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 chromosome abnormalities of chromosomes 13, 18, 21, and certain sex chromosome aneuploidies with high accuracy.

Materials and Methods: 407 maternal blood 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 hypothesis tests (MAP) and can be incorporated into a DNA quality threshold metric to enable determining confidence, or posterior (MAP) hypothesis given the sequencing data. The NATUS method determines confidence, or calculated accuracy, for each sample. Similar confidence scores are produced for the five of five chromosome.

Results: The NATUS algorithm yielded a call at all five chromosome loci, evaluating the abnormal hypotheses T21, T18, T13, and monosomy X. All calls in this group were correct, for all samples that passed quality control (T21, T18, T13, 45, X) and 34 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. This analysis identifies false positive and false negative calls in all cases. NATUS can be used in protocol.

Methods

The Parental Support™ NIPT Method

White Cells = Sequencing = Maternal + Fetal DNA = Genotype = Material + Parental Genotypes = A = Expand hypotheses to include crossovers. B = Generate hypotheses for each fetal chromosome. C = Compare predictions to maternal plasma sequencing data. D = Sum individual likelihoods. E = Identify correct ploidy call.

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

A = Generate hypotheses for each fetal chromosome ploidy state. B = Expand hypotheses to include crossovers. C = Compare predictions to maternal plasma sequencing data. D = Sum individual likelihoods. E = Identify correct ploidy call.

Results

NATUS Performance

<table>
<thead>
<tr>
<th>SNP Type</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T13</td>
<td>100% (44)</td>
<td>100% (373/377)</td>
</tr>
<tr>
<td>T18</td>
<td>100% (11/11)</td>
<td>100% (370/370)</td>
</tr>
<tr>
<td>T21</td>
<td>100% (19/19)</td>
<td>100% (362/362)</td>
</tr>
<tr>
<td>45,X</td>
<td>100% (4/4)</td>
<td>100% (374/374)</td>
</tr>
</tbody>
</table>

Figure 1: The Parental Support™ Non-Invasive Prenatal Test (NIPT) NATUS Method. The NATUS algorithm considers parent genotypes, crossover frequency data, fetal cfDNA fraction, and possible fetal chromosome copy number to calculate expected allele distributions. The method identifies abnormalities in a large number of possible fetal MZ 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 (D), and calls the hypothesis with the maximum likelihood as the ploidy state and fetal fraction (E).

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 expected allele distributions (blue curves), and by definition do not take into account aneuploid distributions (red curves). This results in sub-optimal thresholds that are rarely visualized when the allele distributions are overlaid. The optimal threshold for minimizing false positive and false negative calls is 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 miscalculation errors (especially at low fetal fraction), and is critical for testing at early gestational age, when fetal fractions are typically low.

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. C: Histogram of the 407 samples stratified by fetal fraction, including 34 euploid and 44 aneuploid. 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) reached >95% 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.

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: (allele reads) / (total allele reads). Y-axes: Linear SNP position along each chromosome. The typical pattern depicting trisomy 13 chromosome 13 is depicted in the center trio of green clusters and presence of a 0 and red. The red and blue clusters indicate the presence of two chromosomes. B: The typical pattern depicting trisomy 13. Each plot depicts a single trisomic chromosome 13. The peripheral clusters are unaltered. However, the center trio of clusters has condensed into a dual of clusters. Together, this indicates the presence of three chromosomes.

Figure 5: The combined 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™ NIPPT-NATUS-targeted analysis of polygenic 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, 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 XXX 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.