NEW GUIDELINES FOR DIAGNOSIS AND TREATMENT OF CAH

A. Belgorosky, M.A. Rivarola, G. Guercio, R. Marino
Servicio de Endocrinología
Hospital de Pediatría Garrahan
Pozos 1881
Buenos Aires, Argentina 1245

INTRODUCTION

Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive disorders of one of five enzymes required for the synthesis of cortisol in the adrenal cortex. The most frequent disorder is steroid 21-hydroxylase deficiency, accounting for more than 90 percent of cases [1-2]. The severe form of classical 21-hydroxylase deficiency present two types: one, a combination of cortisol and aldosterone deficiency named salt wasting type and the other, the simple virilizing form, the biosynthesis of adrenal aldosterone is apparently normal. In both, an increment of adrenal androgens synthesis and overproduction of cortisol precursors occurs, secondary to the adrenal cortex stimulation by corticotropin [1-2]. These increments of adrenal androgens during fetal life cause ambiguous genitalia in the affected female fetus. There is also a mild form, a non classical form that may be associated with signs of postnatal androgen excess [2].

Steroid 21-hydroxylase enzyme is a cytochrome P-450 enzyme [3-4], located in the endoplasmic reticulum. It catalyzes first, the conversion of 17-hydroxyprogesterone to 11-deoxycortisol, in the cortisol synthesis pathway in zona fasciculata adrenal cells and second, the conversion of progesterone to 11-deoxycorticosterone, in the aldosterone synthesis pathway, in zona glomerulosa adrenal cells (Figure 1). The CYP21 gene is located in the highly polymorphic HLA histocompatibility complex, on chromosome 6p21.3 (Figure 2). There are two 21-hydroxilase loci, a functional gene formally named CYP21A2 (P450c21B) and a non functional pseudogene named formally CYP21A1P (P450c21A). These two loci are duplicated in tandem with C4A and C4B loci encoding the fourth component of serum complement [4-6]. However, other duplicated genes have been described in the 21-hydroxylase locus, such us, among others, TNXB which encodes for tenascin X and TNXA, which is the corresponding truncated pseudogene [7] (Figure 2).
ADRENAL STEROIDOGENESIS

FIGURE 1. Pathways of adrenal steroidogenesis. Starting from cholesterol, the three main pathways of steroid biosynthesis are shown. These pathways take place in three different zones of the adrenal cortex. The upper horizontal pathway indicates the biosynthesis of aldosterone, in zona glomerulosa, requiring the participation of 21-hydroxylase (P450c21, in red). Pregnenolone is 17-hydroxylated in zona fasciculata. The middle horizontal pathway shows the biosynthesis of cortisol, requiring also, the participation of 21-hydroxylase (P450c21, in red). In zona fasciculata the second function of P450c17, the loss of C20 and C21 in the steroid molecule, produces androstendione. Finally, the vertical pathway on the extreme left, shows the pathway to the synthesis of DHEA and DHEAS, present in the fetal zone (during fetal life) and in zona reticularis of the postnatal adrenal.

When present, 21-hydroxylase deficiency decreases the synthesis of cortisol. As a consequence excessive stimulation of ACTH increases adrenal cortex mass and the accumulation of steroids located before the enzymatic deficiency, such as 17-hydroxy-progesterone, useful for diagnosis, and androgens, responsible for many symptoms, particularly in girls. In cases of severe enzymatic deficiency, aldosterone is not secreted in appropriate amounts, generating sodium loss, potassium retention, hypovolemia and dehydration. During the first months of life, adrenal crisis are usually mineralo and glucocorticoid in nature. In patients under treatment, adrenal crisis is mainly a glucocorticoid deficiency, and it is induced by stress.
FIGURE 2. The complex 21-hydroxylase locus is located in the HLA region of chromosome 6. Both the 21A (pseudogene) and 21B (active gene) are shown in orange, in tandem C4A and C4B complement genes, in pink. Other genes, such as TNXB encoding for tenascin and TNXA, its corresponding pseudogene are shown. Gene transcription in the latter takes place in the opposite direction.
FIGURE 3 shows a cartoon of the 21-hydroxylase gene (CYP21A2) with its 10 exons. It also depicts the most frequent point mutations, but not large conversions or deletions, generated by abnormal events at meiosis, with participation of the pseudogene. Mutations responsible for early onset clinical forms (classical CAH), with the two variants, salt wasting (SW) in yellow, and simple virilizing (SV) in sky blue, are shown above, while mutations responsible for late onset clinical forms (LO, classical) in green, are shown below the gene. Point mutations shown in red are the most frequent mutations in the argentinian population [13], as well as in other populations.

