RESEARCH LETTER

AMERICAN JOURNAL OF medical genetics PART A

Elucidating Discrepant Results in a Prenatal Diagnosis of 48,XXY,+18 (Edwards and Klinefelter Syndromes)

Jess F. Peterson,[1,2]* Kendra Mikulec,[3] John W. Grignon Jr.,[2] Behnaz Behmaram,[1,2] Meredith O. Cruz,[3] and Peter van Tuinen[1,2]

[1]Department of Pathology, Medical College of Wisconsin, Milwaukee, Wisconsin

[2]Wisconsin Diagnostic Laboratories, Milwaukee, Wisconsin

[3]Department of Obstetrics and Gynecology, Medical College of Wisconsin, Milwaukee, Wisconsin

Manuscript Received: 17 March 2015; Manuscript Accepted: 26 June 2015

TO THE EDITOR:

Edwards and Klinefelter syndromes (48,XXY,+18) have been rarely reported with only 12 non-mosaic cases described in the literature to date [Chen et al., 2011]. Among documented cases, male fetuses or infants with concomitant trisomy 18 and an extra X chromosome exhibit phenotypic features of each syndrome, including micrognathia, clenched hands, and congenital heart defects that are observed in Edwards syndrome, in addition to cryptorchidism, commonly observed in Klinefelter syndrome [Chen et al., 2011]. Since the introduction of non-invasive prenatal testing (NIPT) into the clinical setting, screening for aneuploidies of chromosomes 13, 18, 21, X, and Y has been substantially expanded [Bianchi et al., 2014; McCullough et al., 2014; Peters et al., 2015; Song et al., 2015]. However, limitations of NIPT include complications of interpretation due to detection of confined placental mosaicism, maternal mosaicism, and presence of low fetal DNA fraction in maternal plasma, all of which can result in false positive, false negative, and indeterminate results [Wang et al., 2013; Mao et al., 2014; Pan et al., 2014]. We report on a prenatal diagnosis of 48,XXY,+18 (Edwards and Klinefelter syndromes) by classical cytogenetic analysis performed on a cultured chorionic villus sampling (CVS). Interestingly, NIPT and fluorescence in situ hybridization (FISH) only revealed trisomy 18, while karyotyping showed trisomy 18 and an extra X chromosome (48,XXY,+18). Unlike previously reported discrepant NIPT results, we observed what appear to be two non-mosaic cell lines by comparing three testing methodologies.

A 38-year-old G2P0010 female presented for genetic counseling at our institution at 12 $^{2}/_{7}$ weeks of gestation due to a positive trisomy 18 result by NIPT. Importantly, aneuploidy screening for chromosomes 13, 16, 21, X, and Y and seven microdeletion regions (1p, 4p, 5p, 8q, 11q, 15q, and 22q) were negative. After receiving genetic counseling, the proband elected to pursue confirmatory CVS testing. Fluorescence in situ hybridization was performed on interphase cells from a direct CVS preparation for chromosomes 13, 18, 21, X, and Y using the AneuVysion Multicolor DNA probe kit (Abbott Laboratories, Abbott Park, IL). Two technologists independently scored 25 cells each for a total of 50 analyzed cells. All 50 cells revealed one signal each for the X and Y chromosomes, and three signals for chromosome 18 (Fig. 1A). No additional X chromosome signals were observed in any of the interphase cells examined. Classical cytogenetic analysis was performed on 20 metaphase cells from two independent CVS cultures. All 20 cells analyzed had an extra X chromosome and trisomy 18 (Fig. 1B). To rule-out the possibility of an X centromere polymorphism that may account for the absence of a second X-centromere signal by interphase FISH, sequential FISH was performed on metaphase cells from the cultured CVS specimen using the same probe set. Two copies of the X chromosome were observed, both with equally strong hybridization of the X chromosome centromere-specific DNA probes (Fig. 1C). The final karyotype was 48,XXY,+18.nuc ish (DXZ1×1,DYZ3×1,D18Z1×3), (RB1,D21S259/D21S341/D21S342)×2.

Following consultation, the couple decided to terminate the pregnancy. Paraffinized sections of chorionic villi and fetal kidney

How to Cite this Article:
Peterson JF, Mikulec K, Grignon Jr. JW, Behmaram B, Cruz MO, van Tuinen P. 2015. Elucidating discrepant results in a prenatal diagnosis of 48,XXY,+18 (Edwards and Klinefelter syndromes).
Am J Med Genet Part A 167A:2855–2857.

Conflicts of interest: The authors have no conflict of interest to declare.

