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Case Report

A Novel Mutation in 11 Beta -Hydroxylase Combined with 21 Hydroxylase Duplication and Heterozygous Mutation presented with neonatal Salt Wasting 11 Beta Congenital Adrenal Hyperplasia and Adolescent Polycystic Ovarian Syndrome in a Saudi Girl: Case Report and Literature Review

Aida Al Jabri*, Yassin Al Saleh[1], Maram Al Subaiee[2], Sakinah El Sharif[3], Abdulrhman Al Mulla[4], Rania Al Harazi[5]

1. Pediatric Endocrinologist, Department of Pediatrics, Maternaty and children Hospital, Al-Ahsa, Saudi Arabia.
2. Adult Endocrinologist, Department of Medicine, King Abdulaziz Hospital, Ministry of the National Guard-Health Affairs, Al-Ahsa, Saudi Arabia.
3. Pediatric Regestrar, Department of Pediatrics, Security Forces Hospital, Dammam, Saudi Arabia.
4. Adult Endocrinologist, Department of Medicine, King Abdulaziz Hospital, Ministry of the National Guard-Health Affairs, Al-Ahsa, Saudi Arabia.
5. Oby/Gyne, Department of Oby/Gyne, Saudi German Hospital, Dammam, Saudi Arabia.

***Correspondence to:** Aida Al Jabri, Pediatric Endocrinologist, Department of Pediatrics, Al Mossa Specialist Hospital and Research Center, Al-Ahsa, Saudi Arabia.

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Abstract

Congenital Adrenal Hyperplasia (CAH) is one of the commonest inherited disorders, that affects both sexes alike. A 90% of all cases is caused by 21 Hydroxylase Deficiency (21-OHD) and the affected patient will have cortisol and mineralo-corticoid deficiency leads to salt losing, as well as ambiguous genitalia in affected female. The incidence of 5% of all cases of CAH is due to mutations in the gene CYP11B1, and it has a comparable mechanism to those of 21-hydroxylase except because of accumulation of deoxycorticosterone (DOC) before the enzymatic defect, patients with 11β-hydroxylase deficiency may develop hypertension (HTN). The object of this clinical case report is to highlight this unusual presentation of a unique Novel CYP11B1 gene mutation in a Saudi girl, manifested as a typical 21-OHD deficiency who presented with salt-wasting crises during her neonatal period with most likely genetically determined polycystic ovarian syndrome (PCOS) in adolescence. And up to our knowledge, this is the 1st case in our population and worldwide have such combination and this unusual clinical presentation.

Introduction

Congenital adrenal hyperplasia (CAH) is a group of hereditary disorders characterized by adrenal steroid synthesis defects due to various enzymatic deficiencies.(1-13)

21-hydroxylase deficiency is the most common form, which accounts for more than 90% of cases of congenital adrenal hyperplasia. It is due to autosomal recessive mutations in the CYP21A2 gene, which has a significant role in glucocorticoid, mineralocorticoid, and adrenal androgen pathways, leading to metabolic derangements, electrolyte imbalance, and defects in sexual differentiation. The incidence of 5% of all cases of CAH is due to mutations in the gene CYP11B1, and it has a comparable mechanism to those of 21-hydroxylase except because of accumulation of deoxycorticosterone (DOC) before the enzymatic defect, patients with 11β-hydroxylase deficiency may develop hypertension (HTN), hypokalemia virilization, hyperpigmentation, and headache. On the other hand, they usually do not manifest a salt-wasting adrenal crisis.(7, 14-20)

Although it is considered a rare type of CAH, it is relatively frequent among Saudi Arabia's population

compared to reported prevalence from other countries. Our case report describes a unique and Novel CYP11B1 gene mutation in a Saudi girl, manifested with salt-wasting crises during her neonatal period with most likely genetically determined polycystic ovarian syndrome (PCOS) in adolescence.

