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Beyond the basics: A case series from genome-wide non-invasive prenatal testing (NIPT)

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I. Introduction

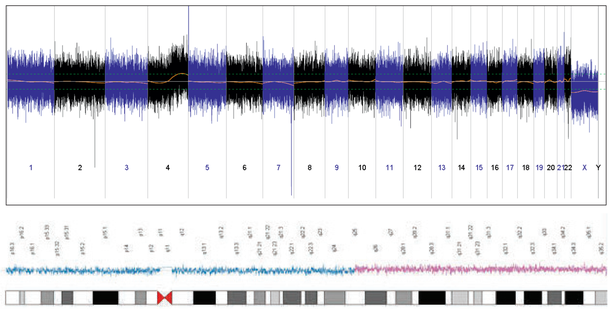
Since the introduction of the first commercially available cell-free DNA screening for Down syndrome in 2011, the content available to patients has expanded to include trisomy 18, trisomy 13, fetal sex and sex chromosome aneuploidies, and select microdeletion syndromes. Most recently, this technology has expanded to allow for genome-wide screening for any aneuploidy and sub-chromosomal events 7 Mb and larger. Genome-wide cfDNA analysis has allowed for more clinically relevant information through screening, but occasionally those results can have additional important implications for the pregnant woman, her partner, or for the fetus. This case series examines some of the complex positive results and important points raised by these cases.

II. Methods

Maternal blood samples submitted to Sequenom Laboratories® for MaterniT® GENOME testing were subjected to DNA extraction, library preparation, and genome-wide massively parallel sequencing as described by Jensen et al.[1] Sequencing data were analyzed using a novel algorithm to detect trisomies and sub-chromosomal, genome wide events 7Mb and larger, as described by Lefkowitz et al.[2] Follow-up information and pregnancy outcomes were elicited from the clinicians as part of routine, ongoing laboratory protocol for positive cases.

III. Cases

Case 1: A primigravida woman in her mid-twenties was referred to maternal fetal medicine at 21+3 weeks due to anomalies identified on a routine anatomy scan with her obstetrician. Her ultrasound at maternal fetal medicine showed left renal agenesis, micrognathia, hypoplastic cerebellum, and generous cerebral ventricles. After declining amniocentesis, MaterniT® GENOME was ordered by a genetic counselor which showed an approximately 80 Mb duplication of 4q25q35.2. Follow-up ultrasound at 26+2 weeks showed growth restriction. A fetal echocardiogram was also performed at this time due to concern for a fetal heart defect, but the cardiac anatomy was determined to be normal. After counseling regarding the cfDNA results and the ultrasound findings, the patient wished to now proceed with amniocentesis. Karyotype was 46,X,der(Y)t(Y;4)(p11.32;q25), indicating that the duplicated piece of chromosome 4 was on the terminal end of the Y chromosome with the Y chromosome apparently intact. Microarray results confirmed the 80.4 Mb duplication of 4q25q35.2. No loss of any Y material was noted on microarray. Paternal chromosomes were ordered and were normal. Non-paternity was denied. Unfortunately, the pregnancy ended in an IUFD at the beginning of the third trimester.



Chromosome 4
Figure 1: MaterniT® GENOME results suggested an approximately 80 Mb gain of chromosome 4.

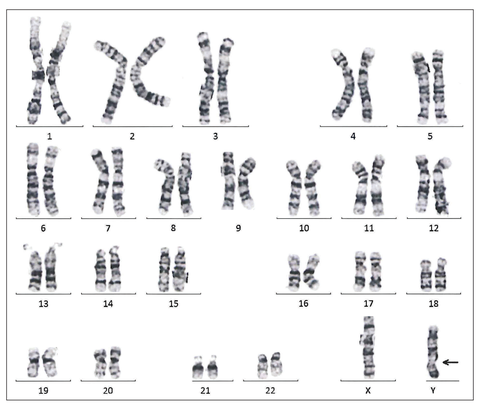
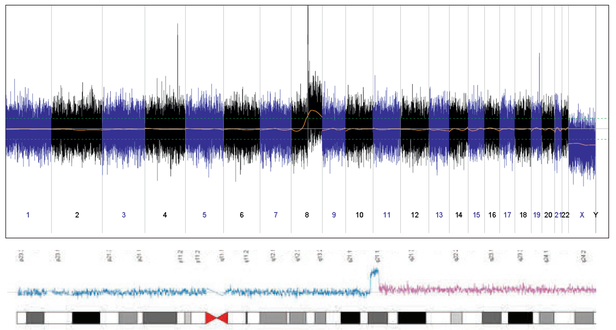


Figure 2: Fetal karyotype at 450-500 band resolution shows an unbalanced translocation creating a derivative Y chromosome with the duplicated portion of chromosome 4.

