Case Report

Divergent Gender Identity in a Phenotypic Male with 46XX Karyotype Caused by a Mutation in CYP21A2 Gene with Congenital Adrenal Hyperplasia

K. C. Pradheep Kumar, Suranjana Banik, Praisy Joy, Sanjukta Sahoo

Department of Anatomy, AIIMS, Bhubaneswar, Odisha, India

Abstract

A male patient in his late twenties presented with ambiguous genitalia to our tertiary specialist unit with complaints of short stature and inadequate copulation. There was no history of consanguinity, and a physical examination raised concerns about possible disorders of sexual development (DSD). Karyotyping and fluorescence *in situ* hybridization results were consistent with the presence of two X chromosomes, revealing the patient to be a genotypic female. Sanger sequencing showed a heterozygous pathogenic mutation in the CYP21A2 gene known to be associated with 21-hydroxylase deficiency, thus confirming the diagnosis of congenital adrenal hyperplasia (CAH), Prader stage V. DSD with CAH is distressing for the patient and their families, and the management needs a multidimensional approach involving diverse medical, genetic, and psychological considerations. Cytogenetic and molecular genetic studies play an essential role in diagnosis and decision-making and should be made affordable in developing countries for better patient care.

Keywords: *Congenital adrenal hyperplasia, diagnostic tool, genetic counseling, genetics*

Introduction

Disorders of sexual development (DSD) present a complex and sensitive challenge for both families and health-care professionals. One of the central issues in managing such cases is the assignment of sex, a decision influenced by a multitude of factors, including cultural background, clinical features, biochemical parameters, imaging reports, parental preference, fertility potential, and the mental well-being of the affected child. However, in third-world countries, the diagnostic process is often complicated, as many children with DSD present late or are diagnosed late, leading to delayed definitive treatment.[1]

A phenotypic sex abnormality known as 46XX females characterizes 46XX DSD with ovaries who do not develop typical external genitalia. The external genitalia may be mildly, moderately, or severely virilized and frequently resemble the external genitalia of a male cryptorchid with a typical phenotypic profile.[2] The overexposure of a female fetus to androgenic hormones throughout intrauterine life is the cause of this disease. Elevated androgen levels may come from the fetus, with congenital adrenal hyperplasia (CAH) being the primary cause.

CAH is a hereditary metabolic disorder brought on by a lack of certain enzymes necessary for the manufacture of cortisol, most frequently a 21-hydroxylase (21-OH) deficit. The prevalence of this specific enzyme deficit is around 95% among affected patients.[3] Female fetuses who are exposed to excessive androgens throughout pregnancy develop higher androgen levels. This can impact primary sexual characteristics by inducing female pseudohermaphroditism, wherein the fetal external genitalia exhibit masculinization.

Molecular genetics delves into the specific genes responsible for DSD, such as those involved in androgen synthesis and action. By combining these investigative approaches, health-care professionals can achieve a more precise diagnosis, allowing for personalized, and timely interventions. However, in resource-constrained settings, the diagnostic journey can be prolonged,

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Address for correspondence:
Dr. Sanjukta Sahoo, Department of Anatomy, Academic Block, AIIMS, Bhubaneswar - 751 019, Odisha, India.
E-mail: anat_sanjukta@aiimsbhubaneswar.edu.in

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impacting the initiation of appropriate treatments. This case aims to explore the crucial role of cytogenetic and molecular genetics in diagnosing individuals with DSD, explicitly focusing on female pseudohermaphroditism and CAH.

Case Report

We reported a case of a male patient in his late twenties who presented with ambiguous genitalia where the presentation was of a phallus with frankly penile configuration, mild penoscrotal hypospadias, and fused labia appearing as empty scrotum [Figure 1] to our tertiary specialist unit. The patient was 140 cm tall and weighed 43 kg in total. After an uneventful full-term vaginal delivery, he was delivered to nonconsanguineous parents [Figure 2]. The patient's voice had a manly timbre. His seated blood pressure was 120/80 mmHg, and his pulse was regular.

Physical examination revealed well-developed secondary male sexual characteristics with no gynecomastia [Figure 3]. External genitalia examination showed a phallus with hypertrophied crus of the clitoris, which raised concerns about a possible DSD. The patient had a single meatus on top of the clitoris that mimicked male external genitalia,[5] a 2 cm long enlarged clitoris, and total union of the posterior scrotal labial folds.

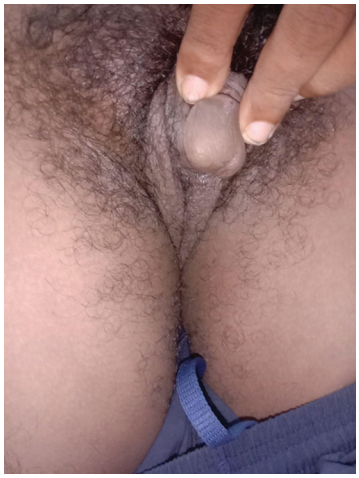

Figure 1: Ambiguous genitalia with presentation of a clitoris with penile configuration, mild penoscrotal hypospadias, and fused labia appearing as empty scrotum

Karyotyping and Fluorescence *in situ* hybridization showed the presence of two X chromosomes [Figures 4 and 5]. The patient was heterozygous positive for CYP21A2:c.293-13C/A>G(I2G) and c.1069>T(R356W) hotspot mutation in Sanger sequencing [Figure 6].

