Clinical Utility of Noninvasive Prenatal Screening for Rare Chromosome Abnormalities in Singleton Pregnancies

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Abstract

Objective: To systematically investigate the clinical utility of noninvasive prenatal screening (NIPS) commercially used for the common fetal aneuploidies as a prenatal screening tool for rare chromosome abnormalities (RCAs). Design: Prospective study. Setting: Hospital-based. Population or Sample: 528 gravidas with positive NIPS results for RCAs. Methods: Gravidas with positive NIPS results for RCAs subsequently underwent amniocentesis for single nucleotide polymorphism array (SNP-array) were recruit. The degrees of concordance between NIPS and SNP-array were classified into full concordance, partial concordance, discordance related and discordance. Main Outcome Measures: The positive predictive values (PPVs) for rare aneuploidies and segmental imbalances, while incidental findings for regions of homozygosity/uniparental disomy (ROH/UPD), were used to evaluate the performance of NIPS. Results: Of the 528 gravidas with positive NIPS results, 29.2% were confirmed with positive prenatal SNP-array results (154/528). The PPVs for rare aneuploidies and segmental imbalances were 6.1% (7/115) and 21.1% (87/413), respectively. ROH/UPDs, as incidental findings, have been identified in 9.5% (50/528) of gravidas with positive NIPS results. The PPV for clinical significant findings was 8.9% (47/528), including 7 cases with mosaic rare aneuploidies, 35 with pathogenic/likely pathogenic copy number variants, and 5 with imprinting disorders. Conclusions: NIPS commercially used for the common fetal aneuploidies yielded low PPV for rare aneuploidies, moderate PPV for segmental imbalances, and incidental findings for ROH/UPD. For the low PPV for clinical significant findings, NIPS has limited clinical utility for RCAs. Prenatal SNP-array should be regarded as the first-tier test for positive NIPS, particularly for those involved imprinted chromosomes.

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Short Title: NIPS for rare chromosome abnormalities

ABSTRACT

Objective: To systematically investigate the clinical utility of noninvasive prenatal screening (NIPS) commercially used for the common fetal aneuploidies as a prenatal screening tool for rare chromosome abnormalities (RCAs).

Design: Prospective study.

Setting: Hospital-based.

Population or Sample: 528 gravidas with positive NIPS results for RCAs.

Methods: Gravidas with positive NIPS results for RCAs subsequently underwent amniocentesis for single nucleotide polymorphism array (SNP-array) were recruit. The degrees of concordance between NIPS and SNP-array were classified into full concordance, partial concordance, discordance related and discordance.

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Results: Of the 528 gravidas with positive NIPS results, 29.2% were confirmed with positive prenatal SNP-array results (154/528). The PPVs for rare aneuploidies and segmental imbalances were 6.1% (7/115) and 21.1% (87/413), respectively. ROH/UPDs, as incidental findings, have been identified in 9.5% (50/528) of gravidas with positive NIPS results. The PPV for clinical significant findings was 8.9% (47/528), including 7 cases with mosaic rare aneuploidies, 35 with pathogenic/likely pathogenic copy number variants, and 5 with imprinting disorders.

Conclusions: NIPS commercially used for the common fetal aneuploidies yielded low PPV for rare aneuploidies, moderate PPV for segmental imbalances, and incidental findings for ROH/UPD. For the low PPV for clinical significant findings, NIPS has limited clinical utility for RCAs. Prenatal SNP-array should be regarded as the first-tier test for positive NIPS, particularly for those involved imprinted chromosomes.

Key Words:

Chromosomal microarray analysis; Noninvasive prenatal screening; Prenatal diagnosis; Rare aneuploidy; Rare chromosomal abnormality; Regions of homozygosity; Segmental imbalance; Uniparental disomy

INTRODUCTION

Noninvasive prenatal screening (NIPS), also referred to as cell-free fetal DNA (cff-DNA) testing, mainly based on massively parallel sequencing (MPS), has been available to screen for the common fetal aneuploidies in more than 60 countries since 2011[1]. NIPS was highly sensitive and specific for detection of trisomy 13, 18, and 21[2,3], which led to a reduction in invasive diagnostic testing requests by up to 40% to avoid procedure-related miscarriage risk[4]. Recently, rare autosomal trisomies, well-known microdeletion/microduplication syndromes (MMS), as well as genome-wide copy number variants (CNVs), have been added by some laboratories as expanded screening items[5-7]. However, while the primary source of cff-DNA in the maternal circulation is apoptosis of placental cells from the cytotrophoblast[8,9], mixed with maternal cell-free DNA, various factors affect the accuracy of NIPS results, including confined placental mosaicism (CPM), maternal

genomic contribution[10]. Thus, all patients with positive NIPS results should be confirmed by invasive diagnostic testing[11,12].

Chromosomal microarray analysis (CMA), a high-resolution genomic technology to detect CNVs, has been recommended as a first-tier test for postnatal evaluation of individuals with unexplained developmental delay, intellectual disability, autism spectrum disorders, or multiple congenital anomalies, as well as for prenatal evaluation of fetuses with structural anomalies observed by ultrasound[13-15]. Furthermore, single-nucleotide polymorphism (SNP) array can additionally identify haploidy, triploidy, and regions of homozygosity (ROH)[16]. The pathogenesis of ROH includes imprinting effects caused by uniparental disomy (UPD)[17], as well as increased susceptibility to complex diseases caused by homozygous mutations of autosomal-recessive genes[18,19].

Several studies expanded the utility of NIPS for specific MMS, including DiGeorge syndrome (DGS), Prader-Willi/Angleman syndrome (PWS/AS), cri du chat (CDC), and 1p36 microdeletion (1p36 del) syndrome with moderate to high positive predictive values (PPVs) for these diseases[20-23]. However, there is still a paucity of research focusing on rare chromosome abnormalities (RCAs) detected by NIPS commercially used for the common fetal aneuploidies. In this study, we conducted a prospective study to systematically evaluate the clinical utility of NIPS as a prenatal screening tool for detection of RCAs, including aneuploidies, segmental imbalances and ROH/UPD, for a cohort of 158,919 singleton pregnancies.

MATERIALS AND METHODS

Patients

From January 2016 to December 2020, singleton pregnancy cases at a tertiary level referral center (West China Second University Hospital, Sichuan University) were recruited for this study. Pretest counseling was performed by trained clinical geneticists. Prior to NIPS or SNP-array analysis, written informed consent was obtained from all gravidas, who agreed to be subjected to NIPS or consecutive amniocentesis due to positive NIPS results. The study was approved by the Medical Ethics Committee of West China Second University Hospital.

For NIPS, inclusion criteria were as follows: (1) advanced maternal age (AMA, [?]35 years) declined invasive procedure; (2) high risk for first or second trimester maternal serum screening (T21 [?]1/270, T18 [?]1/350) declined invasive procedure; (3) intermediate risk for maternal serum screening (T21: 1/270˜1/1000, T18: 1/350˜1/1000); (4) fetuses with soft markers detected by ultrasound, including nuchal translucency (NT) >2.5 mm; (5) positive family history, such as affected offsprings with Down syndrome; (6) pregnancies had no clinical indications. Exclusion criteria were as follows according to current standard practice in China: (1) pregnancy gestation period <12 weeks; (2) fetal structural anomalies detected by ultrasound before NIPS; (3) pregnant women with chromosomal abnormalities; (4) multiple pregnancies or co-twin's demise after 12 weeks; (5) pregnant women who have received stem cell therapy, transplant surgery, allogeneic blood products or immunotherapy with 1 year; (6) pregnant women with malignant tumor. A total of 10 ml blood samples from gravidas were collected in Cell-Free DNA BCT tubes (Streck, Omaha, USA).