The CYP21 genes consist of ten exons (Figure 3), are about 3.4Kb long and differ in only 87 or 88 of their bases (6-7). Approximately 75 percent represent deleterious mutations found in the pseudogene that are transferred to CYP21A2 in gene conversion events. These “point mutations” might represent a loss of a single or few bases, located in exons or introns. A large number of point mutations have been described (Figure 3). About 20 percent of large mutations are meiotic recombinations secondary to an unequal crossover during meiosis that delete a 30 Kb gene segment. They transcribe a non functional chimeric pseudogene (8) (Figure 4).

Several studies in different populations have correlated the genotype and phenotype (9-13) According to the level of enzyme activity predicted from the in
vitro mutagenesis and expression studies (14-16), CYP21 gene mutations can be
grouped in those related to CAH salt wasting or simple virilizing forms, and also in
those related to the mild, late onset form. However, more than one phenotype has
been described for the same CYP21 point mutation, such as it is the case for intron
2 and I172N.

FIGURE 4. Scheme showing, in a simplified form, the theoretical mechanisms
that might involved in large alterations of the CYP21A2 gene. It has been
proposed that abnormal interchanges between the CYP21A1 pseudogene
and the gene might take place during crossing-over of meiotic division. At
the top, the normal arrangement is shown. The 10 purple exons of the
pseudogene are depicted on the left side, and they are separated from the 10
green exons of the active gene, right side, by the C4A complement in sky
blue. The middle horizontal drawing shows an example of a large deletion,
including loss of several exons in the 3' side of the pseudogene and the 5'
side of the active gene. Finally, at the bottom a scheme of a large gene
conversion is depicted, resulting in the replacement of a large area of the
gene by the pseudogene. These large deletion/conversions of CYP21A2 are
responsible for severe enzymatic deficiencies, and they are relatively
frequent within classical forms of CAH.
Figure 5 shows the frequency of different point mutations in the argentinian population. It is similar to the distribution in other populations. The study of point mutations is useful for diagnostic confirmation of 21-hydroxylase deficiency in certain clinical situations, such as hirsutism in post-menarcheal girls, and it is used for pre-natal diagnoses.

![CYP 21 GENE MUTATION IN ARGENTINE POPULATION](image)

Figure 5. The frequency of different mutations of the CYP211A2 in the argentinian population is shown. Mutations associated with the classical forms are depicted in the left panel (prenatal onset). It can be observed that deletion/conversions (17.2 %), Intron 2 (29.1 %), Q318X (11.6 %) and I172N are the most frequent mutations. Mutations associated with the nonclassical forms are depicted in the right panel (postnatal onset). V281L (60.2 %) is, by far, the most frequent mutations.
LINEAR GROWTH

Adult height in CAH patients is an important outcome issue. In some CAH patients, adult height might be stunted despite adequate treatment. The mechanism of this finding is controversial. This discussion will be focus first, on linear growth and second, on the GH-IGF system in CAH patients.

Optimal adult height is influenced by the dose of glucocorticoids used, the degree of hormonal control achieved (level of adrenal androgens suppression), the age at which glucocorticoid therapy, associated or not with mineralocorticoids, is instituted and replacement therapy compliance (17-20). Figure 6 stresses the relationship between glucocorticoid dose and excessive adrenal androgen secretion.

Figure 6. This imaginary balance indicates that there is an equilibrium between the synthesis of adrenal androgens and the dose of glucocorticoids necessary to achieve optimal height in patients with CAH. On one hand, dose should be high enough to inhibit abnormal secretion of androgens, on the other, it should be low enough to avoid adverse effects similar to those of Cushing syndrome, such as growth delay and overweight.
A meta-analysis of data from 18 centers showed that mean weighed final height SD score (S) for 561 patients was −1.37, whereas weighed mean final height SDS-target height SDS for the 204 patients for whom target height was available, was −1.21, and no sex difference was found (20).

In the multicenter retrospective study of Muirhead et al. (19), the distribution of final height, corrected for genetic potential, in 42 CAH patients was shown to be skewed to the left, and 21.4 % of the patients were below or equal to −2 SDS of target range. Since there is considerably heterogeneity in the approach to management of this disorder, and the majority of data available are retrospective analyses, it is difficult to discern in the follow up the weight of each variable implicated in CAH final height. However, there is a consensus that, as it has been mentioned above, early diagnosis, glucocorticoid doses and good treatment compliance appear to improve the outcome.

![Figure 7. A growth curve of a girl with treated CAH is shown. During the first 4 years of life linear growth (in green) was maintained close to the 50th percentile, and bone age (horizontal line) was coincidental with chronological age. For family reasons, treatment became irregular after 4 years old. The consequence of this was reflected in the growth chart, linear growth increased (in orange), it reached a value above the 90th percentile and bone age did accelerate deteriorating the prediction of final height.](image)
Simultaneously, gonadal puberty was initiated as indicated by breast development, going from Tanner’s stage 2 (B2) at 6 years old to stage 4 (B4) at 8 years old.

