

Congenital Adrenal Hyperplasia, the Origin of Combined Infertility: A Case Report and a Review of Literature

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Abstract

Congenital Adrenal Hyperplasia (CAH) – a complex and heterogeneous group of conditions is inherited as Autosomal Recessive (AR) disorders. The resultant deficiencies in one of the five enzymes involved in adrenal steroidogenesis lead to defects in the steroidogenic pathways and biosynthesis of cortisol, aldosterone and androgens. Precursor steroids proximal to the blocked step accumulate and can be shunted into other metabolic pathways, particularly that of androgen biosynthesis.

CAH due to 21-Hydroxylase deficiency is traditionally separated into two clinical groups: the Classical form (C-CAH), which is further separated into salt-wasting (75%) and simple-virilizing (25%) phenotypes, and the Non-classical form (NC-CAH). They are differentiated by their hormonal profile, predominant clinical features and age of appearance.

CAH can affect fertility in females due to inadequate introitus, oligomenorrhea and elevated progesterone levels. Many authors reported an effect on male fertility as well.

This editorial describes a case report of combined infertility due to mutations in the CYP21A2 gene and a review of literature on this subject.

Keywords: Congenital Adrenal Hyperplasia; CYP21 Deficiency

Case Report

A Couple that presented at our infertility clinic with secondary infertility.

The patient was 33 years old, of Ashkenazi origin, generally healthy. Her gynecologic history included menarche at the age of 15 and oligomenorrhea throughout her life.

Two spontaneous pregnancies with her current partner ended in spontaneous early miscarriages. Duration of infertility at presentation – 6 years. A normal salpingohysterogram ruled out mechanical cause.

Her husband was 43 years old, Jewish of North African origin, generally healthy, a father of 3 children from a previous relationship. Last child conceived with fertility treatments due to male factor. He underwent varicocele repair few years earlier.

Current sperm analysis revealed severe Oligo-Terato-Asthenospermia (OTA).

Hormonal investigation for causes of oligomenorrhea demonstrated normal FSH, LH, TSH and prolactin levels. 17-OH-progesterone levels were elevated in repeated tests in a range of: 556-2461 ng/dL.

Corticotropin stimulation test was performed; levels of 17-OH-progesterone an hour after stimulation were 1683 ng/dL diagnostic of late onset Congenital Adrenal Hyperplasia (CAH).

Genetic investigation of CYP21 gene mutations revealed that she was homozygous for V281L mutation. Her husband was diagnosed to be a heterozygous carrier for Q318X mutation in the same gene.

Since there was a 50% chance for birth of a child suffering from NC-CAH which is considered a non-lethal disease, there was no indication for Pre-Implantation Genetic Diagnosis (PGD).

The patient conceived in her second IVF cycle treated with an Antagonist protocol. Twelve ova were retrieved, seven were fertilized using micromanipulation, and two good quality fresh embryos were transferred on day 3 resulting in a single embryo pregnancy. Chorionic Villous Sampling (CVS) was done on 12 weeks of gestation confirming she was carrying a healthy female fetus who was a heterozygous carrier of the V281L mutation in the CYP21 gene.

Pregnancy follow up was normal and the patient delivered a healthy baby girl at term.

Introduction

Adrenal gland enlargement was first described by the Neapolitan anatomist De Crecchio [1]. The adrenal cortex is formed in the 4th week of gestation, functionally secreting steroids by the 6-7th week of gestation [2]. Biosynthesis of the steroids: cortisol, aldosterone, and androgens from cholesterol occurs under the Adrenocorticotropic

Hormone (ACTH) stimulus with the involvement of five key enzymes : P450_{scc}, 3β-OH dehydrogenase (3β-HSD), 17α-hydroxylase (17α-OH), 21-hydroxylase (21-OH), 11β-hydroxylase (11β-OH) [3,4] (Figure 1).

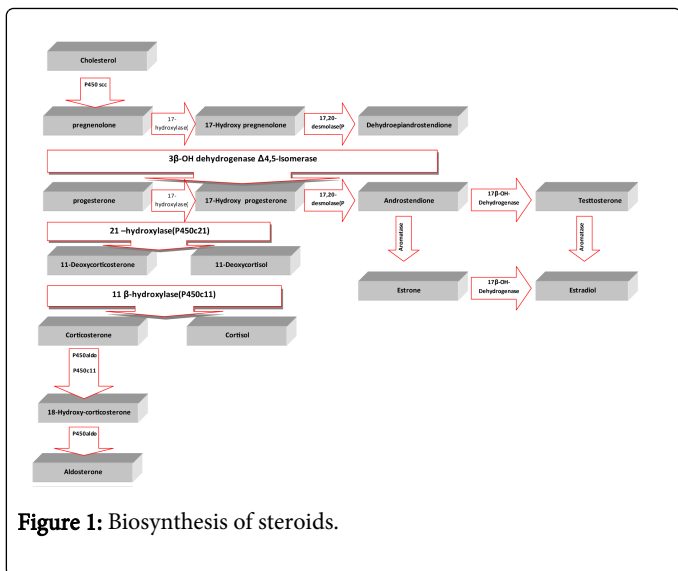


Figure 1: Biosynthesis of steroids.

These steroidogenic enzymes are members of the cytochrome P450 family of oxidases.

Congenital Adrenal Hyperplasia (CAH) – is a complex and heterogeneous group of conditions, inherited as Autosomal Recessive (AR) disorders. The resultant deficiencies in one of the five enzymes involved in adrenal steroidogenesis lead to defects in the steroidogenic pathways and biosynthesis of cortisol, aldosterone and androgens.

Accumulation of steroid precursors as well as a resulting rise in ACTH levels drives enlargement of the adrenal gland and overproduction of adrenal androgens. Precursor steroids proximal to the blocked step accumulate and can be shunted into other metabolic pathways, particularly that of androgen biosynthesis. The biochemical and clinical phenotype depends on the specific enzymatic defect and the impairment of specific enzyme activity [5].

All variants are accompanied with glucocorticoid deficiency but each variant of CAH is characterized by a distinct hormonal milieu reflecting the location of the specific block in the path of steroidogenesis.

Defects of the enzymes 21-OH and 11β-OH only affect adrenal steroidogenesis, whereas 17α-OH and 3β-HSD deficiency also impact steroid biosynthesis in the gonads.

The genes of the different CAH forms are well characterized. Confirmation of the diagnosis by genetic analysis is of major clinical significance, as there is a strong genotype–phenotype association for all CAH variants caused by defects in steroidogenic enzymes [6].

There are five types of CAH (Table 1) [7,8].

Enzyme deficiency	Hormone plasma levels					Sexual ambiguity	
	17-OHP	DHA	Testosterone	Aldosterone	Renin	Males	Females
21-OH NC-CAH	↑	↑/N	↑/N	N	N	-	-

21-OH C-CAH	↑↑↑	↑/N	↑	↓/N	↑↑	-	+
11β-OH	↑/N	↑	↑	↓↓	↓↓	-	+
3β-HSD	↑	↑↑	Females - N/↑ Males - ↓	↓↓	↓↓	+	±
17α-OH	↓↓	↓↓	↓↓	↓↓	↓↓	+	-
P450 _{scc} (lipoid CAH)	0	0	0	0	↑↑↑	+	-

Table 1: Five types of CAH.

C-CAH: Classical Congenital Adrenal Hyperplasia; NC-CAH: Non-Classical Congenital Adrenal Hyperplasia; 3β-HSD: 3β-OH dehydrogenase; 17α-OH: 17α-hydroxylase; 21-OH: 21-hydroxylase; 11β-OH: 11β-hydroxylase; N:Normal

CAH due to 21-hydroxylase (21-OH) (CYP21A2) deficiency

The most common of the enzyme deficiencies and is responsible of 95% cases of CAH [6]. The resulting disease comprises a complex disease entity with a high degree of heterogeneity depending on the gene mutation and the severity of enzymatic defect (Table 2) [9].

