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Original Article

Non-invasive prenatal testing assisted in detecting fetus sex chromosome aneuploidy: a retrospective study of 6,002 singleton pregnancy women cohort

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Abstract: Objectives: The objective of this study was to evaluate the performance of non-invasive prenatal testing (NIPT) in detecting fetus sex chromosome aneuploidy (SCA). Methods: This was a retrospective study in a large patient cohort of 6,002 singleton pregnancy women which underwent NIPT as a prenatal screening test for trisomies 21, 18 and 13, with X and Y chromosomes as secondary findings, in Yantai Yuhuangding Hospital. Results: In the present study, 26 cases were classified as SCA-positive by NIPT. In these cases, karyotyping confirmed 11 cases of the NIPT results (four XXX cases, two XXY cases, three XYY cases and two X cases), giving a positive predictive value of 52.38% (11/21 cases confirmed by karyotyping), ten cases received the examination results and terminated pregnancy, one case decided to continue with pregnancy and there was no abnormality in the appearance of the newborn. In addition, the false positive rate was 38.09% (8/21) and seven cases could not be confirmed by karyotyping. Conclusion: Based on our results, clinical application of NIPT on the non-invasive detection of fetal SCA is feasible. Along with other clinical testing methods, it would provide a simple, safe and convenient clinical way for patients to make decisions.

Keywords: Sex chromosome aneuploidy (SCA), non-invasive prenatal testing (NIPT), fetus, singleton pregnancy women

Introduction

Sex chromosome aneuploidy (SCA) happens with a frequency of 1 in 500, an incidence that is greater than that of trisomy 21 [1]. The most common SCAs include monosomy X (Turner syndrome), XXY (Klinefelter syndrome), XXX (triple X syndrome), and XYY (XYY syndrome). Most cases of triple X and XYY are phenotypically mild without intellectual disability and hence with low clinical evaluation value. However, Turner syndrome and Klinefelter syndrome both have a well-established phenotype which including physical abnormalities, learning delays, and infertility [2-5]. The percentage of most pregnancies affected by Turner syndrome result in spontaneous abortion is up to 90%, but in those that survive, approximately 30% will present with cystic hygroma, thickened nuchal translucency, cardiac defects, and fetal hydrops [6, 7].

Cell-free fetal DNA (cffDNA) has received significant attention for the purposes of prenatal genetic testing in the past decade [8]. Non-invasive prenatal testing (NIPT), based on isolation of cffDNA from maternal blood, is underutilized for many applications including but not limited to prenatal diagnosis, aneuploidy screening, copy number detection (CNV), and prediction of preeclampsia [9-11]. Commercially available NIPT kits yield different sensitivity and specificity for each disease mainly due to difference on the method of isolation, replication, and analysis. Some of the available kits for fetal chromosome abnormalities offer a detection rate of 99% for trisomies, and with 0.15% false positive rates for aneuploidy detection [12-16]. Combined with previous study results, NIPT offers a much better PPV on detection of chromosome aneuploidy abnormalities compared to other available noninvasive screening tests.

Different studies results show that there is a wide range of accuracy and specificity in the detection of sex chromosomal abnormalities by NIPT, which also varies degrees of false positive and false negative. The American College of Obstetricians and Gynecologists (ACOG) reports that NIPT has a sensitivity of 91.0% and specificity of 99.6% to identify sex chromosome abnormalities in patients who receive interpretable results [17, 18]. Some studies have reported positive predictive values depending on the particular sex chromosome variant identified, but in general range from 20 to 40%, around 50% at best [19, 20]. False positive rates have been reported to be between 0.0% and 0.1% [21, 22]. Even so, NIPT provides a resource for families and physicians to correctly identify XXY prenatally, making it beneficial to the medical community and lay public.

Based on these results, we recruited 6,002 singleton pregnancy women samples to further discuss the applicability of NIPT technology in the detection of sex chromosome abnormality, and provide more evidence for the data interpretation.

