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ARTICLE

Maternal age-specific risk for trisomy 21 based on the clinical performance of NIPT and empirically derived NIPT age-specific positive and negative predictive values in Japan

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Abstract

The data collected by nation-wide study of noninvasive prenatal genetic testing (NIPT) for trisomy 21 from 21,610 pregnant women with advanced maternal age in Japan were reported. Among 188 NIPT-positive cases, 180 cases were true positive. The incidence of aneuploidy according to maternal age was estimated using a state-space model. Although, the frequency of trisomy increased exponentially with maternal age as previously reported, the maternal age-specific risk for trisomy 21 that was based on the clinical performance of NIPT was lower than the predicted risk in previous Western cohorts based on the data from invasive prenatal testing (Bayesian two-sided tail-area probability $P = 0.0156$). The empirical positive predictive value (PPV) of NIPT is likely to turn out higher than that of the theoretical PPV calculated from the sensitivity/specificity of the test and the incidence of trisomy 21 from this study.

Introduction

Information regarding the live birth incidence of trisomy is indispensable in prenatal genetic counseling. The incidence at different stages of pregnancy is also useful for identifying necessary prenatal genetic tests [1]. The sensitivity and specificity are used to assess the efficacy of each screening test and the positive predictive value (PPV) and negative predictive value (NPV) are of great concern in clinical testing because they depend on prior probability, or maternal age-specific and gestational age-specific risks of trisomy. The maternal age and gestational age-specific risks have been previously reported [1–3], and are calculated using the

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data from chorionic villus sampling (CVS) (10–14 weeks), amniocentesis (15–20 weeks), and live birth (term) cases.

NIPT is a noninvasive and highly reliable screening test. Unlike invasive procedures, NIPT carries no risk of miscarriage or harm to the unborn child, and the characteristics of NIPT clients at advanced maternal age are similar to those of the natural population.

In Japan, NIPT is not a standard medical test, is permitted only as part of clinical research, and all the collected data are always secured and controlled.

The objective of the present study is to empirically estimate the maternal age-specific risk for trisomy 21 using the data collected by nation-wide NIPT research and maternal age-specific NIPT-PPV and NIPT-NPV, which is based on clinical NIPT data in Japan.

Method

This was a multicenter prospective cohort study conducted in Japan with the approval of the institutional review board of ethics at each respective institution. The data were collected from a total of 21,610 pregnant women undergoing NIPT (massively parallel sequencing: MaterniT21 Plus® and GeneTech NIPT) with advanced maternal age (age: 38.48 ± 0.02, gestational weeks: 13.36 ± 0.01) between April 2013 and December 2015 at 38 medical institutions in Japan. To avoid any biased feedback, the data from the patients who had undergone ultrasound screening before NIPT were excluded.

The estimated risk of trisomy 21 for our data set was compared with that of previously reported data from Western countries [1, 2, 4–7].

The incidence of aneuploidy according to maternal age was estimated using a state-space model [8–10]. The incidence of aneuploidy and observed aneuploidy were modeled according to the following two formulae (where Eq. 1 denotes state process and Eq. 2 denotes observed process):

$$p_t - p_{t-1} \sim p_{t-1} - p_{t-2} + \epsilon_t \tag{1}$$

$$w_t \sim \text{binomial}(p_t), \tag{2}$$

where t was maternal age, $p_t \in (0, 1)$ was incidence to be estimated, $w_t \in \{0, 1, 2\ldots\}$ was the observation count of aneuploidy, and ϵ_t was Gaussian noise. The incidence of aneuploidy generally monotonically increases according to maternal age [1, 2, 4, 6]. Monotonic increase was assumed to occur according to the formula:

$$p_i \leq p_j, (i<j) \tag{3}$$

Besides estimating the incidence of aneuploidy in our data set, we estimated the incidence in other data sets from Australia [2] and Japan [3] as a reference. Estimation was performed using R 3.4.0 (https://cran.r-project.org/) and rstan 2.15.1 (http://mc-stan.org).