All gravidas with positive NIPS results for RCAs, including rare aneuploidies and segmental imbalances, were advised to perform amniocentesis for SNP-array experiments after 16 gestational weeks. Exclusion criteria were as follows: (1) positive NIPS results for common trisomies (T21/T18/T13); (2) positive NIPS results for sex chromosome aneuploidies (SCAs); (3) fetal structural anomalies detected by ultrasound before amniocentesis; (4) pregnant women who declined amniocentesis or who underwent amniocentesis for traditional cytogenetics (e.g. karyotype alone) but declined SNP-array analysis. A total of 20 ml fetal samples were obtained through amniocentesis. Clear amniotic fluid samples were tested directly while blood-stained amniotic fluid samples were cultured before SNP-array experiments. Additionally, peripheral blood samples of the parents were obtained to confirm the fetal CNVs that were inherited or *de novo* , and separate ROH into UPD or consanguinity.

Noninvasive prenatal screening

Plasma of blood samples was isolated within 24 h with a two-step centrifugation. The procedures including cell-free DNA extraction, purification, library construction, and quantification were using the fetal chromosome aneuploidy (T21/T18/T13) test kit (Berry Genomics, Beijing, China). Massively parallel sequencing was performed on the NextSeq CN500 platform (Illumina) with 36-bp single-end reads, resulting in 5 Mb total reads, which corresponds to 0.05× human genome depth. GC-bias were eliminated by using bioinformatics methods combined with a local weighted polynomial regression. Raw reads were aligned to the human reference genome GRCh37 (hg19). Each chromosome with an absolute value of Z-score greater than 3 was marked with chromosome aneuploidies. CNVs were detected using RUPA algorithm developed by Berry Genomics.

Chromosomal microarray analysis

This procedure was described in our previous study[24]. While the limit with which CMA can be expected to detect low-level mosaicism is 10˜20%[25-27], we simultaneously performed fluorescence in situ hybridization analysis (FISH) when mosaicism ([?]10%) was detected by CMA.

Data analysis

Positive results of NIPS for rare RCAs were classified into 2 groups: (1) rare aneuploidies, and (2) segmental imbalances, while positive results of CMA were classified into 3 groups: (1) rare aneuploidies (including mosaic aneuploidies), (2) segmental imbalances, (3) ROH/UPD. Furthermore, segmental imbalances detected by CMA were divided into groups with CNV sizes $<$ 5Mb, 5˜10 Mb, and $>$ 10 Mb.

The NIPS results were compared with those of CMA. The compared results were classified into 4 categories: (1) Full concordance: those with consistent aneuploidy results, or with consistent chromosome arm and copy number gain/loss between NIPS and CMA; (2) Partial concordance: at least one of the findings was consistent but additional findings were detected only by one platform (NIPS/CMA) (For example, NIPS is positive for 20p duplication, CMA detected 20p13p12.1 duplication and 9p24.3p23 deletion); (3) Discordance, related: aneuploidies or segmental imbalances detected by NIPS but ROH/UPD confirmed by CMA; (4) Discordance: none of the findings detected by NIPS and CMA were consistent (For example, 1. NIPS is positive for T6, CMA with negative result; or 2.NIPS is positive for T7, CMA detected 16p11.2 deletion).

Clinical follow-up assessments

Clinical follow-up assessments were performed on all gravidas underwent amniocentesis for SNP-array analysis. This procedure was described in our previous study[24].

Statistical analysis

Statistical analysis was performed using the SPSS Statistics software v24.0 (IBM SPSS, Armonk, NY, USA). Continuous variables were compared with the Student's t -test, and categorical variables were compared with the use of chi-square or Fisher exact analysis, as appropriate. A p -value < 0.05 was considered to indicate statistical significance.

RESULTS

Patient characteristics

A total of 158,919 gravidas were recruited to perform NIPS in the 5-year-period prospective study to evaluate the clinical value of NIPS for rare RCAs, including aneuploidies, segmental imbalances and ROH/UPD. Test failed in 95 cases, with a failure rate of 0.1%. After excluding 508 (0.3%) cases with screen positive results for common trisomies (T21/T18/T13) and 921 (0.6%) cases for SCAs, in the 842 (0.5%) gravidas with screen positive results for RCAs, 528 gravidas were obtained consecutive amniocentesis for SNP-array experiments. The study flow diagram is illustrated in Figure 1. The maternal age ranged from 17 to 44 years (29.1+-4.7 years), 13.8% (73/528) of the gravidas were of advanced maternal age, and the gestational age for amniocentesis ranged from 17 to 30^{+1} weeks (20.5+-3.3 weeks).

Of the 528 positive NIPS results, there were 115 fetuses at high risk for rare aneuploidies, including 10 fetuses involved multiple chromosomes, while the remaining 413 fetuses were at high risk for segmental imbalances, including 16 fetuses involved multiple chromosomes. The SNP-array was successfully performed in all gravidas, while 154 (29.2%) cases were with positive results, including 7 (4.5%, 7/154) fetuses with mosaic rare aneuploidies, 97 (63.0%, 97/154) fetuses with segmental imbalances and 50 (32.5%, 50/154) fetuses with UPD/ROH (Table 1). Concordance between RCAs detected by NIPS and consecutive CMA results was shown in Table 2. No significant difference in maternal age was observed between positive and negetive SNP-array group (28.8+-5.1 years vs 29.2+-4.5, P =0.350).

Rare aneuploidies

In our study, all the rare aneuploidies were confirmed to be mosaicism by SNP-array, accounted for 1.3% (7/528) of positive NIPS results with full concordance (Table 2). The most common aneuploidy was mosaic trisomy 9, while the 5 cases with the mosaic proportion ranged from 15% to 29%. The other 2 cases were mosaic trisomy 15 (26%) and mosaic trisomy 16 (13%). All the mosaic aneuploiedies were simultaneously confirmed by FISH on amniotic fluid. Thus, the PPV for rare aneuploidies was 6.1% (7/115, 95% confidence intervals (CI), 1.7% - 10.5%).

Segmental imbalances

A total of 111 segmental imbalances were detected by SNP-array in 18.4% (97/528) cases with positive NIPS results, including 14 gravidas with multiple CNVs. There were 37 clinical significant CNVs involved 21 pathogenic (P) CNVs and 16 likely pathogenic (LP) CNVs, accounted for 33.3% of segmental imbalances (37/111), with the concordant rate of 78.4% (29/37). Approximate 57.7% (64/111) of CNVs were <5 Mb, 19.8% (22/111) were ranged from 5 to 10 Mb, and 22.5% (25/111) were >10 Mb, with the concordant rate of 75.0% (48/64), 86.4% (19/22), and 88.0% (22/25) between NIPS and SNP-array, respectively (P =0.276).

Parental confirmations by SNP-array were performed in 55.7% (54/97) cases while fetuses were detected with segmental imbalances by CMA, including 34 cases with maternal inheritance, 3 cases with paternal inheritance and 17 cases in *de novo* manner. The proportion of full concordance between NIPS and CMA in cases with maternally inherited CNVs was significantly higher than those with paternally inherited or *de novo* CNVs (85.3% (29/34) vs 55.0% (11/20), P =0.014).

The PPV for segmental imbalances was 21.1% (87/413, 95% CI, 17.1% - 25.0%), composing of the full concordance rate between NIPS and CMA with 18.2% (75/413, 95% CI, 14.4% - 21.9%), and the partial concordance rate with 2.9% (12/413, 95% CI, 1.3% - 4.5%). The details of segmental imbalances detected by SNP-array were shown in Table 3.

For cases with clinical significant CNVs, the PPV was only 7.0% (29/413, 95% CI, 4.5% - 9.5%). For those well-known MMS[20] confirmed by SNP-array, 50% (2/4) of CDC, all DGS, 22q11.2 duplication and 15q11q13 (PWS/AS) duplication were detected, however, 1p36 deletion was ignored by NIPS. There were 3 clinical significant CNVs associated with regions, for which variable expressivity has been demonstrated with incomplete penetrance ranged from 13.1% to 36.9%[28], including deletion of 1q21.1 recurrent region (BP3_BP4 distal)(includes GJA5), deletion of 16p13.11 recurrent region (BP2_BP3)(includes MYH11), and duplication of 22q11.2 recurrent (DGS/VCFS) region (proximal A_B)(includes TBX1).

