Table 4.1
Summary of findings.

Type of samples	Outcome	Number of included studies with low or moderate risk of bias	Comments
NGS for targeted analysis			
NIPT	Monosomy X	9	Few events, inconsistency in data, differences in study design, population and sequencing method applied
NIPT	XXX	4	Few events, inconsistency in data, differences in study design, population and sequencing method applied
NIPT	XXY	5	Few events, inconsistency in data, differences in study design, population and sequencing method applied
NIPT	XYY	4	Few events, inconsistency in data, differences in study design, population and sequencing method applied
NIPT	Trisomies other than T13, T18, T21	3	8 cases of other autosomal trisomies
NIPT	Microdeletions associated with known microdeletion syndromes	1 (2 studies with high risk of bias)	Only cases identified with NGS underwent invasive sampling for confirmation
NIPT	Microduplica-tions associated with known microduplication syndromes	0 (0 studies with high risk of bias)	No studies identified
NGS for whole genome sequencing/whole exome sequencing			
NIPT		2 (0 studies with high risk of bias)	Few events with different genetic abbreviations. Different methods applied in the two studies
Invasive		0 (3 studies with high risk of bias)	Highly selected populations. Only studies with high risk of bias identified.

NGS = Next-generation sequencing; **NIPT** = Non-invasive prenatal testing

Table 4.2 Included studies investigating NIPT with NGS for identification of sex chromosomal aneuploidies (SCA).

Author, year **Reference** **Country**	**Study design**	**Sequencing method**	**Time of study**	**Population** Risk for aneuploidy Mean maternal age (years) Mean gestational age (weeks)	**Outcome**	**Number of included samples** **Number of samples lost to follow-up**
Jiang, 2012 [30] China	Prospective, multicenter (biobanked samples)	MPSS	2009–2010	High risk 20–45 10–34	SCA	903 1
Liang, 2013 [31] China	Prospective, multicenter (biobanked samples)	Not specified	2009–2011	High risk 31 21	SCA and T9	435 23
Porreco, 2014 [29] USA	Prospective, multicenter, (biobanked samples)	MPSS	2009–2011	High risk 35 16	SCA	4 170 969 (892) (different number of non-reportable result for different outcomes)
Bianchi, 2012 [22] USA	Prospective, multicenter, nested case control (biobanked samples)	MPSS	2010–2011	High risk 35 15	Monosomy X	532 (selected from 2 882 samples) 2
Song, 2013 [25] China	Prospective cohort (not biobanked samples)	Not specified	2011	High and low risk 29 (all less than 35) 17	SCA (not all samples underwent invasive reference test)	1 916 184
Yao, 2014 [27] China	Prospective cohort, (not biobanked samples)	Not specified	2011–2012	High and low risk 30 19	SCA (not all samples underwent invasive reference test)	5 950 484
Lau, 2014 [26] China	Prospective cohort, (not biobanked samples)	Not specified	2011–2013	High and low risk 36 14,5 (median)	SCA + other trisomies, deletions and duplications (not all samples underwent invasive reference test)	1 982 1

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Table 4.2 continued

Author, year Reference Country	Study design	Sequencing method	Time of study	Population Risk for aneuploidy Mean maternal age (years) Mean gestational age (weeks)	Outcome	Number of included samples Number of samples lost to follow-up
Song, 2015 [24] China	Prospective cohort (not biobanked samples)	Not specified	2012–2013	High risk 37 (all over 35) 10 (only first trimester)	SCA	213 35
Shaw, 2014 [34] China	Prospective, multicenter cohort (not biobanked samples)	MPSS	2012	High and low risk 35 17	SCA and T16	201 1
Comas, 2015 [28] Spain	Prospective cohort (not biobanked samples)	SNP/ t-MPS	2013	High and low risk 37 15	SCA (only high risk pregnancies at NIPT underwent the invasive reference test)	333 (217 analysed for SCA) 22
Mazloom, 2013 [32] USA	Prospective cohort (biobanked samples)	MPSS	ns	High risk 36 (median) 17 (median)	SCA	411 21
Nicolaides, 2013 [33] United Kingdom	Prospective cohort (biobanked samples)	SNP	ns	High risk 36 (median) 13 (median)	SCA	242 13
Pergament, 2014 [17] USA	Prospective, multicenter cohort, (not biobanked samples)	SNP	ns	High and low risk 30 14	SCA	1 064 98

ns = Not stated; **MPSS** = Massive parallel shotgun sequencing; **SCA** = Sex chromosome aneuploidy; **SNP** = Single nucleotide polymorphism; **T** = Trisomi; **t-MPS** = Targeted massive parallel sequencing

Figur 4.1-4.4 Sensitivitet och specificitet för NIPT med NGS för att identifiera könskromosomavvikelser.

Figure 4.1 Sensitivitet och specificitet för identifikation av monosomi X.

