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Genome wide cfDNA testing: Clinical laboratory experience screening for select microdeletions

Jill Rafalko, MS, LCGC, Kimberly Fanelli, MS, CGC, Theresa Boomer, MS, CGC
Sequenom Inc., a wholly owned subsidiary of Laboratory Corporation of America® Holdings, San Diego, California

Integrated GENETICS
LabCorp Specialty Testing Group
sequenom

I. Introduction

Genome wide cell-free DNA (cfDNA) testing was introduced for clinical use in August 2015. Since its release, over 50,000 samples have been submitted for analysis. We describe the clinical laboratory experience of this screening test for the identification of select chromosomal microdeletions among the initial samples submitted for analysis, and for which sufficient time has elapsed to obtain outcome information from the referring provider.

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 previously described by Jensen et al.[1] Sequencing data were analyzed using a novel algorithm to detect aneuploidies and other subchromosomal events as described by Lefkowitz et al.[2] For positive results, outcome data (e.g. cytogenetic/molecular results and/or birth outcomes) were requested by phone or email from the ordering provider.

III. Results

Analysis of the first 38,573 reportable samples yielded 65 (0.17%) results that were positive for select chromosome microdeletions, specifically: 1p, 4p, 5p, 8q, 11q, 15q, and 22q. This equates to a positive microdeletion result in 1 out of every 593 submitted samples. For reference, the expected incidence of each microdeletion syndrome, based on published literature, is listed in **Table 1**.

The average turn-around time for results was 4 business days (6 calendar days). Indications for testing were as follows: Ultrasound finding (44.6%), advanced maternal age (30.8%), multiple indications (12.3%), personal/family history (3.1%), and "other" (1.5%). Five samples (7.7%) did not indicate a high-risk indication for testing, which could represent screening in an average-risk patient. There were no cases referred due to abnormal serum biochemical screening in the cohort of samples positive for a microdeletion syndrome (**Figure 1**).

The majority (71%) of samples that were positive for a microdeletion were submitted during the second trimester of pregnancy (**Figure 2**).

Diagnostic follow-up testing was available for 63.1% of cases, of which 95.1% of these results confirmed the cfDNA finding (**Figure 3**).

The most commonly identified microdeletion was 22q, accounting for 58% of the positive results, followed by 15q, 4p, 5p, 1p36, 11q, and 8q, respectively (**Figure 4**).

A detailed summary of outcome data available for the 65 positive cases can be viewed in **Table 2**. The estimated positive predictive value (PPV) range for all cases was 86.2–96.9%.

There were 15 cases in which the microdeletion was suspected to be maternal in origin based on the cfDNA signal[4] (14 involving 22q, and 1 involving 8q), which precluded assessment of fetal status for that particular chromosomal region. (**Figure 5**).

Of the 15 suspected maternal deletions, 12 (80%) were confirmed in the mother (by FISH or microarray), and 3 maternal outcomes were unknown (i.e. patient declined testing or results were lost to follow-up) (**Table 2**).

Of the 14 cases positive for a suspected maternal 22q deletion, only one woman had a previous diagnosis of DiGeorge syndrome (as indicated on the test requisition form). Of the remaining 13 women with no prior diagnosis of 22q deletion syndrome: 2 women had a previous affected child, at least 4 women had phenotypes consistent with DiGeorge syndrome (but no previous diagnosis), and 9 women had no overt phenotypic features of 22q deletion syndrome reported to our laboratory. (Please note: these categories are not mutually exclusive.) A summary of the fetal results (for cases with a suspected maternal deletion) is outlined in **Figure 6**.

Based on *ad hoc* feedback from clinicians, there were five "false negative" results reported to our laboratory. All cases were for 22q deletion syndrome, and all fetuses had ultrasound findings which prompted diagnostic evaluation. These cases reinforce the screening nature of cfDNA testing, and the importance of diagnostic testing with microarray analysis for the most comprehensive evaluation of microdeletion syndromes.

