Non-Invasive Prenatal Aneuploidy Testing of Chromosomes 13, 18, 21, X, and Y Using Targeted Sequencing of Polymorphic Loci

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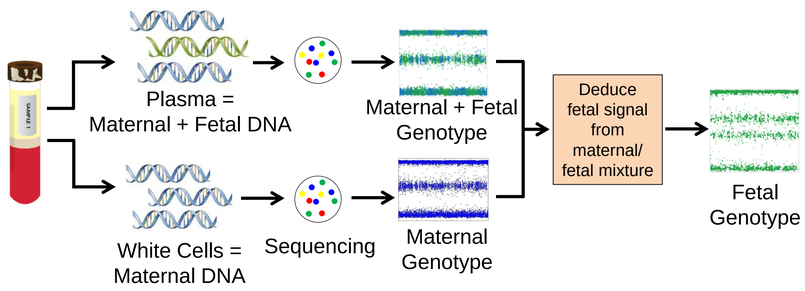
Abstract

Objective: To develop a non-invasive prenatal aneuploidy test based on analysis of cell-free DNA in maternal blood that is capable of detecting all relevant whole chromosomal abnormalities of chromosomes 13, 18, 21, and certain sex chromosome aneuploidies with high accuracy.
Materials and Methods: 407 maternal plasma samples (including 363 euploid and 44 aneuploid samples) were collected from patients at >9 weeks of gestation under an institutional review board (IRB) approved protocol. Cell-free DNA was isolated, and a targeted multiplex PCR amplification of 19,500 loci that includes chromosomes 13, 18, 21, X, and Y was performed. Sequencing data was analyzed using novel Parental Support™ (PS) technology, which includes analysis using the Next-generation Aneuploidy Test Using SNPs (NATUS) algorithm. NATUS employs Bayesian statistics to analyze multiple copy number hypotheses and determine the Maximum Likelihood a posteriori (MAP) hypothesis given the sequencing data. The NATUS method determines confidence, or calculated accuracy, for each sample. Similar confidences are produced for each of the five chromosomes, and can be incorporated into a DNA quality threshold metric to enable draws early in the pregnancy without increasing the probability of false positives or negatives.
Results: The NATUS algorithm yielded a call at all five chromosomes, evaluating the abnormal hypotheses T21, T18, T13, and monosomy X. All calls in this group were correct, for all samples that passed quality control (19 T21, 11 T18, 4 T13, and 4 monosomy X).
Conclusions: NATUS detects fetuses with a chromosomal abnormality from a maternal blood sample with high sensitivity and specificity for T13, T18, T21, 45,X. chromosomes 13, 18, 21, X, and Y. This method calculates a confidence in each call that is personalized for each sample.

