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Case report

Identification of an *AR* mutation in Klinefelter syndrome during evaluation for penoscrotal hypospadias

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ABSTRACT

Genital anomalies, ranging from female genitalia to milder degrees of undervirilization, are rarely reported in Klinefelter syndrome, in which a male is classically expected to be born with male external genitalia. Though androgen insensitivity syndrome (AIS) is one of the possible pathogenic mechanisms also in Klinefelter syndrome with genital anomalies, to date the *AR* gene has not been analyzed in any of the published cases of Klinefelter syndrome of the milder phenotype, except for those patients presenting with a severe phenotype, such as female external genitalia. Lack of interest in considering androgen insensitivity in Klinefelter syndrome with a milder phenotype of genital anomalies may impede its identification through an accurate diagnosis. We present a 14-month-old boy with penoscrotal hypospadias, micropenis, and a ventral penile chordee abnormality who was observed to have both a 47,XXY karyotype and a known missense mutation in the *AR* gene that was inherited from his mother. Although it is recommended that Klinefelter syndrome be considered in the differential diagnosis of penoscrotal abnormalities, mutations in specific genes involved in androgen synthesis or responsiveness should also be investigated.

Key words: Androgen insensitivity syndrome, Androgen receptor, Hypospadias, Klinefelter syndrome

INTRODUCTION

Klinefelter syndrome (KS) is a common chromosomal abnormality with an incidence of 1/600 to 1/1000 live births and is often not diagnosed until adolescence or adulthood.[1] Although different genetic types of KS have been identified, 47,XXY is the most frequent karyotype.[2] KS is clinically characterized by oligo-azoospermia, hypergonadotropic hypogonadism, gynecomastia, and infertility in adults. While KS patients are classically born with normal male external genitalia, a number of patients with KS have been reported to have genital malformations.[1,2] The genital malformations in these patients are thought

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to be related to *in utero* testosterone insufficiency, androgen insensitivity, CAGn trinucleotide polymorphism, and *DAX1* duplication.[1] Androgen insensitivity syndrome (AIS), the most frequent cause of 46,XY disorders of sex development, is due to mutations in the androgen receptor (*AR*) gene, which is located on the X chromosome at Xq12.[3] AIS is associated with a variety of phenotypes, ranging from female genitalia (complete AIS, CAIS) to milder degrees of undervirilization (partial AIS, PAIS).[3,4] While a combination of CAIS and 47,XXY has been documented in nine cases, the combination of PAIS and 47,XXY has not been described to date.[4-12] We present a 14-month old boy with penoscrotal hypospadias, micropenis, and ventral penile chordee who was found to have a 47,XXY karyotype and a known missense mutation in the *AR* gene.

CASE REPORT

A 14-month-old male was first referred to our department due to penoscrotal malformation. The patient was born to a healthy 23-year old mother after successful in vitro fertilization. Prenatal screening tests were normal and his mother had reported no health problems. The parents were nonconsanguineous. The thirty-three year old father had subclinical hypothyroidism and reduced sperm counts in the ejaculate (oligozoospermia). Physical examination of the case revealed a height of 82 cm (SD score 1.50), weight 16.2 kg (SD score 3.4), penoscrotal hypospadias, and ventral chordee abnormality with bilateral normal testes (2/2 ml). Stretched penile length was 2.6 cm (<-2 SDS). Laboratory studies showed normal biochemistry, follicle stimulating hormone 1.37 mIU/mL (normal range, 0.3-4.6 mIU/mL), luteinizing hormone <0.2 mIU/mL (normal range, 0.04-0.42 mIU/mL), total testosterone <0.1 ng/mL (normal range <0.2 ng/mL), 17-hydroxyprogesterone <0.3 ng/ml (normal range <1.8 ng/ml), androstenedione <0.3 ng/mL (normal range <0.9 ng/ml). Pelvic ultrasonography revealed no ovarian or uterine tissue. Following three days of human chorionic gonadotropin administration (1000 U/day) total testosterone increased to 1.32 ng/mL, indicating normal androgen synthesis. Chromosomal analysis revealed a 47,XXY karyotype.

The presence of undervirilization necessitated further genetic analysis and *AR* sequencing was performed. The coding exons, the 5' UTR and the 3' UTR regions, and the exon-intron boundaries of the *AR* gene were sequenced with the MiSeq NGS system using V2 chemistry (Illumia, San Diego, CA, USA) according to the manufacturer's instructions. We detected a previously reported heterozygous missense mutation in exon 1 of the *AR* gene (p.P392S, c.1174C>T).[13-15] While the father had no mutation in the *AR* gene, the mother carried the same *AR* mutation which is consistent with an X-linked pattern of PAIS.

Due to having a heterozygous AR mutation, the mother was evaluated. The twenty-three year old otherwise healthy mother had a normal menstrual cycle (between 28-32 days). She had no hirsutism or acne and the Ferriman-Gallway index score was 6 (normal <8). Hormonal evaluation revealed normal results: follicle stimulating hormone level 3.6 mIU/mL (normal, 3.5-8.7); luteinizing hormone 2.9 mIU/mL (normal, 2.0-9.1); prolactin 7.9 ng/mL (normal, 3.8-26.7), thyroid stimulating hormone 1.5 mIU/mL, free T4 1.46 ng/dL, 17α-hydroxyprogesterone 1.2 ng/mL, dehydroepiandrosterone-sulphate 211.5 µg/dL, total testosterone 0.35 ng/mL (0.10-0.75), and free testosterone concentrations 0.85 pg/mL (0.29-3.18). Pelvic ultrasonography could not be performed.

