

ORIGINAL ARTICLE

Nationwide demonstration project of next-generation sequencing of cell-free DNA in maternal plasma in Japan: 1-year experience

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ABSTRACT

Objective To report the 1-year experience of a nationwide demonstration project to introduce noninvasive prenatal testing of aneuploidy from maternal plasma and discuss how to implement this program in Japan.

Methods The test was conducted to detect aneuploidy in high-risk pregnant women with adequate genetic counseling. The clinical data, test results, and pregnancy outcomes were recorded.

Results Of the 7740 women tested, 142 (1.8%) had positive results, 7594 (98.1%) had negative results, and four (0.1%) had results that were not reportable. Of the 142 women who tested positive, 13 cases resulted in intrauterine fetal death, and three cases refused to undergo the invasive procedure. Of the 126 positive cases confirmed on karyotyping, a normal karyotype was observed for trisomy 21 in three cases, trisomy 18 in eight cases, and trisomy 13 in two cases. In the follow-up study of the negative cases ($n = 1638$), only one false-negative case of trisomy 18 was detected.

Conclusions We described our nationwide 1-year experience with noninvasive prenatal genetic testing. It is expected that the present data will stimulate a debate regarding prenatal genetic testing and hopefully lead to improvements in the perinatal care system with respect to genetic counseling in Japan. © 2014 John Wiley & Sons, Ltd.

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INTRODUCTION

Noninvasive prenatal genetic testing for aneuploidy is currently available using analyses of cell-free DNA in the maternal blood.¹ The clinical application of this test has become widespread worldwide.² In Japan, noninvasive prenatal testing (NIPT) for the detection of trisomy 21, trisomy 18, and trisomy 13 (NIPT) has been applied in clinical research since April 2013 and is limited to use by institutions certified by the Japanese Association of Medical Sciences (JAMS).

In the mid-1990s, there was public controversy when second trimester serum marker tests were first introduced in Japan,³ associated with a dramatic increase in the use of the tests to detect Down syndrome. The ethics of prenatal screening based on serum markers was debated in the context of Japanese law, which does not allow for the termination of pregnancy as a result of fetal abnormalities. The Expert Committee on the Prenatal Diagnosis of the Science Council for Evaluating Advanced Medical Techniques of Japan published the 'View on prenatal serum marker screening' guidelines in 1999, which state that physicians are not required to provide information regarding this test to pregnant women and should not recommend such testing. Thereafter, the number of maternal

serum screening tests decreased.⁴ Prenatal genetic screening tests, such as those using maternal serum markers and/or nuchal translucency examinations, are currently performed in Japan according to the individual patient's request, not as routine care, as no national prenatal screening policies have been adopted in this country. Therefore, the rate of prenatal genetic diagnosis remains extremely low⁴ and care systems to provide prenatal genetic counseling during usual obstetrics care are not well established in Japan.

Concurrent with this background in Japan, the use of NIPT as a commercially available test began in October 2011 in the USA. The introduction of this test in Japan was expected within a few years; however, the debate over prenatal testing in Japan has not advanced from that observed during the 1990's controversy. Many people are apprehensive that women may opt to terminate their pregnancy based on the findings of NIPT without receiving genetic counseling with adequate consideration for the fetus,⁵ including understanding the possible ramifications of a positive outcome. There is concern that introducing the test into general clinical practice in Japan under these circumstances may cause confusion and revive criticism of prenatal genetic testing within society.

For this reason, we planned a clinical study to introduce the test with accompanying adequate genetic counseling at university hospitals and other major institutions. Our aim was to establish adequate circumstances for obtaining a prenatal diagnosis taking into consideration the social and ethical debate in Japan in order to prevent confusion regarding the tests. In responding to the high level of social interest in NIPT, the Japan Society of Obstetrics and Gynecology (JSOG) published a 'policy statement on noninvasive testing of fetal aneuploidy using maternal blood' on March 9, 2013. This JSOG statement notes that: 'The test should not be widely introduced into general obstetric clinical practice in Japan until a system is in place for specialists of obstetrics with knowledge of clinical genetics to provide appropriate genetic counseling to pregnant women who require it. The test should only be carried out in pregnant women with an increased risk for fetal aneuploidy, and conducting the test in mass screening of general pregnant women should be strictly prohibited.' In addition, the statement goes on to say that, 'There should be restrictions in the implementation of the test to a limited number of institutions with the ability to offer adequate genetic counseling.' Therefore, this statement indicates the need for a policy to put forward requirements for institutions to use the test, certify institutions that meet these requirements, and recognize a protocol for implementing the test at such institutions for use in clinical research. The view of the JSOG is echoed by the JAMS, Japan Medical Association, Japan Society of Human Genetics, and Japan Association of Obstetricians and Gynecologists.