Case Presentation

Our patient is a 16-year-old Saudi female from Alhasa in the eastern province but originally from the south of Saudi Arabia. She was born at a term with genital ambiguity and admitted to the neonate intensive care unit for observation and investigation. A karyotype was done, which revealed 46 XX. A pelvic ultrasound was done and showed normal female internal organs. Laboratory investigation (table 1 and 2) at the age of 5 days revealed high 17 hydroxy progesterone (17-OHP), adrenocorticotropic hormone (ACTH) was very high, cortisol was low, electrolyte Na and glucose were low and her K level at that time was high. A genetic study done, a Gene sequencing revealed heterozygous mutation c.952C>T (p.Gln318X) and duplication of the CYP21A2 gene mutation. So, based on the initial presentation, she was treated as a typical case of CYP21A2 CAH with hydrocortisone and fludrocortisone. Reconstruction surgery was done at the age of 1 year, and then her family missed her appointment with the surgeon.

Table 1: Hormonal Workup	
17 hydroxy progesterone	320 nmol/L
ACTH	113 pg/mL
cortisol	25 nmol/L
pH	7.12

Table 2: Basic workup	
Sodium	131 mmol/l
Potassium	5.9 mmo/l
Glucose	2.8 mmol/l
Bicarbonate	8.7 mmol/l

At the age of 3 years, the physician was able to discontinue the fludrocortisone and continue her on hydrocortisone alone. A few months back, at the age of 16 years, the patient was found to have a deepening of her voice, and while she did not yet menstruate. Her physical examination showed signs of high Androgen with virilization, worsening of her acne, her Tanner stage 1 breast development, and Tanner stage 5 pubic hair. Genital examination shows clitoromegaly with a clitoris 5.5 cm in length, a moderate posterior fusion of her labia, and a single perineal orifice. These changes were attributed initially to poor compliance with medication despite her denial. Later, during her close follow-up, all her labs (including 17 OHP and ACTH) were repeated, and the results were within acceptable range, which ruled out the compliance problem.

Suspicious of polycystic ovarian syndrome (PCOS) versus ovarian adrenal rest tumor (OART) was raised.

Hormonal investigation and ultrasound confirmed the diagnosis of PCOS with high androgen levels (testosterone, androstenedione) and cystic ovaries. in the ultrasound.

Interestingly, a relative of hers presented to our clinic was diagnosed as a case of peripheral precocious puberty secondary to nonclassical CAH and was found to have the same mutation of heterozygous mutation c.952C>T (p.Gln318X) and duplication of the CYP21A2 gene mutation.

Back to our patient, she has 21hydroxylase deficiency based on her initial presentation, laboratory, and genetic study along with doubts of non-compliance, but since her carried mutation has never been published with the classical form of CAH with such ambiguity, also in the meantime this mutation could present as precocious puberty with PCOS in adolescents.

Therefore, her primary diagnosis is questionable. We asked to repeat the genetic study. Second genetic studies were done, and it was found that she carries a homozygous variant c.1337G>C p. (Gly446Ala) in the CYP11B1 gene, which is the cause of her initial symptoms and genitalia ambiguity. To the best of our knowledge and intensive research, there was no same variant mutation combined with her initial symptoms as a salt-wasting CAH overall the word.

Finally, our patient went to her 2nd reconstruction surgery at the age of 16 years, and currently, she carries normal female genitalia.

Discussion

Congenital Adrenal Hyperplasia (CAH) is an autosomal recessive disorder characterized by cortisol deficiency due to a block in one of five enzymes along the adrenal steroid synthesis pathway(1). Deficiency of cortisol will result in the reduction of the negative feedback on the hypothalamic-pituitary-adrenal axis. This will lead to ACTH overproduction and subsequent adrenal overgrowth, ending with substrate accumulation before the block with consequent clinical manifestation of electrolyte disturbance, hypoglycemia, and virilization due to excessive androgen synthesis (2-5).

The most common form of CAH is 21 hydroxylase deficiency (21-OHD) due to CYP21A2 mutation on chromosome 6p21.3 (12). It is categorized into classical and nonclassical or late-onset forms. The classical one is further subdivided into salt-wasting and simple virilizing (6-8). Phenotypically, presentation varies according to the degree of severity, which is determined based on the level of functional loss of the enzyme 21 hydroxylase activity genotypically.

