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Commentary

Phenotype-genotype discordance and disorders of sexual differentiation

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Abstract

Noninvasive Prenatal Testing (NIPT) and prenatal ultrasound can identify disorders of sexual differentiation (DSD), although discrepancies between genetic and phenotypic data can complicate diagnoses. This study explores phenotype-genotype discordance within the DSD context, focusing on methodological concerns and biological explanations. Advances in prenatal screening technologies, including cell-free DNA (cfDNA) testing and ultrasound examinations, have improved DSD detection rates. Analysis of a case featuring a 46, XY DSD due to an NR5A1 gene mutation illustrates the importance of integrating cfDNA testing with ultrasound, which enhances detection and early management. The findings underscore the necessity for a multidisciplinary approach in diagnosis and treatment planning, facilitating timely interventions that reduce psychological distress for families. The study recommends refining diagnostic algorithms that combine cfDNA testing and ultrasound to correlate genetic insights with clinical observations, thus improving patient outcomes through informed decision-making and comprehensive care strategies.

Keywords: Phenotype-genotype discordance, Disorders of sexual differentiation, SF1 gene mutation, Molecular genetic testing, Non-invasive prenatal testing, Diagnostic protocols

Introduction

The article "Phenotype-Genotype Discordance and a Case of a Disorder of Sexual Differentiation" describes an infant where discrepancies between genetic sex on NIPT (Noninvasive Prenatal Testing) and phenotypic presentation on prenatal ultrasound led to the eventual diagnosis of a case of DSD. Discordance between the genetic sex identified by NIPT and the phenotype observed on ultrasound can reveal underlying DSD that might otherwise remain undetected until puberty. This commentary explores the phenomenon of phenotype-genotype discordance (PGD) and its methodological and biological explanations. We will also discuss implications for clinical practice and future research.

Advances in Prenatal Screening Technologies

The 2005 Consensus on Disorders of Sex Development (DSDs) developed common terminology enabling advances in the clinical approaches to this diverse set of conditions referred to as DSD [1]. Advances in diagnostic technology have improved the understanding of genetic and phenotypic complexities associated with DSDs, allowing much earlier detection and management of DSD. These advancements allow better patient-centered approaches to improve the quality of life for patients and families. Cell-free DNA (cfDNA)testing, the basis for NIPT, has led to early detection of fetal

sex and common aneuploidies [2]. This testing provides screening but not diagnosis, which must be made by invasive approaches like chorionic villous sampling (CVS) and amniocentesis [3]. Ultrasound examination assesses fetal anatomy, growth, and physiologic markers for well-being [4]. CfDNA testing and ultrasound imaging can combine to improve antenatal detection of previously unrecognized disorders [5]. Bianchi *et al.* discussed how such approaches can reveal differences between genetic and phenotypic sex, enabling the early diagnosis and management of disorders of DSDs [6]. The sensitivity and specificity of ultrasound for detecting fetal gender improve with advancing gestational age. While ultrasound helps confirm normal anatomy, its effectiveness depends on technical considerations, including maternal acoustics, the quality of the ultrasound system, and the training and experience of the sonographer and reading physician [7,8]. The transition from earlier screening methods, like the serum quadruple marker screen, which evaluated placental and fetal markers, to cfDNA testing has broadened the range of detectable conditions and improved the sensitivity, specificity, and positive predictive value for detecting aneuploidies such as trisomy 21, 18, and 13, along with sex chromosome aneuploidies. Some genetic conditions will remain undetected even with the newer technology [9]. cfDNA testing can identify fetuses with potential PGD, which may allow the detection of DSDs before birth [10,11]. Early detection of PGD enables timely evaluation, targeted genetic counseling, and diagnostic follow-ups. This approach allows healthcare providers to develop comprehensive management plans that address the medical and psychosocial needs of both the child and family when a DSD is found. It facilitates earlier interventions that can alleviate the physical and psychological challenges typically associated with delayed diagnoses and provides families with more time for psychological adjustment and preparation for post-birth treatments. Moreover, it diminishes the psychological distress caused by uncertainties about the child's health condition, sparing families the anxiety of awaiting critical information after birth. Several methodological factors can contribute to phenotype-genotype discordance. Smet *et al.* estimate the discordance rate to be 1 in 1,500 to 2,000 pregnancies [3]. According to Migeon *et al.*, 36% of such discrepancies were found to be associated with DSD cases. Based on their findings and considering approximately 4 million babies born per year in the United States, we can extrapolate that there may be roughly between 90 and a couple hundred cases of DSD per year potential detected by phenotype-genotype discordance [12]. Human error remains a significant factor in medical diagnostics, including discrepancies between NIPT and ultrasound results [13]. During prenatal testing, errors may occur in sample collection, labeling, transportation, and analysis [14]. Stringent quality control measures can reduce the frequency of human error in sample acquisition practices and laboratory reporting, but discrepancies can still arise from limitations of ultrasound technology and the sonographer's skill level [15]. The biological explanation for phenotype-genotype discordance during prenatal testing includes conditions such as transplanted organs from the opposite sex, maternal neoplasms, and co-twin demise, which introduce extraneous genetic material into the maternal bloodstream, leading to discordant NIPT results [16,17]. Placental chimerism and confined placental mosaicism, where different genetic cell lines exist within the placenta, can also confuse the interpretation of NIPT and ultrasound findings [18,19].

