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Published in:
Molecular genetics & genomic medicine

DOI:
10.1002/mgg3.1213

Publication date:
2020

Document version
Publisher's PDF, also known as Version of record

Received: 15 November 2019 | Revised: 30 December 2019 | Accepted: 24 February 2020

DOI: 10.1002/mgg3.1213

ORIGINAL ARTICLE

Molecular Genetics & Genomic Medicine Open Access WILEY

Impact of ultrasonography on identifying noninvasive prenatal screening false-negative aneuploidy

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Abstract

Background: To evaluate the impact of ultrasonography on identifying noninvasive prenatal screening (NIPS) false-negative aneuploidy.

Methods: Analysis of large population-based NIPS false-negative aneuploidy data comprising karyotypes, clinical outcomes, and ultrasound results.

Results: From December 2010 to July 2018, a total of 3,320,457 pregnancies were screened by NIPS performed in BGI; among them, 69 NIPS false-negative aneuploidy cases with informed consent were confirmed, and ultrasound examination data for 48 cases were not available. Of the 21 cases with ultrasound results, 19 (90.5%) had various abnormalities on ultrasound, and two (9.5%) cases were shown to be normal on ultrasound. Additionally, six of seven live-born fetuses (approximately 85.7%) were found to have abnormalities on ultrasound. Ventricular septal defects constituted the most frequently observed ultrasound abnormality type among the 21 NIPS false-negative aneuploidy cases.

Conclusion: Application of NIPS has increased rapidly worldwide and now accounts for a large proportion of prenatal screening tests in China. This study suggests that ISUOG guideline should be followed practically, and structural abnormal ultrasound findings should not be neglected, even when NIPS produces a negative result. Combining NIPS with an ultrasound examination can further reduce the incidence of live births with aneuploidy.

KEYWORDS

abnormality, false-negative aneuploidy, NIPS, ultrasonography

1 | INTRODUCTION

Application of noninvasive prenatal screening (NIPS) has increased rapidly worldwide and now accounts for a large proportion of prenatal screening tests for trisomy 21, trisomy 18, trisomy 13, sex chromosomes, and an increasing number of microdeletions (Greene and Phimister, (2014); Van Opstal et al., 2018; Hui, Hutchinson, Poulton, & Halliday, 2017). By means of cell-free DNA genomic sequencing analysis, NIPS for trisomies 21, 18, and 13 achieves much better performance than conventional standard screening tests, which are based on serological markers, maternal age, and maternal

Wei Li and Fanwei Zeng are contributed equally.

history (Hui et al., 2017). The American College of Medical Genetics and Genomics has recommended informing all pregnant women that NIPS is the most sensitive screening option for traditionally screened aneuploidies (i.e., Patau, Edwards, and Down syndromes; Gregg et al., 2016). The potential impact of NIPS on the field of prenatal diagnosis and on the prevalence of live births with chromosomal abnormalities is increasing dramatically since sequencing costs have been gradually decreasing and since government funding has increased (Oepkes et al., 2016).

Although the occurring rate of false-negative cases is very low, discordant findings between NIPS and prenatal diagnoses, which may result from a low fetal DNA fraction or fetoplacental mosaicism (Grati et al., 2014; Scott et al., 2018), are still observed globally. There are case reports that ultrasonography, a powerful method for screening fetal aneuploidy during pregnancy, may contribute to the identification of NIPS false-negative cases (Pan et al., 2014; Yang et al., 2017). Abnormalities on ultrasound (US) are useful markers for detecting trisomy 21, trisomy 18, trisomy 13, sex chromosome aneuploidy (SCA), rare autosomal trisomies (RATs), and even copy number variations, as reported previously (Beulen, Faas, Feenstra, Vugt, & Bekker, 2017; DeVore, 2000; Shaffer et al., 2012). Clinically, the cardiac, neurologic, gastrointestinal, musculoskeletal, and facial defects are the most common ultrasonographic structural abnormalities as reported previously (Ainsworth, Holman, Codsi, & Wick, 2018; Rao & Platt, 2016), and nasal bone abnormalities, nuchal fold thickening, and hyperechoic bowel are useful at a fairly high detection rate for screening fetuses with trisomy 21 (Sonek & Croom, 2014). However, reports on the quantitative contribution of ultrasound toward identifying the genotypes of NIPS false-negative cases are still limited.

