False Low-Risk Single Nucleotide Polymorphism–Based Noninvasive Prenatal Screening in Pentasomy 49,XXXXY

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Abstract

Introduction Pentasomy 49,XXXXY is a sex chromosome anomaly difficult to be diagnosed prenatally. We describe a patient of pentasomy 49,XXXXY with false low-risk results using a noninvasive prenatal screening (NIPS). A 30-year-old G1P0 woman presented at $33^{6/7}$ weeks, secondary to sonographic fetal anomalies. She had low-risk NIPS at $13^{6/7}$ weeks. Anatomy survey showed bilateral clubfeet, clinodactyly of the left fifth digit, micropenis, and echogenic bowel. Cytogenetics analysis revealed pentasomy 49,XXXXY syndrome. We report third-trimester sonographic features of a fetus with pentasomy 49,XXXXY and the importance of thorough pre- and posttest counseling for NIPS.

Keywords
- sex chromosome aneuploidy
- NIPT
- prenatal diagnosis
- screening

The advent of noninvasive prenatal screening (NIPS, sometimes abbreviated as NIPT or NIPS) for aneuploidy has made a substantial impact in maternal–fetal medicine practice by significantly decreasing the number of second-trimester invasive diagnostic tests performed.[1] Nevertheless, the American Congress of Obstetricians and Gynecologists (ACOG) continues to recommend judicious use of NIPS, as this is a nondiagnostic tool and a "negative" (more accurately, low-risk) result does not ensure an unaffected pregnancy.[2]

Pentasomy 49,XXXXY is a rare sex chromosome abnormality with an incidence of 1 in 85,000 male births.[3] The mechanism of this condition is thought to occur due to maternal nondisjunction during both meiosis I and II.[4] Typical characteristics of boys with this condition include short stature, intellectual disability, and various congenital malformations including radioulnar synostosis, hip dysplasia, genitourinary malformation, cleft palate, inguinal hernia, clubfoot, and cardiac anomalies.[3] Prenatal diagnosis of this condition by ultrasound alone is generally difficult due to limited studies describing the sonographic prenatal findings and the nonspecific nature of such. However, reported prenatal features described in the literature include cystic hygroma, microgenitalia, clubfoot, epignathus, nonimmune hydrops, and hypoplastic right heart syndrome.[5] Confirmatory prenatal diagnosis relies on amniocentesis and fetal chromosomal studies.

A single nucleotide polymorphism (SNP)–based NIPS method has been proposed as an accurate method to screen for sex chromosome aneuploidy (with an average calculated accuracy of 99.78%).[6] In this report, we describe a patient with pentasomy 49,XXXXY with false low-risk results (consistent with a normal male fetus) using a SNP-based NIPS.

Case Report

We present a case of 30-year-old gravida 1 para 0 woman presented to our institution for genetic counseling and amniocentesis at $33^{6/7}$ weeks of gestation, secondary to ultrasound-detected fetal anomalies. She had previously undergone SNP-based NIPS at $13^{6/7}$ weeks through her primary obstetric care provider. Results reported a low risk for trisomy 21, trisomy 18, trisomy 13, monosomy X, and

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triploidy/vanishing twin in a male fetus. Per the patient's report, she elected not to attend an 18- to 20-week anatomy ultrasound; NIPS had screened for fetal sex, and she had had an early bedside ultrasound at her obstetric care provider office to establish her estimated due date.

The patient underwent anatomy survey at $30^{6/7}$ weeks, at which time multiple congenital anomalies were noted, including bilateral clubfeet, clinodactyly of the left fifth digit, micropenis, echogenic bowel, and fetal growth restriction (FGR) (►**Fig. 1**). During the genetic counseling session, she reported a family history of a brother with bilateral clubfeet, abnormal hands, and multiple severe impairments of unknown etiology despite extensive genetic work-up.

The patient consented to late-gestation amniocentesis following the genetic consultation. Fetal karyotype and chromosome microarray revealed pentasomy 49,XXXXY syndrome. Ultimately, the couple decided to terminate the pregnancy at ~36 weeks. Physical exam after delivery revealed micropenis and bilateral clubfeet, consistent with prenatal sonographic finding. Autopsy was declined.

Discussion

We present prenatal diagnosis of fetus with pentasomy 49, XXXXY following a false-positive SNP-based NIPS. This report also highlights third-trimester sonographic features of a fetus with pentasomy 49, XXXXY including bilateral clubfeet, clinodactyly of the left fifth digit, micropenis, echogenic bowel, and FGR. Clubfoot was previously noted in four reports.[5] Micropenis was also previously reported.[5] These findings are ultimately nonspecific and should trigger further investigation, including invasive diagnostic testing with prenatal chromosome microarray, as in this case.

Sex chromosome anomaly screening has previously been reported to be difficult using massively parallel shotgun sequencing NIPS methods; however, SNP-based NIPS has been reported to be highly accurate for detection of these particular aneuploidies.[6] Some experts have also alluded to the use of routine NIPS to screen for sex chromosome anomalies, as maternal serum screening does not provide a risk assessment for these conditions.[7] However, in this case, we report a false "low-risk" NIPS result, which ultimately delayed ultrasound screening and resulted in delay of diagnosis.