*Correspondence to:
Jess F. Peterson, M.D., Froedtert/MCW Laboratory Building, 9200 W Wisconsin Ave, Milwaukee, WI 53226.
E-mail: jepeterson@mcw.edu

Article first published online in Wiley Online Library (wileyonlinelibrary.com): 31 July 2015
DOI 10.1002/ajmg.a.37250

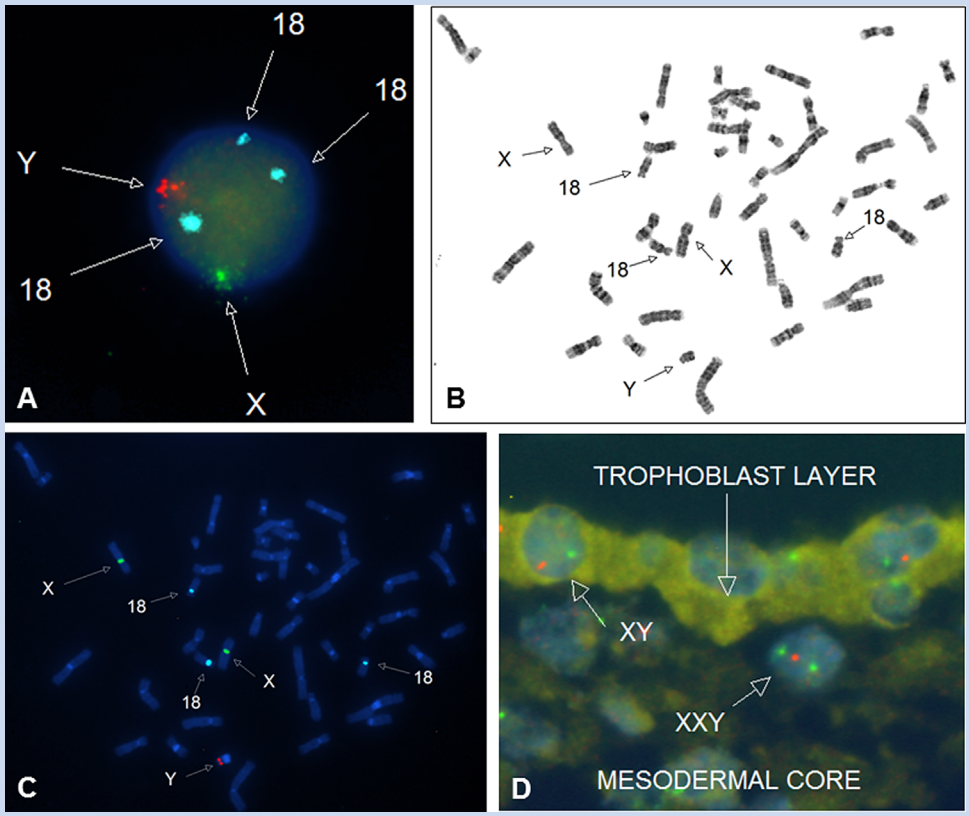

FIG. 1. A: Representative interphase cell from a direct CVS preparation demonstrating centromere-specific probe signals for chromosomes 18, (aqua), X (green), and Y (red). All 50 interphase cells analyzed contained one signal each for chromosomes X and Y, and three signals for chromosome 18. These results are consistent with a male fetus with trisomy 18 (Edwards syndrome). B: Representative metaphase cell from the CVS culture specimen. Arrows point to the sex chromosomes and three copies of chromosomes 18. The karyotype was 48,XXY,+18 (Edwards and Klinefelter syndrome). C: Sequential FISH analysis of the representative metaphase cell (see B) using the same centromere-specific FISH probes used in the direct CVS preparation. All 20 metaphase cells analyzed had two copies of the X chromosome (green), a single Y chromosome (red), and three copies of chromosome 18 (aqua). These results are consistent with a male fetus with an extra X chromosome and trisomy 18. D: FISH analysis of a paraffinized CVS specimen showing the trophoblast layer and mesodermal core. The trophoblast layer is composed of an XY cell line, with one signal each for the X (green) and Y (red) chromosomes. The mesodermal core is composed of an XXY cell line with two signals for the X chromosome (green) and one signal for the Y chromosome (red). Three signals for chromosome 18 were observed in the XY and XXY cell lines (not pictured). (Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/ajmga).

from the products of conception (POC) were subjected to FISH analysis using the same probe sets as previously described. The trophoblast cell layer contained one signal each for the X and Y chromosomes. However, due to truncation artifact, cells were observed with a loss of each signal. Importantly, no cells in the trophoblast layer contained extra X chromosome signals. The mesodermal core and fetal kidney cells contained two signals of the X chromosome and one signal of the Y chromosome. Cells were observed in both tissue types with single signals of the X and Y chromosomes, which most likely represents truncation artifact. Trisomy 18 was confirmed in all tissue types (trophoblast layer, mesodermal core, and fetal kidney cells) by FISH analysis on the paraffinized POC specimen (not pictured).

