Mismatch between fetal sexing and birth phenotype: a case of complete androgen insensitivity syndrome

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Abstract. With advancing maternal age, the number of prenatal genetic tests is increasing in many countries. Prenatal genetic tests, such as amniocentesis, chorionic villus sampling and non-invasive prenatal testing, can disclose fetal chromosomal sex, although these tests were originally designed to prenatally diagnose chromosomal aneuploidies, such as trisomy 21, 18 and 13. Complete androgen insensitivity syndrome (CAIS) is an X-linked recessive disorder caused by an androgen receptor dysfunction leading to hormone resistance. The affected individuals are genetic males as shown by 46,XY but present complete female external genitalia and normal breast development at puberty albeit without menstruation. CAIS is commonly diagnosed in adolescence based on primary amenorrhea or in childhood based on inguinal hernia or testis-like masses in the inguinal region. In the present report, we describe a baby in whom CAIS was diagnosed immediately after birth based on a mismatch between the fetal karyotype detected by amniocentesis and the external genitalia phenotype at birth. We speculate that the increase in the number of prenatal genetic tests is contributing to the early detection of 46,XY disorders of sex development, especially those previously called complete sex reversal, which is supposedly diagnosed during childhood or adolescence. Hence, it is necessary to understand the disease-specific hormone profile at each developmental stage for accurate diagnosis.

Key words: Complete androgen insensitivity syndrome, Disorders of sex development, Prenatal diagnosis, Amniocentesis, Complete sex reversal

CURRENT PRENATAL TESTS are classified roughly into two categories, imaging and genetic test. The former is intended to find morphological abnormalities using ultrasound or MRI, the latter to detect chromosomal aneuploidies, such as trisomy 21, 18 and 13, using amniotic fluid, chorionic villus or cell-free DNA (cfDNA) in the maternal blood. The prenatal diagnosis is performed by one or a combination of these tests. In Japan, with advancing maternal age an increasing number of prenatal tests is being performed, although the rate is still low compared to other countries, such as Australia, Denmark, and England [1]. Since non-invasive prenatal testing (NIPT) with cfDNA was introduced into Japan in 2013, the number of prenatal tests is expected to increase steadily in the coming years.

Fetal sex is always of major interest to prospective parents. Sex determination during pregnancy is usually performed to satisfy parents’ curiosity except in cases of families with serious X-linked recessive disorders. The most common way of ascertaining fetal sex is to check the fetal external genitalia by ultrasound scan. International guidelines for fetal ultrasound state that its accuracy is insufficient for clinical purposes in the first trimester and that fetal gender determination is not considered mandatory in the context of a mid-trimester scan [2, 3]. However, prenatal genetic tests can disclose fetal chromosomal sex although this use differs from its intended purpose.

Complete androgen insensitivity syndrome (CAIS) is an X-linked recessive disorder in which affected individuals present complete female external genitalia and normal breast development at puberty but lack of menstruation. The syndrome was first reported by Morris in 1953 and was described as testicular feminization syn-
drome at that time [4]. Individuals with CAIS are genetically male evidenced by 46,XY. The disease is caused by an androgen receptor (AR) dysfunction leading to hormone resistance. Some 90%–95% of CAIS cases are associated with AR gene mutations [5]. CAIS is commonly diagnosed in adolescence based on primary amenorrhea or in childhood based on inguinal hernia or testis-like masses in the inguinal region [5, 6]. Individuals with CAIS invariably have female gender identity [7].

In the present report, we describe a baby with CAIS diagnosed immediately after birth by the mismatch between fetal karyotype detected by amniocentesis and external genitalia phenotype at birth.