Methods

The Parental Support™ NIPT Method



The Next-generation Aneuploidy Test Using SNPs (NATUS) Algorithm

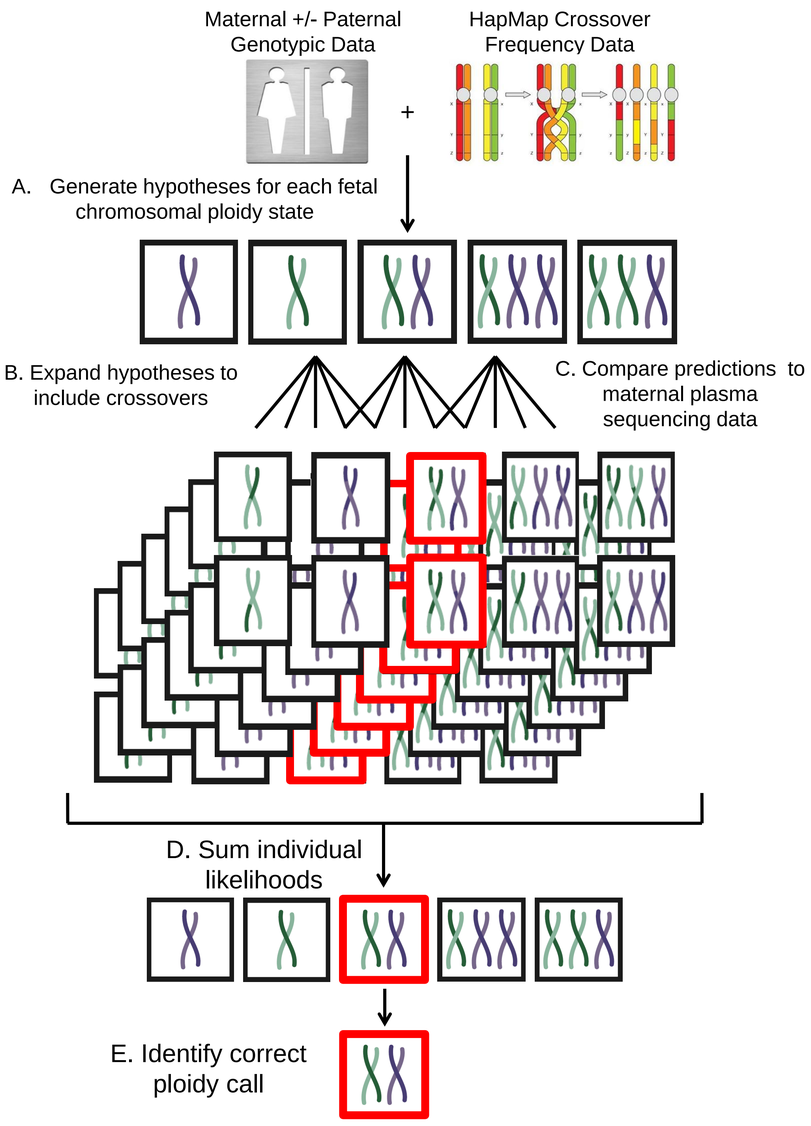


Figure 1: The Parental Support™ Non-Invasive Prenatal Test (NIPT)/NATUS Method. The NATUS algorithm considers parental genotypes, crossover frequency data, fetal cfDNA fraction, and possible fetal chromosome copy number to calculate expected allele distributions for a large number possible fetal ploidy states (A-B). It then compares the various predicted allele distributions to the actual allelic distributions as measured in the cfDNA sample (C), sums the likelihoods of each ploidy state hypothesis (monosomy, disomy, or trisomy) based on the sequencing data (D), and calls the hypothesis with the maximum likelihood as the ploidy state and fetal fraction (E).

Benefits of Maximum Likelihood Estimation

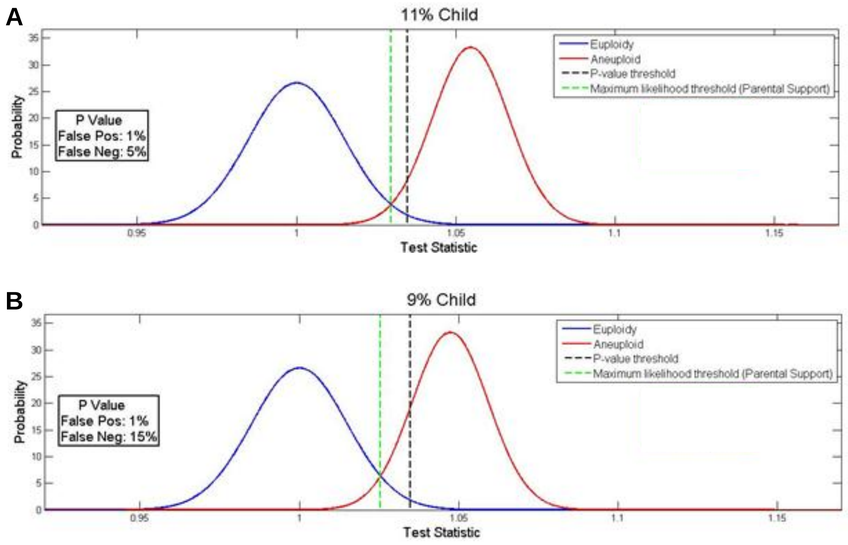
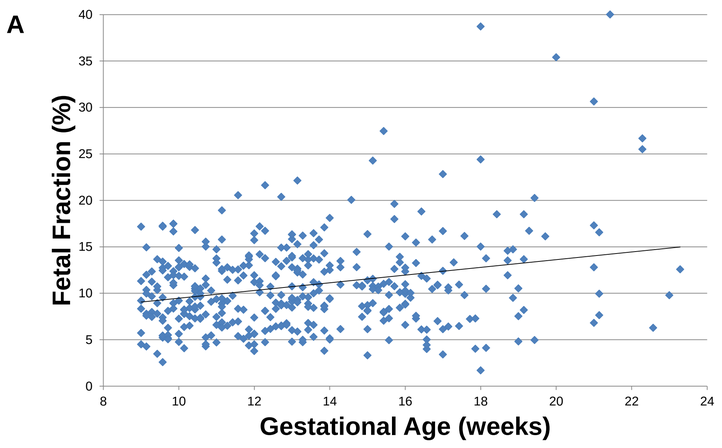
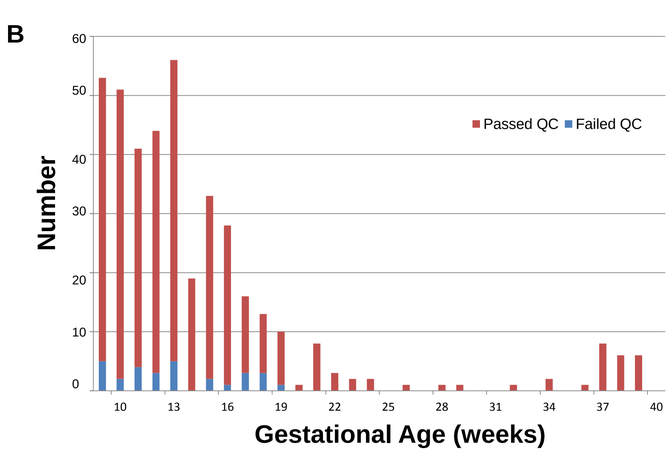


