetuses using NIPT, yielding an aneuploidy rate of 1.1% (26/2320) Sens: 100%, spec: 100%; FPR=0% **Diagnostic Procedures:** NR **Identification of maternal conditions:** NR **Psychosocial outcomes:** NR

Study Information	Population	NIPS	Results
	w/in 1 yr of the pregnancy. **Exclusion criteria** NR **Participant characteristics** Median (range) age: 31 (20-54) yrs AMA, 25% Median (range) GA: 18 (8-31) DCDA, 73.2% MCDA, 25.3% MCMA, 1.2% Unknown, 0.3%		
Zheng et al., 2020 **Country** China **Timeframe** January 2015 to December 2017 **Risk of Bias**	**N** = 13149 NIPS. Voluntary (general risk) N=4675 **Inclusion criteria** Based on national (China) criteria for clinical	**NIPS Platform** In-house **NIPS description** Sequenced by Berry Genomics 28 samples (0.2%) failed QC.	**T21:** 5/4675; 4/4 verified by invasive dx **T18:** n=5; 3 TP/5 invasive **T13:** n=1 TP0/1 invasive **SCA:** n=38, TP8/25 invasive; PPV = 32% (8/25) **CNV:** n=3, TP0/3 invasive

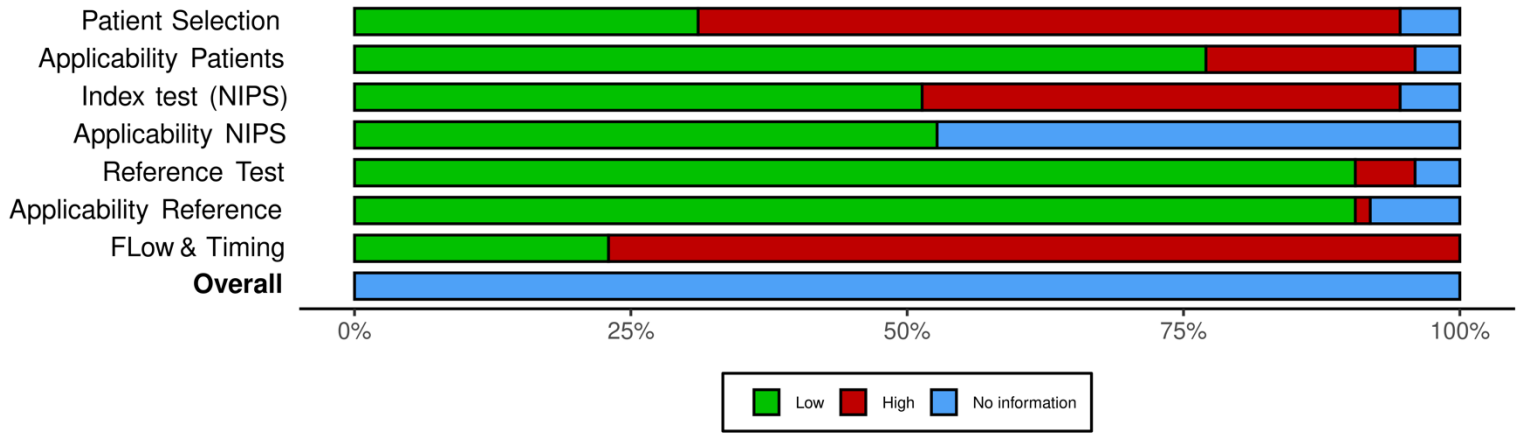
Study Information	Population	NIPS	Results
ROBINS-I: Moderate **Funding/potential COI** None	application of NIPS; GA ≥12 wks **Exclusion criteria** (1) GA < 12 wks; (2) parents w/ definite chrabnormalities; (3) w/in 1 year, receipt of allogeneic blood transfusion, transplantation, allogeneic cell therapy, etc.; (4) fetal US indicated structural abnormalities; (5) having a family history of genetic disease or suggesting a high risk of genetic disease in the fetus; (6) pregnancy with malignant tumor; and (7) other circumstances that the doctor		**RAT:** n=3, TP0/3 invasive **Diagnostic Procedures:** NR **Identification of maternal conditions:** NR **Psychosocial outcomes:** NR

Study Information	Population	NIPS	Results
	thinks have a significant impact on the accuracy of the results. **Participant characteristics** Mean (range) age: 28 (17-48) yrs Mean (range) GA: 17^{+2} (12-29) wks		
Zhou et al., 2017 **Country** China **Timeframe** January 2015 to April 2016 **Risk of Bias** ROBINS-I: Moderate **Funding/potential COI** Multiple authors employed by Berry Genomics	**N** = 112021; 74 w/FP NIPS **Inclusion criteria** Subset of patients with known FP NIPS results confirmed by amnio **Exclusion criteria** NR **Participant characteristics** NR	**NIPS Platform** NR; assumed Berry Genomics **NIPS description** massively parallel sequencing on the NextSeq CN500 platform	**T21:** NR **T18:** NR **T13:** NR **SCA:** NR **CNV:** NR **RAT:** NR **Diagnostic Procedures:** NR **Identification of maternal conditions:** In 6 out of the 74 false positive cases (8.1%), a maternal chromosome CNV was identified. Interrogation of the six CNVs against databases of known genetic variants found no association with known chromosome disease syndromes **Psychosocial outcomes:** NR

