Recent advances in the diagnosis and management of congenital adrenal hyperplasia due to 21-hydroxylase deficiency

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Congenital adrenal hyperplasias (CAH) are inherited defects of cortisol biosynthesis. More than 90% of CAH are caused by 21-hydroxylase deficiency (21-OHD), found in 1:10 000 to 1:15 000 live births. Females with 'classical' 21-OHD, being exposed to excess androgens prenatally, are born with virilized external genitalia. Potentially lethal adrenal insufficiency is characteristic of two-thirds to three-quarters of patients with the classical salt wasting (SW) form of 21-OHD. Non-SW 21-OHD may be diagnosed on genital ambiguity in affected females, and/or later on the occurrence of androgen excess in both sexes. Non-classical 21-OHD, detected in $\geq$1:100 of certain populations, may present as precocious pubarche in children or polycystic ovarian syndrome in young women. 21-OHD is caused by mutations in the *CYP21* gene encoding the steroid 21-hydroxylase enzyme. More than 90% of these mutations result from intergenic recombination between *CYP21* and the closely linked *CYP21P* pseudogene. The degree to which each mutation compromises enzymatic activity is strongly correlated with the clinical severity of the disorder. This close association between genotype and phenotype makes it possible to predict clinical outcome in affected subjects. The risk of SW and prenatal virilization can be estimated, and overtreatment can be avoided in mildly affected cases. Glucocorticoid and mineralocorticoid replacement therapies are the mainstays of treatment, but additional therapies are being developed. A first trimester prenatal diagnosis should be proposed in families in whom molecular studies have been performed previously. The state of heterozygotism can be predicted by hormonal testing and confirmed by molecular studies. Prenatal diagnosis by direct mutation detection in previously genotyped families permits prenatal treatment of affected females in order to avoid or minimize genital virilization. Neonatal screening by hormonal methods identifies affected children before SW crises develop, reducing mortality in this disorder.

Key words: congenital adrenal hyperplasia/genotype−phenotype correlation/21-hydroxylase deficiency/long-term outcome/prenatal diagnosis and treatment

Introduction

The biosynthesis of cortisol, a hormone necessary for survival, occurs in the adrenal glands under the stimulus of the adrenocorticotrophic hormone (ACTH). There are five enzymes necessary for the biosynthesis of cortisol from cholesterol (Figure 1). The biosynthesis of all adrenal steroids is regulated by a negative feedback, but among all steroid hormones produced by the adrenals, cortisol is the only one to exert a significant feedback control on ACTH secretion. Thus, when cortisol secretion is insufficient, whatever the cause, the feedback loop opens and ACTH rises (Figure 1).

Congenital adrenal hyperplasia (CAH) results from inherited defects in one of the five enzymatic steps required for the

biosynthesis of cortisol from cholesterol. These disorders are so named because the adrenal glands are hyperplastic at birth due to unrestrained ACTH stimulation already in fetal life. Indeed, chronic overstimulation of ACTH secretion due to low levels of cortisol results in hyperplasia of the adrenal cortex. In addition, in all forms of CAH, other than lipoid CAH, there is increased output of the steroid precursors above the block, as well as of their urinary metabolites.