Mutation	Severity of enzyme defect (% enzyme activity)	Phenotype
Classical mutations		
G110Δ8nt	Severe (0)	SW
I172N	Severe (0)	SV
Q318X	Severe (0)	SW
R356W	Severe (0)	SW,SV
R483P	Severe(1-2)	SW
Non-classical mutations		
P30L	Mild (30-60)	NC
V281L	Mild (20-50)	NC
R339H	Mild (20-50)	NC
P453S	Mild (20-50)	NC

Table 2: Common mutations in CYP21A2 gene and their phenotypes. SW: Salt Wasting; SV: Simple Virilizing; NC: Non-Classical

Genotype: The gene encoding 21-OH is located on chromosome 6p21.3, within the HLA histocompatibility complex and it exists in two highly homologous forms: the active gene and the inactive pseudogene [10-13]. More than 100 mutations have been described (Table 3) [14-24].

New born	Ambiguous genitalia, Absent testis, Severe shock, Vomiting, Diarrhea, Hyponarmia, Hyperkalemia, solated, clithromegaly, Isolated labial fusion, Penile enlargement, Hyperpigmentation, Failure To Thrive (FTT), Hyperinsulinemia
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	Hypoglycemia
Childhood	Premature adrenarche Precocious puberty (central) Penile enlargement Small testes Tall stature and advanced bone age
Adult	Oligomenorrhea, Clitoromegaly, Hirsutism, Male-pattern Alopecia, Acne, Seborrhea, Short stature, Deepened voice Obesity, Insulin resistance-metabolic syndrome Conflicts in gender identity, Male infertility, Testicular adrenal rest tumours, Adrenocortical tumors

Table 3: Clinical spectrum of 21-OH deficiency.

Prevalence: Incidence of the NC-CAH form is almost 1 : 500–1 : 1,000, and of C-CAH is of about 1 in 9800–16,000 live births in most Caucasian populations [10-12]. Incidence varies among ethnic groups and geographical areas. About 65–75% of the CAH patients are compound heterozygotes [6].

As reported in some studies CAH is the most common AR disorder in humans. In certain populations such as Ashkenazi Jews incidence is as high as 1:27 [11].

The two 21-OH genes – CYP21A2 encoding the active 21-OH; and the inactive pseudogene CYP21A1P are located approximately 30kb apart. The major mechanism by which the active gene acquires defects is via transfer of segments from the pseudogene to the active gene. [12, 13] Disease severity and phenotype are determined by the degree of deficit in 21-OH activity, which is dictated by the least severe CYP21A2 mutation and type of mutation on the allele determining clinical manifestation [5,6].

Phenotype: CAH due to 21-OH deficiency is traditionally separated to two clinical groups: the Classical form (C-CAH), which is further separated into salt-wasting (75%) and simple-virilizing (25%) phenotypes, and the Non-classical form (NC-CAH). They are differentiated by their hormonal profile, predominant clinical features and age of presentation [5,9] (Figure 2) (Table 1).

Different authors found a strong association between genotype and phenotype in cases of homozygosity for severe mutations, especially the salt-wasting form. However, the correlation between the genotype and the virilization phenotype assessed by Prader genital stages is less pronounced [6]. In addition, this association is weaker in cases of compound heterozygotes for two different mutations or those carrying mutations of intermediate severity and as a result prediction of phenotype from genotype tends to become more difficult [25-31].

Clinical presentation: C-CAH represents the most common cause of ambiguous genitalia in 46XX infants with the underlying mechanism being in utero exposure to excessive levels of androgens during critical periods of genital development [5]. The severity of virilization of the external genitalia is traditionally classified according to the Prader staging [32,33]. In classical form of CAH the female fetus is exposed to high levels of adrenal androgens at the critical time of sexual differentiation (9-15 weeks) and is born with virilized external genitalia. Internal female genitalia (uterus, fallopian tubes, and ovaries) are normal.

