Performances of noninvasive prenatal detection for fetal chromosome aneuploidy based on Semiconductor Sequencing Platform (SSP) of difference sequencing depths

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Research Article

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Abstract

Objective: To evaluate the performance of non-invasive prenatal testing (NIPT) for chromosome aneuploidy in different sequencing depths for all chromosome aneuploidies.

Methods: A cohort of 61581 pregnancies were recruited to review the results, which contained 49393 cases of NIPT and 12188 cases of NIPT- plus respectively. Cell-free DNA from plasma samples were sequenced by Ion Proton sequencer, with sequencing depths included 0.15X (3 Mb reads) with NIPT and 0.4X (8 Mb reads) with NIPT- plus. All high-risk cases were recommended to undergo invasive prenatal diagnosis, and all pregnant women were followed up.

Results: A total of 910 cases were high risk of chromosome aneuploidies predicted by NIPT and NIPT-plus. in which NIPT predicted 682 high risk of chromosome aneuploidies , and NIPT- plus predicted 210, The positive rates were 1.38% and 1.72%, respectively. A total of 811 cases accepted prenatal diagnosis with 627 cases of NIPT and 184 cases of NIPT- plus. The PPV of autosomal aneuploidies in NIPT were 81.99%, 69.23%, 25.00%, 4.55%, respectively. and The PPV of autosomal aneuploidies in NIPT- plus were 86.96%, 80.00%, 35.00%, 8.77%,respectively. The PPV of sex chromosome aneuploidies in NIPT- plus was 50%，which consistent with NIPT. In addition, through follow-up, found 1 cases of false negative with trisomy 18 , both of which was detected by NIPT, and termination of pregnancy after prenatal diagnosis due to ultrasound abnormalities.

Conclusion: The PPV of autosomal aneuploidies in NIPT- plus was slightly better than NIPT. It shows that increasing the sequencing depth can improve the detection performance of aneuploidy, which has certain guiding value for clinical application.

Introduction

Since 1997, The discovery of cffDNA opens a new approaches for non-invasive prenatal screening (NIPT) (Lo et al., 1997). NIPT which using massively parallel sequencing (MPS) gradually developed into first-line screening method for fetal chromosomal aneuploidies. NIPT has applied in many countries and has been available clinically for over 10 years (Samura, 2020; van der Meij et al., 2019). NIPT has very satisfactory detection rate not only for trisomies 21, 18, and 13, but also has excellent predictive value for sex chromosomes and chromosome copy number variations (CNVs) (Liang et al., 2019; Liang et al., 2018; Xu et al., 2020; Yang et al., 2021). In China, NIPT is recommended for screening trisomy 21 (T21), trisomy 18 (T18) and trisomy13 (T13) for patients with intermediate risk of serological screening results or serological screening for the single marker value abnormality (AFP, β-HCG,uE3) in the first or second trimester (McCullough et al., 2014), and with ultrasound soft marker abnormalities ("Screening for Fetal Chromosomal Abnormalities: ACOG Practice Bulletin, Number 226," 2020). Some advanced age pregnant women choose NIPT voluntarily. Especially with the implementation of the two-child policy, the number of advanced age pregnant women has gradually increased. Many of them refuse to undergo prenatal

diagnosis and choose NIPT. As more and more knowledge about NIPT publicity and education are popularized, more and more pregnant women actively choose NIPT (Zhu, Ling, Shang, & Huang, 2020).

As well known, NIPT include multiple technical platforms because of deriving from different high-throughput sequencing principles (H. Hu et al., 2016; Shi et al., 2021; Xie et al., 2018). In recent years, expanded NIPT (NIPT-plus) has gradually developed into research focus for clinical applications, expanded NIPT will increase detection performance by increasing sequencing depth (H. Hu et al., 2019). More and more literatures are reported that expanded NIPT is feasible for detection of chromosome copy number variations (CNVs) (Chen et al., 2019; Srinivasan, Bianchi, Huang, Sehnert, & Rava, 2013). However, there are few research reports comparing chromosomal aneuploidy detection efficiency using the same platform with different sequencing depths. Thus, we aimed to retrospectively compare the chromosomal aneuploidy detection efficiency of NIPT using the semiconductor sequencing platform (SSP) with 0.15X (NIPT)and 0.4X (NIPT-plus) sequencing depths. One thing to emphasize is: this research included two detection methods for sequencing depth. NIPT sequencing depth was about 0.15X and the data volume was 3 million reads; and sequencing depth was about 0.4X, and the data volume was 8 million reads.

