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

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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 (PAIS) and 5-alpha-reductase deficiency (5ARD).

5-alpha-reductase is an enzyme for converting testosterone to dehydrotestestrone (dht). Deficiency of this enzyme is an autosomal recessive sex-limited condition call 5-alpha-reductase deficiency (5RD). DHT in utero is necessary for the normal virilization of external genitalia (3). Based on genetic, site of enzyme deficiency and clinical examination, there are three types of 5-alpha-reductase. Type–1 of 5-alpha reductase(4)", is specific for brain, ovary and the entire skin except for genitalia. The epididymis, seminal vesicles, genital skin, uterus, breast, hair follicle and the placenta indicated type 2 of 5-alpha reductase. Both type 1 and type 2 isoenzymes exist in the liver, prostate, and testicles. Patients usually present with a deficiency of type 2. 5-alpha-reductase type 2 deficiency is one of the significant cause of 46,XY disorder of sexual development (DSD) (5, 6)

Classically presentation of this disease includes complete female form of external genitalia , clitoral-like phallus, bifid scrotum, pseudovaginal perineoscrotal hypospadias, and a rudimentary prostate. Occasionally, penile hypospadias occurs as a result of normal secretion of the müllerian-inhibiting 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 described when testes occasionally located in the abdomen. Wolffian duct differentiation is normal with seminal vesicles, vasa differentia, 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 had showed . a 46,XY karyotype. However, repeated ultrasounds mentioned female genitalia. After birth, at 5 months, a first ultrasound mentioned no testis but second ultrasound confirmed the existence of testis in bilateral inguinal.

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

Regarding the lack of electrolyte imbalance and definite female genitalia during first 5 months, less probably it seemed that patient had congenital adrenal hyperplasia. Therefore, based on function and receptor of testosterone, 46XY DSD was noted for this patient. To assess the function and receptor of testestrone to rule out the androgen insensitivity and 5 alfa reductase, human chorionic gonadotropin test with three dosages was performed. Increased testestrone/ dihydrotestrone ratio was noted as…25.. Then, clinicians suspected to 5 alfa redoctase, therefore to confirm the result, a genetic and molecular study was performed and showed a novel homozygous mutation in srd5a2 gene

Genetic testing:

An informed consent form was signed by patient's parent; five ml of peripheral whole blood was taken from the patient. Genomic DNA was extracted using standard salting out protocol. Quantity of DNA was assessed using a spectrophotometer (nanodrop nd2000c; thermo scientific, wilmington, DE, USA).

The coding regions and exon-intron boundaries of srd5a (ng_008365.1; nm_000348.3) were amplified using the forward and reverse primers [available upon request]. Briefly, PCR was performed in a final volume of 50 µl reagents; forward and reverse primers (10 pmol), template DNA (150ng), taq DNA polymerase (0.2 units/µl), mgcl2 (1.5 mm), and dntps (0.4 mm for each nucleotide) were used to amplify the regions by the following PCR program: initial denaturation 5 min at 94°C and 30 cycles for denaturation at 94°C (30 secs), annealing at 62°C (30 secs), extension at 72°C (30 secs) and final extension at 72°C (7 min). The PCR products were directly sequenced using a sequencing analyzer ABI PRISM™ 3500 (PE applied biosystems, foster city, CA, USA) by a bigdye termination method.

Bioinformatics analyses:

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

A multiple amino acid sequence alignment was done using uniprot protein family members (uniprotkb/swiss-prot p31213) to check conservation of the mutated residue protein homology/analogy recognition engine v2.0 (phyre2) and iterative threading assembly refinement (I-TASSER) server were used 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[j1] was performed .Their results were summarized in table 1 that showed normal gonadotropin ,normal electrolytes, slightly increased testosterone and clear increased testestrone / dihydrotestestrone ratio (25)after HCG test. These results suspected as 5 alfa redoctase and to confirm the results genetic assessments was indicated.

One benign homozygous variant, c.265C>G (p.Leu89val) was found in the patient as well as a novel variant of insignificance, c.476T>G (p.Ile159arg). Segregation analysis within the family also showed that parents are heterozygote for this mutation. Bioinformatics analyses were also done and the pathogenicity of this variant was predicted using on line software tools.

Discussion:

Regarding female genitalia with male karyotype and clear increased testestrone / dihydrotestestrone ratio (25)after HCG test. We suspected as 5 alfa redoctase and to confirm the results with genetic molecular assessments . because patient with 5 alfa redoctase deficiency unlike other diseases can be repaired at pubertal age , it is better to pay special attention to this disease so authors suggest genetic molecular assessments in all patient with 5 alfa redoctase deficiency

Recent studies have shown SRD5A2 is localized in reticulum endoplasmic (ER)(9).

Conclusion:

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

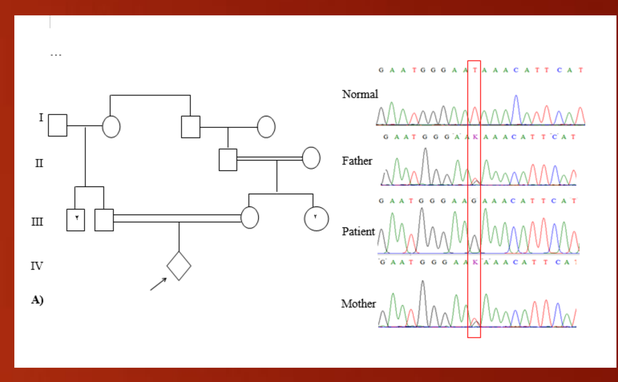

Figure: A) Amino acid alignment of SRD٥A٢ protein among different its orthologous and paralogous members adapted from UniProt protein family members. p.Ile١٥٩ (indicated in the box) is a highly conserved residue in this protein among different species. B) …..

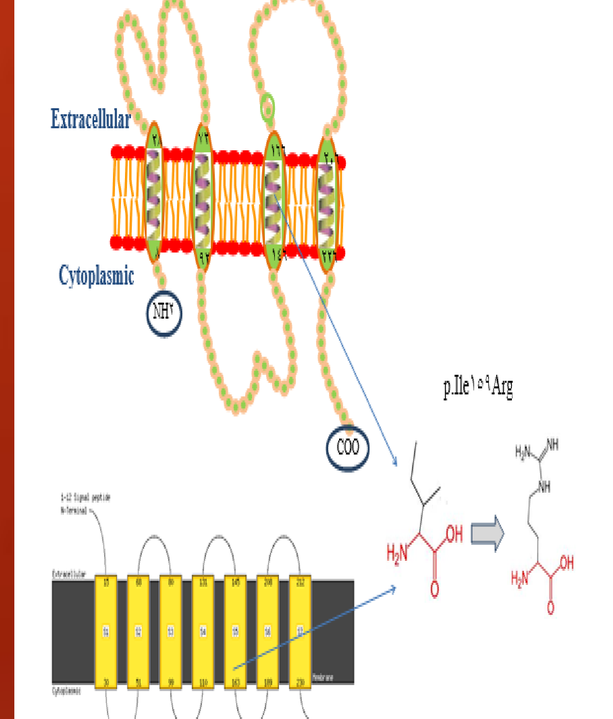

Figure: Schematic possible structure of microsomal ٥-alpha-reductase domains based on A) UniProt database annotation and B) Phyrre ٢ prediction. p.Ile١٥٩Arg is located in a transmembane domain.

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