Case report

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Two Korean girls with complete androgen insensitivity syndrome diagnosed in infancy

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Androgen insensitivity syndrome (AIS) is a rare genetic disease caused by various abnormalities in the androgen receptor (AR). The AR is an essential steroid hormone receptor that plays a critical role in male sexual differentiation and development and preservation of the male phenotype. Mutations in the *AR* gene on the X chromosome cause malfunction of the AR so that a 46,XY karyotype male has some physical characteristics of a woman or a full female phenotype. Depending on the phenotype, AIS can be classified as complete, partial or mild. Here, we report 2 cases of complete AIS in young children who showed complete sex reversal from male to female as a result of *AR* mutations. They had palpable inguinal masses and normal female external genitalia, a blind-end vagina and absent Müllerian duct derivatives. They were both 46,XY karyotype and *AR* gene analysis demonstrated pathologic mutations in both. Because AIS is inherited in an X-linked recessive manner, we performed genetic analysis of the female family members of each patient and found the same mutation in the mothers of both patients and in the female sibling of case 2. Gonadectomy was performed in both patients to avoid the risk of malignancy in the undescended testicles, and estrogen replacement therapy is planned for their adolescence. Individuals with complete AIS are usually raised as females and need appropriate care.

Keywords: Androgen-insensitivity syndrome, Androgen receptors, Disorders of sexual development

Introduction

Androgen insensitivity syndrome (AIS) is defined as a disorder of sexual differentiation caused by complete or partial resistance to the biological action of androgens. AIS patients have a male karyotype, 46,XY, normal testicular development and produce age-appropriate concentrations of androgen but some physical characteristics of a woman or a full female phenotype.[1)]

The male genital phenotype develops via 2 stages. The first stage is the formation of the testes from primitive gonads. It occurs during fetal development under the influence of the sex-determining genetic region located on the Y chromosome (sexual determination). In the second stage, the male characteristics of internal and external genitalia develop, including testicular translocation to the scrotum. It occurs gradually in response to androgen hormones produced in the Leydig cells of the testis (sexual differentiation). If testosterone is absent in the second step or if the function of the androgen receptor (AR) is abnormal, female sexual characteristics develop.[2)] The human AR is an essential steroid hormone receptor that plays a critical role in male sexual differentiation and development, preservation of the male phenotype, and initiation and maintenance of spermatogenesis. Mutations in the *AR* gene cause malfunctions of the AR including loss of function and morphological alterations, many of which are associated with AIS.[3)] AIS is clinically subclassified into 3 categories depending

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on the degree of feminization of the external genitalia: complete (CAIS), partial (PAIS), and mild (MAIS).

Along with AIS, 5 alpha-reductase deficiency (5aRD) is another representative 46,XY disorder of sexual development (DSDs) that manifests with discrepancies between internal and external genitalia. 5aRD is caused by impaired testosterone metabolism, whereas AIS is caused by the resistance to the action of testosterone.[4] 5aRD encoded by the 5-α-reductase type 2 gene (*SRD5A2*) converts testosterone into dihydrotestosterone, which is essential for normal male external genitalia.

The estimated prevalence of AIS based on molecular diagnosis is one in approximately 20,000 to 99,000 genetically 46,XY males.[5] The prevalence of AIS was also estimated as 0.8% to 2.4% when inguinal hernias are palpable in phenotypic females.[6] AIS is an X-linked recessive disease, although sporadic *de novo* mutations are associated with up to 30% of cases.[2] Of the three subtypes, CAIS is usually overlooked at birth because it results in a complete female phenotype, and it can be diagnosed in infancy only when the parents report a palpable inguinal mass. It is more usually identified because of primary amenorrhea during puberty. Clinical examination reveals a short vagina and no uterus. Imaging techniques confirm the absence of the uterus and ovaries, and identify intra-abdominal undescended testes. The karyotype should be confirmed as 46,XY to differentiate AIS from other types of DSD.

In this study, we report two cases of CAIS in 1-month-old and 13-month-old phenotypic girls who presented with inguinal masses. The clinical and pathological aspects and therapeutic strategy for CAIS are also reviewed and discussed.