**Case Presentation**

An amniotic fluid analysis performed at 16 weeks of pregnancy because of advanced maternal age showed a 46,XY karyotype. During pregnancy, a fetal echography did not detect any abnormalities but was unable to identify the scrotum. The patient was born at a local maternity clinic after uncomplicated pregnancy at the gestational age of 40 weeks + 6 days with a birth weight of 3.26 kg. The Apgar score was ten at five minutes. The baby was referred to the hospital after female external genitalia and a bilateral inguinal mass were observed at birth (Fig. 1). There were no other physical findings. Initial investigations at the first day of life showed an elevated testosterone level of 2.10 ng/mL (reference range [9]: 0.63–4.07 ng/mL in male newborn, 0.02–0.77 ng/mL in female newborn). LH and FSH levels were below 0.1 mIU/mL. Testosterone levels decreased to 0.29 ng/mL on day 11. The anti-Müllerian hormone (AMH) level on day 11 equivalent to that of normal male baby (85.8 ng/mL, reference range [10]: 7.46–47.87 ng/mL in male cord blood, 105.46–271.74 ng/mL during male minipuberty). The patient had a normal ACTH and cortisol level, and normal electrolyte balance and glucose level. MRI and echography revealed bilateral testes in the inguinal region, and no uterus or ovaries. Imaging tests did not reveal any abnormalities in the adrenal glands and the kidneys. LHRH stimulation test on day 11 showed little increase from the baseline for LH and FSH (LH 0.28→1.88 mIU/mL, FSH 1.12→2.99 mIU/mL). The human chorionic gonadotropin (hCG) stimulation test (4,000 IU/m² body surface area intramuscular one daily injection for three days with serum androgens measured before first injection and 24 hours after last injection) from day 11 showed that testosterone was markedly elevated from 0.29 ng/mL to 2.25 ng/dL while the ratio of testosterone to androstenedione and of testosterone to dihydrotestosterone after hCG stimulation was within the normal range (4.01 and 3.69, respectively). The presence of the SRY gene was detected by fluorescent in situ hybridization. Genetic analysis identified a known nonsense hemizygous mutation (c.178C>T, p.Q60X) in the AR gene (Fig. 2). The parents decided to assign female gender after full disclosure of the diagnosis and the implications of the disorder by our multidisciplinary team. The gonadectomy postponed until early adolescence, meanwhile the patient is being carefully observed. An informed consent form approved by the Institutional Review Board Committee at the National Center for Child Health and Development was signed by the parents before genetic analyses were conducted.

**Discussion**

There are three main time points for the diagnosis of CAIS. First, patients with CAIS present primary amenorrhea during adolescence despite having spontaneous breast development [11]. Second, they are often referred to a hospital for an inguinal hernia or inguinal mass during infancy or childhood. Previous studies reported that
more than half of individuals with CAIS presented with inguinal hernia and the incidence of CAIS in girls undergoing hernia repair was 1% [6, 12]. Third, more recently fetuses or neonates are being diagnosed prenatally or immediately after birth due to a mismatch between fetal karyotype and external genitalia [13-16]. Individuals with ambiguous genitalia are recognized at birth, but individuals with complete sex reversal, the 46,XY karyotype and complete female external genitalia are usually overlooked at birth and receive diagnosis during adolescence based on primary amenorrhea or delayed puberty. The prenatal disclosure of fetal chromosomal sex enables early diagnosis of complete sex reversal at birth or even during the fetal period. Currently, Japan has a low frequency of prenatal genetic testing. However, we expect to encounter cases like the present one more frequently as NIPT becomes more widespread. Early diagnosis can reduce the risk of gender role change and confusion at puberty and also decrease the risk of dysgerminoma, which can occur even during childhood in these patients.

The new prenatal genetic test by next generation sequencing using cfDNA derived from fetus in maternal blood, which is called NIPT, is now available in many countries. In Japan, NIPT for the detection of trisomy 21, 18 and 13 has been applied in clinical research since April 2013 in certified institutions, and approximately 37,500 pregnant women underwent NIPT by September 2016 [17]. And, NIPT is targeting the prenatal diagnosis of microdeletion syndromes and monogenic diseases in the near future. NIPT has no risk of miscarriage, membrane rupture and hemorrhage involved in the test and has acceptable positive predictive value and very low false negative rate for the chromosomal aneuploidies [17]. However, if NIPT reveals abnormality, invasive prenatal test like amniocentesis is required for definitive diagnosis. And it is important to note that confined placental mosaic or vanishing twin can yield misleading results [18, 19]. Sekizawa et al. reported high accuracy of fetal gender determination using cfDNA since seven weeks of gestation [20]. In Japan, NIPT is not designed to disclose fetal chromosomal sex in this time.

