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Samantha Caldwell

Katelynn Sagaser

Zoe Nelson

Jennifer Frey
Henry Ford Health, jfrey3@hfhs.org

Jenna Wardrop

See next page for additional authors

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Authors

Samantha Caldwell, Katelynn Sagaser, Zoe Nelson, Jennifer Frey, Jenna Wardrop, Theresa Boomer, Ron McCullough, and Stuart Schwartz

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CLINICAL REPORT

Deletion rescue resulting in segmental homozygosity: A mechanism underlying discordant NIPT results

Samantha Caldwell[1] | Katelynn Sagaser[2] | Zoe Nelson[3] | Jennifer Frey[4] | Jenna Wardrop[1] | Theresa Boomer[1] | Ron McCullough[1] | Stuart Schwartz[5]

[1]Sequenom Laboratories, San Diego, California

[2]Division of Maternal Fetal Medicine, Department of Gynecology and Obstetrics, Johns Hopkins University School of Medicine, Baltimore, Maryland

[3]Mount Sinai West Medical Center, New York, New York

[4]Division of Medical Genetics, Henry Ford Health System, Detroit, Michigan

[5]Cytogenetics Laboratory, Laboratory Corporation of America® Holdings, Research Triangle Park, North Carolina

Correspondence
Samantha Caldwell, Sequenom Center for Molecular Medicine, 3595 Johns Hopkins Ct, San Diego, CA, 92121.
Email: samantha.caldwell@integratedgenetics.com

Abstract

With the increasing capabilities of non-invasive prenatal testing (NIPT), detection of sub-chromosomal deletions and duplications are possible. This case series of deletion rescues resulting in segmental homozygosity helps provide a biological explanation for NIPT discrepancies and adds to the dearth of existing literature surrounding segmental UPD cases and their underlying mechanisms. In the three cases presented here, NIPT reported a sub-chromosomal deletion (in isolation or as part of a complex finding). Diagnostic testing, however, revealed segmental homozygosity or UPD for the region reported deleted on NIPT. Postnatal placental testing was pursued in two cases and confirmed the NIPT findings. This discordance between the screening and diagnostic testing is suggestive of a corrective post-zygotic event, such as telomere capture and/or deletion rescue, ultimately resulting in segmental homozygosity and fetoplacental mosaicism. Imprinted chromosomes and autosomal recessive disease genes make homozygosity an important clinical consideration. Amniocentesis with SNP microarray is particularly useful in determining both copy number and UPD issues alike.

KEYWORDS

deletion rescue, fetoplacental mosaicism, NIPT, segmental UPD, telomere capture

1 | INTRODUCTION

Non-invasive prenatal testing (NIPT) is an aneuploidy prenatal screen that analyzes cell free DNA (cfDNA) fragments in maternal plasma, which are derived from the placental trophoblast. For the purposes of NIPT screening, the placenta serves as a proxy for fetal status. The majority of NIPT analysis limits screening to common aneuploidies (21, 18, 13, X & Y). Yet, with increasing capabilities, the detection of deletions and duplications via NIPT is growing. This case series focuses on ≥7 Mb sub-chromosomal deletions detected with genome wide massively parallel sequencing (MPSS) NIPT screening. All detected deletions presented here were >19 Mb (mean = 50 Mb). Despite high sensitivity and specificity, NIPT is limited to genetic information ascertained from placental cfDNA fragments, specifically the placental trophoblast or outermost layer of the placenta. While the cytotrophoblast accurately reflects fetal status in the majority of cases, discrepancies can and do occur. As confirmatory testing is often limited to the fetus, placental investigations are the only way to prove the NIPT finding when discrepant from the fetus. Rescue events leading to fetoplacental mosaicism is one such biological phenomenon underlying discordant NIPT results (Grati, Malvestiti, & Ferreira, 2013).

