Routine NIPT: the rise in fetal sex discordance and earlier diagnosis of disorders of sex development

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ABSTRACT

In Belgium, routine non-invasive diagnostic testing (NIPT) has led to a rise in discordant results between the genetic sex, obtained via NIPT, and the phenotypic sex assessed by ultrasound. This has improved prenatal diagnosis of disorders of sex development (DSD). Here we report on the nine cases of fetal sex discordance encountered in our fetal medicine unit at the CHU de Liège since we began doing NIPT routinely, and share some established protocols to facilitate the management of such discordances. Of the nine discordant cases included in this report, six turned out to be DSD: two cases of 46,XX and four cases of 46,XY DSD. DSDs are still rare, complex conditions and an early diagnosis is a great advantage in clinical practice. Discovering that a fetus has a DSD is a difficult clinical situation, both medically and psychosocially, and being able to perform early prenatal diagnosis and to pre-arrange the various tests needed at birth is invaluable.This highlights the importance of developing and following well-codified multidisciplinary diagnostic protocols in order to improve overall care for both fetus and parents.

KEYWORDS

Routine NIPT, disorder of sex development, 46,XX DSD, 46,XY DSD, prenatal diagnosis, management protocol.

Introduction

Since its introduction into clinical practice in 2011, large-scale non-invasive prenatal diagnosis (NIPT) has become the norm in many countries [1,2]. In Belgium, NIPT has been available and reimbursable for all pregnant women since July 2017. It is offered routinely from 12 weeks of gestation as a first-line screening test for trisomies 21, 18, and 13; it also reports the sex of the fetus. Based on analysis of cell-free fetal DNA in the mother's blood, it is reliable after 12 weeks of gestation. Routine NIPT has led, in recent years, to a rise in the number of reported discordances between the genetic sex – as determined by this test – and the phenotypic sex – as assessed during the second-trimester anatomy ultrasound scan. This, in turn, has significantly increased prenatal diagnosis of disorders of sex development (DSD). Note however, that such mismatches can have other causes such as a maternal history of transplantation, vanishing twin syndrome, or even a laboratory error, which can lead to an erroneous NIPT result. This highlights the importance of having a precise, well-established diagnostic process to ensure appropriate management [3].

DSD is any abnormality in the appearance of the genitalia of the fetus or any mismatch between the genetic, gonadal, and phenotypic sex [4]. We call it a 46, XX DSD when this occurs in a fetus that is genetically female and a 46, XY DSD when the fetus is genetically male [5]. The prevalence of DSD is 5 per 1,000 births, and the most common DSD is hypospadias, which is found in 73% of 46, XY DSD cases [6]. Finding a DSD in a fetus is a difficult clinical situation and requires a rapid and accurate diagnostic process [7]. A multidisciplinary approach is needed to optimize the overall care of both the unborn child and the parents. Caring for these patients is both medically and psychosocially challenging [7,8]. Hence, given the rise in DSD diagnoses with routine NIPT, a precise, well-organized management protocol is essential to preventing diagnostic errors or delays that could lead to devastating situations.

This study reports the cases of genetic/phenotypic sex discordance encountered at our CHU de Liège facility since routine NIPT began. The aim is to share our experience with managing such discordant diagnoses, along with some established protocols to facilitate their management.

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Materials and methods

This was a retrospective study that included all cases of mismatch between the genetic sex found using NIPT and the phenotypic sex assessed via ultrasound that were managed in our fetal medicine unit at the CHU de Liège from 2014 to 2021. Based on a review of all digital medical records, we gathered the results from NIPT, anatomy scans, and the various (invasive and non-invasive) diagnostic tests performed pre- and/or postnatally.

Results

Nine cases of discordance between the NIPT-determined sex and the sonographically- evaluated sex were identified (Table 1). All of the NIPTs yielded normal screening results for trisomies 21, 18 and 13. In three cases the fetal genetic sex came back female, and in the other six cases it came back male.

In two of the XX fetuses determined by NIPT (Cases 1 and 2) the anatomy scan showed male sex with a normal-size penis and no other abnormalities.

In Case 1, repeating NIPT led to the conclusion that a laboratory error had occurred, and the final result was XY genetic sex.

In Case 2, a second NIPT confirmed a XX genetic sex. Then, an amniocentesis with quantitative fluorescent polymerase chain reaction (QFPCR), array-comparative genomic hybridization (array CGH) and testing for the gene mutation responsible for congenital adrenal hyperplasia (CAH) was performed. QFPCR was positive for the SRY marker. Next, SRY probe-targeted fluorescent *in situ* hybridization (FISH) and array CGH were done and confirmed the presence of the SRY gene linked to a t(X;Y)(p22.3;p11.2) (SRY+) translocation in a 46,XX fetus, indicating a probable De La Chapelle syndrome (or XX male syndrome), which would explain the mismatch between the genetic and phenotypic sex of the fetus.