Case report

1. Case 1

A 1-month-old girl was referred to the genetics clinic of our hospital for evaluation of testicle-like masses observed during herniorrhaphy. Bilateral inguinal hernias were noted just after birth and laparoscopic herniorrhaphy was performed at a local clinic when the patient was 1 month old. Laparoscopy showed that there was no uterus, and testicle-like masses were found around the openings of bilateral inguinal canals. This child was born at a gestational age of 38 weeks with a birth weight of 3.8 kg (90th–97th percentile) and had no specific prenatal or perinatal history. She was the second child of healthy nonconsanguineous parents, and her 4-year-old sister was also healthy. There were no abnormal results from the newborn screening for inherited metabolic disorders.

A physical examination performed at her first visit showed that her height and body weight were 58.3 cm (50th–75th percentile) and 5.7 kg (50th–75th percentile), respectively. She showed good activity and did not have either hyperpigmentation or hypertension. A testicle-like mass was palpable in the left but not the right inguinal area. Her external genitalia showed a complete female phenotype and there was only one external opening.

The results of hormonal studies are shown in Table 1. Baseline serum concentrations of adrenocorticotropic hormone (ACTH) and luteinizing hormone (LH), follicle stimulating hormone (FSH), estradiol, and testosterone were no higher than those of normal male infants. Anti-Müllerian hormone (AMH) was also within the normal range for male infants.

No fistula was detected when contrast medium was injected into the common urethra and a blind short vaginal pouch (3.8 cm in length) was found on genitography (Fig. 1A). Pelvis sonography revealed that the right testis (0.87 cm×0.15 cm×0.76 cm) was located in the right lower quadrant of the abdomen and the left testis (1.07 cm×0.45 cm×0.64 cm) was located in the proximal inguinal canal. No uterus, ovaries, or other internal female genital organs were identified (Fig. 1B).

The patient's karyotype was that of a normal male, i.e., 46,XY. Genetic analysis of *AR* and *SRD5A2* was performed, and a hemizygous mutation (c.2324G>A, p.Arg775His) was identified in *AR*. No mutation was found in *SRD5A2*. Family screening for genetic counseling was performed, and the same AR mutation was also found in the child's mother, who was a heterozygous female carrier. The child's elder sister had a normal female karyotype of 46,XX and did not have the *AR* mutation (Fig. 2).

The patient was referred to the pediatric urology clinic for surgical treatment. Laparoscopic bilateral gonadectomy was performed when the child was 8 months old, to avoid the risk of malignancy in the undescended testicles. Both testes were dissected and removed (Fig. 1C). No spermatic fascia was observed in the gross examination of the specimen, although testes and epididymides were present. Histopathological findings revealed an immature testis and epididymis with indistinct lumina and maturation arrest of germ cells (Fig. 3).

The patient was confirmed to have CAIS caused by an AR mutation and is being raised as a girl. She is now 31 months old and shows typical developmental milestones.

Table 1. The results of baseline hormone levels of 2 cases with complest androgen insensitivity syndrome

Case	Hormone (normal range)					
	LH (0.01–7.0 mIU/mL)	FSH (0.16–4.1 mIU/mL)	Estradiol (1.0–3.2 ng/dL)	Testosterone (70–400 ng/dL)	ACTH (0–60 pg/mL)	AMH (15–500 ng/mL)
1	1.1	<0.5	<0.3	<10	51.1	123.7
2	<0.07	0.36	1.86	<10	144.7	ND

LH, luteinizing hormone; FSH, follicle stimulating hormone; ACTH, adrenocorticotropic hormone; AMH, anti-Müllerian hormone; ND, not done.

