Case report /
Prikaz primera

Management of a child with a difference in sex development caused by a *NR5A1* pathogenic variant

Obravnava otroka z motnjo v razvoju spola, kot posledica patološke variacije v genu *NR5A1*

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Abstract

Introduction: Differences of Sex Development (DSD) occur in approximately 1/5000 live births. One of the recently found genetic causes for 46, XY DSD is *NR5A1* gene variants, responsible for a broad phenotypic spectrum.

Case Report: We present a case of a full-term newborn with ambiguous genitalia: one gonad located in the urogenital fold, the other inguinal, absence of wrinkling or hyperpigmentation of the urogenital folds' skin, the short genital tubercle, and labio-scrotal urethral meatus (External Genital Score of 4). Male-type urethra and the absence of a uterus or ovaries were determined by ultrasound. The karyotype was 46, XY, and a pathogenic heterozygous single nucleotide duplication 614dupC in the *NR5A1* gene was found by NGS. The decided gender of rearing was male. Orchidopexy was performed at age 14 months. The histology of the gonad was indicative of a prepubertal testis.

Discussion: *NR5A1* variants have variable expressivity and incomplete penetrance. 46, XY patients with a pathogenic variant in the *NR5A1* gene range from ambiguous genitalia to normal female external genitalia with virilization at puberty. Pubertal development does not strongly correlate to the degree of virilization at birth, with the majority showing signs of virilization at pubertal age.

Keywords: Differences of Sex Development, Next-Generation Sequencing, *NR5A1*

Izvleček

Uvod: Motnje v razvoju spola (DSD) se pojavijo pri približno 1/5000 živorojenih otrok. Med nedavno odkritimi genetskimi vzroki za 46,XY DSD so različice v genu *NR5A1*, ki so odgovorne za širok fenotipski razpon.

Prikaz primera: Predstavljamo primer donošenega novorojenčka z dvoumnimi genitalijami: levostransko tipno gonado v levi urogenitalni gubi volumna 1 ml, ter desnostransko tipno gonado v dimljah, odsotnost gubanosti in hiperpigmentacije kože urogenitalnih gub, kratek genitalni tuberkel dolžine 1,5 cm, labioskrotalni meatus sečnice (ocena zunanjih genitalij 4). Ultrazvok je pokazal prisotnost spolnih žlez, ki so kompatibilne s testisi, moški tip sečnice in odsotnost maternice ali jajčnikov. Kariotip je bil 46,XY in NGS je odkril patogeno heterozigotno podvajanje enega nukleotida 614dupC v genu *NR5A1*. Glede na to je bil določen moški spol in pri 8 mesecih je imel majhno strukturo penisa z dolžino <2 cm, brez razvitega skrotuma in labioskrotalno hipospadijo. Orhidopeksija je bila opravljena v starosti 14 mesecev. Histologija gonad je kazala na prepubertetni testis.

Razprava: Različice *NR5A1* imajo spremenljivo ekspresivnost in nepopolno penetranco. Klinična slika pri bolnikih 46,XY s patogeno različico v genu *NR5A1* sega od dvoumnih genitalij do normalnih ženskih zunanjih genitalij z virilizacijo v puberteti. Zdi se, da razvoj v puberteti ni močno povezan s stopnjo virilizacije ob rojstvu, saj velika večina kaže znake virilizacije v puberteti.

Ključne besede: motnja v razvoju spola, Sekvencioniranje naslednje generacije, *NR5A1*

