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The Revelation of Complex Chromosomal Rearrangements through Genome-Wide cfDNA Screening

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I. Background

Recent adoption of genome-wide cell-free DNA (cfDNA) prenatal screening provides unique insight into placental findings not previously recognized. Here we present data from our first 28,760 clinical samples for expanded cfDNA screening, including genome-wide identification of aneuploidy and subchromosomal copy number variants (CNVs) ≥7 Mb, with specific attention to complex chromosomal rearrangements.

II. Methods

Maternal blood samples submitted to Sequenom Laboratories® for MaterniT® GENOME screening 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

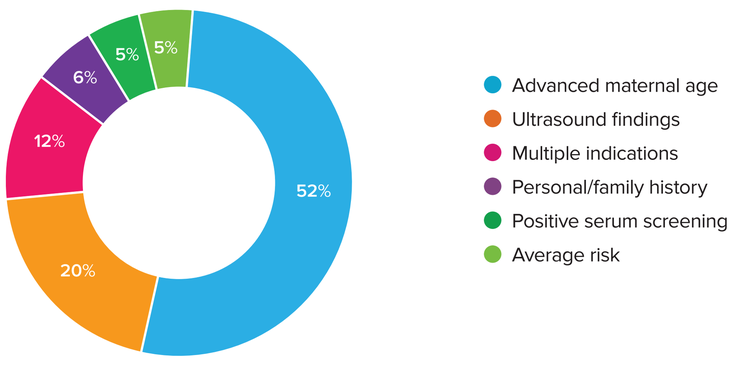


Figure 1: Screening indication(s) per test requisition for all MaterniT GENOME samples submitted to the clinical laboratory through March 2017 (n=28,760).

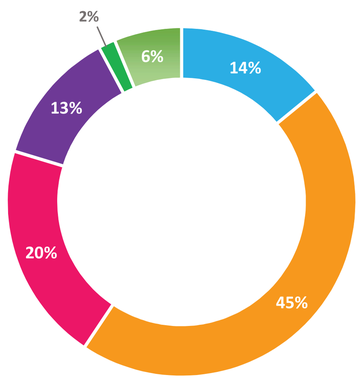


Figure 2: Screening indication(s) per test requisition for the complex chromosomal rearrangement positive results cohort (n=64). Complex rearrangements are defined as ≥2 CNV segments, such as the co-occurrence of a deletion and duplication in the same sample, suggestive of unbalanced chromosomal translocations, recombinant chromosomes, and inversion products.

Complex CNV samples show an enrichment of:

Ultrasound findings

- 45% as a sole indication (vs. 20% in the general MaterniT® GENOME screening population)
- 61% overall, adding in the subset of multiple indications that include ultrasound findings

High risk personal and/or family histories

- 13% as a sole indication (vs. 6% in the general MaterniT® GENOME screening population)
- 34% overall, adding in the subset of multiple indications that include ultrasound findings

Multiple high risk indications

- 20% as a sole indication (vs. 12% in the general MaterniT® GENOME screening population)

Figure 3: Categorization of first 1392 positive MaterniT® GENOME results (4.8% overall positivity rate), including Common Trisomies (T13, T18, T21), Sex Chromosome Aneuploidy (SCA; e.g. XO), Microdeletions (e.g. DiGeorge syndrome), Esoteric Aneuploidies (e.g. Trisomy 7), Isolated del/dup (1 segment), Complex del/dup (≥2 segments, e.g. suggestive of translocations).