Study Information	Population	NIPS	Results
Zhou et al., 2019 **Country** China **Timeframe** January 2012 to December 2017 **Risk of Bias** ROBINS-I: Moderate **Funding/potential COI** None	**N** = 17894; 228 w/NIPS+ results **Inclusion criteria** Pts w/ NIPS+ results **Exclusion criteria** NR **Participant characteristics** GA between 13-27 wks, 100% Age, range: 16-49 yrs; AMA, 33.77% Twins, 3.07%	**NIPS Platform** Illumina Next CN 500 **NIPS description** NR	**Overall:** 91 as T21, 28 as T18, 6 as T13; 95 for fetal sex chromosome aneuploidies (56 as Turner syndrome, 21 as Klinefelter syndrome, 12 as XXX syndrome, 6 as XYY syndrome), and 8 for microdeletion or microduplication involving multiple autosomes or sex chromosomes. Dx verified by additional testing (incl. ultrasound?) & follow-up; out of 174 pts who 'accepted dx'(?), 124 as TP, 50 as FP, NIPT PPV=71.3% **Diagnostic Procedures:** NR **Identification of maternal conditions:** NR **Psychosocial outcomes:** Following prenatal genetic counseling for NIPS+ results, 174 pts (76.3%) accepted the prenatal diagnosis, and 54 pts (23.7%) rejected the diagnosis for various reasons, such as severe ultrasound abnormalities, worry about abortion, etc.
Zhu et al., 2021 **Country** China **Timeframe** cohort 1: between 2015 and 2018; cohort 2: 2018 to 2019 **Risk of Bias** ROBINS-I: Moderate	**N** = cohort 1 = 39134; cohort 2 = 31307 **Inclusion criteria** Singleton pregnancies **Exclusion criteria** NR	**NIPS Platform** In-house **NIPS description** Sequenced on Illumina NextSeq500	**T21:** NR **T18:** NR **T13:** NR **SCA:** NR **CNV:** Cohort 1 total: 39134. T7 = 23 = 0.059%. Diagnostic done on 14. TP = 1/14 (mosaic). Cohort 2 total: 31307. T7 = 16 = 0.051% Diagnostic done on 14. TP = 0/14. **Authors use only Cohort 1 to calculate PPV (7.1%)**

Study Information	Population	NIPS	Results
Funding/potential COI Two authors employees of Xcelom which provides NIPS in Hong Kong and Macau.	**Participant characteristics** Median GA: cohort 1, 14.3 wks; cohort 2, 18.4 wks Median age: cohort 1, 32 yrs; cohort 2, 30 yrs		**RAT:** NR **Diagnostic Procedures:** NR **Identification of maternal conditions:** NR **Psychosocial outcomes:** NR
Abbreviations: CNV, copy-number variant; COI, conflict of interest; FF, fetal fraction; FN, false negative; FP, false positive; mo(s), month(s); NR, not reported; RAT, rare autosomal trisomy; SCA, sex chromosome aneuploidy; T21, trisomy 21; T18, trisomy 18; T13, trisomy 13; TN, true negative; TP, true positive; wk(s), week(s); yr(s), year(s)			

Supplemental Figure 7: Summary Risk of Bias using QUADAS-2



Supplemental Table 24: Summary of all included economic analyses of NIPS

Author, Year, Country	Study Characteristics	Population Characteristics	Analysis Parameters	Results	
				Outcomes	Interpretation/Limitations
Avram et al., 2021 **Country:** United States **Setting**: general population screening **Funding**: one author supported by NIH grant and research funds from Fetal Health Foundation for unrelated research **Conflicts of interest**: NR	**Study objective:** Investigate the costs and outcomes associated with NIPS with and without screening for microdeletions **Perspective**: Societal **Currency, year:** USD, 2019 **Time Horizon**: duration of pregnancy **Discount rate:** 3%/year (maternal lifespan only)	**Source**: theoretical cohort **N** = 4,000,000 pregnant individuals undergoing prenatal genetic screening in the US **Risk**: NR **Age**: NR	**Intervention (I):** NIPS (T21/T18/T13 and 5 pathogenic microdeletion syndromes: 22q11.2, Prader-Willi, Angelman, Cri-du-chat, 1p36 deletion syndrome) **Comparator(s) (C):** NIPS (T21/T18/T13) plus ultrasound **Source of data inputs:** published literature; large population studies **Model:** decision-analysis **Sensitivity analyses**: univariate on probabilities, costs, utilities; incremental costs for microdeletion reporting; specificity; elective termination rates; multivariate sensitivity analyses to evaluate robustness w/10,000 trials in Monte Carlo analysis to simultaneously vary probabilities, costs, & utilities	**Cases identified (n)**: I: 252 C: 335 **Amnio-related losses**: I: 152 C: 4 **Terminations**: I: 805 C: 450 **Spontaneous abortions in 2nd/3rd trimester**: I: 20,327 C: 20,527 **Neonatal deaths**: I: 8,783 C: 8,858 **Cost**: I: $9,207,462,943 C: $9,298,454,727 **Maternal QALYs:** I: 107,950,761	NIPS + microdeletion improved effectiveness by 977 QALYs & decreased cost by $90.9 million vs. NIPS for aneuploidies alone Largest driver of results is the incremental costs of reporting microdeletions in addition to aneuploidies; at cost-effectiveness threshold of $100,000/QALY, intervention is cost-effective until an incremental cost exceeds $47.10. Intervention cost-effective in 92.8% of trials **Limitations:** Lack of some data necessitated the use of comparator syndromes for data for the models; no non-NIPS comparator

Author, Year, Country	Study Characteristics	Population Characteristics	Analysis Parameters	Results	
				Outcomes	**Interpretation/Limitations**
			Measure of effectiveness: Synthesis-based; ICER of $100,000/QALY or less **Outcomes:** clinical outcomes (number of affected cases, pregnancy loss, termination, neonatal death); total costs; maternal QALYs	C: 107,949,784 **ICER:** I: dominant C: dominated	
Burrus et al., 2021 **Country**: United States **Setting:** Austere environment (Cuba/US Military Hospital) **Funding:** None **Conflicts of interest:** None	**Study objective:** Compare direct and indirect costs of conventional serum screening compared to NIPS to detect T21/T18 in a low-resource setting **Perspective:** government (military) payer **Currency, year**	**Source:** theoretical cohort based on historical delivery volume at the military hospital over a 5-to-6-year period **N**: 100 pregnant individuals **Risk**: NR; assumed general risk **Age** (y), mean (range): historical	**Intervention (I):** NIPS (T21/T18) **Comparator(s) (C):** 2-part serum-based screening (two-part integrated screen & 2nd trimester "quad" test) **Source of data inputs:** NIPS performance by a single laboratory; travel costs: Department of Defense; associated medical costs: published Tricare reimbursement rates and pricing from a large health care system; incidence of diagnostic testing: estimated by Maternal-Fetal Medicine	**Upfront costs of testing** (for cohort n=100): I: $44,140.32 C: $8285.01 **Total of upfront & secondary costs:** (e.g., travel, consultations) I: $45,782.35/cohort C: $31,324.10/cohort **Prenatal cost equivalence:** occurs when NIPS upfront cost approx. $341.17/test	In a low-resource military setting, NIPS is more expensive than 2-part serum-based screening per cohort of 100 pts but reaches cost-equivalence when NIPS cost ~$340/test **Limitations**: 1. Models did not include detection of open neural tube defects or other aneuploidies, rate of loss of euploid pregnancies from amnio., number of aneuploid pregnancies averted through