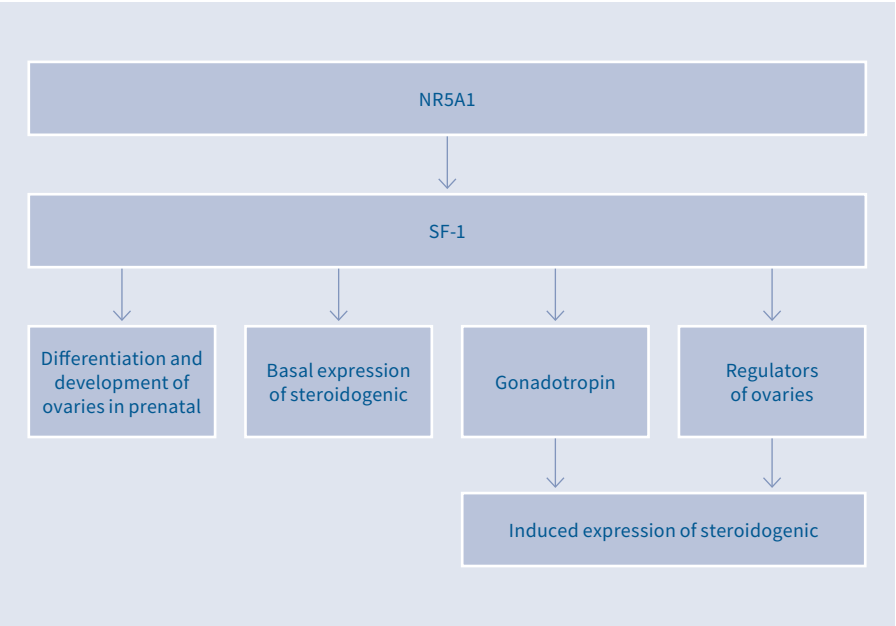

FIGURE 1. CELLULAR FUNCTIONS OF THE STEROIDOGENIC FACTOR-1 (SF-1).
SLIKA 1. FUNKCIJE STEROIDOGENEGA FAKTORJA-1 (SF-1) V CELICI.

Introduction

The term Differences of Sex Development (DSD) encompasses conditions with atypical genital appearance or discordance between phenotypic and chromosomal sex (1). These are uncommon and occur in approximately 1/5000 live births (2, 3)

One of the genetic causes of DSD discovered relatively recently in individuals with 46, XY karyotype is mutations in the *NR5A1* gene (2, 4). It encodes the Steroidogenic Factor-1 (SF-1), a transcription factor vital to steroidogenesis and gonadal and adrenal development (3, 5, 6). During fetal sexual development, SF-1 regulates the bipotential gonad differentiation toward the testes or ovaries (2). In addition, it stimulates the expression of multiple genes responsible for the male differentiation cascade, promoting the differentiation of the precursor cells toward Sertoli cells, virilization through steroidogenesis in Leydig cells, and the regression of the paramesonephric ducts (2, 4, 5).

In patients with 46, XY DSD, mutations in the *NR5A1* gene are responsible for a broad phenotypic spectrum, from isolated hypospadias to ambiguous external genitalia or entirely female external genitalia, with or without adrenal insufficiency (2, 3, 4).

Case report

Neonatal period

We present a case of a full-term newborn from a third spontaneous pregnancy (one abortion) without complications. There was no family history of consanguinity or relevant diseases. The delivery was through elective cesarean section due to a previous cesarean section. Birth weight was on the 10-50th percentile, and birth length was on the 1st-3rd percentile. On physical examination at birth, the following abnormalities of the external genitalia were observed: palpable gonad in the left urogenital fold with a volume of 1 mL, no palpable gonad in the right urogenital fold with the gonad palpable inguinal, absence of wrinkling or hyperpigmentation of the urogenital folds' skin, presence of a penile structure/genital tubercle with a length of 1.5 cm and diameter of 5-6 mm, labio-scrotal urethral meatus and an anogenital distance of 2 cm. The External Genital Score (EGS) was 4 (1). There were no other relevant physical examination findings.

An abdominal and genital ultrasound using the transperineal approach was performed on the fourth day of life. It revealed the presence of gonads in the urogenital folds compatible with testicles, the urethra in the genital tubercle morphologically according to the male type, and the absence of the uterus or ovaries. In addition, hydronephrosis of the left kidney (UTD-P3) with a wide ureter was diagnosed. The total testosterone value was 1.0 nmol/L (reference for male infants under ten days old 1.0-11.5 nmol/L) with free testosterone of 2.3 pmol/L, and AMH was on the lower normal level (8.25 µg/L; reference

range for male infants 10.64 to 161.84 µg /L). Normal basal and stimulated values of cortisol were determined by the ACTH test. The karyotype was available on the 12th day of life and was 46, XY on all examined cells. The intact SRY gene on the Y chromosome was determined.

A Voiding Cystourethrography (VCUG) showed VUR grade IV on the left kidney with the suitably wide urethra, without communication with surrounding structures. A heart ultrasound showed an open oval window and accessory tissue in the outflow tract of the right ventricle, without signs of obstruction, that normalized by the age of 4 months. In addition, the transfontanellar ultrasound was normal.

The multidisciplinary DSD team, together with both of the parents, suggested the gender of rearing to be male.