In addition, For the 10 cases with submicroscopic unbalance rearrangements, except 1 couple refused to perform karyotyping of themselves (No.84), there were 6 cases inherited from parental balanced translocations (No.78-79,81,83,86-87), 1 case inherited from paternal pericentric invertion (No.77), and 2 cases inherited from mother with intellectual disability who was confirmed with derivative chromosome associated with her fetus (No.80,82).

Regions of homozygosity/ uniparental disomy

We incidentally detected 50 (9.5%) fetuses with ROH larger than 10 Mb, while all the findings by SNP-array was relatively consistent with NIPS results, what was mentioned as aneuploidy or segmental imbalance in

the same chromosome. ROHs were observed on autosomes, and chromosome 16 was most frequently involved (9 cases), subsequently with chromosome 1, 2, 6 (6 cases). None of these fetuses was from consanguineous couples.

In total, 30 cases were confirmed as UPD: 19 cases diagnosed with isodisomy as ROHs detected by SNP-array involved the whole chromosome, including 7 cases confirmed the source of ROHs by parental confirmations; all the 11 cases with iso-heterodisomy were verified by parental blood samples. The most frequent UPD was UPD6 (6 cases), subsequently with UPD4 (4 cases), thirdly with UPD1, UPD2 and UPD16 (3 cases).

There were 21 cases with ROHs associated with imprinted chromosomes. Except 4 couples refused to perform parental confirmations, 5 of the 17 cases was confirmed with imprinting disorders, including Transient Neonatal Diabetes mellitus (pUPD6), Silver-Russell syndrome (mUPD11), Beckwith-Wiedemann syndrome (pUPD11), Temple syndrome (mUPD14) and PWS (mUPD15). The details of ROHs detected by SNP-array were shown in Table 4.

Clinical follow-up assessments

Clinical follow-up results were obtained for 90.2% cases (476/528). Except fetuses lost of follow-up, the rates of normal infant, termination of pregnancy (TOP), and birth with defects (including neonatal demises without physical birth defects) without chromosomal aberrations by SNP-array were 94.9% (314/331), 2.4% (8/331), and 2.7% (9/331), while in those with positive results were 49.7% (72/145), 46.9% (68/145), and 3.4% (5/145), respectively (Table 5).

For the 7 fetuses confirmed with mosaic rare aneuploidies, although no significant ultrasound abnormalities were detected, all these families opted for TOP. For the 97 cases with segmental imbalances, except 5 (5.2%) cases lost during at follow-up, the rate of elective TOP in fetuses with P/LP CNVs (74.3%, 26/35) was significantly higher than those with uncertain clinical significance (VUS) (12.9%, 8/62)(P <0.001). Except for 1 fetus treated with TOP due to ultrasound abnormalities, none of the remaining 7 fetuses with VUS were inherited CNVs. The portion of TOP for fetuses with VUS of *de novo* or refused parental confirmations (25.9%, 7/27) was significantly higher than those with inherited VUS (0.0%, 0/30)(P =0.010). For the 50 cases with ROHs, except 4 (8.0%) cases lost at follow-up, the rate of elective TOP in fetuses with UPD (75.9%, 22/29) was significantly higher than those with ROHs (35.3%, 6/17)(P <0.001). There was no significant difference in the rate of elective TOP between fetuses UPD related imprinting disorders (100.0%, 5/5) and those with UPD unrelated imprinting disorders (70.8%, 17/24)(P= 0.222). The foremost reason for elective TOP in fetuses with UPD unrelated imprinting disorders was fetal growth restriction (FGR) (29.4%, 5/17).

DISCUSSION

Main Findings

The screen positive rate of NIPS for RCAs was 0.5% (842/158,824). For the 528 gravidas underwent amniocentesis for SNP-array, NIPS demonstrated low PPV for rare aneuploidies (6.1%, 7/115), and moderate PPV for segmental imbalances (21.1%, 87/413). In addition, ROH/UPDs were related findings associated with positive NIPS results, with the detection rate of 9.5% (50/528).

The PPV for clinical significant findings was low (8.9%, 47/528), including 7 cases with mosaic rare aneuploidies, 35 with pathogenic/likely pathogenic copy number variants, and 5 with imprinting disorders.

Based on chromosome distributions, the PPV for chromosome 6 in our study was high (87.5%), followed by moderate PPVs for chromosome 12, 22, 5, 16, 1 ranged from 57.1% to 43.8%. There were 20.5% (108/528) gravidas detected with positive NIPS results for chromosoome 7, however, the PPV was extremely low (8.3%, 9/108).

Strengths and Limitations

The significant strength of our study is the large size of the cohort, which enables to perform subgroup

analyses to pick up RCAs including aneuploidies, segmental imbalances and ROH/UPD. Those positive NIPS were compared to the SNP-array results from amniocentesis to determine concordance and PPV. While the challenges of expanding the scope of NIPS evaluations are widely discussed, our data are valuable in charting a path forward for patient care.

There are several limitations in our study. Firstly, we expended the clinical utility of NIPS for RCAs, which was worldwide recommended to screen for traditionally screened aneuploidies. The low depth of sequencing influences the PPVs for RCAs compared to NIPS-Plus. Secondly, our study only included gravidas with positive NIPS results of RCAs who subsequently underwent amniocentesis for SNP-array. We did not followed up those gravaidas with negative NIPS results or refused invasive procedures. Thus, we failed to obtain negative predictive value to comprehensively assess clinical utility of NIPS for RCAs. Thirdly, for the cases with negative CMA results, we did not further obtain maternal or placental results to assess the potential proportion to induce unnecessary invasive procedures. Fourthly, for those fetuses with UPD/ROHs, although parental consanguinity was excluded, autosomal recessive disorders, which were associated with ROHs, were not detect regularly.

Interpretation

Currently, NIPS has been widely used for detection of common fetal aneuploidies as well as SCAs, however, expanding the clinical applications to rare RCAs is still controversial [29,30]. According to the current guidelines[31,32], NIPS is not recommended to screen for rare aneuploidies and genome-wide CNVs because the screening accuracy with regard to detection and false-positive rate is not established. The PPVs for these disorders are much lower than for common trisomies, which may lead to unnecessary invasive procedures[28]. In this study, we conducted a prospective study to evaluate the clinical value of NIPS as a prenatal screening tool for RCAs.

Our study showed that the PPV for rare aneuploidies was low (6.1%), which was consistent with previous studies[33,34]. A possible explanation for the high false positive rate is that these rare aneuploidies are less prevalent, while many of which have high rates of CPM whereby a chromosomal abnormality occurs only in the placenta but not in the fetus, with the incidence of around 1-2% in typical CVS[12,34-36]. Interestingly, all the aneuploidies were confirmed to be low-level mosaicisms (13% ˜ 29%) by SNP-array on amniotic fluid, arising from mitotic rescue of a meiotic error or a very early mitotic error[37], which was consistent with previous studies[20,33]. The explainable reason is that cases with RCAs almost all experienced pregnancy loss before amniocentesis, which were excluded from our study. In addition, all the parents decided to terminate the pregnancies even though no significant ultrasound abnormality was detected, which may induce bias to comprehensively evaluate the clinical value of NIPS for rare aneuploidies especially for low-level mosaicisms without postnatal clinical features.