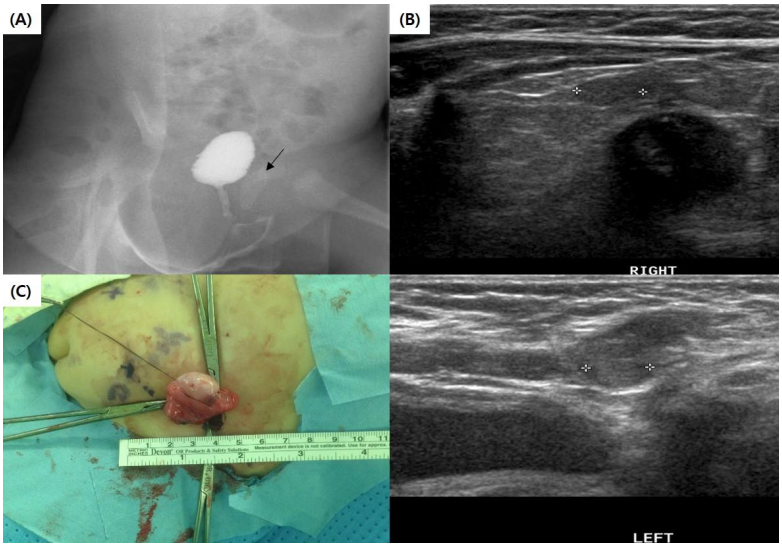

Fig. 1. (A) No fistula was visualized when contrast was injected into the common urethra, and a short blind-ended vaginal pouch (arrow) was found on genitography. (B) Pelvis sonography revealed both testes. (C) Both testes were subsequently dissected and removed.

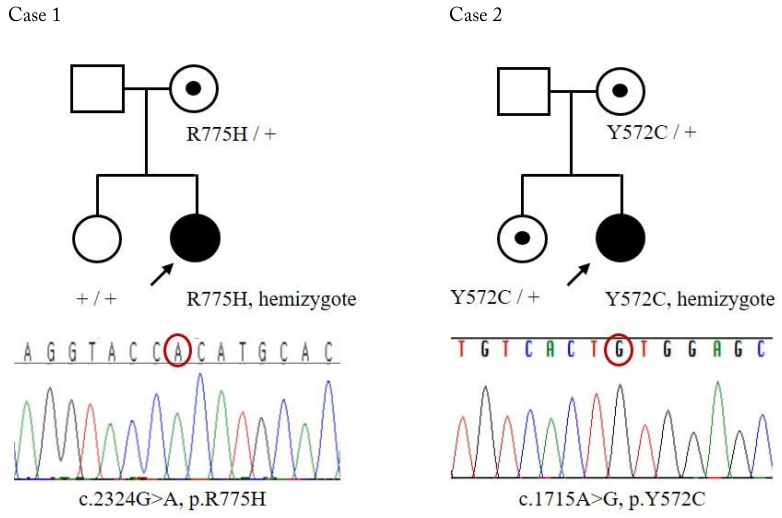

Fig. 2. Pedigrees showed that both the complete androgen insensitivity syndrome patients had pathologic *AR* mutations, p.R775H for case 1 and p.Y572C for case 2. The mutations are inherited from their mothers, who each carried the mutation heterozygously. Both arrows indicate patients in cases 1 and 2.

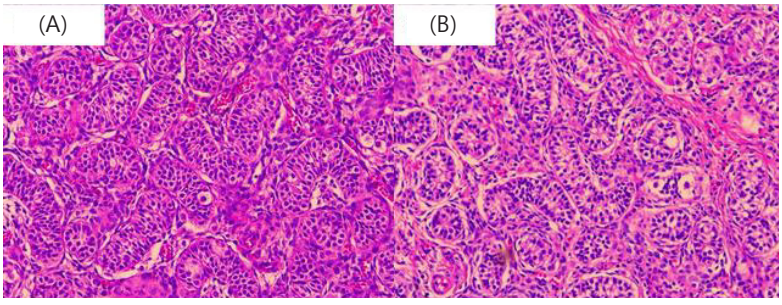

Fig. 3. Histopathological analysis of the gonadectomy specimens revealed immature testis and epididymis with indistinct lumina and maturation arrest of germ cells in 2 cases (A, case 1; B, case 2; A, B, H&E staining, X400). These findings are consistent with androgen insensitivity syndrome.