Fetal Microchimerism

Fetomaternal microchimerism plays a role in both obstetric and postnatal maternal health, and it can cause a multigenerational exchange of genetic information and signaling between the fetus and mother. However, the quantity of fetal cells in maternal circulation is small compared to the placentally derived oligonucleotide fragments providing the basis of NIPT. Bianchi and others have described bidirectional trafficking of cells between the mother and fetus. Microchimeric cells, detectable by six weeks' gestation can persist for years and influence maternal and fetal health beyond the gestational period [20]. In the 1800s, the first descriptions of this phenomenon were presented as placental cells were identified in the lungs of women who had died from eclampsia. In the 1970s, research identified male-derived fetal cells in maternal circulation of women carrying male fetuses. Recently, Sedov *et al.*, highlighted the roles of microchimeric cells in tissue repair and immune system modulation [21]. Initial attempts at prenatal diagnosis using intact cells in maternal serum failed due to the long-lasting nature of the cells and the difficulty of amplifying limited quantities of fetal cells in the maternal serum. The introduction of cfDNA solved all such problems.

Cell-Free DNA Testing Evaluation

cfDNA testing provides a non-invasive method to assess fetal genetic material early in pregnancy. It uses technologies like Massively Parallel Sequencing (MPS) and Single Nucleotide Polymorphism (SNP)-based methods. MPS can detect chromosomal aneuploidies, such as trisomy 21, 18, and 13, by comparing the proportion of sequenced cfDNA from each chromosome against a reference chromosome compliment, looking for statistically improbable excess or deficiencies relative to the reference set. The sensitivity and specificity of MPS for detecting these aneuploidies fall between 95.7% and 99.9%, with a false positive rate of less than 1%, making it a reliable choice for early prenatal screening [22,23]. Unlike MPS, SNP-based methods target specific genetic loci that vary among individuals. This method compares variability between parental genotypes and fetal genotypes from the maternal blood. By comparing known maternal and paternal SNPs to those found in cf DNA, SNP-based tests can identify paternal alleles in the fetus, offering insights into more than just aneuploidies. This can be particularly useful in diagnosing certain genetic conditions not identifiable by MPS alone [24]. cfDNA fetal fraction is the percentage of cfDNA in maternal blood derived from the placenta representing the fetal genome. In a typical 10 cc sample of maternal plasma, there are approximately 30 million oligonucleotide fragments, which comprise a mixture of maternal and placental DNA. Of this total DNA, fetal cell-free fetal DNA generally accounts for about 1.4% to 5.4% in the first trimester [25]. Each laboratory has an internal criterion for the minimum fetal fraction needed for informative results, with higher fractions (generally above 4%) associated with increased screening reliability. Several factors influence the fetal fraction, including maternal weight, gestational age, and certain pathological conditions. Bioinformatics tools play a role in adjusting for these variables to enhance the robustness of the screening process [26].

