

# The long and short of it; NIPT detection of CNVs <7Mb in presence of co-occurring CNVs >7Mb

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### 1. Introduction

Noninvasive prenatal testing (NIPT) of circulating cell-free DNA (cfDNA) has become standard of care in the detection of fetal aneuploidy in high-risk pregnancies. However, this traditional NIPT analysis has been limited to trisomies of whole chromosomes, typically 13, 18, 21, X, and Y. Studies have shown that traditional NIPT can miss more than 20% of clinically significant karyotypic findings.<sup>1</sup> As a result of this clinical need, NIPT expanded to analyze genome-wide events. Currently, genome-wide cfDNA screening (MaterniT® GENOME) is validated to report out copy number variant (CNV) events >7Mb (typical banding resolution of karyotype analysis). In addition to select microdeletions, information on events <7Mb are included when detected in conjunction with an event >7Mb, as they are relevant to data interpretation and clinical management.<sup>2</sup> Here we share our experience with genome-wide cfDNA screening when events <7Mb are reported in conjunction with events >7Mb.

### 2. Methods

Analysis was performed on maternal blood samples submitted to Sequenom Laboratories for the genome-wide cfDNA screening laboratory developed test. Samples processed as described by Jensen et al<sup>3</sup> and analyzed using a novel algorithm to detect copy number variant events.

### 3. Results

This group of 26 samples had at least one reportable event (CNV >7Mb or select microdeletion), as well as an event <7Mb. In all cases where confirmatory testing was pursued, and reported to us (n=19; 73%), the event <7Mb was confirmed. Of the 7 samples with no follow-up information, 5 had abnormal ultrasound findings and one involved a known familial translocation. If cases with ultrasound abnormalities and the known familial translocation are included, the confirmed rate increases to 96%. No discordant positive results from the cohort have been reported. In one case, one of the two reported events was confirmed.

### 4. Conclusions

Previous studies have indicated a sensitivity and specificity of 97.7% and 99.9%, respectively, for whole chromosome and >7Mb sub-chromosomal abnormalities other than T13, T18, T21, and SCA.<sup>1</sup> Events <7Mb had a high rate of confirmation in this cohort. Current limitations include the lack of outcome information on samples where only a <7Mb event, which would not have met reporting criteria, was detected. Also, some <7Mb events may have gone undetected due to lower sensitivity, as estimated by genome-wide cfDNA screening validation, as compared to >7Mb events. From this cohort it appears that NIPT for CNVs can be extended to events <7Mb without a significant impact on PPV. While shown to be technically feasible, isolated events of <7Mb would benefit from additional clinical information and consideration to minimize reporting variants of unknown significance.

### Tables + Figures

| Event 1 Chromosome | Event 1 Size | Event 1 del/dup       | Event 2 Chromosome | Event 2 Size | Event 2 del/dup       | Dx Testing | Finding  |
|--------------------|--------------|-----------------------|--------------------|--------------|-----------------------|------------|--|
| 4                  | 9.9          | duplication           | 1                  | 2.25         | deletion              | POC        | confirmed  |
| 18                 | 44.55        | duplication           | 18                 | 2.15         | deletion              | CVS        | confirmed; patient found to be inversion carrier     |
| 1                  | 43.65        | duplication           | 15                 | 3.75         | deletion              |            | MCA; lost to f/u                                     |
| 22                 | 8            | duplication           | 6                  | 6.55; 1.50   | duplication; deletion |            | ventriculomegaly; lost to f/u                        |
| 1                  | 5.45         | deletion              | 1                  | 3.9          | duplication           |            | MCA; lost to f/u                                     |
| 12                 | 9.3          | deletion              | 2                  | 3.86         | duplication           | amnio      | confirmed  |
| 4                  | 38.7         | deletion              | 4                  | 4.75         | duplication           | post-natal | confirmed  |
| 4                  | 2.35         | deletion              | 2                  | 3.95         | duplication           | amnio      | confirmed  |
| 18                 | 14.8         | deletion              | 18                 | 1.55         | deletion              | cord blood | confirmed; ring chr 18                               |
| 17                 | 12.65        | duplication           | 17                 | 2.8          | deletion              |            | cystic hygroma; lost to f/u                          |
| 3                  | 14.4         | duplication           | 1                  | 5.4          | deletion              |            | lost to f/u  |
| 8                  | 8.85         | duplication           | 6                  | 5.9          | deletion              | amnio      | confirmed  |
| 4                  | 4.2          | deletion              | 8                  | 6.6          | duplication           |            | MCA; lost to f/u                                     |
| 4                  | 19.85        | duplication           | 2                  | 2.9          | deletion              | amnio      | confirmed  |
| 8                  | 47.65        | duplication           | 8                  | 5.8          | deletion              | amnio      | confirmed; FOB found to be recombinant 8 carrier     |
| 11                 | 18.25        | duplication           | 22                 | 3.55         | duplication           | amnio      | confirmed; patient found to be translocation carrier |
| 15                 | 24.1         | duplication           | 17                 | 2.2          | deletion              |            | lost to f/u; patient is known translocation carrier  |
| 2                  | 54.25        | duplication           | 6                  | 2.3          | deletion              | amnio      | confirmed  |
| 11                 | 18.4         | duplication           | 22                 | 3            | duplication           | amnio      | confirmed  |
| 4; 11              | 19.15; 13.8  | duplication; deletion | 11                 | 2.8          | duplication           | amnio      | only del 11q confirmed                               |
| 11                 | 12.45        | duplication           | 11                 | 6.55         | deletion              | amnio      | confirmed  |
| 4                  | 2.35         | deletion              | 2                  | 3.95         | duplication           | amnio      | confirmed  |
| 3                  | 9.9          | duplication           | 4                  | 4.35         | deletion              | amnio      | confirmed  |
| 5                  | 17.45        | duplication           | 14                 | 5.7          | deletion              | post-natal | confirmed  |
| 1                  | 7.45         | deletion              | 1                  | 2.65         | duplication           | amnio      | confirmed  |
| 4                  | 73.2         | duplication           | 15                 | 4.7          | deletion              | post-natal | confirmed; known family translocation                |

### References

- 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.
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- 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.

