Accurate Gender Determination Using a Single-Nucleotide Polymorphism-based Non-invasive Prenatal Test

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Abstract

Background: Accurate gender determination is required in the management of certain x-linked diseases, including medical fetal therapy. In addition, discrepant ultrasound and NIPT gender results may result in unnecessary invasive testing and further unnecessary follow up.
Objective: To determine the ability of single nucleotide polymorphism (SNP)-based non-invasive prenatal testing (NIPT) to determine fetal gender.
Methods: Gender was determined as part of a validation study of a SNP-based NIPT method for fetal whole-chromosomal aneuploidy. [1-5] Cell-free DNA was isolated from maternal blood samples, amplified using a 19,488-plex PCR approach, and sequenced. Sequencing results were analyzed using the NATUS algorithm to determine the fetal chromosomal copy number. Male was defined as presence of the Y-chromosome and female defined as lack of the Y-chromosome. The SNP-based NIPT-determined gender was compared to karyotype results obtained from invasive procedures or at birth.
Results: Gender determination was made on 1,005 samples; Y chromosome was detected in 534 cases, no Y chromosome was detected in 471 cases. All gender determinations (100%; 1,005/1,005) made by NIPT matched karyotype. The cohort contained 12 Monosomy X cases correctly identified as female, including one false negative 45,X case called as 46,XX, and one false positive 46,XX case called 45,X.
Conclusions: This SNP-based NIPT can determine gender with high accuracy. The ability to determine fetal gender early in pregnancy can alter clinical management, particularly in cases of sex-linked disorders. Furthermore, reducing discrepancy between NIPT and ultrasound will result in less unwanted invasive procedures and unnecessary follow up testing.

Background

- In a clinical setting, 85% of patients request fetal sex information.[6]
- Discordant fetal sex findings between NIPT and ultrasound requires additional follow-up and possibly further medical workup for both parent and offspring.
- Accurate fetal sex is particularly important for pregnancies at risk for X-linked disorders, including playing a critical role in medical management – for example administration of dexamethasone in the case of congenital adrenal hypoplasia.
- **Incorporation of parental genotypic information uniquely allows SNP-based NIPT to detect maternal and fetal conditions that may otherwise cause an incorrect fetal sex result:**
  - *Maternal Issues:*
    - Maternal aneuploidy
    - Maternal chimerism/mosaicism
    - Organ/tissue transfer from a male donor
  - *Fetal Issues:*
    - Unidentified multifetal pregnancy
    - Vanishing twin pregnancy
  - *Other issues:*
    - Sample swap

Methods

SNP-based NIPT Methodology

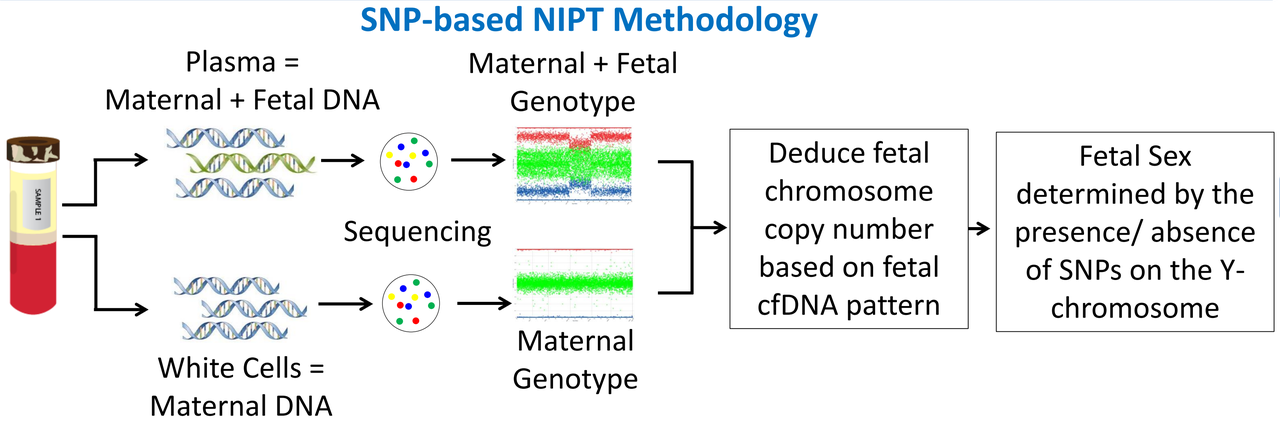

Figure 1: The SNP-based Non-Invasive Prenatal Testing (NIPT)/NATUS Method.
The NATUS algorithm considers parental genotypes, HapMap crossover frequency data, and possible fetal chromosome copy number to calculate expected allele distributions for a large number of hypothetical possible fetal genotypes and ploidy states. The algorithm also determines when cfDNA sequencing results do not match the modeled fetal copy numbers with a high likelihood, and can identify the presence of additional fetal haplotypes that indicate either fetal triploidy or an undetected dizygotic multiple gestation (ongoing or vanishing twins).

