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ORIGINAL ARTICLE

Non-invasive prenatal screening of fetal sex chromosomal abnormalities: perspective of pregnant women

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Objective: **To study whether pregnant women would like to be informed if sex chromosomal abnormalities (SCA) were suspected with the non-invasive prenatal diagnosis of fetal Down syndrome (the NIFTY) test.** ***Methods:*** **Two hundred and one patients carried a singleton pregnancy requesting the NIFTY test were invited to give their preferences if there was suspicion of SCA by the NIFTY test.** ***Results:*** **Over 93.5% were ethnic Chinese, with a mean age of 36. Prior Down screening was positive in 66 (32.8%). Over 50% of subjects considered SCA to be better in terms of disability compared to Down syndrome, and only 5.2% considered SCA to be worse. Yet, the majority (198, 98.5%) indicated that they wanted to be informed if there was suspicion of SCA. Of whom 34.8% would have an amniocentesis for confirmation, while 57.1% were not certain, indicating the possibility of accepting these conditions.** ***Conclusion:*** **Besides screening Down syndrome by NIFTY, most pregnant women would also like to be informed if there was suspicion of SCA. Those screened positive should be counseled by those with experience in genetics to avoid unnecessary pregnancy termination.**

Keywords: fetal DNA, maternal serum, non-invasive prenatal diagnosis, sex chromosomal abnormalities

Introduction

Recently, non-invasive prenatal screening of fetal Down syndrome using massively parallel sequencing of cell free DNA in maternal circulation has been put into clinical use. This test is a highly efficient screening test, with estimated sensitivity and specificity of over 99% [1–5]. Recent studies also suggested that it is also a highly efficient screening test for Trisomy 13 and 18 [6,7]. In addition, aneuploidies of sex chromosomes (SCA) might be detectable by this test. In a small study, all four cases of Turner syndrome and one case of Klinefelter syndrome were correctly identified [4], while in a larger study, 15 of the 16 cases of Turner syndrome were correctly detected [8]. Early experiences based on the initial 10,000 clinical cases from BGI-China suggested that the positive predictive value for fetal SCA is over 50% (unpublished data, personal communication), although the detection rate could not be ascertained because fetal karyotyping was not performed in most of the subjects with a normal screening result. Since the primarily aim of this new test is not for the detection of sex chromosomal problems, and the accuracy of detection is still uncertain, there are some uncertainties as to whether such abnormality should be looked for and, when suspected, conveyed to the pregnant women. It could be argued that pregnant women should not be informed because (i) such conditions are not the primary conditions intended to be screened for, (ii) most of these conditions are non-fatal, and (iii) we may be inducing unnecessary anxiety and pregnancy termination. On the other hand, it could also be argued that pregnant women should be informed because (i) these are findings that may associate with significant clinical features that women have the right to know, (ii) women have a chance to make an informed choice, and (iii) women could have better preparation for the caring of children with potential problems.

The objective of this study was to explore the wishes of pregnant women whether they would like to be informed if sex chromosomal abnormalities are suspected with this new test.

Materials and methods

The non-invasive fetal trisomy (NIFTY) test, a massively parallel sequencing-based screening test, was offered to pregnant women carrying a singleton pregnancy in Hong Kong as a screening test for fetal Down syndrome at or after 12 weeks of gestation. The copy numbers of other chromosomes, including the sex chromosomes, were studied routinely. The test report included the risk assessment for Trisomy 13 and 18 in addition to 21. Further details concerning the clinical and laboratory aspect of this test were as previously reported [9]. Specifically, each pregnancy woman was given written information about the test, had an individual pre-test counseling and an ultrasound scan, and provided a written consent concerning the use of NIFTY as a Down syndrome screening test.

