First-Trimester Combined Screening Is Effective for the Detection of Unbalanced Chromosomal Translocations at 11 to 12 Weeks of Gestation

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First-Trimester Combined Screening Is Effective for the Detection of Unbalanced Chromosomal Translocations at 11 to 12 Weeks of Gestation

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Abstract

The first trimester combined screening, which analyzes fetal nuchal translucency and levels of free β-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein A (PAPP-A) in maternal serum, is routinely used to detect abnormal pregnancies associated with Down syndrome and other trisomy aneuploidies. Based on the hypothesis that major chromosomal translocations could lead to similar biochemical and developmental outcomes during early embryo development, we compared these markers among pregnancies with normal, balanced, or unbalanced fetal karyotypes. Among the parents, 71 (73%) carry balanced reciprocal translocation and 26 (27%) have Robertsonian translocation. Of the 97 pregnancies tested, 39 (40%), 37 (37%), and 22 (23%) fetuses had normal karyotype, balanced chromosomal translocations, and unbalanced chromosomal translocations, respectively. Importantly, we found that pregnancies with an unbalanced translocation had significantly higher free β-hCG multiple of the median (MoM) and larger nuchal translucency thickness than those with normal karyotype or balanced translocations. Analysis showed that the area under a receiver operating characteristic curve (AUC) is 0.716, 0.820, and 0.936 for free β-hCG MoM, PAPP-A MoM, and fetal nuchal translucency, respectively. When these 3 independent factors were combined, the AUC reached 0.976. In addition, logistic regression showed that the most optimal model for predicting an unbalanced chromosomal translocation is a combination of PAPP-A and nuchal translucency with an AUC of 0.980. Therefore, the first trimester combined screening is not only effective in the screening of Down syndrome and other trisomy abnormalities but also has high sensitivity for the detection of unbalanced chromosomal translocations in fetuses.

Keywords

chromosomal translocation, nuchal translucency, first trimester combined screening, β-hCG, PAPP-A.

Introduction

Aneuploidies are common causes of abnormal pregnancies, and first trimester combined screening is routinely used to detect Down syndrome and other trisomies in developed countries. In addition to these common genetic defects, unbalanced chromosome translocations are associated with abnormal pregnancies. In the general population, chromosomal translocations occur in 1 of 500 individuals. Although chromosome translocations between nonhomologous chromosomes in gametes can lead to chromosome abnormality, such translocations (ie, balanced translocations) are usually harmless even though carriers of balanced reciprocal translocations have increased risks of creating gametes with unbalanced chromosome translocations (where the exchange of chromosome material is unequal resulting in extra or missing genes), leading to miscarriages or children with abnormalities.[1-3]

Currently, obstetricians in most developed countries screen for fetal Down syndrome using a first trimester combined screening that combines measurements of fetal nuchal translucency and levels of free β-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein A (PAPP-A) in maternal serum. With a fixed false-positive rate of 5%, the first trimester combined screening method detects 90% of

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pregnancies with fetal chromosomal aneuploidy including trisomy at chromosomes 21, 13, and 18.[4-6] On the other hand, current standard clinical guidelines recommend that pregnant couples in which 1 partner carries a chromosomal translocation to have prenatal genetic testing using amniocentesis or chorionic villous sampling (CVS).[3] Although it has been reported that measurement of fetal nuchal translucency can be used to detect unbalanced chromosomal translocations,[7] however, whether unbalanced chromosomal translocations can be specifically identified with the first trimester combined screening is not clear.

Accordingly, in this study we examined whether the first trimester combined screening is useful for differentiating pregnancies with unbalanced fetal karyotypes from those with normal or balanced translocation fetal karyotypes. We analyzed nuchal translucency and maternal serum markers of pregnancies in which a parent has a balanced chromosomal translocation.

Materials and Methods

Patients were recruited from the Chang Gung Memorial Hospital Fetal Medicine Center, the Taipei City Hospital Women and Children Health Center, and their associated private clinics. All women were counseled and provided informed consent before the first trimester combined screening was performed. The study was approved by the institutional review board of the Taipei City Hospital and the Chang Gung Memorial Hospital Research and Ethics Committee. From October 2004 to August 2011, singleton pregnancies in which 1 parent has a balanced chromosomal translocation were enrolled at 11 to 12 weeks of gestation (calculated by early ultrasound measurement of the fetal crown-rump length). In addition, all couples were advised to undergo CVS with fetal karyotyping due to the presence of balanced chromosomal translocation in one of the parents.