Trisomy or monosomy rescue can be a source of false positive and false negative NIPT results due to chromosomal differences between the fetus and placenta. This is a well-established biologic explanation for fetoplacental discordance and confined placental mosaicism (CPM) of an aneuploid cell line (Conlin, Thiel, Bonnemann et al., 2010; Grati et al., 2013). In a trisomy rescue event one of the three chromosome copies is lost, leaving the typical diploid state. One third of the time, the two remaining copies will be from the same

parent resulting in uniparental disomy (UPD) for that chromosome. The embryological time point of a trisomy rescue will inform the distribution of aneuploid/euploid cells lines in both the fetus and placenta. UPD may pose clinical consequence if it involves an imprinted chromosome or contains a homozygous mutated recessive disease gene. Trisomy/monosomy rescue, however, is not the only mechanism in which UPD may arise.

Similar to full aneuploidies, we propose a sub-chromosomal deletion may undergo a post-zygotic correction subsequently resulting in segmental UPD for that chromosome. Cases of segmental UPD subsequent to deletions remain rarely reported in the literature (Johnson et al., 2014; Knijnenburg et al., 2017; Kotzot, 2008; van Opstral, van Veen, Joosten, et al., 2019). However, these will become more apparent with NIPT screening and subsequent diagnostic follow-up. Segmental UPD events are relatively rare and the underlying mechanisms are inherently complex and not yet well defined (Kotzot, 2008). Complex rescue mechanisms may help explain discrepancies between NIPT and diagnostic testing results and inform additional testing recommendations. Here, we describe three cases with a large sub-chromosomal deletion (>19 Mb) detected by NIPT. Fetal diagnostic testing revealed homozygosity associated with segmental UPD for the region of interest while placental testing, when completed, confirmed the deletion as reported by NIPT.

1.1 | Cases

In the following three cases, NIPT reported a sub-chromosomal deletion in isolation (Cases 1 and 2) or as part of a complex finding (Case 3). Confirmatory diagnostic microarray testing (amniocentesis or postnatal) revealed segmental UPD for the region reported as deleted by NIPT. Microarray analysis includes reporting of large regions of homozygosity (ROH) regardless of the chromosome due to autosomal recessive risk. The cases presented here all involved chromosomal segments which met microarray ROH reporting criteria. As NIPT is an evaluation of placental trophoblast cfDNA, postnatal placental testing was completed for Cases 1 and 3. In both cases, placental testing confirmed the sub-chromosomal deletion NIPT finding.

1.2 | Patient 1

A 31 year old patient (G7P1) elected a subsequent NIPT screening at 16 weeks gestation after receiving an uninformative aneuploidy NIPT screening result from a different laboratory. Her first pregnancy resulted in a spontaneous abortion (SAB) at 8 weeks gestation. The remaining six pregnancies included a naturally conceived blighted ovum, three IVF-conceived SABs (all between 6 and 8 weeks), and one healthy daughter conceived through IVF. The current pregnancy was conceived via timed intercourse. This patient's medical history is significant for PCOS diagnosed in adolescence.

The initial uninformative NIPT from another laboratory suggested the clinician consider an abnormality in a chromosome besides Chromosomes 21, 18, and 13 or to consider maternal malignancy in the differential diagnoses. Due to a family history of breast cancer, the patient was referred to cancer genetics. Concurrent to repeated NIPT screening, the patient also pursued SNP-chromosomal microarray analysis by amniocentesis, and peripheral blood karyotype (due to personal history of recurrent miscarriage).

Data from the second NIPT suggested a terminal 13q21.1-q34 deletion (Figure 1 and Figure S1). NIPT Z scores for Chromosome 13 were notably depressed due to an apparent segmental deletion (>50 Mb) on Chromosome 13 at 13q21.1-q34. Amniocentesis SNP array revealed normal copy number but a terminal 57.4 Mb stretch of homozygosity at 13q21.1-q34. Maternal karyotype was normal. Microsatellite studies confirmed maternal segmental uniparental isodisomy for 13q21.1-qter and biparental inheritance for the remainder of Chromosome 13. A custom sequencing panel of autosomal recessive disease genes within the 13q21.1-q34 region was negative. Ultrasound was unremarkable throughout the pregnancy. The patient spontaneously delivered a healthy male at 38 weeks who was discharged home at day four of life and was reportedly doing well at 6 months of age.

