#70

# Genome Wide Non-Invasive Prenatal Testing: 2,000 Samples Outcome Experience

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## I. Introduction

Noninvasive prenatal testing (NIPT) of circulating cell-free DNA (cfDNA) has become part of the standard of care in the screening for fetal aneuploidies in high-risk pregnancies. However, this traditional NIPT analysis has been limited to analysis of chromosomes 13, 18, 21, X and Y. The MaterniT® GENOME test can report on trisomies, monosomies, select microdeletions, as well as genome-wide copy number variants larger than 7 Mb. Here we present our experience collecting cytogenetic, molecular and/or birth outcomes on our first 1,997 clinical samples received by the laboratory.

## II. Methods

Maternal blood samples submitted to Sequenom Laboratories® for MaterniT® GENOME testing were subjected to DNA extraction, library preparation, and whole genome massively parallel sequencing as previously described.[1,2] For results reported with a positive screening result, we followed up with the ordering clinician at approximately 22 weeks gestational age to obtain cytogenetic and/or microarray results in addition to any ultrasound abnormalities detected. We also followed up with the ordering clinician at approximately 42 weeks gestational age to obtain birth outcome (clinical exam, cytogenetics, molecular testing, etc.). All outcome information was logged and categorized in a secure database.

## III. Results

In the first 1997 clinical samples, with follow up on over 80% of cases, we observed a high positive predictive value for subchromosomal events as well as the core trisomies (Trisomy 21, 18, 13) with no discordant positives noted for subchromosomal events and only one for Trisomy 13. No discordant negative results were reported to the laboratory among the 1,856 negative results. In the discordant positive Trisomy 13 case, a vanishing twin was reported to the laboratory after results were issued, the genetic counselor discussed that the increased chromosome 13 material could be from the vanishing twin. The remaining discordant cases involved trisomy for other autosomes (esoteric trisomies) or monosomy X. This data is in line with placental studies suggesting that both esoteric trisomies and monosomy X are commonly found in placental tissue and often exhibit confined placental mosaicism (CPM).3 Consequential placental compromise or dysfunction from CPM can manifest as abnormal serum screening results, IUGR, and/or pre-term delivery. Additionally, other biological explanations for discordant cfDNA results include co-twin demise, and maternal events. Many of the discordant positive cases in this cohort had clinical findings suggestive of these biological explanations. Collectively, the clinical utility of genome-wide cfDNA screening may extend beyond concordant fetal results, to also include discordant fetal results that may otherwise put the pregnancy at risk.

**Results Continued:** Additionally, other biological explanations for discordant cfDNA results include co-twin demise, and maternal events. Many of the discordant positive cases in this cohort had clinical findings suggestive of these biological explanations. Collectively, the clinical utility of genome-wide cfDNA screening may extend beyond concordant fetal results, to also include discordant fetal results that may otherwise put the pregnancy at risk.

Image 1. Breakdown of results

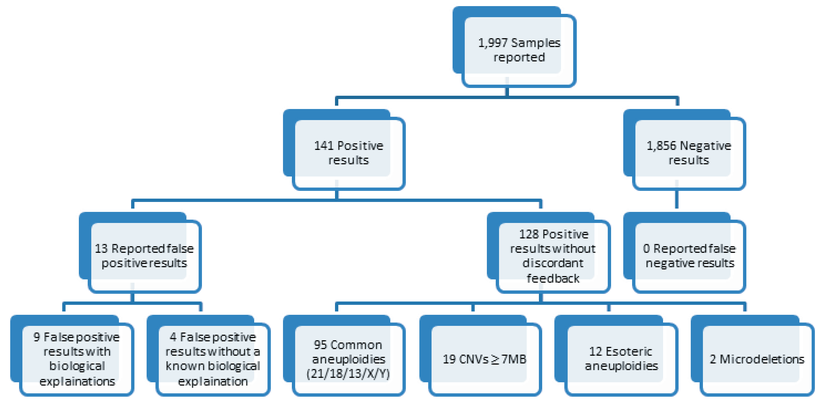


Table 1. False Positive Results

| Result | Indication for testing | Mosaic Data | Vanishing Twin | IUGR | Stillbirth | Maternal Suspicion |
|---|---|---|---|---|---|---|
| Trisomy 3 | Advanced maternal age | X | | | | |
| Trisomy 7 | Ultrasound Finding (heart defect/echogenic intracardiac focus) | X | | | | |
| Trisomy 8 | Advanced maternal age | | | | | |
| Trisomy 8 | Ultrasound Finding | | | | | X |
| Trisomy 13 | Advanced maternal age | | X | | | |
| Trisomy 15 | Advanced maternal age | X | | | | |
| Trisomy 16 | Advanced maternal age | X | | X | | |
| Trisomy 16 | Ultrasound Finding (heart defect/intrauterine growth restriction) | | | X | X | |
| Trisomy 22 | Ultrasound Finding (heart defect/absent nasal bone) | | | X | | |
| Trisomy 22 | Advanced maternal age | X | X | | | |
| Monosomy X | Advanced maternal age | | | | | |
| Monosomy X | Advanced maternal age | | | | | |
| Monosomy X | Previous child with 22qdel | | | | | |

## IV. Conclusion

In the first 1997 clinical samples, with follow up on over 80% of cases, we observed a high positive predictive value for subchromosomal events as well as the core trisomies (Trisomy 21, 18, 13) with no discordant positives noted for subchromosomal events and only one for Trisomy 13. No discordant negative results were reported to the laboratory among the 1,856 negative results. In the discordant positive Trisomy 13 case, a vanishing twin was reported to the laboratory after results were issued, the genetic counselor discussed that the increased chromosome 13 material could be from the vanishing twin. The remaining discordant cases involved trisomy for other autosomes (esoteric trisomies) or monosomy X. This data is in line with placental studies suggesting that both esoteric trisomies and monosomy X are commonly found in placental tissue and often exhibit confined placental mosaicism (CPM).[3] Consequential placental compromise or dysfunction from CPM can manifest as abnormal serum screening results, IUGR, and/or pre-term delivery. Additionally, other biological explanations for discordant cfDNA results include co-twin demise, and maternal events. Many of the discordant positive cases in this cohort had clinical findings suggestive of these biological explanations. Collectively, the clinical utility of genome-wide cfDNA screening may extend beyond concordant fetal results, to also include discordant fetal results that may otherwise put the pregnancy at risk.

## V. References

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

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3. Grati FR, Malvestiti F, Ferreira JC, et al. (2014) Fetoplacental mosaicism: potential implication for false-positive and false-negative noninvasive prenatal screening results. Genet Med. 2014 Aug;16(8):620-4. doi: 10.1038/gim.2014.3.
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