



Approaching fertility in congenital adrenal hyperplasia: exploring P30L mutation-induced 21-hydroxylase deficiency with a presentation between non-classical and simple virilizing phenotypes. A case report

Department of Endocrinology, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

Alina Maria Herdean, Adana Maria Radulescu, Ioana Rada Popa Ilie

Abstract

Congenital adrenal hyperplasia (CAH) is determined in the vast majority of cases by mutations in the *CYP21A2* gene, which cause the deficiency of the 21 hydroxylase enzyme, which is involved in the synthesis of cortisol and aldosterone. Generally, CAH phenotype and disease severity can be predicted with the genotypes and is related to the residual activity of 21 hydroxylase enzyme. It is divided into classical CAH with salt wasting and simple virilizing forms and non-classical or late-onset CAH forms, respectively. Patients with 21 hydroxylase deficiency, including those with non-classic forms face immense challenges to their fertility. Glucocorticoid therapy has been shown to be useful in obtaining and maintaining a pregnancy among these patients, but it must be used with caution. Given the relevance of CAH in reproductive medicine as well as the diagnostic challenges posed by the phenotypic overlap with polycystic ovary syndrome and by overlap of its own phenotypes (classic CAH-nonclassic CAH), we present the case of a woman with CAH due to 21 hydroxylase deficiency caused by the P30L mutation with a clinical and biochemical presentation between the non-classical form and the classic simple virilizing form. Further, the successful fertility management in this patient and an overview of fertility management in CAH is depicted, as well.

Keywords: congenital adrenal hyperplasia, virilism, p30l mutation, fertility

Introduction

Congenital adrenal hyperplasia (CAH) represents a group of autosomal recessive disorders that result from impaired steroidogenesis due to mutations in genes encoding certain steroidogenic enzymes.

In over 90% of cases, CAH is due to 21-hydroxylase deficiency (21-OHD), an enzyme which catalyzes the conversion of 17-OH progesterone to 11-deoxycortisol and that of progesterone to deoxycorticosterone, which represent precursors in the pathway of cortisol and aldosterone synthesis, respectively. Mutations in the *Cytochrome P450*

(*CYP21A2* (*CYP21A2*)) gene cause the deficiency of 21 hydroxylase. *CYP21A2* gene is located on chromosome 6p21.3, with most affected individuals being compound heterozygotes, having a mild and a severe mutation. The phenotype is usually determined by the milder mutation.

CAH can clinically be manifested in two major forms: classical (C-CAH) and non-classical (NC-CAH or late-onset), depending on the amount of the functioning enzyme.

Furthermore, C-CAH can be categorized into the salt-wasting form (SW) and the simple virilizing form (SV),

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Address for correspondence:
Adana Maria Radulescu
adanaradulescu@gmail.com

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with <1% and respectively 2-10% 21-OH residual enzyme activity. The residual activity of 21-OH in NC-CAH is estimated to be around 30 to 50%.

NC-CAH is much more common than C-CAH, with a prevalence of around 0.1-0.4% in the general population. Patients with NC-CAH typically become symptomatic anywhere from late childhood until adulthood. Affected females present hyperandrogenic symptoms resembling polycystic ovary syndrome (PCOS) such as hirsutism, acne, menstrual disorders, androgenic alopecia and impaired fertility. Unlike the case of C-CAH, few studies have assessed the issue of fertility in NC-CAH and the guidelines for management in pregnancy leave room for interpretation. Though little data are available, it appears that many patients with NC-CAH can conceive spontaneously, but the incidence of miscarriage is significantly higher than that of unaffected females [1,2].

Case report

A 26-year-old female, with a recent diagnosis of late-onset CAH, presented for the first time to the Endocrinology Department in September 2017, complaining of hirsutism and weight gain. She had no significant family or past medical history, except for secondary amenorrhea. She was repeatedly found with high blood concentrations of total testosterone (4.82; 6.84 nmol/L, normal values (NV) <1.67) and of DHEAS. Therefore, she had been diagnosed with polycystic ovary syndrome (PCOS) at the age of 15. It was only 4 months before her first admission to our clinic when she first had a 17-OH progesterone (17OHP) determined, which proved to be high (17OHP of 41.59 ng/ml) and so she was concluded to actually have late-onset CAH (21-OHD). She had been taking on and off the oral

contraceptive pill (OCP) Diane-35 between ages 19 and 26. At the time of presentation, she had been on 0.25 mg dexamethasone daily for 3 months and oral contraceptive pills (OCPs) Diane 35 for 4 months.

On physical examination: height: 165 cm (+0,15 DS); body weight: 69 kg, BMI of 25.3 kg/m², a Ferriman-Gallwey score of 24 consistent with moderate hirsutism and no other pathological findings. Hormonal evaluation (Table I) revealed a high 17OHP (102.3 ng/ml, NV:0.2-1.3), high total testosterone (1.86 ng/ml, NV:0.2-0.75), high-normal DHEA-S (3.12 µg/ml, NV:0.9-3.6), whereas prolactin and thyroid function tests were within normal ranges. We decided to stop dexamethasone and to substitute the OCP Diane-35 for Yasmin; since hirsutism was her main complaint, we also added an antiandrogen: spironolactone 50 mg twice daily.

