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Positive NIPT: An indicator for complex genetic findings

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I. Introduction

The majority of current non-invasive prenatal testing (NIPT) uses next-generation sequence analysis of cell-free DNA (cfDNA) in maternal circulation to identify fetal aneuploidy risk for chromosomes 13, 18, 21, X, and Y[1]. More extensive NIPT screening is available for additional aneuploidies and microdeletions[1], as well as any abnormality in the entire genome (> 7 Mb)[2], but the basic aneuploidy panel can also provide the initial indicator of more complex genetic findings. Here we report three cases in which NIPT positive screening for the basic aneuploidies led to the identification of chromosome abnormalities and/or genetic disorders other than simple aneuploidy.

II. Materials and Methods

- Our laboratory received amniotic fluid specimens from three patients with positive NIPT results as the primary clinical indication for diagnostic testing.

  Case 1: NIPT positive for monosomy X
  - 27 years old, G2 P1
  - Fetal ultrasound indicated male genitalia

  Case 2: NIPT positive for trisomy 18
  - 28 years old, G8 P3
  - Fetal ultrasound showed enlarged cisterna magna, club feet, ventricular septal defect, ventriculomegaly

  Case 3: NIPT positive for monosomy X
  - 29 years old, G1 P0
  - No ultrasound findings reported

- Interphase fluorescence in situ hybridization (FISH) analysis for chromosomes 13, 18, 21, X, and Y, was performed on uncultured amniotic fluid. At least 50 cells were examined for each probe (AneuVysion/Abbott Molecular, Inc.).

- Tissue culture and chromosome analysis was performed by standard cytogenetic methods.

- For cases 1 and 3, SNP chromosome microarray (CMA) analysis was performed using the Affymetrix Cytoscan® HD platform (743,000 SNP probes and 1,953,000 NPCN probes with a median spacing of 0.88 kb). 250ng of total genomic DNA was digested with NspI and then ligated to NspI adaptors, respectively, and amplified using Titanium™ Taq with a GeneAmp® PCR System 9700. PCR products were purified using AMPure beads and quantified using NanoDrop™ 8000. Purified DNA was fragmented, biotin labeled and hybridized to the Affymetrix Cytoscan HD GeneChip. Data was analyzed using Chromosome Analysis Suite. The analysis was based on the GRCh37/hg19 assembly.

- For Case 2, DNA was extracted from cultured amniocytes. Subsequent molecular analysis of the *SNRPN* gene was performed by methylation-specific PCR and gel electrophoresis.

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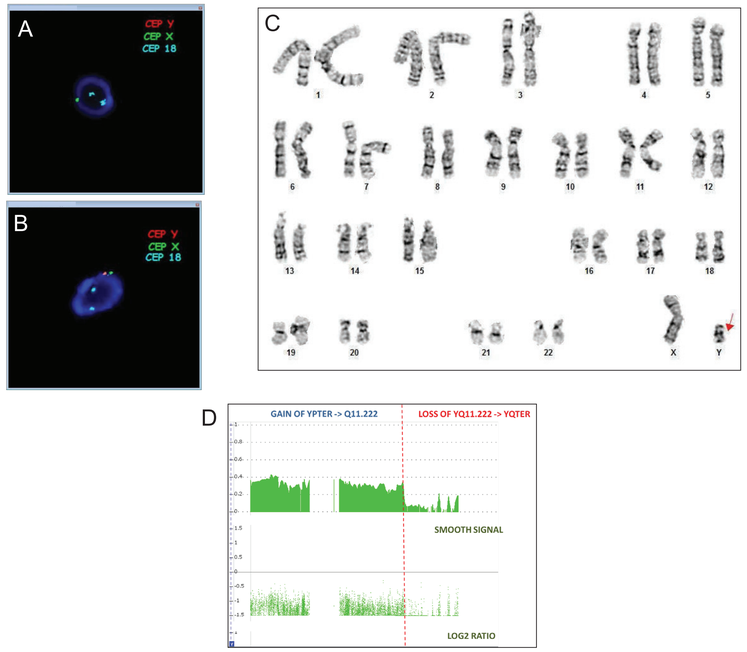
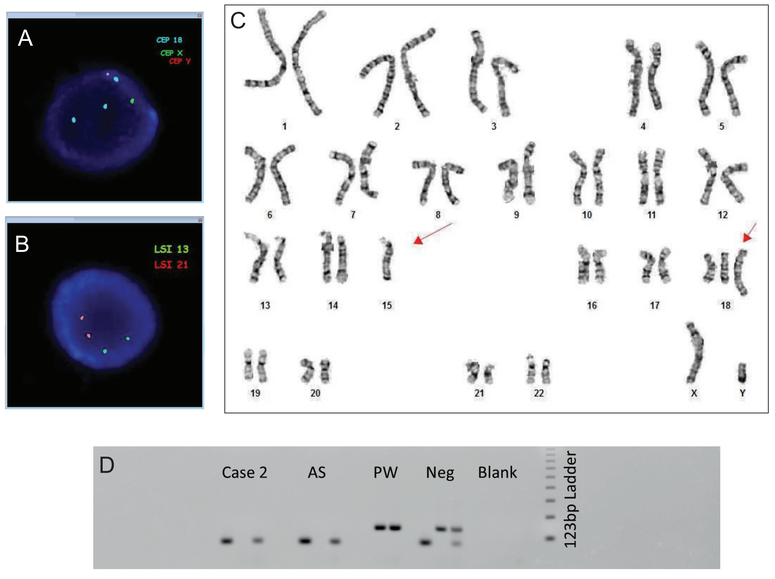
III. Results