Follow-up

The genetic diagnosis was available at the age of 2 months. A heterozygous single nucleotide duplication 614dupC in the *NR5A1* gene was found by Next-Generation Sequencing (NGS), representing a premature transcription termination previously described as pathogenic (4). The same variant was found in the healthy mother.

At three months, a GnRH test showed a basal LH of <0.1U/L and basal FSH of 4.0 U/L, increasing to 0.8 and 23.5 U/L, respectively, by 60 minutes. Inhibin B was normal, and AMH low (7.88 µg/L).

The decided gender of rearing was male, and at eight months of age, the child presented with urogenital folds (without developed scrotum), a small penile structure with <2 cm length and 5-6 mm diameter, and underdeveloped root, labio-scrotal hypospadias and inguinal positioned gonads (Figure 2).

The child had a bilateral orchidopexy. The goal of the procedure was to fix the gonads inside the urogenital folds outside of the abdominal cavity. A biopsy of the right gonad was performed during the procedure. Histologically, spermatogonia in the seminiferous tubules were described alongside clusters of Leydig cells in the interstitium.

Discussion

Variants in the *NR5A1* gene are predominantly inherited dominantly with variable expressivity and incomplete penetrance. 90% of patients have 46, XY karyotype (5). There is no concise phenotype–genotype correlation, with a spectrum varying from complete female to complete male appearance (5).

Case series of 46, XY patients with *NR5A1* variants report a wide variety of presenting phenotypes, ranging from ambiguous genitalia and low EGS at birth to normal female external genitalia, but with signs of virilization at puberty or pubertal delay. Even if external genitalia is apparently female, most patients have no residual Müllerian structures (uterus and fallopian tube) (2, 4, 5). AMH is responsible for their regression during prenatal development. In XY fetuses, it is secreted from the Sertoli cells of the testis. Patients with 46, XY DSD may present normal AMH levels at birth, without a uterus or fallopian tubes, or low concentrations of AMH and detectable Müllerian structures. The reported patient presented without Müllerian structures and a low normal AMH value at birth and low value at age two months, suggesting that AMH was secreted prenatally, with a decrease in secretion postnatally (4). Inhibin B levels in male subjects reflect the function of the Sertoli cell in the testis. The levels in patients with *NR5A1* pathogenic variations are mostly very low (2). In the presented case, inhibin B was normal. Inhibin B and AMH values below the male reference range despite the absence of Müllerian structures and decrease in testicular volume throughout puberty suggest progressive Sertoli cell failure over time and highlight the importance of keeping the possibility of early spermiogram and cryopreservation in patients with an *NR5A1* mutation in mind (2, 4, 7).

In most cases with pathogenic variants in the *NR5A1* gene, testosterone level is low during the neonatal period, which was also determined in the present case (4). Therefore, hypogonadotropic hypogonadism with normal/low normal testosterone levels is expected, but low and extremely low testosterone concentrations may also be determined (2, 4, 5). Monig et al. described that despite normal testosterone levels, LH levels were elevated in the vast majority of patients and postulated that this could be due to Leydig cells having a relevant preserved function, but higher LH levels are needed to maintain enough testosterone, possibly because of impaired SF-1 stimulation activity or partial gonadal dysgenesis due to the *NR5A1* mutations (2). Increased FSH concentrations and low LH/FSH ratios have also been reported (4), with our patient presenting this hormonal pattern.

Pubertal development does not strongly correlate to the degree of virilization of the external genitalia at birth in 46, XY DSD patients with *NR5A1* mutations. The great majority of patients, even with very low EGS, show signs of virilization at pubertal age, though in many cases, the gonadal volume stays below average (2, 4). In a few cases, virilization might not occur, and spontaneous breast development might even be present (2). Patients with ambiguous genitalia at birth and male sex assignment who showed spontaneous puberty and normal testosterone values have been reported, supporting the idea of preserved Leydig cell function later in life (4, 8, 9).

