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CASE REPORT

A Girl with 45,X/46,XX Turner Syndrome and Salt Wasting Form of Congenital Adrenal Hyperplasia Due to Regulatory Changes

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SUMMARY

The incidence of Congenital Adrenal Hyperplasia (CAH) is 1:10,000 - 16,000 worldwide, of which 90% occurs in the CYP21A2 gene coding for steroid 21-hydroxylase. On the other hand, Turner's syndrome, with an incidence of 1:2500, is a form of gonadal dysgenesis which leads to early ovarian failure and other phenotypic changes such as webbed neck, widely-spaced nipples and short stature. Here, we present a girl suffering from both 45,X/46,XX Turner's syndrome and salt wasting (SW) form of CAH.
Clinical and biochemical examinations were performed for the patient. Cytogentic studies and molecular testing such as allele specific PCR for eight mutations in the CYP21A2 gene, multiplex ligation probe amplification (MLPA) and direct sequencing confirmed the clinical diagnosis. Heterozygous mutations in the regulatory region at positions -316 to -264 verified SW form of 21-hydroxylase deficiency. 45,X/46,XX mosaicism proved Turner's syndrome. The SW presentation of the patient may be due to the CYP21A1P microconversion. The study of regulatory changes of the CYP21A2 and gender differentiation pathways would be possible using such patients.
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KEY WORDS

CYP21A2 gene, 21-hydroxylase deficiency, Turner syndrome

INTRODUCTION

Ambiguous genitalia are commonly seen in females born with classical CAH due to 21-hydroxylase deficiency (21-OHD), which is necessary in the synthesis of cortisol as well as aldosterone. In the severe salt wasting (SW) form, cortisol and aldosterone secretion is negligible. Cortisol, which normally regulates adrenocorticotropic hormone (ACTH) via negative feedback, is decreased [1]. Excess ACTH stimulates the synthesis of precursor adrenal hormones which become shunted into the adrenal androgen pathway. *In utero,* the excess androgens virilize the female external genitalia. After birth, lack of mineralocorticoids causes a life-threatening SW crisis typically seen within the first few weeks of life. Patients present with hyperkalemia, hyponatremia, diarrhea, vomiting, and shock needing urgent care with aggressive intravenous resuscitation with sodium containing fluids [2]. These patients may have various degrees of phenotypic expression and ambiguity of genitalia generated by genotypic variability. Age manifestation of the disease also depends on the genotypes.
Different genes participating in the steroidogenesis pathway may cause different phenotypes. In addition, alternative splicing, receptor mutations and modifier genes may influence the phenotypic variability. However, other genetic defects may be carried by the patients that show different symptoms [2].

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Turner's syndrome is a disorder of 46,XX gonadal dysgenesis involving a chromosomal abnormality due to loss of one X chromosome. Some patients have a mosaic karyotype e.g. 45,X/46,XX. Mosaic karyotypes are usually formed due to chromosome lag in a post-zygotic mitotic division. Phenotype of patients with Turner's syndrome is variable and typically includes short stature, webbed neck, widely-spaced nipples, and infertility due to early ovarian failure [3]. Here, we report about a girl with CAH and SW crises having mosaic Turner's syndrome.

MATERIALS AND METHODS

Case Presentation

A thirteen year old girl is under clinical management for CAH. She is the third child of a first cousin marriage from the northern part of Iran (Mazandaran province). Their ethnicity is Persian-Caucasian. When she was three months old, she was referred to the Children's Medical Center Hospital, Tehran University of Medical Sciences due to diarrhea, vomiting, and lethargy. Examination on admission showed signs of hyperpigmentation, hyponatremia, and hyperkalemia, dehydration and shock. Her weight was 2500 g and length 50 cm. Blood pressure was 60/40 mmHg. Her heart rate was 130/ minute. Therefore, she needed intravenous fluid therapy. Birth weight was 2900 g, length 47 cm and head circumference 34 cm. External genitalia were ambiguous with clitoromegaly, labia fusion, and no palpable gonads in labia. Sonographic examination showed normal ovaries, fallopian tubes, uterus, upper vagina and cervix. In addition, physical examination by her pediatrician revealed short neck, webbed neck with low posterior hair line, high arched palate, pectus excavatus, widely-spaced nipples and non-pitting edema on hands and feet which was diagnosed as Turner's syndrome stigma.

Biochemical tests revealed a high 17-hydroxyprogesterone (17-OHP) concentration of 20 nmol/L (normal range [NR]: 0.2 - 2.3 nmol/L), base cortisol of 2 µg/dL (NR: 5 - 23 µg/dL), testosterone 10 ng/dL (NR: 3 - 10 ng/dL), dehydroepiandrosterone sulfate (DHEA-s) 300 µg/L (NR: 3 - 83 µg/L), Δ^4-androstenedione 2 ng/dL, (NR: 0.28 - 1.75 ng/dL), ACTH 1000 pg/mL (NR: 25 - 100 pg/mL), and plasma renin activity 300 ng/mL/hour (NR: 0.2 - 2.5 ng/mL/hour). Plasma Na 120 mEq/L (NR: 139 - 146 mEq/L) and K 6 mEq/L (NR: 3.5 - 5 mEq/L) levels were assessed. 21-hydroxylase deficiency was diagnosed by an endocrinologist according to published criteria. Karyotype analysis using GTG banding revealed mosaicism 45,X [10] /46,XX [20] and confirmed mosaic Turner's syndrome. Thus, both signs of congenital adrenal hyperplasia and Turner's syndrome were supported clinically and cytogenetically.