2. Case 2

A 13-month-old girl was referred to our hospital for further evaluation of palpable masses in both inguinal areas. She was born at 37^{+5} weeks gestation, at a birth weight of 2.92 kg (25th–50th percentile). A swelling in the inguinal area was first observed when she was 10 days old; ultrasonography was subsequently performed when she was 2 months old. It showed structures suspected to be testes on both sides of the inguinal area, and no uterus or ovaries were seen. Karyotype analysis using her peripheral blood demonstrated a 46,XY karyotype. Baseline hormone tests were performed, and neither sex hormones nor gonadotropin levels were higher than the normal ranges for infants (Table 1). Slightly elevated levels of 17 alpha-hydroxyprogesterone (5.05 ng/mL; normal range, 0.4–2.0 ng/mL) and elevated levels of ACTH (144.7 pg/mL; normal range, 0–60.0 pg/mL) were detected and hydrocortisone treatment was initiated at that time and had been continued until her visit to our hospital.

The patient was the second-born child of healthy nonconsanguineous parents and her 5-year-old sister was healthy. Physical examination at the first visit showed that her body weight and height were 10.3 kg (50th–75th percentile) and 75 cm (50th–75th percentile), respectively, and she had met normal developmental milestones. Systolic and diastolic blood pressures were within the normal ranges and she had no hyperpigmentation. Her external genitalia showed a complete female phenotype, and there were 2 separated orifices (urethral and vaginal orifices) with a short vagina. Bilateral testes were palpable in the inguinal area. Sonographic examination showed that the inguinal masses were 0.97 cm×0.71 cm×1.49 cm on the right side and 0.92 cm×0.83 cm×1.41 cm on the left. No uterus or ovaries were detected.

Analysis of *AR* identified a hemizygous mutation c.1715A>G, p.Tyr572Cys. There was no mutation in *SRD5A2*. The same *AR* mutation was also present heterozygously in her sister, whose karyotype was 46,XX. Therefore, the patient's sister was confirmed to be a female heterozygous carrier (Fig. 2).

The patient underwent laparoscopic removal of both undescended testes at the age of 21 months. Pathologic examination identified immature seminiferous tubules and vas deferens with a few germ cells, findings consistent with AIS (Fig. 3). After surgery, her dose of hydrocortisone was tapered, and a low-dose ACTH stimulation test revealed normal adrenocortical responses, including cortisol production. The patient is currently 42 months old and is being raised as a girl and shows typical developmental milestones.

Written informed consent to participate in this study was obtained from the patients' parents. An Institutional Review Board approved this study (H-1712-081-907).

Discussion

DSD in individuals with a 46,XY karyotype can be divided into 3 main groups: (1) disorders of gonadal development, (2) disorders of testosterone synthesis, and (3) disorders of metabolism or action of testosterone. In this classification, AIS is a rare genetic disorder, which occurs because of resistance to the actions of androgen hormones, and it results in a female phenotype.[4]

This disease was first described as "testicular feminization" in a 1953 review of 82 cases by Morris.[7] These patients were characterized by normal breast development and minimal pubic and axillary hair. The external genitalia were female in phenotype, but the vagina was absent or rudimentary and there was no uterus. Gonads were found in the labia majora, the inguinal ring or intra-abdominally. Urinary 17-ketosteroid and androgen metabolite levels were within the normal range, and there was no therapeutic response to methyltestosterone. This suggested androgen resistance rather than hormone deficiency, and the nomenclature was changed from testicular feminization to AIS.[8]

The *AR* gene that induces AIS is situated on the proximal long arm of the X chromosome, specifically locus Xq11-Xq12.[9] The *AR* gene encodes four functional domains: the N-terminal domain, which serves as a transcriptional activator, a central DNA-binding domain rich in cysteine residues, a hinge region containing a nuclear-targeting signal and a C-terminal ligand-binding domain (LBD). In addition to ligand binding, the LBD plays an important role in nuclear localization, receptor dimerization, and interaction with other proteins. In humans, AR is a 110-kDa protein composed of 919 amino acids.[10] The majority of *AR* mutations are X-linked recessive, and there are approximately 500 known *AR* mutations. An *AR* mutation is found in more than 95% of CAIS patients; therefore, if there is no *AR* mutation and the patient has a phenotype consistent with CAIS, it is necessary to investigate whether there is an abnormality in AR protein expression.[11] Both of the mutations found in our patients have been reported previously in AIS patients.[12,13]