Figure 2: Importance of using Maximum Likelihood Estimation. P-value calculations versus Maximum Likelihood Estimates at **(A)** high and **(B)** low fetal fractions. P-value thresholds (black dashed lines) are determined based on single hypothesis rejection tests, which only consider euploid distributions (blue curves), and by definition do not take into account aneuploid distributions (red curves). This results in sub-optimal thresholds that are readily visualized when the aneuploid distributions are overlaid; the optimal threshold for minimizing false positive and false negative calls in indicated by green dashed lines. This is especially important at low fetal fractions, where using a single-hypothesis rejection-based method results in much higher false negative rates. Thus, Maximum Likelihood Estimation improves call accuracy, flags likely miscalls (especially at low fetal fraction), and is critical for testing at early gestational age, when fetal fractions are typically low.

Results

NATUS Performance





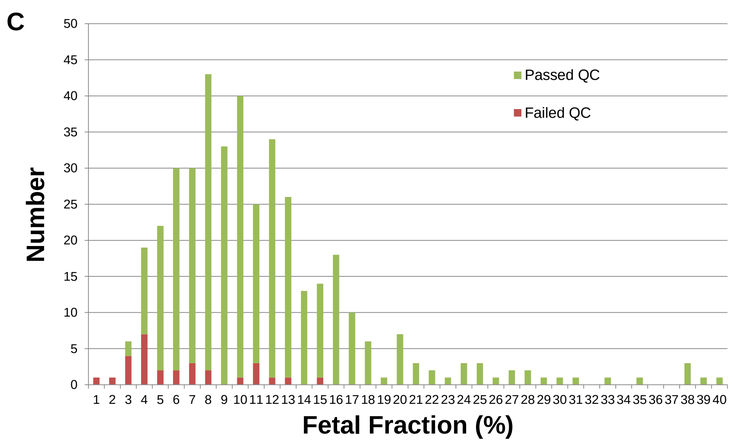


Figure 3: The NATUS method, samples and performance. **A.** Fetal fraction plotted as a function of gestational age. **B.** Histogram of the 407 samples stratified by gestational age. Average gestational age = 14.9 weeks. **C.** Histogram of the 407 samples stratified by fetal fraction, including 363 euploids and 44 aneuploids. Average fetal fraction = 11.8%. Samples that passed quality control are indicated in green and samples that failed quality control are indicated in red. All samples that passed QC (381/407) returned >99% accuracy for all 5 chromosomes, for 1905/1905 correct copy number calls. Truth was verified on all samples by karyotype of amniocentesis, CVS, cord blood, or child buccal samples.