Methods and materials

A total of 6,002 women from January 2015 to September 2017 with singleton pregnancies were recruited from the prenatal diagnostic center of Yantai Yuhuangding Hospital, with written informed consent and Institutional Ethics Committee approval. A total of 5-6 mL of maternal peripheral blood samples was collected processed, and analyzed using validated molecular biology and bioinformatics methodology. All samples were analyzed for fetal copy number of chromosomes 13, 18, 21, X and Y. Note that informed consent for the standard NIPT assay was only limited to fetal risk of chromosomes 13, 18 and 21 trisomy aneuploidy. Whole blood samples were collected in either EDTA tubes within 8 hours or cell-free DNA BCT tubes (Streck Inc.; Omaha, NE) within 72 hours or on processed plasma that was shipped and received frozen. Cell free DNA (cfDNA) extraction, library construction, quality control and pooling were performed according to instrument of JingXin Fetal Chromosome Aneuploidy (T21, T18, T13) Testing Kits (CFDA registration permit No. 0153400300). Then for DNA sequencing, 15~20 libraries were pooled and sequenced with ~200 bp reads by JingXin BioelectronSeq 4000 System (CFDA registration permit NO. 20153400309) that was a kind of semiconductor sequencer. Sequencing reads were filtered and aligned to the human reference genome (hg19) [23]. Fetal DNA concentration was calculated as quality control using our previously mentioned method [24]. A combined GC-correction and Z-score testing methods were used to identify fetal autosomal aneuploidy of trisomy 21, 18, 13, X and Y as described previously. Additionally, fetal and maternal chromosome copy number variations (CNVs) were classified using our modified Stouffer's Z score method as described previously.

Results

In the present study, a total of 6,002 singleton pregnancy women were recruited to display NIPT, finding that 26 samples of sex chromosome abnormality, with positive rate of 0.43%. The NIPT results for the above mentioned 26 samples were as follows; four XXX cases, eight XXY cases, five XYY cases, and nine monosomy X cases. The information about the 26 sex chromosome abnormality samples is shown in **Table 1**. Among the 26 cases with sex chromosome abnormality, seven cases failure to be confirmed by karyotyping which including three XXY cases, four monosomy X cases. The positive predictive value for detection of sex chromosome abnormalities was 52.38% (11/21, confirmed by karyotyping), and false positive rate was 38.09% (8/21).

Among of eleven cases (four XXX cases, two XXY cases, three XYY cases and two X cases) of positive results, one case (No. 4 in **Table 1**) was diagnosed of XXX by NIPT, and also detected a 14M deletion on chromosome 21. Additionally, karyotyping showed XXX result, and finally this case was listed as true positive. Furthermore, two cases (No. 5 and No. 6 in **Table 1**) with NIPT results were XXY, confirmed by karyotyping analysis of XYY; while diagnosed XXY/XYY chimera with the placenta tissue and XYY with neonatal peripheral blood, these two cases were also listed as true positive. In addition, one case (No. 18 in **Table 1**) was monosomy X, that confirmed as 45,X0[70]/46,XX[30] chimera by karyotyping was listed as true positive, too. However, one case (No. 15 in **Table 1**) of