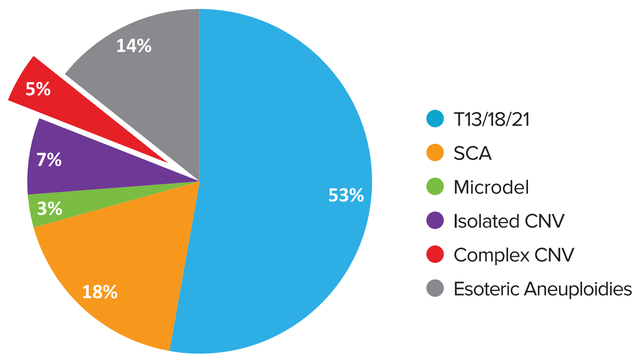
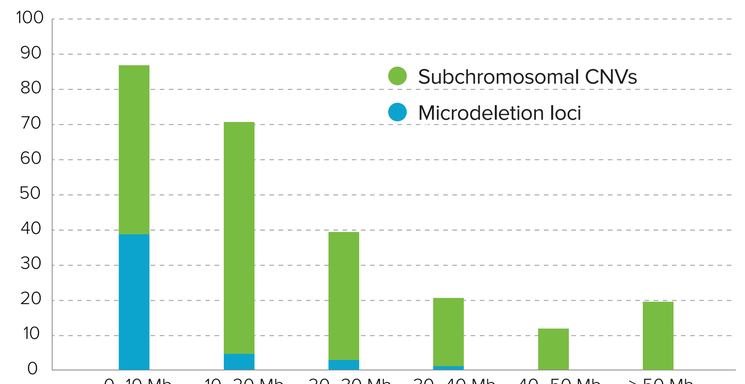


Figure 4: Size distribution of all subchromosomal CNVs seen in the first 1,392 positive results, excluding whole chromosome aneuploidies (n=209 samples). CNV sizes ranged <10 Mb to ~100 Mb, with the majority of non-microdeletion CNVs falling between 10-20 Mb.



Complex CNV Reported	Total Reported Positive	Full Concordance (all reported segments confirmed)	Partial Concordance (1 segment confirmed in fetus)	Likely Concordant (USF and/or Consistent Family Hx)	Unknown	Discordant	Relative PPV Lower - Upper estimate (%)
Unbalanced Translocation	49	24	5	18	2	0	96% - 100%
Recominant Chromosome	12	5	1	3	0	3	75%
Inverson Byproduct	3	3	0	0	0	0	100%
Total	64	32	6	21	2	3	92% - 95%

Table 1: Subsequent fetal confirmation was reported in the majority (59%, 38/64), with 33% (21/64) pending and likely concordant, 3% (2/64) unknown/lost to follow-up, and 5% (3/64) discordant.

Complex CNV Reported	Total Reported Positive	Carrier parent known prior to screening	Carrier parent subsequently identified post screening	*de novo*	Parent carrier status unknown or pending full investigation
Unbalanced Translocation	49	14	8	3	24
Recombinant Chromosome	12	0	2	1	9
Inverson Byproduct	3	0	2	0	1
Total	64	14	12	4	34

Table 2: Complex CNV report interpretations
Prior knowledge of a parental rearrangement (e.g. translocation, insertion, inversion) was true for 22% (14/64) of complex results, while 19% (12/64) were identified post screening. A minority of cases (6%, 4/64) were *de novo*, with the remaining subset pending full parental assessment (53%, 34/64).

Full Concordance: All reported unbalanced segments in the cfDNA results were confirmed by diagnostic testing in fetus (or rarely in mother) by karyotype and/or microarray analysis on samples from CVS, amniocentesis, POC, or peripheral blood.

Partial Concordance: One reported unbalanced segment in the cfDNA results was confirmed by diagnostic testing in the fetus by karyotype and/or microarray analysis on samples from CVS, amniocentesis, POC, or peripheral blood. Confined Placental Mosaicism (CPM) for the remaining segment is possible and presumed likely, representing partial fetal rescue.

Likely Concordant: Suspected cases in which diagnostic testing was declined, but clinical findings (e.g. ultrasound abnormalities, consistent family history) support the abnormal cfDNA result.

Unknown: No additional information was available from the ordering provider.

Discordant: Positive cfDNA results with a normal fetal karyotype or microarray. Diagnostic studies were performed by amniocentesis (n=2) and postnatal peripheral blood (n=1). Concomitant maternal fibroids were noted or suspected in 2 of the 3 cases.