Case 2: A G2P1 woman in her early 20's was referred to maternal fetal medicine at 24 weeks due to ventriculomegaly and a single umbilical artery identified on routine ultrasound. Ultrasound at maternal fetal medicine confirmed ventriculomegaly and the single umbilical artery and also identified a bilateral cleft lip and palate, growth restriction, and hypoplastic cerebellar vermis. Cardiac views were poor and she was scheduled for a fetal echo. She met with a genetic counselor, at which time she declined amniocentesis. She accepted MaterniT® GENOME, which reported a 63.70 Mb duplication of 8q, and discussion with the laboratory indicated concern for an adjacent maternal duplication of 1.95 Mb. Although below the limit of detection for MaterniT® GENOME, this 1.95Mb finding was verbally relayed to the clinician due to potentially increased risk for recurrence if the maternal duplication was confirmed. The patient was non-compliant and did not keep her second trimester appointment for echocardiogram. She eventually returned for echocardiography at 33+4 weeks, at which point tetralogy of fallot with severe pulmonary stenosis was identified. Fetal growth was below the 1st percentile and polyhydramnios had developed. The patient accepted amniocentesis at this time, given the additional findings. Karyotype showed 46,XY,inv(9)(p12q13)der(8;15)(q21.13;p11.2) indicative of an unbalanced reciprocal translocation (along with a common benign chromosome 9 inversion). Microarray confirmed the 65.6 Mb duplication of 8q21.13q24.3. Neither microarray nor MaterniT® GENOME suggested chromosome 15 imbalance, as the implicated segment of chromosome 15 is comprised of non-coding p-arm satellite material. Maternal microarray was subsequently ordered and confirmed a 1.9 Mb duplication of 8q21.13, which was classified as a variant of uncertain significance that was likely benign. Two OMIM genes are present in the region, *HEY1* and *ZFAND1*. While the patient herself was phenotypically normal, her other child had questionable developmental delays. Family history was otherwise unremarkable as reported by the patient. Parental karyotypes were not completed but were recommended. Delivery was induced at 38+2 weeks due to fetal anomalies. Additional neonatal findings included hypertelorism, low-set ears, severe retromicrognathia, undescended testes, hypertonia, a sacral dimple, long and broad big toes, and feeding dysfunction requiring G-tube placement. The child spent nearly 2 months in the NICU and was placed for adoption after birth. No additional follow-up information is available for the patient or the infant after discharge from the hospital.

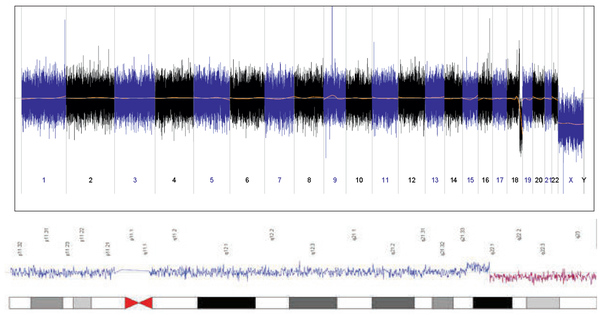


Chromosome 8
Figure 3: MaterniT® GENOME suggested a 63.70 Mb duplication of chromosome 8. The magnitude of the large duplication was proportionate to the fetal fraction of the sample. An adjacent 1.95 Mb duplication is also noted with a greater magnitude compared to that predicted by the fetal fraction, suggesting that this smaller event may be maternal.

Case 3: An 18-year-old G1P0 was referred to maternal fetal medicine at 23+1 weeks for a level II ultrasound after routine ultrasound showed questionably enlarged lateral ventricles in the brain and poor views of the cardiac anatomy. Her ultrasound scan at maternal fetal medicine showed a significantly abnormal facial profile with severe frontal bossing and midface hypoplasia in a male fetus. The fetal profile was noted to be similar to that of the maternal grandmother, who attended the appointment with the patient. Ultrasound also noted minimal bilateral cranial ventriculomegaly and the cardiac anatomy was not well visualized due to maternal body habitus. A subsequent fetal echocardiogram at 25 weeks was normal. The patient received genetic counseling and testing options were discussed. She declined amniocentesis but accepted MaterniT® GENOME, which reported a 13.95 Mb deletion of 18q22.1-q23. Both the patient and her maternal grandmother had possible learning difficulties based on the observations of the genetic counselor. The patient did not display the same facial profile as the fetus and maternal grandmother. The rest of the patient's history and family history was unremarkable. The remainder of the pregnancy was unremarkable and she delivered a term infant via an uncomplicated Caesarean section. The delivery was scheduled at a higher-level facility than it might have otherwise been due to the cfDNA results, as the patient lived in a very rural area. While the baby initially did well and was admitted to the well-baby nursery, on day of life 2 he began having seizures and was transferred to the neonatal intensive care unit (NICU) at the local children's hospital. He spent 2.5 months in the NICU due to severe seizures before being discharged home on palliative care. He has since done well and has followed up with genetics and a complex care team. He requires nasal oxygen, G-tube feedings, and hearing aids. His seizures are well controlled with medications and the care team is considering surgery to treat his craniosynostosis.