Case 1: NIPT positive for monosomy X



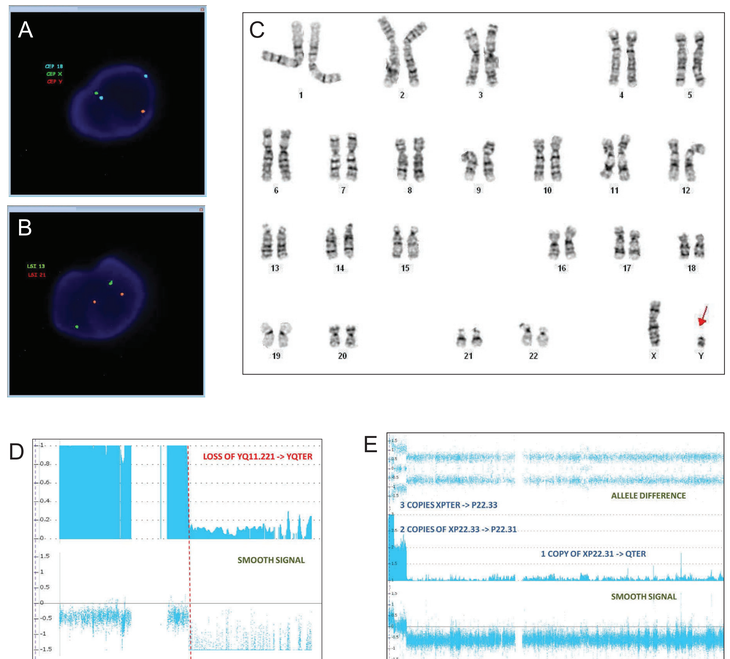
- FISH showing A) monosomy X and B) male pattern. Result is uninformative.
- C) Karyogram with isodicentric Y. Karyotype is 45,X[11]/46,X,idic(Y)(q11.2)[4].
- D) CMA showing a mosaic 22.05 MB gain of Ypter->q11.222 and a 37.33 MB terminal deletion of Yq11.221->qter.

Case 2: NIPT positive for trisomy 18



- FISH showing A) trisomy 18 in a male and B) disomy 13 and 21.
- C) Karyogram with monosomy 15 and derivative 18. Karyotype is 46,XY,-15,+der(18)t(15;18)(q14;q21.1).
- D) Methylation analysis gel showing Case 2 is positive for Angelman syndrome.

Case 3: NIPT positive for monosomy X



- FISH showing A) male pattern, disomy 18 and B) disomy 13 and 21.
- C) Karyogram with derivative Y. Karyotype is 46,X,del(Y)(q11.22).
- CMA showing D) a 43.2 MB terminal deletion of Yq11.221->qter and E) a 2.69 MB terminal gain of Xpter->Xp22.33 and a 5.80 MB interstitial duplication of Xp22.33->p22.31.

IV. Conclusion

These cases illustrate situations where positive NIPT screening for the common aneuploidies ultimately led to the diagnosis of complex genetic conditions:

- An isodicentric Y chromosome
- A derivative chromosome causing partial trisomy for chromosome 18 and partial deletion of 15q resulting in Angelman syndrome
- A derivative Y chromosome resulting from a translocation involving the X and Y chromosomes

Although such complex genetic findings might not be anticipated following positive NIPT screening for common aneuploidies, we demonstrate that it may lead to the detection of unexpected genetic abnormalities that require diagnostic follow-up and genetic counseling.

V. References

1. Allyse M, Minear MA, Berson E, et al. Non-invasive prenatal testing: a review of international implementation and challenges. International Journal of Women's Health. 2015;7:113.

2. Lefkowitz RB, Tynan JA, Liu T, et al. Clinical validation of a noninvasive prenatal test for genomewide detection of fetal copy number variants. Am J Obstet Gynecol 2016;215:227.