The PPV for segmental imbalances was moderate (21.1%), consistent with studies of Zhu *et al* (28.9%)[33] and Chen *et al*(29.0%)[34], but extremely lower than Liang *et al*(40.8%)[20]. The depth of sequencing may be attributable to the difference as the Liang *et al* [20] performed NIPS-Plus with 20 Mb reads per sample, which was approximate 4 times our data. Additionally, NIPS-Plus used combinatorial data analysis algorithms to additionally call genome-wide CNVs associated with MMS[20]. We detected most of the well-known MMS (DGS, 22q11.22 microduplication, PWS/AS and CDC) recommended by NIPS-Plus with moderate to high PPVs [20]. However, the positions of CNVs detected by NIPS in our study could only be located to the chromosome arms, while NIPS-Plus was able to locate the cytobands and co-ordinates of CNVs. No matter NIPS or NIPS-Plus, the techniques have limited power in regions with high repeat content, thus some MMS, such as 1q21.1 recurrent microduplication/microdeletion, 16p13.11 recurrent microduplication/microdeletion, 16p11.2 microduplication/microdeletion, were susceptible to be ignored, even though the prevalence of which was similar to those well-known MMS recommended by NIPS-Plus.

In our study, compared the results of NIPS to SNP-array, there was no significant difference among the concordant rates for subgroups of CNVs <5 Mb (75.0%), ranged from 5 to 10 Mb (86.4%), and >10 Mb (88.0%). The results opposes to the empirical hypothesis that NIPS yielded a higher positive rate for larger

segmental imbalances than smaller ones. This could be attributed to optimization and validation of regions of well-known MMS. It is exemplified that all the 4 detected CNVs involved 22q11.2 recurrent (DGS/VCFS) region in our study were less than 3.5 Mb, which was consistent to previous studies[33,38,39]. For the full concordant segmental imbalances between NIPS and SNP-array, parental confirmations showed that the rate of maternally inherited CNVs (72.5%, 29/40) was significantly higher than those with paternal inheritance or in *de novo* manner (27.5%, 11/40). Thus, it is reasonable to suspect that for gravidas with positive NIPS but negative CMA results of segmental imbalances, maternal CNVs may be detected, which also could reduce the PPVs of NIPS. Although confirmatory chromosome testing was not performed for all those gravidas in our study, it has been reported by Kaseniit *et al* in a large-scale study[40].

For cases with clinical significant CNVs, the PPV was only 7.0% (29/413). Except 9 cases with parental chromosome rearrangements, the PPVs for clinical significant CNVs was extremely low (4.8%, 20/413). All the fetuses with parental-inherited VUS, even with incomplete penetrant P/LP CNVs, were born, however, 46.4% (45/97) families refused parental confirmations, 2 of which opted for TOP. VUS accounted for 63.9% (62/97) of imbalanced segments in our study, thus, the detection of VUS following positive NIPS is bound to accompany with increased family economic burden, maternal anxiety or even panic, and potential risk to terminate pregnancy. It should be prudent whether NIPS is an effective way to screen for clinical significant CNVs.

ROHs, termed as copy number neutral segments showing continuous homozygosity with no intervening heterozygosity[41], were incidental findings (9.5%) in our study, while the NIPS results involved aneuploidies or segmental imbalances related to the chromosome. Consistent to previous studies[42,43], as we excluded positive NIPS results from sex chromosomes, ROHs most frequently involved chromosome 16 and 2, followed by chromosome 1 and 6. UPD is defined as both homologous chromosomes are inherited from one parent, with no contribution (for that chromosome) from the other parent[44]. The common mechanisms resulting in UPD involving trisomy rescue, monosomy rescue, and somatic mitotic crossing over[45]. After further parental confirmation, 60.0% ROH were diagnosed as UPD, with maternal to paternal rate of 14:4, which is consistent to previous studies due to the higher propensity for maternal non-disjunction[46,47]. Thus, we recommend prenatal SNP-array for gravidas with positive NIPS results of RCAs, especially for those involved imprinted chromosomes.

Benefit from the incidental findings of NIPS, 5 fetuses with imprinting disorders were detected. As the results of prenatal diagnosis were obtained before detailed second trimester fetal anomaly scans, these families opted for TOP prior to typical ultrasound presentation of these disorders were shown. While imprinting disorders were excluded, UPD is almost without clinical consequence[45]. However, it was reported that ROH/UPD fetuses with ultrasound abnormalities showed worse prognoses than those without abnormalities[42]. In our study, 12.0% (6/50) cases showed FGR, one of the common ultrasound abnormalities for fetuses with ROH/UPD, which indicated for adverse perinatal outcomes, and those families opted for TOP. Interestingly, the fetus (No.123) with mUPD15 and fetus (No.126) with mUPD16 was subsequently confirmed with placental trisomy 15 and trisomy 16, respectively, which further verified the mechanism of CPM.

CONCLUSIONS

In summary, this prospective study demonstrates that NIPS commercially used for the common fetal aneuploidies yielded low PPV for rare aneuploidies (6.1%), moderate PPV for segmental imbalances (21.1%), and incidental findings (9.5%) for ROH/UPD. This study provided valuable information for genetic counselling and management of gravidas with positive NIPS results of RCAs. For the low PPV for clinical significant findings, NIPS has limited clinical utility for rare RCAs. Prenatal SNP-array should be regarded as the first-tier test for positive NIPS, particularly for those involved imprinted chromosomes.

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DISCLOSURE OF INTERESTS

The authors report no conflict of interest.

Contribution to authorship

The study was conceived and designed by TH, HW, XN and SL. Patient recruitment and sample collection were under taken by TH and JW. Experiments and data collection were performed by TH, JW, RH, LX, NL and SL. Data analyses and interpretation were performed by TH, JW, QZ and ZZ. All figures and tables were generated by TH and JW. The manuscript was written by TH and JW. All authors critically reviewed the manuscript and approved the final manuscript for publication.

Details of ethics approval

The study was approved by the Medical Ethics Committee of West China Second University Hospital (IRB no. 20160029, approval date 28 December 2016).

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Table 1 Summary of the CMA results of 528 fetuses with positive NIPS results

Chromosome	NIPS (n)	CMA (n) Rare aneuploidies	CMA (n) Segmental imbalances	CMA (n) ROH/UPD	CMA (n) Normal	PPV
Chr1	16	_	1	6	9	43.8,
Chr2	28	_	5	6	17	39.3,
Chr 3	25	_	4	2	19	24.0,
Chr 4	15	_	2	4	9	40.0,

Chr 5	19	_	8 (1)[?]	_	10	42.1,
Chr 6	8	_	1	6	1	87.5,
Chr 7	108	_	5 (2)[?]	2	99	6.5, 1.
Chr 8	48	_	8	5	35	27.1,
Chr 9	20	5*	2	3	10	50.0,
Chr 10	16	_	6	_	10	37.5,
Chr 11	19	_	3(1)[?]	2	13	26.3,
Chr 12	7	_	3	1	3	57.1,
Chr 13	14	_	5	1	8	42.9,
Chr 14	18	_	1 (3)[?]	1	13	11.1, -
Chr 15	20	1*	5 (1)[?]	1	12	35.0,
Chr 16	33	1*	5	9	18	45.5,
Chr 17	7	_	1	1	5	28.6, -
Chr 18	22	_	8 (1)[?]	_	13	36.4,
Chr 19	1	_	_	_	1	_
Chr 20	26	_	3	_	23	11.5, -
Chr 21	21	_	3	_	18	14.3, -
Chr 22	11	_	6	_	5	54.5,
Multiple chromosome	26	_	2 (1)[?]	_	23	7.7, -3
Total (n)	528	7	87 (10)[?]	50	374	27.3,

* mosaic aneuploidies; [?] The positive results discordant with NIPS.