The first trimester combined screening included ultrasound fetal nuchal translucency measurement at 11 to 12 weeks of gestation, and the analysis of free β-hCG and PAPP-A in maternal serum at 9 to 12 weeks of gestation. Ultrasound measurement was performed using a transabdominal approach with a Voluson 730 Expert scanner (GE Medical system, Zipf, Austria) according to the criteria of the Fetal Medicine Foundation London, United Kingdom,[7] by experienced obstetricians who had obtained the Fetal Medicine Foundation Certificate of Competence for scanning at 11 to 14 weeks of gestation. Three nuchal translucency thickness measurements were obtained for each fetus, and the largest values were used for risk calculation.

Free β-hCG and PAPP-A levels in maternal serum were analyzed using a Kryptor immunoanalyzer (Brahms AG, Berlin, Germany).[5,6] The serum levels of these factors were entered into the first-trimester combined screening program database as multiples of the median (MoMs) of the underlying reference values for pregnant women of the same gestational age. Down syndrome risk was calculated using the algorithm provided by the Fetal Medicine Foundation, with correction for maternal smoking status and the weight. A calculated risk of >1 in 270 was deemed positive according to regional guidelines.

The CVS was carried out transabdominally with a 1.1-mm outer-diameter steel needle under ultrasound guidance using the free-hand technique under aseptic conditions. Karyotyping of fetal chorionic villi samples was performed with both the short- and long-term culture methods.

Statistical Analysis

Analysis of variance was conducted to compare maternal age, gestational age, maternal weight, and median fetal nuchal translucency as well as levels of free β-hCG and PAPP-A among fetuses with normal karyotype, balanced chromosomal translocation, or unbalanced chromosomal translocation. Fisher Least Significant Difference (FLSD) test was conducted as a post hoc analysis. Statistical analysis was performed using SAS version 8.2 (SAS Institute, Cary, North Carolina). All P values were 2-sided and considered statistically significant at <.05. Power analysis indicated that 20 patients in each of the 3 test groups would be sufficient to provide a 90% power for the detection of significant differences between fetuses with normal or balanced karyotypes or between those with normal karyotype or unbalanced chromosomal translocations.

The risk of having an unbalanced chromosomal translocation in relation to levels of free β-hCG MoM, PAPP-A MoM, and nuchal translucency was derived based on logistic regression analysis. Sensitivity, specificity, and the estimate of the area under the curve (AUC) were calculated based on the receiver operating characteristic (ROC) curve analysis. To assess the performance of these biomarkers for the prediction of unbalanced chromosomal translocation, positive and negative likelihood ratios (LRs), positive predictive value (PPV), and negative predictive value (NPV) were calculated based on the optimal cutoff points selected for each biomarker. In addition, models that combined multiple biomarkers were calculated in order to derive the optimal model for the prediction of pregnancies with an unbalanced chromosomal translocation.

Results

A total of 122 singleton pregnancies were recruited for the present study from 2004 to 2011 (Figure 1), and 97 pregnancies with confirmed balanced or Robertsonian translocation in one of the couples were included in the analysis. Among the couples studied, 71 (73%) patients carried balanced reciprocal translocation and 26 (27%) patients had Robertsonian translocation. Among these patients, 61% (59 of 97) of the translocations had a maternal origin, whereas 39% (38 of 97) were of paternal origin (Table 1). Karyotyping analysis showed that 39 (40%) fetuses had normal karyotypes. Among the remaining patients, 36 (37%) had balanced chromosomal translocations, and 22 (23%) had unbalanced chromosomal translocations (Table 2). In these 22 patients of unbalanced chromosomal translocations, only 4 patients involved the 3 chromosomes most common associated with human aneuploidies (13, 18, and