Placental testing was completed after delivery. Five placental biopsies (one from each quadrant and center) were pooled for analysis. Direct placental SNP array revealed a 57.5 Mb mosaic (37%) terminal 13q deletion (same deletion observed on NIPT) & allelic mosaicism of 13q21.1-qter, consistent with segmental UPD for this region.

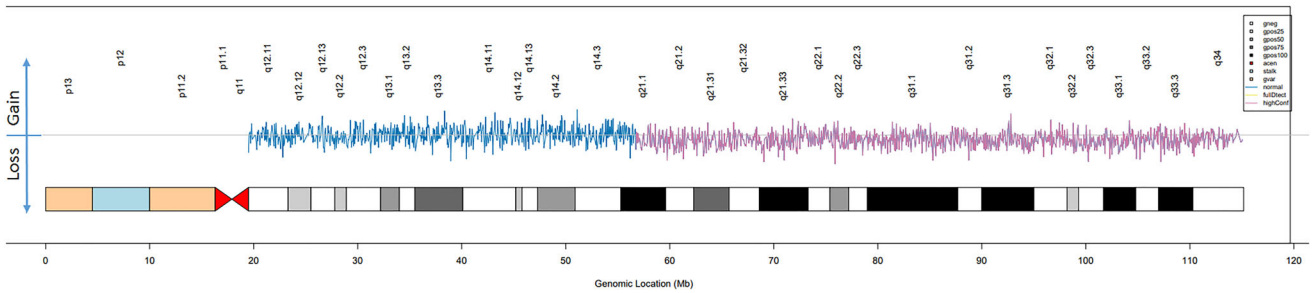

FIGURE 1 NIPT Chromosome 13 ideogram. This ideogram represents the Chromosome 13 NIPT data for Case 1. The pink segment is depressed and denotes the deletion event starting at band 13q21.1, while the blue sequence data is copy number neutral [Color figure can be viewed at wileyonlinelibrary.com]

1.3 | Patient 2

A 41 year old patient (G2P0) pursued NIPT screening at 12 weeks gestation due to advanced maternal age. This pregnancy was naturally conceived and first trimester serum screening was low risk. Her NIPT result was positive for a >20 Mb terminal deletion at 8p21.3 (Figure S2). Subsequent to this positive NIPT result, the patient pursued amniocentesis with FISH (8p and 8q telomere FISH probes), karyotype and SNP array. Amniocentesis FISH and karyotype were normal, as was the early anatomy ultrasound. SNP array by amniocentesis revealed a terminal 19.0 Mb region of homozygosity at 8p21.3-pter, consistent with the region reported deleted by NIPT. Cord blood was considered to assess for occult mosaicism, but ultimately all additional testing was declined. A healthy baby was delivered by C-section due to breech position at full term.

1.4 | Patient 3

A 22 year old patient (G2P1) was drawn for NIPT at 31 weeks gestation due to ultrasound abnormalities including IUGR (<2nd percentile) and cardiac malformations. This was a naturally conceived pregnancy and prenatal diagnostic testing had been declined. Her NIPT screening was positive for an approximate 42.15 Mb 8p11.21-pter deletion and an approximate 20.75 Mb 20p11.23-pter duplication, the latter of which was suggestive of mosaicism (Figure 2). Due to advanced gestational age and increased risk for pre-term labor, prenatal diagnosis was declined and instead confirmatory testing was completed at birth.

Postnatal karyotype revealed 46,XX,r(19), consistent with non-mosaic ring 19 and unremarkable Chromosomes 8 and 20. Subsequent postnatal SNP array showed homozygosity for most of 8p (41.5 Mb at 8p11.21-pter) and a low mosaic gain of the majority of Chromosome 19. Chromosome 8 distal telomere ends showed normal representation, as did Chromosome 20. Postnatal FISH helped elucidate the mosaic Chromosome 19 gain seen an array. The FISH studies showed 90% of cells with 46,XX,r(19). In the remaining 10% of cells, the ring 19 was actually composed of double Chromosome 19 material, resulting in a single larger ring 19. While all cells had one normal Chromosome 19 and one ring 19, in 10% of the cells the ring 19 was composed of double Chromosome 19 material. No material associated with the ring appeared to be deleted. Tetralogy of Fallot (TOF), supernumerary nipple, and a single digit contracture were observed at birth.