Author, Year, Country	Study Characteristics	Population Characteristics	Analysis Parameters	Results	
				Outcomes	Interpretation/Limitations
	NR; assumed USD **Time Horizon**: duration of pregnancy **Discount rate:** NR	cohort (n=48): 26.9 (19-39)	clinicians within the Military Health System; productivity costs: 2005 RAND Corp report, adjusted for inflation **Model**: cost-of-care analysis **Sensitivity analyses:** NR **Measure of effectiveness:** NR **Outcomes:** direct and indirect costs of testing; cost-equivalence for NIPS		termination, or postnatal care of an infant w/an aneuploidy. 2. The specific setting used in this analysis may not be generalizable to other low-resource settings 3. Limited description of key variables and sensitivity analyses
Xie et al., 2020 **Country:** Canada **Setting:** general population screening **Funding:** Health Quality Ontario	**Study objective:** Determine the cost-effectiveness and budget impact of primary NIPS in average-risk individuals **Perspective**: provincial public payer in Canada	**Source:** theoretical cohort based on estimated number of pregnancies in Ontario from 2018-2022 **N**: 142,000-148,000 **Risk**: average risk (<0.008 at 12 weeks gestation)	**Intervention**: 1st-tier NIPS (T21/T18/T13) **Comparator**: conventional screening (TPS) **Source of data inputs:** multiple published studies, European Registry; NIPS detection and false positive rates from a single study; NIPS failure rate from a review; uptake of diagnostic testing after positive NIPS or positive TPS+NIPS was estimated; cost data from a	**Incremental cost of NIPS** (CDN$ (95% CrI)**:** • Contingent NIPS vs TPS: —866,301 (—1,549,974; —286,869) • 1st-tier NIPS vs contingent NIPS: 33,036,595 (25,523,479; 40,574,118) **Difference in diagnostic tests,** (n (95% CrI)): • Contingent NIPS vs TPS: —2447 (—3342; —1669)	2nd-tier NIPS dominated conventional screening; detecting more affected fetuses, reducing number of diagnostic tests performed, reducing total screening costs 1st tier NIPS was dominated by 2nd tier NIPS strategy; finding an additional 80 affected fetuses and costing an additional $33 million.

Author, Year, Country	Study Characteristics	Population Characteristics	Analysis Parameters	Results	
				Outcomes	Interpretation/Limitations
Conflicts of interest: one author receives research materials from PerkinElmer and education funds to attend workshops by Thermo Fisher Scientific	**Currency, Year** CDN$, 2017 **Time Horizon**: length of full-term pregnancy (12 weeks to term) **Discount rate**: NA	**Age**: target population age <40 yrs	hospital, Ontario Schedule of Benefits, Ontario Case Costing Initiative, or published study **Model**: decision analysis; probabilistic simulation analysis w/5000 repetitions • TPS • Contingent NIPS following positive TPS result **Sensitivity analyses:** NIPS price, WTP thresholds, uptake rate for 1st tier NIPS, NIPS FPR, trisomy prevalence; acceptance rate for further testing; SCA & 22q11.2 deletion screening in 1st tier NIPS **Measure of effectiveness:** (1) numbers of chromosomal anomaly cases detected and confirmed; (2) number of diagnostic tests performed; (3) number of pregnancy losses related to diagnostic testing; (4) number of live births with T21/T18/T13	• 1st-tier NIPS vs contingent NIPS: —91 (—200; 26) **Difference in diagnostic procedure-related pregnancy losses**, n (95% CrI): • Contingent NIPS vs TPS: —5 (—11; 0) • 1st-tier NIPS vs contingent NIPS: 0 (—2; 3) **Difference in affected live births**, n (95% CrI): • Contingent NIPS vs TPS: —12 (—34; 12) • 1st-tier NIPS vs contingent NIPS: —29 (—51; —8) **Incremental cost of NIPS per additional affected fetus (T21/T18/T13)**, CDN$: • Contingent NIPS vs TPS: Dominant • 1st-tier NIPS vs 2nd-tier NIPS: $412,411 **Incremental cost of NIPS per additional affected fetus**	TPS was not the optimal screening strategy at any WTP threshold; at WTP threshold >$415000, 1st tier NIPS was optimal strategy Analyses including SCA and 22q11.2 deletion screening were preliminary **Limitations**: Included costs of nuchal translucency ultrasound scans & GC in cost of 1st tier NIPS; data for the SCA and 22q11.2 deletion are sparse & results of their analyses should be considered in this context; did not consider the societal perspective; estimated cost/affected fetus instead of cost/QALY over a lifetime horizon; input parameters may not be robust estimates; did not include additional costs for women who receive a

