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Validation of Fetal and Maternal Spinal Muscular Atrophy (SMA) and Hemoglobin (Hb) Bart's Screening with FirstGene, a Combined Non-Invasive Prenatal cfDNA Assay for Fetal Aneuploidy, Recessive Diseases, and Serological Screening

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Background

Spinal muscular atrophy (SMA) is a serious genetic condition typically caused by deletion in the *SMN1* gene. Hemoglobin (Hb) Bart's is a nearly fatal condition typically caused by homozygous deletions in *HBA1* and *HBA2*. Determining fetal copy number for both the *SMN1* and the *HBA1* and *HBA2* gene regions is complicated by their high homology to other parts of the genome. Guidelines support routine screening for both conditions, yet the necessity of screening both reproductive partners to identify the risk of an affected fetus is logistically challenging. The FirstGene assay addresses this challenge by predicting fetal recessive disease status without prior knowledge of maternal or paternal carrier screening results.

Objective: We describe the analytical validation of the fetal and maternal SMA and Hb Bart's screening components of FirstGene.

Methods

- *SMN1* and the *HBA1* and *HBA2* region copy number calling in FirstGene utilizes multiple bases differentiating homologous regions (diffbase) and high frequency SNPs to estimate the expected depth of various maternal and fetal copy number states **(Figure 1)**.
- The final copy number call utilizes fetal fraction enrichment to assess copy number at various fetal fractions (depth trajectory) **(Figure 2, 3)**.
- Genomic DNA (gDNA) mixtures from 5 trios with SMA carrier mothers (*SMN1* copy number 1) and one trio with an Hb Bart's carrier mother (1 double-cis deletion allele) were included in this validation study. One proband from each set of trios was affected by the corresponding diseases.
- Differentially fragmented gDNA from the proband and the mother were mixed at 3%, 5%, 10%, 15% and 20% ratios to represent a range of fetal fractions (samples below 3% fetal fraction are routinely failed in FirstGene). These mixed samples were run on FirstGene and assessed for concordance with results of the corresponding single source gDNA samples run on a validated orthogonal assay.
- In addition to contrived cell line mixtures, two simulation approaches were developed to estimate the analytical sensitivity and specificity for fetal calls at diverse fetal fraction levels representative of a general population.
  1. Utilized observed plasma data to simulate affected and carrier fetuses.
  2. Used subsampled data from *RHD,* a gene known to have many copy number changes, to mimic SMA and Hb Bart's data.

Conclusions

- FirstGene utilizes multi-site depth calculation to accurately identify maternal and fetal SMA and Hb Bart's disease status.
- This screening approach streamlines the identification of at-risk pregnancies, especially in cases where carrier status is unknown for the reproductive partner.

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

Results

- FirstGene had 100% sensitivity and specificity for identifying maternal SMA and Hb Bart's status.
- In cell line experiments, FirstGene had 100% sensitivity for fetal SMA and Hb Bart's status and 100% specificity for fetal SMA status **(Figure 4)**.
  - There were 3 SMA and 1 Hb Bart's fetal no-calls, which were all at the 3% contrived fetal fraction **(Figure 4)**.
  - Simulations demonstrated that the expected analytical sensitivity and specificity were >96% for fetal SMA status and >98% for fetal Hb Bart's status in cases where pregnant person is a carrier for each disease **(Table 1)**. A separate set of simulations demonstrated specificity of >99.9% in the general population.

Figure 1. *SMN1* copy number calling based on multiple probes

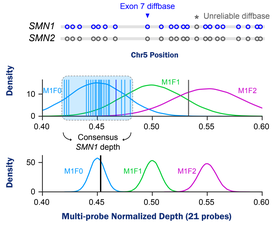

Figure 1: *SMN1* copy number calling based on multiple probes. The top panel illustrates 22 selected single nucleotide sequence differences (diffbases) between *SMN1* and *SMN2* that are often in cis with the corresponding Exon 7 alleles. Asterisk denotes an unreliable diffbase where the allele typically *in cis* with the *SMN2* Exon 7 diffbase allele is present in *SMN1* in the fetus. The middle panel shows normalized depth values of 22 diffbases relative to the single-probe expected depth distributions. Depth value from the unreliable diffbase was excluded by the FirstGene algorithm from the consensus depth calculation (blue box). The bottom panel shows the consensus depth (black vertical line) and the aggregated expected depth distributions, constructed by the median values of iteratively sampled single-probe depth distributions with a sample size of 21 (i.e., to match the size of the selected *SMN1* diffbase set).