In this study, 201consecutive subjects who carried a singleton pregnancy requesting the NIFTY test between 26 January and 23 February 2012 were asked whether they would like to be informed if there were suspicions of SCA by the NIFTY test. At the time of registration to the clinic, an information sheet was provided explaining (i) what sex chromosomes are, and (ii) the clinical symptoms of the four most common SCA, including 45 XO, 47 XXY, 47 XXX, and 47XYY. A summary was given at the end of the leaflet, emphasized that

- The primary aim of the test was not to screen for fetal SCA, although many of those were detectable;
- based on previous experience, the suspicion of SCA was confirmed in about 50% while the rest were normal;
- individuals affected by SCA without ultrasound abnormalities:

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- Are not hermaphrodites but true male or females;
- they are compatible with life;
- some types, but not all, are associated with infertility;
- mental development in general is within normal range, although some may have mild delay in specific areas of development.

At the end of the information sheet, pregnant women were asked to rate their perceived degree of disability for each of the four common SCA syndromes in comparison to Down syndrome, and whether they would like to be informed if abnormalities were suspected. They were explained that their answers would be the basis of the action in case a SCA was suspected from the NIFTY test. In case they do, there were also asked about their likely action, that is, whether they would have an amniocentesis for confirmation.

The completed questionnaires were collected before subjects left the clinic. Descriptive statistics were summarized, subjects were divided into four groups: Group A: "want to be informed, probably will have an amniocentesis for a definite diagnosis"; Group B: "want to be informed, not sure whether they will have an amniocentesis yet"; Group C: "want to be informed, but probably will not have an amniocentesis but a postnatal confirmation"; Group D: "do not want to be informed.". Between groups comparisons were performed by χ^2 test for categorical variables, or ANOVA for continuous variables. A p value <0.05 was considered statistically significant.

Results

During the study period, 201 patients were invited and completed the questionnaire. Table I showed the basic maternal and pregnancy characteristics. Over 93.5% of the subjects were ethnic Chinese. The mean maternal age was36 ± 4, and over 68.6% were 35 or above. The mean and median gestation age at NIFTY test was 15 and 14 weeks respectively, with 48.3% between 12 and 13 weeks of gestation. Overall, there was no prior Down syndrome screening test in 62 (30.9%), and the prior screening test was positive in 66 (32.8%), negative in 46 (22.9%), and was not available yet in 27 (13.4%).

The NIFTY test was positive for Trisomy 21 in four cases, and all were subsequently confirmed by prenatal karyotyping. There was no suspicious of fetal SCA or other aneuploidies in any of the remaining subjects in this cohort.

Table II showed the perceived degree of disability of various SCA compared to Down syndrome. About one quarter of the subjects were not able to make a committed answer (i.e. don't know). About 22–27% considered SCA were much better in terms of disability compared to Down syndrome, and 52–60% considered SCA were either slightly or much better. There was a slight tendency for a poorer rating for Turner syndrome.

The overwhelming majority (n = 198, 98.5%) indicated that they would want to be informed if the NIFTY test was suspicious of SCA, irrespective of their perceived significance of the SCA (Table III). Only three subjects (1.5%) did not want to be informed. Of those who want to be informed, 69 (34.8% of 198) indicated that they would probably performed an amniocentesis for a definite prenatal diagnosis when they were so informed, 113 (57.1%) were not sure, while 16 (8.1%) probably would not have an amniocentesis but would only confirm the suspicion by postnatal cord blood karyotyping because they would accept these babies anyway. The decision of subjects were not related to maternal age (p = 0.11), the perception of severity of SCA (p = 0.31), or result of prior screening test (p =0.52).

Table I. Basic patient characteristics.

Characteristics	Number of cases (%) N = 201
Maternal age	
20–24	1 (0.50%)
25–29	9 (4.48%)
30–34	53 (26.37%)
35–39	89 (44.28)
40–44	44 (21.89%)
≥45	5 (2.49%)
Gestation at NIFTY test	
12 weeks–13 weeks 6 days	97 (48.26%)
14 weeks–15 weeks 6 days	56 (27.86%)
16 weeks–20 weeks 6 days	39 (19.40%)
21 week and above	9 (4.48%)
Previous trisomy 21 pregnancies	0 (0%)
Family history of trisomy 21	5 (2.49%)
Ethnicity	
Chinese	188 (93.53%)
Caucasian	10 (4.98%)
Others	3 (1.49)
Prior Down syndrome screening test	
None	62 (30.85%)
Combined 1st Trimester NT + Biochemistry	111 (52.22%)
1st Trimester NT (±other USG markers) only	10 (4.98%)
1st trimester biochemistry only	3 (1.49%)
2nd biochemistry only	12 (5.97%)
Other tests, or more than one test	3 (1.49%)
Result of prior screening tests (n = 139)	
High risk	66 (47.78%)
Low risk	46 (33.09%)
Result not available at time of NIFTY test	27 (19.42%)