The NIPS result represents the genotype of the fetus, whereas the ultrasound finding reflects the phenotype of the fetus. We aimed to assess the impact of ultrasound on NIPS false-negative aneuploidy based on real-world data from 3,320,457 pregnancies screened from 2010 to 2018.

2 | METHODS

2.1 | Study population and sample collection

From December 2010 to July 2018, a total of 3,320,457 pregnancies were screened by NIPS performed by BGI, among which 69 NIPS false-negative aneuploidy cases (with written informed consent for research purposes) were confirmed by G-banded karyotyping or a chromosomal microarray (CMA) diagnosis. This study was approved by the institutional review board of BGI (BGI-IRB). The article was previously published as a preprint on BioRxiv, https://doi.org/10.1101/748269.

NIPS and ultrasound examination were recommended for pregnancies according to the standard screening process (Bianchi et al., 2014). Whole-genome shallow massively parallel sequencing was performed in all cases at a depth of about 0.1 time. The z-score cutoff was set as 3 for calling trisomies. And, we are not using an assay to detect microdeletions due to the low sequencing depth. Thereafter, G-banded karyotyping or CMA diagnosis was highly recommended for cases that were high risk.

2.2 | Validation and follow-up of NIPS false-positive or false-negative aneuploidy cases

The NIPS-positive results were followed up and further validated by G-banded karyotyping or CMA diagnosis, or follow-up of clinical outcomes.

Meanwhile, both false-positive and false-negative NIPS results (for trisomy 21, 18, 13) were encouraged to be reported by offering each participant an insurance as a part of the test. In the case of a false-positive NIPS result, the insurance policy requires refunding the cost of the invasive tests. For each false-negative NIPS case with clinically confirmed aneuploidy before or after live birth, the insurance policy requires paying CNY 20,000 or CNY 400,000 to the patient, respectively.

The ultrasound examination results, if available, could be traced from the insurance materials. The unavailability of ultrasound information meant either that ultrasound was not performed or that the ultrasound results were not available from the insurance materials.

2.3 | Statistical analyses

Statistical analysis was performed using Student's *t*-test, and $p < .05$ was considered statistically significant. All statistical analyses were conducted using R, version 3.4.3.

2.4 | Data availability statement

All data generated or analyzed during this study are included in this published article (and its supplementary information files).

3 | RESULTS

The enrollment, clinical follow-up, and outcomes of the false-negative cases participating in NIPS and ultrasound are presented in Figure 1. Of the 69 NIPS false-negative pregnancies (Table S1), 21 cases had available ultrasound examination information, and 48 cases did not have available information. The median gestational age (GA) of the 21 NIPS