In response, we conducted a clinical research study of this test beginning in April 2013 at major institutions with clinics for prenatal genetic counseling. Our research covered almost all cases of NIPT performed in Japan. The aim of the current report is to describe the 1-year experience of a Japanese nationwide demonstration project of NIPT and discuss how to implement such testing against a background in which no prenatal screening policies have been adopted.

METHODS

Prenatal genetic testing for trisomy 21, trisomy 18, and trisomy 13 using cell-free DNA in maternal plasma was carried out among pregnant women who requested testing at institutions authorized by the JAMS between April 2013 and March 2014. The details of the study protocol, including the recruitment of pregnant women who requested testing, are provided on the Internet (<http://www.nipt.jp/>). The number of participating institutions was 15 in April 2013 and rose to 37 by March 2014. The test was performed at 10 to 18 weeks of gestation in women with a singleton pregnancy at increased risk of aneuploidy. The study participants included women 35 years of age or older, fetuses with ultrasonographic or maternal serum marker findings indicating an increased risk of aneuploidy, women with a history of children affected by trisomy, or a parent carrying a balanced Robertsonian translocation with an increased risk of trisomy 13 or trisomy 21. Genetic counseling was provided by a genetic specialist or a certified genetic counselor. The sessions were structured to discuss the referral indications, preexisting risks, features of the various forms of trisomy, implications of the test results, benefits and limitations of prenatal genetic

testing, and a review of all testing options. In the sessions, we used common counseling materials comprising 80 pages with numerous graphs and tables. The materials were used to maintain a certain degree of uniformity for genetic counseling at each institution. In addition, after the initial counseling session, a leaflet summarizing the content of the counseling program was distributed. A total of 20 mL of blood was collected from the pregnant women after obtaining their informed consent at each institution, and the samples were subsequently sent to Sequenom, Inc. (San Diego, CA, USA) for MaterniT Plus tests, which include molecular analyses of trisomy 21, trisomy 18, and trisomy 13.^{6,7} The cost of testing (approximately \$2000) was paid for by the pregnant women, without any accommodations or insurance coverage. The results of the tests were explained at each institution in the genetic counseling sessions. If the results were positive, then either amniocentesis or chorionic villus sampling, in which the cost was included in the initial cost of the testing, was performed for karyotyping, as was previously explained to the women. The karyotype results were also explained in genetic counseling sessions, and continuous genetic counseling with a pediatrics geneticist was also offered. This study is a multicenter prospective cohort study. The clinical data, test results, and pregnancy outcomes were collected and aggregated every month at the data center of the secretariat. This study is a part of a clinical trial registered with the University Medical Information Network clinical trials registry (UMIN000009338) that includes a pre-NIPT and post-NIPT questionnaire study of genetic counseling. The clinical research was approved by the institutional ethics committee of each institution.

RESULTS

In 1 year, 7740 tests were carried out. More than 95% of the tests were performed in women 35 years of age or older (Table 1). The mean (range) age of the pregnant women was 38.3 (21–48) years, the mean gestational age at the time of testing was 13.3 (10.0–19.9) weeks, and the mean body mass index (BMI) was 20.9 (14.1–37.0). The mean turnaround time of testing was 5.2 calendar days, taking more than 10 days in only 0.9% of cases.