Author, Year, Country	Study Characteristics	Population Characteristics	Analysis Parameters	Results	
				Outcomes	**Interpretation/Limitations**
			Outcomes: incremental cost, incremental effectiveness, incremental cost per additional affected case detected	**(T21/T18/T13; SCAs [expected prevalence])**, CDN$: 1st-tier NIPS vs contingent NIPS: $154,839 **Incremental cost of NIPS per additional affected fetus (T21/T18/T13; SCAs [expected prevalence], 22q11.2 deletion)**, CDN$: 1st-tier NIPS vs contingent NIPS: $183,120	positive NIPS result but decline further testing
Gomes et al., 2019 **Country**: Portugal **Setting**: 1st-trimester screening in a low-risk population **Funding**: NR **Conflicts of interest**: None	**Study objective:** assess performance of contingent NIPS one year after clinical implementation **Perspective**: NR **Currency, Year** Euro, NR **Time Horizon**: NR	**Source:** theoretical cohort based on clinical cohort (n=1272) receiving 1st-trimester screening between March 2017-February 2018 **N**: 10 000 **Risk**: intermediate risk (1:100-1:500); low risk (<1:500) **Age, mean (SD)**: 30.05 (5.9) years	**Intervention (I)**: Contingent screening w/NIPS (T21, T18, T13) **Comparator (C)**: 1st-trimester combined screening **Source of data inputs**: historical clinical data; costs and rate of hospital admissions from a single publication **Model**: cost-effectiveness **Sensitivity analyses**: NR **Measure of effectiveness**: direct costs	**Total costs**: I: 322 290 € C: 309 760 € **Incremental cost of contingent NIPS**: 1.25 € per patient **Rate of invasive tests**: Contingent NIPS: 2.44% vs. 1st-trimester screening: 3.52%; p = 0.086	NIPS does not substantially raise the costs of a screening program compared to no NIPS and reduces the rate of invasive tests **Limitations**: Extremely limited reporting of statistical model and inputs, perspectives, etc.; unclear generalizability

Author, Year, Country	Study Characteristics	Population Characteristics	Analysis Parameters	Results	
				Outcomes	Interpretation/Limitations
	Discount rate: NR		**Outcomes**: rate of invasive tests performed; performance of aneuploidy screening; incremental cost associated with contingent NIPS		
Kostenko et al., 2019 **Country**: Belgium **Setting**: general pregnancy population of Belgium **Funding**: Roche Sequencing Solutions funded the study **Conflicts of interest**: multiple authors are	**Study objective:** Evaluate the clinical and economic impact of NIPS as a 1st-line screening for T21/T18/T13 in general-risk pregnancy population **Perspective**: public health system **Currency, Year** Euro, 2018 **Time Horizon**: prenatal screening period	**Source:** theoretical cohort based on estimated annual number of pregnancies reaching 10 weeks gestation in Belgium **N**: 131,567 (range: 105, 254-157,880) **Risk**: general risk **Age**: NR	**Intervention**: NIPS as 1st tier screening (Harmony® prenatal test, Roche) (T21, T18, T13) **Comparator(s)**: Conventional screening (FTS, STS) **Source of data inputs:** costs from government registries; published studies, expert review **Model:** decision-analysis **Sensitivity analyses**: one-way; FPRs, test performance; extreme case analysis (assume best and worst performance) **Measure of effectiveness**: incremental costs; incremental effectiveness	**Incremental cost/trisomy detected** (€) based on FPR (%) 0.1%: 3617 0.3% 4199 0.6% 4889 1% 5808 **Estimated cost/trisomy dx**, based on NIPS cost of 260€: 3617 **Number of invasive tests**, n, (I vs. C): 797 vs. 8709; difference: —7,912 (—90.8%) **Number of procedure-related miscarriages**, n (I vs C): 4 vs 44; difference: —40 (—90.8%) **Total trisomies detected**, n (%), I vs C: 411 (99%) vs 318 (81%) **Detection rate T21**, n (%), I vs C: 293 (100%) vs 221 (79%)	NIPS as the primary screening strategy substantially decreased the number of invasive tests and treatment-related miscarriages; the incremental cost per trisomy diagnosed varied by the FPR of the test; the authors state that at a NIPS cost of 260€/NIPS test, the effectiveness and decreases in numbers of invasive tests come at a 'reasonable cost' **Limitations**: limited time horizon that does not account for lifetime costs of a child with a trisomy; input costs may not be generalizable or change over time

Author, Year, Country	Study Characteristics	Population Characteristics	Analysis Parameters	Results	
				Outcomes	Interpretation/Limitations
employees of Roche Sequencing Solutions or GfK consultancy; multiple authors have previously received consulting fees from Roche	**Discount rate**: NA		**Outcomes**: number of invasive tests, procedure-related miscarriages or other complications, missed trisomies, total number of trisomies detected	**Detection rate T18**, n (%), I vs C: 87 (97%) vs 74 (87%) **Detection rate T13,** n (%), I vs C: 31 (94%) vs 24 (75%)	
Le Bras et al., 2019 **Country**: France **Setting**: general population screening **Funding**: French Ministry of Health	**Study objective:** Evaluate the cost-effectiveness of multiple screening strategies compared to NIPS **Perspective**: healthcare provider **Currency, Year** Euro, 2017	**Source:** theoretical cohort based on expected number of annual pregnancies in France **N**: 652 653 **Risk**: variable **Age**: NR	**Intervention (I)**: NIPS in the general population (T21/T18/T13, other UBCA) **Comparators (C**): invasive testing following 1st-trimester screening **Source of data inputs**: 2016 data from French Biomedicine Agency (published and unpublished); French National Health Insurance tariff; French Ministry of Health; published data from single studies **Model**: cost-effectiveness 1. Contingent NIPS for pts w/a risk following FTS of ≥1/250	**Cost (€)** I: 287 610 817 C1: 12 004 022 C2: 39 969 156 C3: 12 610 144 C4: 43 053 119 **ICER per additional UBCA detected**: I vs C2, €9,166,689 **T21 detected** (n): I: 1070 C1: 876 C2: 1025 C3: 879 C4: 1028	NIPS in a general population to detect all unbalanced chromosomal anomalies (trisomies, SCAs, and other) was not cost-effective at costs ranging from €188-496, compared to risk-based strategies. **Limitations**: did not include the ability of NIPS to detect SCA or other UBCAs; input values may vary over time and other variables (e.g., location); results may not be generalizable to different health care