After delivery four placental sections were taken and a microarray was performed on tissue established from the third section (central full thickness section) which was consistent with the originally reported NIPT findings revealing an 8p deletion and mosaic 20p duplication with normal Chromosome 19. Neither the placental array nor the NIPT detected the Chromosome 19 abnormality. This could be due to the inability of either test to detect very low level placental mosaicism. Alternatively, it is possible the Chromosome 19 abnormality was not present in placental tissue or was not present in the specific biopsy taken, as site-specific patterns of placental mosaicism are well-documented (Henderson, Shaw, Barret, et al., 1996).

2 | DISCUSSION

In all three cases, a deletion event detected by NIPT was observed as a region of homozygosity upon diagnostic testing (amniocentesis or postnatal). Placental testing was completed in Cases 1 and 3 and was consistent with the NIPT findings, including the reported segmental deletions, confirming the NIPT findings were true results and not technical artifact. Collectively, this suggests the occurrence of corrective post-zygotic events subsequent to a deletion, ultimately resulting in segmental UPD and feto-placental discordance. This novel phenomenon leading to mitotic segmental UPD along with mechanisms like telomere capture stabilization (Meltzer, Guan, & Trent, 1993) may

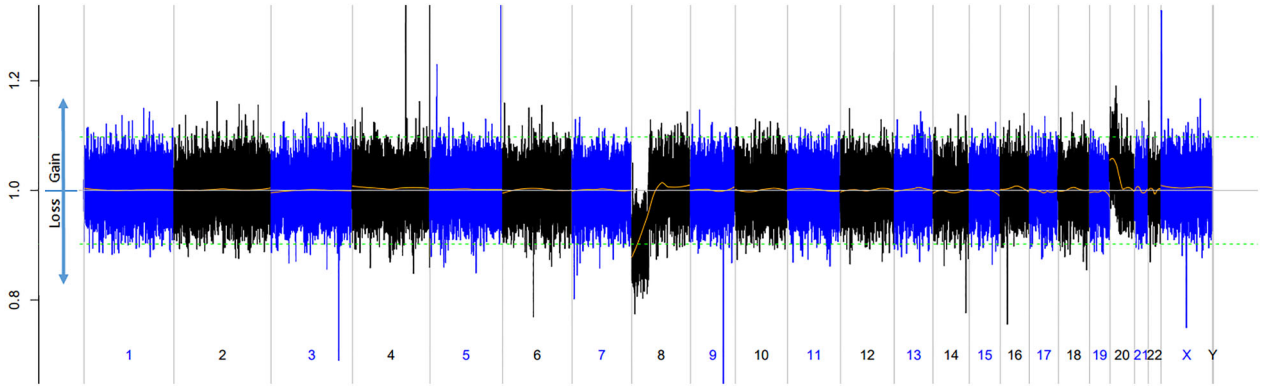

FIGURE 2 Genome-wide NIPT sequencing profile showing segmental underrepresentation of Chromosome 8 and mosaic segmental overrepresentation of Chromosome 20. This is a genome-wide profile view of the NIPT data for Case 3. The sequence data (orange line) is normalized to the 1.0 line denoting disomy for the chromosome. The dashed green lines above and below the 1.0 line are set by the sample's fetal fraction. The depression for terminal 8p reaches the below dashed green line indicating the underrepresentation of this segment is present in the full fetal fraction. The elevation on chromosome 20p, however, does not reach the above dashed green line, indicating the duplication event is not observed in the full fetal fraction and suggests possible mosaicism for that finding [Color figure can be viewed at wileyonlinelibrary.com]

help explain the discrepancies between NIPT (sampling a "pre-rescue" source) and diagnostic testing (sampling a "post-rescue" source).