Methods

Visualization of SNP Data Used in Fetal Sex Determination

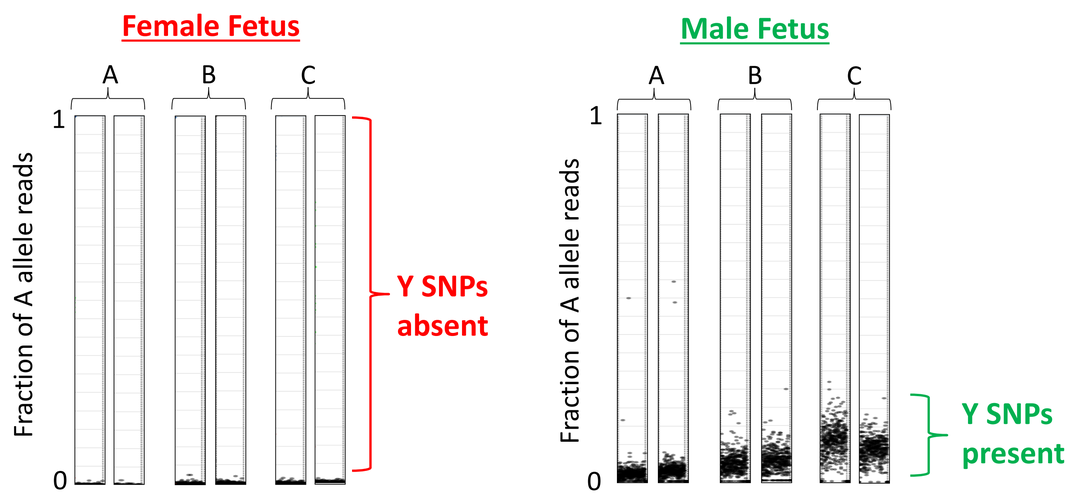

Figure 2: Y-Chromosome SNP data for female and male fetuses. Y-axis indicates the magnitude of measured Y chromosome SNPs. A male fetus is characterized by the presence of a cluster of black dots (SNPs) on the Y chromosome. The absence of Y chromosome dots indicates a female fetus. Representative SNP plots shown at 5% (A), 10% (B), and 20% (C) fetal fractions; the exclusive presence of Y-chromosome SNPs in male fetuses is evident across a range of fetal fractions. For male fetuses, as fetal fraction increases, the spread of the Y-chromosome dots increases and they migrate further up from the X-axis. Note that this is not how the algorithm makes ploidy calls, but is one method for visualizing the data.

Results

Validation Studies: 100% Accuracy for Fetal Sex

		Fetal Sex and Karyotype	
		Male 46,XY; 47,XX +21/+18/or +13	**Female** 45,X; 46,XX; 47,XX +21/+18/or +13
NIPT Call	**Male** 46,XY; 47,XY +21/+18/or +13	**534**	0
	Female 45,X; 46,XX; 47,XX +21/+18/or +13	0	**471**

Table 2: Comparison of NIPT fetal sex results with fetal karyotype.

Conclusions

This SNP-based NIPT accurately determined fetal sex, as defined by the presence or absence of the Y chromosome. Accurate sex determination is required in the management of X-linked disorders. Incorrect fetal sex determination can lead to incorrect identification of sex chromosome abnormalities and gender, resulting in unnecessary diagnostic follow-up testing and potential diagnostic odyssey.

References

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3. Nicolaides KH, Syngelaki A, Gil MD, et al. Prenatal Detection of Fetal Triploidy from Cell-Free DNA Testing in Maternal Blood. Fetal Diagn Ther 2014;35(3):212-7.
4. Samango-Sprouse C, Banjevic M, Ryan A, Sigurjonsson S, Zimmermann B, Hill M, et al. SNP-based non-invasive prenatal testing detects sex chromosome aneuploidies with high accuracy. Prenat Diagn. 2013;33(7):643-9.
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