Studie, år	Sensitivitet (95% KI)	Specificitet (95% KI)
Bianchi, 2012	0,94 (0,70; 1,00)	1,00 (0,99; 1,00)
Jiang, 2012	0,75 (0,19; 0,99)	1,00 (0,99; 1,00)
Liang, 2013	1,00 (0,48; 1,00)	1,00 (0,99; 1,00)
Mazloom, 2013	0,94 (0,73; 1,00)	1,00 (0,99; 1,00)
Nicolaides, 2013	1,00 (0,03; 1,00)	1,00 (0,98; 1,00)
Pergament, 2014	0,92 (0,62; 1,00)	1,00 (0,99; 1,00)
Porreco, 2014	1,00 (0,66; 1,00)	1,00 (0,99; 1,00)
Shaw, 2014	0,75 (0,19; 0,99)	1,00 (0,96; 1,00)
Song, 2015	0,00 (0,00; 0,98)	0,99 (0,97; 1,00)

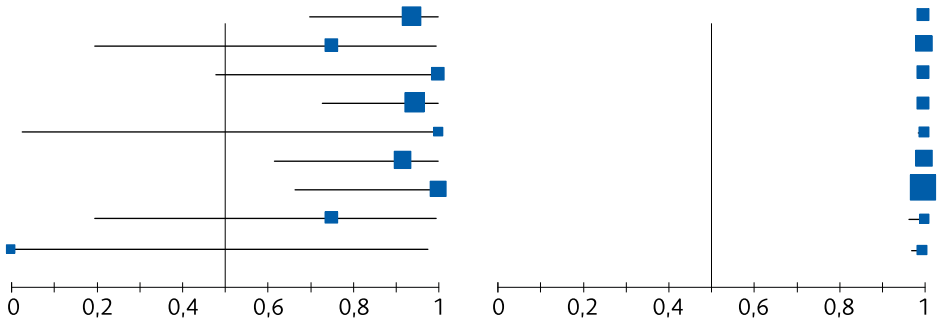

Figure 4.2 Sensitivitet och specificitet för identifikation av XXX.

Studie, år	Sensitivitet (95% KI)	Specificitet (95% KI)
Liang, 2013	1,00 (0,03; 1,00)	1,00 (1,00; 1,00)
Mazloom, 2013	1,00 (0,03; 1,00)	1,00 (0,99; 1,00)
Porreco, 2014	1,00 (0,40; 1,00)	1,00 (1,00; 1,00)
Song, 2015	Inte specificerat	0,99 (0,97; 1,00)

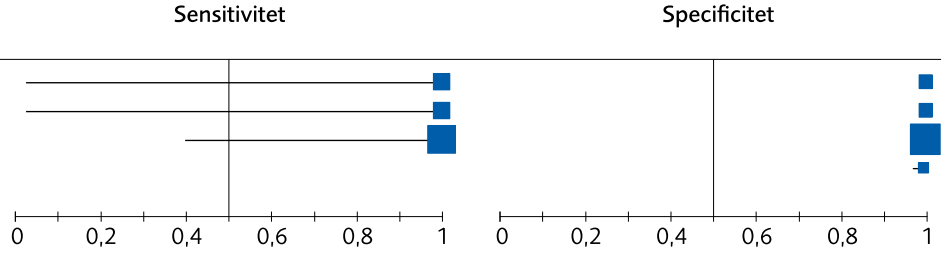

Figure 4.3 Sensitivitet och specificitet för identifikation av XXY.

Studie, år	Sensitivitet (95% KI)	Specificitet (95% KI)
Jiang, 2012	1,00 (0,16; 1,00)	1,00 (0,99; 1,00)
Liang, 2013	1,00 (0,03; 1,00)	1,00 (0,99; 1,00)
Mazloom, 2013	1,00 (0,48; 1,00)	1,00 (0,99; 1,00)
Porreco, 2014	1,00 (0,03; 1,00)	1,00 (0,99; 1,00)
Song, 2015	Inte specificerat	0,99 (0,97; 1,00)

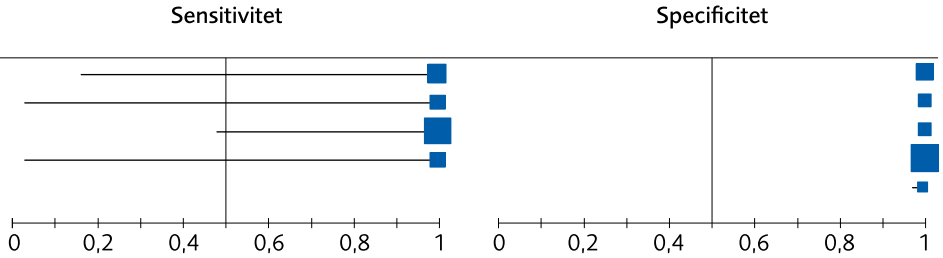

Figure 4.4 Sensitivitet och specificitet för identifikation av XYY.