Table 1. Incidence of microdeletion syndromes and number of positive MaterniT® GENOME cases

Microdeletion Syndrome	Chromosome Region	Disease Incidence (approximately)[3]	Actual number of positive MaterniT® GENOME cases (of 38,573 reportable samples)
DiGeorge Syndrome	22q	1 in 4,000	38 24 suspected fetal, 14 suspected maternal
Prader-Willi Syndrome	15q	1 in 10,000–30,000	8
Angelman Syndrome	15q	1 in 12,000–20,000	
Wolf-Hirschhorn Syndrome	4p	1 in 50,000	7
Cri-du-chat Syndrome	5p	1 in 20,000–50,000	6
1p36 Deletion Syndrome	1p	1 in 5,000–10,000	3
Jacobsen Syndrome	11q	1 in 100,000	2
Langer-Giedion Syndrome	8q	Rare*	1 suspected maternal
Combined disease incidence		Expected: ~1 in 1,400–1 in 2,065	Observed: ~1 in 593 (overall) ~1 in 771 (only suspected fetal)

*For calculation purposes, disease incidence of 1 in 1,000,000 was used.

Figure 1. Testing indications provided for MaterniT® GENOME samples positive for a microdeletion

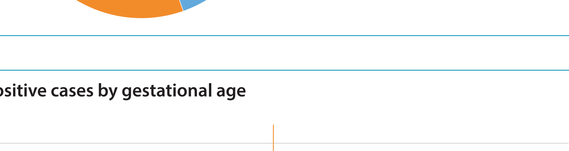


Figure 2. Number of positive cases by gestational age

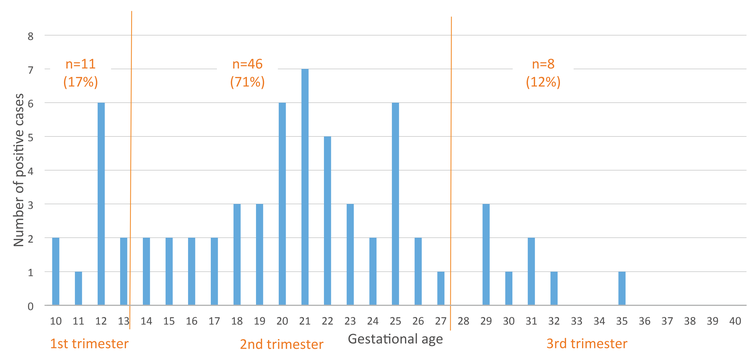


Figure 3. Outcome data for cases positive for a microdeletion

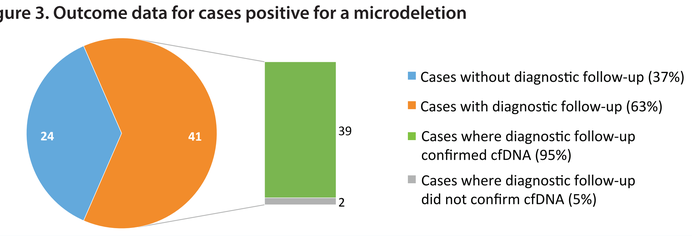


Figure 4: Positive results by microdeletion region (n=65)

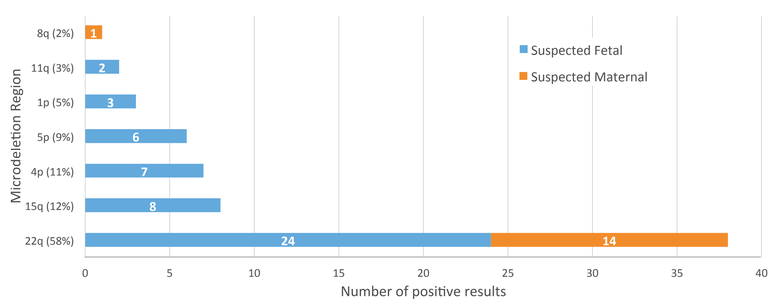


Figure 5. Ideogram of chromosome 22 demonstrating underrepresentation of 22q11.21 in a sample with a deletion suspected to be fetal in origin (top) and maternal in origin (bottom)

Figure 5 shows data from a positive sample in which the deletion was suspected to be fetal in origin (top), compared to a positive sample in which the deletion was suspected to be maternal in origin (bottom).