Of the 7740 women tested, 141 (1.8%) had positive results, 7581 (98.0%) had negative results, and 18 (0.2%) had results that were not reportable at the first test. Of the 18 women for whom judgment was deferred, 16 were retested, including one woman found to be positive for trisomy 18, 13 women found to be negative, and two women again with unreportable results (Figure 1). The rate of positive findings was higher among the women with fetuses exhibiting ultrasonographic findings indicative of an increased risk of aneuploidy (Table 1).

Of the 142 women who tested positive, 13 (9.2%) and 2 (1.4%) cases resulted in intrauterine fetal death (IUFD) before and after the test results were reported, respectively. Of the cases of IUFD diagnosed before the invasive procedures, karyotyping was carried out in four cases using chorionic villus tissues of the miscarriage, with trisomy confirmed in each case (Table 2). Of the 126 positive women who underwent invasive procedures, chromosomal abnormalities of trisomy 21, 18, and 13 were confirmed in 70, 34, and 9 cases, respectively.

Table 1 Indications for testing and rates of positive findings

Indication	No tested (%)	No positive results			Total	Positive rate (%)
		Trisomy 21	Trisomy 18	Trisomy 13		
Maternal age	7387 (95.4)	68	41	9	118	1.6
Previous history	226 (2.9)	0	3	1	4	1.8
Ultrasonography	108 (1.4)	11	6	3	20	18.5
Serum marker	16 (0.2)	0	0	0	0	0
Translocation	3 (0.04)	0	0	0	0	00
Total	7740	79	50	13	142	1.8

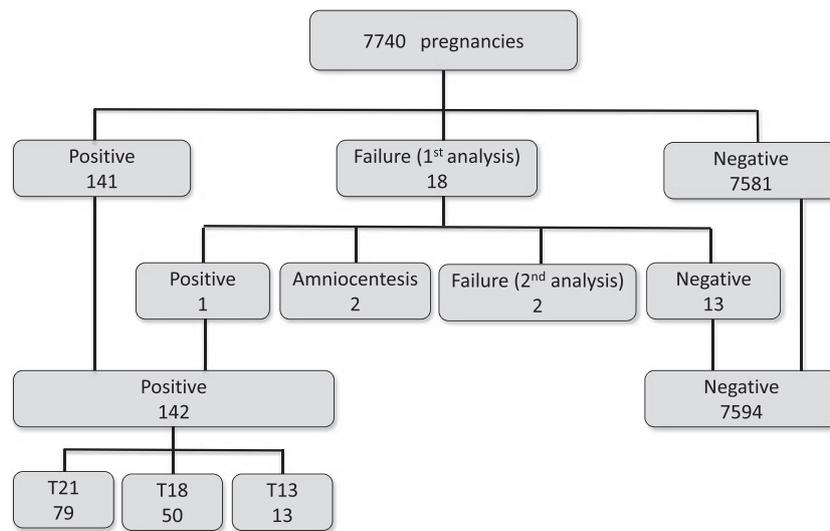


Figure 1 NIPT results for 7740 women tested between April 2013 and March 2014 in Japan

Table 2 Karyotype and pregnancy outcomes in the cases with positive NIPT results

	Trisomy 21	Trisomy 18	Trisomy 13	Total
Positive results	79	50	13	142
Invasive procedure performed (n = 126)	Normal karyotype	3	8	2
	Trisomy	70	34	9
	IUFD	0	1	1
	Termination of pregnancy	69	33	8
Continuation of pregnancy	1	0	0	1
Invasive procedure not performed (n = 16)	IUFD	3	8	2
	Karyotype analyzed	1	2	1
	Karyotype not analyzed	2	6	1
	Withdrawal	3	0	0

IUFD, intrauterine fetal death.

The positive predictive value in this study population was 95.9% (70/73) for trisomy 21, 81.0% (34/42) for trisomy 18, and 81.8% (9/11) for trisomy 13, respectively. Each case that was found to have trisomy 18 and trisomy 13 resulted in IUFD before the results of amniocentesis were reported. Among the cases confirmed to involve fetal aneuploidy (n = 111), 110

women opted to terminate their pregnancy, while one woman opted to continue the pregnancy. Although the genetic counseling sessions were carried out with thoughtful consideration, three women who tested positive for trisomy 21 refused to undergo invasive procedures and opted to terminate the pregnancy.