In case 3, a XX fetus was determined with NIPT, and the anatomy ultrasound scan showed labia minora hypertrophy with what appeared to be central fusion of the labia at the perineum (Figure 1). Second-line diagnostic ultrasound helped confirm the isolated nature of the genital abnormality. This guided the diagnostic process to look first at the history for an extrinsic iatrogenic cause of virilization. An intrinsic source of virilization was ruled out by measuring the mother's androgen levels. That left two other differential diagnoses: CAH or hypertrophy as a simple normal variant. The patient did not consent for amniocentesis. The Guthrie card test, which measures the 17-OH progesterone level (among other things) at birth, ruled out CAH and led to the conclusion of simple, isolated labia minora hypertrophy.

Of the six fetuses that were XY determined with NIPT, four exhibited phenotypically female genitalia on the anatomy ultrasound scan, with no abnormalities (Cases 4-7).

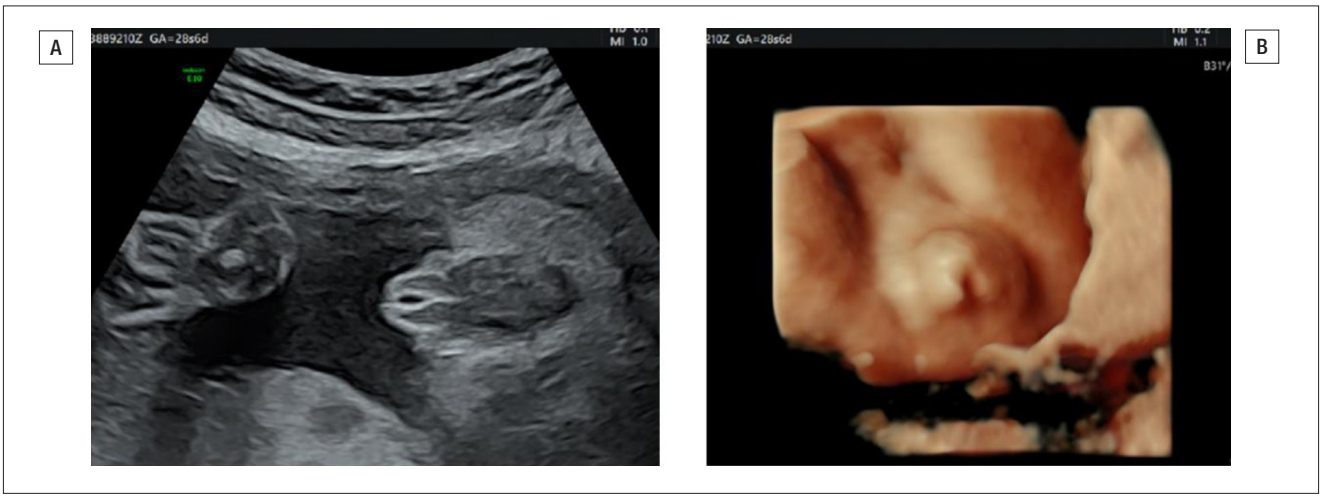
In Case 4, the fetus was from what was initially a twin pregnancy, with early demise of one of the twins at 8 weeks of gestation. The most likely hypothesis to explain the genotype/phenotype discordance was that the NIPT result represented a male vanishing twin, and this was confirmed by a second NIPT

Table 1

	Relevant maternal, obstetrical and family history	Genetic sex 1st NIPT / 2nd NIPT		Phenotypic sex assessed during the second-trimester anatomy scan – +/- associated abnormalities	Amniocentesis	Other antenatal diagnostic investigations	Postnatal diagnostic investigations	Final diagnosis of genotype/phenotype mismatch
Case 1	–	XX	XY	Male sex with no abnormalities	–	–	–	**Laboratory error**
Case 2	–	XX	XX	Male sex with no abnormalities	-**QFPCR**: positive for the SRY marker -**SRY probe-targeted FISH + array CGH**: presence of the SRY gene linked to a t(X;Y)(p22.3;p11.2) (SRY+) translocation in a 46,XX fetus	–	–	**De La Chapelle syndrome (or XX male syndrome)**
Case 3	No evidence in favor of extrinsic iatrogenic virilization (e.g. taking testosterone or progestogens)	XX	XX	Labia minora hypertrophy with apparent central fusion at the perineum (Figure 1)	Refused by parents	**Mother's blood test: normal androgen levels**	–	**Simple, isolated labia minora hypertrophy** (The Guthrie card test ruled out CAH)
Case 4	Twin pregnancy with early demise of one of the twins at 8 weeks LMP	XY	XX	Female sex with no abnormalities	–	–	–	**Male vanishing twin**
Case 5	–	XY	Low number of "Y reads", consistent with a female fetus	Female sex with no abnormalities	–	–	–	**Male vanishing twin**