Studie, år	Sensitivitet (95% KI)	Specificitet (95% KI)
Jiang, 2012	1,00 (0,03; 1,00)	1,00 (1,00; 1,00)
Liang, 2013	1,00 (0,03; 1,00)	1,00 (0,99; 1,00)
Mazloom, 2013	1,00 (0,16; 1,00)	1,00 (0,99; 1,00)
Porreco, 2014	1,00 (0,03; 1,00)	1,00 (1,00; 1,00)
Shaw, 2014	1,00 (0,03; 1,00)	1,00 (0,96; 1,00)

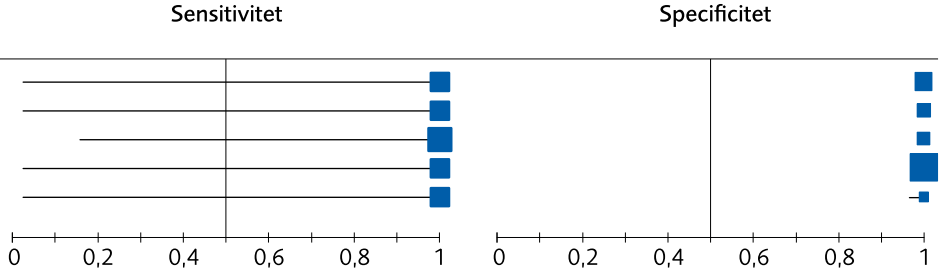

Table 4.3 Distribution of positive events in studies using NGS and non-invasive samples (NIPT).

Outcome	Number of included studies	Only true positive events: Number of studies Number of events (number of successful analyses)	Only false positive events: Number of studies Number of events (number of successful analyses)	Both true and false positive events: Number of studies Number of events (number of successful analyses)
Monosomy X	13	4 7 (332)	2 2 (179)	7 81 (6 379)
XXX	6	3 4 (804)	1 1 (178)	2 17 (3 280)
XXY	7	3 8 (1 705)	2 2 (179)	2 10 (3 208)
XYY	6	5 6 (5 006)	0	1 1 (2*)

* Only cases with positive NIPT results underwent confirmation from invasive samling.

Table 4.4 Number of analyses, true and false positive events, and the proportion of false positive events in the studies where both true and false positive event were identified.

Outcome	Number of Studies	Total number of successful analyses	Number of true positive events Median (range)	Number of false positive events Median (range)	Proportion of false positive events Median (range)
Monosomy X	7	6 379	9 (3–17)	1 (1–11)	16.7% (6.3–55%)
XXX	2	3 280	5.5 (4–7)	3 (3–3)	36,4% (30.0–42.9%)
XXY	2	3 208	2 (1–3)	3 (2–4)	61,9% (57.1–66.7%)
XYY	1	2	1	1	50%

Table 4.5 Distribution of negative events in studies using NGS and non-invasive samples (NIPT).

Outcome	Number of included studies	Only true negative events: Number of studies (Total number of successful analyses)	Only false negative events	Both true and false negative events: Number of studies Number of false negative events (Number of successful analyses)
Monosomy X	9	3 (3 911)	0	6 5 (2 870)
XXX	4	4 (4 250)	0	0
XXY	5	5 (5 084)	0	0
XYY	5	5 (5 006)	0	0

Table 11.1 Included studies Next-generation sequencing from NIPT sample to detect sex chromosome aneuploidies and trisomies other than T13, T18 and T21.

First author Year Reference Country	Study design	Population	Index test platform Reference test	Results for diagnosed trisomy or sex chromosome aneuploidies	Study quality Comments
Bianchi 2012 [22] USA	**Study design** Nested case control study within a prospective multicenter, observational cohort Blinded **Time of study** June 2010 to August 2011	**Population** High risk for fetal aneuploidy n=2 882 (cohort) n=534 (selected) Successful samples n=433 **Samples** Biobanked samples **Inclusion criteria** Age 38 years or older Prior aneuploid pregnancy Anomaly detected by USS Positive maternal serum screen **Exclusion criteria** Ineligible samples, no karyotype recorded or multiple gestation **Gestational age at sampling** 10–23 weeks **Maternal age** Mean 35 years **Drop-outs** Did not pass quality control n=2 No fetal DNA detected n=16 Censored complex karyotype n=37 Unclassified =49 (when checked number should read 46)	**Platform** NIPT MPSS **Reference test** Karyotype	**Monosomy X** TP 15 FP 1 FN 1 TN 416 Sensitivity: 93.8 (95% CI, 69.8; 99.8) Specificity: 99.8 (95% CI, 98.7; 99.9)	Moderate **Commercial partner** Verinata

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Table 11.1 continued

First author Year Reference Country	Study design	Population	Index test platform Reference test	Results for diagnosed trisomy or sex chromosome aneuploidies	Study quality Comments
Comas 2015 [28] Spain	**Study design** Prospective cohort study Not blinded **Time of study** January to December 2013	**Population** Mainly low risk for fetal aneuploidy (84%) n=333 (total) n=311 (analysed) Samples Not biobanked **Inclusion criteria** Singleton pregnancies Pregnancies referred by anxiety or high risk in maternal serum screen combined with nuchal translucency **Exclusion criteria** Anomaly detected by USS Pregnancies at high risk of other genetic conditions **Gestational age at sampling** 9–23 weeks **Maternal age** Mean 37 years (21–46) **Drop-outs** No test result n=4 Repeated sampling and analysis n=6 Lost to follow up n=18 (pregnancies that were still in progress)	**Platform** NIPT SNP/T-MPS **Reference test** Karyotype or neonatal data Karyotyping only in patients with high risk in NIPT analysis	**Monosomy X** (1 identified with NIPT, 1 analysed with karyotype) TP 0 FP 1 Lost to follow-up: 0	Moderate **Commercial partner** Natera