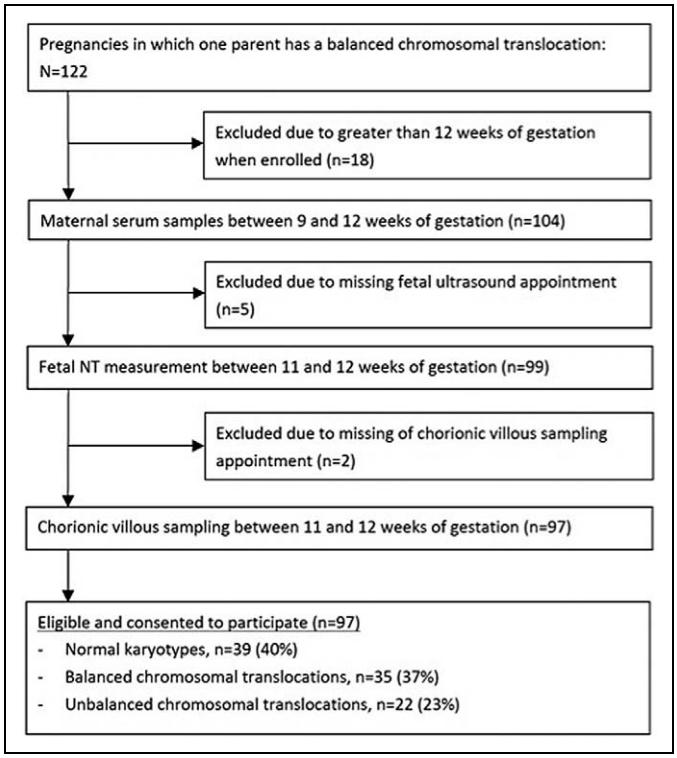

Figure 1. Flowchart of the study design. NT indicates nuchal translucency.

21); one had chromosome 18 translocation, 3 had chromosome 13 translocation; whereas, no chromosome 21 translocation was detected.

Among the 3 karyotype groups, there were no differences in maternal age, gestational age, or maternal body weight. Interestingly, measurements of fetal nuchal translucency indicated that nuchal translucency thickness of the unbalanced translocation group is significantly higher than those of normal karyotype ($P < .001$) and balanced translocation groups ($P < .001$, FLSD test). In addition, levels of free β-hCG MoM of the unbalanced translocation group were significantly higher as compared to those of normal karyotype group ($P = .012$) or balanced translocation group ($P = .025$). By contrast, the unbalanced translocation group had significantly lower PAPP-A MoM than the normal karyotype group ($P < .001$) and balanced translocation group ($P < .001$, Table 3).

On the other hand, no significant differences in free β-hCG MoM ($P = .710$), PAPP-A MoM ($P = .592$), or fetal nuchal translucency ($P = .493$) were observed between the normal karyotype group and the balanced translocation group.

Based on the ROC analysis, >1 in 270 risk ratio was selected as the cutoff to evaluate the risk of unbalanced chromosomal translocation based on the first-trimester combined screening, which comprises measurements of free β-hCG, PAPP-A, and fetal nuchal translucency. With this cutoff, 20 of the 22 unbalanced chromosomal translocation pregnancies, 2 of the 36 balanced translocation pregnancies, and 2 of the 39 normal karyotype pregnancies were considered positive (Table 4). This result indicated that the first trimester combined screening has a detection rate of 91% (20 of 22) for patients with unbalanced chromosomal translocations with a 5% (4 of 75) false-positive rate (Table 4). Likewise, analysis of sensitivity, specificity, PPV, NPV, LR+, and LR− indicated that the first trimester combined screening is effective for differentiating pregnancies with unbalanced chromosomal translocation from those with balanced translocation or normal karyotype (Table 4).

Table 1. The Distribution of Balanced and Unbalanced Chromosomal Translocations Among the Affected Parents.

	Balanced Reciprocal Translocation	Robertsonian Translocation	Total
Maternal origin	45	14	59 (61%)
Paternal origin	26	12	38 (39%)
Total	71 (73%)	26 (27%)	97 (100%)

In addition, ROC curve analyses showed that the AUC is 0.716 (95% CI 0.616-0.803), 0.820 (95% CI 0.729-0.891), and 0.936(95% CI 0.868-0.976) for free β-hCG MoM, PAPP-A MoM, and fetal nuchal translucency, respectively. Collectively, the first-trimester combined screening has an AUC of 0.976 (95% CI 0.923-0.996). Furthermore, logistic regression analysis showed that the most optimal model for predicting pregnancies with unbalanced chromosomal translocations was a combination of PAPP-A MoM and fetal nuchal translucency; it has an AUC of 0.980 (95% CI 0.928-0.997; Table 5, Figure 2).