Materials And Methods

Participant recruitment

This study was approved by the Ethics Review Committee of Guangdong Women and Children Hospital (No. 2013102301). All clinical workflows and methods were carried out in accordance with the relevant guidelines and specifications. Pregnant women who were willing to accept NIPT were informed by consulting doctors of the objective, significance, accuracy, risk and limitations, every participant had signed written informed consent.

From January 2015 to December 2020, the retrospective study enrolled high-risk pregnancies who undergone NIPT in Guangdong Woman and Children Hospital. we recruited a total of 61581 consecutive pregnant women attending the Prenatal Diagnosis Center, which contained 49393 cases of NIPT From January 2015 to December 2020 and 12188 cases of NIPT- plus From January 2019 to December 2020 respectively. According to Chinese fetal cell-free DNA testing guidelines, the pregnancy characteristics of the pregnant women were divided to 5 groups.: (i) advanced maternal age (≥35 years, AMA); (i) ultrasound soft marker abnormality; (iii) Serological screening for high or intermediate risks; (iv) Serological screening for the single marker value abnormality (AFP, β-HCG, uE3). Pregnant women within the scope of the indication chose NIPT or NIPT-plus on to their own wishes. Although pregnant women who comply the test indications choose to be tested voluntarily, they have a tendency to choose due to different charging prices.

Samples preparation and sequencing

For each patient, Peripheral blood sample (5 ml) was withdrawn from cubital vein using EDTA-K2 tubes. and centrifuged at 4 ℃ and 1,600 × g for 10 min within 6 hr first of all and secondly centrifuged at 4°C

and 16,000 × g for 10 min for isolating plasma by Eppendorf 5810R and 5424 centrifuge (Eppendorf) . The samples were stored frozen at -20℃ as soon as possible until Genomic DNA extraction. Whole-genome sequencing was performed by Semiconductor sequencing technique on the Bioelectronseq 4000 sequencing platform (CFDA registration permit NO. 20153400309). Following the DNA library constructing, 9~23 libraries were pooled and then sequenced within ~ 200 bp reads, see our previous article for details (Yin et al., 2015). A combined GC-correction and Z-score testing methods were used to identify fetal chromosome aneuploidy of trisomy 21, 18 and 13 described previously (Liao et al., 2014). Meanwhile, fetal and maternal chromosome aneuploidy were classified using our modified Stouffer's Z score method as described previously.

Prenatal diagnosis and pregnancy follow-up

Pregnant women of high risk of NIPT were accepted genetic counseling, and were fully informed to performed prenatal diagnosis, which obtain fetal cells through (, amniotic fluid, umbilical cord blood) puncture for fetal chromosome analysis. The chromosomal detection techniques include karyotyping (The resolution of G-banding was 400 bands) and chromosome microarray analysis (CMA) (CytoScanTM 750K, available from Affymetrix, USA). To obtain information about neonatal outcome and newborn growth, we followed up all participants via telephone interviews.

Statistics

Excel and R language were used for data statistical analysis. The positive predictive values (PPVs) of fetal chromosomal aneuploidies detected by NIPT were calculated based on prenatal diagnosis results. Fisher exact probability tests were used for comparing fetal chromosomal aneuploidy PPVs for NIPT among different groups. Results with p values of less than 0.05 were considered statistically significant.

Results

Population profiles

From January 2015 to December 2020, 61581cases were successfully carried out for the NIPT detection in Prenatal Diagnosis Center of Guangdong Women and Children Hospital. There were 49393 cases opted 3 Mb reads sequencing depth detection, which was NIPT. And 12188 cases opted 8 Mb reads sequencing depth detection, which was NIPT.-plus The average age of all pregnant women who performed NIPT were 31.0±10.3 years. The majority (29.42%) of pregnant women were high risk of serological screening. Of these 61581 pregnant women, 7271were advanced maternal age women (AMA, age≥35 years), and 1781 were twin pregnancies. In addition, there were 4190 in-vitro fertilization (IVF) pregnancies. Table 1 showed the basic demographic and clinical characteristics, and Figure. 1 showed the study flow.