A defect in a particular step may be manifested clinically not only because cortisol and other steroid hormones are not synthesized effectively but also because precursor steroids proximal to the blocked step accumulate and can be shunted into other metabolic pathways, particularly that of androgen biosynthesis.
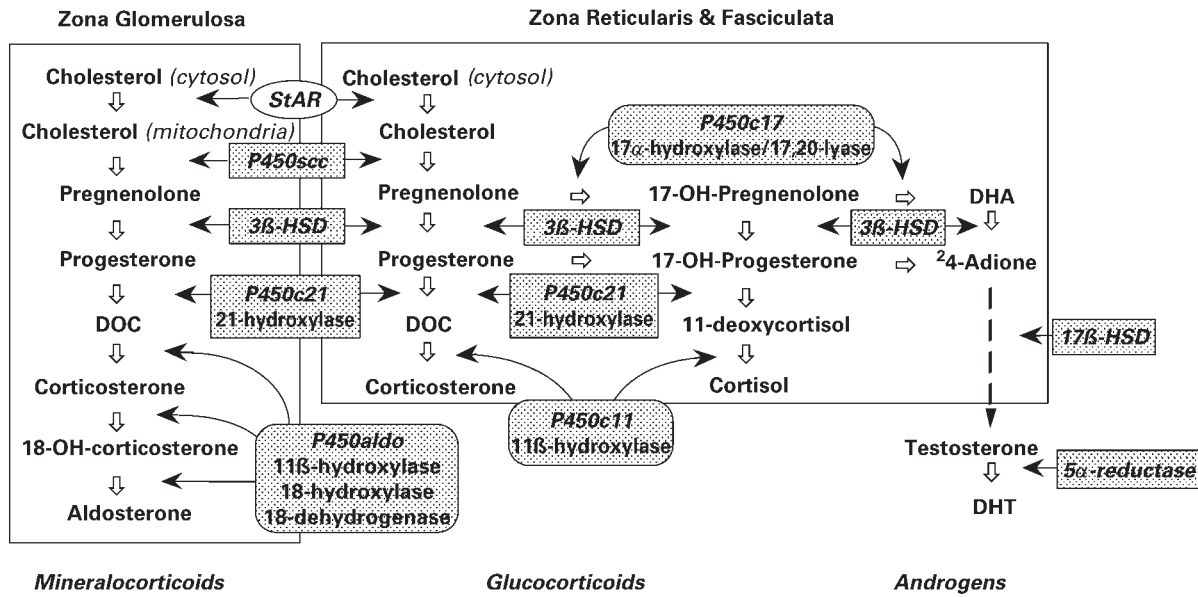
Figure 1. Steroid pathways in the three functional zones of the adrenal cortex (glomerulosa, reticularis and fasciculata) are shown in the frames. Extra-adrenal androgens biosynthesis are outside the frames. DOC = 11-deoxycorticosterone; Δ^4-adione = Δ^4-androstenedione; DHA = dehydroepiandrosterone; DHT = dihydrotestosterone. The 17β-hydroxysteroid dehydrogenase (17β-HSD) type III synthesizes testosterone in the Leydig cells of the testes, mainly from Δ^5-androstendiol, or from Δ^4-androstendione.

Each enzymatic defect produces a distinctive hormonal profile and clinical picture. The two most common CAH, 21-hydroxylase (>90%) and 11β-hydroxylase deficiencies (~5%), are due to enzymes (P450c21, and P450c11) expressed exclusively in the adrenal glands; classical and NC forms must be differentiated before genetic counselling. In the classical forms of 21-hydroxylase and 11β-hydroxylase deficiency, the androgens produced in excessive amounts virilize the female fetuses causing female pseudohermaphroditism (FPH). In contrast, the three other CAH (≤ 5%) are due to deficiencies in enzymes (P450scc, 3β-hydroxysteroid dehydrogenase and P-450c17) expressed in both adrenal and gonadal tissues, resulting in decreased biosynthesis of both cortisol and testosterone (Figure 1). Therefore, the impaired testosterone biosynthesis results in the absence or incomplete masculinization of male fetuses (male pseudohermaphroditism or MPH) affected with any of the other three enzyme deficiencies (Figure 1).

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 (P450scc deficiency) in which almost no steroids are produced (Table I). Nowadays clinical diagnostics are re-evaluated in the light of genetic studies. Indeed, all CAH are genetic disorders with autosomal recessive inheritance. The genes involved in all defects have been isolated and characterized, and specific mutations have been identified.

21-Hydroxylase deficiency (see reviews: Morel and Miller, 1991; Miller, 1994; New, 1998; White and Speiser, 2000)

Steroid 21-hydroxylase (*CYP21*; P450c21; E.C.1.14.99.10) (also termed 21-mono-oxygenase) is a microsomal cytochrome P450 enzyme that converts progesterone to deoxycorticosterone (DOC) and 17α-hydroxyprogesterone (17-OHP) to 11-deoxycortisol,

Table I. Clinical and hormonal data allowing a differential (aetiological) diagnosis of the five adrenal steroid enzyme defects