Discussion

Based on studies of pregnancies of carriers with chromosomal translocations, we were able to test the whether the hypothesis that tests of the first-trimester combined screening are effective for the prediction of unbalanced chromosomal translocations. With a 90% power, our studies demonstrated that fetal nuchal translucency and maternal serum biomarkers of the first trimester combined screening are capable of differentiating fetuses with unbalanced chromosomal translocations from those with normal karyotype or balanced chromosome translocations with >95% sensitivity. Thus, the first-trimester combined screening should be incorporated with current clinical practice and other noninvasive prenatal diagnostic program to detect fetal unbalanced chromosomal translocations before analysis of amniocentesis or CVS.

In an earlier study, we have found that measurements of nuchal translucency thickness at 11 to 12 weeks of gestation alone are capable of detecting pregnancies with unbalanced chromosomal translocations resulting from carriers with balanced chromosomal translocation.[7] However, how sensitive the measurement of nuchal translucency thickness in detecting unbalanced chromosomal translocations has not been defined. In the present study, we analyzed nuchal translucency thickness together with maternal serum levels of free β-hCG and PAPP-A in pregnancies with 3 different karyotypes in the first

Table 2. Pregnancy Characteristics of the 3 Karyotype Groups.[a,b]

	Normal Karyotype (n = 39)	Balanced Chromosomal Translocation (n = 36)	Unbalanced Chromosomal Translocation (n = 22)	*F*	*P*
Maternal age, years	29.9 (4.4)	29.5 (3.0)	30.8 (3.3)	.877	.42
Gestational age, days	80.2 (5.3)	80.4 (6.5)	81.3 (4.4)	.417	.660
Maternal body weight, kg	54.83 (6.43)	55.11 (8.13)	55.32 (9.58)	.852	.427

Abbreviation: SD, standard deviation.
[a] Values are given as mean (SD).
[b] No significant differences of these variables among groups were observed.

Table 3. Median Nuchal Translucency Values and Maternal Serum Levels of Free β-hCG and PAPP-A.[a]

	Normal Karyotype (n = 39)	Balanced Chromosomal Translocation (n = 36)	Unbalanced Chromosomal Translocation (n = 22)	*F*	*P*
Free β-hCG, IU/L	71.97 (55.40)	63.31 (34.38)	108.9 (66.13)	5.665	.005
Free β-hCG, MoM	1.29 (1.01)	1.21 (0.84)	1.86 (0.99)	3.647	.03
PAPP-A, IU/L	3.85 (3.36)	3.51 (2.12)	2.43 (2.22)	1.978	.144
PAPP-A, MoM	1.26 (0.73)	1.18 (0.59)	0.60 (0.42)	8.558	<.001
NT, mm	1.70 (0.47)	1.79 (0.54)	3.09 (0.83)	43.668	<.001

Abbreviations: NT, nuchal translocation; β-hCG, β-human chorionic gonadotropin; PAPP-A, pregnancy-associated plasma protein A; MoMs, multiples of the median; SD, standard deviation.
[a] Values are given as mean (SD).

Table 4. Screening of Unbalanced Chromosomal Translocation Based on the First Trimester Combined Screening.

	Normal Karyotype (n = 39)	Balanced Chromosomal Translocation (n = 36)	Unbalanced Chromosomal Translocation (n = 22)	Predicting Power
Screening positive[a] (n = 24)	2	2	20	
Screening negative (n = 73)	37	34	2	
Sensitivity % (95% CI)				90.91 (70.84-98.88)
Specificity % (95% CI)				94.67 (86.90-98.53)
PPV % (95% CI)				83.33 (62.62-95.26)
NPV % (95% CI)				97.26 (90.45-99.67)
+LR (95% CI)				17.05 (6.51-44.63)
−LR (95% CI)				0.10 (0.03-0.36)

Abbreviations: CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value; LR, likelihood ratio.
[a] Calculated risk when >1 in 270 chance was deemed positive.