Table 1. The information for 26 cases of sex chromosome abnormalities by NIPT

No.	NITP Result	Z Value	Age (years)	Gestation (weeks)	Serological	Ultrasound	Karyotying analysis	Medical History	Pregnancy Outcome
1	47,XXX	(chrX) 5.293 (chrY) 0.402	39	18	/	NCAG	47,XXX	/	Pregnancy terminated
2		(chrX) 10.673 (chrY) -2.475	40	17	/	NCAG			
3		(chrX) 7.611 (chrY) -0.261	37	15	/	NT=1.0			
4		(chrX) 5.673 (chrY) -0.241	43	15	/	NT=2.3			
5	47,XXY	(chrX) -0.779 (chrY) 44.576	40	17	T21:1/10	NCAG	47,XXY	/	Pregnancy terminated
6		(chrX) 0.006 (chrY) 50.280	35	13	/	NT=2.7-3.6			
7		(chrX) 1.015 (chrY) 13.254	38	15	/	NT=1.3	Normal	/	Continued gestation
8		(chrX) 1.003 (chrY) 12.094	31	18	/	NT=1.38		/	
9		(chrX) 1.897 (chrY) 26.694	36	13	/	NT=1.4		Thyroid papillary carcinoma	
10		(chrX) 0.59 (chrY) 25.33	38	15	/	NT=1.9	/	/	
11		(chrX) 1.002 (chrY) 34.904	36	15	/	NCAG			
12		(chrX) 1.745 (chrY) 21.552	38	14	/	NT=1.4			
13	47,XYY	(chrX) 1.553 (chrY) 16.087	29	17	T21:1/65	NCAG	47,XYY	Threatened abortion history	Pregnancy terminated
14		(chrX) 1.524 (chrY) 2.382	41	17	/	/	47,XYY	/	No abnormality in the appearance of the newborn
15		(chrX) -1.116 (chrY) 28.862	39	17	/	NT=1.5	chr 14 microduplication	/	Continued gestation
16		(chrX) 1.246 (chrY) 27.939	38	17	/	NCAG	47,XYY	/	Pregnancy terminated
17		(chrX) -5.19 (chrY) 56.422	36	14	/	NT=2.0	Normal	Thyroid papillary carcinoma	Continued gestation

18	45,MX	(chrX) -4.276 (chrY) -1.614	29	17	T2:1/566 T18:1/976	Normal	45,XO[70]/46,XX[30]	/	Pregnancy terminated
19		(chrX) -17.609 (chrY) -0.015	32	18	T21:1/618 T18':1/32817	/	Normal	/	Continued gestation
20		(chrX) -30.361 (chrY) 0.884	35	15	/	/		/	
21		(chrX) -4.282 (chrY) 0.268	41	15	/	/		Spontaneous abortion history (3 times)	
22		(chrX) -19.038 (chrY) -0.491	33	22	T21:1/549	NT=1.3	/	threatened abortion	
23		(chrX) -3.801 (chrY) -1.132	31	17	/	Normal		/	
24		(chrX) -4.549 (chrY) 2.129	28	18	T21:1/79	NT=1.1			
25		(chrX) -3.941 (chrY) 0.292	31	17	T21:1/376 T18:1/15148	NCAG	45,X	Pregnant women and her parents in short stature	Pregnancy terminated
26		(chrX) -6.215 (chrY) 0.641	38	17	/	Fetal hydrops	/	/	

NCAG: nothing abnormal detected.

XYY, which was detected by karyotyping only had microdeletion on chromosome 14, was listed as a false positive result. Of the eleven positive cases, ten cases received the examination results and terminated pregnancy, one case decided to continue with pregnancy and there was no abnormality in the appearance of the newborn.

In addition, among the eight cases of false positive, two pregnant women had thyroid papillary carcinoma and had undergone thyroid resection.

Discussion

Non-invasive prenatal test of fetal sex using cell-free fetal DNA provides an alternative to invasive techniques in families at risk for sex-linked disorders since 2011. Subsequent studies demonstrated that the ability to analyze sex chromosome sequences using massively parallel sequencing to classify XX, XY and monosomy X with a high degree of sensitivity and specificity and also to detect other SCA, including XXX; XXY and XYY [25, 26].