NIPT results

In NIPT (3 Mb reads and 8 Mb reads), a total of detected 910 positive and 60671 negative cases of fetal chromosomal aneuploidies, including 682 positive cases of NIPT with a positive rate of 1.38%, 210 positive cases of NIPT-plus with a positive rate of 1.72%. Among them of positive pregnancies 811 (89.12%) were followed up by amniocentesis and prenatal diagnosis, and the remaining 81ositive pregnancies (8.91%) refused prenatal diagnosis after genetic counseling on results. There were trisomy 21 (T21) 257 cases, trisomy 18 (T18) 67 cases, trisomy 13 (T13) 60 cases respectively. In addition, there were 211 cases of rare autosomal aneuploidies and 216 cases of sex chromosome aneuploidies. In addition, 18 cases lost pregnancy-related information due to refusal to follow up.

Comparison of PPVs between NIPT and NIPT-plus for aneuploidies

A total of 910 cases were high risk of aneuploidies that predicted by NIPT and NIPT-plus in 61581 samples, the total positive rate was 1.48%. PPVs of NIPT for T21/T18/T13 were 81.99%, 69.23%, 25.00% respectively; On the other hand, PPVs of NIPT-plus for T21/T18/T13 were 86.96%, 80.00%, 35.00% respectively. NIPT-plus had raised the PPV for trisomy 21/18/13, especially for trisomy 18 (68.52% vs 80.00%, $p<0.001$) (Table 2, Figure 1). The PPV of NIPT and NIPT-plus for other autosomal aneuploidies were 4.55% and 8.77% respectively, the difference is not statistically significant. The PPV of NIPT for Sex chromosome aneuploidy as same as NIPT-plus was 50.00%.

Comparison of PPV for aneuploidies between NIPT and NIPT-plus according to different pregnancy characteristics

At the same time, we analyzed and compared the PPVs of NIPT and NIPT-plus in different pregnancy characteristics group. PPV of the group of serological screening for high or intermediate risks between NIPT and NIPT-plus was difference, which had statistically significant. but there was no difference in PPV for another groups (Table 3).

Comparison of detection efficiency for rare trisomy

A total of 218 cases were detected for rare trisomies aneuploidies by NIPT and NIPT-plus (Figure 2. The positive rate was 0.03% and 0.57% for NIPT and NIPT-plus. The PPV was 4.55% for NIPT that was similar to 8.77% for NIPT-plus. Among 218 positive cases, 12 true positive cases were confirmed through invasive prenatal diagnosis, which included 2 cases of partial segment loss of heterozygosity, 9 cases of low-proportion mosaic trisomy, and 1 case of trisomy 20 (Table 4).

Follow-up low risk pregnancies and pregnancies who decline prenatal diagnosis

According to the NIPT technical guideline of chinese. All pregnancy women were followed-up to 3 months after the newborn’s delivery. In this study, all cases have been followed up. All pregnancy women had given birth. One trisomy 18 false negative case (Yang et al., 2017) were foundand 63 cases who refused prenatal diagnosis were followed up. All pregnancy women had given birth successfully. No visible abnormality was found in the newborn screening.

Disscussion

In this study, we analysised efficiency of NIPT to detect Chromosome aneuploidies based on SSP,and compared the performance of NIPT and NIPT-plus for detecting all fetal chromosome aneuploidies. The results revealed that there was no difference of detection performance between NIPT and NIPT-plus for aneuploidies. NIPT-plus had a better performance in detecting chromosome aneuploidies in terms of the total positive rate (1.72 vs 1.38), and NIPT-plus had a better performance in detecting T21/T18/T13. It is worth mentioning that PPVs of NIPT for other autosomal aneuploidies and sex chromosome aneuploidy was better than NIPT-plus. This would be related to the subjective will of the result reviewer.

We used PPV to evaluate the detecting performance in this study. The PPV of NIPT for T21/T18/T13 was 81.99%, 69.23%, 25.00% respectively. Results were basically consistent with the Liu' paper (Liu et al., 2020). The PPV of NIPT-plus for T21/T18/T13 was 86.96%, 80..%, 35.00% respectively, which was better than NIPT. Sequencing depth had been increased, PPVs would be increased obviously, and further improved the detection efficiency. The PPV for other chromosome aneuploidy was 4.55%of NIPT and 8.77% of NIPT-plus. The difference was not statistically significant (Chen et al., 2019). The PPV for Sex chromosome aneuploidy was 50% of NIPT as same as NIPT-plus, This is an unexpected discovery. Similarity, zheng's paper also showed this results (Zheng et al., 2020). Sex chromosomes became target disease. The awareness of preventing missed diagnosis directly affected the subjective judgment of result reviewers.