NIPS: noninvasive prenatal screening; CMA: chromosomal microarray analysis;

ROH: regions of homozygosity; UPD: uniparental disomy; PPV: positive predictive value;

CI: confidence intervals

Table 2 Concordance between RCAs detected by NIPS and consecutive CMA results

Chromosome	**CMA (n)**	**CMA (n)**	**CMA (n)**	**CMA (n)**	**CMA (n)**	**CMA (n)**	**CMA (n)**	**CMA (n)**	**CMA (n)**	**CMA (n)**	**CMA (n)**	**CMA (n)**	**CMA (n)**	**CMA (n)**	**CMA (n)**	**CMA (n)**	**CM (n)**
	Rare aneuploidies		**Segmental imbalances**	**Segmental imbalances**	**Segmental imbalances**	**Segmental imbalances**	**Segmental imbalances**	**Segmental imbalances**	**Segmental imbalances**	**Segmental imbalances**	**Segmental imbalances**	**Segmental imbalances**	**Segmental imbalances**	**Segmental imbalances**	**Segmental imbalances**	**Segmental imbalances**	**Segmental**
	Full concordance		**Full concordance**	**Full concordance**	**Full concordance**	**Full concordance**		**Partial concordance**	**Partial concordance**	**Partial concordance**	**Partial concordance**		**Discordance**	**Discordance**	**Discordance**	**Discordance**	**Discordan**
			mat	**pat**	**de novo**	**NA**		**mat**	**pat**	**de novo**	**NA**		**mat**	**pat**	**de novo**	**NA**	
Chr 1	_		1	_	_	_		_	_	_	_		_	_	_	_	
Chr 2	_		1	_	_	4		_	_	_	_		_	_	_	_	
Chr 3	_		2	_	1	_		_	_	1	_		_	_	_	_	
Chr 4	_		_	_	1	1		_	_	_	_		_	_	_	_	

Chr 5	_	3	_	_	5	_	_	_	_	_	1	_	_
Chr 6	_	1	_	_	_	_	_	_	_	_	_	_	_
Chr 7	_	1	1	1	2	_	_	_	_	_	_	1	1
Chr 8	_	2	_	_	4	_	_	1	1	_	_	_	_
Chr 9	5*	_	_	_	_	_	_	2	_	_	_	_	_
Chr 10	_	3	_	_	3	_	_	_	_		_	_	_
Chr 11	_	2	_	1	_	_	_	_	_	1	_	_	_
Chr 12	_	_	1	_	1	_	_	1	_	_	_	_	_
Chr 13	_	3	_	_	2	_	_	_	_	_	_	_	_
Chr 14	_	1	_	_		_	_	_	_	1	_	_	2
Chr 15	1*	2	_	_	3	_	_	_	_	1	_	_	_
Chr 16	1*	1	_	_	4	_	_	_	_	_	_	_	_
Chr 17	_	1	_	_	_	_	_	_	_	_	_	_	_
Chr 18	_	1	_	1	3	2	_	_	1	_	_	_	1
Chr 19	_	_	_	_	_	_	_	_		_	_	_	_
Chr 20	_	_	_	_	1	_	_	1	1	_	_	_	_
Chr 21	_	2	_	1		_	_	_	_	_	_	_	_
Chr 22	_	1	_	3	2	_	_	_	_	_	_	_	_
Multiple chromosome		1	_	_	_	_	_	1	_	_	_	_	1
PPV (n (%, 95% CI))	**7* (1.3,0.3 _- 2.3)**	**29**	**2**	**9**	**35**	**2**	_	**7**	**3**	**3**	**1**	**1**	**5**
		75 (18.2, 14.4 _- 21.9)	**75 (18.2, 14.4 _- 21.9)**	**75 (18.2, 14.4 _- 21.9)**	**75 (18.2, 14.4 _- 21.9)**	**12 (2.9, 1.3 _- 4.5)**	**12 (2.9, 1.3 _- 4.5)**	**12 (2.9, 1.3 _- 4.5)**	**12 (2.9, 1.3 _- 4.5)**	**-**	**-**	**-**	**-**

87 (21.1, 17.1_-25.0)[?]	**87 (21.1, 17.1_-25.0)[?]**	**87 (21.1, 17.1_-25.0)[?]**	**87 (21.1, 17.1_-25.0)[?]**	**87 (21.1, 17.1_-25.0)[?]**	**87 (21.1, 17.1_-25.0)[?]**	**87 (21.1, 17.1_-25.0)[?]**	**87 (21.1, 17.1_-25.0)[?]**	**87 (21.1, 17.1_-25.0)[?]**	**87 (21.1, 17.1_-25.0)[?]**	**87 (21.1, 17.1_-25.0)[?]**	**87 (21.1, 17.1_-25.0)[?]**	**87 (21.1, 17.1_-25.0)[?]**	**87 (21.1, 17.1_-25.0)[?]**	**87 (21.1, 17.1_-25.0)[?]**

* mosaic aneuploidies; [?] The positive results discordant with NIPS.

RCAs: rare chromosome abnormalities; CMA: chromosomal microarray analysis; NA: not available; ROH: regions of homozygosity; UPD: uniparental disomy; PPV: positive predictive value; CI: confidence intervals

Table 3 Prenatal CNVs detected by CMA among the 528 gravidas with positive NIPS results

No.	NIPS	CNVs (GRCh37)	Size of CNVs (kb)	Copy number	HI/TS region	HI/TS gene	Inherited or de novo	ACMG Classification	Outcomes
1	chr7p dup	7p22.2(2966567_3334799)x3	368	Gain	/	/	NA	VUS	Born
2	chr5p del	5p14.3(19076278_19448955)x1	373	Loss	/	/	NA	VUS	Born
3	chr13q dup	13q12.11(22688245_22999366)x3	311	Gain	/	/	NA	VUS	Born
4	chr3p dup	3p26.2p26.1(4076046_4273489)x3	197	Gain	/	/	Inherited from normal mother	VUS	Born
5	chr15q dup	15q13.3(31994832_32428066)x3	433	Gain	/	/	NA	VUS	Born
6	chr7q del	7q11.21(64615280_65148399)x1	533	Loss	/	/	NA	VUS	Born
7	chr20q del	20q13.13(46470867_47125819)x1	655	Loss	/	/	NA	VUS	Born
8	chr5p dup	5p15.31(6752757_7429552)x4	677	Gain	/	/	Inherited from normal mother	VUS	Born
9	chr7p dup	7p21.1(17592351_18327081)x3	735	Gain	/	/	Inherited from normal father	VUS	Born
10	chr13q dup	13q12.11(21542089_21972234)x3	430	Gain	/	/	NA	VUS	Born
11	chr22q del	22q11.21(21049669_21800471)x1	750	Loss	/	/	Inherited from normal mother	VUS	Born
12	chr22q del	22q11.21(21049669_21800471)x1	750	Loss	/	/	*de novo*	VUS	TOP
13	chr17q dup	17q11.2(28746821_29516669)x3	769	Gain	/	/	Inherited from normal mother	VUS	Born

14	chr18p dup	18p11.32(124558_-1802917)x3	Gain	/	/	NA	VUS	Born (Premature delivery)
15	chr15q del	15q13.3(31967770_-32914239)x1	Loss	15q13.3 recurrent region (D_-CHRNA7 to BP5) (includes CHRNA7 and OTUD7A)	/	NA	P	Born
16	chr8q dup	8q24.11(117902212_-119012676)x3	Gain	/	/	Inherited from normal mother	VUS	Born
17	chr13q dup	13q31.1(81924087_-83110879)x3	Gain	/	/	Inherited from normal mother	VUS	Born
18	chr2p dup	2p12(78631719_79851089)x4	Gain	/	/	NA	VUS	Born
19	chr13q del	13q12.12(23533511_-24970361)x1	Loss	/	/	Inherited from normal mother	VUS	Born
20	chr10q dup	10q24.32q25.1(104583879_-106039196)x3	Gain	/	/	NA	VUS	Born
21	chr13q dup	13q32.1(95340570_-96874757)x3	Gain	/	/	Inherited from normal mother	VUS	Born
22	chr2q del	2q12.2q12.3(107856366_-108527327)x1	Loss	/	/	NA	VUS	Born
23	chr16p dup	16p13.12p13.11(14770633_-16538596)x3	Gain	/	/	NA	VUS	Born
24	chr1q del	1q21.1q21.2(146106724_-147933973)x1	Loss	1q21.1 recurrent region (BP3_-BP4 distal) (includes GJA5)	/	Inherited from normal mother	P	Born