As demonstrated in the figure, a suspected fetal event shows only a slight downward deviation from the normal data; whereas, the suspected maternal event shows a significant downward deviation from the normal line, related to the percentage contribution of cfDNA from the fetus alone versus the maternal cfDNA fraction. An algorithm is also applied to these results to determine the mosaicism ratio[4] associated with the abnormal finding. This mosaicism ratio calculation helps to determine which events may be fetal vs. maternal in origin.

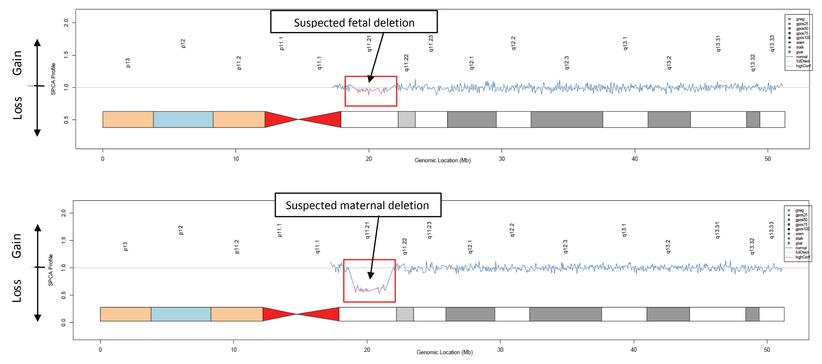
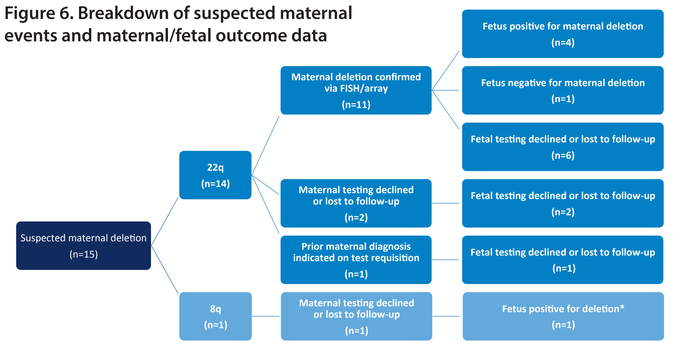


Table 2. Outcome data for microdeletions identified by MaterniT® GENOME for the first 38,573 specimens

Deletion (Associated Syndrome)	Total Reported Positive	Concordant	Likely Concordant	Unknown	Discordant	PPV lower-upper estimate (all cases) (%)	PPV lower-upper estimate (fetal only cases) (%)
22q11.2 (DiGeorge)	38 24 suspected fetal 14 suspected maternal*	26 14 confirmed fetal 12 confirmed maternal	8 8 suspected fetal	4 2 suspected fetal 2 suspected maternal	0	89.5–100%	91.7%–100%
1p36	3	2	1	0	0	100%	100%
15q (Prader-Willi/Angelman)	8	4	2	2	0	75.0–100%	75.0–100%
5p (Cri-du-chat)	6	0	4	1	1	66.7–83.3%	66.7–83.3%
4p (Wolf-Hirschhorn)	7	5	2	0	0	100%	100%
11q (Jacobsen)	2	1	0	0	1	50%	50%
8q (Langer-Giedion)	1 suspected maternal*	1 confirmed fetal	0	0	0	100%	N/A
Totals	65 50 suspected fetal 15 suspected maternal	39 27 confirmed fetal 12 confirmed maternal	17 17 suspected fetal	7 5 suspected fetal 2 suspected maternal	2 fetal	86.2–96.9% (suspected fetal + suspected maternal)	86.0–96.0% (suspected fetal cases only)