Although the company was not validated to diagnose pentasomy cases, it did report "male" as the fetal sex. Due to the suspected maternal origin of the extra X chromosomes in pentasomy 49, XXXXY,[4] we would have expected the SNP profile to at least be suggestive of Klinefelter's syndrome (as both of the maternally derived X chromosome SNP profiles would be represented in the fetoplacental circulating cell-free DNA). If the SNP profiles were indeed reflective of at least Klinefelter's syndrome, we would expect at the very least a "noninformative" or "no-call" result. This false low-risk result could theoretically result from confined placental mosaicism involving aneuploidy rescue in an early

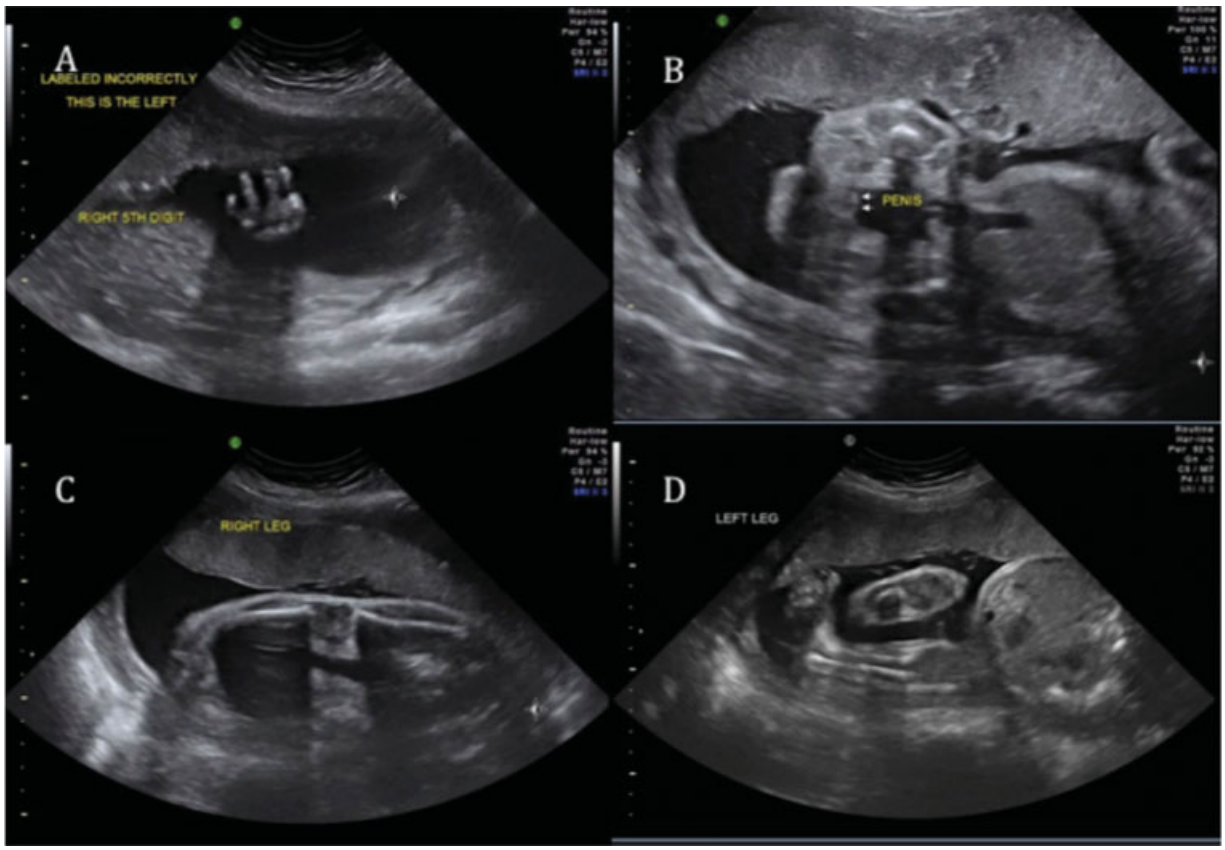

Fig. 1 Sonographic findings of pentasomy 49,XXXXY in our patient. (**A**) Right fifth digit clinodactyly, (**B**) micropenis, and (**C**) and (**D**) bilateral club feet.

trophoblastic progenitor.[6,8] Placental pathology with karyotyping was not performed to explore this theory in this case.

Our report also highlights the importance of thorough pre- and posttest counseling for noninvasive DNA screening. It is a common misconception among patients (and even providers) that NIPS is diagnostic or "near diagnostic"; to mitigate this, pretest counseling points have been suggested to assist clinicians in performing this important task.[9,10] Emphasizing the screening nature of NIPS is paramount prior to undergoing testing, and it should not replace the use of routine ultrasound to evaluate for fetal anomalies. Routine anatomy ultrasound may have resulted in earlier detection in this case.

It is worth noting that some commercial companies who provide NIPS will return "noninformative," "no-call," or "failed" results in the event of an unexpected cell-free DNA profile. In this case, such result could have prompted an anatomy ultrasound or invasive diagnostic testing and thus an earlier diagnosis. Noninformative results have been reported to be associated with an increased risk of aneuploidy; amniocentesis is often recommended by the reference laboratory in these cases. In addition, it is important to mention that ACOG and Society of Maternal-Fetal Medicine have also continued to recommend conventional screening methods for the low-risk population given limited data of accuracy among them.[2]

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