RESEARCH LETTER

Noninvasive prenatal genetic testing for fetal aneuploidy detects maternal trisomy X

Hong Yao[1], Lei Zhang[2], Hongyun Zhang[2], Fuman Jiang[2], Hua Hu[1], Fang Chen[2], Hui Jiang[2], Feng Mu[2], Lijian Zhao[2], Zhiqing Liang[1]* and Wei Wang[2]*

[1]Prenatal Diagnosis Center, Department of Gynaecology & Obstetrics, Southwest Hospital, The Third Military Medical University, Chongqing, China
[2]Beijing Genomics Institute (BGI), Shenzhen, China
*Correspondence to: Wei Wang. E-mail: wangw@genomics.cn; Zhiqing Liang. E-mail: zhiq.lzliang@gmail.com

Funding sources: None
Conflicts of interest: The co-authors, Lei Zhang, Hongyun Zhang, Fuman Jiang, Fang Chen, Hui Jiang, Feng Mu, Lijian Zhao and Wei Wang are employed by BGI. The other authors, Hong Yao, Hua Hu and Zhiqing Liang are employees of the Third Military Medical University and have no financial relationship with BGI.

Trisomy X is a sex chromosomal abnormality with a variable phenotype caused by the presence of an extra X chromosome in females (47,XXX instead of 46,XX). It is the most common female chromosomal abnormality, occurring in approximately 1 in 1000 female births.[1] There is considerable variation in the phenotype, from asymptomatic and very mildly affected to significant physical and psychological features, leading only 10% of diagnosis ratio for those individuals with trisomy X.[2–4] Current prenatal diagnosis of trisomy X relies on invasive prenatal tests such as karyotyping analysis. Although such tests allow accurate diagnosis, wide clinical use is limited by its complex operations and invasive nature with a 0.5% to 1% of procedure-related miscarriage.[5,6] Recently, a new method based on massively parallel sequencing for cell-free DNA in maternal plasma was developed to detect fetal trisomy disorders. A rapid response statement from a committee on behalf of the Board of the International Society for Prenatal Diagnosis showed that the test currently available is only for fetal trisomy 21 and trisomy 18, which constitutes only about half of the fetal aneuploidy that would be identified through amniocentesis or CVS.[7] Prior studies have shown that this massively parallel sequencing based noninvasive fetal trisomy test (the NIFTY test, which has no relationship with the 'NIFTY' trial on noninvasive prenatal diagnosis that was sponsored by the US National Institutes of Health) can not only detect trisomy 21 and trisomy 18, but also sex chromosomal aneuploidies.[8,9] However, the effect of the maternal genetic background on the performance of the NIFTY test is not clear. Here we present a case in which maternal chromosome X materials affect the performance of the NIFTY. This case may provide a useful complement for the clinical application of the NIFTY test.

CASE PRESENTATION

The female patient, aged 25 years, is about 165 cm high and 42 kg in weight, with normal phenotype. The patient had secondary education and was employed as a worker at a factory in southwest China. Her menarche started at about 12 years old, with a regular menstrual cycle about 5 days/28 days. She has normal breast development and nipple spacing. Pubic hair and armpit hair are in adult type. The patient was married at 23 years old. In 2010, she had a natural abortion because the fetus stopped growing at the stage of 40-day intrauterine embryo. The same kind of disease and other genetic or familial disease histories were not found in her family members. She is the only child of her parents and no infaust history was reported during the pregnancy of her mother. For this pregnancy, her last menstrual period was reported on 29 April 2011. No sign of abortion was found during the early pregnancy. At the 17^{+4} gestational week, maternal serum screening test of Down syndrome was performed and the result showed a high risk of T21. After the maternal serum screening test, NIFTY test was accepted by the patient based on an informed consent at 19^{+4} gestational weeks following the published protocols.[9] The NIFTY test showed that the risk of fetal T21 and T18 is low, but was highly suspected with trisomy X. To make a further analysis of this trisomy X-suspicious case, we recruited ten pregnant women with singleton euploid male fetuses and ten with singleton euploid female ones from the same center as the control group. The informed written consent and local approval were obtained. All those participants were in the gestation weeks synchronous with the trisomy X-suspicious case (19 gestational weeks). Relationship between *Z*-score and cell free fetal DNA (cffDNA) ratio was used to assay the status of the fetal chromosome X. According to prior studies, cffDNA amount to 10% to 20% of the total DNA circulating in the maternal plasma.[10] We found that the level of chromosome X in the plasma of this trisomy X-suspicious patient was significantly higher than that of the control group (Figure 1). Hence, we speculated that the aberration most likely was contributed by the pregnant patient