Author, Year, Country	Study Characteristics	Population Characteristics	Analysis Parameters	Results	
				Outcomes	Interpretation/Limitations
Conflicts of interest: NR	**Time Horizon**: period from completion of FTS to completion of testing **Discount rate**: None		2. Contingent NIPS for pts w/a risk of ≥1/1000 3. invasive testing for pts w/a risk of ≥1/250 4. invasive testing for pts w/a risk of ≥1/1000 **Sensitivity analyses**: (one-way) 1. Cost of NIPS €100; 2. Deducted cost of FTS in general risk pop.; 3. Difference on rate of miscarriage for women w/a risk of ≥1/250 and ≥1/1000; 4. NIPS (Panorama™, cost €427) detected trisomies and SCAs **Measure of effectiveness**: incremental costs **Outcomes**: direct costs; number of UBCAs detected; estimated number of miscarriages; incremental cost per additional UBCA detected	**T21/T18/T13 detected**, (n): I: 1168 C1: 959 C2: 1121 C3: 963 C4: 1125 **All UBCA detected**, (n): I: 1168 C1: 959 C2: 1121 C3: 1138 C4: 1330	systems; miscarriage rate CI was reported but not shown w/analysis
Nshimyumukiza et al., 2018 **Country**: Canada	**Study objective:** Evaluate the cost-effectiveness of NIPS (1st-tier or	**Source:** theoretical cohort	**Intervention (I):** NIPS as 1st tier test (T21/T18/T13)	**Base analysis, Serum integrated + NIPS (dominant) vs universal NIPS**: **Costs** (CAD$): $9,534,059 vs $66,596,727	Contingent NIPS after serum screening was the dominant screening strategy across most analyses until the cost of

Author, Year, Country	Study Characteristics	Population Characteristics	Analysis Parameters	Results	
				Outcomes	Interpretation/Limitations
Setting: general screening in Quebec **Funding**: supported by PEGASUS project (Genome Canada, Canadian Institutes of Health) **Conflicts of interest**: four authors receive research materials from commercial NIPS labs or equipment that can be used to perform NIPS	contingent screening) with traditional prenatal screening strategies **Perspective**: payer (public health system (Quebec)) **Currency, Year** CAD, 2014-2015 fiscal yr **Time Horizon**: duration of pregnancy **Discount Rate**: None	**N**: 1,879,872 (range: 1,870,000 – 1,900,000) **Risk**: 1:300 cut-off for traditional screening **Age**: NR	**Comparator(s) (C)**: current screening strategies recommended by SOGC **Source of data inputs:** single published studies and assumptions for population and probabilities data; one to a few studies for costs and screening performance **Model:** decision-analysis; semi-Markov agent and population-based model simulations performed 1000 times • C1-6: No NIPS Current strategies recommended by SOGC • C7-12: Contingent NIPS following a positive result from C1-6 **Sensitivity analyses** (one-way & probabilistic; 1000x w/different virtual populations): Costs and event probabilities; risk cut-offs **Measure of effectiveness:** cost per T21 case detected;	All strategies w/contingent NIPS (C7-12) were less expensive than current screening strategies (C1-6) **Cost/case T21 detected**: $63,139 vs $308,318 **ICER/case of T21 detected**: Universal NIPS, $1,553,615 **Base analysis, Serum integrated + NIPS (dominant) vs universal NIPS:** **Invasive tests** (n): 259 vs 539 **Euploid fetal losses** (n): 0.0122 vs 0.495 **T21 detected**: 151 vs 216 **T18 & T13 detected**: 69 vs 98	NIPS dropped below $400, when QUAD + NIPT became the dominant strategy. NIPS as 1st tier test was dominated by other strategies unless (1) cost of NIPS set at $240 and the cost per T21 case detected equaled cost of integrated screening strategy; or (2) cost of NIPS set at $184 and cost per T21 case detected equaled the cost of serum screening strategy **Limitations**: compared many screening recommendation strategies across a variety of WTP thresholds and other variables; results may not be generalizable outside of the perspective and assumptions; limited time horizon that does not take into consideration costs associated w/clinical mgmt. of pts w/a chromosomal aneuploidy; does not

