NEW MUTATION IN 5 ALFA REDUCTASE: A five-month-old infant with a karyotype of 46 xy

Authors: Setilla Dalil1, Nejat Mahdie2

1. Pediatric growth disorders research center, 17 th shahriar hospital, department of pediatrics, school of medicine, guilan university of medical sciences, Rasht, Iran.

2. Rajaee cardiovascular disorders and research center, Iran university of medical sciences, Tehran, Iran.

Introduction: disorders of sex development (DSD) or disorders of sex differentiation or differences of sex development, are medical “congenital conditions in which development of chromosomal, gonadal, or anatomical sex is atypical” (1, 2).

The most common DSD are congenital adrenal hyperplasia (CAH), complete androgen insensitivity syndrome (CAIS), partial androgen insensitivity syndrome (PAS) and 5-alfa-reductase deficiency (5-AR).

1. Alpha reductase deficiency is an enzyme for converting testosterone to dihydrotestosterone (DHT). Deficiency of this enzyme is an autosomal recessive sex-linked condition and 5-alfa-reductase deficiency (5-AR) cell in testes is necessary for the normal development of external genitalia (3). Based on genetic, this is one of the most common reasons for cryptorchidism and hypospadias (4). The diagnosis of 5-alfa-reductase deficiency (5-AR) is confirmed by measuring testosterone levels in the serum. Lack of testosterone in the serum results in external genitalia that do not correspond to the internal sex except female genotype. The epiphyses, seminal vesicles, genital skin, labia, breast, hair follicles, and the placenta indicated type 2 of 5-alfa reductase. Both type 1 and type 2 of 5-alfa-reductase disease result in the pseudo-hermaphrodite phenotype. Patients usually present with a deficiency of type 2 5-alfa reductase. Type 2 deficiency is one of the cause significant of 46 XY disorder of sexual development (5). (4, 6).

Classically presentation of this disease includes complete female form of external genitalia, (florid-like phallic, bilateral scarlet, pseudovaginal parietalcostal hypertrophy, and a rudimentary gonad). Occasionally, venous hypoplasia occurs as a result of normal regression of the müllerian-differentiation factor and lack of uterus and fallopian tubes. In these patients, testes are intact and are usually found in the inguinal canal or scrotum. However, cryptorchidism is frequently detected at birth, and type 1 deficiency trait is occasionally located in the abdomen. Ultrasound duct differentiation is normal with vaginal vessels, vas deferences, epididymides, and ejaculatory ducts. (7, 8).

Materials and methods:

A five-month-old infant with female genitalia referred to endocrinology clinic, prenatal screening (amniocentesis) of down syndrome in the family medical history was showed 46.XY karyotype. However, operated ultrasound mentioned female genitalia. After birth of 6 months, a first ultrasound mentioned no tests but second ultrasound confirmed the existence of testis in bilateral inguinal.

No ovary and uterus was noted and there was a pseudovagina.

Regarding the lack of electrolyte imbalance and definite female genitalia during first 5 months, less probably it is thought that patient had congenital adrenal hyperplasia. For checking the function and receptor at androstenedione (A5.1) DSD was noted for this patient to assess the function and receptor of testosterone to rule out the androgen insensitivity and 5-alfa-reductase, human chorionic gonadotropin test with three dosages was performed. Increased testosterone, dihydrotestosterone was noted on 25. Then, clinicians suspected to 5 alfa reductase, therefore, to confirm the result, a genetic and molecular study was performed and showed a novel homozygous mutation in 5alpha-gene.

Genetic testing:

An informed consent form was signed by patient’s parent (s). Five milliliter of whole blood was taken from the patient. Genomic DNA was extracted using standard salting out protocol. Quantity of DNA was assessed using spectrophotometer (Transcriptor 2000; thermo scientific, Wilmington, DE, USA).

The coding regions and exon-intron boundaries of 5alpha (NM_000248.1; nm_000248.3) were amplified by the forward and reverse primers (available upon request). PCR was performed in a final volume of 50 µl reaction, forward and reverse primer (5 µm), genomic DNA (50 ng), 2.5 mmol/L MgCl2, and 1.25 units of Taq polymerase (Roche, Germany) for 35 cycles with denaturation at 94°C for 40 seconds, annealing at 55°C (30 seconds), extension at 72°C (30 seconds) and final extension at 72°C (7 min). The PCR products were directly sequenced using a sequencing analyzer ABI PRISM 3500 (4x applied 4.0 bp, labile city; CA, USA) by a big dye termination method.

Bioinformatics analyses:

Available on the software tools including sorting intolerant from tolerant (sift), provean, combined annotation dependent deplot (cadd) and polymorphism phenotyping (polyphen-2 v2.1) were applied to predict pathogenic score of the variant.

A multiple protein acid sequence alignment was done using uniprot protein family members (uniprot/psicid p231213) to check conservation of the mutation of 355 amino acids. IDR converges in the core region of the 5alfa reductase domain and was evaluated to predict the effects of variant on the function and structure of protein.

Results:

Regarding female genitalia with male karyotype biochemical tests for the patient(1) was performed. Their results were summarized in table (1). These patients showed high-dosage testosterone, high levels of dihydrotestosterone and increased levels of androstenedione. After performing genetic analysis for the patient(1) to confirm the function and receptor of testosterone to rule out the androgen insensitivity and 5 alfa-reductase, the patient is positive with 5 alfa reductase deficiency. The results show the 5 alfa reductase does, and to confirm the results genetic assessment was indicated.

Discussion:

Regarding female genitalia with male karyotype biochemical tests for the patient(1) was performed. Their results were summarized in table (1). These patients showed high-dosage testosterone, high levels of dihydrotestosterone and increased levels of androstenedione. After performing genetic analysis for the patient(1) to confirm the function and receptor of testosterone to rule out the androgen insensitivity and 5 alfa-reductase, the patient is positive with 5 alfa reductase deficiency. The results show the 5 alfa reductase does, and to confirm the results genetic assessment was indicated.

One benign homogyous variant, c.2463G>C (p.Leu811Pro) was found in the patient as well as a novel variant of insignificance, c.4767G>T (p.Leu1592Arg). Sequential analysis, based on the patient’s family analysis, showed that the variant is inactivating 5alfa reductase (25) for HGC test. These results suggest a 5 alfa reductase and to confirm the results genetic assessment was indicated.

Recent studies have shown 18K542A is localized in reticulum endoplasmic (ER).

Conclusion:

Because patient with 5 alfa reductase deficiency unlike other disorders can be repaired at puberty age, it is better to pay special attention to this disease so authors suggest genetic molecular assessments in all patient with 5 alfa reductase deficiency.

References:


