P2-17

Segmental UPD to the Rescue: A biological mechanism underlying discordant NIPT results

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I. Introduction

Trisomy/monosomy rescue, which carries risk for uniparental disomy (UPD), is a familiar biological mechanism in the context of aneuploidy. With the increasing capabilities of non-invasive prenatal testing (NIPT), the detection of subchromosomal events, beyond full trisomies, is now possible. Similar to full aneuploidies, these subchromosomal events may undergo post-zygotic correction, resulting in segmental UPD. Complex rescue mechanisms may help explain discrepancies between NIPT results and diagnostic testing. Four cases with a subchromosomal deletion detected by NIPT in which the diagnostic testing revealed UPD for the region of interest are described.

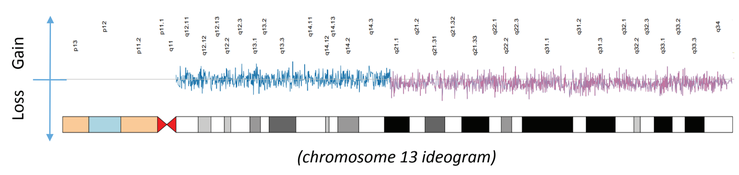
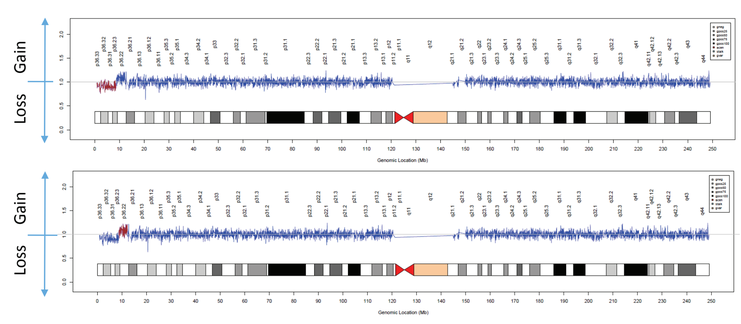
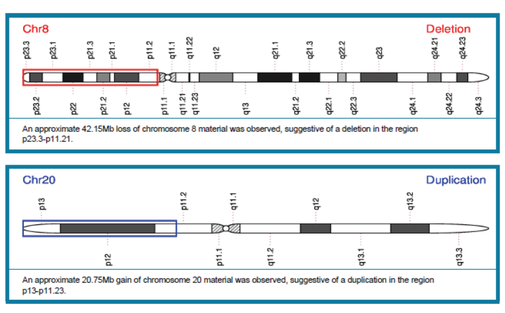
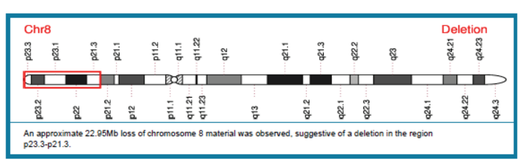
II. Methods

Maternal blood samples submitted to Sequenom Laboratories® for MaterniT® 21 PLUS or MaterniT® GENOME testing were subjected to DNA extraction, library preparation, and whole genome massively parallel sequencing as described by Jensen et al and Lefkowitz et al.[1,2] Postnatal placental testing was facilitated by the ordering provider and completed by an external laboratory (**Case 3**) or by Integrated Genetics (**Cases 1 & 2**). Follow up information and pregnancy outcomes were elicited from the clinicians as part of routine, ongoing laboratory protocol for positive cases.

III. Results

In the four cases below, NIPT screening reported a subchromosomal deletion in isolation (**Case 1 & 4**) or as part of a complex finding (**Case 2 & 3**). Confirmatory microarray testing (amniocentesis or postnatal) revealed segmental UPD for the precise region reported as deleted by NIPT. As NIPT is an evaluation of placental trophoblast cfDNA, postnatal placental testing was completed in three cases (**Case 1 - 3**). In all cases, placental testing was consistent with the reported NIPT findings.