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Table 11.1 continued

First author Year Reference Country	Study design	Population	Index test platform Reference test	Results for diagnosed trisomy or sex chromosome aneuploidies	Study quality Comments
Jiang 2012 [30] China	**Study design** Multicenter prospective cohort study Blinding unclear **Time of study** June 2009 to August 2010	**Population** High risk for fetal aneuploidy n=903 (total) n=903 (analysed) **Samples** Biobanked samples **Inclusion criteria** Pregnant women undergoing invasive test **Exclusion criteria** Not specified **Gestational age at sampling** 10–34 weeks **Maternal age** Range 20–45 years **Drop-outs** 1 due to failure in gender classification (calculated as ITT)	**Platform** NIPT MPSS **Reference test** Karyotype	**Monosomy X** TP 3 FP 1 FN 1 TN 898 Sensitivity: 75% Specificity: 99.9% **XXY** TP 2 FP 0 FN 0 TN 901 Sensitivity: 100% Specificity: 100% **XYY** TP 1 FP 0 FN 0 TN 902 Sensitivity: 100% Specificity: 100%	Moderate **Commercial partner** BGI

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Table 11.1 continued

First author Year Reference Country	Study design	Population	Index test platform Reference test	Results for diagnosed trisomy or sex chromosome aneuploidies	Study quality Comments
Lau 2014 [26] Hong Kong	**Study design** Prospective cohort single center study Blinded **Time of study** August 2011 to February 2013	**Population** n=1 982 (total) n=1 981(analysed) **Samples** Not biobanked samples 1 929 singleton, 30 twin fetuses, 23 internal controls (anomaly detected by USS) **Inclusion criteria** Physician request of NIPT, pregnancy >12 weeks **Exclusion criteria** Major anomaly detected by USS **Gestational age at sampling** Median 14.5 weeks **Maternal age** Mean 36 years (20–46) **Drop-outs** Failed analysis n=1 Repeat blood sample n=23 Lost to follow-up 14.4%	**Platform** NIPT Not specified **Reference test** Karyotype, QF-PCR or CMA (only NIPT positive & on 23 internal controls) NIPT negative cases were contacted within 3 months after expected delivery for a clinical outcome follow up	**Monosomy X** (1 identified with NIPT, 1 analysed with karyotype) TP 1 FP 0 Lost to follow-up: 0 **XXX** (4 identified with NIPT, 2 analysed with karyotype) TP 2 FP 0 Lost to follow-up: 2 **XXY/XYY** (3 identified with NIPT, 2 analysed for karyotype) TP 2 FP 0 Lost to follow-up: 1 **Autosomal trisomy** 4/6 detected trisomies with NIPT were analysed with karyotyping TP 0 FP 4 **Triple trisomy** 1 detected with NIPT but confirmed confined to placental mosaicism **Deletion/duplication** 1 partial trisomy 18 1 partial monosomy 18 Confirmed for fetal or maternal origin	Moderate **Commercial interests** BGI

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Table 11.1 continued

First author Year Reference Country	Study design	Population	Index test platform Reference test	Results for diagnosed trisomy or sex chromosome aneuploidies	Study quality Comments
Liang 2013 [31] China	**Study design** Multicenter prospective cohort study Blinded **Time of study** March 2009 to June 2011	**Population** High risk for fetal aneuploidy n=435 (total) n=412 (analysed) **Samples** Biobanked samples **Inclusion criteria** Positive maternal serum screen n=217 Anomaly detected by USS n=67 Prior aneuploidy pregnancy n=4 AMA ≥35 n=84 More than one indication n=63 **Exclusion criteria** Not specified **Gestational age at sampling** Median 21 weeks (11–39 weeks) **Maternal age** Mean 31 years **Drop-outs** n=11 karyotypes missing n=12 failed sequencing	**Platform** NIPT Not specified **Reference test** Karyotype	**Monosomy X** (3 mosaic 0X treated as diploid: 1 detected and 2 mosaic missed in sequencing) TP 5 FP 1 FN 0 TN 406 Sensitivity: 100% Specificity 99.8% **XXX** TP 1 FP 0 FN 0 TN 411 Sensitivity and Specificity: 100% **XXY** TP 1 FP 0 FN 0 TN 411 Sensitivity and Specificity: 100% **XYY** TP 1 FP 0 FN 0 TN 411 Sensitivity and Specificity: 100% **Trisomy 9** TP 1 FP 0 FN 0 TN 411 Sensitivity and Specificity: 100%	Moderate **Commercial partner** Berry Genomics

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Table 11.1 continued

First author Year Reference Country	Study design	Population	Index test platform Reference test	Results for diagnosed trisomy or sex chromosome aneuploidies	Study quality Comments
Mazloom 2013 [32] USA	**Study design** Prospective cohort study Blinded **Time of study** Not reported	**Population** High risk for fetal aneuploidy Validation set only population n=411 Successful samples n=390 **Samples** Biobanked samples **Inclusion criteria** Invasive sampling for karyotyping due to high risk for fetal aneuploidy **Exclusion criteria** Multiple gestation, mosaicism for sex chromosomes; no documented karyotype reported **Gestational age at sampling** 8–29 weeks **Maternal age** Median 36 years (19 to 47) **Drop-outs** Non reportable results n=21 (5%)	**Platform** NIPT MPSS **Reference test** Karyotype/FISH or both	**Monosomy X** TP 17 FP 1 FN 1 TN 371 **XXX** TP 1 FP 0 FN 0 TN 389 **XXY** TP 5 FP 0 FN 0 TN 385 **XYY** TP 2 FP 0 FN 0 TN 388	Moderate **Commercial partner** Sequenom Inc