PPV and NPV were calculated exclusively based on the data from the patients whose birth outcomes were confirmed.

In this study, "theoretical PPV" (PPV_t) and "empirical PPV" (PPV_e) were defined as following:

$$\text{PPV}_t = \frac{pS_n}{pS_n + (1-p)(1-S_p)}, \tag{4}$$

$$\text{PPV}_e = 100\frac{w_{\text{true}}}{w_{\text{NIPT}}}, \tag{5}$$

where p was incidence of trisomy, S_n was sensitivity, S_p was specificity, w_{true} was the number of the true trisomy confirmed by diagnostic test, and w_{NIPT} was the number of positive cases in NIPT data set.

The theoretical PPV and NPV for trisomy 21 were calculated based on a sensitivity of 99.1% and specificity of 99.9% that were reported by Palomaki et al. in the validation study of MaterniT21 Plus® [11].

Results

The number of NIPT-positive cases for trisomy 21 was 188, of which 180 cases were confirmed by invasive testing or karyotyping of POC (PPV 95.7%) (Table 1, Fig. 1). The

Table 1 Incidence and positive predictive values of trisomy 21

Maternal age	*N*	NIPT positive	True positive	Risk	PPV (%)
34	721	2	2	0.28	100
35	2126	5	5	0.24	100
36	2496	9	7	0.28	77.8
37	2785	10	9	0.32	90.0
38	2967	13	12	0.40	92.3
39	3091	30	29	0.94	96.7
40	2803	29	29	1.03	100
41	2021	26	24	1.19	92.3
42	1325	33	32	2.42	97.0
43	733	19	19	2.59	100
44	355	7	7	1.98	100
45	127	4	4	3.15	100
46	38	0	0	0	ND
47	16	1	1	6.25	100
48	3	0	0	0	ND
49	2	0	0	0	ND
50	1	0	0	0	ND
Total	21,610	188	180	0.83	95.7