Enzyme deficiency	Sexual ambiguity		Symptoms		Hormonal findings (plasma levels)					
Classical forms	Girls (FPH)	Boys (MPH)	Salt loss	Hypertension	OHP	Δ^4	DHA	T	Aldo	Renin
21-OH with salt loss	yes	no	yes	no	↑↑↑↑	↑↑	N or ↑	↑	↓	↑↑
21-OH simple virilizing	yes	no	no	no	↑↑↑	↑↑	N or ↑	↑	N	± ↑
11β-OH[a]	yes	no	no	yes	N or ↑	↑↑↑	↑	↑	↓↓	↓↓
3β-HSD	±	yes	yes	no	↑	± ↑	↑↑↑	↑ ↓[b]	↓↓↓	↓↓↓
17α-OH	no	yes	no	yes	↓↓↓	↓↓↓	↓↓↓	↓↓↓	↓↓↓	↓↓↓
Lipoid CAH	no	yes	yes	no	~0	~0	~0	~0	~0	↑↑↑↑

[a]The marker of this enzyme defect is a very marked rise in 11-deoxycortisol (compound S).
[b]Normal or elevated for normal girl, or decreased in boys.
21-OH = 21-hydroxylase; 11β-OH = 11β-hydroxylase; 3β-HSD = 3β-hydroxysteroid dehydrogenase; 17α-OH = 17α-hydroxylase; lipoid CAH = lipoid congenital adrenal hyperplasia; OHP = 17α-hydroxyprogesterone; Δ^4 = Δ^4-androstenedione, DHA = dehydroepiandrosterone, T = testosterone; Aldo = aldosterone; N = normal; ~0 = practically undetectable.

in the glomerulosa and fasciculata zones of the adrenal cortex respectively (Figure 1). This enzyme is the product of a gene (*CYP21*) located on the short arm of chromosome 6. The zona fasciculata and glomerulosa are the primary sites of *CYP21* gene expression. However, numerous reports have shown that steroid 21-hydroxylation also occurs elsewhere, as suggested for instance by the finding of urinary or serum aldosterone in patients in whom a complete genetic defect was demonstrated (Koppens *et al.*, 1998). The use of sensitive RT–PCR demonstrated low levels of *CYP21* expression in the human skin (Slominski *et al.*, 1996), lymphocytes (Zhou *et al.*, 1997), heart (Kayes-Wandover and White, 2000), and hippocampus (Beyenburg *et al.*, 2001), as well as a putative unrelated 21-hydroxylase activity in skin keratinocytes (Rogoff *et al.*, 2001). Obviously, the extra-adrenal 21-hydroxylation by *CYP21* (likely isoenzymes) may complicate the diagnosis of adrenocortical biosynthesis defects and possibly alter the phenotype of the CAH-affected patients (Speiser *et al.*, 1991).

The disease has two major consequences, a state of cortisol deficiency and a hyperproduction of adrenal androgens due to ACTH overstimulation (Figure 2). The consequence is the excessive synthesis of adrenal sex hormone precursors and their metabolic by-products. These steroids are not 21-hydroxylated, and include progestins, androstenedione, and testosterone, dihydrotestosterone (DHT) and, to a lesser extent, estrogens, the three latter being produced by peripheral conversion from androstenedione. 21-Deoxycortisol (11β-hydroxy-17-OHP) is a compound normally produced in minute amounts because 17-OHP is not a good substrate for the 11β-hydroxylase enzyme (Figure 2).

Steroid 21-hydroxylase deficiency is a common monogenic defect that follows the prototypic pattern in that a gene encodes a protein that results in a metabolism that produces phenotype. However, the disease has a wide spectrum of clinical variants. These are not different diseases but represent points on a spectrum of disease severity directly related to the degree of enzymatic compromise conferred by a given genetic defect.
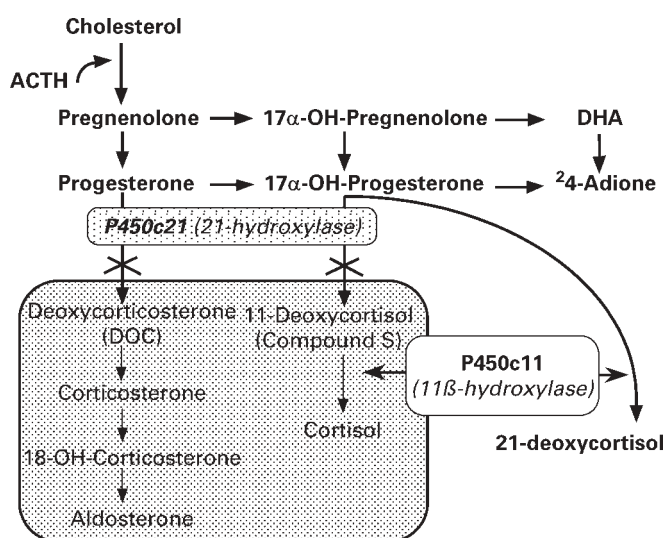

Figure 2. Steroid 21-hydroxylase deficiency. Steroids above the enzymatic block are produced in high amounts, whereas those below the block are abnormally low (shaded box). The biosynthetic pathway of 21-deoxycortisol is illustrated.