The deletion event seen by both NIPT and placental testing coupled with the segmental homozygosity on amniocentesis suggest that a deletion existed within the placental trophoblast but was subsequently corrected and stabilized by copying the missing region from the opposite parental copy (Figure 3). In Case 1, it is inferred a break occurred in the paternal Chromosome 13 resulting in the 13q deletion. It is proposed this was subsequently stabilized by copying and acquiring the homologous maternal Chromosome 13 sequence. This is supported by the microsatellite studies and trio microarray analysis, which confirmed maternal segmental uniparental isodisomy for 13q21.1-qter and biparental inheritance for the remainder of the chromosome. Microsatellite testing was not completed in Cases 2 and 3. Segmental UPD of 13q subsequent to a deletion was also observed in Van Opstal et al., where NIPT reported a terminal 13q deletion. A mosaic terminal 13q deletion was found in placental cytotrophoblast (same tissue origin as NIPT cfDNA) but maternal uniparental isodisomy for that segment was found in placental mesenchyme and the fetus, suggesting telomere capture from the normal homolog before trophectoderm and inner cell mass differentiation.

Johnson et al. presented a similar case in which a recurrent familial 11q23-qter Jacobsen deletion of maternal origin was observed in two affected brothers. The mother, however, was homozygous for all loci in the deleted region on lymphocytes and mosaic for the 11q terminal deletion on fibroblasts (Johnson et al., 2014). This suggests maternal germline mosaicism for the 11q deletion and development of mitotic UPD, which restored disomy from a chromosomal deletion. This family is also the first report of what was dubbed a "'deletion rescue' by presumed UPD" (Johnson et al., 2014). One of the affected brothers showed mosaicism for the 11qter deletion (~90%) and paternal UPD (~10%) for the deleted segment. While no fetal mosaicism for the deletion was observed in the cases presented in this case series, occult mosaicism cannot be dismissed. Distribution of the deletion vs. segmental UPD and any deletion-related phenotype would be dependent on when in development the "deletion rescue" occurred.

While Case 3 in this series is more complex overall, it is likely the 8p deletion underwent a similar "deletion rescue" in fetal tissues but went a different route in the placenta. If the 8p deletion is presumed to be the original genetic insult, perhaps the placental and fetal tissues each took different means of attempted rescue/correction. Telomere capture is a well-documented rescue mechanism in which a terminal chromosome segment is acquired to stabilize an open deletion (Conlin et al., 2010; Kostiner, Nguyen, Cox, & Cotter, 2002; Meltzer et al., 1993; Yu & Graf, 2010). It's proposed the placental tissue acquired its sequence from another chromosome (in this case 20p) in effort to stabilize the "open" terminal 8p deletion. This would explain the mosaic 20p gain observed on both NIPT and placental biopsy. However, karyotype was never completed on placental tissues, so fusion of the 20p material to the deleted 8p arm cannot be definitively known. Alternatively, in fetal tissue the 8p segmental homozygosity suggests that the missing 8p region was repaired by the opposite parent's copy, similar to Cases 1 and 2. Perhaps acquiring sequence from the opposite homolog vs. another chromosome was driven by fetal developmental selection, as homozygosity is presumed to be better tolerated than a partial trisomy during cell division (Robinson, 2000).