In the present study, 26 singleton pregnancy women with sex chromosome abnormalities were detected by NIPT results. The positive predictive value and false positive rate for detection of sex chromosome abnormalities was 52.38% (11/21, confirmed by karyotyping) and 38.09% (8/21), respectively. In all, these were eleven cases of true positive value confirmed by karyotyping, eight cases with false positive value, seven cases hadn't confirmed by karyotyping. Among of the seven cases, one case detected as fetal hydrops (No. 26 in **Table 1**) refused invasive diagnosis, and six cases unknown. Among the eleven positive cases, there were four XXX cases, two XXY cases, three XYY cases and two X cases. All (four) cases were diagnosed with XXX and confirmed by karyotyping. Whether this phenomenon suggested that the experimental kit used in this study was superior to the screening of chromosome abnormality XXX, requires more samples for confirmation. There are several reasons for the failure outcome of six unknown cases as followed, 1) The pregnant women refused to receive the interventional prenatal diagnosis, 2) The newborn was too young to find any significant abnormalities, 3) Lost contact.

Based on our study, two cases (No. 5 and No. 6 in **Table 1**) with NIPT results of XXY, while confirmed by karyotyping of XYY and diagnosed XXY/XYY chimera with the placenta tissue and XYY with neonatal peripheral blood. According to the results of previous studies, XXY and XYY were difficult to judge in NIPT detection. The unique biology of SCA presents different challenges than autosomal trisomy for prenatal detection via the analysis of cfDNA. A primary consideration is the incidence of mosaicism of both maternal and fetal origin. As women age, they experience a natural loss of the X chromosome resulting in a baseline rate of mosaicism that would generate maternal cfDNA with fewer X chromosome fragments than expected [27]. The potential presence of fetal mosaicism must also be considered, as 10-15% of XXY and XXX cases are mosaic, and up to 50% of surviving cases of Monosomy X display multiple cell lines [2-4]. In addition to the potential for mosaicism, undiagnosed maternal SCA may also complicate testing. Approximately 90% of XXX cases never come to clinical attention despite an overall incidence of 1 in 1000, meaning that many women with this condition would be unaware of their status prior to testing [2]. In addition to SCA, expansion of the testing platform to include analysis of the Y chromosome allows for the prenatal determination of fetal sex based on the presence or absence of Y.

Furthermore, we learned that the false positive rate was 38.09% (8/21) in our study, and among the eight cases of false positive, two pregnant women had thyroid papillary carcinoma and had undergone thyroid resection. Whether tumor-free DNA could play a role on the false positive results will require additional experimental data to prove.

From our results, we learned that the positive predictive value for detection of sex chromosome abnormalities was 52.38%, which could be explanted as mild phenotypic presentation, high rates of fetal mosaicism, risk of identifying maternal aneuploidy, and inefficiency of NIPT to properly identify XXY without cytogenetic confirmation are several associated concerns and may contribute to why this screening is not yet offered routinely [28]. Comprehensive information including the variability of the phenotype and response to intervention is often limited [28]. Current counseling for a XXY diagnosis

focuses on long-term implications involving health, physical development and intellectual capacity [29]. Nevertheless, positive results of NIPT should be heeded with caution and an invasive diagnostic procedure should be performed, especially for rare chromosomal abnormalities and sex chromosome abnormalities where NIPT performs subpar compared to its performance for detection of trisomy 21.

Conclusion

In the present retrospective study, a large patient cohort of 6,002 singleton pregnancy women were analyzed. A total of 26 cases were classified as SCA-positive by NIPT. In these cases, karyotyping confirmed 11 cases of the NIPT results (four XXX cases, two XXY cases, three XYY cases and two X cases), giving a positive predictive value of 52.38% (11/21 cases confirmed by karyotyping), ten cases received the examination results and terminated pregnancy, and one case decided to continue with pregnancy and there was no abnormality in the appearance of the newborn. In addition, the false positive rate was 38.09% (8/21) and seven cases hadn't been confirmed by karyotyping. NIPT is now playing an increasing important role on pregnancy screening, when the result prompted for risk. NIPT should strengthen the ultrasound examination in the middle of pregnancy, in order to detect abnormalities early and took measures to minimize damage to the pregnant. Based on our results, clinical application of NIPT in the non-invasive detection of fetal SCA is feasible. Along with other clinical testing methods, it could provide a simple, safe and convenient clinical way for patients to make decisions.

Disclosure of conflict of interest

None.

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