Detection of genomic copy number abnormalities from circulating single fetal cells using next generation sequencing (NGS) offers a promising non-invasive alternative for prenatal diagnosis. Towards this goal, we have established a method for performing fetal cell-based, non-invasive prenatal testing (CB-NIPT) during the first trimester. CB-NIPT for prenatal diagnosis has **dramatic potential advantages** over the currently available cell-free DNA-based tests (CF-NIPT) because it enables analysis of **pure fetal DNA**. Here we show that we can successfully repeatedly recover **individual fetal cells** during the first trimester and perform NGS to detect clinically important copy number variants.

**METHODS**

Fetal cell enrichment was carried out using methods developed by the commercial author organization for blood preservation, density based enrichment, immunostaining, custom high-resolution scanning and analysis, and integrated **single-cell picking**. Whole genome amplification was performed on recovered single fetal cells and single nucleotide polymorphism-based genotyping studies were carried out for confirmation of fetal origin. NGS on an Illumina platform with approximately 5 million reads per cell (~0.1x haploid genome) was used to generate **genome-wide copy number data**.

**RESULTS**

**FIGURE 1. Fetal cell identification**

Eight fetal cells were identified from the same study subject and demonstrate the varied appearance of fetal cells. Positive immunofluorescence for cytokeratin (green) is a key attribute of fetal cells. The WBC marker, CD45, is also routinely used as a negative marker (not shown).

**FIGURE 2. NGS data from single fetal cells is reproducible and concordant with diagnostic array CGH data**

**A. 4 cells from a trisomy 21 pregnancy**

**B. 1 cell from a fetus with a 2.7 Mb deletion**

**FIGURE 3. Two cells from a pregnancy with a positive cell-free NIPT for Trisomy 13 and a normal CVS**

CB-NIPT has many potential advantages over CF-NIPT including the ability to analyze **pure fetal DNA** free of contamination by maternal DNA and avoid detecting maternal findings. CB-NIPT has the potential to detect **most clinically significant cytogenetic abnormalities** and even, in the future, deleterious point mutations. Optimization of fetal cell recovery and **validation studies** on larger numbers of samples from pregnant women are underway to evaluate the clinical validity of this test.

**CONCLUSION**

This work has been recently published:

Breman et. al, Prenatal Diagnosis 2016, 36, 1-11