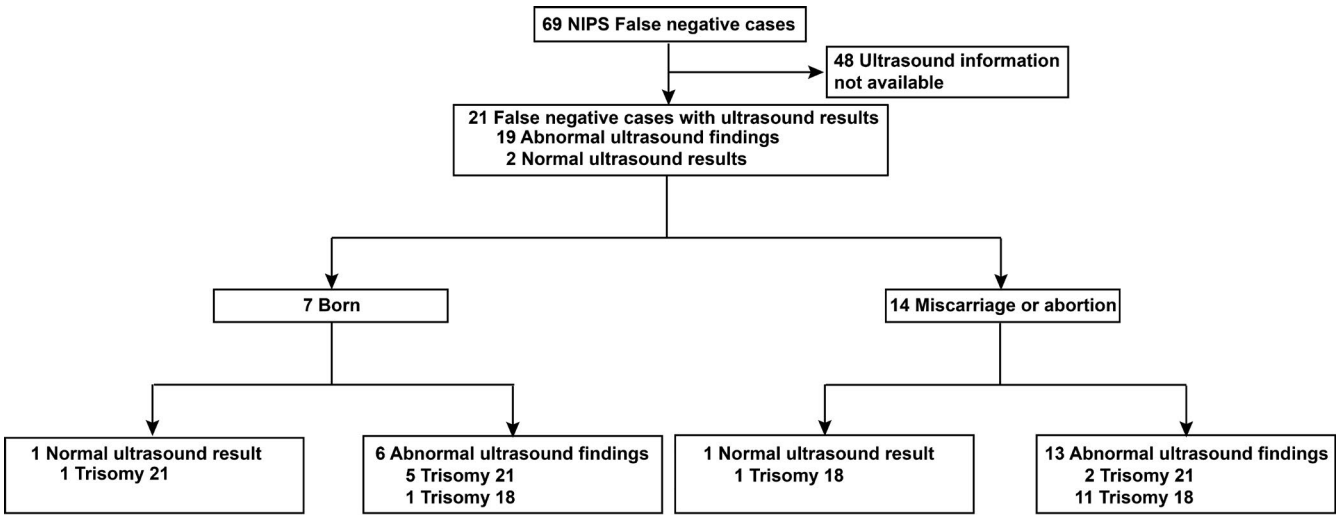

FIGURE 1 Enrollment, clinical follow-up, and outcome classification of the false-negative cases participating in the NIPS and ultrasound examinations

false-negative cases that underwent ultrasound examinations was 24.8 weeks; herein, the second trimester GA group (66.7%) was the most predominant (Table S2). The median maternal age was 30 years, and most of the women, 12 of 21 cases (57.1%), were not more than 30 years old (Table S2).

Regarding the 21 cases with ultrasound information (Figure 1; Table 1), a total of 19 pregnancies were found to have abnormalities by ultrasound examination, whereas only two pregnancies were normal by ultrasound examination (Figure 1). That is, 90.5% of the cases with ultrasound information (19 of 21) and at least 27.5% of the total cohort (19 of 69) were NIPS false-negative cases that could potentially be avoided by means of a combination of NIPS and an ultrasound examination, followed by G-banded karyotyping or CMA diagnosis, which would probably not increase the risk of fetal loss.(Malan et al., 2018) It did not escape our notice that six of seven children born had abnormal ultrasound findings during pregnancy; that is, six of seven NIPS false-negative cases born could have potentially been identified by means of the ultrasound examination (Figure 1; Table 1). The abnormal ultrasound findings for the six children born included small head circumference, lateral ventriculomegaly (11.2/10.2 mm), ventricular septal defect, aortarctia, tricuspid moderate regurgitation, nasal bones dysplasia, short femora, short humeri, bilateral renal pelvis dilation (4.9/4.0 mm), polyhydramnios, and single umbilical artery (Table 1).

The features of abnormal ultrasound findings and the corresponding karyotyping genotypes for the 19 cases (Table 1) were analyzed and summarized in Figure 2. Congenital cardiovascular defects, skeletal malformations, and brain and nervous system defects were the most frequently detected organic system abnormalities (Figure 2a), and ventricular septal defects (7 of 19 cases) were the most frequently observed ultrasound abnormalities (Figure 2a) among the NIPS false-negative aneuploidy cases. There is a preponderance of the trisomy 18 genotype over the trisomy 21 genotype among all of the top six ultrasound-based systematic abnormalities, including the cardiovascular, skeletal, brain, and nervous, urinary, fetal appendage, and craniofacial systems (Figure 2a). Among the defects in the cardiovascular system, ventricular septal defect, pulmonary artery enlargement, dilated right heart, hypoplastic left heart, and double outlets of the right ventricle could be strong ultrasonic markers for trisomy 18, whereas an atrioventricular septal defect suggests trisomy 21. Additionally, tricuspid regurgitation and aortarctia could be ultrasonic findings for either trisomy 18 or trisomy 21. As shown in Figure 2b, the fetuses with trisomy 18 tended to show abnormal findings by ultrasound at an earlier average GA (24.5 versus. 27.8 weeks) than those with trisomy 21; however, the difference was not significant (p value was .19).

4 | DISCUSSION

With the large population-based data, this study analyzes the impact of prenatal ultrasound examination on NIPS false-negative cases. This report focuses on cases between December 2010 and July 2018 in China, and these data may also have significance in other countries as a reference. Even though the occurring rate of NIPS false-negative cases was low, we aimed to investigate the false-negative case data and explore a viable method to supplement NIPS to further reduce the live birth prevalence of aneuploidy, or help the families to plan for care after birth.

