Clinical performance of Non-invasive prenatal testing for sex chromosomal aneuploidies in Northeast China

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Research

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Abstract

Background: Along with the discovery of cell-free DNA (cfDNA) and the invention of next-generation sequencing (NGS), non-invasive prenatal testing (NIPT) had appeared and been applied for detecting common aneuploidies such as trisomy 21, 18, and 13, with low false-negative and false-positive rates. Recently, it had also been used for sex chromosome aneuploidies (SCAs). To assess the clinical utility of NIPT for SCAs in Northeast China, we collected NIPT data from BGI 500 sequencing platform in the Center for Reproductive Medicine, Center for Prenatal Diagnosis of the First Hospital of Jilin University, and calculate the positive predictive value (PPV) and false positive rate (FPR).

Results: A cohort of 14936 samples were analyzed by NIPT, and revealed 70 cases with SCAs high-risk, among them, 40 women agreed to undergo amniocentesis, but as many as 30 ones refused further diagnose. Based on verified fetal karyotype, 30.0% (12/40) were confirmed to be a true positive. Unluckily, the PPV for monosomy X performed 0%. Besides, positive 47,XXX were 46.67% (7/15), 40.00% (2/5) were positive for 47, XYY, and 42.86% (3/7) were positive for 47, XXY.

Conclusions: In conclusion, our present results confirmed that NIPT sequenced by BGI 500 demonstrated lowest PPVs for 45,X, but the more accurate prediction for other SCAs, it is still a potential method for SCAs screening. Henceforth, we should focus on how to improve the test utility and provide better services for pregnant women in need.

Background

With high sensitivity and specificity, non-invasive prenatal testing (NIPT) is an unparalleled screening method for fetal aneuploidies [1]. As we know, it has now been widely used in the clinical screening for trisomy diseases, such as Patau, Edwards, and Down syndrome [2,3], and the analysis of cell-free DNA (cfDNA) in maternal blood showed high accuracy in trisomy 21, 18, and 13 [4]. However, false-positive, false-negative, and failure-reportable cases can take place, and which may have some biological reasons.

Besides these goal aneuploidies, the aneuploidies occurred in the X and Y chromosomes should also be taken seriously. In 2012, NIPT of sex chromosome aneuploidy (SCAs) was introduced in the United States. At first, there was little information on the effects of these trials; however, its development was rapid [5, 6]. The influence of SCAs containing Turner (45, X), Klinefelter (47, XXY), Jacob syndromes (47,XYY) and Triple X syndrome (47,XXX) on new births is about 0.3% [7,8,9]. Turner, Klinefelter and some cases of 47,XXX could lead to delayed or abnormal sexual growth, and infertility, especially Turner and Triple X syndrome might show mental retardation [10]. To grasp the correct diagnostic information of neonatal SCAs is very important for early treatment. For many cases of SCAs, initial screening results in a better prognosis [11].

Nowadays, most laboratories provided the results of the SCAs as part of the report. However, recent researches had shown that the NIPT is less accurate in SCAs detection than autosomal aneuploidy [12,13]. But, a few of false-positive cases and low positive predict value still existed.

Our institution set up BGISEQ-500 sequencing platform in 2018, which was powered by Combinatorial Probe-Anchor Synthesis (CPAS) and modified DNA Nanoballs (DNBs) methods. In previous studies, BGISEQ-500 is a recently launched high-through put sequencing protocol; NIPT based on CPAS seemed to be a suitable method for

routine clinical screening for SCAs [14]. Therefore, in our study, we try to investigate the accuracy of NIPT in SCAs screening among pregnancies admitted to our lab from Oct 2018 to May 2020, to evaluate our clinical performance of CPAS in detecting SCAs.

Materials And Methods

Subjects

A number of 14936 samples from pregnant women who visited at our institution, between Oct 2018 and May 2020 accepted NIPT. After the report, 40 patients of 70 SCAs high-risked cases were undergone amniocentesis for further prenatal diagnosis.

Written informed consent had been signed after the nature of the procedures had been fully explained before the study. The Medical Ethics Committee of First Hospital of Jilin University approved our research (No.2018-441).