At the 1-year follow-up, she admitted a significant amelioration of hirsutism, regular withdrawal bleeding, but she complained of dizziness, tinnitus and affirmatively repeated episodes of low blood pressure (as low as 70/40 mmHg). She decided to stay on the same treatment at that moment. Hormonal evaluation performed 28 months later (Jan 2020) showed a significantly decreased 17OHP, DHEA-s above the upper limit of normal, a normal total testosterone (Table I) and elevated 8 a.m. cortisol. We decided to discontinue spironolactone because of dizziness; she remained on the OCP Yasmin (until September 2020, when OCPs were also discontinued since she decided to have a child). We advised her to have a genetic testing and she did. Genetic testing was carried out by DNA extraction, followed by PCR and then reverse-hybridization on the Vienna-Lab strip. The test identified the mutation P30L (c.89C>T) in the simple heterozygote form.

Table I. The evolution of the hormonal profile of our patient.

	September 2017	January 2020	January 2021	May 2021	June 2021	April 2022 (week 28 of gestation)
Ongoing treatment at the moment of presentation	Dexamethasone 0.25 mg /day for 3 months Diane ₃₅ ® for 4 months.	Yasmin® for 28 months Spironolactone 50 mg*2/day for 28 months	Without treatment	HC 20 mg/day for 4 months	HC 20 mg/day for 5 motnhs	HC 25 mg/day for 9 motnhs
17 OH progesterone	102.3 ng/ml	6.89 ng/ml	65.37 ng/ml	99.5 ng/ml	12 ng/ml	60.6 ng/ml
Testosterone	1.86 ng/ml (NV:0.2-0.75)	2.31 nmol/L (NV:0.25-2.75)	1.74 ng/ml (NV:0.1-0.9)	2.8 nmol/L (NV:0.25-2.75)	0.63 nmol/L (NV:0.25-2.75)	1.43 nmol/L (NV:0.25-2.75)
DHEA's	3.12 mcg/ml (NV:0.9-3.6)	634.4 ug/dl (NV: 8 -511.7)	5.16 mcg/ml (NV:0.18-3.91)	246.3 ug/dl (NV: 8 -511.7)	N/A	44.74 ug/dl (NV: 8 -511.7)
ACTH	N/A	N/A	87.44 pg/ml (NV: 7.2-63.3)	107.2 pg/ml (NV: 7.2-63.3)	33.12 pg/ml (NV: 7.2-63.3)	60.84 pg/ml (NV: 7.2-63.3)
8 am cortisol	N/A	577.6 nmol/L (NV: 172-497)	185.1 nmol/L (NV: 172-497)	113 nmol/L (NV: 172-497)	22.09 nmol/L (NV: 172-497)	140.3 nmol/L (NV: 172-497)
Progesterone	N/A	N/A	16.84 nmol/L	16.88 nmol/L	2.17 nmol/L	N/A

In January 2021, 4 months after stopping OCP, hormonal evaluation showed an elevated 17OHP (65.37 ng/ml), high total testosterone, high DHEA-s (Table 1), FSH=5.45 mU/ml (NV:3.5-12.5), LH=1.98 mU/ml (NV:2.4-12.6), E_2 =426.1 pmol/L (NV:45.4-854), high progesterone (16.84 nmol/L) in the early follicular phase and, for the first time since the diagnosis, low-normal 8 a.m. cortisol levels; An ACTH was therefore ordered which proved to be high. Since Synacthen 250 is not available in Romania, the adrenal reserve was not assessed. Due to the discovery of partial glucocorticoid insufficiency and her wish to become pregnant, glucocorticoid (GC) replacement treatment in the form of hydrocortisone (HC) 20 mg daily, divided into two doses was started. After 6 months of treatment, in June 2021, she had had regular menstrual cycles and on presentation we found significantly decreased levels of 17OHP (12 ng/ml) and of progesterone (2.17 ng/ml) in the early follicular phase, in addition to an ACTH of (33.12 pg/ml), a morning cortisol of (22.09 nmol/L) and a normal androstendion (2.19 ng/ml, NV: 0.4-3,4). The dose of HC was increased to 30 mg daily for a month, followed thereafter by 25 mg daily. 3 months later the patient reported a positive pregnancy test. She remained on 25 mg of HC daily and at 28 weeks of gestation the dose was increased to 30 mg daily. At 39 weeks of gestation she gave birth to a healthy boy by caesarean section, who she then managed to breastfeed. During labor we recommended intravenous stress doses of HC after which she returned to chronic oral HC treatment, tapering to 10 mg HC/day at 2 weeks after delivery.