Postnatally, a karyotype and microarray were ordered. The microarray showed a terminal loss of 18p11.32-p11.31 (3.17Mb) and a terminal loss of 18q22.1-q23 (14.81Mb). The smaller deletion is below the limit of detection for MaterniT® GENOME. Karyotype showed each cell with one normal chromosome 18 and two ring 18s. Combining rings includes genetic material from 18p11.32 to 18q22.1 with no overlapping segments. One ring consists of some centromeric material to 18q21.1 and the other contains the p arm (minus the terminal deletion), some centromeric material, and the portion of the q arm between 18q21.1 and the terminal deletion at 18q22.1. See figure 5.

No testing on the patient or her mother (the fetus's maternal grandmother) has been performed, although the possibility of an inherited rearrangement has been discussed with them. Such studies may give additional insight into the etiology of the rearrangement as well as clarify recurrence risk for the family.



Chromosome 18
Figure 4: MaterniT® GENOME reported a 13.95 Mb deletion of chromosome 18.

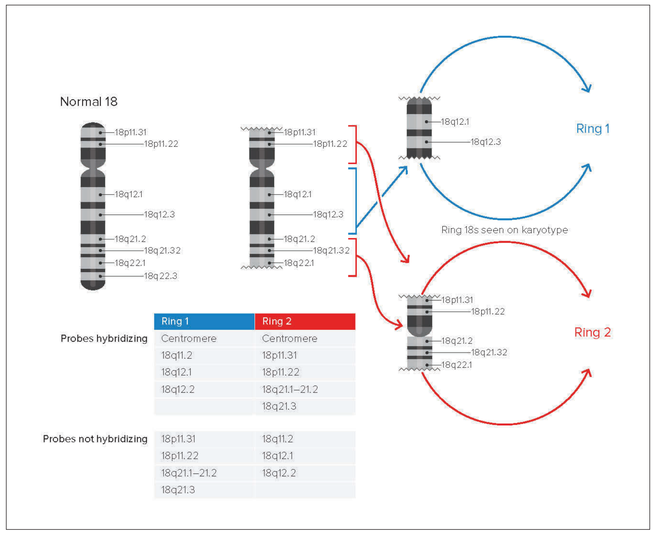


Figure 5: Illustration of the chromosome 18 rings.

IV. Discussion

In case 1, the abnormality identified by MaterniT® GENOME prompted the provider to order a karyotype in addition to microarray when the patient wished to pursue diagnostic testing. When the diagnostic testing showed involvement of the Y chromosome, the finding directed the clinician to only pursue paternal karyotype when insurance and logistical issues might have otherwise prompted starting with maternal karyotype. When paternal karyotype was normal, the clinician had a conversation with the family about non-paternity. Although non-paternity was denied, unbalanced, non-reciprocal Y-autosome translocations are rare[3] and recurrence risk would be significant if the Y-chromosome translocation were inherited from a man carrying a balanced arrangement. The clinical and psychosocial implications of the fetal results impacted how the provider managed the follow-up in this case.

In case 2, the cfDNA results directed additional testing for the pregnant woman, providing important information about recurrence risk for future pregnancy.

In case 3, what initially appeared to be a simple deletion ended up being part of a very complicated result. Given the abnormal phenotype in another family member, the provider did discuss testing for both the pregnant patient and her mother. However, without additional testing of these individuals, it is not possible to know the exact recurrence risk for future pregnancies for the patient or for other members of the family.

MaterniT® GENOME has allowed for screening for fetal chromosome abnormalities beyond the traditional aneuploidies. The results can be complex but also valuable, both clinically and psychosocially. Much like other genetic tests, results from this cfDNA test may have clinical relevance for individuals beyond the fetus. This is an important counseling consideration.

V. 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. American Journal of Obstetrics & Gynecology. doi: http://dx.doi.org/10.1016/j.ajog.2016.02.030
3. Powell C. Sex chromosomes and sex chromosomes abnormalities. in Gersen SL, Keagle MB (eds) The Principles of Clinical Cytogenetics New Jersey, Totowa: Humana Press, 2013, pp 175-211.

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