Of the 1638 women who tested negative and for whom follow-up was provided after birth at the time of May 2014, only one false-negative case of trisomy 18 was detected.

DISCUSSION

NIPT for trisomy 21, 18, and 13 was first applied in April 2013 in Japan as a nationwide trial conducted by the Japan NIPT consortium. At the end of March 2014, the test was carried out at 39 institutions, of which 37 were participants in our study and two institutions are currently less active. Therefore, our findings represent the results of realistic nationwide data for NIPT in Japan. In the present study, a total of 7740 women underwent NIPT with prenatal genetic counseling sessions provided by genetic specialists in the first year. More than 95% of the subjects were women 35 years of age or older, and the rate of positive findings was 1.8%. It is unreasonable that pregnant women with an advanced maternal age be primarily subjected to the test. However, because information regarding screening tests for fetal aneuploidy is not routinely provided to pregnant women and such testing not common in Japan, the main indication among the study subjects was an advanced maternal age. We believe that it is too late to establish a screening strategy that includes maternal serum or ultrasound markers in Japan. As the cost of NIPT decreases dramatically in the near future, NIPT will become a standard screening method for pregnant women who request testing for fetal chromosomal abnormalities.

There were some women with a normal karyotype fetus among those who tested positive in this study. Consequently, the positive predictive value in this study population was 95.9% (70/73) for trisomy 21, 81.0% (34/42) for trisomy 18, and 81.8% (9/11) for trisomy 13, respectively. These rates are within the expected range, and invasive prenatal examinations are mandatory to confirm positive results. Two percent of the women (3/142) who tested positive refused to undergo invasive confirmatory procedures and opted to terminate their pregnancy. In this study, all participants were provided genetic counseling sessions before and after testing by genetic specialists at authorized institutions. The genetic counseling sessions included a discussion of the implications of the results and the need for invasive procedures in cases that test positive. Without adequate genetic counseling, more women who test positive may refuse to undergo invasive procedures and opt to terminate their pregnancy. Therefore, providing high-quality prenatal genetic counseling is a key issue in order to successfully conduct NIPT.

Of the 142 positive cases, 15 (10.6%) resulted in IUFD. The rate of IUFD in the positive cases was 3.8% (3/79) for trisomy 21, 18% (9/50) for trisomy 18, and 23.1% (3/13) for trisomy 13. Of the 111 trisomy cases confirmed using invasive procedures, excluding IUFD, 110 (99.1%) women opted to terminate their pregnancy. Suzumori *et al.* reported that women electing NIPT tend to have higher rates of depression and anxiety.⁸ Women with psychological distress may often terminate their pregnancy. IUFD is sometimes observed in the NIPT-positive cases and leads to increase the psychological distress. Careful attention regarding the implications of IUFD in NIPT-positive cases and pregnancy termination is therefore needed when providing genetic counseling for women who undergo NIPT.

Among the women who tested negative out of a total of 7740 patients, we obtained follow-up data after birth in only 1638 cases and confirmed one false-negative case of trisomy 18. The false-negative rate of testing was less than 0.1%. Because this rate was originally very low, more cases should be included in order to determine the actual false-negative rate of testing, and it is necessary to continue collecting follow-up data in the study population. Although the blood samples were sent to the USA from Japan in this study, the rate of analysis failure was very low compared with that observed in other reports⁶, namely, 0.2% in the first analysis and 0.05% (4/7740) in the first and second analyses. The rate of analysis failure differs according to the methodology of the companies, with a low fetal fraction and destruction of cell-free DNA suspected to be the main causes of this phenomenon. Although the fetal fraction of cell-free DNA tends to be low in the plasma from obese women,⁹ the mean BMI of the current participants was 20.9, which implies that Japanese pregnant women are less obese. We therefore believe that the main factor accounting for the low rate of analysis failure is the low prevalence of obesity in our study population. In addition, the procedures for handling the blood samples at authorized institutions are suspected to be effective in preventing the destruction of cell-free DNA. Therefore, the rate of analysis failure is very low, even when the samples are shipped from abroad, using appropriate blood handling.