Clinical manifestations

Although this disorder can be viewed as a continuum from salt wasting (SW) forms to the moderate forms, they are divided into two broad groups: classical (severe, ex-congenital) and non-classical (NC) (less severe, formerly termed late onset or cryptic) forms. This classification is convenient, and moreover is supported by the evidence of specific genotypes associated with each group (see later).

Classical forms

In these cases, androgen excess causes external ambiguity and FPH in the affected females. Steroid 21-hydroxylase deficiency is by far the most frequent cause of FPH. The degree of masculinization of the external genitalia is variable, and classified into five Prader stages (Prader and Gurtner, 1955). Stage 1 applies to the presence of clitoromegaly, without labial fusion. Stage 2 describes clitoromegaly and posterior labial fusion. Stage 3 involves a greater degree of clitoromegaly with almost complete labial fusion and the presence of a urogenital sinus. In Stage 4 the clitoris has a phallic appearance, the urethral orifice is at the base of the clitoris and there is chordee with otherwise completely fused labial fold. Stage 5 describes a male phenotype due to the penile transformation of the clitoris and urethra and complete fusion of the labial folds. Such infants would be mistaken for under-developed males, but close examination reveals a lack of palpable gonads. The internal genitalia are normal. Therefore, pelvic sonography reveals a uterus, Fallopian tubes and ovaries. Karyotype is 46,XX. Boys have no overt signs of androgen excess and have normally formed penis, scrotum, testicles and internal Wolffian ducts. They generally have hyperpigmentation of the genitalia, but may also look perfectly normal, and the SW crisis may be the first sign of the disorder. In both sexes, adrenal sonography may show enlarged and misshapen adrenal glands, but this is not always the rule.

Classical 21-hydroxylase deficiency is characterized by impaired adrenocortical function with a decrease in cortisol and aldosterone secretion and an increase in androgen secretion. The complete form with clinical SW is characterized by a salt loss crisis in the first (at least by the 4th) week of the life associated with a prenatal virilization. In >75% of patients with classical 21-hydroxylase deficiency, salt wasting occurs because they cannot produce sufficient aldosterone to maintain normal sodium balance. The symptoms include weight loss and lack of appetite, dehydration, vomiting, diarrhoea, acidosis, and a general failure to thrive. Lack of cortisol impairs cardiac output and the ability to cope with stress. Patients may succumb if undiagnosed (particularly boys) from life-threatening hyponatraemia, dehydration and shock. The degree of virilization is not always dependent on the severity of the salt loss. The same clinical form is usually found in affected siblings, but a few exceptions have been reported (Stoner *et al.*, 1986), likely due to complex pedigrees (see below).

In the other 25% or so, the patients are believed to have adequate aldosterone production and normal sodium balance, yet show signs of virilization, hence the term of simple virilizing (SV) form. Nevertheless, they almost always present an increased plasma renin activity (or active renin) which suggests a slight (compensated) deficiency in aldosterone synthesis.

Delayed diagnosis in this severe form is invariably associated with progressive virilization (precocious pseudo-puberty, with markedly advanced bone maturation), which in late childhood will induce central precocious puberty. Indeed, when advanced bone maturation has reached the average age of the onset of puberty, the latter begins. In other circumstances, the hypothalamo-pituitary axis may be down-regulated by high levels of testosterone produced by the peripheral metabolism of the adrenal precursors. When treatment is started testosterone is suppressed, thus the feedback loop opens and central puberty starts.

Apart from fundamental abnormalities in adrenocortical steroid production, adrenomedullary function is also compromised due to developmental defects in the formation of the adrenal medulla, leading to decreased production of catecholamines, mainly epinephrine (Merke *et al.*, 2000; Weise *et al.*, 2004). This is a possible factor for unstable cardiovascular status leading to shock and the characteristic 'adrenal crisis'. It has even been proposed that measuring plasma free metanephrine and molecular genotype predict phenotype with similar accuracy (Charmandari *et al.*, 2002b).