	Relevant maternal, obstetrical and family history	Genetic sex 1st NIPT / 2nd NIPT		Phenotypic sex assessed during the second-trimester anatomy scan – +/- associated abnormalities	Amniocentesis	Other antenatal diagnostic investi-gations	Postnatal diagnostic investigations	Final diagnosis of genotype/ phenotype mismatch
Case 6	–	XY	XY	Female sex with no abnormalities	-**Standard karyotype + array CGH**: normal 46,XY -**Small DSD panel** (SRY, WT1, SF1 et AR): hemizygous mutation in the AR gene	–	–	**Complete androgen insensitivity syndrome (CAIS)**
Case 7	–	XY	XY	Late diagnostic ultrasound at 37 weeks LMP: female sex with no apparent abnormalities	–	–	-**Clinical exam**: sexual ambiguity (clitoromegaly + urethra placed below the clitoral hood) -**Abdominal ultrasound**: presence of uterus but no gonads, and a left renal ptosis -**WT1 gene sequencing** (because of the infant developed severe acute renal failure): heterozygous pathogenic mutation	**Denys-Drash syndrome**
Case 8	-Mother's Type 1 diabetes -Paternal uncle with an intellectual disability who died at age 10 years from unknown causes	XY	XY	"Buried penis" appearance with a visible scrotum and a probable micropenis (Figure 2), IUGR + short but not bowed femurs (<P1)	-**array CGH**: normal 46,XY -**Amniotic fluid steroid levels**: normal -**DHCR7 and SOX9 genes sequencing**: normal -**Small DSD panel**: normal -**Large DSD panel**: normal -**Mendeliome**: normal	–	-**array CGH** : normal 46,XY -**Blood hormone** panel : confirmed minipuberty with a satisfactory testicular response	**Idiopathic DSD with normal testicular function due to a severe IUGR**
Case 9	–	XY	XY	Hypospadias + IUGR	-array CGH : normal 46,XY	–	-array CGH : normal 46,XY -AR gene testing normale -Blood hormone panel : confirmed minipuberty with a satisfactory testicular response	**Isolated hypospadias, which is often associated with IUGR**

Figure 1 2D (a) and 3D (b) ultrasound of case 3 showing hypertrophy of the labia minora with apparent central fusion at the perineum.



done at 25 weeks and 6 days of gestation, ultimately concluding that the fetus was XX.

Case 5 was similar to the previous one, but in this case a second NIPT done at 22 weeks and 4 days of gestation was unable to accurately determine the sex. It did, however, yield a low number of "Y reads", consistent with a female fetus, as the ultrasound suggested. The assumed etiology was thus a male vanishing twin.

In Case 6, follow-up NIPT confirmed a genetic male. Amniocentesis with a "small" DSD panel (sequencing the genes most often responsible for 46,XX DSD, i.e., SRY, WT1, SF1 and AR) showed a mutation, during the hemizygous state, in the androgen receptor (AR) gene, resulting in the diagnosis of complete androgen insensitivity syndrome (CAIS).

The Case 7 patient was referred to us late, at 37 weeks of gestation, for management of a genotype/phenotype discordance. The ultrasound done upon admission showed no other abnormalities and confirmed the absence of any evidence suggesting male sex. Diagnostic testing was done, in this case, at birth. The mother delivered a phenotypically female fetus at 38 weeks and 6 days of gestation, although the clinical exam noted sexual ambiguity. Abdominal ultrasound confirmed that the infant had a uterus but no gonads, and a left renal ptosis. In the immediate postnatal period, the infant developed severe acute renal failure (ARF). This combination of signs was suspicious for Denys-Drash syndrome, and WT1 gene sequencing confirmed the diagnosis. After a month of birth, the infant died of ARF.

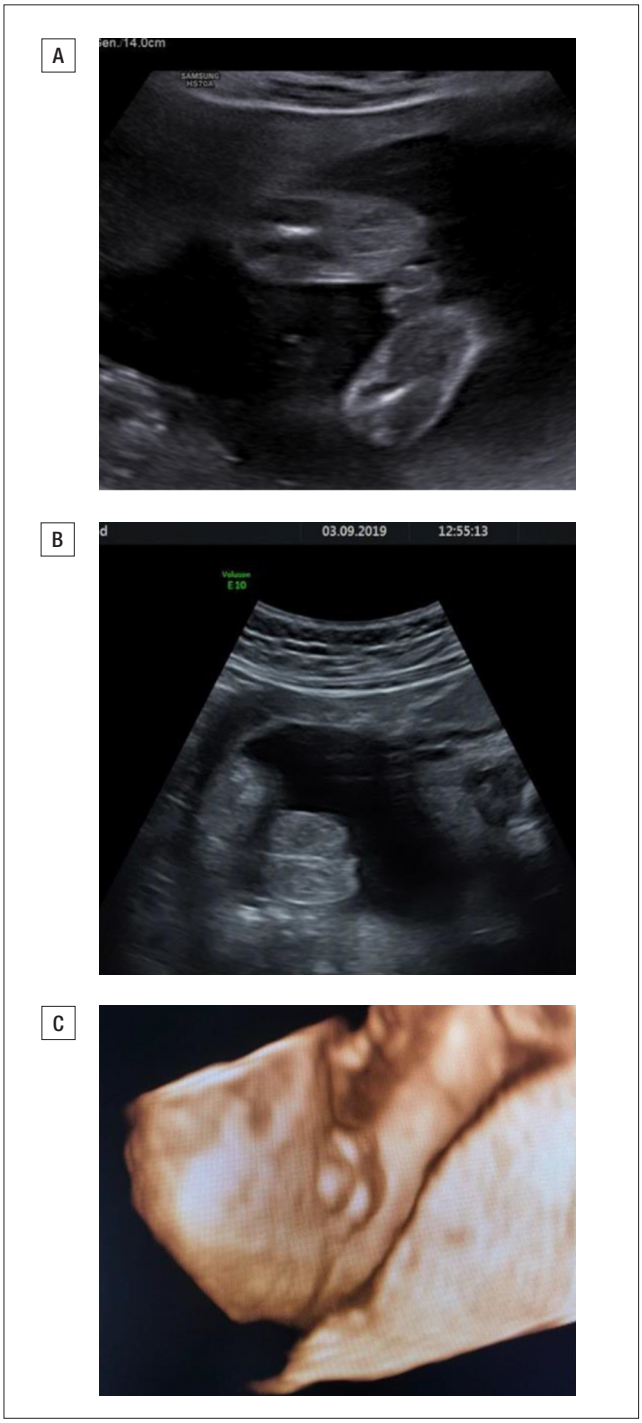
In Cases 8 and 9, (both XY determined by NIPT), the anatomy ultrasound scan showed a combination of intrauterine growth restriction (IUGR) and sexual ambiguity in the form of a "buried penis" appearance with a visible scrotum and a probable micropenis (Case 8; Figure 2), and suspected hypospadias (Case 9; Figure 2), raising the possibility of a syndromic origin. Amniocentesis was performed in both cases.

