

CASE REPORT

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Received: 31.07.2017
Acceptance: 14.09.2017
DOI: 10.18521/ktd.341688

Konuralp Medical Journal
e-ISSN1309-3878
konuralptipdergi@duzce.edu.tr
konuralptipdergisi@gmail.com
www.konuralptipdergi.duzce.edu.tr

A Novel p.Arg179Ser (c.537 G>T) Heterozygotes Mutation on Exon 3 of SRD5A2 Gene Accompany with Biotinidase Deficiency in Case with Ambiguous External Genitalia

ABSTRACT

The SRD5A2 gene that encodes SRD5A2 enzyme is placed on chromosome 2p23. The gene includes 5 exons that are translated into a 254-amino acid protein. To evaluate SRD5A2 gene that encodes SRD5A2 enzyme is placed on chromosome 2p23 accompany with biotinidase deficiency in case with ambiguous external genitalia. We investigated a case with ambiguous external genitalia for cytogenetic and gene mutation analysis. Gene mutation analysis and cytogenetic analysis were performed according to a standard DNA sequencing method and the present international standard nomenclature (ISCN), respectively. A Novel p.Arg179Ser (c.537 G>T) heterozygotes mutation on exon 3 of SRD5A2 gene accompany with biotinidase deficiency was detected. The chromosomal analysis result is 46, XY. This is the first case with biotinidase deficiency and novel R179S p.Arg179Ser (c.537 G>T) mutation of the SRD5A2 gene, which cause 5-alpha reductase deficiency.

Keywords: SRD5A2, Ambiguous External Genitalia, 5α-Reductase Type 2 Deficiency.

Belirsiz Dış Genitali Bir Hastada, Biotinidaz Eksikliği Eşlik Eden SRD5A2 Geninin Ekzon 3'ünde Yeni Bir p.Arg179Ser (c.537 G> T) Heterozigot Mutasyonu

ÖZET

SRD5A2 enzimini kodlayan SRD5A2geni kromozomun 2p23 bölgesinde yerleşir. Genin kodlayan bölgesi 254 amino asitlik bir proteine çevrilen 5 ekzon bölgesi içerir. Çalışmanın amacı, belirsiz dış genitali vakada biyotinidaz eksikliğinin eşlik ettiği kromozom 2p23 bölgesinde yerleşen SRD5A2 enzimini kodlayan SRD5A2genini değerlendirmektir. Biz belirsiz dış genitali bir vakayı sitogenetik ve gen mutasyonu analizi için inceledik. Gen mutasyon analizi ve sitogenetik analizler sırasıyla, PCR temelli bir standart DNA sekans metodu ve uluslararası standart terminolojiye (ISCN) göre yapıldı. Biotinidaz eksikliğinin eşlik ettiği, SRD5A2 geninin ekzon 3'ünde yeni bir heterozigot mutasyonu pArg179Ser (c.537 G>T) tespit edildi. Kromozomal analiz sonucu 46, XY idi. Bu biyotinidaz eksikliği olan ve 5 alfa redüktaz eksikliğine neden olan SRD5A2 geninde yeni bir mutasyon R179S p.Arg179Ser (c.537 G>T) olan ilk olgudur.

Anahtar Kelimeler: SRD5A2, Belirsiz Dış Genital, 5α-Redüktaz Tip 2 Eksikliği.

INTRODUCTION

Ambiguous genitalia is an important instance of a disorders of sex development (DSD) with different etiologies, potential difficulties for diagnosis, and many challenges with immediate and longterm administration. Its' incidence is 1-2 for every thousand live births (1). The examination of ambiguous genitalia has a medical, surgical and psychological emergency in the newborn. The most important decision is the choice of sex assignment in this situation. Therefore rapid and precise diagnosis is crucial (2). Cases with 46,XY and DSD are characterized by female external or ambiguous genitalia because of incomplete intrauterine masculinization. Different types of 46,XY disorders of sex differentiation may have similar clinical findings with 5 α -reductase type 2 deficiency insensitivity, 17 β -hydroxysteroid dehydrogenase deficiency and steroidogenic factor mutations sometimes can be difficult (3). Differential diagnosis with partial androgen in XY newborns with normal/high plasma testosterone (T) is performed and partial androgen insensitivity syndrome is commonly the first diagnosis evoked that implies an androgen receptor deficiency (2). 5 α -Reductase disorder is rare an autosomal recessive enzymatic disorder that occurred as a specific form of 46,XY disorder of sexual development (4,5). Abnormally high baseline and hCG-stimulated T to dihydrotestosterone (DHT) ratio is the hallmark of 5 α -reductase type 2 deficiency (4,5). Nevertheless after hCG stimulation especially in cases with partially enzyme dysfunction, common increased level of T to DHT ratio does not refute the diagnosis (6, 7). 5 α -reductase type 2 gene (SRD5A2) encodes the 5 α -reductase enzymes, which catalyzes the conversion of T to DHT. DHT also mediates great number important steps of masculinization at puberty and modulates psych-orientation of the prenatal human brain. Residual activity of abnormal 5- α -reductase type 2 enzyme cause to different stages of virilization at birth and has an impact on lifelong psychological functioning (8). Males affected by this disorder generally present with ambiguous external genitalia, perineoscrotal hypospadias, microphallus, inguinal or cryptorchid testes and prostatic hypoplasia (9). However, an extensive variety of clinical phenotypes range from complete feminization to undermasculinization or isolated infertility. It was known that some cases are sufficiently masculinized at birth to be raised as boys, but cases with predominantly female external phenotype structures are frequently increased as girls. Nevertheless, those raised as girls express spontaneous virilization at puberty (4,5). Early molecular diagnosis of the disorder is essential for the crucial decision of the newborn's sex of rearing. In the article, we reported a case with novel R179S heterozygotes mutation on exon 3 of SRD5A2 gene that accompany with biotinidase deficiency.

