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Complex chromosomal rearrangements revealed through Genome-wide cfDNA: 40,000 samples

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I. Background

Genome-wide cell-free DNA prenatal screening continues to increase our insight into placental findings not previously recognized. Here we present data from the first two years of clinical testing for expanded cfDNA screening, including genome wide aneuploidy detection and subchromosomal copy number variants (CNVs) larger ≥7 Mb, with specific attention to complex chromosomal rearrangements.

II. Methods

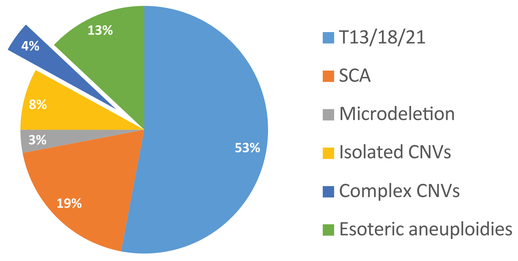
Maternal blood samples submitted for genome-wide cfDNA testing were subjected to DNA extraction, library preparation, and whole-genome massively parallel sequencing as described by Jensen et al.[1] Sequencing data were analyzed using a novel algorithm as described by Lefkowitz et al.[2]

III. Results

41,634 samples were submitted to the clinical laboratory between August 2015 and November 2017.

MaterniT® GENOME: Overview of positive cases | Aug 31,2015 - Nov 2, 2017 | (n=1,957 positives)

Figure1. Categorization of the first 1957 positive MaterniT® GENOME results (4.7% overall positivity rate), including common trisomies (T13, T18, T21), Sex Chromosome Aneuploidies (SCA; e.g. monosomy X), Microdeletions (e.g. DiGeorge Syndrome), Esoteric Aneuploidies (e.g. Trisomy 7), Isolated Copy Number Variants (CNVs; e.g. single deletion or duplication), Complex Copy Number Variants (CNVs; e.g. ≥2 deletions and/or duplications, such as with unbalanced translocations).



MaterniT® GENOME Complex CNVs: Testing indications of positive cases | (n=83 positives)

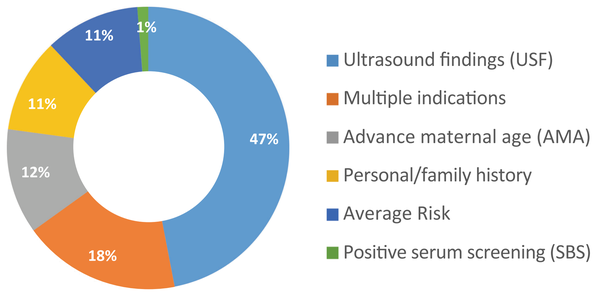
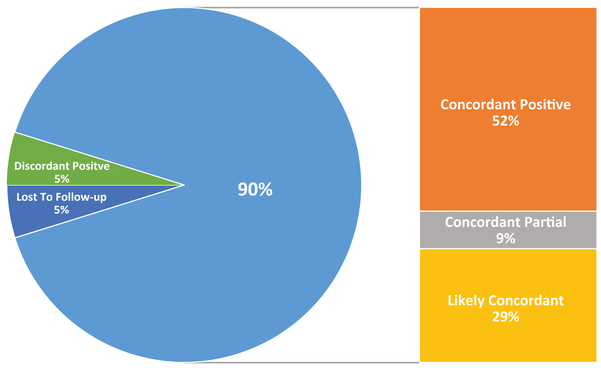


Figure 2. According to test requisitions, complex CNV positive samples have consistently shown enrichment for ultrasound findings (USF), with 62% of complex CNV samples reporting USF alone or in combination with other high risk indications.[3] In comparison, ultrasound findings are reported alone or in combination in only 22% of all samples submitted for MaterniT® GENOME (n=41,634).[4] Similarly, the indication of personal or family history alone or in combination is more common among the complex CNV cohort (29%), compared to only 8% for all MaterniT® GENOME testers.[4] These findings are rather intuitive and consistent with translocation-like phenotypes and typical family histories. However, it should be noted that nearly a quarter (24%) of complex CNV samples were submitted with only advanced maternal age or average risk as the testing indication.