The fetus in Case 8 also had short unbowed femurs (<P1), giving rise to two main diagnostic hypotheses: Smith-Lemli-Opitz (SLO) syndrome and a acampomelic campomelic dysplasia. However, sequencing the genes in question – DHCR7 and SOX9, respectively – ruled these out.

For the other samples (detailed in Table 1), no abnormalities were detected. Similarly, all of the tests performed at birth turned out to be normal, resulting in a diagnosis of idiopathic DSD with normal testicular function due to a severe IUGR of vascular origin most likely related to the mother's type 1 diabetes.

Case 9 was one of the team's first cases of prenatal DSD diagnosis involving a genotype/phenotype discordance (2014). Only an array CGH had been ordered on the amniocentesis sample which showed a normal 46,XY profile. The diagnostic process was predominantly postnatal, with another array CGH (whose results were more complete than the prenatal one) and AR gene testing, which ruled out androgen insensitivity (we know that the AR gene is mutated in some cases of severe hypospadias). Those tests failed to find any abnormalities. A blood hormone panel was also performed on the newborn and rule out gonadal dysgenesis. The final diagnosis was isolated hypospadias, which is often associated with IUGR.

Figure 2 2D (a) and 3D (b) ultrasound of case 8 showing the "buried penis" appearance with visible scrotum and probable micropenis.



Discussion

Of the nine discordance cases included in our study, six ultimately turned out to be DSDs: two cases of 46,XX (Cases 2 and 3) and four cases of 46,XY DSD (Cases 6-9).

To understand the different etiologies of DSD and their new karyotype-based classification (Table 2), it is essential to remember that normal fetal sexual differentiation is a series of four major embryological steps, during which a set of cellular and hormonal signals interact, in a precise order, to create the male or female reproductive system (Figure 3). Therefore, DSDs occur during the embryonic and fetal life, and have five major causes: an abnormal number or structure of sex chromosomes; a mutation in one of the many genes involved in gonadal development; a disturbance in androgen production or activity; intrinsically- or extrinsically-caused virilization by maternal androgens; or the activity of certain endocrine disruptors [6,9].