Non-classical forms

Non-classical (NC) 21-hydroxylase deficiency refers to the condition in which partial deficiencies of 21-hydroxylation permits a late onset, a less extreme hyperandrogenism and milder clinical symptoms or even no symptom at all. However, NC forms are allelic variants of the classical forms (Migeon *et al.*, 1980; Kohn *et al.*, 1982).

Symptomatic forms. These are defined by the absence of prenatal virilization (normal vaginal and urethral orifices and no labial fusion at the time of the diagnosis), and uneventful course throughout childhood, except for the manifestation of mild androgen excess symptoms such as postnatal onset of mild clitoral enlargement, accelerated growth rate with eventual bone age advancement. They are expressed as non-specific hyperandrogenism in females, particularly in the peri-pubertal period, and more rarely of pseudo-precocious puberty in children of both sexes (Forest, 1996). The clinical signs (premature pubarche, tall stature, and advanced bone age, menstrual disturbances, infertility, slowly progressive hirsutism, acne) occur in later childhood, adolescence or after puberty. At the most, a clitoromegaly is observed, but the presence of the fusion of the labia minora in some cases shows the difficulty of distinguishing the borderline between a SV form diagnosed in childhood and a NC form of rather early onset. Actually, genetic and hormonal studies of CAH families and the common use of 17-OHP measurements increase these difficulties as they move back the age of diagnosis of these forms.

Asymptomatic forms. Diagnosis is usually made in the course of genotyping and/or hormonal testing of families with classical 21-hydroxylase deficiency. The relatives present the same biochemical profiles as patients with a NC symptomatic form, but no symptoms. These asymptomatic forms are named cryptic forms. Variations of phenotypic appearance in the same family may depend on mechanisms other than the mutations of *CYP21* gene. Other factors should be taken into account in explaining the variability in clinical expression. These patients may either produce less adrenal androgens or have inactive androgen activity in target tissues such as the skin. It has been suggested that increased sensitivity of the androgen receptor (AR) may explain the poor growth spurt and rapidly advanced bone age in adolescence. However, the observation of a family presenting with both CAH and AR gene mutation suggests that AR gene mutations or polymorphisms are not a common factor influencing the degree of hyperandrogenic symptoms displayed by CAH girls (Giwercman *et al.*, 2002). It is also possible that the symptoms of mild 21-hydroxylase deficiency may wax and wane depending on the degree of stress, providing variable stimuli for corticotrophin releasing hormone (CRH)–ACTH release.

Heterozygotes

Subjects heterozygous for any clinical form of 21-hydroxylase deficiency (carriers, detected by HLA in the past and by DNA studies nowadays, such as parents, siblings, relatives of affected offspring) are clinically normal. However, they manifest a very mild degree of 21-hydroxylase deficiency as evidenced by slightly increased ACTH-stimulated 17-OHP levels (Forest, 1992). The increase is significantly greater in groups of heterozygotes compared to groups of normal controls, but because of a large overlap between the two groups, the test cannot be used on an individual basis because only one-half to one-third of heterozygotes have a normal 17-OHP response to ACTH stimulation (Forest, 1996). On the contrary, during the same test the rise in 21-deoxycortisol levels is quite discriminating, and very useful to detect the heterozygous state in the family members of CAH patients and in the general population.

As mentioned above, the present classification is convenient, but the distinction between each form is sometimes not easy. For example, it may be difficult to distinguish the clinical presentation of males with a SV form from males with NC 21-hydroxylase deficiency. In contrast, the distinction between NC forms and heterozygote subjects is easier if an ACTH test is performed (see below).

Incidence

In recent reports, estimates based on case surveys have varied between 1:12 099 to 1: 23 044, except in Asian countries (1:43 764). In the worldwide experience of neonatal screening reported by Pang and Clark (1993), the incidence was estimated as 1:14 199 live births for homozygous patients, 1:60 for heterozygous subjects, with a gene frequency of 0.0083. Two populations, the Yupik Eskimos (Alaska) and the people of La Réunion (France) have been reported with greater than usual frequency (1:282 and 1:2141 respectively).