Case Discussion

Snipes *et al.* present a PGD case involving a 46, XY disorder of sexual differentiation attributed to a mutation in the NR5A1 gene. In the case description, a NIPT initially identified the fetus as male through NIPT at 12 weeks of gestation, but a 20-week anatomic ultrasound examination showed the fetus exhibited female external genitalia [27]. Subsequent postnatal genetic testing confirmed a mutation in the NR5A1 gene [28,29]. Steroidogenic factor 1 (SF1,

NR5A1) is a nuclear receptor that plays a role in the development and function of the adrenal gland and gonads [30]. Mutations in the NR5A1 gene can result in a range of phenotypes from complete sex reversal to milder forms of DSD. Variants in NR5A1 have been linked to a wide phenotypic spectrum ranging from 46, XY gonadal dysgenesis to milder forms of disorders of sexual development and infertility. These mutations can disrupt normal androgen synthesis, which is essential for typical male sex differentiation and the progression of puberty. Patients with NR5A1 mutations often experience incomplete or atypical pubertal development, which may lead to infertility due to impaired gonadal function. Recent research suggests that phenotypic variability in patients with NR5A1 mutations might be explained by additional genetic factors. For instance, Mazen *et al.* identified two pathogenic NR5A1 mutations in patients with 46, XY gonadal dysgenesis. Interestingly, one of these patients also carried a missense mutation in the MAP3K1 gene, suggesting possible digenic inheritance, which may contribute to the observed variability in clinical presentation. This highlights the complexity of DSD cases associated with NR5A1 mutations, where interactions between multiple genes involved in sex determination pathways may modulate the severity of androgen insufficiency, further complicating the onset of puberty and fertility outcomes [31]. Effective communication and clinical information handoff across medical teams improve the management of such cases. After birth, the pediatric team assigned the child a female gender based on the external genitalia; however, antenatal discordance findings between phenotype and genotype led to consultation with the pediatric endocrinologist that led to a reassessment of the child's condition [32]. This interdisciplinary approach produced a correct diagnosis, underscoring the importance of clinical teamwork when dealing with PGDs [33].

Clinical and Diagnostic Implications

Prenatal DSD detection and diagnosis can improve patient outcomes by enabling healthcare providers to offer appropriate interventions and support to affected families, reducing the potential for psychological distress and improving overall quality of life [34]. Identifying DSD *in utero* or at birth allows for a proactive, multidisciplinary approach that integrates medical, surgical, and psychological care from an early stage, providing families ample time to understand the diagnosis and prepare for future decisions. In contrast, diagnosis at puberty often presents a more reactive scenario, where medical intervention may be urgent, and the adolescent may face greater emotional and identity challenges due to the delayed diagnosis, potentially complicating both medical management and psychosocial outcomes [35]. Comprehensive genetic testing, including gene panels and exome sequencing, can provide precise diagnoses, guide clinical management in many cases [36], and facilitate early interventions [37]. Many clinical labs offer gene panels that range from a couple of dozen to more than 200 candidate genes. In addition to known pathogenic variants, there will be novel mutations of uncertain pathogenic significance, such as those seen in the Snipes report, which will need to be explored to assess their likely contribution to the phenotype. Genetic databases such as Varisome are helpful in identifying other cases with a similar mutation. As our understanding of the complex interplay between multiple genes in the pathways of sexual differentiation evolves, there is a better understanding of the nuances of DSD cases present. Examples of such clinical nuances come from case reports of siblings inheriting the same mutation but exhibiting different phenotypes. However, as genetic testing advances, researchers must consider the tests' ethical, legal, and social implications. Genetic panels and exome sequencing explore potential underlying genetic causes of suspected DSD cases [38]. These are complex tests best interpreted by specialists in these disorders and may be considered during the antenatal or neonatal assessment of PGD cases. Integrating exome sequencing with targeted gene panels has advanced diagnostic accuracy in DSD [39]. These genetic tools facilitate the analysis of a broad spectrum of genes related to sexual development, significantly enhancing the likelihood of identifying common and rare pathogenic variants that traditional methods might overlook [40,41]. Before delivery, a PGD will often lead to invasive testing, and a structured sequence of genetic tests can be offered, starting with a karyotype analysis, can detect chromosomal abnormalities. Recommended assessments include reflex testing to Chromosomal Microarray Analysis (CMA), along with specific gene testing for the SRY gene, which is involved in sex determination and can help evaluate PGD. Further exploration with targeted gene panels, such as those assessing genes involved in gonadal development and adrenal function, is available after consultation with genetics and pediatric endocrinology if the initial investigative results are uninformative or ambiguous [42]. Once identified, pediatric endocrinologists must be involved early in the diagnostic process of DSD, assessing, and managing hormonal balance and endocrine function, and proscribing life-threatening electrolyte imbalances such as those seen in congenital adrenal hyperplasia (CAH). Urology should evaluate for anatomical urinary abnormalities and subsequently, psychologists should help support the patient's and family's mental health [43-46].