Relative PPV: Lower estimate is calculated by combining 'full concordance', 'partial concordance', and 'likely concordant', divided by the total number of positives (presuming that all patients with no additional information are confirmed to be false positives). Upper estimate is calculated by combining 'full concordance', 'partial concordance', and 'likely concordant', divided by the total number of positives (presuming all patients with no additional information are confirmed to be true positives).

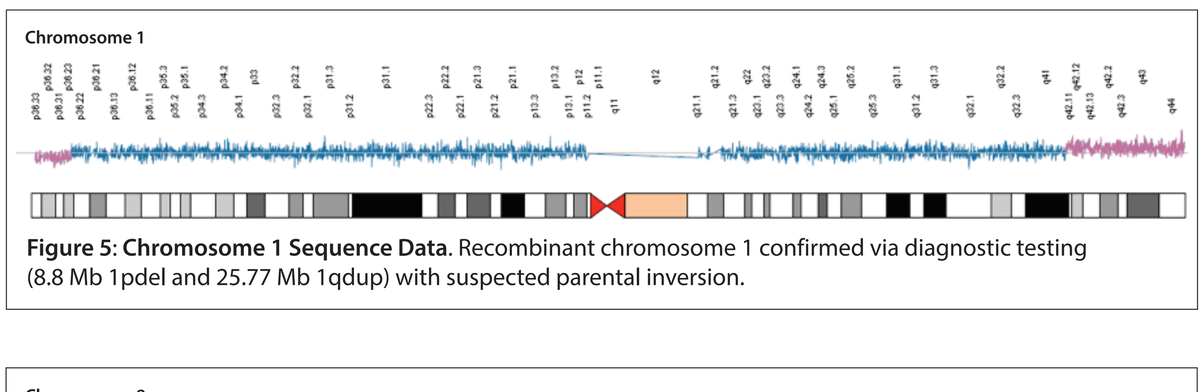


Figure 5: Chromosome 1 Sequence Data. Recombinant chromosome 1 confirmed via diagnostic testing (8.8 Mb 1pdel and 25.77 Mb 1qdup) with suspected parental inversion.

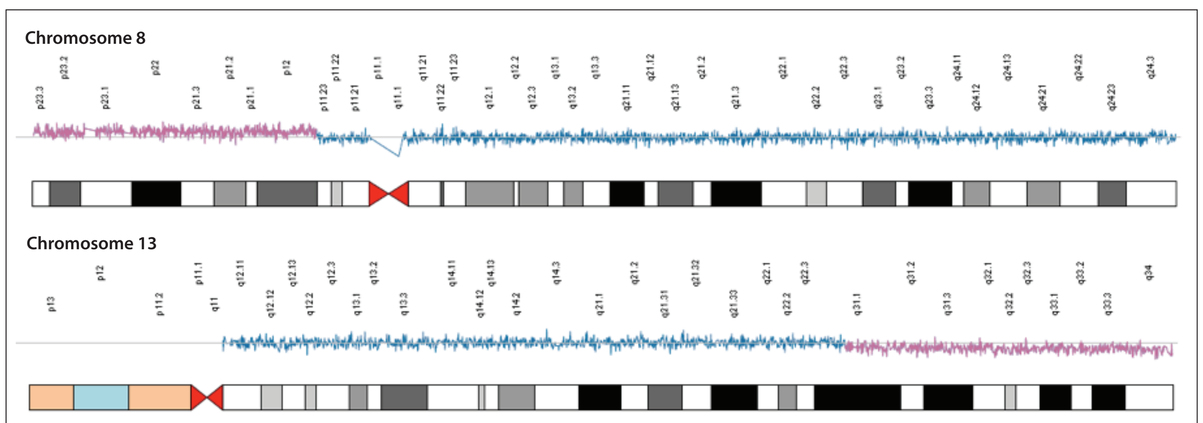


Figure 6: Chromosomes 8 & 13 Sequence Data. Chromosomal translocation t(8;13)(p11.2;q31) confirmed via diagnostic testing with subsequent maternal carrier identified.