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Table 11.1 continued

First author Year Reference Country	Study design	Population	Index test platform Reference test	Results for diagnosed trisomy or sex chromosome aneuploidies	Study quality Comments
Nicolaides 2013 [33] United Kingdom	**Study design** Prospective cohort study Blinded **Time of study** Not reported	**Population** High risk for fetal aneuploidy n=242 (total) n=229 (analysed) **Samples** Biobanked samples **Inclusion criteria** AMA Prior aneuploid pregnancy Positive maternal serum screen **Exclusion criteria** Not specified **Gestational age at sampling** 11–14 weeks **Maternal age** Median 36 years (18–47) **Drop-outs** n=13 failed quality control	**Platform** NIPT SNP **Reference test** Karyotype	**Monosomy X** TP 2 FP 0 FN 0 TN 227 **Triploidy** TP 1 FP 0 FN 0 TN 228	Moderate **Commercial partner** None reported (personal communication)

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Table 11.1 continued

First author Year Reference Country	Study design	Population	Index test platform Reference test	Results for diagnosed trisomy or sex chromosome aneuploidies	Study quality Comments
Pergament 2014 [17] USA	**Study design** Prospective cohort multicenter study Blinded **Time of study** Not reported	**Population** n=1 064 (total) n=966 (analysed) **High risk for fetal aneuploidy** n=543 (total) n=492 (analysed, mosaic monosomy X cases included) **Low risk for fetal aneuploidy** n=521 (total) n=474 (analysed, mosaic monosomy X cases included) **Samples** Not biobanked samples **Inclusion criteria** >18 years Singleton pregnancy >7 weeks **Exclusion criteria** Confirmed sex chromosome aneuploidy, triploidy or fetal mosaicism **Gestational age at sampling** Median 14 weeks (7–40) **Maternal age** Median 30 years (18–47) **Drop-outs** Did not pass quality control n=85 Fetal mosaic monosomy X n=2	**Platform** NIPT SNP **Reference test** Karyotype or FISH	**Monosomy X** TP 9 FP 1 FN 1 TN 953 Sensitivity: 90 (95% CI, 55.5; 99.8) Specificity: 99.9 (95% CI, 99.4; 100) **Monosomy X including mosaic karyotypes** TP 11 FP 1 FN 1 TN 953 Sensitivity: 91.7 (95% CI, 61.5; 99.8) Specificity: 99.9 (95% CI, 99.4; 100)	Moderate **Commercial partner** Natera

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Table 11.1 continued

First author Year Reference Country	Study design	Population	Index test platform Reference test	Results for diagnosed trisomy or sex chromosome aneuploidies	Study quality Comments
Porreco 2014 [29] USA	**Study design** Prospective multicenter observational study Blinded **Time of study** September 2009 to April 2011	**Population** High risk for fetal aneuploidy n=4 170 (total), n= 3 430 (selected) Successful samples for outcome of X or XXX n=3 278 Successful samples for outcome of XXY or XYY n=3 201 **Samples** Biobanked samples **Inclusion criteria** AMA, Personal or family history Positive maternal serum screen Anomaly detected by USS , Singleton pregnancy ≥18 years of age **Exclusion criteria** Inability to give written informed consent, multiple gestation, fetal demise of additional embryo during current pregnancy Insufficient sample volume Outside time window for laboratory processing **Gestational age at sampling** Mean 16 weeks (9–37) **Maternal age** Mean 35 years (18–50) **Drop-outs** Low quantity of fetal DNA or complex karyotypes: Outcome X or XXX n=152 (4.6%) Outcome XXY or XYY n=229 (7.1%)	**Platform** NIPT MPSS **Reference test** Karyotyping	**Monosomy X** TP 9 FP 11 FN 0 TN 3 258 Sensitivity: 100 (95% CI, 66.4; 100) Specificity: 99.7 (95% CI, 99.4; 99.8) **XXX** TP 4 FP 3 FN 0 TN 3 271 Sensitivity: 100 (95% CI, 39.8; 100) Specificity: 99.9 (95% CI, 99.7; 100) **XXY** TP 1 FP 2 FN 0 TN 3 198 Sensitivity: 100 (95% CI, 2.50; 100) Specificity: 99.9 (95% CI, 99.8; 100) **XYY** TP 1 FP 0 FN 0 TN 3 200 Sensitivity: 100 (95% CI, 2.5; 100) Specificity: 100 (95% CI, 99.9; 100)	Moderate **Commercial partner** Sequenom