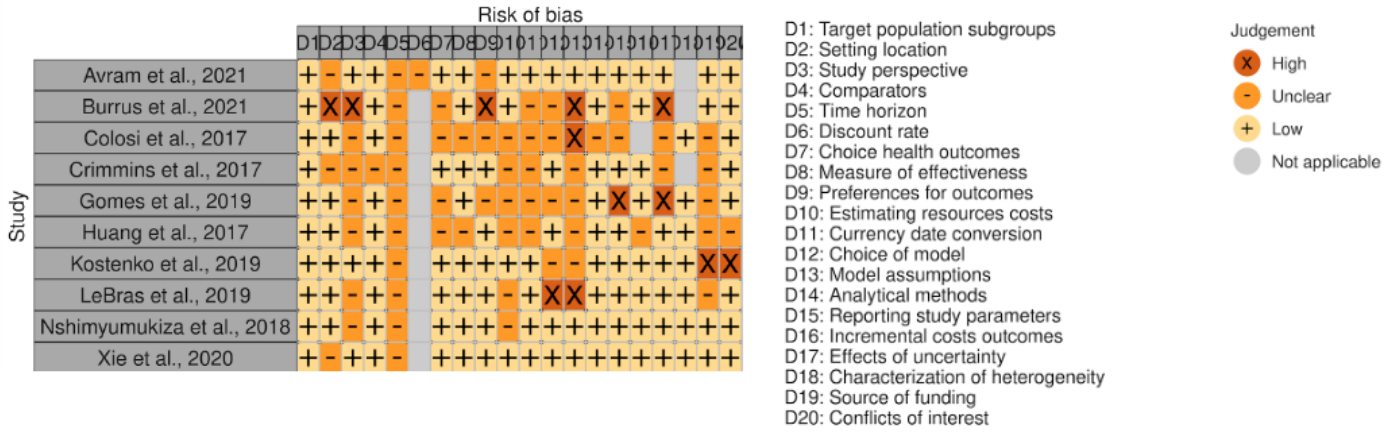
Author, Year, Country	Study Characteristics	Population Characteristics	Analysis Parameters	Results	
				Outcomes	Interpretation/Limitations
			incremental cost per additional T21 case detected **Outcomes:** total direct costs to health care system; number of affected fetuses detected; number of invasive procedures; number of euploid fetal losses		include SCAs or other chromosomal abnormalities that may be detected by NIPS; costs from 2014-2015 may not reflect current costs
Colosi et al., 2017 **Country**: Italy **Setting**: patients undergoing 1st-trimester screening at single hospital in Italy between November 2011 to May 2015 **Funding**: NR **Conflicts of interest**: None	**Study objective:** Determine optimal (best value/costs) screening strategy for NIPS **Perspective**: public payer **Currency, Year** Euro, NR **Time Horizon**: NR **Discount Rate:** NR	**Source:** clinical population **N**: 20 831 **Risk**: intermediate 1:251 to 1:1000; low >1:1000 **Age, median**: 32.3 yrs	**Intervention (I):** contingent NIPS (T21/T18/T13) **Comparator (C):** combined test **Source of data inputs**: clinical population (effectiveness and costs of screening for trisomies, test performances) **Model**: cost-effectiveness • Combined test (no NIPS) • Primary NIPS • Contingent NIPS if risk between 1:10-1:1000 • Contingent NIPS if risk between 1:10-1:1000 and nasal bone evaluation **Sensitivity analysis**: None	**Total estimated costs (€)**: Combined test: 2,385,473 Primary NIPS: 5,796,060 Contingent NIPs: 2,834,213 Contingent NIPS + nasal bone eval: 2,338,433 **Invasive procedures** (n): Combined test: 1313 Primary NIPS: 760 Contingent NIPs: 188 Contingent NIPS + nasal bone eval: 188 **Detection rate for T21/T18/T13**: Combined test: 94.92% Primary NIPS: 97.82% Contingent NIPs: 97.82% Contingent NIPS + nasal bone eval: 97.82%	Contingent NIPS after a combined test that includes evaluation of nasal bone is the least costly screening option and yields the lowest number of invasive procedures and highest detection rate for the trisomies **Limitations**: minimal reporting of key cost-effectiveness metrics (e.g., time horizon); did not perform sensitivity analyses to evaluate uncertainty

Author, Year, Country	Study Characteristics	Population Characteristics	Analysis Parameters	Results	
				Outcomes	Interpretation/Limitations
			Measurement of effectiveness: NR **Outcomes**: detection rate; final costs; invasive test rate		
Crimmins et al., 2017 **Country**: United States **Setting**: urban population receiving 2nd-trimester screening **Funding**: NR **Conflicts of interest**: None	**Study Objective:** Determine the threshold point at which NIPS would be at least cost equivalent to QUAD screening **Perspective**: NR (presumed health care provider) **Currency, Year** USD, NR **Time Horizon**: NR **Discount rate:** NR	**Source**: clinical population **N**: 590 **Risk**: NR **Age** (yrs), median (range): 23.9 (15-44)	**Intervention (I)**: NIPS (T21) **Comparator (C)**: QUAD screen for T21 **Source of data inputs:** Cost inputs from published literature, local costs (e.g., GC session), or Medicaid data **Model:** decision-analysis; cost-sensitivity **Sensitivity analyses**: Cost of NIPS ($0-$3000) **Measure of effectiveness**: cost-equivalence **Outcomes**: rate of invasive procedures; rate of procedure-related loss; number of patients meeting with the genetic counselor	**Change in rate of invasive procedures** (%), I vs C: —55.4% **Change in rate of procedure-related loss** (%), I vs C: —57% **Pts meeting w/GC** (%), I vs C: 2.9% vs 14.7% (—78%) **Cost-equivalence between primary NIPS and QUAD**: $360.66	At a cost of $360.66, NIPS as the primary screen to detect T21 is cost-equivalent to QUAD screening and results in substantial reductions in the number of invasive procedures, the number of procedure-related losses, and the number of patients needing to meet with GCs for risk assessment in patients presenting in the 2nd trimester. **Limitations**: very limited reporting of key cost-effectiveness variables (e.g., time horizon, perspective); inputs based in part on local data which may not be generalizable to other locations or may vary

Author, Year, Country	Study Characteristics	Population Characteristics	Analysis Parameters	Results	
				Outcomes	**Interpretation/Limitations**
					over time; narrow focus on T21
Huang et al., 2017 **Country**: Canada **Setting**: general population screening **Funding**: NR **Conflicts of interest**: NR	**Study objective:** Identify a screening strategy for T21 that maximized performance and minimized costs **Perspective**: public payer **Currency, Year** NR; NR **Time Horizon**: NR	**Source:** theoretical cohort based on historical cohort of pregnant individuals in Ontario from April 2011 to March 2012 **N**: 97385 **Risk**: variable; 1:200; 1:1500; 1:1000 **Age**: NR	**Intervention (I)**: primary NIPS (T21) **Comparator (C)**: conventional screening strategies **Source of data inputs:** published studies for NIPS performance and failure rate; outcomes derived from actual outcomes of historical cohort **Model:** cost-effectiveness • Integrated screening (IPS) • Contingent NIPS after 1st-trimester screening (FTS) • Contingent NIPS after enhanced 1st-trimester screening (EFTS) [includes serum placental growth factor and alpha fetoprotein] **Sensitivity analyses**: Extremes of choice for uptake of diagnostic testing after failed NIPS; cost of NIPS ($550, $400, $200)	**(NIPS cost $550)** **Total program costs:** IPS: $17,385,291 FTS+NIPS: $21,821,010 EFTS+NIPS: $18,583,611 Primary NIPS w/100% uptake of diagnostic test after NIPS failure: $59,384,682 **Cost per individual screened:** IPS: $179 FTS+NIPS: $224 EFTS+NIPS: $191 Primary NIPS w/100% uptake of diagnostic test after NIPS failure: $610 **Cost per T21 case detected**: IPS: $129,114 FTS+NIPS: $91,605 EFTS+NIPS: $78,014 Primary NIPS w/100% uptake of diagnostic test after NIPS failure: $236,833 **NIPS cost $200:** **Total program costs:**	With NIPS ≤$400, contingent NIPS after EFTS was the dominant screening strategy. In all scenarios, universal NIPS for T21 was dominated. **Limitations:** did not report key cost-effectiveness data (e.g., time horizon); analysis of extremes for uptake of dx testing after NIPS failure; inputs may not reflect current estimates; overall results may not be generalizable to other locations or health system structures; narrow focus on T21