The patient would present with salt wasting and virilization in complete gene inactivation. In contrast, the preserved gene activity of around 1-2% will result in simple virilization only. However, those with 20-70% activity usually display a nonclassical form with a mild phenotype of virilization (5, 9-12).

The second most common enzymatic cause of CAH is 11beta -hydroxylase deficiency (11β-OHD), which accounts for about 5-8% of CAH cases. (14) 11 beta-hydroxylase (CYP11B1) gene is situated on chromosome 8q21-q22.

Deficiency of 11-beta-hydroxylase action in the zona fasciculata in the adrenal gland blocks the conversions of 11-deoxycortisol to cortisol and 11-deoxycorticosterone (DOC) to corticosterone. The consequences are an accumulation of steroids with mineralocorticoid activity and an overproduction of androgens.

To date, more than 100 different mutations in human CYP11B1 distributed throughout the gene have been recorded in the Human Gene Mutation Database (21-24)
The Mutation described includes missense mutations which diminish enzymatic action (21-24) frameshift mutation(25), and nonsense mutations(26, 27) which inhibit the synthesis of the enzyme.

In a large Cohort study (published in 2010), they found the incidence of 11β-OHD in Moroccan Jews is 1:15,000–57,000 live births, which is mainly due to p.R448H mutation. (16, 17) R448H is certainly the most common mutation, which is why we have a high prevalence of 11OHD in Jews of Moroccan origin (21)
Another frequent mutation in the Tunisian population is p.Q356X, which is also common in African Americans and North Africans. (28)
CYP11B1 and CYP11B2 (gene encoding aldosterone synthase required for aldosterone synthesis) showed 95% sequence homology in their coding regions and 90% in intronic sequences.(15)
In 2001, Two patients from different case reports were found to have 11 beta-hydroxylase deficiency, which occurred as a result of unequal recombination between the CYP11B1 and CYP11B2 genes,(29, 30), which is suggestive of the genetic event leading to glucocorticoid-remediable aldosteronism (GRA).

Another combination that can present as NCCAH or be misdiagnosed as polycystic is combined CYP21A2 and CYP11B1 heterozygous mutations, which have been reported in more than one study(31, 32).
Furthermore, 25-50% of the nonclassical CAH patients were diagnosed with either a homozygous or a

compound heterozygous having 2 mild alleles affection, while the majority around 50-75% were diagnosed with compound heterozygous having one of the alleles with severe mutation, which manifest earlier with higher 17-hydroxyprogesterone (17 OHP) level in comparison to homozygous group(12, 13, 33, 34)

In some cases of CYP21A2 mutation, clinical and hormonal findings are not consistent with the diagnosis of CAH. In such cases, duplication of CYP21A2 should be suspected. As reported in a case presentation by O Lekarev et al.(35).

They described a patient born to an affected mother of salt-wasting CAH due to 21OHD. A genetic test was conducted antenatally and revealed the fetus carrying exon 8 mutation (Q318X) from the father and exon 8 (R356X) from the mother. Therefore, CAH was suspected in the fetus. After birth, the newborn screen was negative, and the clinical and biochemical profile did not support the diagnosis of CAH, so the baby was retested for genetic DNA and found to have a paternal CYP21A2 gene duplication with Q318X mutation on one copy of CYP21A2.(35)

Another study conducted in Vienna in 2009 detected that more than 80% of the individuals with Q318X mutation were carriers of duplicated CYP21A2.(36) This duplication categorized the patient as a mutation carrier rather than affected by CAH.