DISCUSSION

In the present study, we describe for the first time, to our knowledge, a combination of KS, manifesting with penoscrotal hypospadias, micropenis, and ventral penile chordee, and a point mutation in the first exon of the *AR* gene indicative of *AR*-mutation positive PAIS. The diagnosis of KS was confirmed via the identification of the 47,XXY karyotype. Although some cases of KS with CAIS have been documented, a combination of KS and PAIS has not previously been described, nor has the pathophysiological mechanism behind the coexistence of these two latter diseases as yet been investigated. KS patients classically present with normal external male genitalia. However, those with genital abnormalities ranging from mild anomalies (isolated hypospadias, micropenis, cryptorchidism) to moderate undervirilization (penoscrotal abnormalities) have rarely been published in the literature.[1,16-18] In these reports, though androgen insensitivity has

been postulated as a possible mechanism, the *AR* gene was not analyzed in any of them. However, in three patients with both 47,XXY and genital abnormalities, Lee et al[1] demonstrated normal androgen-binding characteristics in genital skin fibroblasts. It is thus evident, as also demonstrated in our case, that the *AR* gene should be analyzed in KS patients with genital abnormalities.

The few published cases with the combination of KS and AIS had female external genitalia, which is compatible with CAIS, and most of them had high FSH, LH and total testosterone levels.[4-12] Uehera et al reported a case with 47,XXY and CAIS that had both homozygous nonsense mutations in exon 4 (c.2280C >T, p.Q640X) and in exon 5 (c.2615G>A, p.W751X) of the *AR* gene.[12] Girardin et al identified a homozygous known missense mutation in exon 5 of the *AR* gene (c.2560A>T, p.Q733L) in a patient with a 47,XXY karyotype and CAIS.[4] In our KS patient with features including penoscrotal hypospadias, micropenis, and ventral penile chordee abnormalities, we have described a known missense mutation in exon 1 in the *AR* gene (p.P392S). To date, the p.P392S mutation has been identified in 8 cases (4 penile hypospadias, 1 penoscrotal hypospadias, 1 isolated micropenis, and 2 isolated infertility), which indicated that this mutation, in line with the current case, mostly leads to a mild phenotype in patients with AIS.[13-15] The mutation in exon 1 of the *AR* gene is located within an important region for transcriptional activity of the receptor. However, in contrast to the above data, Hiort et al[19] showed that the p.P392S mutation did not lead to gross alterations in the transcriptional activity of the androgen receptor. This mutation may have different in vivo and in vitro activity and it is generally thought that the p.P392S mutation normally leads to a mild phenotype in male external genitalia.

The X-linked *AR* gene mutation is the most common cause of 46,XY disorders of sex development (DSD). Usually, hemizygous mutations in the *AR* gene lead to AIS in individuals with a 46,XY karyotype. However, in males with two X alleles, as is the case in individuals with a 47,XXY karyotype, the occurrence of *AR* gene inactivation could be explained differently. Partial AIS can occur in XXY when a non-mutant X is inactivated or a mutant allele is preferentially expressed.[10] CAIS in Klinefelter syndrome can also occur with uniparental disomy (UPD), as described by Jacobs et al.[20] Therefore, it is likely that the mutant X allele expression could be responsible for the moderate PAIS presentation in our patient with the heterozygous *AR* gene mutation.

While *AR* gene mutations lead to various degrees of genital abnormalities in males, CAG repeat length variation (polyglutamine) that encodes on the 1st exon of the *AR* gene and heterozygous *AR* mutations have been shown to be associated with hyperandrogenism or development of polycystic ovary syndrome in adult women.[21,22] Hyoun et al[22] reported a 37-year old woman who gave birth to a child with CAIS diagnosed with polycystic ovary syndrome (PCOS), probably due to a heterozygous *AR* mutation. In contrast, the mother of our patient had a heterozygous *AR* mutation not previously described in polycystic ovary syndrome. Since there are limited data demonstrating the relationship between heterozygous *AR* mutations and PCOS in adult females, there is a need for further studies to clarify this association.

Due to long-term testosterone deficiency, comorbid conditions such as bone and muscle mass loss, obesity, deterioration of glucose metabolism, and type 2 diabetes may occur in the progressive stages of puberty in cases with KS.[23,24] In addition, patients with PAIS who were assigned male have relative testosterone deficiency as well.[25] Thus, in both diseases, testosterone replacement is recommended to induce puberty, to enhance virilization post puberty and to prevent such comorbidities associated with hypogonadism. However, the treatment approach is not clear in patients who have both PAIS and KS and who are raised male. We consider that testosterone replacement could be appropriate during the follow-up period of puberty in our case with both KS and PAIS. The long-term outcome will help us to improve our approach to the treatment of these patients.

In conclusion, this is, to our knowledge, the first description of a male case with the combination of the 47,XXY and PAIS phenotype. As far as we know, the patients previously reported with both 47,XXY and AIS all had a CAIS phenotype. Random X inactivation of the healthy allele probably caused the PAIS phenotype in our KS patient. *AR* gene muta-

tions, which are the most common cause of 46,XY disorders of sex development, must be excluded in undervirilized males with KS.

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STATEMENT OF ETHICS

Written informed consent of the parents was obtained for this case report.

DISCLOSURE STATEMENT

The authors have nothing to disclose.

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