NIPT

Maternal peripheral blood samples were collected in Cell-Free DNA Storage Tube (CWBIO, Taizhou, China), and centrifuged within 96 hours. The plasma was separated and stored at -80°C until cfDNA was extracted. cfDNA was obtained from 200 µl plasma with Nucleic Acid Extraction Kit by using BGISP-300 (BGI, Shenzhen, China). And library DNA was constructed by Fetal Chromosome Aneuploidy (T21, T18, T13) Detection Kit (Combined probe-anchored polymerization sequencing method). The products were quantified by Qubit® 3.0 Fluorometer (Life Technologies, Invitrogen USA) using Qubit™ dsDNA HS Assay Kits and the concentration $\geq 8\text{ng}/\mu\text{l}$ was qualified standards. Each sample's volume was calculated according to the concentration, to make sure the same mass is mixed into a pool. After pooling, the qualified DNBs (appropriate range from 8 to 40 ng/µl) were produced and loaded onto chips, then sequenced on BGISEQ-500 (BGI, Shenzhen, China).

Karyotype and Chromosome microarray analyses of amniotic fluid cells

NIPT positive patients were recommended undergoing amniocentesis to examine fetal chromosomes. Amniocentesis was performed at 16-23 weeks of gestation to extract amniotic fluid cells, 25ml amniotic fluid cells were cultured in amniotic membrane medium (Irvine Scientific, Santa Ana, CA, USA), then treated with colcemid. G-banding was performed on metaphase chromosomes by standard method. Each individual had at least 30 metaphase cells counted, and at least 5 cells analyzed. Chromosomal abnormalities were described on the basis of the criteria, which were established by International System for Human Cytogenetic Nomenclature (ISCN 2013). Simultaneously, 10ml uncultured amniotic fluid cells were used to detect by Chromosome microarray analyses based on Affymetrix CytoScan 750K array chips (Affymetrix, Santa Clara, CA). Copy number variations (CNVs) and heterozygosity loss (LOH) in the human genome were revealed by ≥ 50 probe markers and $\geq 200\text{Kb}$ resolution, the probe located on 22 pairs of autosomes and sex chromosomes. The results of CNVs and LOH were analyzed by comparing with the public databases or published works of literature.

Statistics

Online software (<http://vassarstats.net/clin1.html>) was used to calculate the FPR and PPV, the amniocentesis results regarded as the gold standard, based on the standard normal distribution, the 95% confidence interval (CI) was calculated by the clopper-Pearson method.

Results

NIPT was used to evaluate our fetal DNA in detecting SCAs performance. As a result, the analysis of 14936 pregnancies revealed 70 cases with SCAs high-risk results; the total frequency for the SCAs-positive was 0.50% (70/14936). Among the 70 abnormal cases, NIPT detected 24 cases with Monosomy X (45,X), 27 cases with Trisomy X (47,XXX), 8 cases with 47,XYY and 11 cases with 47,XXY. To verify the accuracy of NIPT positive results, amniocentesis was recommended for verification of abnormal samples. However, of the 70 SCAs-positive cases, 40 women agreed to have a fetal karyotype, but the other 30 refused further examinations for some reasons. The next confirmation according to fetal karyotyping and CMA was available for 40 positive cases (13 for 45,X; 15 for 47,XXX; 5 for 47, XYY; and 7 for 47,XXY;) and the other 30 patients (11 for 45,X; 12 for 47,XXX; 3 for 47,XYY; and 4 for 47,XXY) were unconfirmed because the women denied amniocentesis. Compared to the gold standard, none true positive results were found in 13 cases of monosomy X; of the 15 patients with trisomy X, 7 were true positive, with PPV of 46.67%(7/15). Of the 5 cases of the 47,XYY, 2 cases were true positive, so the PPV of 47,XYY by NIPT was 40.00% (2/5). At last, NIPT revealed 47,XXY with a PPV of 42.86% (3/7), 3 cases of NIPT results were coincident with the diagnose of amniocentesis (Table 1).