We have also analyzed the different PPV in different pregnancies characteristics group, and also compared the difference of PPV between NIPT and NIPT-plus. The difference in high-risk or intermediate-risk serological screening of PPV was statistically significant between NIPT and NIPT-plus (P = 0.03). The reason maybe that the proportion of T18 and T13 in NIPT-plus group was higher and PPV for T18 and T13 was relatively lower, which decreased the PPV. PPV of NIPT-plus for aneuploidy in ultrasound soft marker abnormalities was higher than NIPT, which was similar to other literature reports (T. Hu et al., 2021; Wang et al., 2018). Regardless of whether in China or international ("Screening for Fetal Chromosomal Abnormalities: ACOG Practice Bulletin, Number 226," 2020), the applicable population of NIPT had Clearly defined. However, PPV had slightly different in different clinical characteristics group. This study provided a certain reference value for clinicians about NIPT detection efficiency. In addition, characteristic of the two groups mainly due to the large difference in sample size between the two groups. Fetal fraction for NIPT-plus was higher than NIPT, because we used a method to increase the fetal fragment fraction from 2018 (P. Hu et al., 2019). While enrichment was not used earlier than 2018. NIPT-plus was started from 2019, which was all used this enrichment.

During recent years, other rare autosomal aneuploidies (RAAs) were often reported in clinical practice as the unexpected findings of NIPT. However, genetic counseling was difficult, because the limited clinical information on the incidence and pregnancy outcomes of suspected RAAs. The PPVs for other chromosome aneuploidies were lower at 8.39% and similar to those reported also in Lu's paper (ref. (Chen et al., 2019). But compared with Liang's paper (Liang et al., 2019), the PPV was slightly lower. The

possible reason was that RAAs were less prevalent, and the incidence is relatively low, most of the RAAs fetuses had spontaneous abortions in the early stages (Li et al., 2020). And another important reason was that these aneuploidies had high rates for confined placental mosaicism (CPM). Due to cell-free fetal DNA was mainly derived from placental trophoblast cell apoptosis. Confined placental mosaicism would cause the false positive results of NIPT (Eggenhuizen, Go, Koster, Baart, & Galjaard, 2021; Hartwig, Ambye, Sørensen, & Jørgensen, 2017). Among all the rare autosomal aneuploidies, Trisomy 7 was the most common, but PPV was still low like other rare autosomal aneuploidies. The main reason was due to CPM (Qi et al., 2019). Simultaneously, the prognosis of perinatal infants was very good (Gou et al., 2020). It is worth noting that the 2 true positive cases were confirmed to be loss of heterozygosity. it was suspected to be a uniparental diploid of chromosome 16 (mixed type) and a uniparental diploid of chromosome 14 respectively. Two fetus occurred intrauterine growth retardation, but the case of uniparental diploid of chromosome 16 had intrauterine death, and the case of uniparental diploid of chromosome 14 had a normal delivery and live birth.

Follow-up was very important part of NIPT. Except refused to be followed up, all other cases were followed up for pregnancy outcome. According to the guideline of National Health Commission of the People's Republic of China, follow-up began at week 12 after delivery. Follow-up content included complications during pregnancy and the pregnancy outcomes and the health of the newborn. The shortcoming of this study was that false-positive cases have not been verified for the placenta after delivery. On the one hand, the reason was that pregnant women were not cooperative, and on the other hand, it was relatively lack of funds.

Conclusion

In conclusion, this study compared two sequencing depth based on SSP. The PPV of autosomal aneuploidies in NIPT- plus was slightly better than NIPT. It shows that increasing the sequencing depth can improve the detection performance of aneuploidy, which has certain guiding value for clinical application. Chromosomal aneuploidy abnormalities detected by NIPT; further interventional prenatal diagnosis is required. The disadvantage of this study is that the NIPT-plus samples are relatively more less, which may lead to bias in this study.

Abbreviations

NIPT Noninvasive prenatal testing; PPV positive predictive values;RAAs Rare autosomal aneuploidy; CNV copy number variations; cffDNA cell-free fetal DNA; ; CMA chromosome microarray analysis ; NGS next-generation sequencing; SSP semiconductor sequencing platform;

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Guangdong Women and Children Hospital.

