587/PT

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

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I. 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.[1] 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.[2] Here we share our experience with genome-wide cfDNA screening when events <7Mb are reported in conjunction with events >7Mb.

II. 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[3] and analyzed using a novel algorithm to detect copy number variant events.

III. 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.

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

* All event sizes are given as Mb

IV. 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.[1] 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.

V. References

1. 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 Gynec*. doi:http://dx.doi. org/10.1016/j.ajog.2016.02.030.
2. Boomer T, et al. Complex chromosomal rearrangements revealed through genome-wide cfDNA: 40,000 samples. Poster presented at: *2018 NSGC Annual Education Conference*. 2018 Nov 14-17; Atlanta, GA.
3. 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.

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