trimester. This study clearly showed that pregnancies with unbalanced translocations lead to significant increases of fetal nuchal translucency thickness and free β-hCG when compared to pregnancies with normal karyotype or balanced chromosome translocations. By contrast, unbalanced translocations resulted in a significant decrease of PAPP-A in maternal serum. In addition, we found that nuchal translucency thickness and PAPP-A level are more sensitive than the free β-hCG for the detection of the unbalanced translocation group. Importantly, fetal nuchal translucency together with the PAPP-A level has a 95% sensitivity and 96% specificity for the detection of unbalanced chromosomal translocation pregnancies. This efficiency is comparable to that of the first-trimester combined screening based on a cutoff of 1 in 874 generated from ROC analysis for 100% sensitivity and 95% specificity and to that of the traditionally used algorithm for the detection of Down syndrome and other trisomies (91% sensitivity and 95% specificity with a 1 in 270 cutoff).

Although it is not clear how unbalanced chromosomal translocations lead to changes in levels of PAPP-A and free β-hCG in the first trimester, these data suggest that unbalanced chromosomal translocations have biochemical and pathological consequences similar to those of trisomy 21 and trisomy 18. The PAPP-A, produced by the placental trophoblast, can increase the bioavailability of insulin-like growth factors, which in turn stimulates trophoblast invasion and modulates glucose and amino acid transport in the placenta.[8,9] Fetal chromosomal aberrations, including common trisomies (21, 18, and

Table 5. Performance of Individual and Combinations of Biomarkers for the Prediction of Unbalanced Chromosome Translocations.

Testing Method (cutoff values)	Sensitivity, % (95% CI)	Specificity, % (95% CI)	+LR, % (95% CI)	−LR, % (95% CI)	AUC (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)
Free β-hCG, MoM (>0.992)	86.36 (65.09-97.09)	60 (48.04-71.15)	2.16 (1.56-2.98)	0.23 (0.08-0.66)	0.716 (0.616-0.803)	38.78 (25.20-53.76)	93.8 (82.80-98.69)
PAPP-A, MoM (<0.605)	72.73 (49.78-89.27)	84 (73.72-91.45)	4.55 (2.55-8.10)	0.32 (0.16-0.65)	0.82 (0.729-0.891)	57.14 (37.18-75.54)	91.3 (82.03-96.74)
NT, mm (>2.1)	95.45 (7.16-99.88)	81.33 (70.67-89.40)	5.11 (3.16-8.27)	0.06 (0.01-0.38)	0.936 (0.868-0.976)	60.00 (42.11-76.13)	98.39 (91.34-99.96)
First trimester combined screen (>1/874)	100 (84.56-100)	94.67 (86.90-98.53)	18.75 (7.23-48.65)		0.976 (0.923-0.996)	84.62 (65.13-95.64)	100 (94.94-100)
Log odd (PAPP-A+ NT)	95.45 (77.16-99.88)	96.00 (88.75-99.17)	23.86 (7.84-72.59)	0.05 (0.01-0.32)	0.98 (0.928-0.997)	87.5 (67.64-97.34)	98.63 (92.60-99.97)

Abbreviations: NT, nuchal translocation; β-hCG, β-human chorionic gonadotropin; PAPP-A, pregnancy-associated plasma protein A; MoMs, multiples of the median; LR, likelihood ratio; AUC, the area under the curve; PPV, positive predictive value; NPV, negative predictive value.