Broader Implications and Future Directions

Current research indicates shifting trends in sex assignment practices for infants with DSDs and the need for flexible, patient-centered care that prioritizes both medical outcomes and psychosocial development [30]. Ultimately, well-crafted policy frameworks can optimize clinical outcomes and maintain high ethical standards amidst the rapid evolution of genetic diagnostic technologies [47]. The resulting collaborative care model in DSD management will help the family navigate issues like gender dysphoria, hormonal medication management, gonadal management, addressing cancer risk factors, and sex assignment practices [40]. The ACCORD alliance offers standardized information and support for families facing DSDs, providing resources that help manage the psychological distress associated with these diagnoses, including confusion, anxiety, and depression [48,49].

Algorithm

1. **History and physical examination**

a. **Medical history**: Gather detailed family and personal medical history to identify potential genetic patterns.

b. **Physical examination**: Conduct a thorough physical assessment for DSD-associated traits.

2. **Laboratory and prenatal screening**

a. **Routine ultrasound**: Perform during the first trimester to check for physical indicators of DSDs.

b. **NIPT (Non-invasive prenatal testing)**: Screen for chromosomal anomalies and potential PGD.

c. **Invasive tests (if indicated)**: If anomalies are suspected or

patient desires to proceed with definitive amniocentesis or CVS for detailed fetal genetic analysis.

3. **Genetic analysis**

a. **Initial screening**: Utilize targeted gene panels focusing on mutations known to cause DSDs, such as SF-1 (NR5A1).

b. **Extended analysis**: If initial results are inconclusive, proceed with whole exome or genome sequencing.

c. **Bioinformatics**: Apply advanced computational tools to interpret genetic data and predict phenotypic impacts.

4. **Interpretation of results**

a. **Diagnostic integration**: Combine results from all tests to form a comprehensive understanding of the genetic and phenotypic data.

b. **Multidisciplinary consultation**: Discuss findings with a team including endocrinologists, geneticists, and ethicists to evaluate the implications.

5. **Plan and decision-making**

a. **Counseling**: Provide detailed genetic counseling to explain the nature of DSDs, test results, and potential outcomes.

b. **Shared decision-making**: Engage with the family to decide on further diagnostics, management options, and long-term care strategies.

6. **Postnatal confirmation and follow-up**

a. **Confirmatory testing**: Verify prenatal diagnoses with postnatal evaluations.

b. **Management plan development**: Create a personalized care plan based on confirmed diagnosis, including hormonal, surgical, and psychological support.

7. **Ongoing support and monitoring**

a. **Educational resources**: Offer continuous education and support to the family.

b. **Support group facilitation**: Help the family connect with relevant support groups and participate in research opportunities if interested.

Conclusion

The exploration of PGD in prenatal screening and the integration of cfDNA testing and detailed ultrasound evaluations have enhanced our diagnostic accuracy and emphasized the necessity for a multidisciplinary approach to managing DSDs. By aligning genetic insights with phenotypic observations, healthcare providers can devise comprehensive management plans that cater to the condition from the earliest stages of detection. Furthermore, transitioning from traditional screening methods to more sophisticated genomic analyses such as whole exome and genome sequencing advances our understanding. It enables the detection of nuanced genetic variations that contribute to DSDs. This shift necessitates ongoing adjustments to clinical guidelines and ethical frameworks to address the evolving landscape of prenatal diagnostics, ensuring that such advancements improve patient outcomes while adhering to high moral standards.

As research continues to advance our capabilities in genetic testing, it is important to integrate these findings with clinical management to enhance the quality of life for individuals with DSDs and their families.

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