Figure 2. *SMN1* depth trajectory differentiates maternal and fetal copy number

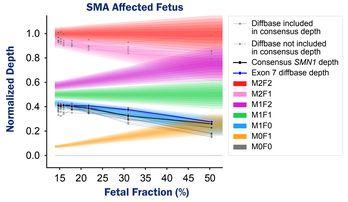

Figure 2: Example depth trajectories of *SMN1* copy number calling in cell-line mixture samples with SMA affected fetuses. The plots show the depth shifts as FF increases with *in silico* size selection, where the solid black line is consensus depth, grey lines are the depths from individual diffbases, and the solid blue line is the depth of the exon 7 diffbase. The individual diffbases that are plotted with "x" are excluded from the final set of diffbases to produce the *SMN1* copy number call. The colored distributions represent the aggregated expected depth distributions at different fetal-maternal copy number combinations as a function of FF.

Figure 3. Fetal and maternal genotype prediction using an AB trajectory-based Gaussian Mixture Model (GMM)

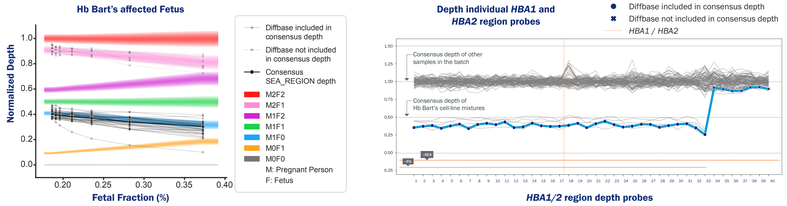

Figure 3: (left) Example depth trajectory plot of a cell-line mixture sample with Hb Bart's syndrome affected fetus. The plot shows the depth shifts in common double-cis deletion region as FF increases with *in silico* FF enrichment. Colors as in Figure 2. **(right)** Consensus normalized depth values at all probes within the double-cis deletion region for all cell-line mixture samples at a single fetal fraction. Sample from the left plot is highlighted in blue. In this set of cell-line mixtures, the fetus inherited a –FIL allele from the pregnant person and a –SEA allele from the reproductive partner. When calculating consensus depth in this region, the caller correctly excluded the probes outside the overlapping region of the two alleles.

Figure 4. Fetal *SMN1* and *HBA1/2* copy number calling performance in gDNA mixtures with carrier mothers

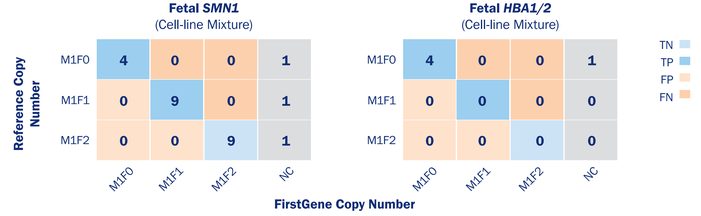

Table 1. *SMN1* and *HBA1/2* fetal and maternal copy number calling performance

Sample Type		Region	Sensitivity (%) 95% CI	Specificity (%) 95% CI
Fetal results when maternal carrier	Cell line mixture	*SMN1*	100 (77.19-100)	100 (70.09-100)
		HBA 1/2	100 (51.01-100)	N/A
	Plasma (via Simulation)	*SMN1*	99.2 (98.43-99.59)	96.1 (94.71-97.13)
		HBA 1/2	99.8 (99.27-99.95)	99.7 (99.12-99.90)
	Plasma (via Emulation)	*SMN1*	97.9 (96.81-98.62)	97.2 (95.98-98.06)
		HBA 1/2	98.4 (97.42-99.01)	99.9 (99.44-99.99)
Maternal results	Plasma	*SMN1*	100 (80.84-100)	100 (98.49-100)
		HBA 1/2	100 (72.25-100)	100 (98.53-100)