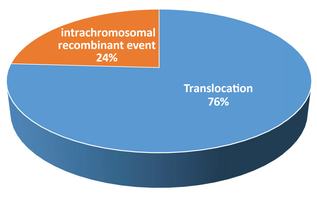
MaterniT® GENOME Complex CNVs: Testing outcomes of positive cases | (n=83 positives)

Figure 3. Results were considered concordant/likely concordant in 90% of complex CNV cases. Specifically, MaterniT® GENOME complex screening results were fully confirmed (all segments) in over half of the patient cohort (52%), with an additional 9% receiving partial diagnostic confirmation (one segment); likely due to confined placental mosaicism (CPM) and subsequent fetal rescue.[6] An additional 29% of the cohort were deemed "likely concordant" because diagnostic studies were declined, not possible due to pregnancy loss, or the patient transferred care; but the presence of multiple congenital anomalies (MCA) on ultrasound, and/or at birth, and/or a consistent family history (prior affected pregnancies, known parental translocation carriers) were considered consistent with the presence of a complex chromosomal rearrangement. A small minority of the cohort (5%) were truly lost to follow-up without any clinical details provided, and an equal minority (5%) of results yielded discordant diagnostic results and thus considered "false positives". Of note, each of the 'discordant' results were accompanied by notable case histories, including a mother with large fibroids (known to yield abnormal cfDNA)[7], clearly mosaic cfDNA data[8], and a consistent family history that may suggest parental (gonadal) mosaicism.



MaterniT® GENOME Complex CNVs: Rearrangement types

Predicted rearrangement type | (n = 83 positives)



Known parental testing outcomes | (n = 36 positives)

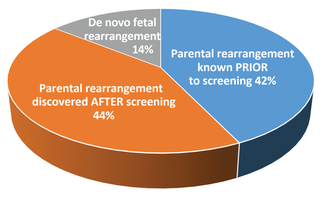
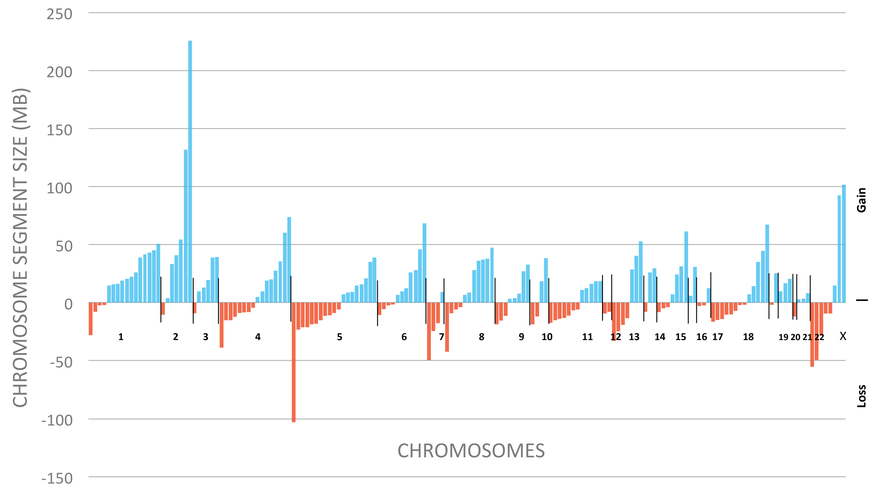
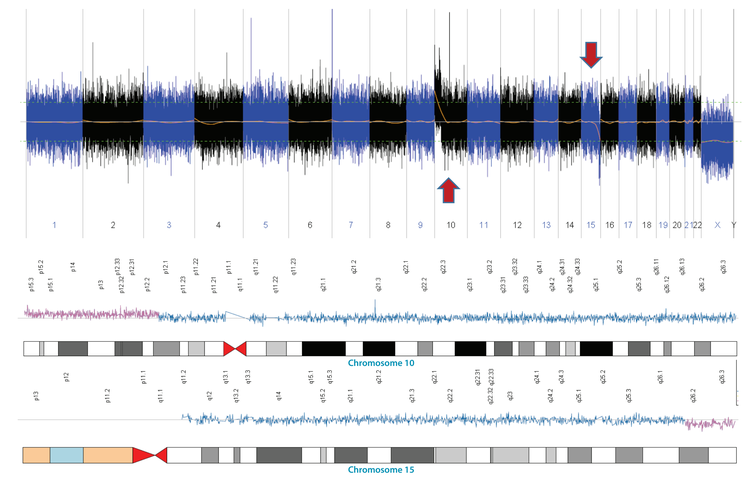