Cytogenetic and Gene Mutation Analysis:

Metaphase chromosomes were obtained from the peripheral blood cultures. Karyotype analysis was carried out by Giemsa banding. Chromosomal anomalies were not detected.

From the case, 2 cc peripheral blood samples were collected in tubes containing EDTA for DNA isolation. Genomic DNA was isolated and sequenced for the related gene.

CASE REPORT

A boy 4 months old presented with biotinidase deficiency and R179S p.Arg179Ser (c.537 G>T) mutation on exon 3 of the SRD5A2 gene, which cause 5-alpha reductase deficiency (5-ARD). He had ambiguous genitalia. According to genital area examination, microphallus, penoscrotal hypospadias, bifid scrotum were detected. His dorsal and ventral phallus were 1.8 and 0.5 cm in seventeen days. He had bilateral palpable gonads. His genital ambiguity was stage 3 according to Sinnecker's classification. In pelvic USG of the patient, solid appearances consistent with testicular tissue were observed in the distal of bilateral inguinal canal as 6x7 mm sized in the right and 5x7 mm sized in the left but uterus and ovaries were not detected. His sistemic findings were normal. When the patient was 17 days old, DHT level was 4.93 ng/dl and total T was found 135.66 ng/dl. When the patient was 4 months old, after the hCG stimulation test, the results were 45.9ng/dl and 631ng/dl for total T. Due to the patient's serum T / DHT (13.74) was high (normal value < 12), 5-ARD was suspected. Thus SRD5A2 gene was sequenced and R179S heterozygotes mutation was detected. His karyotype was 46,XY according to the present international standard nomenclature. 5-ARD was diagnosed for the case with these findings. He was able to keep her head in the second month, onset sit unsupported in the six month, walk and speech at his age. His length, weight and head circumference were 81.2 cm (50-75p), 11kg (50p) and 47 cm (25-50p) at fifteenth month, respectively. The phallus response to exogenous testosterone is instructive for both diagnosis and gender decision. 25 mg of testosterone was given to the case as intramuscular for 3 times with 4 weeks apart and phallus response was evaluated in the twelfth month. An increase to 3.5 cm from 1.8 cm was detected. Her hearing, vision and pediatric echocardiography examination were normal.

Family History: The patients' parents were first cousins. The parents have not chronic diseases. His mother's and father's age were 22 and 29 years old, respectively. His mother has not been used alcohol and cigarette. No anomaly was detected at USG controls during pregnancy. The mother had not used any drug during pregnancy. No asphyxia story and admission in a couveuse at birth. He was born a 37 week of gestation with a birth weight of

2.1 kg by normal vaginal birth from 21 year old mother with G2P1. He had not a sibling (Figure 1).

Individual informed consents for genomic analyses and case presentations were obtained from patients.