At the same time, the comparison of the whole results between NIPT and amniocentesis for fetal SCAs was showed in Table 2. We had known that NIPT screening for Monosomy X in our study showed the highest FPR of all SCAs types, exaggeration to 100%. However, case 1 showed a fetal karyotype of sex chromosome abnormalities (46,XX?) after conventional G-banding, and CMA result explained the abnormality of X chromosome was a 0.356 Mb deletion of Xq27, a 0.237 Mb duplication of Xp22 and 0.338 Mb duplication of Xq13. Also, case 2, case 7 and case 13 were all detected microduplication or microdeletion of X chromosome or autosomes. Case 14-28 in table 2 displayed the comparison of Trisomy X, NIPT, fetal karyotype by G-banding and CMA showed consistent trends in 7 fetuses. About cases with 47, XYY high-risk, in case 29, amniocentesis result not only showed the same karyotype with NIPT but also detected chromosome polymorphism and a microduplication of chromosome 2q. Among 47,XXY high-risked cases 34-40, the false-positive case 39 and case 40 revealed CNVs of autosomes nevertheless. Otherwise, 2 healthy fetuses proved by CMA showed chromosome polymorphism karyotypes of 46,XN,1qh+ and 46,XN,21pss in case 8 and case23.

Discussion

The sensitivities for T21, T18, and T13 had reached greater than 99% [15]. However, as far as sex chromosomes were concerned, aneuploidy detection remained problematic, as some previous studies had shown [5,6,16,17]. This time, we focused on SCAs detected by BGISEQ-500 sequencing platform. Among the 14936 cases, 70 cases showed sex chromosome abnormality by NIPT. Karyotype analysis of 40 SCAs cases; the consistency rate of amniotic fluid was about 30.00%. The detection rate was different from previous tests, so the positive predictive value was used as a more accurate method. And thus, concrete SCAs' PPV is all be counted (45,X=0%, 47,XXX=46.67%, 47XYY=40%, 47,XXY=42.86%), from the results, 45,X detection seemed to be none accurate. Similarly, in 2018, Li et al. reported a series of SCAs' PPV by the same methods with us, ranged from 47,XXX (90%, CI₉₅,55.95-98.46%), 47,XXY (75%, CI₉₅, 49.25-90.27%), 47,XYY (50%, CI₉₅, 12.37-87.63%) to 45,X (36.84%, CI₉₅, 25.00-50.52%). The PPV of 45,X was the lowest, too[18]. Wang et al. also showed their PPV of SCAs sequenced by BGISEQ-500, the PPV in total SCAs was 19/33(57.6%), the specific PPV of 45,X was 3/14(21.4%), 47,XXX 3/4(75.0%), 47,XXY 10/11(90.9%), 47,XYY 3/4(75.0%) [19]. All data indicated that the FPR of 45,X was still the highest based on BGISEQ-500, and the accuracy needed to be improved.

Several reasons might explain the poor PPV of monosomy X: at first, as we know, there were 1098 genes located on the X chromosome, but Y chromosome contained only 78 genes, among them, there were also homologous 58 genes on both X chromosomes and Y chromosomes, and 29 genes were at the ends of sex chromosomes. Secondly, the sequencing read length was only 35 bases, which might easily lead to the misplacing of X and Y chromosome. The non-random inactivation of X chromosomes in placental tissues may be the third reason, in XX female trophoblast cells, X chromosomes from father tended to inactivate more easily [20,21,22].

Although, the current NIPT could detect fetal 21, 18 and 13 trisomies, with high sensitivity and specificity [23,24]. But, a small number of tests could give false-positive results or a complete failure for several reasons, including maternal chromosomal mosaicism [25], confined placental mosaics [26], maternal malignancy [2,27,28,29], co-twin demise [30], and so on. In the same way, these causes could also lead to false positive results for SCAs. Generally speaking, our results of all types SCAs performed on the lower side than the above mentioned, because of fewer subjects (57.14%, 40/70) would to accept invasive surgery, who afraid of miscarriage or intrauterine infection, its small size limited our study, in the future, we'll keep watching and updating the results.

Otherwise, it is worth mentioning that among our revealed false-positive SCAs cases, 2 cases were found CNVs of the X chromosome, and there were 3 cases found with autosomal CNVs detected by CMA, including microdeletion and microduplication. It is worth considering whether other fetal CNVs will cause different NIPT risk values to increase, and it is necessary to carry out CMA and karyotype analysis simultaneously, if possible.

Conclusion

NIPT powered by BGI 500 sequencing platform yielded the lower PPVs for SCAs, especially for 45,X. Forecast of other SCAs was more accurate. However, this technique needed further research. Invasive prenatal karyotype analysis should be recommended for pregnant women with fetal SCAs, and CMA ran in parallel if possible. Clinicians should fully inform patients of the limitations of NIPT and make it clear that it is only a prenatal screening technology. Fetal health should be evaluated in combination with other measures.