Author, Year, Country	Study Characteristics	Population Characteristics	Analysis Parameters	Results	
				Outcomes	Interpretation/Limitations
			Measure of effectiveness: NR **Outcomes**: number of detected T21 cases; detection rate; number of invasive tests; procedure-related fetal loss (unaffected); total costs and costs per individual screened, per additional T21 case diagnosed	IPS: $17,385,291 FTS+NIPS: $15,242,641 EFTS+NIPS: $14,834,281 Primary NIPS w/100% uptake of diagnostic test after NIPS failure: $25,299,867 **Cost per individual screened:** IPS: $179 FTS+NIPS: $157 EFTS+NIPS: $152 Primary NIPS w/100% uptake of diagnostic test after NIPS failure: $260 **Cost per T21 case detected**: IPS: $129,114 FTS+NIPS: $63,989 EFTS+NIPS: $62,274 Primary NIPS w/100% uptake of diagnostic test after NIPS failure: $100,899	

Supplemental Figure 8. Risk of bias of individual studies included in the economic analyses of NIPS.



Supplemental Table 25. Exclusion rationale for studies excluded after full-text review.

Study	Published Year	Covidence #	Exclusion reason
Haidar 2018	2018	#131	Exclusion reason: abstract only
Ju 2021	2021	#508	Exclusion reason: case report
García-Pérez 2018	2018	#612	Exclusion reason: systematic evidence review/meta-analysis
Palomaki 2018	2018	#669	Exclusion reason: systematic evidence review/meta-analysis
Huijsdens-vanAmsterdam 2018	2018	#641	Exclusion reason: systematic evidence review/meta-analysis
Gil 2017	2017	#565	Exclusion reason: systematic evidence review/meta-analysis
Badeau 2017	2017	#598	Exclusion reason: systematic evidence review/meta-analysis
Cernat 2019	2019	#677	Exclusion reason: systematic evidence review/meta-analysis
Benn 2019	2019	#703	Exclusion reason: systematic evidence review/meta-analysis
Zaami 2021	2021	#545	Exclusion reason: systematic evidence review/meta-analysis
Bianchi 2014	2014	#59	Exclusion reason: systematic evidence review/meta-analysis
Liang 2020	2020	#729	Exclusion reason: Unable to obtain full text
Saes 2019	2019	#682	Exclusion reason: Unable to obtain full text
Cai 2017	2017	#606	Exclusion reason: Unable to obtain full text
Bevilacqua 2019	2019	#640	Exclusion reason: Unable to obtain full text
Kane 2021	2021	#528	Exclusion reason: wrong intervention
Wang 2014	2014	#399	Exclusion reason: wrong intervention
Sullivan 2019	2019	#348	Exclusion reason: wrong intervention
Vinante 2018	2018	#609	Exclusion reason: wrong outcomes
Scott 2018	2018	#568	Exclusion reason: wrong outcomes
Fujimoto 2020	2020	#746	Exclusion reason: wrong outcomes
Miltoft 2018	2018	#616	Exclusion reason: wrong outcomes

Study	Published Year	Covidence #	Exclusion reason
Birko 2019	2019	#675	Exclusion reason: wrong outcomes
Balaguer 2020	2020	#748	Exclusion reason: wrong outcomes
Morano 2018	2018	#653	Exclusion reason: wrong outcomes
Gammon 2018	2018	#611	Exclusion reason: wrong outcomes
Agatisa 2018	2018	#645	Exclusion reason: wrong outcomes
Lund 2018	2018	#635	Exclusion reason: wrong outcomes
Yang 2021	2021	#525	Exclusion reason: wrong outcomes
Akiel 2020	2020	#494	Exclusion reason: wrong outcomes
Kater-Kuipers 2021	2021	#538	Exclusion reason: wrong outcomes
Melcer 2021	2021	#543	Exclusion reason: wrong outcomes
Ravitsky 2021	2021	#502	Exclusion reason: wrong outcomes
Chen 2019	2019	#81	Exclusion reason: wrong outcomes
Dhamankar 2020	2020	#113	Exclusion reason: wrong outcomes
Crabbe 2019	2019	#97	Exclusion reason: wrong outcomes
Cheng 2019	2019	#85	Exclusion reason: wrong outcomes
Tiller 2015	2015	#371	Exclusion reason: wrong outcomes
Tan 2016	2016	#363	Exclusion reason: wrong outcomes
Bayindir 2015	2015	#44	Exclusion reason: wrong outcomes
Agatisa 2015	2015	#31	Exclusion reason: wrong outcomes
Barrett 2017	2017	#43	Exclusion reason: wrong outcomes
Pariente 2016	2016	#283	Exclusion reason: wrong outcomes
Farrell 2015	2015	#417	Exclusion reason: wrong outcomes
vanSchendel 2015	2015	#384	Exclusion reason: wrong outcomes
D'Aversa 2018	2018	#102	Exclusion reason: wrong outcomes
Okmen 2020	2020	#734	Exclusion reason: wrong patient population
Ehrich 2017	2017	#559	Exclusion reason: wrong patient population