Discussion

SCA is a group of conditions, not a single disease. Each type of SCA is associated with different clinical features, ranging from normal phenotype (such as Triple X syndrome) to *in utero* fetal death (such as Turner syndrome associated with huge cystic hygroma and cardiac anomalies). Nonetheless, the prognoses of those with SCA without prenatal sonographic anomalies in general are good, compatible with life, with overall normal mental function. Therefore, prenatal screening for this group of conditions has not been suggested as a clinical routine. However, SCA may be detected as an incidental finding during prenatal karyotyping such as after amniocentesis. Under this circumstance, it was recommended that "All (Medical/Genetic) test results relevant to genetic disorders or fetal malformations should be disclosed. These include sex chromosome abnormalities and disorders that may not be considered serious [10]." Although such an approach resulted in pregnancy termination in a significant proportion, about 40–72%, of patients [11–13], the general consensus is that couples have the right to know when this information is known, even incidentally. In general, Turner syndrome and Klinefelter syndrome are the two types of SCA that were associated with higher percentage of parental decision for pregnancy termination, with an average of 76 and 61% of cases, respectively [14].

Table II. Pregnant women's perception of degree of disability in individuals affected by sex chromosomal abnormalities (SCA), compared to those affected by Down syndrome.

SCA	Much better	Slightly better	Similar	Slightly worse	Much worse	No comment	Total (*N*)
45 XO	44 (21.9%)	61 (30.3%)	36 (17.9%)	7 (3.5%)	4 (2.0%)	49 (24.4%)	201
47 XXY	53 (26.4%)	67 (33.3%)	22 (11.0%)	6 (3.0%)	4 (2.0%)	49 (24.4%)	201
47 XXX	55 (27.4%)	64 (31.8%)	21 (10.5%)	6 (3.0%)	4 (2.0%)	51 (25.4%)	201
47 XYY	55 (27.4%)	67 (33.3%)	19 (9.5%)	6 (3.0%)	5 (2.5%)	49 (24.4%)	201
Overall	207 (25.7%)	259 (32.2%)	98 (12.2%)	25 (3.1%)	17 (2.1%)	198 (24.6%)	804

Table III. Characteristics of subjects stratified by their wishes to be informed of sex chromosomal abnormalities.

	Group A (n = 69)	Group B (n = 113)	Group C (n = 16)	Group D (n = 3)	P*
Maternal age (mean ± SD)	36 ± 4	37 ± 4	38 ± 4	34 ± 3	0.11**
Prior screening test					
Positive (%)	21 (30.4%)	39 (34.5%)	4 (25.0%)	2 (66.7%)	
Negative (%)	19 (27.5%)	25 (22.1%)	2 (12.5%)	0 (0%)	0.52***
No/not available (%)	29 (42%)	49 (43.4%)	10 (62.5%)	1 (33.3%)	
Consider SCA is worse than Down syndrome (%)	7 (10.1%)	7 (6.2%)	0 (0%)	0 (0%)	0.31***

Group A: want to be informed, probably will have an amniocentesis for a definite diagnosis; Group B: want to be informed, not sure whether they will have an amniocentesis yet; Group C: want to be informed, but probably will not have an amniocentesis but a postnatal confirmation; Group D: do not want to be informed.
*Comparison was performed, excluding Group D because of the small number.
**ANOVA test.
***χ^2 test.