Consent for publication

The authors declare that they have no competing interests and the patients in this case report had provided their consent for publication.

Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare no conflicts of interest.

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Authors' contributions

All authors have materially participated in the study and manuscript preparation. J.-x. Y. collected all clinical data and drafted the manuscript; Jing Wu and Dongmei Wang carried out all the molecular genetic analyses; Yixia Wang,Haishan Peng,Fangfang Guo,Yaping Hou and Haoxin Ouyang participated in the data analysis; A.-h. Y. designed the work revised the manuscript. All authors have approved the final article.

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Tables

Tables 1 to 4 are available in the Supplementary Files section

Figures

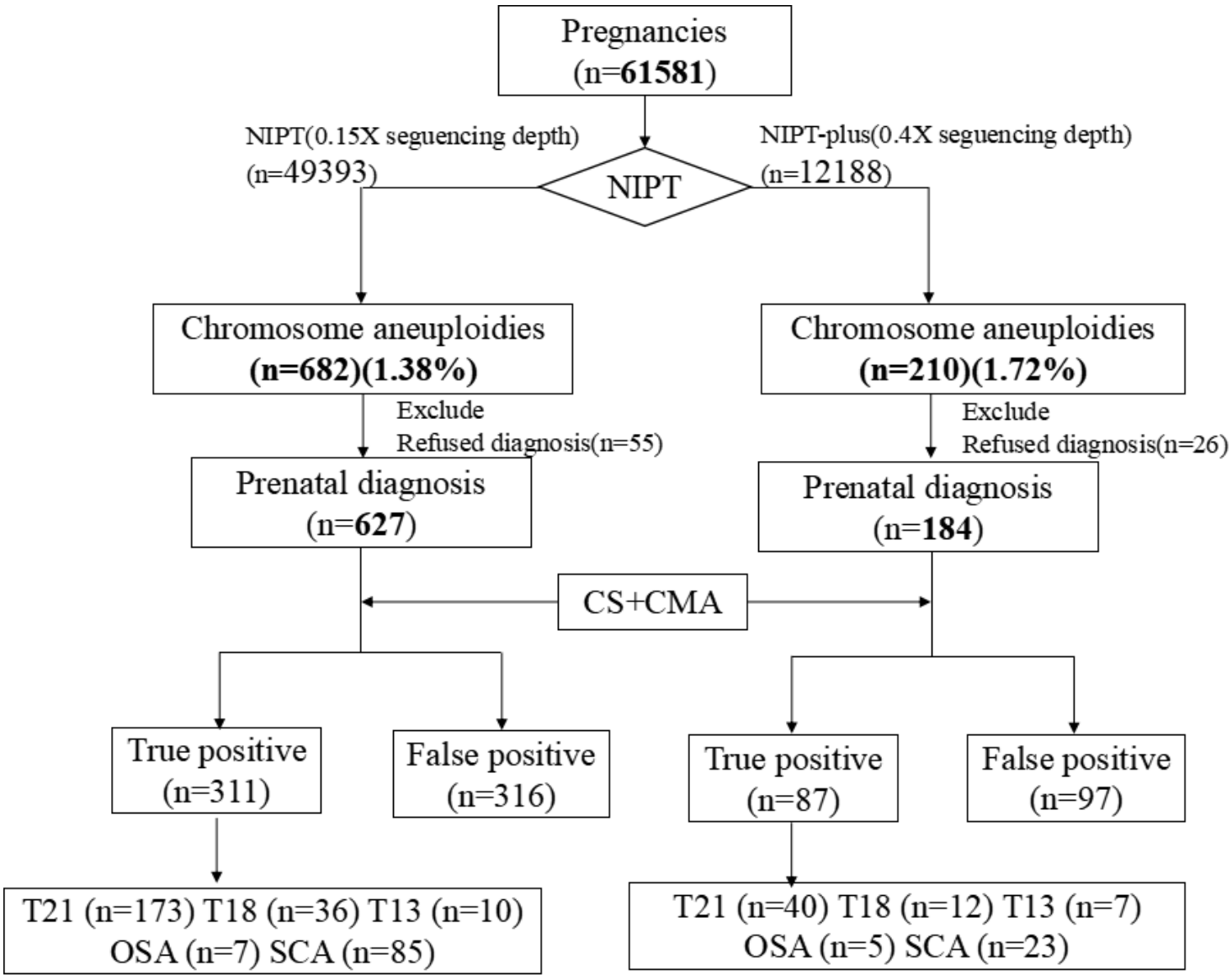

Figure 1

Flowchart of the study.