The frequency of NC forms has been evaluated to be 1:27 for Ashkenazi Jews, 1:53 for Hispanics, 1:63 for Yugoslavia, 1:333 for Italians and 1:1000 for other Caucasians. By a different approach, this group confirmed a high frequency for the NC gene (10%) (Sherman *et al.*, 1988). These studies suggest that >1% of the population is heterozygous carrier for a NC allele. However, the incidence may significantly vary between populations. On the other hand, prevalence of NC among hirsute women is ≤10% in all studies made whatever the population, in France (personal data), Italy, or Central Anatolia (Kamel *et al.*, 2003), and was similar in children presenting with premature pubarche (Forest and Morel, 1992).

Diagnosis

The diagnosis of classical 21-hydroxylase deficiency is based on markedly elevated serum levels of 17-OHP, which is the main substrate for the enzyme. Baseline values are >300 nmol/l (~1000 ng/ml) in severely affected infants as compared to those of normal newborns which are <3–6 nmol/l (~10–20 ng/ml). Other findings are inappropriate low serum or urinary aldosterone, in the face of hyponatraemia, hyperkalaemia and hyperreninaemia. The hormonal diagnosis can be further defined by performing an ACTH (cosyntropin) stimulation (Forest, 1992), and comparing precursor:product ratios, thus differentiating 21-hydroxylase deficiency from other forms of CAH (Table I). In practice, the finding of basal 17-OHP levels >600 nmol/l (>2000 ng/ml) is diagnostic of classical forms. SV patients have somewhat lower 17-OHP levels, although the range overlaps that seen in SW patients (>300–600 nmol/l). Androstenedione offers similar information but the differences are less pronounced (New et al., 1983). Testosterone is elevated in prepubertal boys and in females at all ages before diagnosis. Basal cortisol is very low (still measurable) in both SW and SV forms, with no significant rise after ACTH stimulation.

Other steroid analysis methods have been described, the most prominent of which is gas chromatography–mass spectrometry of urinary or serum steroids (Wudy et al., 2000), but this costly material is not available everywhere, and the diagnostic profile is more evident after 1 week of life (Shackleton, 1976).

Diagnostic criteria and management are still a matter of controversy in patients presenting with NC forms of 21-hydroxylase deficiency. They may have mildly elevated hormone levels, especially in early infancy. However, random measurements of 17-OHP are often normal in NC patients. This is why we and others (Ibanez et al., 1995; Torok et al., 2003b) believe that an ACTH stimulation test must be performed to establish the accumulation of specific markers in the NC forms. In most NC patients, stimulated levels of 17-OHP are >60 nmol/l (200 ng/ml). However, the threshold value between NC and heterozygotes is also a matter of discussion, because 17-OHP levels vary markedly between patients, e.g. 45–600 nmol/l (150–1000 ng/ml) (stress effect). Comparison of 17-OHP-stimulated levels and genotyping have shown that ACTH-stimulated values of 17-OHP between 30 and 51 nmol/l seem to have overestimated the diagnosis (Bachega et al., 2000). Recent protocols with low dose ACTH seem to be equivalent to the classical high dose test for making the diagnosis. Androgens are variably elevated. Basal and ACTH-stimulated levels of cortisol are normal in NC patients.

Molecular genetic basis of the disease

Normal gene

The 21-hydroxylase activity is mediated by cytochrome P450-C21. The structural gene encoding P450c21 (now named CYP21, ex-CYP21B) and a pseudogene (now named CYP21P, ex-CYP21A) are located on chromosome 6p21.3, within the major human histocompatibility complex (HLA), ~30 kb apart, adjacent to and alternating with the C4B and C4A genes encoding the fourth component of serum complement, and two genes (XA and XB) for an extracellular matrix protein termed tenascin-X (Bristow et al., 1993). These genes are located in tandem and in an array (C4A, CYP21P, XA, C4B, CYP21 and XB). Genes C4A, C4B, CYP21 and XB all encode functional proteins, while CYP21P and XA genes are transcribed in the adrenal cortex, but do not encode proteins. The relationship between CYP21 and X genes is most unusual since these genes overlap one another on opposite strands of DNA (Miller, 1994). This duplicated region of 35 kb has an extremely high degree of nucleotide identity, indicating frequent crossover events and making genetic distinction of the loci difficult (Gitelman et al., 1992).