Study	Published Year	Covidence #	Exclusion reason
Qian 2019	2019	#643	Exclusion reason: wrong patient population
Grati 2017	2017	#581	Exclusion reason: wrong patient population
Galeva 2019	2019	#690	Exclusion reason: wrong patient population
Galeva 2019	2019	#666	Exclusion reason: wrong patient population
Richardson 2017	2017	#602	Exclusion reason: wrong patient population
Chan 2018	2018	#595	Exclusion reason: wrong patient population
Chan 2018	2018	#617	Exclusion reason: wrong patient population
Ravi 2018	2018	#629	Exclusion reason: wrong patient population
Zheng 2019	2019	#697	Exclusion reason: wrong patient population
Yaron 2020	2020	#733	Exclusion reason: wrong patient population
Lee 2018	2018	#627	Exclusion reason: wrong patient population
Pasquini 2019	2019	#668	Exclusion reason: wrong patient population
Suzumori 2021	2021	#755	Exclusion reason: wrong patient population
Gil 2017	2017	#562	Exclusion reason: wrong patient population
Flöck 2017	2017	#586	Exclusion reason: wrong patient population
Al-Ibraheemi 2017	2017	#577	Exclusion reason: wrong patient population
Martínez-Payo 2018	2018	#636	Exclusion reason: wrong patient population
Guy 2019	2019	#683	Exclusion reason: wrong patient population
Huang 2018	2018	#622	Exclusion reason: wrong patient population
Lu 2018	2018	#619	Exclusion reason: wrong patient population
Cheng 2018	2018	#599	Exclusion reason: wrong patient population
ElKhattabi 2019	2019	#659	Exclusion reason: wrong patient population
Chibuk 2020	2020	#743	Exclusion reason: wrong patient population
Vifçifá 2017	2017	#610	Exclusion reason: wrong patient population
VanOpstal 2018	2018	#558	Exclusion reason: wrong patient population
Lund 2021	2021	#540	Exclusion reason: wrong patient population

Study	Published Year	Covidence #	Exclusion reason
Togneri 2020	2020	#491	Exclusion reason: wrong patient population
Junhui 2021	2021	#515	Exclusion reason: wrong patient population
Wu 2020	2020	#498	Exclusion reason: wrong patient population
Zou 2021	2021	#497	Exclusion reason: wrong patient population
Wan 2020	2020	#394	Exclusion reason: wrong patient population
Zheng 2020	2020	#465	Exclusion reason: wrong patient population
Mesoraca 2020	2020	#245	Exclusion reason: wrong patient population
Iwarsson 2020	2020	#163	Exclusion reason: wrong patient population
Cai 2018	2018	#71	Exclusion reason: wrong patient population
Togneri 2019	2019	#372	Exclusion reason: wrong patient population
Verma 2018	2018	#386	Exclusion reason: wrong patient population
Shiv 2017	2017	#328	Exclusion reason: wrong patient population
Ericsson 2019	2019	#122	Exclusion reason: wrong patient population
Holzer 2019	2019	#148	Exclusion reason: wrong patient population
Kellogg 2014	2014	#177	Exclusion reason: wrong patient population
How 2019	2019	#150	Exclusion reason: wrong patient population
Wang 2015	2015	#396	Exclusion reason: wrong patient population
Lefkowitz 2016	2016	#201	Exclusion reason: wrong patient population
Takeda 2018	2018	#359	Exclusion reason: wrong patient population
Chetty 2013	2013	#87	Exclusion reason: wrong patient population
Ramdaney 2018	2018	#296	Exclusion reason: wrong patient population
Lee 2019	2019	#701	Exclusion reason: wrong patient population
Scibetta 2017	2017	#583	Exclusion reason: wrong patient population
He 2018	2018	#649	Exclusion reason: wrong study design
Liang 2018	2018	#670	Exclusion reason: wrong study design
McKanna 2019	2019	#652	Exclusion reason: wrong study design

Study	Published Year	Covidence #	Exclusion reason
Kaseniit 2018	2018	#665	Exclusion reason: wrong study design
Bevilacqua 2018	2018	#642	Exclusion reason: wrong study design
Dahl 2018	2018	#632	Exclusion reason: wrong study design
Zhang 2019	2019	#681	Exclusion reason: wrong study design
Lee 2018	2018	#605	Exclusion reason: wrong study design
Lin 2020	2020	#753	Exclusion reason: wrong study design
Schmid 2018	2018	#601	Exclusion reason: wrong study design
Abousleiman 2019	2019	#688	Exclusion reason: wrong study design
Kagan 2017	2017	#576	Exclusion reason: wrong study design
Jones 2018	2018	#604	Exclusion reason: wrong study design
Post 2017	2017	#593	Exclusion reason: wrong study design
Suzumori 2014	2014	#353	Exclusion reason: wrong study design
Aziz 2020	2020	#489	Exclusion reason: wrong study design
Kim 2018	2018	#183	Exclusion reason: wrong study design
Gadsbøll 2020	2020	#422	Exclusion reason: wrong study design
Yin 2015	2015	#443	Exclusion reason: wrong study design
McNamara 2015	2015	#239	Exclusion reason: wrong study design
Li 2015	2015	#206	Exclusion reason: wrong study design
Wang 2015	2015	#398	Exclusion reason: wrong study design
Ji 2018	2018	#167	Exclusion reason: wrong study design
Yin 2019	2019	#445	Exclusion reason: wrong study design
Eswarachari 2019	2019	#124	Exclusion reason: wrong study design
Farrell 2014	2014	#418	Exclusion reason: wrong study design
Xing 2018	2018	#414	Exclusion reason: wrong study design
Futch 2013	2013	#421	Exclusion reason: wrong study design
Friel 2014	2014	#420	Exclusion reason: wrong study design

Study	Published Year	Covidence #	Exclusion reason
Bettencourt 2014	2014	#9	Exclusion reason: wrong study design
Anazi 2017	2017	#17	Exclusion reason: wrong study design/article type

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