Table 1. Case series of segmental UPD and associated NIPT deletion

Case	NIPT Result	Diagnostic Testing	Placental Testing	Clinical Details
1	13q21.1-q34 deletion  *(chromosome 13 ideogram)*	**Amnio SNParray:** 57.4Mb LOH at 13q21.1-q34	**Postnatal Placental Array:** 57.5Mb mosaic (37%) terminal 13q deletion (consistent with NIPT) & allelic mosaicism of 13q21.1-qter, consistent with segmental UPD for this region	Fetal anatomy normal throughout pregnancy. Mildly elevated AF-AFP of 2.49 MoM with a normal AChE. Microsatellite studies: maternal segmental uniparental isodisomy for 13q21.1-qter and biparental inheritance for 13pter-q21.1. Negative custom sequencing panel for evaluation of autosomal recessive disease genes in LOH region. Baby doing well and discharged 4 days after delivery.
2	1p36.33-p36.23 deletion & 1p36.23-p36.22 duplication  *(chromosome 1 ideograms)*	**Amnio Karyotype:** 46,XX **Amnio SNParray:** 16.3Mb LOH 1p36.33-p36.13 (*female with probable 1p segmental UPD secondary to somatic correction of germ line imbalance*)	**Postnatal Placental Array:** 8.72Mb 1p36.33-p36.23 deletion & 3.44Mb 1p36.23-p36.22 duplication (consistent with NIPT)	2VC, reportedly unremarkable neonatal period
3	20p13-p11.23 duplication & 8p23.3-p11.21 deletion  *(MaterniT® GENOME report: ideogram of events)*	**Postnatal Karyotype:** 46,XX,r(19) **Postnatal Array:** 41.5Mb LOH at 8p23.3p11.21. Mosaic gain of almost entire chromosome 19 due to presence of interstitial duplication of r(19) in 9% of cells.	**Postnatal Placental Array:** 41.5Mb deletion at 8p23.3p11.21, mosaic loss of 2.8Mb at 8p11.21 and mosaic gain of 20.8Mb at 20p13p11.23. Normal chromosome 19 (consistent with NIPT).	Small for age (antenatal & postnatal), tetralogy of fallot, supernumerary nipple, digit contracture
4	8p23.3-p21.3 deletion  *(MaterniT® GENOME report: ideogram of chromosome 8)*	**Amnio FISH and Karyotype:** 46,XY **Amnio SNParray:** 19.0Mb LOH at 8p23.3-p21.3	All additional testing declined.	Normal serum screening; NT 1.3mm. Early anatomy scan was unremarkable. Considered cord blood to assess for potential occult mosaicism. Healthy baby delivered full term.

IV. Discussion

In all four cases, a deletion event detected by NIPT was seen as segmental UPD upon diagnostic testing (amniocentesis or postnatal). All placental testing completed is consistent with the reported NIPT findings, including the reported subchromosomal deletions. Collectively this is suggestive of corrective post-zygotic recombination subsequent to a deletion, ultimately resulting in segmental UPD and fetoplacental discordance. Complex somatic mechanisms, like telomere capture stabilization or homolog-templated correction[3] (**Image 1**), in which the missing region is replicated from the homologous chromosome, may help explain the discrepancies between NIPT ('pre-rescue') and diagnostic testing ('post-rescue').

Image 1: Homolog-Templated Deletion Correction

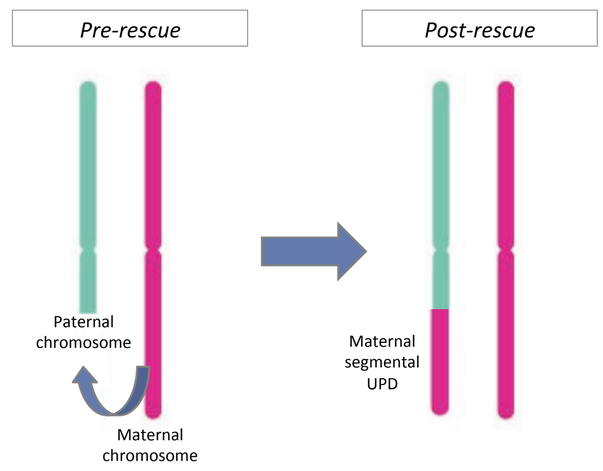


Image inspired by O'Keefe, C., et al. 2010

V. Conclusion

Rescue events that carry risk for UPD are important to consider when faced with discordant NIPT results. This is particularly poignant for imprinted chromosomes and autosomal recessive disease genes. These four cases highlight the importance of careful test selection in confirmatory prenatal diagnosis of positive NIPT results, as many of these findings eluded standard karyotype analysis alone. Microarray utilizing SNP technology may be particularly useful to determine both copy number and potential UPD issues alike, which could have additional clinical implications directly impacting pregnancy management.

Key points:

- Post-zygotic rescue events can result in segmental UPD and fetoplacental discordance, which may help explain discrepancies between cfDNA findings and diagnostic testing results.
- Segmental UPD may have clinical implications for imprinted chromosomes and autosomal recessive disease genes.
- Deliberate test selection following abnormal cfDNA screening is important. SNP microarray may be particularly useful to determine both copy number and potential UPD issues.

VI. References

1. Jensen TJ, Zwiefelhofer T, Tim RC, et al. High-throughput massively parallel sequencing for fetal aneuploidy detection from maternal plasma. *PLoS One*. 2013;8(3):e57381. doi: 10.1371/journal.pone.0057381. Epub 2013 Mar 6.

2. Lefkowitz RB, Tynan J, Liu T, et al. Clinical validation of a non-invasive prenatal test for genome-wide detection of fetal copy number variants. *Am J Obstet Gynecol*. doi: http://dx.doi.org/10.1016/j.ajog.2016.02.030.

3. O'Keefe C, et al. Copy neutral loss of heterozygosity: a novel chromosomal lesion in myeloid malignancies. *Blood*. 2010. doi: https://doi.org/10.1182/blood-2009-10-201848.

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