However, the situation is quite different concerning the NIFTY test. Firstly, the detection of SCA is not "incidental". During karyotyping, the first step is the proper arrangement of all chromosomes and therefore any SCA will be apparent and its diagnosis is unavoidable. On the other hand, the first step of the NIFTY test generates a large set of sequencing data, and the detection of copy number abnormalities of a particular chromosome requires specific bioinformatics analysis for that chromosome. Therefore, it is possible to limit the analysis to the status of chromosome 21 only but not others. In other words, the diagnosis of SCA requires specific bioinformatics analysis but not an incidental finding. Secondly, the detection rate or the false positive rate of SCA by the NIFTY test is not certain at this moment. Thirdly, current experience suggested that any suspicion of SCA on NIFTY carries a positive predictive value of about 50%. It is not diagnostic and therefore requires an invasive test for confirmation if a definitive prenatal diagnosis is needed.

During the pre-testing counseling for the NIFTY test, one of the commonest questions pregnant women asked was whether the test would detect other abnormalities or whether other chromosomes would be tested. Most patients appeared to be very happy when they got a positive answer. The objective of this study was to report on the preference of pregnant women whether they would like the NIFTY test to report on SCA, when some background information about this group of conditions was provided. The authors had tried to make the information as factual as possible. The results showed that the overwhelming majority would like to know this information, given the limitations as stated before, irrespective of their understands and perception of the clinical significance of these conditions. It is important to note that over 25% of subjects believe that the disability associated with SCA are much better than fetal Down syndrome, yet virtually all of them still wanted to be informed if there is any suspicion that their fetuses were affected. However, the inclusion of SCA in the NIFTY report will inevitably increase slightly the overall false positive rate. Further study is required to confirm the magnitude of the effect. Nonetheless, the overall false positive rate of the NIFTY test, even all 23 pairs of chromosomes were studied, is still likely to be below 1% which is much lower than that of any existing conventional Down syndrome screening tests.

It is an important finding that the majority (57.1%) were uncertain whether they would have an amniocentesis in case the NIFTY test was positive for SCA. Only 34.8% indicated that they probably would have an amniocentesis. This contrast sharply with the previous finding that 92.6% of pregnant women would opt for amniocentesis if they were screened positive for Down syndrome [14]. This probably reflexes that subjects considered SCA to be a less serious condition and might consider accepting these babies even if confirmed. Previous studies have repeatedly demonstrated that one of the most significant factors association with pregnancy termination of prenatally diagnosed SCA is the health care provider's genetic experience [15]. Therefore, if SCA is to be included in the NIFTY test, those who are screened positive for SCA should be counseled by specialists with sufficient genetic experience.

Early studies on non-invasive prenatal diagnosis have been focused on fetal Down syndrome. Experts suggested that when planning for implementation of this new test, one must balance against the potential loss of information about chromosomal abnormalities other than Down syndrome compared with full karyotyping with traditional prenatal diagnosis [16]. However, such a worry may not be necessary; because there are increasing evidences that this new approach is likely to be equally effective in detecting aneuploides involving other chromosomes [8], and even chromosomal structural abnormalities [17]. However, the desire to "know more" should be balanced against the slight increase in false positive rate and the possibility of misuse of the test resulting in the eradication of potentially normal babies. Further ethical and social studies are urgently required in view of the rapid development of our diagnostic abilities.

In conclusion, maternal plasma DNA sequencing is able to detect fetal SCA in addition to Down syndrome. Most pregnancy women would like to be informed if SCA is suspected. Since the test was primarily designed for Down syndrome, extensive

pre-test counseling on SCA is probably not cost-effective nor worthwhile. However, those screened positive for SCA should be counseled by experts with extensive experience in medical genetics, so as to avoid biased or directive counseling.

Declaration of Interest: The authors report no conflicts of interest.