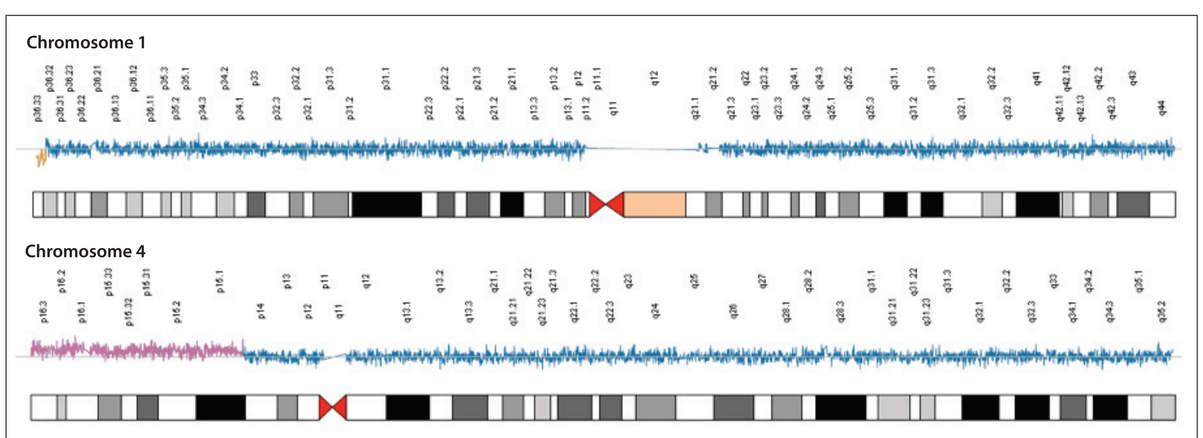


Figure 7: Chromosomes 1 & 4 Sequence Data. Suggestive chromosomal translocation t(1;4)(p36.33;p15.1) partially confirmed in fetal tissue, as only 3.4 Mb 1p deletion found on amniocentesis SNP microarrray. However, chromosome 4p (34.90 Mb) duplication subsequently found to be 51% mosaic in post-delivery placenta testing, confirming confined placental mosaicism (CPM), partial fetal rescue, and a possible 'telomere capture' mechanism.[3]

IV. Conclusions

Identification of complex chromosomal rearrangements via cfDNA prenatal screening marks a new era in prenatal testing. These findings tend to segregate with significant high risk prenatal indications, however it should be noted that 22% of these pregnancies had no known family history or overt ultrasound findings at the time of screening.

Many known familial rearrangements not previously amenable to cfDNA screening may now benefit from early identification or added reassurance. Providers have relayed that these high risk patients often use such information for diagnostic testing plans, deciding to wait for an amniocentesis if normal vs. chorionic villus sampling if abnormal, for example. Similarly, the mental and emotional preparedness that such early screening results allow is a new and valued asset for providers and patients alike.

In addition, new discovery of families at risk of carrying a recombinant event often helps to explain past pregnancy complications, as well as clarify future reproductive risks.

Identification of both balanced translocation and inversion carriers earlier in their reproductive lives can assist with improved pregnancy surveillance, maximizing patient options and overall medical management.

Key points:

- Patients at risk for familial unbalanced chromosomal rearrangements during pregnancy (e.g. translocations, insertions, inversions) can benefit from early cfDNA genome-wide screening.
- Nearly a quarter of the patients yielding positive complex chromosomal rearrangements had no known family or personal history, nor overt ultrasound 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

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. Yu S, Graf W, D, Telomere Capture as a Frequent Mechanism for Stabilization of the Terminal Chromosomal Deletion Associated with Inverted Duplication. *Cytogenet Genome Res* 2010; 129:265-274.

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