25	chr8p dup	8p23.2p23.1(1882453_-6204870)x3	Gain	/	/	Inherited from normal mother	VUS	Born
26	chr22q dup	22q11.22q11.23(22997928_-24995256)x3	Gain		/	*de novo*	VUS	Born
27	chr7q dup	7q11.21q11.22(65785467_-68970684)x4	Gain	/	/	Inherited from normal mother	VUS	Born
28	chr5p dup chr14q dup	5p15.31(8827707192228_-9798033)x3 14q31.3(85359235_-87586936)x4	Gain Gain	/ /	/ /	Inherited from normal mother Inherited from normal mother	VUS VUS	Born
29	chr16q dup	16q21(61996302306_-64301745)x3	Gain	/	/	Inherited from normal mother	VUS	Born
30	chr22q del	22q11.21(18624855_-21062134)x1	Loss	22q11.2 recurrent (DGS/VCFS) region (proximal A_B) (includes TBX1)	/	*de novo*	P	TOP
31	chr18q dup	18q22.1(64283871_-66769260)x3	Gain	/	/	NA	VUS	NA
32	chr21q dup	21q22.3(43942592_-46523623)x3	Gain	/	/	Inherited from normal mother	VUS	Born
33	chr22q dup	22q11.21(18626761_-21283290)x3	Gain	22q11.2 recurrent (DGS/VCFS) region (proximal A_B) (includes TBX1)	/	NA	P	TOP

34	chr12q del	12q24.32q24.33(127391909_130111679)x1	Loss	/	/	Inherited from normal father	VUS	Born (Premature delivery)
35	chr14q del	14q21.2q21.3(48010321_49016299)x1	Loss	/	/	Inherited from normal mother	VUS	Born (hydronephr
36	chr22q dup	22q11.21(18628856_21461017)x3	Gain	22q11.2 recurrent (DGS/VCFS) region (proximal A_B) (includes TBX1)	/	NA	P	TOP
37	chr16p dup	16p13.11p12.3(15325073_18157612)x3	Gain	/	/	NA	VUS	Born
38	chr22q del	22q11.21(18631364_21800471))x1	Loss	22q11.2 recurrent (DGS/VCFS) region (proximal A_D) (includes TBX1)	/	NA	P	TOP
39	chr6p dup	6p12.3(46466834_50480392)x3	Gain	/	/	Inherited from normal mother	VUS	Born
40	chr2q del	2q12.2q13(104340325_111370025)x1	Loss	/	/	Inherited from normal mother	VUS	Neonatal death
41	chr16q dup	16q11.2q12.1(46403969_51098261)x3	Gain	/	/	NA	VUS	Born
42	chr21q dup	21q21.1(18498857_23347274)x3	Gain	/	/	Inherited from normal mother	VUS	Born

43	chr15q dup	15q11.2q13.1(22832678_-28560664)x3	Gain	15q11q13 recurrent (PWS/AS) region (BP2_-BP3 Class 2)	/	Inherited from normal mother	P	Born
44	chr5q dup	5q22.1q23.1(112896866_-116195651)x3	Gain	/	/	NA	VUS	TOP
45	chr8q dup	8q23.1q23.3(114273153_-115684011)x3	Gain	/	/	NA	VUS	Born
46	chr11p dup	11p15.1p14.3(23253705_-25684613)x3	Gain	/	/	Inherited from normal mother	VUS	Born
47	chr11p dup	11p15.1p14.3(23277669_-25713381)x3	Gain	/	/	Inherited from normal mother	VUS	Born
48	chr10q del	10q11.22q11.23(46293590_-51817663)x1	Loss	/	/	Inherited from normal mother	VUS	Born
49	chr15q dup	15q26.1q26.3(96404310_-98968661)x3	Gain	/	/	NA	VUS	Born
50	chr16p del	16p13.13p12.3(12548052_-18242713)x1	Loss	16p13.11 recurrent region (BP2_-BP3) (includes MYH11)	/	NA	P	Born
51	chr15q dup	15q11.2q13.1(22670421_-28526905)x3	Gain	15q11q13 recurrent (PWS/AS) region (BP1_-BP3 Class 1)	/	Inherited from normal mother	P	NA
52	chr12q del	12q21.2q21.31(79313475_-85441579)x1	Loss	/	PPP1R12A	NA	P	Born
53	chr8q del	8q21.13q21.3(80275606_-87340145)x1	Loss	/	/	NA	VUS	TOP

54	chr10q dup	10q22.3q23.2(78374867_-88957815)x3	Gain	/	/	Inherited from normal mother	VUS	Born
55	chr10q del	10q22.3q23.2(78630469_-88957815)x1	Loss	10q22.3q23.2 recurrent region (LCR_-3/4_-flanked) (includes BMPR1A)	BMPR1A/PTEN	NA	P	TOP
56	chr10q dup	10q22.3q23.2(78630469_-88973570)x3	Gain	/	/	Inherited from normal mother	VUS	Born
57	chr7p del	7p21.3p21.1(8974812_-17191226)x1	Loss	/	/	*de novo*	VUS	TOP
58	chr11q del	11q24.2q25(129647772_-134937416)x1	Loss	/	/	*de novo*	VUS	TOP
59	chr3q dup	3q11.1q12.3(93209465_-101839691)x3	Gain	/	/	Inherited from normal mother	VUS	NA
60	chr8p del	8p23.3p23.1(8552_-9010029)x1	Loss	/	/	NA	LP	TOP
61	chr5q dup	5q21.1q22.1(101322649_-110634622)x3	Gain	/	/	Inherited from normal mother	VUS	Born
62	chr5p dup	5p11q11.2(46625514_-55986750)x3	Gain	/	/	Inherited from normal mother	VUS	Born
63	chr2p del	2p23.1p22.1(30732152_-41085497)x1	Loss	/	SPAST	NA	P	Born
64	chr8p del	8p23.3p23.1(165289_-10685851)x1	Loss	/	/	NA	LP	TOP
65	chr3q dup	3q27.2q29(185499997_-197851444)x3	Gain	/	/	NA	LP	TOP
66	chr5q del	5q21.3q23.1(108832795_-118713574)x1	Loss	/	APC	NA	P	TOP
67	chr21q del	21q11.2q21.3(14726487_-29188153)x1	Loss	/	/	*de novo*	VUS	NA
68	chr4q del	4q13.1q21.1(62322426_-76675789)x1	Loss	/	/	*de novo*	LP	TOP

69	chr18q del	18q22.1q23(6[illegible]-78013728)x1	Loss	/	/	Inherited from mother with intellectual disability	VUS	NA
70	chr18p del	18p11.32(136[illegible]-1331930)x1 13827 18p11.32p11.21(1343954_-15170636)x3	Loss Gain	/ /	/ /	NA NA	VUS LP	Born
71	chr18p dup	18p11.32q23([illegible]-15181207)x2.15	Gain (mosaic)	/	/	NA	LP	TOP
72	chr2q dup	2q23.3q31.1([illegible]-170720261)x3	Gain	/	/	NA	LP	TOP
73	chr5p del	5p15.33p14.3([illegible]-18727376)x1	Loss	5p15 terminal (Cri du chat syndrome) region	TRIO	NA	P	TOP
74	chr4q del	4q31.3q34.2([illegible]-176868942)x1	Loss	/	/	NA	LP	TOP
75	chr5p del	5p15.33p14.1([illegible]-26243789)x1	Loss	5p15 terminal (Cri du chat syndrome) region	TRIO	NA	P	TOP
76	chr18p dup	18p11.22([illegible]-10139732)x31322 22q11.23q12.1(25116001_-26437690)x3	Gain Gain	/ /	/ /	Inherited from normal mother *de novo*	VUS VUS	TOP (hydrocepha
77	chr8p del	8p23.3p23.2([illegible]-3220759)x1 69180 8q21.11q24.3(77115706_-146295771)x3	Loss Gain	/ /	/ /	Paternal invertion: 46,XY,inv(8)(p23.2;q21)	VUS LP	TOP
78	chr8p del	8p23.3p23.2([illegible]-4745371)x1 22284 9p24.3p21.3(208454_-22492876)x3	Loss Gain	/ /	/ /	Maternal balanced translocation: 46,XX,t(8;9)(p23;p21.3)	VUS LP	TOP