4.1 | Combination of NIPS and ultrasound

NIPS is the most accurate and powerful prenatal screening method for Patau, Edwards, and Down syndromes to date,

TABLE 1 Characteristics of the 21 NIPS false-negative cases

Case	Age (years)	Gestational age[a] (weeks)	Ultrasound results	Genotype	Outcome
1	28	23.0	Bilateral renal pelvis dilation (4.9/4.0 mm)	47,XN,+21	Born
2	31	31.6	Small head circumference; aortarctia, tricuspid moderate regurgitation; short femora	47,XN,+21	Born
3	28	N.A	Nasal bones dysplasia	47,XN,+21	Born
4	27	27.0	Lateral ventriculomegaly (11.2/10.2 mm)	47,XN,+21	Born
5	32	25.0	Short humeri	47,XN,+21	Born
6	29	N.A	Normal	47,XN,+21	Born
7	30	N.A	Ventricular septal defect; polyhydramnios; single umbilical artery	47,XN,+18	Born
8	27	27.3	Absence of both the fingers and the metacarpal bones; intrauterine growth restriction	47,XN,+21	Abortion
9	27	24.4	Completely atrioventricular septal defect	mos 47,XN, +21[45]/46,XN[40]	Abortion
10	28	23.0	Multiple malformation (Not specified)	47,XN,+18	Abortion
11	35	28.4	Bilateral choroid plexus cyst, lemon sign; ventricular septal defect; left wrist drop, abnormal position of fingers, scoliosis; left dysplastic kidney	47,XN,+18	Abortion
12	35	23.3	Multiple malformation (Not specified)	47,XN,+18	Abortion
13	33	24.9	Cauda cerebelli dysplasia; ventricular septal defect, dilated right heart; pulmonary artery enlargement; single umbilical artery	47,XN,+18	Abortion
14	30	16.0	Low-set ears, edema in the posterior head and neck	47,XN,+18	Abortion
15	29	24.7	Cauda cerebelli dysplasia, absence of the splenium corpus callosum; ventricular septal defect, aortarctia; overriding fingers	47,XN,+18	Abortion
16	37	25.1	Multiple malformation (Not specified)	47,XN,+18	Abortion
17	28	25.3	Cauda cerebelli dysplasia; ventricular septal defect; pulmonary artery enlargement; micromandible; rocker-bottom foot, overriding fingers; single umbilical artery	47,XN,+18	Abortion
18	31	24.1	Ventricular septal defect, tricuspid slight regurgitation, hypoplastic left heart, double outlets of right ventricle; overriding fingers; unilateral hydronephrosis; cyst of left ureter tube; single umbilical artery	47,XN,+18	Abortion
19	35	29.9	Small transverse cerebellar diameter, bilateral choroid plexus cyst, cavum veli interpositi dilatation; short femora, short humeri; intrauterine growth restriction	47,XN,+18	Abortion
20	35	24.3	Ventricular septal defect; cross-foot	47,XN,+18	Abortion
21	24	12.3	Normal	47,XN,+18	Miscarriage

Abbreviation: N.A, not available.

[a]The examination test date of ultrasound.

according to a previous global report (Chitty et al., 2016; Gregg et al., 2016). Nevertheless, NIPS can still produce false-negative chromosomal aneuploidy results for fetuses, as well as live births when the probability of a live birth (approximately 20% of trisomy 21 fetuses may progress to term delivery; Antonarakis, Lyle, Dermitzakis, Reymond, & Deutsch, 2004) is taken into account. NIPS false-negative findings may occur due to various reasons, including a low fetal DNA fraction and fetoplacental mosaicism. As was reported previously, mosaic trisomy can be accurately detected