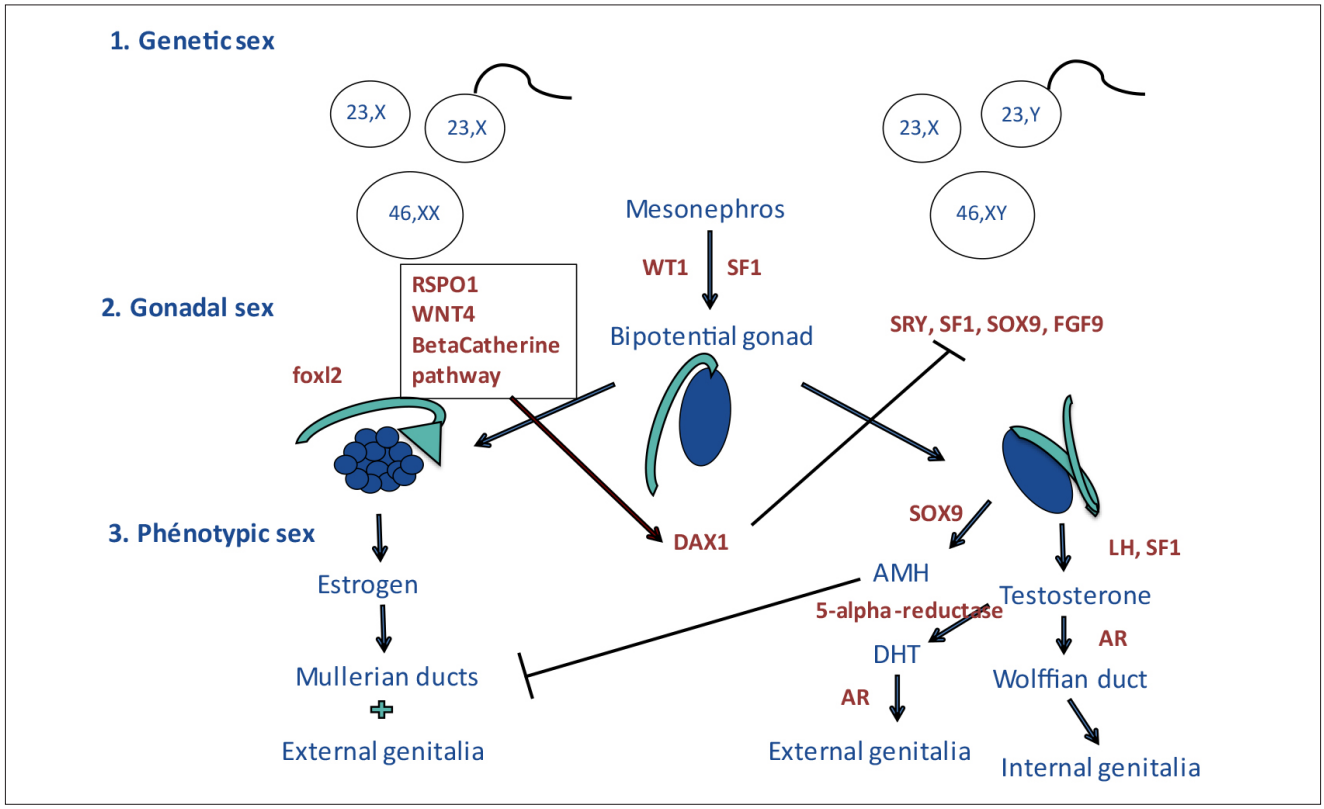
For a long time, most DSDs were not diagnosed until birth, when abnormalities in the newborn's external genitalia became evident [1,7]. This can be explained by the technical difficulty of prenatal ultrasound diagnosis of genital abnormalities, especially when they are isolated and the genetic sex is not known to the sonographer [9,10].

Table 2 An example of DSD classification proposed by the Chicago Consensus [2].

DSD 46, XY	DSD 46, XX	Specificity
A. Disorders of testicular development - Complete or partial gonadal dysgenesis (e.g. SRY, SOX9, WT etc) - Gonadal regression - Ovotesticular DS	A. Disorders of gonadal development - Ovotesticular DSD - Testicular DSD (e.g. SRY+, dup SOX 9) - Gonadal dysgenesis	A. 45,X (Turner syndrome and variants) B. 47,XXY (Klinefelter syndrome and variants) C. 45,X/46,XY (mixed gonadal dysdenesis, ovotesticular DSD) D. 46,XX/46,XY (chimerism, ovotesticular DSD)
B. Disorders in androgen sybthesis or action - Defect in androgen biosynthesis (e.g. 5α-reductase deficiency) - Defect in androgen action (CAIS, PAIS) - LH receptor defect (e.g. Leydig cell hypoplasia) - Disorders of AMH and AMH receptor	B. Androgen excess - Fetal (e.g. 21-hydroxylase deficiency (CYP21A2), 11-hydroxylase deficiency (CYP11B1)) - Fetoplacental (e.g. aromatase deficiency (CYP19) - Maternal (maternal virilizing tumors (e.g. luteoma), exogenous (androgenic drugs)	
C. Other - Cloacal extrophy, severe hypospadias	C. Other - Cloacal extrophy, vaginal atresia, Müllerian agenesis/hypoplasia (e.g. MURCS), other syndromes	

DSD: disorder of sex development; **CAIS**: complete androgen insensitivity syndrome; **PAIS**: partial androgen insensitivity syndrome; **LH**: luteinizing hormone; **AMH**: anti-müllerian hormone; **MURCS**: müllerian duct aplasia; renal aplasia and cervicothoracic somite dysplasia.

Figure 3 Diagram of the steps of sexual differentiation, by Dr. Laterre Marie, from an introduction to the protocol for managing DSDs (the list of genes is not exhaustive).



Hence, it is understandable that the number of DSDs diagnosed prenatally has been rising since NIPT became routine. Indeed, knowing the genetic sex when performing the second trimester anatomy ultrasound scan has – thanks to a quasi-systematic correlation with the observed phenotypic sex – improved prenatal screening for urogenital abnormalities and increased the diagnosis rate for genotype/phenotype discordance. One recent series estimated the incidence of discordance to be 1 out of every 1500 to 2000 pregnancies [3,11].

The increase in antenatal diagnosis of DSD represents a real advantage. Indeed, their earlier detection allows clinicians to anticipate the diagnostic announcement and organize mutidisciplinary care to help families navigate the medical, surgical, social and psychological decisions unique to these diagnoses [3]. Indeed, these situations are always imbued with a heavy emotional charge for the parents of the affected child.

