Cell based non-invasive prenatal testing using NGS-based copy number assessment of fetal cells

#3299T

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BACKGROUND

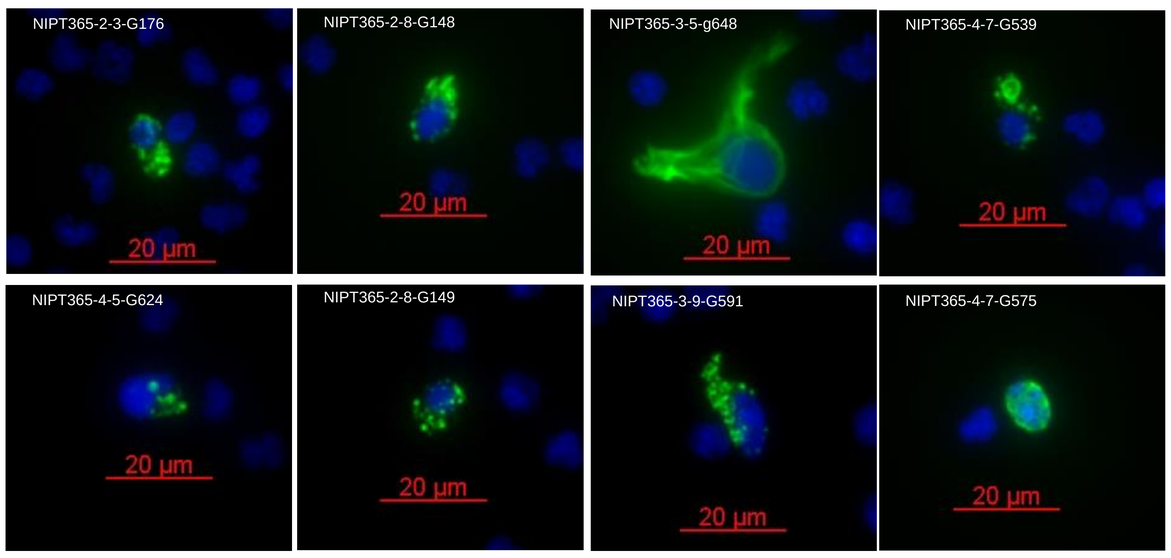
Detection of genomic copy number abnormalities from circulating single fetal cells using next generation sequencing (NGS) offers a promising non-invasive alternative for prenatal diagnosis. Towards this goal, we have established a method for performing fetal cell-based, non-invasive prenatal testing (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

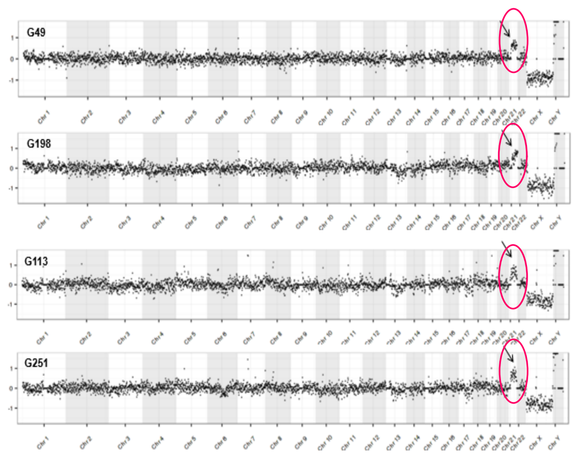


Eight fetal cells were identified from the same study subject and demonstrate the varied appearance of fetal cells. Positive immunofluorescence for cytokeratin (green) is a key attribute of fetal cells. The WBC marker, CD45, is also routinely used as a negative marker (not shown).

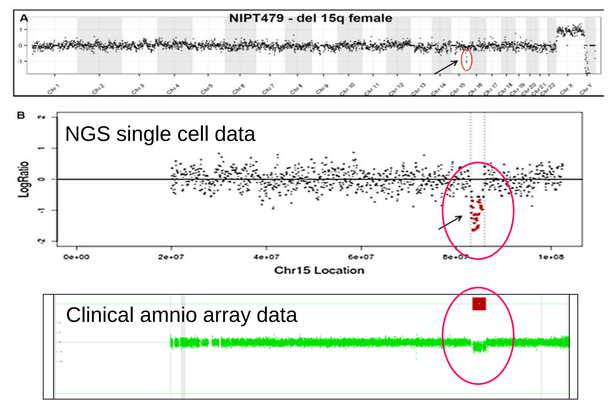
RESULTS

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



A. Comparison of NGS analysis of four trisomy 21 cells from a single case. Subject 511 showing four out of eleven cells with similar results. All four plots show the gain of chromosomes 21 (black arrows). Additionally, the plots show a loss of X and gain of Y due to comparison with a normal female reference. NGS data is displayed as 1000 kb bins across the genome.

B. NGS analysis of a fetal cell with a 2.7 Mb deletion of chromosome 15q25. Top: Whole genome plot from a single fetal cell derived from a fetus harboring a 2.7 Mb deletion of chromosome 15q (subject 479). **Middle:** Enlarged plot of chromosome 15 showing the deleted region in red. NGS data is displayed as 1000 kb bins for the whole genome plot and 30 kb bins for the chromosome 15 plot. **Bottom:** Clinical array CGH plot from amniotic fluid.

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

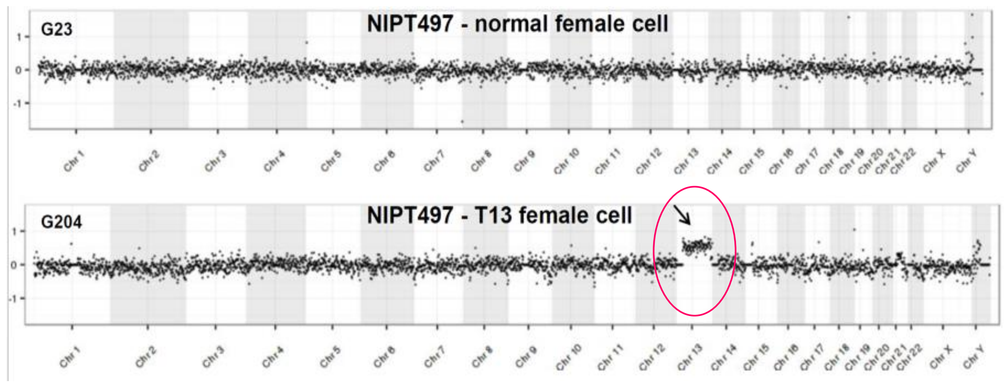


Figure 6. Demonstration of mosaicism with a normal and one trisomy 13 cell. NGS whole genome plots from two single fetal cells derived from a female pregnancy suspected to have trisomy 13 by plasma-based NIPT studies (subject 497). One of three normal cells is shown above and the only trisomy 13 cell is shown below. Clinical cytogenetic studies on CVS tissue from this pregnancy showed a normal female FISH result and a 46,XX chromosome complement with no evidence of trisomy 13 mosaicism. NGS data is displayed as 1000 kb bins across the genome.

CONCLUSION

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.

This work has been recently published:
Breman et. al, Prenatal Diagnosis 2016, 36, 1-11