In the present study, 98% of the pregnant women with a high risk for aneuploidy were able to avoid more invasive procedures, with a risk of a false-negative result of 0.06% (1/1638). In addition, there are great advantages for women 35 years of age or older and those with a history of having a child with a trisomy. Recently, the number of births in Japan has decreased yearly, while the number of pregnant women 35 years of age or older has markedly increased, exceeded 25% of total pregnancies. This reflects the annual increase in the number of invasive genetic tests.⁴ However, insufficient information regarding maternal serum screening tests and ultrasonographic markers is currently provided to pregnant women. Against this background, the present results imply that offering NIPT for pregnant women who wish to undergo prenatal screening of aneuploidy is a reasonable strategy for reducing the number of invasive procedures.¹⁰ Furthermore, it is expected that the present nationwide demonstration project of NIPT will stimulate debate regarding prenatal genetic testing and hopefully lead to improvements in the perinatal care system with respect to genetic counseling in Japan.

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WHAT'S ALREADY KNOWN ABOUT THIS TOPIC?

- The clinical application of noninvasive prenatal testing of fetal aneuploidies from cell-free DNA in maternal plasma began in the USA in 2011, and the use of this test has since become widespread worldwide. However, there is controversy as to how to implement the test as a prenatal genetic screening test.

WHAT DOES THIS STUDY ADD?

- This study describes the findings of a nationwide demonstration project to introduce noninvasive prenatal testing of fetal aneuploidies from maternal plasma in Japan, based on the historical background of prenatal diagnosis. In settings in which no prenatal screening policies have been adopted, it is reasonable to offer this test to pregnant women who wish to undergo prenatal screening of aneuploidy in order to reduce the number of invasive procedures.

REFERENCES

1. Chiu RW, Chan KC, Gao Y, *et al.* Noninvasive prenatal diagnosis of fetal chromosomal aneuploidy by massively parallel genomic sequencing of DNA in maternal plasma. *Proc Natl Acad Sci U S A.* 2008;105(51):20458–63.
2. Bianchi DW, Platt LD, Goldberg JD, *et al.* Genome-wide fetal aneuploidy detection by maternal plasma DNA sequencing. *Obstet Gynecol* 2012;119(5):890–901.
3. Onda T, Kitagawa M, Takeda O, *et al.* Triple marker screening in native Japanese women. *Prenat Diagn* 1996;16(8):713–7.
4. Sasaki A, Sawai H, Masuzaki H, *et al.* Low prevalence of genetic prenatal diagnosis in Japan. *Prenat Diagn [Letter]*. 2011;31(10):1007–9.
5. Yotsumoto J, Sekizawa A, Koide K, *et al.* Attitudes toward non-invasive prenatal diagnosis among pregnant women and health professionals in Japan. *Prenat Diagn* 2012;32(7):674–9.
6. Palomaki GE, Deciu C, Kloza EM, *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 [Clinical TrialMulticenter StudyResearch Support, Non-U.S. Gov't]*. 2012;14(3):296–305.
7. Palomaki GE, Kloza EM, Lambert-Messerlian GM, *et al.* DNA sequencing of maternal plasma to detect Down syndrome: an international clinical validation study. *Genet Med [Clinical TrialMulticenter StudyResearch Support, Non-U.S. Gov'tValidation Studies]*. 2011;13(11):913–20.
8. Suzumori N, Ebara T, Kumagai K, *et al.* Non-specific psychological distress in women undergoing noninvasive prenatal testing because of advanced maternal age. *Prenat Diagn* 2014;34(11):1055–60.
9. Wang E, Batey A, Struble C, *et al.* Gestational age and maternal weight effects on fetal cell-free DNA in maternal plasma. *Prenat Diagn* 2013;33(7):662–6.
10. Larion S, Warsof SL, Romary L, *et al.* Uptake of noninvasive prenatal testing at a large academic referral center. *Am J Obstet Gynecol* 2014 PII : S0002-9378(14)00611-5. doi: 10.1016/j.ajog.2014.06.038.

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