The patient decided to keep his social identity as a man, so a total abdominal hysterectomy bilateral salpingo-oophorectomy was done, followed by hypospadias repair in two steps with a successful penienic urethra restoration.

Discussion

The present case is a case of female pseudohermaphroditism. The failure of the gonadal hormones to function appropriately in female pseudohermaphroditism associated with 21-CAH may be interpreted as hypogonadism, with adrenal hyperandrogenism being responsible for the interference with sexual differentiation.[4] The karyotyping was 46, XX. The patient was advised for molecular endocrinology evaluation and was found to have pathogenic disease in 21-OH deficiency. The results have been validated with Sanger sequencing. However, parental screening is required to confirm the compound heterozygosity of these two mutations identified in the heterozygous state. Further recommendations were made for family screening and genetic counseling. The most frequent biochemical cause of CAH is decreased cortisol synthesis due to diminished or lost 21-OH enzyme function. Female pseudohermaphroditism and virilization are most commonly caused by CAH.[6]

The development of male secondary sexual characteristics in cases of female pseudohermaphroditism can be attributed to the presence of the SRY gene, which is usually associated with male differentiation. The presence of male or ambiguous genitalia characterizes

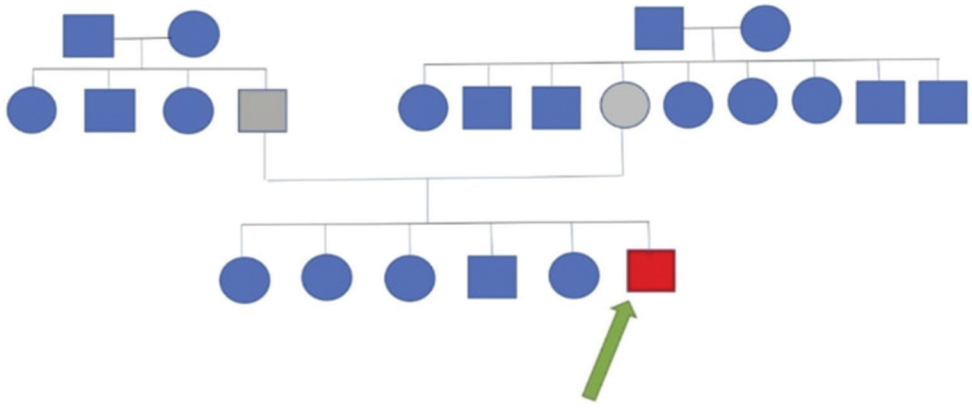

Figure 2: Pedigree of proband (Arrow shows the proband)

female pseudohermaphroditism. In about 80% of cases of XX males (individuals with two X chromosomes), the SRY gene is expressed, leading to male development. Y chromosome is the location of this gene and is responsible for initiating the male reproductive pathway.[7] However, some instances of XX males exist where the SRY gene is absent (SRY-negative XX males), leading to atypical development. Male secondary sexual characteristics can be influenced by the presence or absence of the SRY gene in individuals with female pseudohermaphroditism. However, cases of vanishing testis have been reported, and in such cases, Inhibin B serum level with a diagnostic value of <1 pg/ml has confirmed the diagnosis.[8] The test can be of great importance in present situations where secondary sexual characters are pretty evident despite the absence of the Y chromosome and testis absence. The probable anomalies associated with 46XX DSD should be kept in mind by fertility specialists, urologists, and andrologists as they conduct proper screening, suitable general physical examinations, and local examinations with a focus on detecting cryptorchidism, hypospadias and gynecomastia. Mutation in other genes involved in the sex determination cascade, such as SOX9, SOX3, DAX1, WT1, FGF9, and SF1, should also be evaluated. In 46, XX CAH patients raised as boys, breast growth is especially noticeable during puberty. Patients who are not candidates for steroid therapy may still grow breasts, in which case breast surgery may be necessary.[9,10] In the present case, the patient did not present with breast development at all, unlike other cases. Most fertility-eliminating surgeries were carried out without a complete gender identification assessment. The age at installation and the rate of testicular prosthesis placement varied widely.[11] In the present case, the patient underwent surgery for chordee correction plus stage 1 urethroplasty without postoperative complications. The patient chose to be male and reported having comfortable sexual relationships with women postsurgery. The rate of erectile dysfunction posturethroplasty may range from 0% to 40% generally.[12] However, in the present case, no history of erectile dysfunction was mentioned.