NATUS Performance

NIPT/NATUS Sensitivity and Specificity (1,2)

	Sensitivity	Specificity
T13	100% (4/4)	100% (377/377)
T18	100% (11/11)	100% (370/370)
T21	100% (19/19)	100% (362/362)
45,X	100% (4/4)	100% (374/374)

False Positive Rate: 0%
False Negative Rate: 0%
Redraw Rate, Overall: 7.1% (5.1% with father, 8.8% without father)

Note that this methodology identified Klinefelter (47,XXY) and 47,XYY in a previous dataset .[1,3] However, this sample cohort was not analyzed for and did not include any sex chromosome trisomies.

Graphical Representation of NATUS Results

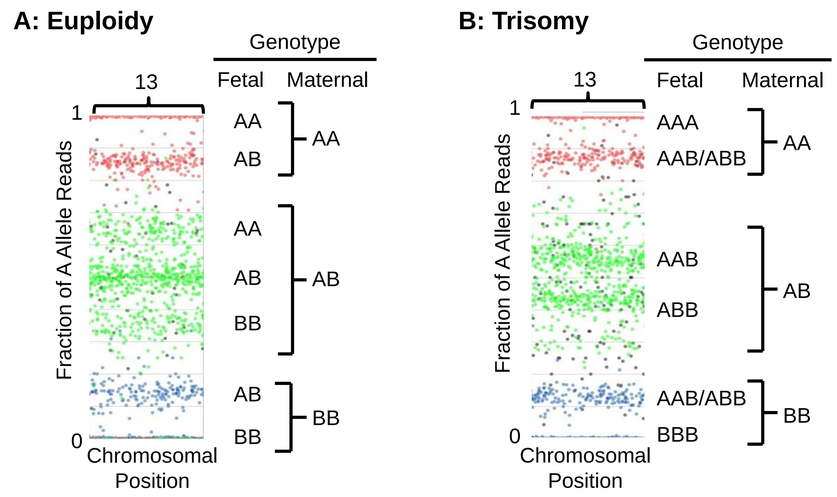


Figure 4: The NATUS-generated data presented in a simplified fashion as plots depicting the ratios of the two most likely alleles, labeled as A and B. Note that this is not how the algorithm makes ploidy calls, but is one method for visualizing the data. X-axes: (A allele reads)/(Total allele reads). Y-axes: Linear SNP position along each chromosome, as indicated above the plots. Fetal and maternal genotypes are indicated to the right. Each spot represents the sum of maternal and fetal cfDNA A allele read proportion. Red: maternal AA alleles, Blue: maternal BB alleles, Green: maternal AB alleles. **A.** The typical pattern depicting euploidy on chromosome 13. The center trio of green clusters and presence of red and blue peripheral clusters indicate the presence of two chromosomes. **B.** The typical pattern depicting trisomy. This plot depicts a single trisomic chromosome 13. The peripheral clusters are largely unaffected. However, the center trio of clusters has condensed into a duo of clusters. Together, this indicates the presence of three chromosomes.

The Importance of Increased Clinical Coverage

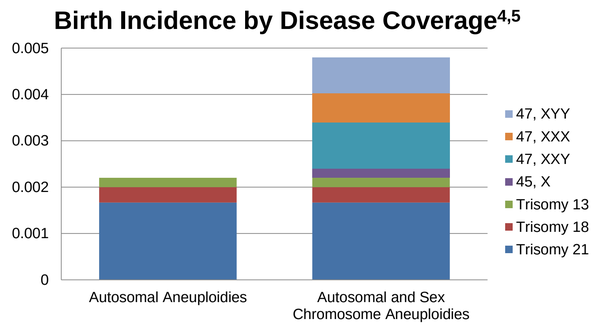


Figure 5: The combined at-birth incidence of sex chromosome anomalies is higher than that of the autosomal trisomies. There are currently no routine screening methods to detect sex chromosome anomalies.

Conclusions

Parental Support™ NIPT/NATUS-targeted analysis of polymorphic regions of the genome represents a novel method for non-invasive prenatal aneuploidy testing. Here, the NATUS method identified chromosome copy number at chromosomes 13, 18, 21, X, and Y, detecting T13, T18, T21, and 45,X with 100% sensitivity and 100% specificity for all samples that passed the quality test. The method also detects 47,XXY and 47,XYY (data not shown) and is expected to detect triploidy and copy-number neutral abnormalities. This method also obviates issues with amplification variation and generates a more powerful sample-specific calculated accuracy for samples with low fetal fractions of cfDNA. Together, this holds promise for the development of a non-invasive screening test with scope comparable to current invasive testing.

References

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