Figure 4 & Figure 5. Of the 83 complex CNVs reported, 63 were interpreted as possible translocations and 20 as possible intrachromosomal recombinant events (e.g. inversion byproducts, inverted deletion/duplications).[5] Parental rearrangements (e.g. translocation, insertion, inversion) were previously known for 18% of these results, 19% were consequently identified post positive cfDNA screening, 6% proven *de novo*, and 57% pending full parental assessment. It should be noted that parental follow-up testing information is generally limited when soliciting fetal outcomes, as testing is often delayed, declined altogether, highly dependent on insurance coverage, and generally skewed toward maternal testing only.

MaterniT® GENOME Complex CNVs: Individual Chromosome Findings | (n=83 positives) | (n=168 segments)

Figure 6. Size distribution of complex CNV cases from the first 1,957 positive results, including 83 individual patient results and 168 imbalanced segments. CNV sizes widely ranged from 1.5 Mb to 225.9 Mb, with a median deletion size of 11.5 Mb and duplication size 22.2 Mb. Of note, MaterniT® GENOME is specifically validated to report on CNVs ≥7 Mb. Any CNVs included in this cohort that were reported below that threshold 'discordant' size either overlap a validated microdeletion loci or were accompanied by a larger (≥7 Mb) CNV and included for context and overall interpretation of the collective findings, with careful notation that the smaller segment fell below validated threshold.





Images 1-3. Translocation Sequence Data Example. 50Kb Genome-wide view illustrating gain on 10p and loss on 15q. Individual chromosome trace data details t(10;15)(p12.1;q26.2) with unbalanced segments offset and colored red. Mother was a known balanced translocation carrier prior to testing.

Table 1. MaterniT® GENOME Complex CNV samples' key metrics.

Key Metrics	Average	Median
Turn-around Time	4.9 business days	4.5 business days
Gestational Age	18.0 weeks	16.5 weeks
Fetal Fraction	11.1%	10.1%
Duplication Size	30.1 Mb	22.2 Mb
Deletion Size	-15.3 Mb	-11.2 Mb

IV. Conclusion

Genome-wide cfDNA prenatal screening with subchromosomal CNV detection has allowed noninvasive technology to reach the subset of patients at highest risk for chromosomal imbalance, many previously unaware. These high risk families can benefit from early identification or added reassurance, prior to diagnostic testing. While the nature of cfDNA placental screening can find and report CPM, certain complex chromosomal rearrangements have an extremely high fetal concordance rate, with 90% being diagnostically confirmed, partially confirmed, or highly likely given supportive clinical details and family histories. Collectively, the stellar performance of cfDNA screening in this unique subset of high risk patients speaks to the clinical feasibility and utility of including CNVs in early cfDNA screening in pregnancy.

Key Points:

- Patients at risk due to familial chromosomal rearrangements (e.g. translocations, insertions, inversions) can benefit from early cfDNA genome-wide screening.
- Nearly a quarter of the patients yielding positive complex CNV results had no known family or personal history, nor overt fetal findings at the time of screening.
- New discovery of families at risk of carrying a recombinant chromosomal event via cfDNA screening can clarify future reproductive risks as well as maximize surveillance options.

V. References

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