An understanding of embryology is instructive in interpreting the discrepant results of NIPT and FISH (XY,+18) when compared to the classical cytogenetic studies (XXY,+18). Following conception, the conceptus undergoes successive rounds of mitosis to produce the morula, which eventually cavitates to produce an inner cell mass [Gardner et al., 2012]. The outer layer of what is now referred to as the blastocyst is composed of trophoblast, which surrounds the chorionic villi [Gardner et al., 2012]. The inner cell mass that protrudes into the blastocyst cavity gives rise to two cellular layers, the epiblast and the hypoblast [Gardner et al., 2012]. The epiblast will develop into the embryo, while the hypoblast will develop into the extra-embryonic mesoderm, which includes the mesodermal core of the chorionic villi [Gardner et al., 2012]. Considering that the trophoblast cell layer of chorionic villi contributes the majority of cell-free fetal DNA in NIPT [Faas et al., 2012], and to interphase cells used for FISH analysis on a direct CVS preparation, the original genetic constitution of the conceptus was likely 47,XY,+18. This hypothesis is supported by positive trisomy 18 results by both NIPT and FISH analysis. However, as the inner cell mass of the blastocyst

differentiated into the epiblast and hypoblast cell layers, a non-disjunction event likely occurred which gave rise to a 48,XXY,+18 cell line. This series of events would account for the non-mosaic 48, XXY,+18 karyotype observed by CVS culture. An exceedingly unlikely explanation is a 48,XXY,+18 conceptus with postzygotic loss of the extra X chromosome in all cells that constitute the morula.

Unique to this case is the presence of two cell-lines, one representing the original genetic constitution of the conceptus (47,XY,+18) that appears to be restricted to the trophoblast layer of the chorionic villi and the second cell line observed in the mesodermal core that is thought to have arisen from an additional non-disjunction event resulting in 48,XXY,+18 (Edwards and Klinefelter syndromes) (Fig. 1D). Therefore, all three methodologies utilized in this case correctly identified the abnormal cell line that was present in the respective tissue components, with no evidence of mosaicism within either tissue. This contrasts with previously reported discrepant NIPT results that have been attributed to varying degrees of mosaicism within tissue components [Wang et al., 2013; Mao et al., 2014; Pan et al., 2014]. Our case also highlights the potential discrepancies that can exist between direct FISH analysis performed on trophoblastic cells from a CVS specimen, and metaphase cells obtained from the cultured mesodermal core. In summary, this case illustrates the limitations that are inherent to each testing methodology and reinforces the need to perform confirmatory testing such as classical cytogenetic studies.

REFERENCES

Bianchi DW, Parker RL, Wentworth J, Madankumar R, Saffer C, Das AF, Craig JA, Chudova DI, Devers PL, Jones KW, Oliver K, Rava RP, Sehnert AJ, CARE Study Group. 2014. DNA sequencing versus standard prenatal aneuploidy screening. N Eng J Med 370:799–808.

Chen CP, Chern SR, Chen CY, Wu PC, Chen LF, Pan CW, Wang W. 2011. Double aneuploidy with Edwards-Klinefelter syndromes (48,XXY,+18) of maternal origin: Prenatal diagnosis and molecular cytogenetic characterization in a fetus with arthrogryposis of the left wrist and aplasia of the left thumb. Taiwan J Obstet Gynecol 50:479–484.

Faas BH, de Ligt J, Janssen I, Eggink AJ, Wijnberger LD, van Vugt JM, Vissers L, Geurts van Kessel. 2012. Non-invasive prenatal diagnosis of fetal aneuploidies using massively parallel sequencing-by-ligation and evidence that cell-free fetal DNA in the maternal plasma originates from cytotrophoblastic cells. Expert Opin Biol Ther 12:S19–S26.

Gardner RJ, Sutherland GR, Shaffer LG. 2012. Chromosome abnormalities and genetic counseling. New York: Oxford University Press. p 442.

Mao J, Wang T, Wang BJ, Liu YH, Li H, Zhang J, Cram D, Chen Y. 2014. Confined placental origin of the circulating cell free fetal DNA revealed by a discordant non-invasive prenatal test result in a trisomy 18 pregnancy. Clin Chim Acta 433:190–193.

McCullough RM, Almasri EA, Guan X, Geis JA, Hicks SC, Mazloom AR, Deciu C, Oeth P, Bombard AT, Paxton B, Dharajiya N, Saldivar JS. 2014. Non-invasive prenatal chromosomal aneuploidy testing—clinical experience: 100,000 clinical samples. PLoS ONE 9:e109173.

Pan Q, Sun B, Huang X, Jing X, Liu H, Jiang F, Zhou J, Lin M, Yue H, Hu P, Ning Y. 2014. A prenatal case with discrepant findings between non-invasive prenatal testing and fetal genetic testings. Mol Cytogenet 7:48.

Peters DG, Yatsenko SA, Surti U, Rajkovic A. 2015. Recent advances of genomic testing in perinatal medicine. Semin Perinatol 39:44–54.

Song Y, Huang S, Zhou X, Jiang Y, Qi Q, Bian X, Zhang J, Yan Y, Cram DS, Liu J. 2015. Non-invasive prenatal testing for fetal aneuploidies in the first trimester of pregnancy. Ultrasound Obstet Gynecol 45: 55–60.

Wang Y, Zhu J, Chen Y, Lu S, Chen B, Zhao X, Wu Y, Han X, Ma D, Liu Z, Cram D, Cheng W. 2013. Two cases of placental T21 mosaicism: Challenging the detection limits of non-invasive prenatal testing. Prenat Diagn 33:1207–1210.