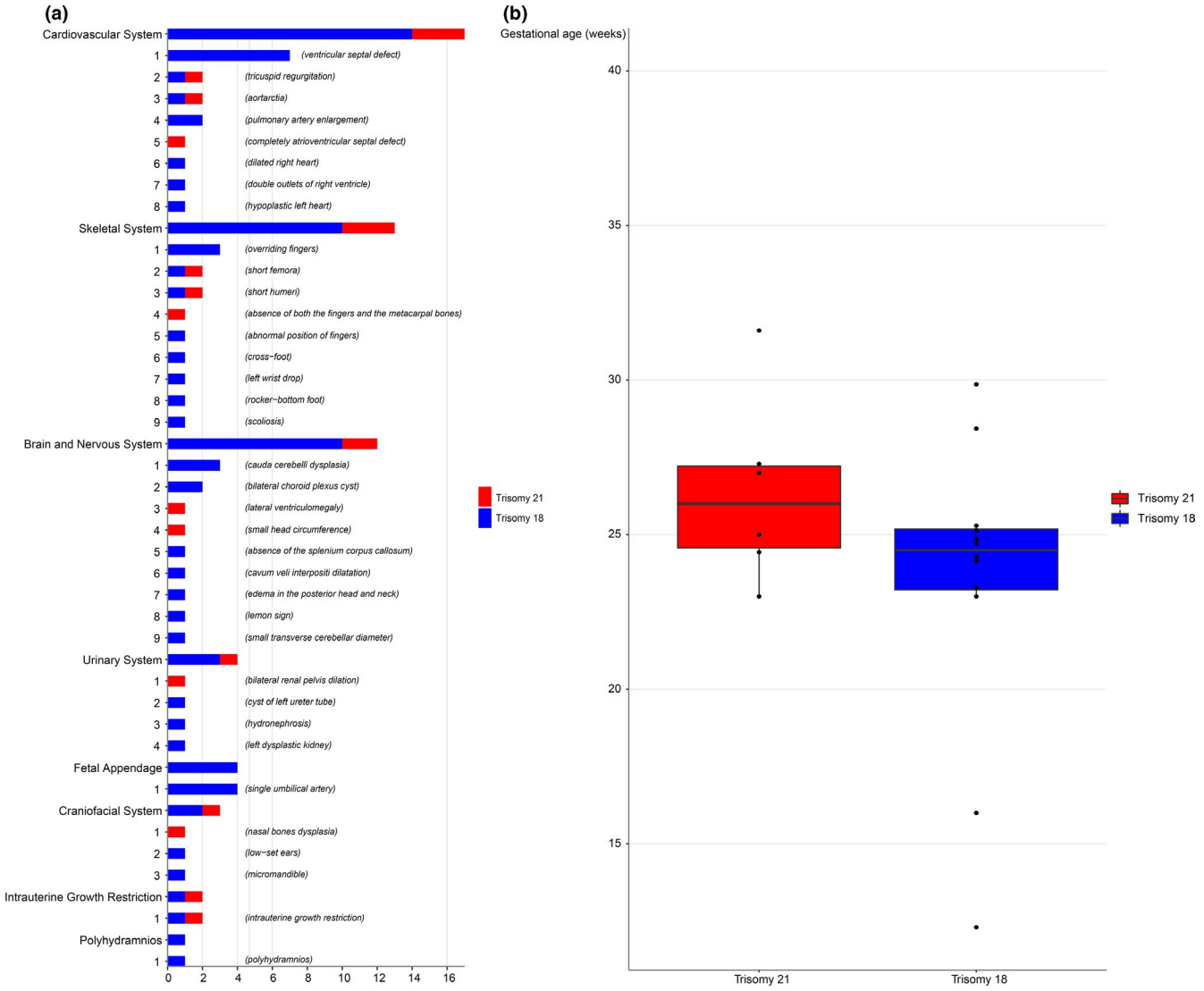

FIGURE 2 The distribution of abnormal ultrasound phenotypes. (a) Histogram showing the number of distinct ultrasound abnormal phenotypes based on affected organ systems, as well as the distinct ultrasound findings and corresponding genotypes. (b) Boxplot comparison of the gestational age for the abnormal ultrasound findings among five trisomy 21 fetuses and 11 trisomy 18 fetuses. Statistical analysis was performed using Student's *t*-test, and the *p* value was .19

by NIPS only when the fraction of fetoplacental mosaicism is higher than 70% (Grati et al., 2014).

The combination of NIPS and an ultrasound examination may further reduce the risk for false-negative fetuses and live births. We observed a significant proportion (90.5%, 19 of 21 cases with ultrasound data) of NIPS false-negative cases (at least 27.5% of the total cohort [19 of 69], if all of the US cases without available data had normal ultrasound results or 97.1% [67 of 69], if all of the ultrasound cases without available data had abnormal ultrasound results) could be potentially avoided by means of a combination of NIPS and an ultrasound examination, followed by G-banded karyotyping or CMA diagnosis, which would probably not increase the risk of fetal loss, according to an updated large population study (Malan et al., 2018). Clinicians should pay additional attention to the top ultrasound findings indicating congenital cardiovascular defects, skeletal malformations, brain and nervous system defects, urinary malformations, and especially ventricular septal defects, which are the most common abnormal findings on ultrasound among NIPS false-negative cases (Figure S2). In addition, special attention should be paid to abnormal signs on ultrasound, namely small head circumference, lateral ventriculomegaly (11.2/10.2 mm), ventricular septal defect, aortarctia, tricuspid moderate regurgitation, nasal bones dysplasia, short femora, short humeri, bilateral renal pelvis dilation (4.9/4.0 mm), polyhydramnios, and single umbilical artery, since, surprisingly, six NIPS false-negative fetuses (five trisomy 21 cases and one trisomy 18 case) with these ultrasound signs were born alive because these abnormal ultrasound findings were overlooked (Table 1). Five of these cases (cases 2, 3, 4, 5, and 7) with strong