Declarations

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Author's contribution

Lu Wang, Rulin Dai and Yuting Jiang performed the clinical data collection and analysis. Qingyang Shi performed genetic counseling and amniocentesis. Karyotype analysis was performed by Hongguo Zhang. The first draft was written by Lu Wang, and Ruizhi Liu was responsible for revision, at last, Qi Xi submitted this article.

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Availability of data and materials

All data generated or analyzed in this study are presented in the article.

Competing interest

The authors state that they have no conflict of interest.

Ethics approval and consent to participate

Written informed consent was obtained from all patients participating, and our study was authorized by the Ethics Committee of the First Hospital of Jilin University. (No.2018-441)

Consent for publication

All patients agreed to authorize our institution to use their samples, phenotypic information and testing data.

Abbreviations

NIPT: non-invasive prenatal testing; cfDNA: cell-free DNA; NGS: next-generation sequencing; SCAs: sex chromosome aneuploidies; PPV: positive predictive value; FPR: false positive rate. CPAS: Combinatorial Probe-Anchor Synthesis; DNBs: DNA Nanoballs; CMA: Chromosome microarray analyses; CNVs: Copy number variations; LOH: heterozygosis loss; ISCN: International System for Human Cytogenetic Nomenclature.

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Tables

Table 1 Efficiency of NIPT in SCAs screening.

SCAs types	NIPT positive	Karyotype validated	True positive	False positive	Positive predictive value(PPV)(CI ₉₅)	False positive rate (FPR)(CI ₉₅)	Unknown
45,X	24	13	0	13	0.00% (0.00%-28.34%)	100% (71.66%-100%)	11
47,XXX	27	15	7	8	46.67% (22.28%-72.58%)	53.33% (27.42%-77.72%)	8
47,XYX	8	5	2	3	40.00% (7.26%-82.96%)	60% (17.04%-92.74%)	3
47,XXY	11	7	3	4	42.86% (11.81%-79.76%)	57.14% (20.24%-88.19%)	4
Total	70	40	12	28	30% (17.09%-46.71%)	70% (53.29%-82.91%)	30

Table 2 The result comparison among NIPT, conventional fetal karyotype by G-banding and CMA.

Case	NIPT result	Fetal karyotype	CMA result
1	45,X	46,XX?	delXq27(0.356 Mb); dupXp22(0.237 Mb) dupXq13(0.338 Mb)
2	45,X	Normal	delXp22.33(0.694 Mb)
3	45,X	Normal	Normal
4	45,X	Normal	Normal
5	45,X	Normal	Normal
6	45,X	Normal	Normal
7	45,X	Normal	dup20p13(0.413Mb)
8	45,X	46,XN,1qh+	Normal
9	45,X	Normal	Normal
10	45,X	Normal	Normal
11	45,X	Normal	Normal
12	45,X	Normal	Normal
13	45,X	Normal	dup8q11.23(0.24Mb)
14	47,XXX	Normal	Normal
15	47,XXX	47,XXX	47,XXX
16	47,XXX	Normal	Normal
17	47,XXX	Normal	Normal
18	47,XXX	Normal	Normal
19	47,XXX	47,XXX	47,XXX
20	47,XXX	Normal	Normal
21	47,XXX	47,XXX	47,XXX
22	47,XXX	47,XXX	47,XXX
23	47,XXX	46,XN,21pss	Normal
24	47,XXX	47,XXX	47,XXX
25	47,XXX	Normal	Normal
26	47,XXX	Normal	Normal
27	47,XXX	47,XXX	47,XXX
28	47,XXX	47,XXX	47,XXX
29	47,XYX	47,XYX,inv(9)	47,XYX,dup2q13(0.48Mb)

		(p11q13)	
30	47,XXY	Normal	Normal
31	47,XXY	Normal	Normal
32	47,XXY	Normal	Normal
33	47,XXY	47,XXY	47,XXY
34	47,XXY	Normal	Normal
35	47,XXY	47,XXY	47,XXY
36	47,XXY	47,XXY	47,XXY
37	47,XXY	Normal	Normal
38	47,XXY	47,XXY	47,XXY
39	47,XXY	Normal	dup18p11.31(0.471Mb)
40	47,XXY	Normal	del6q26(0.348Mb)