In Sweden, duplicated CYP21A2 were reported in 1.6% of the population; also, 1.7 % of healthy Caucasians carried duplication of CYP21A2.(37) Furthermore, 7% of healthy individuals in Spain were found to have a duplicated CYP21A2 gene, with one copy affected with a Q318X mutation.(36)

Harrat M et al. demonstrate a high prevalence of the Q318X mutation in the CYP21A2 in patients from the Tunisian population. Also, they found a very high frequency of carriers with duplicated CYP21A2 gene haplotype in a healthy population. So, they recommend that once a Q318X is detected, we should discriminate between the severe Q318X mutation and the normal Q318X variant.(38)

Regarding the Clinical features of the disorder of sex differentiation (DSD) is considered the most common presentation in female patients with 11β-OHD, while male patients may present with peripheral precocious puberty in the form of penile enlargement and early pubic hair development and hyporeninaemic hypokalemic hypertension, 11β-hydroxylase is also classified to classical and nonclassical form. Premature adrenarche, hyperandrogenism, menstrual disorders, and hypertension characterize the Nonclassical form of 11β-hydroxylase deficiency. The nonclassical form is milder and rarer than the classical one. Therefore, the

diagnosis may be delayed or misdiagnosed as polycystic ovary syndrome or primary hypertension.(7, 18-20)

In neonates, the diagnosis can be established based on elevated basal and ACTH-stimulated serum 11-deoxycortisol concentrations with low cortisol or increased urinary excretion of tetrahydro-11-deoxycortisol with low cortisol metabolites.(14, 39, 40) In young adults, basal serum 11-deoxycortisol values may be normal. Therefore, cosyntropin stimulation testing is often required for diagnosis establishment.(41, 42) Diagnosing nonclassical 11OHD requires normal or near-normal cortisol in addition to elevated 11-deoxycortisol with a level of >1800 ng/dL. Nevertheless, diagnoses need to be confirmed with a genetic study.

17OHP is often moderately elevated in 11OHD, 17OHP will accumulates above the block of 11-beta-hydroxylase. So, for a young girl with mild virilization, androgen excess, and moderate 17OHP elevations. The differential diagnosis includes nonclassical 21OHD, which is very common, followed by the less common 11OHD, and the last 3HSD3B2 deficiency which is extremely rare.(43) To differentiate between these, we need to measure the other steroids, like DOC, 11-deoxycortisol, and 17-hydroxypregnenolone, to determine the enzymatic defect.

High androgen levels in CAH patients in the presence of androgen-responsive external genitalia will lead to ambiguity in the female genitalia. On the other hand, Mullerian structure, including the uterus and ovaries, will be spared and develop normally. Hence, females with classical and nonclassical CAH have the chance for fertility.(44)

The majority of females with classical CAH developed menarche late, hirsutism, androgenic alopecia, oligomenorrhea, and primary or secondary amenorrhea are not unusual among those with poor compliance to medication, but it can happen in an inadequately treated or excessively treated patient.(44)

Fertility is reduced among patients with CAH, and several reasons have been proposed, including androgen overproduction, hypersecretion of progesterone, ovarian adrenal rest tumors, outcomes of genital surgery, polycystic ovarian syndrome, and psychosexual issues.(45)

Polycystic ovaries contribute to subfertility, a common finding seen in ultrasonography in patients with classical and nonclassical CAH. More than this, it fulfills the criteria of Rotterdam to diagnose PCOS.(45)

Several reasons have been suggested that may play a role in the development of what is called secondary polycystic syndrome, including Androgen overproduction, hypersecretion of luteinizing hormone (LH) hypersecretion, as well as excess androgen exposure while in utero.(45)

Hague WM et al. investigated around 77 female patients with CAH (36 adults and 41 children under 16) using hormonal tests, pelvic ultrasound, and HLA typing. Also, they studied 46 close female relatives by ultrasound for ovarian abnormalities after assessment of their heterozygous state using HLA typing. The coexistence of polycystic ovaries (PCO) by Ultrasound in CAH patients was as follows: in an adult patient (83%) adult patients, in post-pubertal girls (40%) while in pre and peripubertal girls (3%), overall, 35/46 (76%) postmenarcheal patients. 6/9 (67%>) pre-menopausal mothers of patients with PCO and 8/10 (80%) sisters of patients with PCO also had PCO.