79	chr20p dup	20p13p12.1(16622_-13546848)x310936 9p24.3p23(208455_-11144684)x1	Gain Loss	/ /	/ SMARCA2	Paternal balanced translocation: 46,XY,t(9;20)(p23;p12.1)	LP P	TOP
80	chr9p dup	9p24.3p22.3(208455_-15608372)x31528 5p15.33(113577_-1641914)x1	Gain Loss	/ /	/ /	Inherited from mother with intellectual disability: 46,XX,der t(5;9)(p15.3;p22)	VUS VUS	TOP
81	chr12q dup	12q24.21q24.33(1[illegible]-133777562)x3287 2q37.3(240495629_-242782258)x1	Gain Loss	/ /	/ /	Maternal balanced translocation: 46,XX,t(2;12)(q37;q24)	LP VUS	TOP
82	chr18q dup	18q21.33q23(6[illegible]-78013728)x33641 4q35.2(187316147_-190957460)x1	Gain Loss	/ /	/ /	Inherited from mother with intellectual disability: 46,XX,der t(4;18)(q35;q22)	VUS VUS	TOP
83	chr17q dup chr11p del	17q23.3q25.3(6[illegible]-81041823)x32928 1p36.33p36.32(849466_-3777765)x1	Gain Loss	/ 1p36 terminal region (includes GABRD)	/ /	Maternal balanced translocation: 46,XX,t(1;17)(p36;q23)	LP P	TOP
84	chr18q del	18q21.32q23(5[illegible]-78013728)x11113 1q43(238949246_-240062389)x1	Loss Loss	/ /	/ /	NA	LP VUS	TOP
85	chr9p dup	9p24.3p13.2(2[illegible]-37055141)x31369 9p24.3(208454_-1577575)x1	Gain Loss	/ /	/ /	NA	LP VUS	TOP
86	chr20p dup	20p13p11.21(4[illegible]-24487341)x38545 11q24.2q25(126392021_-134937416)x1	Gain Loss	/ /	/ /	Maternal balanced translocation: 46,XX,t(11;20)(q24.2;p11.2)	LP VUS	TOP

87	chr3p dup	3p26.3p22.2(2[illegible]-37597219)x3 8637 5p15.33p15.31(113577_-8750244)x1	Gain Loss	/ 5p15 terminal (Cri du chat syndrome) region	/ TRIO	Paternal balanced translocation: 46,XY,t(3;5)(p24;p15.3)	VUS P	TOP
88	chr14q del	Yq11.21q11.2[illegible](1446077[illegible]-15220682)x0	Loss	/	/	NA	VUS	Born
89	chr7q del	16p11.2(294[illegible]-30190029)x1	Loss	16p11.2 recurrent region (proximal BP4_-BP5) (includes TBX6)	/	*de novo*	P	TOP
90	chr1p del chr4q dup	4q35.2(1901[illegible]-190957460)x1	Loss	/	/	NA	VUS	Born
91	chr15q dup	10p13p12.33([illegible]-18286639)x3	Gain	/	/	Inherited from normal mother	VUS	Born
92	chr7q del	Xp22.31(644[illegible]-8143509)x1	Loss	Xp22.31 recurrent region (includes STS)	STS	NA	P	Born
93	chr18p dup	4q34.3(1781[illegible]-179860825)x3	Gain	/	/	NA	VUS	Born
94	chr5q dup	8q24.12(119[illegible]-122337637)x3	Gain	/	/	Inherited from normal father	VUS	Born
95	chr11p del	1q25.3q31.1([illegible]-187563410)x1	Loss	/	/	Inherited from normal mother	VUS	Born
96	chr14q dup	5p15.1p14.3([illegible]-21148212)x3	Gain	/	/	Inherited from normal mother	VUS	Born

97	chr14q del	5p15.33p15.32(21[illegible]3577_-6138632)x1	Loss	5p15 terminal (Cri du chat syndrome) region	TRIO	NA	P	TOP

CNV: pathogenic copy_number variant; CMA: chromosomal microarray analysis; NIPS: noninvasive prenatal screening; HI: haploinsufficiency; TS: triplosensitivity; ACMG: American College of Medical Genetics and Genomics; NA: not available; P: pathogenic; LP: likely pathogenic; VUS: uncertain clinical significance; TOP: termination of pregnancy

Table 4 Prenatal ROHs detected by CMA among the 528 gravidas with positive NIPS results

No.	NIPS	CNVs (GRCh37)	Size of ROHs (kb)	Copy number	Disorder	Source	Outcomes
98	chr1 dup	1p36.33p11.2(888[illegible]9-121339317) hmz 1q21.2q44(149879544_-249198164) hmz	86589-120451	UPD1 (isodisomy)	/	NA	TOP
99	chr1 del	1p36.33p11.2(888[illegible]9-121339317) hmz 1q21.2q44(149879544_-249198164) hmz	86589-120451	UPD1 (isodisomy)	/	NA	TOP (FGR)
100	chr1dup	1p36.33p11.2(888[illegible]9-121339317) hmz 1q21.2q44(149879544_-249198164) hmz	86589-120451	UPD1 (isodisomy)	/	NA	Born (Methylmalonic acidemia)
101	chr2 del	2p25.3p11.2(508[illegible]-87053152) hmz 2q11.1q37.3(95550957_-242773583) hmz	87002-147223	UPD2 (isodisomy)	/	NA	TOP (FGR)
102	chr2 dup	2p25.3p11.2(508[illegible]-87053152) hmz 2q11.1q37.3(95550957_-242773583) hmz	87002-147223	mUPD2 (isodisomy)	/	maternal	TOP (hydrops fetalis)

103	chr2 dup	2q14.2q24.1(121274207_158756848) hmz 2q31.1q34(175042562_213345197) hmz 2p24.1p16.1(19693805_59685825) hmz	38303 39992	mUPD2 (iso_-heterodisomy)	/	maternal	Born
104	chr3 dup	3q11.1q13.11(93587216_105429152) hmz 3p26.3p25.1(73602_16294894) hmz 3p12.3p11.1(74601403_88586090) hmz	16221 13985	mUPD3 (iso_-heterodisomy)	/	maternal	NA
105	chr4 dup	4p16.3p11(75174_49063479) hmz 4q11q35.2(52696791_190921709) hmz	18988 138225	UPD4 (isodisomy)	/	NA	TOP
106	chr4 del	4p16.3p11(75174_49063479) hmz 4q11q35.2(52696791_190921709) hmz	18988 138225	pUPD4 (isodisomy)	/	paternal	TOP (FGR)
107	chr4 dup	4p16.3p11(75174_49063479) hmz 4q11q35.2(52696791_190921709) hmz	18988 138225	UPD4 (isodisomy)	/	NA	Born
108	chr4 dup	4p16.3p15.33(75174_15121280) hmz 4p14p11(38705256_49063479) hmz 4q28.2q34.2(129685157_177412472) hmz	10358 47727	mUPD4 (iso_-heterodisomy)	/	maternal	TOP
109	chr6 dup	6p25.3p11.1(203723_58726706) hmz 6q11.1q27(61972917_170896644) hmz	108924	pUPD6 (isodisomy)	Transient Neonatal Diabetes mellitus (TNDM)	paternal	TOP (FGR)