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Table 11.1 continued

First author Year Reference Country	Study design	Population	Index test platform Reference test	Results for diagnosed trisomy or sex chromosome aneuploidies	Study quality Comments
Shaw 2014 [34] Taiwan	**Study design** Prospective multi-center cohort study Blinded **Time of study** June to December 2012	**Population** n=201 (total) n=200 (analysed) **High risk** n=100 (total and analysed) **Low risk** n=101 (total) n=100 (analysed) **Samples** Not biobanked samples **Gestational age at sampling** High risk: Mean 17 weeks Low risk: Mean 16 weeks **Inclusion criteria** High risk: Risk of 1:30, NT >3.0 mm Low risk: Risk of 1:1 500 **Exclusion criteria** Not specified **Maternal age** High risk: Mean 35 years Low risk: Mean 35 years **Drop-outs** Due to early gestational age n=1	**Platform** NIPT MPSS **Reference test** Karyotype and phenotype at birth In the high risk group all samples were analysed with karyotype	**High risk group** **Monosomy X** TP 3 FP 0 FN 1 TN 96 **XYY** TP 1 FP 0 FN 0 TN 99 **Trisomi 16** TP 1 FP 0 FN 0 TN 99 **Low risk group 45X, 47XYY or Trisomi 16** TP 0 FP 0 FN 0 TN 100	Moderate **Commercial partner** Berry genomics

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Table 11.1 continued

First author Year Reference Country	Study design	Population	Index test platform Reference test	Results for diagnosed trisomy or sex chromosome aneuploidies	Study quality Comments
Song 2013 [25] China	**Study design** Prospective cohort study Not Blinded **Time of study** April to December 2011	**Population** High risk for fetal aneuploidy n=1 916 (total) n=1 741 (analysed by DNA sequencing) n=202 (karyotype) **Samples** Not biobanked samples **Inclusion criteria** Singleton pregnancies Maternal age less than 35 years Positive maternal serum screen **Exclusion criteria** Not specified **Gestational age at sampling** Mean 17 weeks (11–21) **Maternal age** Mean 29 years **Drop-outs** Failed sequencing quality control n=73 Lost to follow-up, birth outcome n=111	**Platform** NIPT Not specified **Reference test** Karyotyping	**Monosomy X** (2 identified with NIPT, 2 analysed with karyotype) TP 2 FP 0 Lost to follow-up: 0 **XXY** (1 identified with NIPT, 1 analysed with karyotype) TP 0 FP 1 Lost to follow-up: 0	Moderate **Commercial partner** None reported

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Table 11.1 continued

First author Year Reference Country	Study design	Population	Index test platform Reference test	Results for diagnosed trisomy or sex chromosome aneuploidies	Study quality Comments
Song 2015 [24] China	**Study design** Prospective cohort study Blinded **Time of study** May 2012 to August 2013	**Population** High risk for fetal aneuploidy n=213 (total) n=178 (analysed by both methods) **Samples** Not biobanked samples **Inclusion criteria** AMA >35 years Singleton pregnancy **Exclusion criteria** Not specified **Gestational age at sampling** 8–12 weeks **Maternal age** Mean 37 years (35–45) **Drop-outs** Failed quality control n=1 No karyotype results n=34	**Platform** NIPT Not specified **Reference test** Karyotype	**Monosomy X** TP 0 FP 1 FN 1 (mosaic 45X/47XXX) TN 176 **XXY** TP 0 FP 0 FN 1 (mosaic 45X/47XXX) TN 177 **XXX** TP 0 FP 0 FN 1 TN 177	Moderate **Commercial partner** Berry Genomics Co, Ltd

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Table 11.1 continued

First author Year Reference Country	Study design	Population	Index test platform Reference test	Results for diagnosed trisomy or sex chromosome aneuploidies	Study quality Comments
Yao 2014 [27] China	**Study design** Retrospective analysis, cohort study Not blinded **Time of study** June 2011 to December 2012	**Population** High risk for fetal aneuploidy n=5 950 (analysed by DNA sequencing) n=24 (karyotyping) n=5 466 (pregnancy outcome) **Samples** Not biobanked samples **Inclusion criteria** Singleton pregnancy Pre-test counseling **Exclusion criteria** Not specified **Gestational age at sampling** Mean 19 weeks **Maternal age** Mean 30 years **Drop-outs** Lost to follow-up, pregnancy outcome n=408 Of the 33 with a positive NIPT result for SCA 8 declined invasive confirmatory test One karyotype which was indicated as monosomy X failed	**Platform** NIPT Not specified **Reference test** Karyotyping or pregnancy outcome	**Monosomy X** (7 identified with NIPT, 5 analysed with karyotype) TP 2 FP 3 Lost to follow-up: 2 **XXX** (14 identified with NIPT, 10 analysed with karyotype) TP 7 FP 3 Lost to follow-up: 4 **XXY** (9 identified with NIPT, 7 analysed with karyotype) TP 3 FP 4 Lost to follow-up: 2 **XYY** (3 identified with NIPT, 2 analysed with karyotype) TP 1 FP 1 Lost to follow-up: 1	Moderate **Commercial partner** BGI-Shenzehen

AMA = Advanced maternal age; **CI** = Confidence interval; **CMA** = Chromosomal microarray analysis; **CNV** = Copy number variations; **DNA** = Deoxyribonucleic acid; **FISH** = Fluorescent in situ hybridization; **FN** = False negative; **FP** = False positive; **ITT** = Intention to treat; **MPSS** = Massive parallel shotgun sequencing; **n** = Number; **NIPT** = Non-invasive prenatal testing; **QF-PCR** = Quantitative fluorescence polymerase chain reaction; **SCA** = Sex chromosome; **SNP** = Single nucleotide polymorphism; **T-MPS** = Targeted massive parallel sequencing; **TN** = True negative; **TP** = True positive; **USS** = Ultrasound screening

Table 11.2 Studies with moderate risk of bias investigating Next-generation sequencing on microdeletion/duplications or whole genome coverage.