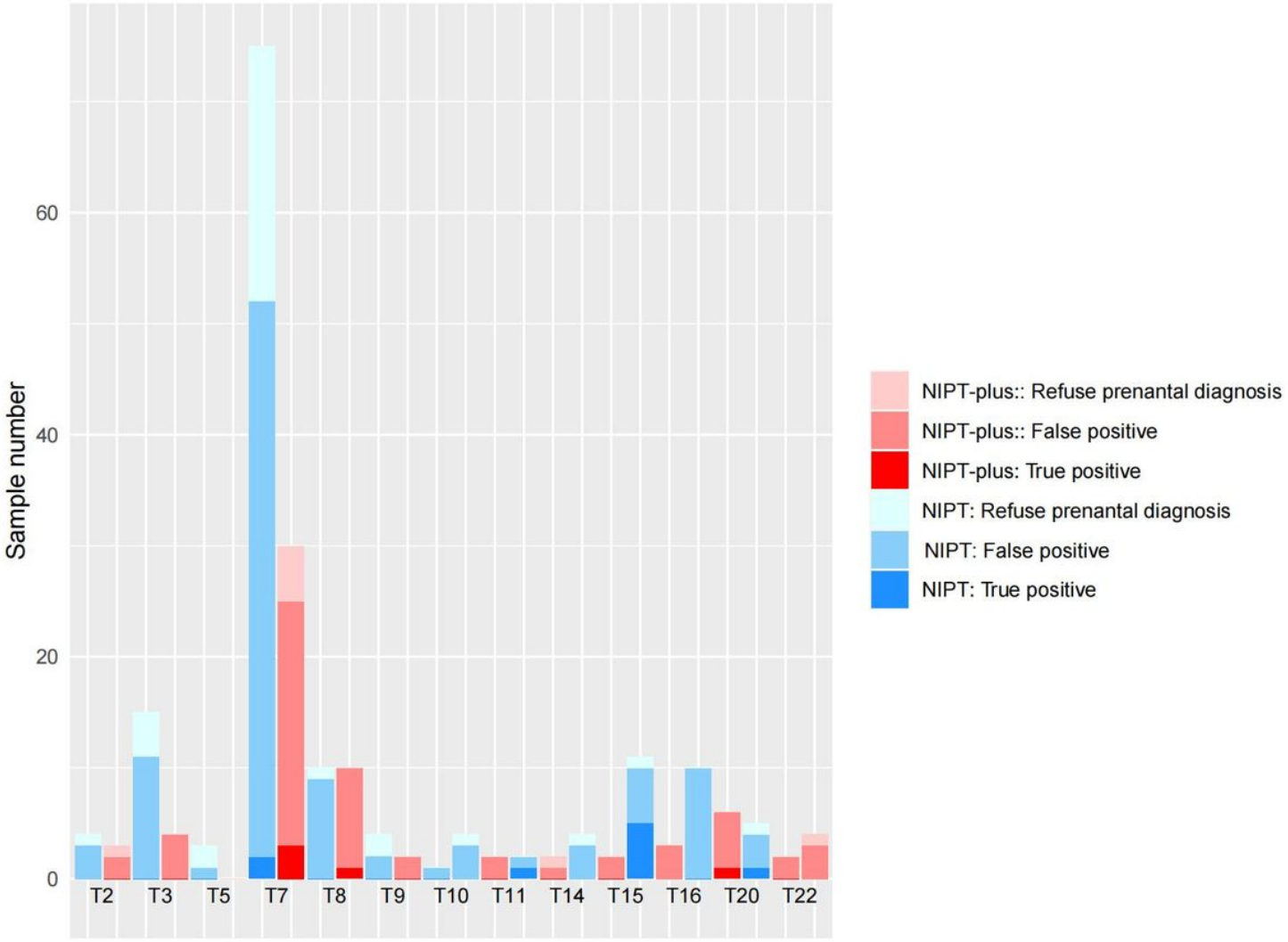

Figure 2

Distribution of Other autosomal aneuploidies between NIPT and NIPT-plus

Supplementary Files

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