CAIS is often diagnosed in girls at puberty because of primary amenorrhea. However, as was the case in our patients, it may be diagnosed earlier when testes are found during an inguinal hernia repair in a female infant. It may also be identified during genetic screening because of a family history, or because of a mismatch between the presence of a Y chromosome in fetal genetic analysis and a female sex phenotype at birth. In AIS, AMH secretion from Sertoli cells in the gonads is normally maintained, thus preventing the Müllerian system from developing into a uterus and other Müllerian-derived internal structures.[14] Therefore, a blind-ending vagina is present and the uterus is absent. The gonads migrate independently of androgen and are found in the lower abdomen or inguinal canal.[15] In adolescent women with AIS, breast and female adiposity develop and a normal growth spurt occurs. It is because estrogens are converted from androgens by the normal functioning of the P450 aromatase enzyme. However, pubic and axillary hair is absent or sparse. The height of these girls is greater than the average female height, but shorter than the average male height;

this is thought to be an effect of the Y chromosome.[16]

Unlike CAIS, PAIS presents with varying degrees of masculinization of the external genitalia depending on the degree of residual function of AR. These individuals usually have a micropenis, severe hypospadias and a bifid scrotum with or without cryptorchidism. Individuals with MAIS generally show normal male development or an isolated micropenis and are diagnosed because of gynecomastia at puberty or infertility in adulthood.[16]

Women with CAIS are hormone resistant, have serum testosterone concentrations that are within or higher than the normal range for men or boys, and have higher LH concentrations than normal. Their concentrations of FSH and inhibin are usually normal. Serum estrogen levels are higher than those in normal males but lower than those in normal females. After gonadectomy, the concentration of serum gonadotropin increases and is partly suppressed again with estrogen therapy. However, these patterns of hormone resistance are not evident during early infancy. Normal infants and PAIS infants show postnatal surge of plasma LH and testosterone concentrations at 1 month of age, but it is not shown in CAIS infants. It is because there is no negative feedback of androgen on the gonadotropic axis and, consequently, no hormone resistance in CAIS infants.[17]

5aRD and PAIS are representative 46, XY DSDs with ambiguous genitalia. These 2 disorders are difficult to distinguish by phenotype alone, so additional hormonal or genetic evaluations are needed. The human chorionic gonadotropin (hCG) stimulation test is a useful test to evaluate impaired testicular tissue function and testosterone biosynthesis. PAIS can be distinguished from 5aRD or other DSDs with a similar phenotype by the elevated levels of LH and testosterone seen in response to hCG stimulation. Although a positive hCG test excludes biosynthetic defects of testosterone, a low testosterone value at baseline or following hCG stimulation does not always exclude AIS.[18] Identification of the carrier of the mutation in the *AR* gene is clinically more important for the diagnosis of CAIS, as was the case in our patients, whose external genitalia had a complete female phenotype.[19]

When a patient is diagnosed with AIS, it is necessary to determine whether they should be reared as male or female. Most cases of CAIS are raised as females. In addition, gonadectomy should be performed because patients with 46,XY DSDs have a higher risk of developing germ-cell tumors in the gonads. However, the tumor risk before adolescence is considered to be as low as 0.8% to 2.0%, and spontaneous puberty occurs when gonads are present in patients with CAIS. Hence, some recent studies have recommended gonad removal after puberty.[20] In our patients, the gonads were removed before puberty reflecting the wishes of the parents to escape confusion for the patients' gender identity. They will require hormonal induction of puberty and subsequent sustained hormone replacement therapy. Their hypoplastic vaginas may require self-dilatation therapy and vaginoplasty procedures after adolescence.

Management of AIS requires a multidisciplinary approach. Providing appropriate information about diagnosis is very important. Several issues need to be considered when monitoring the patient's puberty, determining the timing of gonadectomy, and maintaining appropriate sexual function and optimal quality of life. Psychological support is very helpful and is critical to decision making. A team approach involving endocrinologists, clinical geneticists, urologists, gynecologists, and psychologists is required.[2]

As described above, AIS is mostly found at puberty with primary amenorrhea. However, as in these cases, 0.8% to 2.4% of girls at infancy are diagnosed with AIS when the inguinal mass is palpable. Therefore, additional consideration and diagnostic procedures for AIS is needed for girls at infancy with inguinal mass.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

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