Use of targeted sequencing of SNPs to achieve highly accurate non-invasive detection of fetal aneuploidy of chromosomes 13, 18, 21, and sex chromosomes.

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Abstract

Objective: To non-invasively detect all chromosome abnormalities on chromosomes 13, 18, 21, X and Y in the fetus through analysis of cell-free fetal DNA in maternal blood.

Study Design: 763 maternal plasma samples (including 673 euploid and 90 aneuploid samples) were collected from patients at 9 weeks of gestation under institutional review board (IRB) approved protocols and analyzed using Natera's Panorama NIPT test. 351 samples were externally blinded, and the remainder were internally blinded where the number and identity of aneuploidies were not known. 138 of the samples had a paternal genomic sample available, which was included in the analysis. Aneuploid samples included Trisomy 21, Trisomy 18, Trisomy 13, and Monosomy X. Cell-free DNA was isolated, amplified using multiple PCRs targeting 19,468 SNP loci covering chromosomes 13, 18, 21, X, and Y and sequenced. Sequencing data was analyzed using the informatics enhanced Next-generation Aneuploidy Testing Using SNPs (NATUS). The algorithm uses Bayesian statistics to analyze multiple copy number hypothesis tests and performs a Maximum Likelihood Estimate (MLE) over the various predicted allele distributions to meet.

The NATUS algorithm considers parental genotypes, HapMap crossover frequency data, and possible fetal chromosome copy number to calculate expected allele distributions for a large number of hypothetical parental trios and ploidy states (A-B). It then calculates a likelihood for each hypothesis by comparing the various predicted allele distributions to the actual allele distributions (A+B). The algorithm calculates the log likelihood for each hypothesis corresponding to the three ploidy states (monosomy, disomy, or trisomy) based on the sequencing data (D), and calls the ploidy state with maximum likelihood as the actual ploidy state, also giving the fetal fraction in the Maximum Likelihood estimation (E).

Methods

The next-generation aneuploidy test using SNPs (NATUS) algorithm

The NATUS algorithm identifies all whole chromosome abnormalities on chromosomes 13, 18, 21, X, and Y with sensitivity of 100% at all gestational ages. This method also obviates the need for a second sample; greater than 90% of these redraws are expected to give a result. Other methods are more likely to make a wrong call in this region.

Advantages of Redraws

Overall, 45 / 763 (5.9%) samples did not pass NATUS’s quality control thresholds, and could have had a second sample requested had they been clinical samples. The NATUS QC thresholds are significantly more sophisticated than other reported methods, and include not only the number of targeted reads and fetal fraction, but also metrics indicative of the amount of input DNA, the presence of haplotype blocks due to consanguinity or multiple gestations, uninformative SNPs, possible contamination, and fetal fraction performance. Data are processed on the NATUS algorithm and sample is called aneuploid with giving NATUS unprecedented insight into the DNA composition of each sample.

Conclusions

Panorama™/NATUS®-targeted analysis of SNPs represents a novel method for non-invasive prenatal aneuploidy testing. Here, the NATUS method identifies chromosome copy number at chromosomes 13, 18, 21, X, and Y, detecting T13, T18, T21 with 100% sensitivity; 45,X and 47,XX with 92% sensitivity, and 100% specificity for all samples that passed the quality test. The method also detects 47,XXY, 47,XY, and triploidy (data not shown) and is expected to be able to detect sub-chromosomal abnormalities. This method also obviates issues with amplification variability caused by GC bias, and generates a more powerful sample-specific calculated accuracy for samples with fetal fractions of cfDNA. Together, this holds promise for a non-invasive screening test with unparalleled accuracy and scope, close to the ceiling of accuracy defined by mosaic.

References