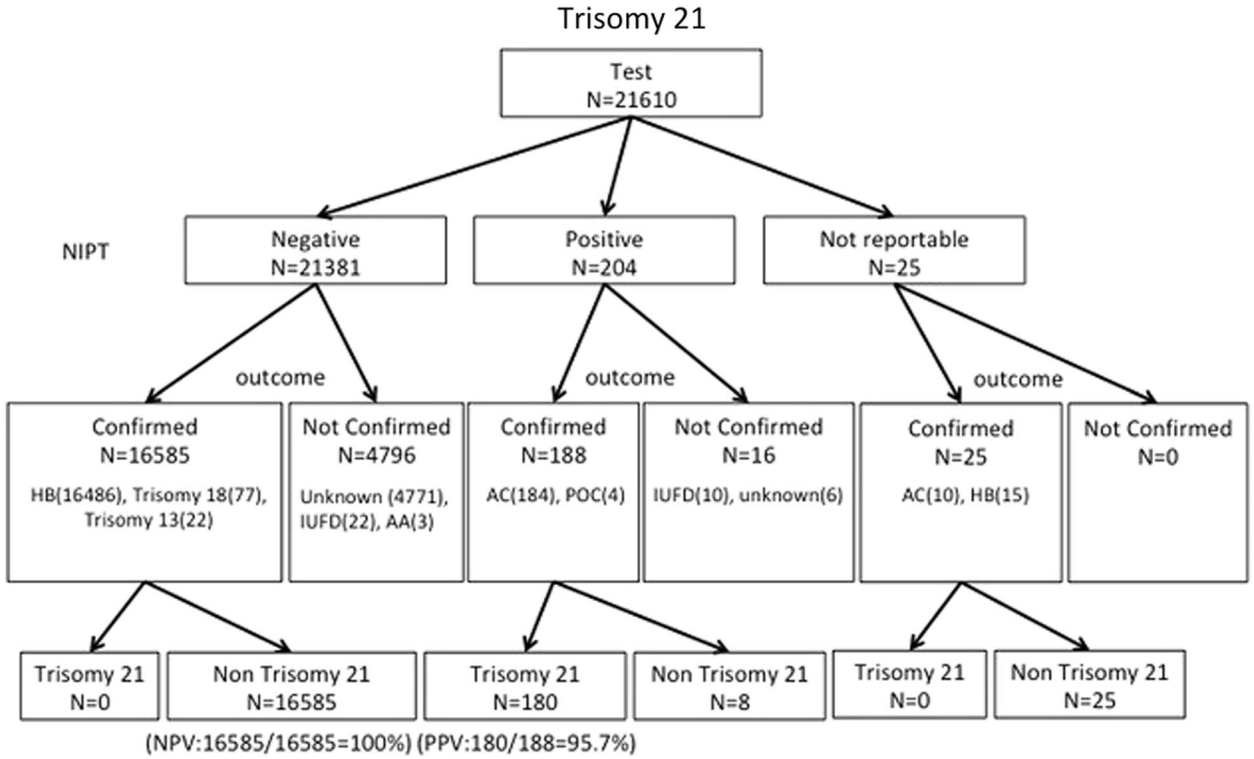

Fig. 1 NIPT results and outcomes for detection of trisomy 21 in 21,610 women. 'Confirmed outcomes' represents clinical confirmation of a healthy baby, or actual karyotyping. IUFD intra uterine fetal death without karyotyping, AA artificial abortion without karyotyping, HB healthy baby, AC karyotyping with amniocentesis, POC karyotyping with product of conception after IUFD

negative predictive value was 100% because there were no false-negatives identified in this cohort (Fig. 1). Among 21,381 NIPT-negative cases, the cases of which outcomes were confirmed were 16,585. There was no false-negative case in 16,585 cases. In another paper of our study group [12], there were two false-negative cases (one trisomy 21 and one trisomy 18) in 13,491 cases. These cases were not included in this study because the indication of these cases was abnormal findings of ultrasound. The frequency of trisomy increased exponentially with maternal age, as previously reported [1–7] (Table 1, Fig. 2a). However, the maternal age-specific risk for trisomy 21 during the CVS period (10–14 weeks) that was based on the clinical performance of NIPT was lower than the predicted risk [1] in Western cohorts (Bayesian two-sided tail-area probability $p = 0.0156$) (Fig. 2a), while the risk for trisomy 18 and 13 were approximately the same (data not shown).

We then calculated the maternal age-specific PPV curve from the maternal age-specific risk for trisomy 21, the detection rates, and the test specificities (Fig. 3). The risk according to the empirical data was higher than that according to the theoretical data, especially among younger age groups.

Discussion

The first aim of the present study was to empirically estimate the maternal age-specific risk for trisomy 21 using NIPT data in Japan. For evaluating the obtained risk for trisomy 21, we compared our data with previous studies. The maternal age and gestational age-specific incidence of trisomy as reported by Snijders et al. [1] is often cited in established textbooks [13]. However, our data of maternal age-specific risk for trisomy 21 was much lower than that of Snijders et al. [1]. We re-evaluated the available data by comparing other independent studies. Although the maternal age-specific risk of trisomy reported by Kratzer [5] was similar to that reported by Snijders et al. [1], Halliday [2] confirmed the results of the present study. In comparing studies based on amniocentesis period (Fig. 2b), and term (Fig. 2c) with other studies, large variation was observed among studies, especially in mothers older than 41 years, while the maternal age-specific risks reported by Snijders was much higher. The risk of trisomy 21 might be lower in Japanese people, as the Japanese risk seems to be the lowest based on CVS (our NIPT data) and amniocentesis periods [3]. Other studies [2–7], have also indicated that the incidence of trisomy 21 at each gestational period is lower than