The salt-wasting form presents with severe renal salt loss as a consequence of aldosterone deficiency that can manifest with a life-threatening salt-losing crisis in the neonatal period.

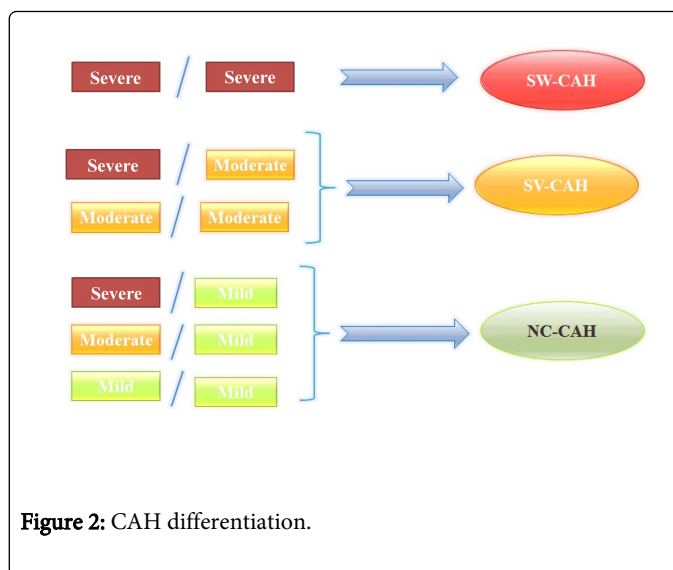


Figure 2: CAH differentiation.

The salt-wasting form presents with severe renal salt loss as a consequence of aldosterone deficiency that can manifest with a life-threatening salt-losing crisis in the neonatal period.

Interestingly, the degree of salt-wasting does not correlate with the degree of virilization [34].

The simple-virilizing form is characterized by virilization of the external genitalia in newborn females and by hypocortisolism and precocious pseudopuberty due to reactive androgen overproduction in both sexes. Early subtle evidence of C-CAH in males which include hyperpigmentation and penile enlargement may go unnoticed [35].

Adequately treated male patients have normal: pubertal development, testicular function, spermatogenesis and fertility. However, small testes and reduced sperm count can occur in patients as a result of inadequately treated disease. Fertility in males with 21-OH deficiency is frequently impaired due to a high incidence of gonadal adrenal rest tumors. These tumors most probably arise from cells having mixed adrenal and Leydig cell properties [15,36].

Steroidogenic cells of both the adrenal and the gonad obviously derive from a common primordium during embryogenesis. The adrenal-like cells express steroidogenic enzymes such as 11β-OH, 17α-OH or 21-OH and respond to ACTH, however, they do not express Leydig cell specific markers. Such a cell population has not been identified yet in human testis [37]. The tumors express adrenal enzymes and ACTH as well as angiotensin receptors. In theory, the atypical local steroid hormone milieu produced by the tumor in the testis results in oligozoospermia. Long-acting glucocorticoids may reverse fertility, but testis-sparing surgery may be necessary in cases where testicular function cannot be restored hormonally. Semen conservation should be advocated in late adolescence or early adulthood because of the high incidence of such tumors [38].

In addition to impaired adrenocortical function, patients suffering from 21-OHD show a compromised adrenomedullary function [39]. This is due to developmental defects in the formation of the adrenal medulla, leading to depletion of epinephrine stores and decreased production of metanephrine [40]. It seems that normal cortisol secretion by the adrenal cortex is necessary for adrenomedullary organogenesis, because glucocorticoids stimulate the expression of phenylethanolamine-N-methyltransferase, the enzyme that converts

norepinephrine to epinephrine [41]. Epinephrine may play a role not only in hypoglycemia observed in children with CAH, but also in leptin concentration (epinephrine inhibits leptin) and probably in insulin resistance that is observed in CAH cases [38,42].