There are differential diagnoses for the case with 46,XY and female phenotype. Swyer syndrome, also known as complete gonadal dysgenesis, have unambiguous female external genitalia and Müllerian structures. A deletion in the DNA-binding region of the SRY gene accounts for 10%-20% [21-23]. The diagnosis of this syndrome is challenging for two reasons: genetic analysis is not helpful in most cases and hypoplastic uterus is not easily visualized prior to estrogen exposure [24-26]. Laparoscopy can be more useful for visualizing Müllerian structures and gonads compared to a pelvis sonography. Imaging at the first few days may reveal the uterus more readily due to residual estradiol stimulation. It should be noted that previous articles have reported the Müllerian remnant in CAIS cases [27, 28]. 17β-hydroxysteroid dehydrogenase type 3 (17βHSD-3) is an enzyme catalyzing the conversion of androstenedione into testosterone. It is crucial to make a distinction between 17βHSD-3 deficiency and CAIS, because a change to male gender at puberty has been reported in individuals with 17βHSD-3 deficiency, while those with CAIS invariably have female gender identity [7]. 5α-reductase 2 deficiency (5αRD), which leads to defective synthesis of dihydrotestosterone, the strongest factor in external genitalia virilization, is also an important differential diagnosis. However, virilization to varying degrees can usually be observed in such individuals. The hCG test is useful for distinguishing among these three disorders. Previous studies reported that the ratio of testosterone to dihydrotestosterone in CAIS might be increased due to secondary 5αRD and a low ratio of testosterone to androstenedione before and after hCG might not be evident in 17βHSD-3 deficiency [29, 30]. Therefore, genetic diagnosis should be performed for confirmation. Severe form of Leydig cell hypoplasia also manifest phenotypically female appearance. An exaggerated LH level by LHRH stimulation test, a low testosterone level and a normal AMH level may be useful for diagnosis.

Transient activation of the hypothalamic-pituitary-gonadal (HPG) axis is observed in infants during the first few months. This period, also referred to as minipuberty, is a short window of opportunity for the evaluation of the HPG axis. Bouvattier et al. reported that infants with CAIS did not show a postnatal testosterone and LH surge during minipuberty probably because of an impaired pituitary feedback mechanism [31]. Our patient had an elevated testosterone level and a suppressed LH and FSH.
levels at the first day of life, and low testosterone and LH levels at the second week of life. To the best of our knowledge, this is the first CAIS case to show an elevated testosterone level and suppressed LH and FSH levels at birth. A typical hormone profile for CAIS patients has the following features: low LH/FSH and elevated testosterone levels at birth, low LH/FSH and low testosterone levels at minipuberty, low LH/FSH and low testosterone levels during infancy and childhood, and elevated LH, normal or mild elevated FSH, and elevated testosterone levels together with an elevated estradiol levels for male reference range after the onset of puberty [11, 31, 32]. A recent publication reported that basal testosterone and estradiol levels in postpubertal women with CAIS were found in the usual adult male reference ranges [33]. It is necessary to understand the disease-specific hormone profile at each developmental stage for accurate diagnosis.

The consensus statement on management of disorders of sex development (DSD) was published in 2006 based on the Chicago Consensus Meeting in 2005 [34]. Prophylactic gonadectomy has been recommended in CAIS due to increased risk of germ cell tumor during adulthood in the statement. Early gonadectomy is not necessary before onset of puberty, because CAIS has relatively low tumor risk compared with other DSDs, such as mixed gonadal dysgenesis, and no cases with tumor have been reported before onset of puberty in CAIS individuals [34, 35]. Recently, surgical procedures in DSD individuals, including gonadectomy, has also been discussed in terms of ethics, which means that first-person informed consent based on ethical implications must be obtained, at least with regards to suspendable procedures. Taking these situations into account, we decided to defer gonadectomy under careful observation.

**Conclusions**

The increasing number of prenatal diagnoses can contribute the early detection of 46, XY DSD, especially cases previously referred to complete sex reversal.

**Disclosure**

The authors declare no conflict of interest.

**Author contributions**

K.Y. evaluated the patient clinically and wrote the manuscript. M.F. performed AR gene analysis and revised the manuscript. Y.T. also evaluated the patient clinically. Y.N. and R.H. acted as consultant clinicians and assisted in writing the manuscript. All the authors read and approved the final version of the manuscript.

**References**