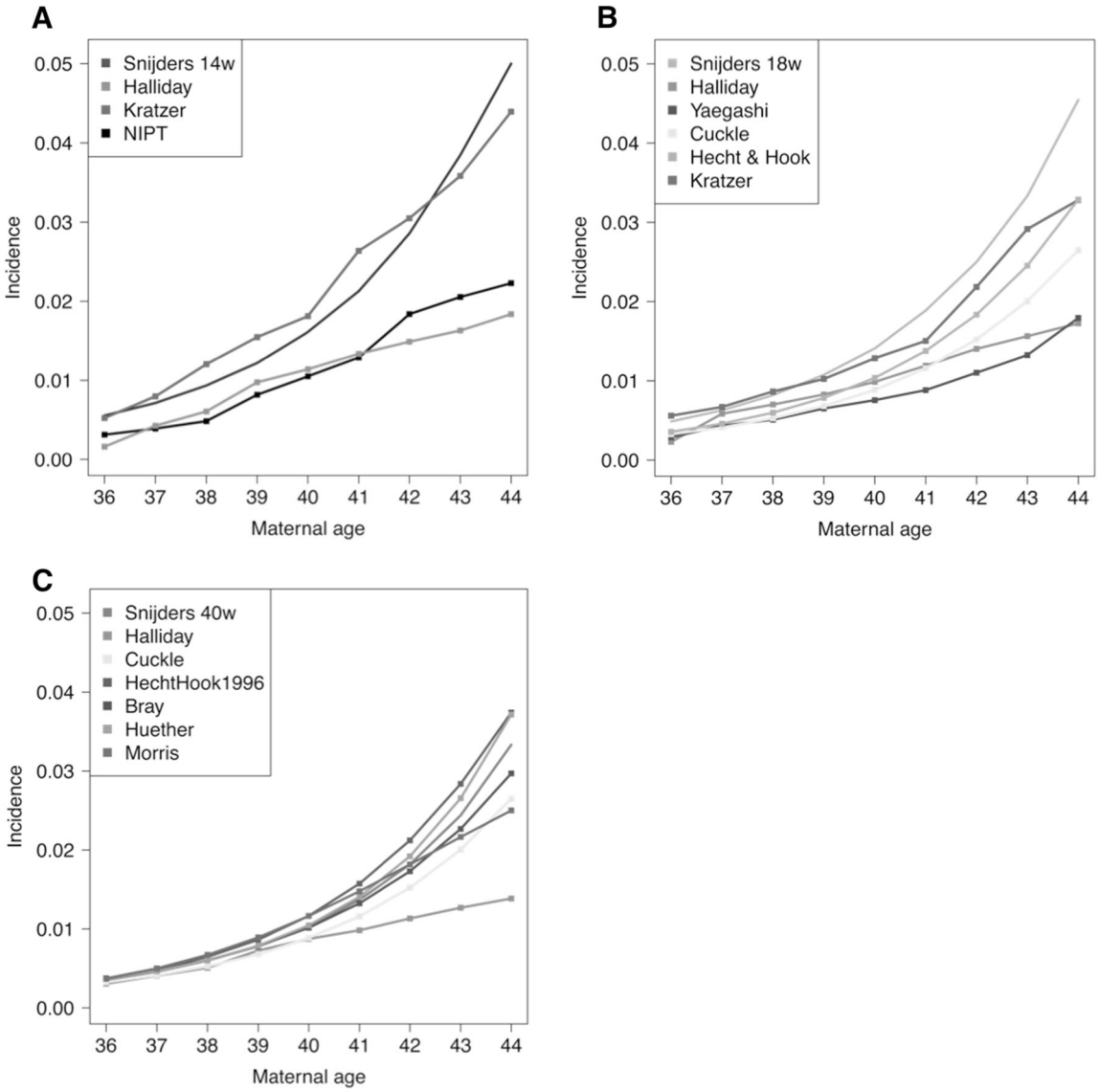

Fig. 2 The maternal age-specific risk for trisomy 21 based on the clinical performance of NIPT in the chorionic villus sampling period (10–14 weeks) (**a**), amniocentesis period (15–20 weeks) (**b**) and live birth period (term) (**c**)

that reported by Snijders et al. [1]. Our data might therefore represent a more accurate depiction of the trisomy risk than that of Snijders [1]. The results of the present study could be used to evaluate the significance of NIPT data as well as that of other screening tests (maternal serum markers, combined testing, etc.). In Japan, the artificial reproductive technology is prevalent, especially among advanced maternal age. Limitations of this study include that the age of oocyte retrieval was not considered in the analysis.