The sex phenotypic prognosis is often imprecise, making it difficult to assign fetal sex at birth. In the most complex situations, the pediatrician can ask the deputy responsible for Civil Status to suspend the declaration of birth (normally compulsory in France within 5 days of the birth) until a multidisciplinary decision determining of sex is taken, with the parents, and in

consultation with the DSD reference centre. Therefore, earlier detection of a sex discordance creates opportunity for additional support and counseling for the family as they make decisions surrounding their child's sexual and gender development. Today, the vast majority of people with DSD remain with the sex assigned at birth [12].

One of the other advantages in the prenatal detection of a sex discrepancy is to be able, in the face of virilization of the female genitalia, to diagnose an early CAH. Indeed, this diagnosis requires a special management and prevention to avoid an adrenal dysfunction. A delivery plan that involves early electrolyte analysis and evaluation by pediatric endocrinology is recommended to avoid salt-wasting crisis. Being able to anticipate this care reduces the stress of the parents and the medical team [3].

Therefore, when ultrasound evaluation finds an abnormality and/or discordance with the genetic sex as determined by NIPT, it is recommended that the patient be referred to an experienced sonographer who will do a second, more complete and detailed, ultrasound anatomy scan. This is one step in the diagnostic process for DSDs, discussed below, and helps guide the various tests to be performed [10].

Management of NIPT/ultrasound discordance

A discordant result between the genetic and phenotypic sex can have many causes. It is therefore important to proceed step by step to examine potential etiologies and yield a diagnosis. Given the rise in such discordances, the CHU de Liège genetics department has developed and implemented a management protocol aimed at standardizing the practices at its facility (Figure 4).

1.Take the medical history, review the prenatal ultrasounds, and collect a second NIPT sample

The first step is to rule out an erroneous NIPT result. The medical history should ask about an organ transplant, a stem cell transplantation or recent (within the past 3 months) blood transfusions, which can interfere with the NIPT by creating an additional source of cell-free DNA, and potentially alter the sex result [13].

First trimester ultrasounds should be reviewed to look for a vanishing twin, since the latter's DNA can persist in the mother's bloodstream for several weeks and cause an erroneous NIPT result when the surviving twin is of the opposite sex. Actually, the period during which a placenta of a deceased co-twin can release cell-free DNA into the maternal bloodstream is not really defined. Some studies report a duration of 7 to 8 weeks but further studies are needed to understand in more detail the dynamics of the vanishing or absorption process and the impact on NIPT results [14].

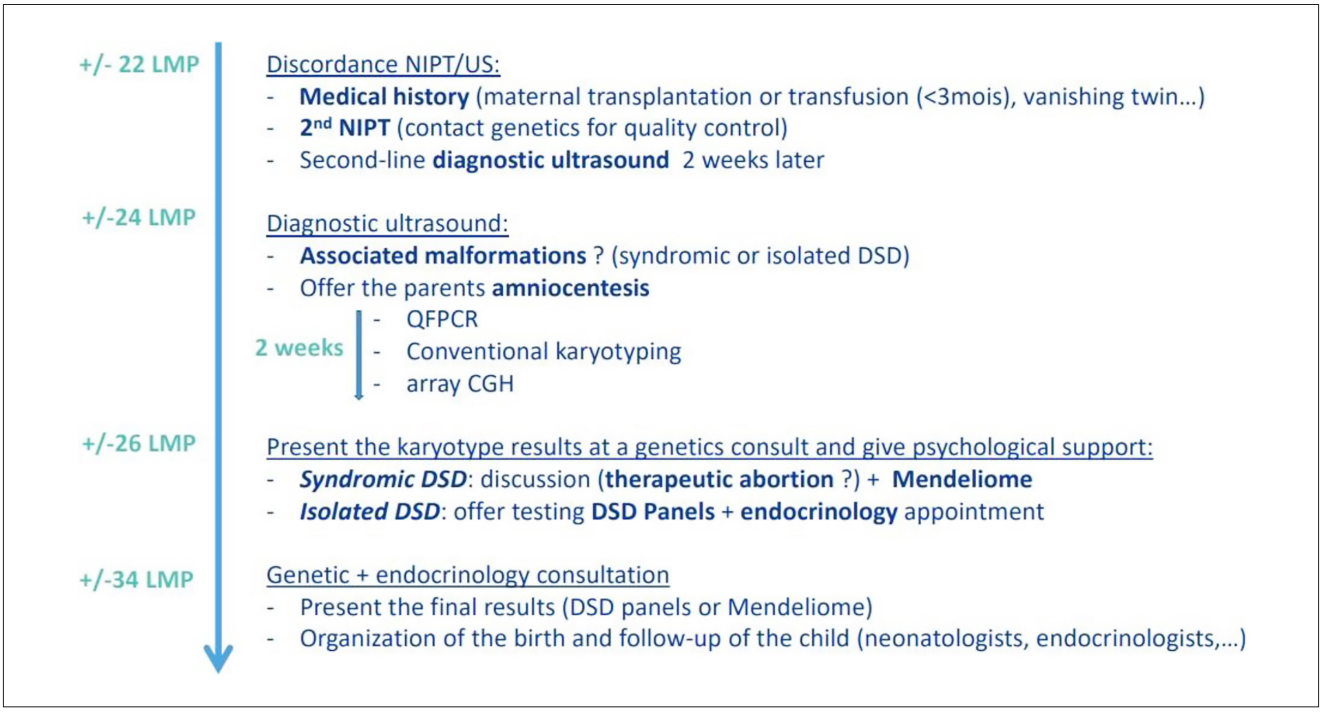
Lastly, a second NIPT sample should be collected to rule out lab error (switched tubes, coding error, etc.) [3].