Notably, the prevalence of adrenal insufficiency incidence is low (0.05%) in these patients (5). Most cohorts show no adrenal insufficiency (2, 4,

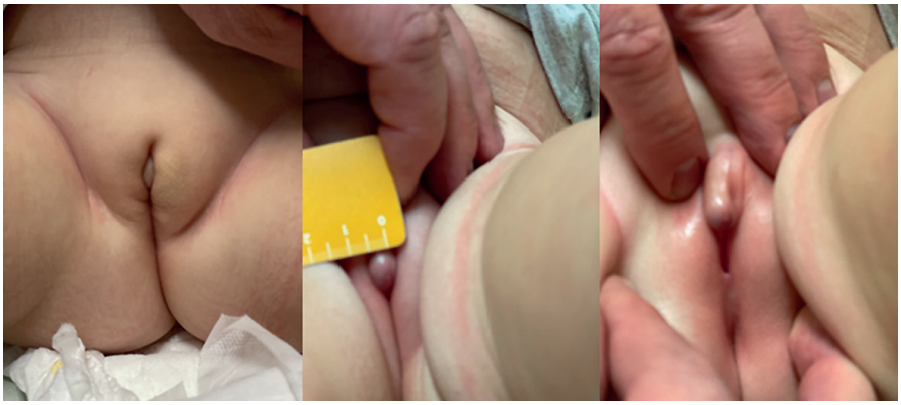

FIGURE 2. THE EXTERNAL GENITALIA OF THE PATIENT AT EIGHT MONTHS OF AGE. SMALL GONADS WERE PALPABLE IN THE UROGENITAL FOLDS THAT DID NOT DEVELOP INTO THE SCROTAL FOLDS. THE PHALLIC STRUCTURE WAS POORLY DEVELOPED. THE ANOGENITAL DISTANCE WAS REDUCED. THESE FEATURES INDICATE SUBOPTIMAL DEVELOPMENT OF THE EXTERNAL MALE GENITALIA.

SLIKA 2. ZUNANJE SPOLOVILO V STAROSTI 8 MESECEV. MAJHNE GONADE SO BILE TIPNE V UROGENITALNIH GUBAH, KI SE NISO RAZVILE V SKROTUM. FALIČNA STRUKTURA JE SLABO RAZVITA. ANOGENITALNA RAZDALJA JE BILA ZMANJŠANA. NAVEDENO KAŽE NA SUBOPTIMALEN RAZVOJ ZUNANJEGA MOŠKEGA SPOLOVILA.

5), although sporadic cases have been reported (6). In heterozygous *Nr5a1*+/– mice, adrenal insufficiency was determined only during stress conditions. It was associated with significant adrenal hyperplasia, demonstrating that a normal gene dosage of SF-1 is required for mounting an adequate stress response (10). One must be aware of possible acute adrenal insufficiency in *NR5A1* subjects during stress conditions (4).

Two additional features were determined in the presented case: VUR grade IV on the left kidney and open oval window and accessory tissue in the outflow tract of the right ventricle. Both features do not seem to be associated with *NR5A1* mutation (2–5).

The gender of rearing of DSD patients is a topic of debate. An experienced multidisciplinary team should make optimal clinical management of individuals with DSD in close communication with the caregivers, and all patients should receive a gender assignment (11). Factors influencing gender assignment include etiology, genital appearance, reproductive anatomy, surgical options, the need for lifelong replacement therapy, the potential for fertility, and parental/cultural factors (11, 12).

Most 46, XY subjects with *NR5A1* mutations reared as girls will undergo progressive masculinization if the gonads are not removed, and boys with *NR5A1* mutations can undergo spontaneous puberty as well as preserved fertility, suggesting that a 46, XY individual with an *NR5A1* mutation reared as a boy has certain advantages (4, 8, 9, 13). In a literature review, 46% of patients with *NR5A1* mutations were assigned female, and 54% were assigned male (5). Female-to-male sex reassignment was referred in 15% of patients, but only 1 case underwent male-to-female sex reassignment. In another cohort, 16 out of 19 patients assigned female gender at birth decided to reassign to male gender (4). Given all these factors, the reported child was assigned male gender at birth after extensive discussions and approval by the parents. The specific etiology of the DSD and reported evidence for future outcomes in these patients, the presence of male gonads without Müllerian structures, and the hormonal profile lead us to believe this could be the best option for the patient.

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prispelo / *received*: 12. 1. 2023
sprejeto / *accepted*: 10. 2. 2023

João Gaia M, Kavčič M, Debeljak M, Kordič R, Kotnik P. Management of a child with a difference in sex development caused by a NR5A1 pathogenic variant. Slov Pediatr 2023; 30(3): 74–78. https://doi.org/10.38031/slovpediatr-2023-2-04.