Empirical NIPT/PPV is likely to turn out higher than the theoretical PPV extracted from the lower trisomy 21 incidence, which our study showed, and this is especially true for younger generation. Although the etiology remains unknown, we can postulate a possible explanation. For the calculation of theoretical PPV, we used the sensitivity (99.1%) and specificity (99.9%) reported by Palomaki et al. [11]. However, the actual specificity might be much higher now than it was 5 years ago. When we assume the specificity is 99.98%, the theoretical PPV curve rises and is similar to the curve based on the empirical data (Fig. 3). At the least, this is an area that can use further research; A larger sample size in NIPT positive will allow empirical PPVs to be more accurate, but it is safe to say that what is found in this study is rather significant.

In Japan, NIPT is not a standard medical test, but a clinical research performed for patients in higher risk groups. NIPT is permitted only for high-risk pregnant women, including those with advanced maternal age, because the utility of NIPT for low-risk pregnant women had not been established when the Japanese guidelines on

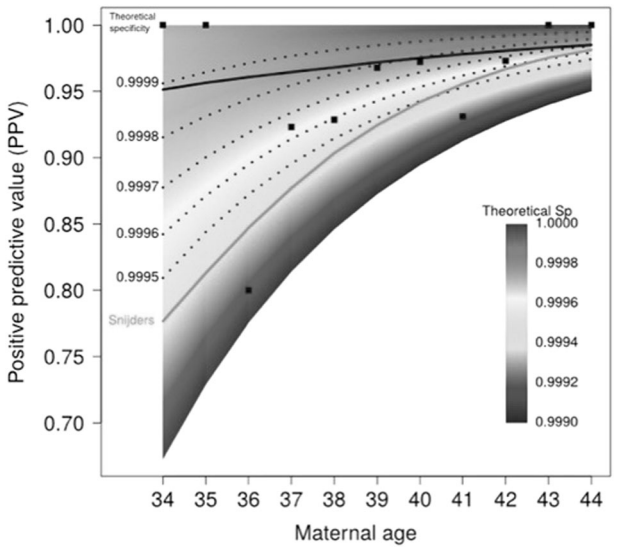

Fig. 3 Maternal age-specific positive predictive value (PPV) of trisomy 21 at 14 weeks of gestation. The color gradation represents the variation in NIPT specificity. The dark blue line located in the bottom of the graph is the PPV curve, which was calculated from the specificity reported by Palomaki et al. (99.90%) [11]. The pink line is the PPV curve, which was calculated based on Snijders et al. [1]. The black line is the regression curve of the outlier model from the empirical data obtained in the present study. The empirical PPV was much higher than the theoretical PPV, especially among younger age groups. The theoretical PPV increased with increasing specificity

new prenatal genetic testing methods using maternal blood were released [12]. As a result, young women may not receive NIPT even if they request it. Recently, in some non-approved clinics, some low-risk pregnant women have received NIPT without proper genetic counseling, resulting in serious problems for patients who received positive results. These patients do not receive the appropriate genetic counseling, and suffer from persistent anxiety, often visiting our hospitals and demanding proper genetic counseling and care. Although some evidence of the efficiency of NIPT in low-to-middle-risk women [14, 15] has been reported, since the release of the guidelines, the guidelines have not been updated. The present study provides further evidence to support the utility of NIPT for low-risk pregnant women, and prompt the revision of the guidelines.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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