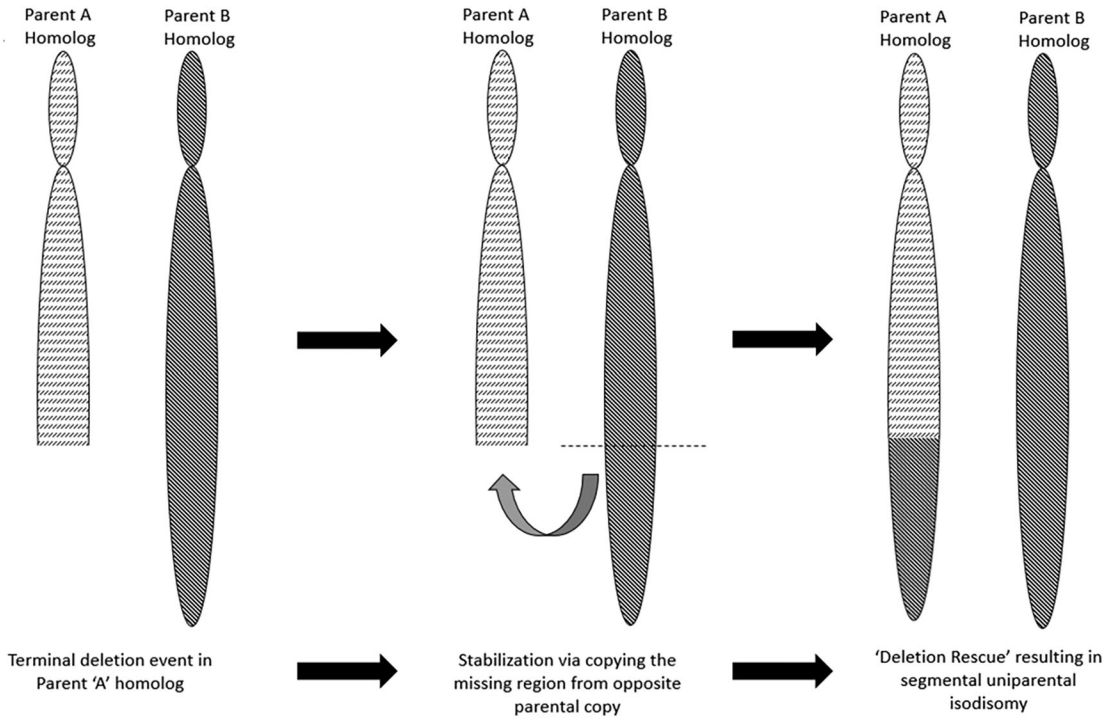

FIGURE 3 "Deletion rescue" mechanism resulting in segmental UPD illustrates the stabilization of a terminal deletion via copying the opposite parental homolog

Rescue events that carry risk for UPD are important to consider when faced with discordant NIPT and diagnostic testing results. This is particularly poignant for imprinted chromosomes, autosomal recessive disease genes, and the risk for occult mosaicism in fetal tissue. Counseling challenges are inherent when faced with discordant results or results of uncertain clinical significance, underscoring the need for genetic counseling. These three cases highlight the importance of amniocentesis and careful test selection following positive NIPT results, as many of these findings eluded standard karyotype analysis alone. Microarray utilizing SNP technology is particularly useful to determine both copy number and UPD issues alike, which could have additional clinical implications directly impacting pregnancy management.

While segmental UPD is collectively rare, case reports are increasing and early post-zygotic events are likely more complex than previously thought (Kotzot, 2008). By studying these different tissue types (placental/fetal) and utilizing different methodologies (NIPT/SNP array/karyotype), we are able to capture a chronological glimpse into fetoplacental biology and the accompanying cytogenetic cascade. As most segmental UPD detection seems to occur by chance (Kotzot, 2008), it is possible there are other patients with mosaic deletion and/or segmental UPD. We hope this case series adds to the understanding and presumed occurrence of segmental UPD via deletion rescue.

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CONFLICT OF INTEREST

Authors Caldwell, Boomer, Wardrop and McCullough are or were full time employees of Sequenom Laboratories when this study was conducted. Stuart Schwartz is an employee of Laboratory Corporation of America.

AUTHOR CONTRIBUTIONS

Conceptualization: Samantha Caldwell, Katelynn Sagaser, Theresa Boomer, Ron McCullough. Data curation and analysis: Samantha Caldwell, Katelynn Sagaser, Zoe Nelson, Jennifer Frey, Stuart Schwartz. Supervision: Jenna Wardrop, Stuart Schwartz, Ron McCullough. Writing—original: Samantha Caldwell, Stuart Schwartz. Writing—review and editing: Samantha Caldwell, Katelynn Sagaser, Zoe Nelson, Jennifer Frey, Jenna Wardrop, Theresa Boomer, Ron McCullough, Stuart Schwartz.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Samantha Caldwell https://orcid.org/0000-0001-9210-4752

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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