***= Suspected fetal/maternal:** The microdeletion identified by cfDNA testing was suspected to be present in the fetus vs. the mother based on the cfDNA signal. Refer to **Figure 5** for further details.
Concordant: "True positive" – The positive cfDNA result was confirmed in the fetus or mother by diagnostic studies (karyotype/FISH/microarray) on samples from CVS, amniocentesis, POC, or peripheral blood.
Likely Concordant: "Suspected" – Cases in which diagnostic testing was declined, but clinical findings (e.g. ultrasound abnormalities, maternal phenotype, etc.) support the abnormal cfDNA result.
Unknown: No additional information was available from the ordering provider, typically because patient declined further testing or results were lost to follow-up.
Discordant: Positive cfDNA results with normal diagnostic studies (karyotype/FISH/microarray) on samples from CVS, amniocentesis, POC, or peripheral blood. Possible reasons for discordance include vanishing twin or confined placental mosaicism[5] for the microdeletion.
PPV lower-upper estimate: Lower estimate is calculated by combining 'concordant' and 'likely concordant', divided by the total number of positives. This presumes that all patients with no additional information ('unknown') are false positives. Upper estimate is calculated by combining 'concordant,' 'likely concordant', and 'unknown', divided by the total number of positives. This presumes that all patients with no additional information are true positives.
PPV lower-upper estimate (fetal only cases): PPV calculated using above formula, but excluding positive cases suspected to be maternal in origin.

Figure 6. Breakdown of suspected maternal events and maternal/fetal outcome data



*= Indication for testing: AMA; Gestational age: 16 weeks; Amniocentesis with microarray confirmed 1582kb deletion on 8q24.12 (containing 12 genes) – deletion not expected to be pathogenic; Follow-up ultrasound and fetal echo: normal; Maternal testing declined/lost to follow-up.

IV. Conclusion

Genome wide cfDNA testing allows for the identification of select microdeletion syndromes throughout all trimesters of pregnancy (starting at 9 weeks gestation), and provides results in a timely manner (on average, within 6 calendar days). A positive result may be seen in approximately 1 out of every 600 samples submitted for testing. This observed microdeletion frequency is higher than the expected disease incidence of ~1 in 1,400 to 1 in 2,065 live births (for all 8 syndromes combined). The difference in observed vs. expected rate could be because: 1) expected disease frequencies may underestimate actual disease frequency (i.e. individuals may have a mild, subclinical phenotype and go undiagnosed); 2) the prenatal disease incidence may differ from postnatal incidence (i.e. affected pregnancies may not continue to term); 3) patients screened with MaterniT® GENOME may represent a population enriched for chromosome abnormalities, as the majority of referrals were due to ultrasound findings. When a microdeletion is identified from this test, the positive predictive value is estimated to be between 86–97%. Most patients with a positive microdeletion result are referred for testing due to abnormal ultrasound findings, though almost one-third of abnormal results were seen in women referred simply because of advanced maternal age. It is important to note that cfDNA testing may also reveal previously undiagnosed maternal copy number variants (CNVs). Though fetal status for the deletion cannot be determined in these cases, the knowledge of a potential maternal deletion should prompt additional evaluation of the fetus, as the risk for the fetus to have the same CNV would be as high as 50%. Though the uptake of diagnostic testing in microdeletion-positive cases is limited, knowledge of a potential chromosome abnormality in these pregnancies may help families arrange for appropriate diagnostic testing at birth.

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. *Am J Obstet Gynecol*. doi: http://dx.doi.org/10.1016/j. ajog.2016.02.03
3. Genetics Home Reference. Retrieved August 10, 2018, from https://ghr.nlm.nih.gov/.
4. Wardrop J et al. Mosaicism Ratio in cfDNA Testing: A Potential Tool to Identify Discordant Results. Poster presented at: *2017 ACMG Annual Clinical Genetics Meeting*. 2017 Mar 21-25; Phoenix, AZ.
5. Caldwell S et al. Telomere capture: An underlying mechanism of copy number variant (CNV) confined placental mosaicism (CPM). Poster presented at: *2018 ACMG Annual Clinical Genetics Meeting*. 2018 Apr 10-14; Charlotte, NC.

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Poster presented at the *NSGC Annual Conference*; 2018 November 14-17; Atlanta, GA.