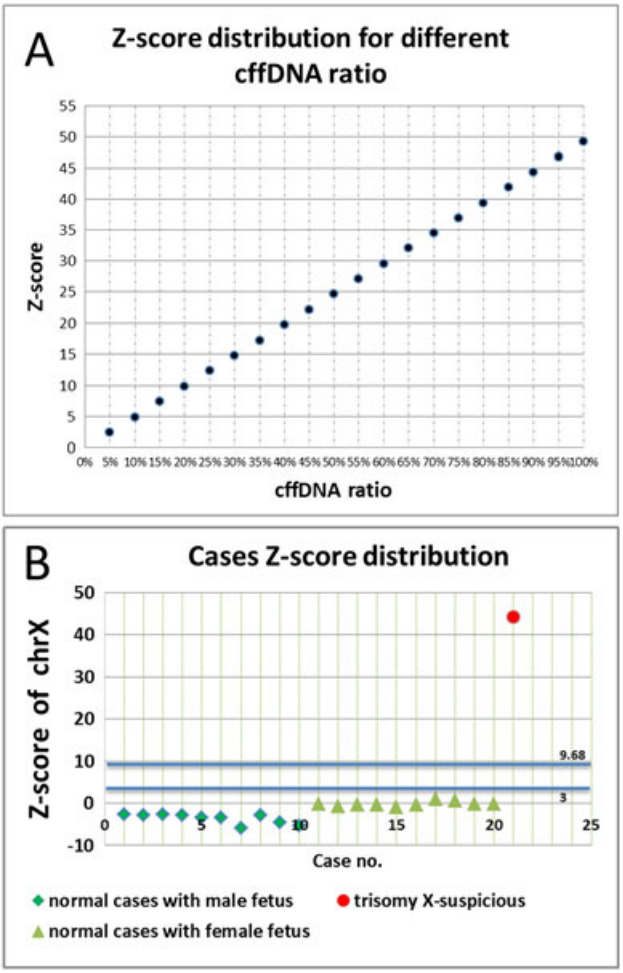

Figure 1 Data analysis for the trisomy X-suspicious case. (A) *Z*-score distribution for different cffDNA ratio, (B) *Z*-score distribution for the trisomy X-suspicious case. Relationship between *Z*-score and cffDNA ratio was used to assay the status of the fetal chromosome X. The *Z*-scores of those pregnancies with normal female fetus are between −2 and +2 while the *Z*-scores less than −2 are corresponding to pregnancies carrying normal male fetus. A *Z*-score >3 (lower blue line, corresponding fetus cell free DNA concentration in maternal serum equals 5%) and <9.68 (upper blue line, corresponding fetus cell free DNA concentration in maternal serum equals 20%) indicates a supernumerary X pregnancy. The chromosome X *Z*-score for the trisomy X-suspicious was 44.21 (corresponding fetus cell free DNA concentration in maternal serum equals 90%), which is obviously beyond the high limitation of fetus sourced supernumerary X pregnancies

herself, but whether the fetus is normal or not were unclear. A NIFTY post-test genetic counseling was given to the pregnant patient and her families by a senior genetic counselor on the following points, including (1) the formation mechanisms of the trisomy X, possible phenotypic abnormalities, the influence of fertility and treatment; (2) the sensitivity and specificity of the NIFTY test on sex chromosomal abnormalities assessment; (3) current diagnostic approaches for trisomy X in clinical practices. The pregnant patient then decided to take the diagnostic procedures after the counseling, and her peripheral blood cells and amniotic fluid were obtained to perform the karyotyping and fluorescence *in situ* hybridization analysis at 22^{+3} gestational weeks. Both karyotyping and fluorescence *in situ* hybridization results showed that this pregnant patient carries a trisomy X condition (Figure 2A) whereas her fetus has normal karyotype (46,XX) (Figure 2B). At 24^{+5} gestational weeks, ultrasound examination of the fetus was performed and no abnormalities were found: biparietal diameter 62 mm, head circumference 219 mm, abdomen circumference 196 mm, length of femur 42 mm, length of humerus 40 mm and cerebellum trans diameter 26 mm; with normal heart, liver, kidney, kidney pelvis, stomach, and bladder; two umbilical arterial; maximum amniotic fluid depth 47 mm. The fetus was born on 8 February 2012 with normal phenotype with body length of 49 cm, apgar score 10, and there were no other complications.

DISCUSSION

Patients with trisomy X display significant variability in developmental delays, learning disabilities, and psychological characteristics. So far, it is not yet possible to predetermine which child will exhibit any or all of these concerns. Prenatal screening and diagnosis of trisomy X is important for parents and physicians to make further education and medical care plans for the trisomy X patients. Patients diagnosed in the prenatal period may have better developmental and educational outcomes and more typical peer relationships in comparison to patients diagnosed in the postnatal period. Recently, the International Society for Prenatal Diagnosis has provided recommendations for best practices in prenatal screening for aneuploidy.[11] The development of the NIFTY test may offer substantial new opportunities to improve performance of prenatal screening of sex chromosomal abnormalities. Here, we demonstrated that the NIFTY test can not only indicate the problems sourced from the fetus side but also the maternal side therefore may affect the performance of the NIFTY test. Even though the NIFTY test plays an increasingly important role in noninvasive prenatal screening, this technique needs further development to meet the complex clinical requirement. Our experience suggests that the fetus chromosomal status cannot be solely determined by the NIFTY test if the parents have chromosomal abnormalities. Alternative diagnosis solutions, such as karyotyping and ultrasound examination, should be applied before final prenatal diagnosis results were given. In the meantime, patients who might be considering the NIFTY test need to receive detailed pretest counseling that explains the benefits, informed consent and some potential genetic condition that may obscure the NIFTY test results may be identified. For those trisomy X patients or patients with trisomy X fetus pregnancies, a post-test genetic counseling is necessary. Couples should be informed of the high frequency of trisomy X and that most patients go undiagnosed, to support them in understanding and accepting that their diagnosis is not an isolated case with a predetermined outcome.