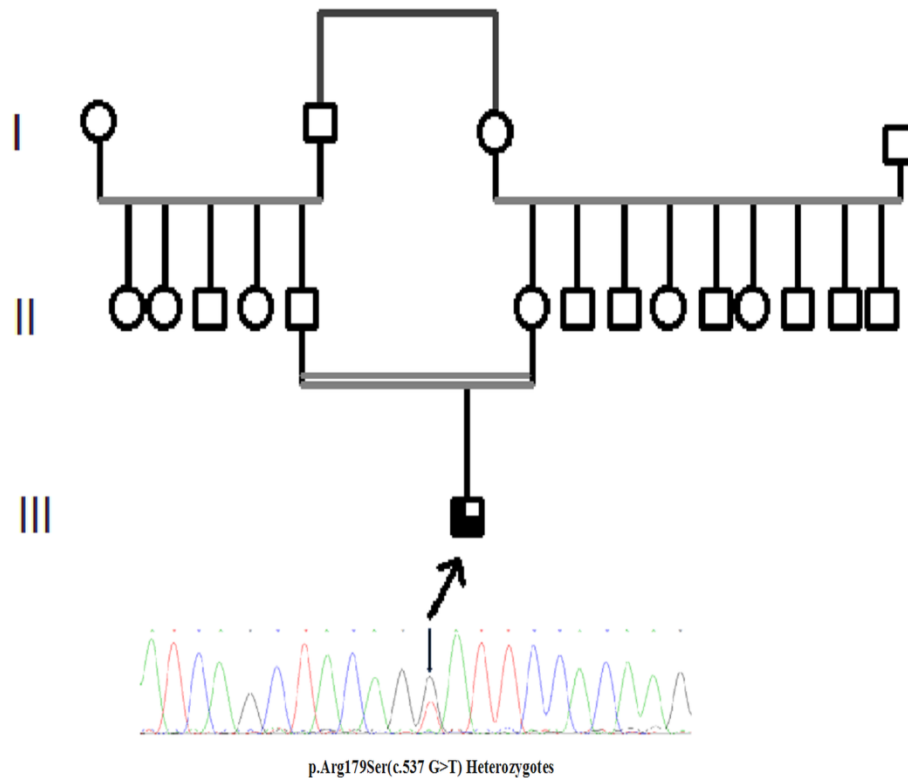


Figure 1. Family pedigree of the case with a novel p.Arg179Ser (c.537 G>T) heterozygotes mutation of SRD5A2 gene

DISCUSSION

Steroid 5- α -reductase deficiency (SRD) is a rare autosomal recessive form of 46,XY disorder of sex development, which cause incomplete virilization (10). The defect of enzyme converting T to DHT cause SRD. DHT is responsible for the differentiation of male external genitalia. SRD has two isoenzymes that contains SRD5A1 and SRD5A2. The SRD5A2 isoenzyme is expressed in the genital skin tissue during fetal development. Deletions and mutations of SRD5A2 gene result in undervirilized external genitalia. Physical examination, biochemical features and radiological findings are important for diagnosis of SRD (10). During early infancy and puberty period, the diagnosis is generally based on an increased ratio of T to DHT, with or without hCG stimulation. One study indicated that T/DHT ratio greater than 10 detected almost 75% of the patients; hCG stimulated T/DHT ratios were also used (11). However, SRD5A2 mutations have been reported in cases with a non-significant increase in this ratio, so DNA analysis is crucial to obtain the correct diagnosis (12). It was known that the phenotypic variability is a striking feature of 5 α -reductase deficiency. Clinical features of SRD is ranging from male phenotype with hypospadias to female phenotype with bilateral testes, normal Wolffian

structures and a blind vaginal pouch (12,13). The phenotypic variability may be owing to the residual 5 α -reductase type 2 activity and consequently because of mutation type and environmental factors or the action of 5 α -reductase type 1. Commonly, the following are increased as females but the increase of serum T and 5 α -reductase type 1 (SRD5A1) enzyme activity at puberty, cause growth of musculature mass, phallic enlargement and deepening of the voice (14,15). SRD5A1 located on chromosome 5p15 is expressed in post-pubertal skin and scalp. The SRD5A2 gene that encodes SRD5A2 enzyme is placed on chromosome 2p23. The coding region of the gene includes 5 exons that are translated into a 254-amino acid protein (16). Approximately 96 different genetic variants of SRD5A2 including 13 small deletions, two gross deletions, 70 missense/nonsense mutations and six splicing mutations have been reported (17). Our patient has p.Arg179Ser (c.537 G>T) mutation in the SRD5A2 gene and in congenital metabolic disease screening revealed accompanied by biotinidase deficiency (Figure 1). To the best of our knowledge, the R179S mutation has not been detected in the literature. In addition to this, biotinase enzyme deficiency that accompany with 5-alpha reductase gene mutation has not been

reported to date. Due to the extensive heterogeneity in the clinical features, phenotype/genotype correlations are not well established. Microphallus, penoscrotal hypospadias, bifid scrotum was detected in the genital examination of our patient at birth. These symptoms of abnormal sex development are similar to 17 β -hydroxysteroid dehydrogenase deficiency and partial androgen insensitivity.

For the diagnosis of a SRD5A2 deficiency, elevated T/DHT ratio after hCG stimulation for 3 consecutive days was reliable (11). The description of gender is a complex biological and psychological events, which had pre-natal component. A possible biological factor is the severity of the mutation in terms of the in vitro enzyme production disorder, causing less or more (prenatal) exposure of the brain to androgens (14), but it was reported that a gender role change is not only associated with the mutation severity (18). Cultural or environmental pressures have also been reported as potential determinants (14). Prenatal exposure of the brain to androgens has increasingly been mentioned as a crucial factor in the development of gender identity (19). The brain is prenatally exposed to normal male T levels in 5 α -reductase deficiency and also in severe undervirilization of

external genitalia (20,21). Due to the gender identity is crucial and may have severe psychological effects in the life of individuals; early diagnosis of gender and well-planned management of the severe under virilization of external genitalia and genetic counselling for parents are important after a definite diagnosis.

CONCLUSIONS

We reported a novel p.Arg179Ser (c.537 G>T) heterozygotes mutation of SRD5A2 gene that accompany with biotinidase deficiency in newborn with ambiguous external genitalia. Molecular analysis in cases with suspicious SRD is important for correct diagnosis. The combination of testosterone treatment to response and molecular results along with family and cultural values should be consideration for sex selection. To obtain more certain knowledge about the relation among the R179S mutation and biotinase enzyme deficiency and ambiguous external genitalia, additional studies about the current topic should be performed.

Declaration of interest: The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding: There is no funding.

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