This first step made it possible to determine the cause of the NIPT/ultrasound discordance in three of the fetuses in our study (Cases 1, 4, and 5). A male vanishing twin was the cause of the discordance in two of the fetuses initially found to be 46,XY on NIPT (Cases 4 and 5). In Case 1, a second NIPT showed that the apparent discordance was in fact a lab error.

2. Diagnostic ultrasound

When the causes discussed in step 1 have been ruled out and a second NIPT has confirmed the initial results, the patient should be referred for a second-line diagnostic ultrasound. An experienced sonographer will perform a detailed anatomical assessment of the genitalia in order to look for any abnormalities

Figure 4 Protocol for managing NIPT/ultrasound discordances, developed by the CHU de Liège Genetics Department



and specify the type. Fetal magnetic resonance imaging (MRI) can sometimes be useful in providing additional, more precise information regarding the anatomy of the external and internal fetal genitalia [15]. The sonographer will also look for other associated malformations, which helps guide the diagnosis toward either an isolated or syndromic origin for the DSD, an important step for deciding what tests should come next.

In our series, in Case 3 (46,XX DSD), the study of the genitalia from the anatomy ultrasound scan revealed a labia minora hypertrophy-type malformation, apparently showing centrally-fused labia at the perineum (Figure 1). A second-line diagnostic ultrasound then confirmed that the genital abnormality was an isolated finding, guiding the rest of the diagnostic process (isolated DSD).

Among the four 46, XY DSD cases (Cases 6-9), ultrasound found a genital malformation in two fetuses (Cases 8 and 9). It also showed severe IUGR in those two fetuses, accompanied, in Case 8, by short, unbowed femurs (<P1), suggesting a syndromic origin and helping to orient subsequent diagnostic testing (syndromic DSD).

3. Amniocentesis

The next step in the diagnostic process is to offer the parents amniocentesis, first to confirm the genetic sex and place the DSD into one of the three main existing subclasses: 46,XX DSD, 46,XY DSD, or sex chromosome DSD (e.g., sex chromosome mosaicism) (Table II), and then to look for a chromosomal abnormality. Amniocentesis can also be used to measure the level of certain hormones in the amniotic fluid, which can be a useful adjunct to the other genetic tests.

The following techniques are used: QFPCR, conventional karyotyping, and array CGH which is now the gold standard at many centers for prenatal chromosomal testing [16].

If a sex chromosome abnormality like 46,XX/46,XY mosaicism is found after performing these tests, the DSD can be classified as a sex chromosome DSD and no further genetic testing is needed [6].

When karyotyping and array CGH yield a normal result – i.e., 46,XX or 46,XY – the rest of the testing is guided by whether or not other abnormalities are found on ultrasound. The diagnostic process will be different for isolated versus syndromic DSDs.

4. Present the karyotype results at a genetics consultation, give psychological support, and offer further testing, depending on whether the DSD is isolated or syndromic

Isolated DSD

For isolated 46,XX DSDs, the first thing to rule out when there is no history of maternal treatment with androgenic substances – thus ruling out iatrogenic virilization – is 21-hydroxylase deficiency CAH. The deficiency is due to a mutation in the CYP21A2 gene (autosomal recessive transmission) and causes fetal virilization via excess androgen production by the adrenal gland. It can be diagnosed prenatally by CYP21A2 gene testing [5].

In Case 2 of our series (two NIPTs showing 46,XX but phenotypically normal male sex on ultrasound), amniocentesis was done first to rule out CAH. However, the different analyzes finally made it possible to make the diagnosis of De La Chapelle syndrome, another differential diagnosis to consider in cases of 46,XX DSDs.

A maternal hormone panel should be done in parallel with the CYP21A2 gene testing to rule out excess placental or maternal androgen production as a cause of fetal virilization. Aromatase deficiency is one of the most common causes of virilization of placental origin, and is diagnosed based on very high maternal levels of androstenedione and testosterone and plummeting estrogen levels, in particular estriol, during pregnancy.

If the mother's blood test shows hyperandrogenism, she should be investigated for an androgen-secreting tumor, the most common benign ovarian tumors or hCG-dependent luteomas [5].