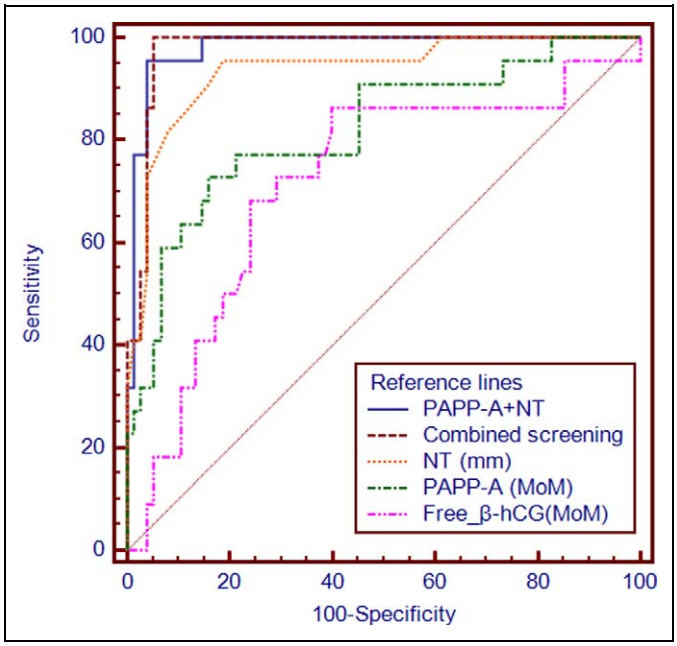

Figure 2. The sensitivity and 100-specificity of individual and combinations of biomarkers for the prediction of the pregnancies with unbalanced chromosomal translocations. Best cutoff values for free β-hCG: 0.992 MoM, PAPP-A: 0.605 MoM, and NT: 2.1 mm were determined based on the ROC curve analysis. The 45 line (deep-red color) indicates the theoretical plot of a test with no discrimination between unbalanced chromosomal translocations and control. First trimester combined screening (free β-hCG, PAPP-A, and NT), 1 in 874. β-hCG indicates β-human chorionic gonadotropin; MoM, multiple of the median; PAPP-A, pregnancy-associated plasma protein A; NT, nuchal translucency; ROC, receiver operating characteristic.

13) and sex chromosome aneuploidy, have been associated with low levels of maternal serum PAPP-A.[10-15] Likewise, low maternal serum PAPP-A levels at the first trimester have been associated with an increased frequency of adverse obstetrical outcomes.[16,17] Therefore, a low level of PAPP-A in circulation may result in abnormal placental function and represents a sensitive indicator of different types of aneuploidies ranging from triploidies to unbalanced chromosomal translocations.

Free β-hCG is mainly produced by placental villous trophoblasts,[18,19] and its biosynthesis is linked to differentiation of the cytotrophoblast.[20] Free β-hCG levels have been shown to elevate in fetal trisomy 21[21-24] but are markedly decreased in trisomy 18.[21,25,26] Thus, changes in free β-hCG in maternal serum depend on the types of chromosome abnormality. Although the mechanisms underlying increases in free β-hCG in pregnancies with unbalanced chromosome translocations remain to be investigated, the regulation of transcription of hCG genes in these pregnancies is likely impaired leading to global changes in fetoplacental development.[25]

Chromosomal translocations originate from chromosome cleavages followed by rejoining of parts between nonhomologous chromosomes. Phenotypic features of unbalanced chromosomal rearrangements varied among patients due to different origins of the chromosomal fragments being altered and the associated genes loss or gain.[27-30] Therefore, it is important to note that while pregnancies with unbalanced chromosome translocations exhibit a similar pattern of changes in fetal nuchal translucency and levels of β-hCG and PAPP-A, genetic alterations found in fetuses analyzed here could vary significantly. Given the potential heterogeneity in patients of unbalanced chromosome translocation, our data suggest that most of the unbalanced genetic alterations can lead to altered fetoplacental function, and this is reflected in the imbalance of placental protein secretion, similar to the hormonal changes associated with trisomy.[21,22] This conclusion is also supported by the observation that measurements of nuchal translucency and levels of β-hCG and PAPP-A in normal and balanced translocation pregnancies in the current study were in good agreement with those reported in normal pregnant population during the same measurement period. Furthermore, the prospective consecutive cohort study design we adopted here has minimized potential selection bias.

Finally, it is also important to note that while the number of patients in our study was relatively low when compared to major investigations that studied common chromosomal aneuploidies, power analysis indicated that the study is sufficiently powered, and that our observation with regard to unbalanced chromosome translocation is likely a common phenomenon. However, to give a better estimate of the risk of unbalanced chromosome translocations, future studies need to take into account the prior risk of maternal age risk for unbalanced chromosome translocation, similar to that have been provided for the Down syndrome.

In conclusion, our study has demonstrated that the traditional first-trimester combined screening is effective for the detection of common unbalanced chromosome translocations that would likely not be detected with the current developing noninvasive prenatal testing technique. Our results suggest that these noninvasive tests should be further incorporated into the prenatal counseling process and the decision-making process with regard to the timing and methods of prenatal diagnosis in the future.

Authors' Note

This work was performed at the Department of Obstetrics and Gynecology, Chang Gung Memorial Hospital-Linkou Medical Center, Taoyuan, Taiwan, ROC.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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