Discussion

The mutation P30L in exon 1 of CYP21A2 gene is classified as a mild missense single base pair mutation (c89C>T). It decreases 21-hydroxylase activity to 20-60%, frequently causing NC-CAH. However, according to others, patients carrying mutations in P30L present with stronger virilization, clitoromegaly and even with classic-simple virilizing forms of the disease [5-8]. Studies of CAH have confirmed the uniqueness of P30L mutation such as difficulties in phenotype classification, different fertility or growth issues in comparison with other genotypes, biochemical and clinical features straddling the classic/nonclassic boundaries [3].

The mechanisms by which mutations at the P30L level cause a more severe form of the disease have not yet been concretely elucidated. Although in general in CAH the genotype correlates quite well with the phenotype, it appears that the correlation weakens with a diminished severity of the disease, or it depends on the subject's background with respect to other genes regulating androgen and estrogen metabolism [9].

The P30L mutation is more frequent in India and in central and southern Europe, having the highest prevalence

in Romania and Macedonia [3,10]. Interestingly, our patient presents the P30L (c89C>T) mutation in the simple heterozygous form, which means a single, and usually mild mutation. Nevertheless, we have to be aware, that the method used in this case has limitations. One of them is the fact that the genetic strip test used is able to identify only the most frequent 11 mutations in the CYP21A2 gene that can cause CAH. Actually, none of the techniques used for 21-OHD genotyping are able to identify 100% of possible variants due to the complexity of the CYP21A2 locus. However, the best practice 21-OHD genotyping is considered nowadays the PCR-based sequence analysis along with MLPA, which would detect the majority of types of potential alterations responsible for the complex spectrum of CAH disease [9].

Data on fertility and the role of GC treatment in patients with NC-CAH who wish to conceive are not yet sufficiently clarified [11-14]. Excess androgen hormones can inhibit folliculogenesis and interfere with normal gonadotropin secretion. Increased levels of adrenal-derived progesterone perturb the menstrual cycle, sperm penetration and folliculogenesis, leading to empty follicles, thin endometrium and implantation difficulties [15,16]. In the case presented by Kawarai et al. [16] substitution with GCs reduced the persistently elevated levels of follicular-phase progesterone and led to the normal restoration of folliculogenesis. Thus, in vitro fertilization was successfully achieved, after repeated unsuccessful attempts. Furthermore, for women who fail to conceive or experience a delay in conception, a follicular phase progesterone level of around 0.6 ng/ml (2 nmol/L) should be achieved with GCs [16,17], a much tighter control than for women not attempting to conceive.

It is interesting that our patient developed cortisol deficiency, a partial form though, suggested by the increased values of ACTH and low-normal basal cortisol, unexpected in NC-CAH, but probably explained by the P30L mutation. As a result, in January 2021, treatment with GC was initiated, especially because she expressed her wish to become pregnant. Consequently, we could observe a marked reduction in the levels of progesterone, of androgens and the normalization of ACTH and a normal pregnancy was obtained several months later.

Women diagnosed with NC-CAH, with clinical +/- biochemical hyperandrogenism and infertility, who want to conceive, should receive GC treatment, preferably HC, prednisone, or prednisolone [18]. Ovulation inducers such as clomiphene citrate or other assisted reproduction techniques can be used if GC therapy is not effective [19].

HC has no effect on the fetus because it is metabolized by the placental enzyme 11 beta (OH) steroid dehydrogenase II. The dose should be increased to 20-25 mg/day at the time of conception, and changed every 6-8 weeks in order to keep testosterone values at the upper

limit of normal specific to the pregnancy trimester [20]. The glucocorticoid dose should be increased by 20–40%, particularly during the third trimester.

In patients with NCCAH, the Endocrine Society guideline (18) suggests HC stress dosing for major surgery, trauma, or childbirth only if the patient has a suboptimal cortisol response to cosyntropin or iatrogenic adrenal suppression. Those with CAH-SV forms should also receive GC replacement therapy during periods of stress.

Otherwise, therapy in patients with P30L mutations should depend on the clinical picture, and should consist mostly of improving symptoms, not biochemical findings. Hyperandrogenemia and oligomenorrhea can be successfully treated with OCPs +/- antiandrogens + laser therapy for hirsutism [18]. On the other hand, long-term GC administration, in particular chronic use of more potent or long-acting GCs was associated with adverse side effects.

Genotyping is highly recommended for women with NC-CAH who wish to conceive, for purposes of genetic counseling [20-22].

In conclusion, we present the case of a young woman diagnosed with CAH due to 21 OHD caused by P30L mutation, with a clinical and biochemical phenotype between C-CAH SV form and NC-CAH. Though, diagnosed in adolescence with an apparently late-onset form, she had manifestations of pronounced hyperandrogenism, with moderate hirsutism and secondary amenorrhea and she was found with partial glucocorticoid deficiency, typical of SV-CAH form.

Therefore, although the P30L allele is still categorized as a non-classical mutation, our findings support the role of the P30L mutation in causing more complex CAH phenotypes and more pronounced androgen excess.

Women diagnosed with NC-CAH who fail to get pregnant spontaneously should be treated with GC, preferably HC. The genetic analysis in women with NC-CAH trying to conceive should be performed because it may give us additional information about the phenotype and the need for genetic testing of the partner.

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