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Validation of Fetal Aneuploidy Detection with FirstGene: a Combined, Non-Invasive Prenatal cfDNA Assay for Fetal Aneuploidy, Recessive Diseases, and Serological Screening

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Background

- Screening for genetic risk in pregnancy is typically achieved by performing prenatal cell free DNA (pcfDNA) screening to assess for fetal aneuploidy risk in conjunction with carrier screening from both reproductive partners.
- Collection of the required samples to achieve a complete risk assessment can be logistically challenging.
- The FirstGene assay addresses this challenge by screening for fetal aneuploidy, fetal and maternal recessive disease, and fetomaternal Rh compatibility from a single maternal blood draw in an integrated molecular workflow involving a single round of sequencing.

Objective: Here we describe the analytical validation of the fetal aneuploidy component of FirstGene.

Methods

- 478 plasma samples from 459 pregnant patients were analyzed on FirstGene and Prequel, a previously validated whole genome sequencing (WGS)-based pcfDNA screen with Fetal Fraction Amplification (FFA), to evaluate concordance of calls between the assays.[1] The output of Prequel was interpreted as clinical truth when evaluating sensitivity and specificity.
- Cohort included:
  - 24 cases of Trisomy 21 (T21)
  - 22 cases of Trisomy 18 (T18)
  - 11 cases of Trisomy 13 (T13)
  - 6 cases of 22q11.2 microdeletion
  - 12 cases of Monosomy X
  - 28 other sex chromosome abnormalities (SCAs)
- FirstGene uses a depth-based aneuploidy calling model **(Figure 1)** paired with a novel "trajectory analysis" that uses covariance of read depth and insert size to differentiate fetal from maternal anomalies **(Figure 2)**.

Conclusions

- Performance of fetal aneuploidy screening on the FirstGene assay is comparable to that of standalone WGS-based pcfDNA screening with FFA.
- The unique features of the FirstGene assay may improve positive predictive value for the detection of fetal Monosomy X.

Reference: 1. Welker, N.C., Lee, A.K., Kjolby, R.A.S. et al. High-throughput fetal fraction amplification increases analytical performance of noninvasive prenatal screening. Genet Med 23, 443450 (2021)

Disclosures: All authors were employees of Myriad Genetics, Inc. at the time of this study and received salaries and stock as compensation

Results

- As shown in **Figure 3** and **Table 1**: Sensitivities for common aneuploidies (T13, T18, T21) were 100%. Specificities for both T13 and T21 were 100%. T18 specificity was 99.78%, with a single false positive call. No call rates were inflated due to the inclusion of low volume plasma samples that are below the standard input of the assay.
- All 6 samples identified as screen-positive for 22q11.2 microdeletion on Prequel were also detected by FirstGene, and one additional sample was correctly identified as a maternal 22q11.2 microdeletion.
- FirstGene and Prequel had concordant detection of a fetal Y chromosome, with 99.6% sensitivity and 99.5% specificity.
- All 12 Monosomy X (MX) samples on Prequel had MX signal identified correctly by FirstGene, but 4 of the 12 were no-called due to the trajectory analysis in FirstGene that suggested they were mosiac anomalies from the pregnant person (diagnostic results were available for 2 of 4 such samples and confirmed their pregnant-person origin). Omitting these 4 putative non-fetal mosaic MX samples, FirstGene achieved 100% sensitivity and 99.77% specificity on fetal SCAs

Figure 1. Aneuploidy calling with a fetal-fraction-informed depth model

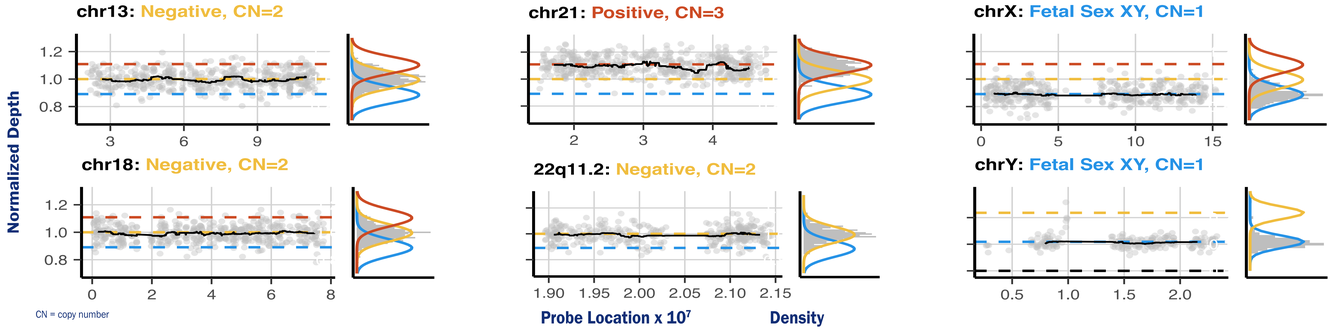

Figure 2. Depth trajectories differentiate fetal and maternal anomalies

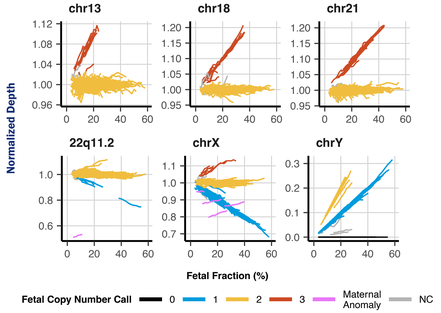

Figure 3. Aneuploidy calling concordance analysis

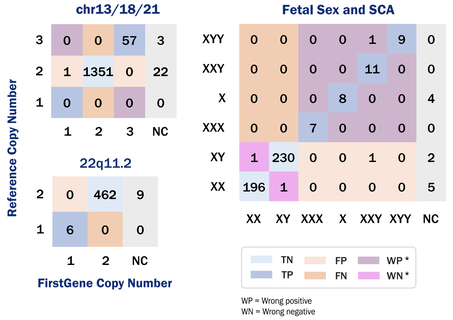

Table 1. Summary of fetal aneuploidy calling performance in FirstGene

Region	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)
chr13	100 (67.86 – 100)	100 (98.97 – 100)
chr18	100 (81.5 – 100)	99.78 (98.57 – 99.99)
chr21	100 (82.83 – 100)	100 (98.93 – 100)
22q11.2 microdel	100 (51.68 – 100)	100 (98.96 – 100)
Presence of Y	99.57 (97.25 – 99.98)	99.49 (96.77 – 99.97)
SCA	100 (87.99 – 100)	99.77 (98.5 – 99.99)