110	chr6 del	6p25.3p11.1(203887 58726706) hmz 6q11.1q27(61972917_-170896644) hmz	23 108924	mUPD6 (isodisomy)	/	maternal	Born
111	chr6 dup	6p25.3p11.1(203887 58726706) hmz 6q11.1q27(61972917_-170896644) hmz	23 108924	UPD6 (isodisomy)	/	NA	TOP
112	chr6 dup	6p24.1p11.1(133 58726706) hmz 6q16.1q22.31(94446431_-124307093) hmz 6q25.1q27(150710446_-170896644) hmz	20186	mUPD6 (isodisomy)	/	maternal	TOP (FGR)
113	Chr6 dup	6p25.3p11.1(203887 58726706) hmz 6q11.1q27(61972918_-170896644) hmz	23 108924	mUPD6 (isodisomy)	/	maternal	Born
114	chr7 del	7p22.3p11.1(509 58019983) hmz 7q11.21q36.3(62569501_-159118443) hmz	69 96549	UPD7 (isodisomy)	/	NA	Born (short stature)
115	chr7 dup	7p22.3p11.1(509 58019983) hmz 7q11.21q36.3(62569501_-159118443)	69 96549	UPD7 (isodisomy)	/	NA	TOP
116	chr8 dup	8p23.1p11.1(811 43776564) hmz 8q11.1q24.3(46919156_-146292734) hmz	99374	mUPD8 (isodisomy)	/	maternal	TOP
117	chr8 dup	8p23.1p11.1(811 43776564) hmz 8q11.1q24.3(46919156_-146292734) hmz	99374	UPD8 (isodisomy)	/	NA	TOP

118	chr9dup	9p24.3p13.1(216[illegible]6 69998 38771831) hmz 9q21.11q34.3(71013799_-141011581) hmz	UPD9 (isodisomy)	/	NA	TOP
119	chr9 dup	9p24.3p13.1(216[illegible]6 69998 38771831) hmz 9q21.11q34.3(71013800_-141011581) hmz	pUPD9 (iso_-heterodisomy)	/	paternal	TOP
120	chr11 dup	11q23.2q25(112[illegible]5_-134930689) hmz	mUPD11 (iso_-heterodisomy)	Silver–Russell syndrome (SRS)	maternal	TOP
121	chr11 dup	11q14.2q25(881[illegible]9_-134930689) hmz	pUPD11 (iso_-heterodisomy)	Beckwith–Wiedemann syndrome (BWS)	paternal	TOP
122	chr14 dup	14q23.1q32.11(6[illegible]461_-91714413)	mUPD14 (iso_-heterodisomy)	Temple syndrome	maternal	TOP
123	chr15 dup	15q21.1q22.2(48[illegible]91_-62709924) hmz	mUPD15 (iso_-heterodisomy)	Prader–Willi syndrome (PWS)	maternal	TOP Placental trisomy 15 confirmed by FISH
124	chr16 dup	16p13.3p13.13(9[illegible]-12360 11870494) hmz 16q23.1q24.3(77786018_-90146366) hmz	mUPD16 (iso_-heterodisomy)	/	maternal	TOP
125	chr16 dup	16p13.3p12.3(9[illegible]5 20285 20050658) hmz 16q22.1q24.3(69860932_-90146366) hmz	mUPD16 (iso_-heterodisomy)	/	maternal	Born
126	chr16 dup	16p13.3p13.13(9[illegible]-11667 11219041) hmz 16q22.3q24.3(73469057_-90146366) hmz	mUPD16 (iso_-heterodisomy)	/	maternal	TOP (demise) Placental trisomy 16 confirmed by FISH

127	chr17 del	17p13.3p11.2(1822052 - 22170994) hmz 17q11.1q25.3(25309336 - 81041760) hmz	55732	UPD17 (isodisomy)	/	NA	TOP (FGR)
128	chr1 dup	1p13.3p11.2(108528362 - 121339317) hmz 1q21.2q24.3(149879544 - 172597553) hmz 1q41q43(218237293 - 241346599) hmz	22718 23109	ROH	/	NA	Born
129	chr1 dup	1p32.2p21.3(58259467 - 99399687) hmz 1q25.3q42.12(182520611 - 226528744) hmz	44008	ROH	/	NA	NA
130	chr1 dup	1p13.2p11.2(115835056 - 121339317) hmz 1q21.2q23.3(149879545 - 165480347) hmz	601	ROH	/	NA	Born
131	chr2 dup	2p25.3p22.2(19362889 - 37998488) hmz 2q36.1q37.3(223571444 - 242773583) hmz	19202	ROH	/	NA	Born (short stature)
132	chr2 dup	2p13.2p11.2(72842771 - 87053152) hmz 2q11.1q12.3(95550957 - 109616111) hmz	14065	ROH	/	NA	Neonatal death
133	chr2 dup	2p12p11.2(75698754 - 87053152) hmz 2q11.1q12.2(95550957 - 106174659) hmz	10624	ROH	/	NA	Born

134	chr3 dup	3p26.3p26.1(736818 - 6891874) hmz 3p22.2p13(39251141 - 69830674) hmz 3q25.1q27.2(150678233 - 185795060) hmz	30580 35117	ROH	/	NA	TOP
135	chr6 dup	6p25.3p11.1(203723 - 58726706) hmz		ROH	/	NA	TOP (LVOTO)
136	chr8 del	8q11.1q12.2(46914936 - 61854841) hmz 8p12p11.1(31079982 - 43776564) hmz	12697	ROH	/	NA	Born
137	chr8 dup	8p21.3p11.23(20 - 36638339) hmz 8q12.3q22.1(65927736 - 93643205) hmz	2810	ROH	/	NA	NA
138	chr8 dup	8p12p11.1(34070737 - 43776564)x2 hmz 8q11.1q13.2(46919157 - 68771501)x2 hmz	21852	ROH	/	NA	Born
139	chr9 dup	9p24.3p21.3(216 - 20570700) hmz 9q31.3q34.3(112814078 - 141011581) hmz	20355	ROH	/	NA	TOP
140	chr12 dup	12p13.33p11.22(3 - 30396571) hmz		ROH	/	NA	Born
141	chr13 dup	13q31.1q34(844 - 115095705) hmz		ROH	/	NA	Born
142	chr16 dup	16p13.3p13.13(9 - 12292798) hmz 16q23.2q24.3(80394565 - 90146366) hmz	9752	ROH	/	NA	NA
143	chr16 dup	16q23.1q24.3(78 - 90146366) hmz		ROH	/	NA	Born

144	chr16 dup	16p13.3p13.12(9480859-14053831) hmz	ROH	/	NA	TOP
145	chr16 dup	16p13.3p12.3(9480836-19331243) hmz	ROH	/	NA	Born
146	chr16 dup	16p13.3(94808_-7059 8206 7154181) hmz 16q23.3q24.3(81940867_-90146366) hmz	ROH	/	NA	Born
147	chr16 dup	16p13.3(94808_-5405 16958 5500174) hmz 16q21q23.3(66159040_-83117017) hmz	ROH	/	NA	fetal loss after amniocentesis

CMA: chromosomal microarray analysis; NIPS: noninvasive prenatal screening; UPD:uniparental disomy; ROH: regions of homozygosity; NA: not available; TOP: termination of pregnancy; FGR: fetal growth restriction; LVOTO: left ventricular outflow tract; FISH: fluorescence in situ hybridization

Table 5 Clinical follow-up assessment of the 528 fetuses detected by CMA

SNP-array	SNP-array	Total	Loss of follow-up	TOP	TOP	TOP	Birth	Birth
				Chromosomal abnormalities	Ultrasound abnormalities	Other	Normal	Birth defect
Rare aneuploidies		7	-	7	-	-	-	-
Segmental imbalances	P/LP CNVs	35	1	26	-	-	8	-
	VUS	62	4	7	1	-	49	-
ROH/UPD	UPD	30	1	14	7	1	5	2
	ROH	20	3	3	1	1	10	1
Normal		374	43	-	6	2	314	9

CMA: chromosomal microarray analysis; SNP: single-nucleotide polymorphism; TOP: termination of pregnancy; P: pathogenic; LP: likely pathogenic; VUS: uncertain clinical significance; UPD:uniparental disomy; ROH: regions of homozygosity

Figure 1