ultrasound markers (in isolation or with additional anomalies) would be advised by clinicians to undergo an invasive diagnosis (Gilmore et al., 2008), while the other case (case 1) with a soft ultrasound marker may not be advised to undergo an invasive diagnosis by the clinician in China according to the ISUOG guidelines (Salomon et al., 2017; Society for Maternal-Fetal Medicine, Biggio, & Kuller, 2017). Reasonably, all five fetuses with soft markers (ultrasound findings), including nasal bone dysplasia, lateral ventriculomegaly (11.2/10.2 mm), polyhydramnios, and bilateral renal pelvis dilation (4.9/4.0 mm), successfully progressed to term delivery, indicating that these types of ultrasound findings are inclined to be overlooked more frequently (Table 1).

This implies the complementary role of ultrasonography with NIPS to achieve a better prenatal screening performance. Clinically, abnormal ultrasound findings should not be neglected when performing G-banded karyotyping or the CMA diagnosis, even when NIPS results in a negative result.

Our study had the following limitations. First, the ultrasound results were not available for all of the 69 cases, so we could not accurately evaluate the quantitative contributions of ultrasound toward identifying NIPS false-negative cases. The rate of abnormal ultrasound findings among the NIPS false-negative cases may be approximately 90.5% (19 of 21 cases with ultrasound data), fluctuating between 27.5%, (at least 19 of 69, if all of the ultrasound cases without available data had ultrasound normal results) and 97.1% (at most 67 of 69, if all of the ultrasound cases without available data had ultrasound abnormal results). Second, accurate follow-up of some clinical information was not performed. Specifically, the reasons that six cases with prenatal abnormal findings on ultrasound were born were undetermined in our study, and these reasons may be that the clinicians overlooked the findings or that the patients declined pregnancy termination. On the latter situation, ultrasound may not be helpful for further reducing the live birth incidence of false-negative aneuploidy cases, but could be helpful for the families to plan for care after birth. Lastly, detailed clinical ultrasound information, such as the presence of multiple malformations (Table 1), was not available for all the cases because these details were not specified in the insurance materials.

5 | CONCLUSION

Application of NIPS has increased rapidly worldwide and now accounts for a large proportion of prenatal screening tests in China. We observed that 19 of the 69 NIPS false-negative pregnancies were found to have abnormal findings on ultrasound, indicating that between at least 27.5% (if all of the ultrasound cases without available data had normal ultrasound results) and at most 97.1% (if all of the ultrasound cases without available data had abnormal ultrasound findings) false-negative cases could be potentially avoided by means of a combination of NIPS and ultrasonography, followed by G-banded karyotyping or a CMA diagnosis. Clinically, we should follow the ISUOG guidelines more strictly when abnormal ultrasound findings were accompanied with NIPS negative results. Based on all the information presented above, clinically, we may be more cautious about the abnormal ultrasound findings even though NIPS shows negative results.

ACKNOWLEDGMENTS

We thank all medical centers, participants, and the families in this study for their cooperation and support.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

All listed authors have each made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; Wei Li participated in drafting the manuscript and revising it critically for content; Fanwei Zeng and Nan Yu collected patients' materials; Sheng-li Li and Zhiyu Peng have approved the final version of the submitted manuscript. Baitong Fan, Hui Huang, Yun Yang, and Jing Wu accepted responsibility for the integrity of the data analysis. All authors reviewed the manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Li W, Zeng F, Fan B, et al. Impact of ultrasonography on identifying noninvasive prenatal screening false-negative aneuploidy. *Mol Genet Genomic Med*. 2020;8:e1213. https://doi.org/10.1002/mgg3.1213