NC-CAH results from mild to moderate enzyme deficiency. It presents postnatally with signs of hyperandrogenism. Females with the NC form are not virilized at birth and do not present with genital ambiguity. Males with NC-CAH were also reported to suffer from: gonadal dysfunction, oligospermia, subfertility, subtle hyperpigmentation, penile enlargement.

Diagnosis: The diagnosis of classical 21-hydroxylase deficiency is made by detecting significantly high levels of 17-OHP, which is the main substrate of the enzyme. Baseline values are >3,500 ng/dL in severely affected infants as compared to those of normal newborns which are <100 ng/dL [43,44].

In practice, the finding of basal follicular-phase morning serum 17-OHP levels >800ng/dL is considered diagnostic for CAH.

The hormonal diagnosis can be further defined by performing ACTH (cosyntropin) stimulation test. Comparing precursor: product ratios can guide differentiating 21-hydroxylase deficiency from other forms of CAH [7]. Nevertheless, in cases of C-CAH ACTH stimulation test is not absolutely necessary for diagnosis.

In most NC patients, stimulated levels of 17-OHP are >1,500 ng/dL. However, the threshold value between NC and heterozygotes is a matter of discussion [43].

Diagnosis must be based on the increased levels of ACTH-stimulated steroid precursor(s) that accumulate above the enzymatic block, with the exception of lipoid adrenal hyperplasia (P450sc deficiency) in which almost no steroids are produced [7].

Neonatal screening: Earlier diagnosis and treatment of CAH can be lifesaving. This is achievable by the simple method of measuring 17-OHP levels on dried blood samples. The screening process, however, is less reliable among low birth weight or preterm infants.

Neonatal screening for CAH can be falsely negative in the event of neonatal dexamethasone treatment. Finally, neonatal screening must be confirmed by genotyping [7].

Prenatal prevention: In pregnancies with offspring at risk of being affected with CCAH, prenatal diagnosis and treatment should be carried out. If the CAH patient's partner is a carrier of a severe mutation, placing the fetus at risk for CCAH, the patient should be referred to prenatal diagnosis and treatment with dexamethasone until CVS or amniocentesis is performed. The goal is to prevent virilization in 46XX fetuses. Suppressing the active fetal hypothalamic-pituitary-adrenal axis by administering dexamethasone to the mother prevents androgen overproduction and inappropriate masculinization. Dexamethasone binds minimally to Cortisol Binding Globulin (CBG) in the mother and escapes inactivation by placental 11 β hydroxysteroid dehydrogenase enzyme; thus crosses the placenta and may suppress adrenal androgen overproduction. The recommended dose is 20 μ g dexamethasone/kg of weight body/day, divided into three doses [7,8,21,35,45-48].

Treatment: The diagnostic evaluation in 21-OHD has become more sophisticated, the standard medical treatment still consists of the same principles which Lawson Wilkins and his colleagues used back in the 1950s [49]. Treatment with glucocorticoid, with or without mineralocorticoid and salt replacement, is directed to prevent adrenal

crises and ensuring normal childhood growth by alleviating hyperandrogenism.

Obtaining levels of 17-OHP between 3 and 10 ng/ml usually indicate the efficacy of treatment [13].

Other Less Frequent Forms of CAH

CAH caused by 11 β -hydroxylase (CYP11B1) deficiency

About 5–8% of CAH cases are estimated to be caused by 11 β -hydroxylase deficiency. This is equivalent to an incidence of 1 in 100,000–200,000 live births. The highest incidence is reported to occur among Jewish population from Morocco (about 1 in 5000–7000 live births) [6].

11OHD results in decreased cortisol secretion and accumulation of the glucocorticoid precursor 11-deoxycortisol and the mineralocorticoid precursor Deoxycorticosterone (DOC). Since DOC can activate the mineralocorticoid receptor, patients can subsequently suffer from significant hypertension, a hallmark feature of this CAH variant [6]. Accumulated precursors are shifted into the androgen synthesis pathway, leading to hyperandrogenism. Classic 11OHD most frequently results in severe virilization of the external genitalia in newborn females, and precocious pseudopuberty in both sexes [50].