Vaginoplasty was performed when she was 9 months old. Abdominal sonography and echocardiography were normal. She was under replacement therapy with fludrocortisone acetate (salt is retained in the body) and hydrocortisone until now. Now, at 13 years of age, height is 144 cm and weight 44 Kg. She shows poor growth and -4SD on growth chart (below 3 percentile). She is on cyclic therapy with oral contraceptive pills.

After informed consent was given by the family, molecular diagnosis was carried out. 5mL of the peripheral blood was taken for DNA extraction. Allele specific PCR for eight *CYP21A2* gene mutations [4], multiplex ligation probe amplification (MLPA) using SALSA MLPA kit P050B2 (MRC-Holland, Amsterdam, The Netherlands) and direct sequencing (ABI 3730XL, PE Applied BioSystems, Foster City, CA, USA) were done.

Molecular analysis

Typical of autosomal recessive disorders, consanguinity is present in the pedigree. No other family members within the pedigree described signs of CAH.

Allele specific PCR of the CYP21A2 gene showed no common mutations [5]. Sequence analysis of the entire *CYP21A2* gene (RefSeq NG_007941.2) including all exons and exon/intron boundaries showed no significant exonic changes except for some polymorphisms (e.g. g.118C > T, g.138C > A). Intronic changes were observed at g.+630-631CA > GG and g.+2375G > A. Direct sequencing of the regulatory region of the *CYP21A2* gene revealed mutations at position -295T > C, -294A > C, -283A > G, -281T > G, -196A > C in heterozygous state.

MLPA analysis of the *CYP21A2* gene revealed no deletion or duplication of the *CYP21A2* gene but the 5'*CYP21A1* probe displayed regulatory changes at position -316 to -264 which is influenced by the microconversion of the 5'*CYP21A2* gene which belongs to both alleles. Also, dosage analysis of chromosome X revealed one copy of the probe instead of two copies compared to the control samples by GeneMarker Version 1.85 (SoftGenetics LLC, State College, PA, USA) (Figure 1).

In silico analysis by Human Splicing Finder Version 2.4 (HSF) [6] of the intronic changes showed new splicing sites as being acceptor sites at position g.+630-631CA > GG and g.+2375G > A.