References

1. Chiu RW, Akolekar R, Zheng YW, Leung TY, Sun H, Chan KC, Lun FM, et al. Non-invasive prenatal assessment of trisomy 21 by multiplexed maternal plasma DNA sequencing: large scale validity study. BMJ 2011;342:c7401.
2. Ehrich M, Deciu C, Zwiefelhofer T, Tynan JA, Cagasan L, Tim R, Lu V, et al. Noninvasive detection of fetal trisomy 21 by sequencing of DNA in maternal blood: a study in a clinical setting. Am J Obstet Gynecol 2011;204:205.e1–205.11.
3. Sehnert AJ, Rhees B, Comstock D, de Feo E, Heilek G, Burke J, Rava RP. Optimal detection of fetal chromosomal abnormalities by massively parallel DNA sequencing of cell-free fetal DNA from maternal blood. Clin Chem 2011;57:1042–1049.
4. Lau TK, Chen F, Pan X, Pooh RK, Jiang F, Li Y, Jiang H, et al. Noninvasive prenatal diagnosis of common fetal chromosomal aneuploidies by maternal plasma DNA sequencing. J Matern Fetal Neonatal Med 2012; 25:1370-1374.
5. Palomaki GE, Kloza EM, Lambert-Messerlian GM, Haddow JE, Neveux LM, Ehrich M, van den Boom D, et al. DNA sequencing of maternal plasma to detect Down syndrome: an international clinical validation study. Genet Med 2011;13:913–920.
6. Chen EZ, Chiu RW, Sun H, Akolekar R, Chan KC, Leung TY, Jiang P, et al. Noninvasive prenatal diagnosis of fetal trisomy 18 and trisomy 13 by maternal plasma DNA sequencing. PLoS ONE 2011;6:e21791.
7. Palomaki GE, Deciu C, Kloza EM, Lambert-Messerlian GM, Haddow JE, Neveux LM, Ehrich M, et al. DNA sequencing of maternal plasma reliably identifies trisomy 18 and trisomy 13 as well as Down syndrome: an international collaborative study. Genet Med 2012;14:296–305.
8. Bianchi DW, Platt LD, Goldberg JD, Abuhamad AZ, Sehnert AJ, Rava RP; MatErnal BLood IS Source to Accurately diagnose fetal aneuploidy (MELISSA) Study Group. Genome-wide fetal aneuploidy detection by maternal plasma DNA sequencing. Obstet Gynecol 2012;119:890–901.
9. Lau TK, Chan MK, Salome Lo PS, Connie Chan HY, Kim Chan WS, Koo TY, Joyce Ng HY, Pooh RK. Clinical utility of noninvasive fetal trisomy (NIFTY) test – early experience. J Matern Fetal Neonatal Med. 2012 Apr 28. [Epub ahead of print].
10. Wertz DC, Fletcher JC, Berg K. Review of Ethical Issues in Medical Genetics. Report of Consultants to WHO. World Health Organization Human Genetics Programme. 2003. p. 73
11. Balkan M, Kalkanli S, Akbas H, Yalinkaya A, Alp MN, Budak T. Parental decisions regarding a prenatally detected fetal chromosomal abnormality and the impact of genetic counseling: an analysis of 38 cases with aneuploidy in Southeast Turkey. J Genet Couns 2010;19:241–246.
12. Hamamy HA, Dahoun S. Parental decisions following the prenatal diagnosis of sex chromosome abnormalities. Eur J Obstet Gynecol Reprod Biol 2004;116:58–62.
13. Holmes-Siedle M, Ryynanen M, Lindenbaum RH. Parental decisions regarding termination of pregnancy following prenatal detection of sex chromosome abnormality. Prenat Diagn 1987;7:239–244.
14. Chan LW, Chau MC, Leung TY, Fung TY, Leung TN, Lau TK. Expectation and knowledge of women undergoing first-trimester combined screening for Down syndrome in a Chinese population. Prenat Diagn 2005;25:1248–1252.
15. Jeon KC, Chen LS, Goodson P. Decision to abort after a prenatal diagnosis of sex chromosome abnormality: a systematic review of the literature. Genet Med 2012;14:27–38.
16. Susman MR, Amor DJ, Muggli E, Jaques AM, Halliday J. Using population-based data to predict the impact of introducing noninvasive prenatal diagnosis for Down syndrome. Genet Med 2010;12:298–303.
17. Peters D, Chu T, Yatsenko SA, Hendrix N, Hogge WA, Surti U, Bunce K, et al. Noninvasive prenatal diagnosis of a fetal microdeletion syndrome. N Engl J Med 2011;365:1847–1848.