CAH caused by 17 α -hydroxylase (CYP17A1) deficiency

Steroid 17 α -hydroxylase deficiency (17OHD) is a rare form of CAH which accounts for about 1% of all CAH cases. 17OHD results in both glucocorticoid deficiency and sex steroid deficiency. In addition, the mineralocorticoid precursor's corticosterone and DOC accumulate, exerting significant, albeit weak, glucocorticoid activity. Therefore, 17OHD does not necessarily manifest with adrenal crisis. However, accumulation of corticosterone and DOC also results in mineralocorticoid excess, causing severe hypokalaemic hypertension. Sex steroid deficiency caused by loss of 17,20-lyase activity results in under virilization in male newborns and in primary amenorrhea in 46XX individuals. There is a lack of pubertal development due to hypergonadotropic hypogonadism in both sexes [51].

CAH caused by 3 β -hydroxysteroid dehydrogenase type 2 (HSD3B2) deficiency

CAH due to 3 β -hydroxysteroid dehydrogenase type - 2 deficiency represents another rare autosomal recessive CAH variant. Data on population-based incidence are lacking. The clinical spectrum shows a wide variety of disease expression but clinical presentation can be largely divided into salt-wasting and non-salt wasting. Genital virilization among female newborns varies variably and as a result diagnosis could be delayed [52].

P450 oxidoreductase deficiency (ORD)-(Antley-Bixler syndrome)

Recently, mutations in the electron donor enzyme P450 oxidoreductase were identified as the cause of CAH. P450 oxidoreductase deficiency (ORD) has a complex phenotype including two unique features not observed in any other CAH variant, skeletal malformations and severe genital ambiguity in both sexes. Apparent paradox of female virilization despite concurrently low circulating androgens observed in this CAH variant suggests the existence of an

alternative pathway in human androgen synthesis [53,54]. The biochemical profile suggests partial deficiency of both 17 α -hydroxylase and 21-hydroxylase activities. Typical findings include elevated levels of 17OHP, though not to the extent observed in 21OHD. In contrast to 21OHD, sex steroids are low and there is no mineralocorticoid deficiency. Analysis of serum steroids may be confusing and non-diagnostic of the other CAH subtypes [55,56].

Alternative Treatments, New Approaches

Many advances have been made in the management of 21-hydroxylase deficiency during the past 60 years. Despite these advances, the clinical management of patients with CAH is often complicated by abnormal growth and development, iatrogenic Cushing's syndrome, inadequately treated hyperandrogenism, and infertility [57].

New treatment approaches to classical CAH represent potential solutions to these unresolved issues. These approaches include for example regimens combining a reduced hydrocortisone dose, an anti-androgen, and an aromatase inhibitor [58].

Another approach aims to improve final height in 21-OHD is a combined treatment with GnRH agonists (GnRHa) and Growth Hormone (GH) together with the standard replacement therapy. This approach does not try to avoid high glucocorticoid doses but tries to improve directly poor growth velocity and central precocious puberty directly. Combination therapy with GH and GnRHa appears to improve final height in 21-OHD, but long term randomized, controlled trials are lacking. Obviously, this regimen treats the side effects of glucocorticoid therapy and does not treat the underlying cause of the disease [56].

Other treatment approaches investigated include combination therapy to block androgen action and inhibit testosterone production, and bilateral adrenalectomy in the most severely affected patients [59]. Adrenalectomy will not improve problems caused by gonadal adrenal rests.

Further, even more experimental or theoretical methods of treatment include: corticotrophin-releasing hormone receptor antagonists to avoid adrenal hyperstimulation, carbenoxolone to increase *in vivo* cortisol concentrations and gene therapy [60].

The applicability and success of these approaches await the results of current research.

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