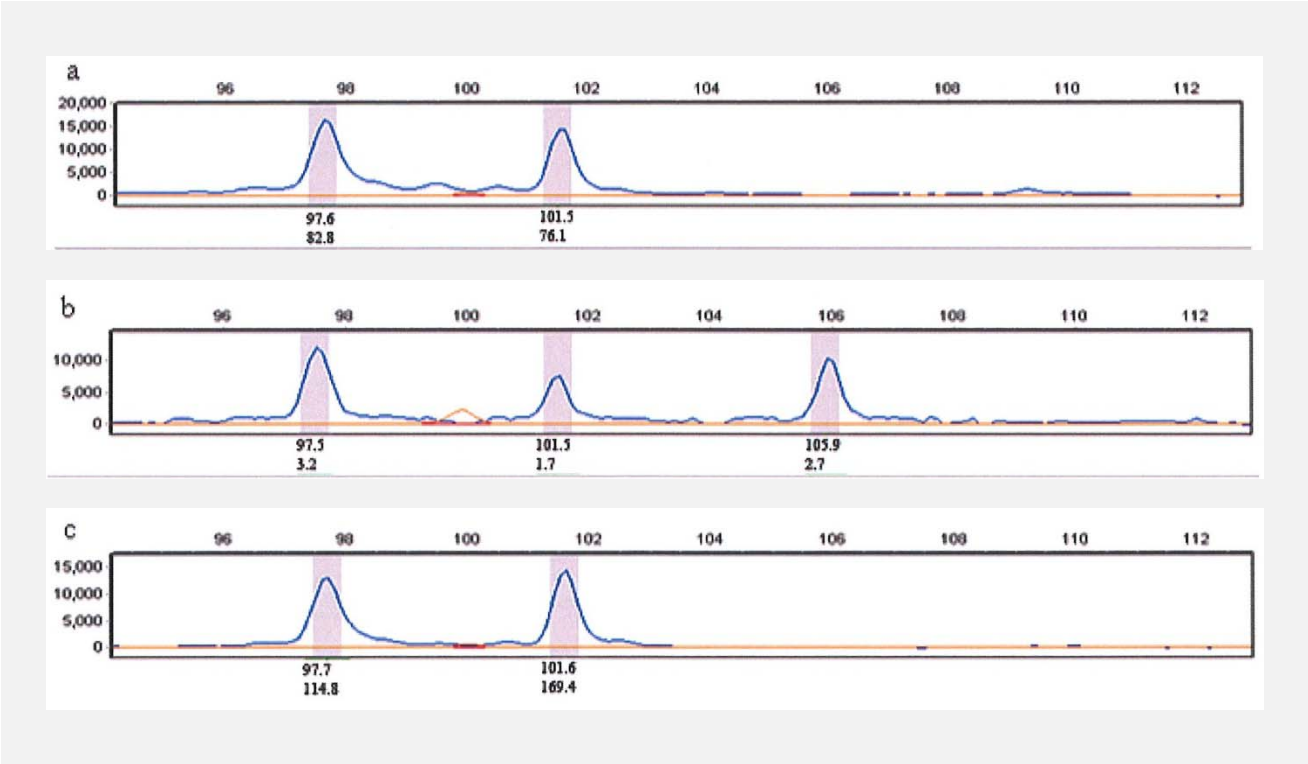

Figure 1. Diagram indicates part of electropherograms of MLPA analysis of a) case, b) negative male control, and c) negative female control, showing the position of X and Y chromosome at 101 bp and 105 bp fragments, respectively. The X chromosome of case (a) has approximately the mid peak area of a female (c) and higher peak area than a male X chromosome (b).

DISCUSSION

Patients with multiple disorders have previously been reported but the occurrence of two disorders together is a rare event. Many reports of CAH have been published and a few associated with Turner's syndrome [7,8,9]. Turner's syndrome occurs in 1 in 2500 [10] female births; mostly due to non-disjunction of the homologous chromosomes during parental meiosis. Approximately 20% of the cases have mosaicism. However, considering the frequency of CAH, the prevalence of such a case with two diseases would be 1:2,500 × 1:10,000 to 16,000, = 1 in 25,000,000 to 40,000,000, which indicates its rarity. In a first cousin marriage, which is frequent in the Iranian population [5,11,12], the coincidence occurrence of classic CAH and Turner's Syndrome may be much higher. Assuming a CAH heterozygote frequency of 1/50, we may expect 1/50 × 1/8 (fraction of shared genes in first cousins) × ¼ (affected offspring in recessive disorders) × 1/2,500 = 1 in 4,000,000 female births which is also rare. Mosaicism is usually the result of an anaphase lag in post zygotic divisions. CAH is a monogenic disorder transmitted as an autosomal recessive trait owing to the *CYP21A2* gene mutations. Consequently, diagnostic tests noticed that 1) the patient had two diseases, which is a rare event, 2) Turner's syndrome was the result of post zygotic divisions due to chromosome lag at anaphase, and 3) CAH was due to regulatory changes. Sequencing analysis and MLPA results indicated that the presence of regulatory changes at positions -295T > C, -294A > C, -283A > G, -281T > G, -196A > C [13] would influence the *CYP21A2* gene function. No sign of gene duplication or deletion was detected in the patient. Other studies have reported the association of promoter mutations with non-classic (NC) and simple virilizing (SV) forms of CAH [14-16]. To our knowledge, this is the first report of a SW form with regulatory changes. In addition, other reports have demonstrated the influence of other regions e.g. intron 35 C4B gene as a novel cAMP-dependent regulatory element [17,18]. Mutations at regulatory sites of a gene may alter the gene expression, which should be functionally analyzed.

Further sequencing results of the patient for all coding regions, intronic and splice sites of the CYP21A2 gene, revealed heterozygous changes at position p.L39 (g.118 C > T) and g.138C > A at p.P45 which are carried in different forms of chimeras [18]. Here, we concluded that these changes might be associated with microconversions of the regulatory regions. Also, these changes may affect expression of the gene at the RNA level. *In silico* analysis by HSF [6] of the intronic changes showed new splicing sites as being acceptor sites at position g.+630-631CA > GG and g.+2375G > A, which acted as enhancer motifs that constitute the matrices for SRp40, SC35, SF2/ASF, and SRp55 proteins; it may al-

so act as exonic splicing regulatory sequences at position +630/631. These changes may lead to cryptic splice mutations, thus, pathologic effects on hnRNA splicing should be investigated by more functional studies. The pathogenicity of these mutations was only evaluated *in silico*. The HSF bioinformatic program was used to score the mutations as deleterious. The *in silico* analysis tool could be used for predicting the functional influence of a mutation or polymorphism. However, *in vivo* studies should be performed to evaluate the impact of the mutation.

In conclusion, the SW presentation of the patient may be due to the *CYP21A1P* microconversion and leaky splicing site of the intronic region. Furthermore, this patient would be beneficial for the study of regulatory changes of the *CYP21A2* gene and gender differentiation pathways, since it is an SW patient with regulatory changes. Also, the combination of new splicing sites could be important for the leaky mutation evaluation.

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Declaration of Interest:
The authors have declared no conflict of interests.

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