Nevertheless, the cause of short adult stature in patients with CAH is unknown. On one hand, long-term androgen excess might be a risk factor for short stature due to an advance of epiphyseal maturation and premature epiphyseal fusion, as well as the presence of early or precocious puberty. In CAH patients, the latter clinical condition is frequently observed, secondary to delayed age of starting glucocorticoid therapy and/or lack of compliance (21, 22). On the other hand, overtreatment with glucocorticoids is also a risk factor for short stature, since disturbances of the GH-IGF system, of calcium adsorption in intestine and kidney, and of the function of the growth plate have been described (23). Regarding glucocorticoid doses, three critical susceptible age periods have been proposed: 1) between the age of 6 and 12 months, 2) between the age of 8 and 10 years and 3) during the pubertal growth spurt (18, 19, 24, 25). There is no doubt that in salt wasting CAH patients, mineralocorticoid treatment should be used. However, according to the LWPES and ESPE consensus (26), in order to reduce glucocorticoid dose at least during the first year of postnatal life, all CAH newborn patients should be treated with mineralocorticoids. The need for continuing mineralocorticoids afterwards should be assessed based on plasma renin activity and blood pressure. Finally, the use of GnRH analog for therapy of central precocious puberty, potentially combined with growth hormone therapy, might be useful to improve final height in some of these patients (27). Recently, Dr. Maria New’s group from New York, has published final height data from 14 patients [28] combining a mean of 4.4 years of treatment with rhGH with a mean of 4.2 years of treatment with a GnRH analog. They found a 1 SDS (mean) increment of final height comparing with the control group.

THE GH-IGF AXIS

The relationship between GH and cortisol is dynamic, and a mutual bidirectional interaction between the GH/IGF axis and the hypothalamic pituitary–adrenal axis has been reported (29, 30). It has been also described that there is a positive relationship between the spontaneous secretion of cortisol and GH, and the modulation of serum IGFBP-1, IGFBP-2 and IGFBP-3 in normal children (31).

The information about the GH-IGF system in CAH patients is scarce. Charmandari et al (32) have shown that children with CAH have a more regulated pattern of GH secretion and a more synchronous joint GH-cortisol secretory dynamics than their normal counterparts. Probably, this is a result of the exogenous administration of hydrocortisone at fixed doses and specific time interval. Even though 24 hour GH secretion was not elevated in comparison to the control group, these results need to be confirmed, since the control group in this study were idiopathic short stature patients with normal growth.

Serum levels of IGF-1 in CAH patients are not clearly established (32-35). In Charmandari et al. (32), serum IGF-1 levels were elevated but a similar IGF-1/height ratio was observed. However, the biological implication of this ratio has
not been established. Cavallo et al (33) have described that serum IGF-1 levels were quite variable and inconsistent with treatment control in CAH patients. Besides, as it is known that high levels of androgens might be associated with high serum IGF-1 and IGFBP-3 levels (35), it is logical to expect that in CAH with suboptimal glucocorticoid treatment or lack of compliance, serum IGF-1 and IGFBP-3 levels should be elevated.

Lack of adrenarche has been described in treated CAH patients (36). It has been proposed that conventional glucocorticoid therapy probably impairs adrenal androgen maturation. It has been also described that children with CAH have high serum leptin and insulin concentrations and an increment in the insulin resistance index (37). It has been proposed that these results might be secondary to chronic adrenomedullary hypofunction and glucocorticoid therapy. We have hypothesized that the inhibition of DHEAS induced by glucocorticoid treatment at the time when adrenarche takes place might influence the GH-IGF system and insulin sensitivity.

Finally, our challenge as pediatric endocrinologists is to increase our knowledge about additional factors that might be beneficial to improve final height. It has to be pointed out that we still do not have a clear and precise consensus on which are the most important variables to be considered.

REFERENCES

8. Tusie-Luna MT, White PC. Gene conversions and unequal crossovers between CYP21 (steroid 21-hydroxylase gene) and CYP21P involve different mechanisms. Proc Natl Acad Sci USA 92:10796-800, 1995
in congenital adrenal hyperplasia due to 21-hydroxylase deficiency. J Clin Invest 90:584-95, 1992
22. Penny R, Olambiwonnu NO, Frasier DS. Precocious puberty following treatment in a 6-year-old male with congenital adrenal hyperplasia: studies of serum luteinizing hormone (LH), serum follicle stimulating hormone (FSH) and plasma testosterone. J Clin Endocrinol Metab. 36:920-4, 1973


32. Charmandari E, Pincus SM, Matthews DR, Johnston A, Brook CG, Hindmarsh PC.
Oral hydrocortisone administration in children with classic 21-hydroxylase deficiency leads to more synchronous joint GH and cortisol secretion. J Clin Endocrinol Metab. 87:2238-44, 2002


35. Juul A, Flyvbjerg A, Frystyk J, Muller J, Skakkebaek NE. Serum concentrations of free and total insulin-like growth factor-I, IGF binding proteins -1 and -3 and IGFBP-3 protease activity in boys with normal or