They conclude that the proportions of CAH patients and heterozygote subjects with PCO were significantly greater than that of a normal population. However, they described two homozygous non-CAH-affected adult sisters with PCO. On the opposite side, we have heterozygous adult relatives and postmenarcheal CAH patients with normal ovaries, which may indicate that PCO may be independent of CAH.(46) Another study found that the prevalence of PCOS among nonclassical CAH carriers is high compared to the general population.(47)

Furthermore, in one study, they found that 8.4% of the women with clinical and biochemical features of PCOS could be assumed to have 11 beta-OH deficiency based on high 11-DOC level after ACTH stimulation.(48)

Another dilemma is how to differentiate between primary PCOS and secondary type. Insulin resistance and the polycystic ovarian morphology on ultrasound are believed to be markers of PCOS also present in NCAH. Also, Obesity is not helpful to differentiate since many nonclassical CAH females were found to be obese.(49)

Regarding our patient, she presented in the neonatal period with genital ambiguity and salt-wasting, which is extremely rare in CAH patients due to 11-beat hydroxylase deficiency with few reports. Al Jurayyan, in his study, concludes that in the Saudi Arabian 11 beta-hydroxylase deficiency is relatively frequent among the population (50, 51), but given that 21 hydroxylases are far more common and are found to have CYP21A2 heterozygous and duplication status in a molecular genetic patient was labeled as 21 hydroxylase deficiency.

Later on, when the patient presented with primary amenorrhea, virilization, clitoromegaly, hirsutism, and PCOS finding on ultrasound in addition to lack of need for mineralocorticoid medication, her primary diagnosis was reviewed due to the diagnostic uncertainty, subsequent genetic study showed a novel mutation in CYP11B1. Interestingly, another study from Saudi Arabia in 2017 found that 11 β-OHD in Saudi Arabia has a unique genotype with a high rate of novel mutations. Also, they found that the p. R448P mutation is the most common in the Saudi Arabian population.(52) While in our patient, we found (c.1337G>C p.Gly446Ala) mutation in the CYP11B1 gene, which supports the idea of a high rate of a new mutation in our population.

Our patient presented with a picture of salt-wasting CAH /PCOS. Double heterozygous mutation of CYP21A2 and CYP11B1 was reported before with the same presentation.(31) Still, to the best of our knowledge, this is the first time to report homozygous CYP11B1 together with heterozygous duplication in CYP21A2, causing classical salt-wasting CAH and PCOS.

A gene sequencing in our patient revealed heterozygous mutation p.Gln318X (Q318X) and duplication of the CYP21A2 gene mutation due to a lack of a definitive tool to differentiate between the normal variant and severe pathogenic Q318X mutation. (53) We are unable to judge whether this mutation added to clinical presentation or just coexistence.

Conclusion

This case showed a patient with a clinical picture, laboratory investigation, ultrasonographic features, and molecular genetic results suggestive of nonclassical CAH And PCOS. It was misdiagnosed initially as classic CYP21A2 CAH with primary PCOS. However, it was found to have a unique and novel combined mutation in the form of homozygous CYP11B1, heterozygous, and duplication mutation in CYP21A2, causing classical features of salt-wasting CAH. Q318X mutation is identified in our case with no apparent association to the clinical picture; identifying pathogenic one from normal variant Q318X mutation is straightforward but very important, especially for future pregnancy and genetic counseling.

A high index of suspicion is the first step to proper diagnosis. CAH should be considered in all females with amenorrhea, virilization, and infertility. This study highlights the importance of the molecular genetic study to confirm the diagnosis to manage the patient properly, for genetic counseling, and to put a plan for subsequent pregnancy.

Given the higher prevalence of PCOS in this patient population, clinicians should consider the diagnosis of PCOS in patients who are found to be heterozygous for CYP21A2 gene mutations on expanded carrier

screening, which makes our patient presented with ambiguous genitalia and salt-wasting 11beta CAH and this variant never been published in the literature.

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