First author Year Reference Country	Study design	Population	Index test platform Reference test Verification test	Results	Study quality Comments
Chen 2013 [19] China	**Study design** Cohort study Blinded **Time of study** Not reported	**Population** Total n=1 451 140 control samples with normal karyotype to develop algorithm Tested n=1 311 **Samples** Biobanked NIPT **Gestational age at sampling** Mean 21 weeks (10–28) **Inclusion criteria** Women undergoing invasive prenatal diagnosis after maternal blood samples had been collected **Exclusion criteria** Not specified **Maternal age** Mean 32 years **Drop-outs** Not specifed	**Platform** FCAPS **Reference test** Karyotype	**Diagnosed CNV of >10 kb** TP 3 FP 1 Sensitivity: 100% Specificity: 99.92%	Moderate **Commercial partner** BGI-Shenzehen

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Table 11.2 continued

First author Year Reference Country	Study design	Population	Index test platform Reference test Verification test	Results	Study quality Comments
Helgeson 2015 [35] USA	**Study design** Prospective cohort study Not Blinded **Time of study** October 2013 to October 2014	**Population** n=175 393 n=123 096 analysed for deletions associated with 22q11.2 deletion syndrome, Cri du chat syndrome, Prader Willi and Angelman syndrome only **Samples** High risk for fetal aneuploidy (NIPT) **Inclusion criteria** High risk pregnancies including AMA, anomaly detected by USS, positive maternal serum screening, personal or family history **Exclusion criteria** Not specified **Gestational age at sampling** Not specified **Maternal age** Not specified **Drop-outs** Of the 55 that received a diagnosis based on the NIPT test, 2 were lost to follow-up. The number of dropouts and analysis failures from the entire cohort is not reported	**Platform** Not specified **Verification** CMA or FISH, clinical findings	**Diagnoses n=55** 1p36 deletion n=5 (TP 3, FP 1, 1 lost to follow-up) Wolf-Hirschhorn (4p16.3) n=1 (TP 1) Cri du chat (5p15) n=6 (TP 4, FP 2) Prader Willi/Angelman (15q11.2–q13) n=9 (TP 8, 1 positive based on clinical findings) 22q11.2 deletion n=32 (both of maternal and fetal origin) (TP 23, 8 positive based on clinical findings 1 lost to follow-up) Jacobsen (11qter) n=1 (TP 1) Langer-Giedion (8q24.1) n=1 (1 positive based on clinical findings)	Moderate **Commercial partner** Authors employees at Sequenom laboratories

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Table 11.2 continued

First author Year Reference Country	Study design	Population	Index test platform Reference test Verification test	Results	Study quality Comments
Li 2016 [36] China	**Study design** Retrospective cohort study Blinded **Time of study** September to December 2014	**Population** n=117 (total) n=117 (analysed) **Samples** NIPT on biobanked samples CMA on invasive samples CVS n=20 AF n=67 Cord blood n=38 **Inclusion criteria** Women where invasive samples were analysed using CMA Anomaly detected by USS AMA Maternal anxiety **Exclusion criteria** Twin pregnancies Fetuses with common trisomies or SCA **Gestational age at sampling** Mean 21.3 weeks (12–34) **Maternal age** Mean 30 years (19–40) **Drop-outs** n=0	**Platform** Ion Proton sequencer (Life Technologies) **Library** Ion Plus Fragment Library Kit (Life Technologies) **Reference** CMA Cytoscan 750K Affymetrix reporting threshold 100kb	**Diagnosed CNV ≥1 Mb** TP 11 FP 4 TN 95 FN 7 Sensitivity: 61.1% Specificity: 95% **False positive rate** 5%	Moderate **Commercial partner** None reported

AMA = Advanced maternal age; **AF** = Amniotic fluid; **CMA**= Chromosomal microarray analysis; **CNV** = Copy number variations; **CVS** = Chorionic villus sampling; **FCAPS** = Fetal copy number analysis through maternal plasma sequencing; **FISH** = Fluorescent in situ hybridization; **FN**= False negative; **FP** = False positive; **n** = number; **NIPT**=Non-invasive prenatal testing; **SCA** = Sex chromosome aneuploidies; **TN**= True negative; **TP** = True positive; **USS** = Ultrasound screening

Table 11.3 Studies with high risk of bias investigating Next-generation sequencing on microdeletion/duplications or whole genome coverage.