That was the diagnostic process used in Case 3 (Table I), once the ultrasound had confirmed an isolated 46,XX DSD. We first reviewed at the history for an extrinsic iatrogenic cause of virilization, such as the mother taking testosterone or progestogens. By measuring the mother's serum androgen levels, we then ruled out an intrinsic cause of virilization. Two differential diagnoses were then considered: CAH or hypertrophy as a normal variant. Those abnormalities were investigated postnatally. With isolated 46,XY DSDs, the first diagnosis to consider is androgen insensitivity syndrome (AIS) due to a mutated AR gene. Two forms have been described: the complete form (CAIS), which results in female external sex characteristics, and the partial form (PAIS), often associated with a micropenis and severe hypospadias [5]. In both forms there is a problem with peripheral receptivity to normally-secreted androgens from the testes. All cases of isolated 46,XY DSD therefore require AR gene testing. In our series, AR gene sequencing was done in the three cases of 46,XY DSD for which amniocentesis was performed (Cases 6, 8, and 9), leading to a diagnosis of CAIS in one of the cases (Case 6).

The AR gene was actually part of the gene panels mentioned earlier. At our institution, these panels are analyzed at the Ghent University Hospital's Center for Medical Genetics (UZ Gent), with whom we work closely, since it is our reference center for DSDs. Sometimes we start with a "small" DSD panel that sequences the four genes most often responsible for isolated 46,XY DSDs (SRY, WT1, SF1, and AR). If that fails to show any mutations, a "large" DSD panel is done to study a broader selection of genes (130) known to be possibly involved in 46,XY DSDs. These panels are part of the diagnostic process used in our department. However, in our series, except for the diagnosis of SICA made in case 6, the analysis of these panels in the fetus of case 8 and of the AR gene in the fetus of case 9, did not reveal any genetic abnormality. We ultimately concluded that the causal factor in both of those cases of 46,XY DSD was IUGR. Several retrospective studies have shown a higher rate of hypospadias in low birthweight male fetuses. The most likely cause is thought to be placental dysfunction in the form of abnormal hCG-controlled testosterone secretion during the first 14 weeks of gestation [5].

Syndromic DSDs

To date, several syndromes involving a DSD are well known. In our series, 2 syndromes were suspected in the fetus of case

8 due to the association of the genital anomaly, severe IUGR and short unbowed femurs (<P1). The first was a SLO syndrome, mainly associating facial dysmorphism, 2-3 syndactyly, polydactyly and genital anomalies with sexual ambiguity. This syndrome is linked to a mutation of the DHCR7 gene causing a deficiency in 7- dehydrocholesterol reductase (enzyme of the endogenous cholesterol synthesis chain) [17]. The second was an acampomelic campomelic dysplasia linked to the mutation of the SOX 9 gene, and characterized by the variable association of skeletal abnormalities (abnormalities of the long bones without curvature, abnormalities of the pelvis and thorax) and extraskeletal abnormalities, including in particular the presence of sexual ambiguity. However, these 2 hypotheses could be ruled out by the diagnostic investigations that followed.

Other syndromic DSDs exist, for example the WAGR syndrome (or 11p deletion syndrome) described as (W)ilms' Tumor, (A)niridia, (G)enitourinary abnormalities (such as undescended testicles or hypospadias in males, or internal genital or urinary anomalies in females), and Mental (R)etardation), as well as certain mutations in the GATA 4 gene, identified as being responsible for mild testicular dysgenesis and congenital heart disease.

Management of syndromic DSDs is a bit more complex, and is routinely discussed as part of genetic counseling. Therapeutic abortion has a legitimate role in these situations, where it is hard to determine the precise etiology and where the child's long-term prognosis is uncertain. Parents are also offered Mendeliome or exome sequencing in cases of syndromic DSD in an attempt to achieve a diagnosis by testing a broader spectrum of genes. These tests are done either prenatally – when therapeutic abortion is not indicated based on the sonographic evidence or not desired by the parents, with results taking roughly 8 weeks – or postnatally after fetal autopsy when therapeutic abortion was performed. Results in the latter case can take up to four months.

5. Present the final results (DSD panel or Mendeliome) at a genetic consultation in addition to endocrinology appointment

Again, the final results are presented at a genetic consultation. A visit with an endocrinologist is also routinely scheduled. Other appointments are arranged with various specialists, depending on the results, in order to provide comprehensive care to the child at birth and to prepare the expectant parents.

Even after all of these steps, a molecular diagnosis can be made in only 30 to 50% of 46,XY DSD cases. Yet achieving at a diagnosis is important so that parents can be given all available information on their child's pathology and the risk of recurrence in future pregnancies can be assessed [6]. Close collaboration with existing DSD reference centers and protocols like the one outlined in this study are thus critical to standardizing care practices for these rare disorders.

Conclusion

Over the past few years, routine NIPT has increased *in utero* diagnosis of DSDs. Earlier diagnosis is an asset in our clinical practice because it helps us prepare what we will tell parents regarding both diagnosis and prognosis and facilitates full, clear communication using appropriate terms. Another advantage is that is makes it possible to pre-arrange – with the appropriate medical and allied health professionals – the various tests and treatments that will be needed at the time of birth and for the child's long-term follow-up. However, these rare and complex disorders are still a challenge for clinicians. Despite all of the advances in genetic testing, the etiologies for a large number of DSDs are still unknown. This highlights the importance of developing clear, well-organized, multidisciplinary management protocols that improve the diagnostic process and the overall care for both fetus and parents.

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