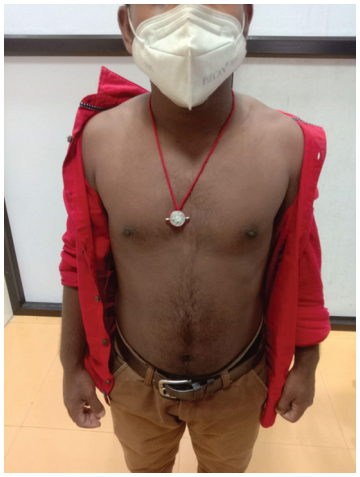

Figure 3: Well-developed secondary sexual characteristics with no gynecomastia

A diagnosis of female pseudohermaphroditism can be emotionally distressing for individuals and their families. It may lead to feelings of confusion, shame, anxiety, and depression. Psychiatric counseling can help individuals and families understand the condition, its implications, and the available treatment options. It provides a safe space for expressing emotions and concerns. Psychiatric counseling in the case of female pseudohermaphroditism serves as a valuable resource for addressing the psychological challenges that individuals and their families may face.[13] It fosters understanding, resilience, and emotional well-being, helping individuals navigate the complexities of their condition and lead fulfilling lives. However, it is important to note that each individual's

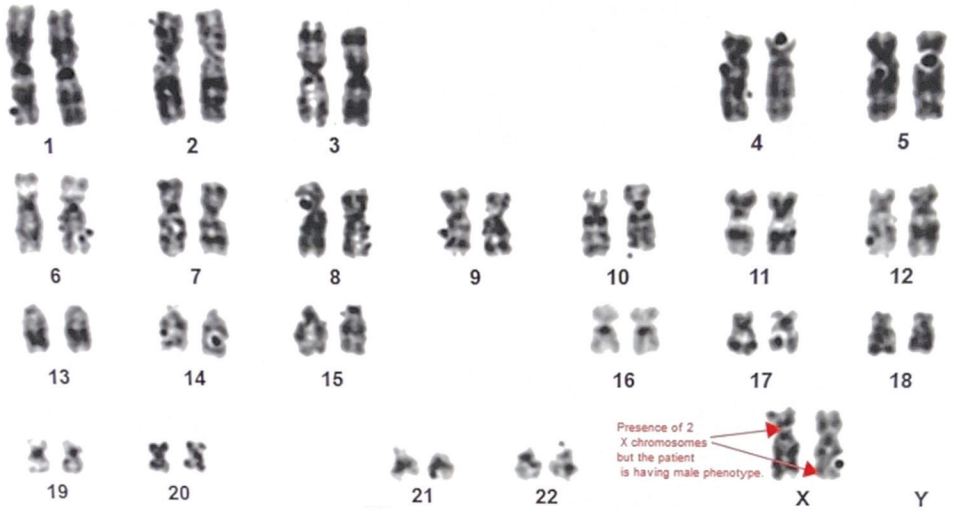

Figure 4: Karyotype showing a 46XX

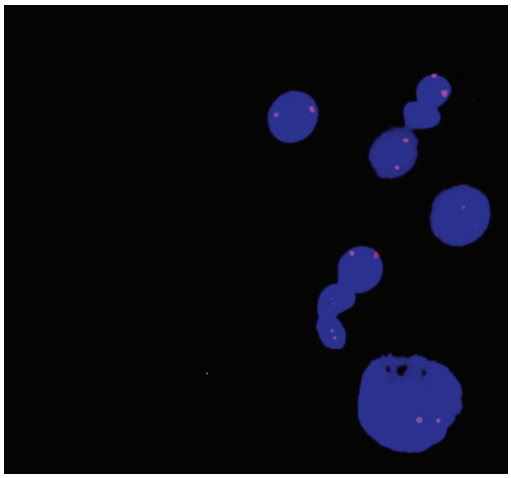

Figure 5: Fluorescence *in situ* hybridization done using X and Y probes showing the presence of two X chromosomes. The region visible with blue mark represents the nucleus and the red dots are X chromosomes

Result summary:

Utilizing the in-house standardized Allele Specific PCR approach, the patient was found to be heterozygous positive for *CYP21A2*:c.293–13C/A>G (I2G) and c.1069C>T (R356W) hotspot mutations

The results have been validated with Sanger sequencing.

Figure 6: Sanger sequencing report

experience is unique, and counseling approaches were tailored to meet their specific needs and preferences. The multidisciplinary approach included physicians, endocrinologists, geneticists, and surgeons involved in treating the patient.

Conclusion

The management of DSD, particularly female pseudohermaphroditism, and CAH, is a multidimensional process involving diverse medical, genetic, and psychological considerations. Cytogenetic and molecular genetic studies play a vital role in unraveling the underlying causes of DSD, enabling better-informed decision-making and more effective patient care. Bridging the diagnostic gap in third-world countries requires increased awareness, resources, and collaborative efforts to ensure timely and accurate diagnosis, thereby improving the lives of individuals affected by these conditions.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient has given his consent for his images and other clinical information to be reported in the journal. The patient understands that his name and initials will not be published and due efforts will be made to conceal his identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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