DETAILS OF ETHICS APPROVAL

Ethics approval was obtained from the ethics committee of BGI-Shenzhen on 20 August 2010.

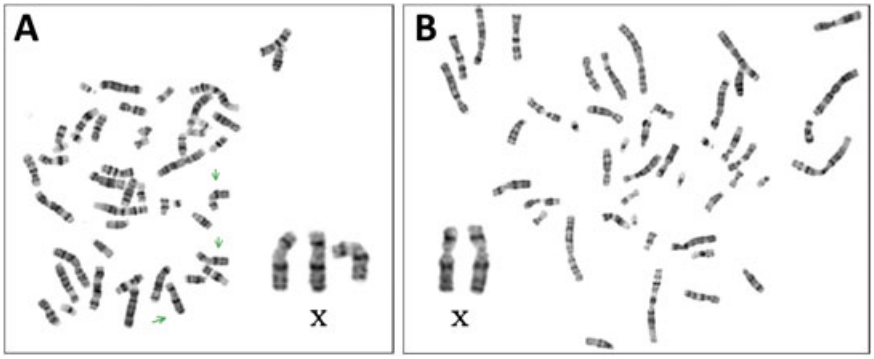

Figure 2 **Results of karyotyping analysis. (A) Peripheral blood cells and (B) amniotic fluid cells. The pregnant patient's peripheral blood cells and amniotic fluid cells were used to perform the karyotyping analysis on 22^{+3} gestational weeks. The results showed this pregnant patient with a karyotype of 47,XXX and the fetus with normal karyotype (46,XX)**

WHAT'S ALREADY KNOWN ABOUT THIS TOPIC?

- Non-invasive fetal trisomy (NIFTY) test, whichis based on massively parallel sequencing for cell-free DNA of maternal plasma, may offer substantial new opportunities to improve performance of prenatal screening of chromosomal abnormalities.

WHAT DOES THIS STUDY ADD?

- This study demonstrated for the first time that the maternal genomic materials can affect the performance of the NIFTY. This provides useful information for further development of the NIFTY technique to meet the complicated clinical requirements, especially when sex chromosomal aneuploidies are involved.
- Noninvasive prenatal genetic testing for aneuploidy detected trisomy X in the mother.

REFERENCES

1. Jacobs PA. The incidence and etiology of sex chromosome abnormalities in man. Birth Defects Orig Artic Ser 1979;15:3–14.
2. Linden MG, Bender BG, Harmon RJ, *et al.* 47,XXX: what is the prognosis. Pediatrics 1988;82:619–30.
3. Ratcliffe SG. Longitudinal growth studies on children with sex chromosome abnormalities. Prog Clin Biol Res 1985;200:301–9.
4. Nielsen J, Wohlert M. Sex chromosome abnormalities found among 34,910 newborn children: results from a 13-year incidence study in Arhus, Denmark. Birth Defects Orig Artic Ser 1990;26:209–23.
5. Pitukkijronnakorn S, Promsonthi P, Panburana P, *et al.* Fetal loss associated with second trimester amniocentesis. Arch Gynecol Obstet 2011;284:793–7.
6. Tabor A, Alfirevic Z. Update on procedure-related risks for prenatal diagnosis techniques. Fetal Diagn Ther 2010;27:1–7.
7. Benn P, Borrell A, Cuckle H, *et al.* Prenatal Detection of Down Syndrome using Massively Parallel Sequencing (MPS): a rapid response statement from a committee on behalf of the Board of the International Society for Prenatal Diagnosis, 24 October 2011. Prenat Diagn 2012;32:1–2.
8. Bianchi DW, Simpson JL, Jackson LG, *et al.* Fetal gender and aneuploidy detection using fetal cells in maternal blood: analysis of NIFTY I data. National Institute of Child Health and Development Fetal Cell Isolation Study. Prenat Diagn 2002;22:609–15.
9. Lau TK, Chen F, Pan X *et al.* Noninvasive prenatal diagnosis of common fetal chromosomal aneuploidies bymaternal plasma DNA sequencing. J Matern Fetal Neonatal Med 2012; Feb 24.
10. Lo YMD, Tein MS, Lau TK, *et al.* Quantitative analysis of fetal DNA in maternal plasma and serum: implications for noninvasive prenatal diagnosis. Am J Hum Genet 1998;62:768–75.
11. Benn P, Borrell A, Crossley J, *et al.* Aneuploidy screening: a position statement from a committee on behalf of the Board of the International Society for Prenatal Diagnosis, January 2011. Prenat Diagn 2011;31:519–22.