First author Year Reference Country	Study design	Population	Index test platform Reference test Verification test	Results	Study quality Comments
Drury 2015 United kingdom [37]	**Study design** Proof of principle cohort study Blinded	**Population** n=24 (total) n=24 (analysed) **Samples** Invasive samples AF or CVS **Inclusion criteria** Anomaly detected by USS, including NT >3.5 **Exclusion criteria** Not sufficient DNA, abnormal karyotype, fetuses were CNVs had been identified by CMA **Gestational age at sampling** Not specified **Maternal age** Not specifed **Drop-outs** Outcome of pregnancy: 2 cases lost to follow-up	**Platform** HiSeq 1 000 or HiSeq 2 500 (Illumina) or outsourcing to BGI Genomics **Library** TruSeq Exome (Illumina), Nextera Rapid exome kit (Illumina) or SureSelect All exon V (Agilent Biosystems) **Reference** Karyotype, CMA **Verification** Sanger sequencing	**Diagnoses** 5 definitive diagnoses (Milroy disease, hypophosphatasia, achondrogenesis type 2, Freeman-Sheldon syndrome and Baraitser-Winter Syndrome) 1 plausible diagnosis (orofaciodigital syndrome type 6)	Low **Commercial partner** Not reported

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Table 11.3 continued

First author Year Reference Country	Study design	Population	Index test platform Reference test Verification test	Results	Study quality Comments
Fan 2012 [20] USA	**Study design** Proof of principle case control study	**Population** **Case 1** Normal karyotype **Case 2** 22q11.2 microdeletion Maternal plasma and cord blood **Gestational age at sampling** Patient 1: First, second and third trisemester Patient 2: Third trimester	**Platform** GAII and Hiseq (Illumina) **Library** SeqCap EZ Human Exome Kit v2.0 (Roche) **Reference test** Karyotype	**Diagnoses** 22q11.2 microdeletion	Low **Commercial partner** BGI-Shenzehen
Jensen 2012 [21] USA	**Study design** Proof of principle case control study Not Blinded **Time of study** Not reported	**Population** **Cases** 2 fetuses with known 22q11.2 microdeletions **Control** 14 fetuses low risk for chromosomal aberration **Samples** NIPT **Gestational age at sampling** Not specified **Maternal age** Not specified **Drop-outs** Not reported	**Platform** Hiseq 2 000 (Illumina) **Library** TruSeq (Illumina) **Coverage** 3.1-fold, 4.4-fold **Repeated** 15.87-fold, 16.77-fold **Reference test** Karyotype one of the two cases only	**Diagnoses** 22q11.2 microdeletion Both affected fetuses were detected	Low **Commercial partner** Sequenom laboratories

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Table 11.3 continued

First author Year Reference Country	Study design	Population	Index test platform Reference test Verification test	Results	Study quality Comments
Wapner 2014 [23] USA	**Study design** Proof of principle case control study Blinded	**Population** n=469 (total) Cases n=6 Control n=352 Artificial DNA n=111 (both cases and controls) **Samples** Biobanked maternal plasma or artificial DNA **Drop-outs** Did not pass quality control n=23	**Platform** PCR based, using SNPs, NATUS algorithm	**Diagnoses** 1p36 microdeletion n=1 TP 1, FP 0 Cri du chat (5p15) n=24 TP 24, FP 1 Prader Willi/Angelman (15q11.2–q13) n=36 TP 36, FP 0 22q11.2 deletion n=46 TP 45, FP 3	Low **Commercial partner** Natera

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Table 11.3 continued

First author Year Reference Country	Study design	Population	Index test platform Reference test Verification test	Results	Study quality Comments
Yu 2013 [18] Hong Kong	**Study design** Proof of principle case control study Not blinded	**Samples** 6 cases (3 post-invasive, 3 pre-invasive) **Case 1** Post-invasive,cordocentesis 22q11.2 microdeletion, FISH **Case 2** Post-invasive, cordocentesis 22q11.2 microdeletion, FISH **Case 3** Post-invasive amniocentesis 22q11.2 microdeletion, QF-PCR and FISH **Case 4** Pre-invasive Chorionic villus sampling 22q11.2 microduplication (2.4 Mb) Array CGH **Case 5** Pre-invasive Amniocentesis 22q11.2 microduplication (2.4 Mb) Array CGH **Case 6** Pre-invasive amniocentesis 3q29 microduplication (5.1 Mb); 4q32.1-q35.2 microdeletion (32.9 Mb) Array CGH 8 controls	**Platform** HiSeq 2 000 (Illumina) **Library** TruSeq (Illumina) **Coverage** 3 Mb Resolution **Reference test** Karyotype CMA FISH QF-PCR	**Diagnoses** All 6 cases detected	Low **Commercial partner** None reported

AF = Amniotic fluid; **CGH** = Comparative genomic hybridization; **CMA** = Chromosomal microarray analysis; **CNV** = Copy number variations; **CVS** = Chorionic villus sampling; **DNA** = Deoxyribonucleic acid; **FISH** = Fluorescent in situ hybridization; **FP** = False positive; **Mb** = Megabases; n = Number; **NIPT** = Non-invasive prenatal testing; **NT** = Nuchal translucency; **PCR** = Polymerase chain reaction; **QF-PCR** = Quantitative fluorescence-polymerase chain reaction; **SNP** = Single nucleotide polymorphism; **TP** = True positive; **USS** = Ultrasound screening

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