Mutations in the CYP21 gene

The major mechanism by which the active gene acquires defects is via transfer of segments from the pseudogene to the active gene (Morel et al., 1992; Wedell and Luthman, 1993; Wedell, 1998; White et al., 1994). So far, all mutations causing 21-hydroxylase deficiency apparently result from either a complete deletion of the genes C4B and CYP21B (a product of misalignment and unequal crossing-over between chromatids during meiosis). This results in large 30 kb deletions and a single non-functioning CYP21P/CYP21 fusion gene (termed macroconversion). Southern blot studies show that ~20% of CAH chromosomes carry large alterations of the CYP21 gene: deletions or macroconversion of CYP21 into CYP21P gene. However, the frequency of the gene deletion(s) varies between populations (11–35%), the highest being found in the Northern European population. Nevertheless, rearrangements of the HLA class III region and the polymorphism of the gene C4 might be useful for antenatal diagnosis.

However, it soon appeared that in the majority of the patients (~70–75%), the enzyme defect resulted from deleterious point mutations. These mutations apparently result from partial non-reciprocal sequence transfer from CYP21P to CYP21, a mechanism termed microconversion (Morel et al., 1989). Twelve of the 15 mutations of the CYP21 gene represented in Figure 3 are normally present in CY21P, and can be identified directly on the CYP21 gene specifically amplified by PCR.

The wide range of CAH phenotypes is associated with multiple mutations known to affect 21-hydroxylase enzyme activity. To date, ~100 different CYP21 mutations have been reported, mostly point mutations, but small deletions or insertions have also been described, as well as complete gene deletions. As mentioned above, 15 mutations, constituting 90–95% of alleles, are derived from intergenic recombination of DNA sequences between the CYP21 gene and the highly homologous CYP21P, while the remaining are spontaneous mutations. A reliable and accurate detection of CYP21 mutations is not only important for clinical diagnosis, but also for carrier detection as there is a high variability in the basal level of 17-OHP between normal and heterozygous individuals.

There is an increasing number of reports concerning the genetics of 21-hydroxylase deficiency from various countries in the world. The percentage of given mutations varies, and some rare mutations have been demonstrated (see review in White and Speiser, 2000). For instance, there is a high prevalence of the mutation Q318X in Tunisia (Kharrat et al., 2004), or three novel mutations due to a founder effect in Brazil (Billerbeck et al., 2002). A cluster of four pseudogene-derived point mutations in exons 7 and 8 on a single allele seems particular to the Dutch
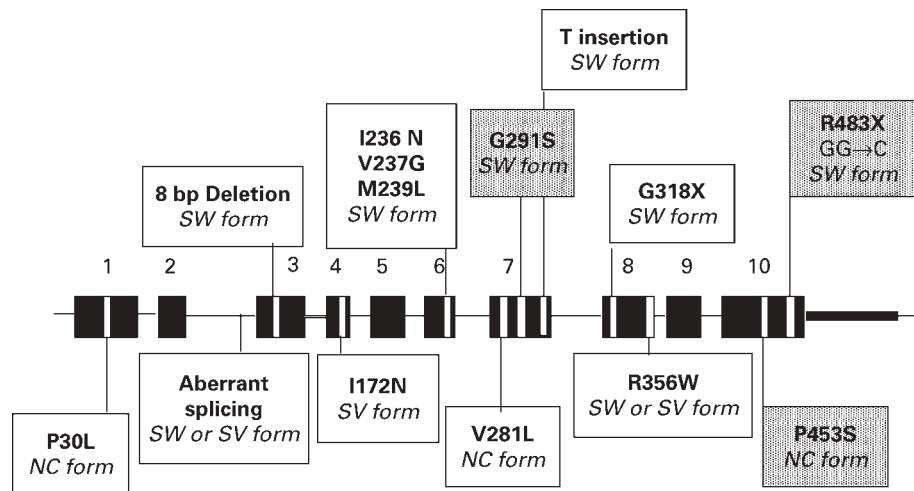
Figure 3. Mutations in the *CYP21* gene causing 21-hydroxylase deficiency. All these mutations (except the three on the shaded background) are present in the pseudogene *CYP21P*, a phenomenon termed genetic microconversion. Genotype association with clinical forms (phenotype) are observed: severe mutations are associated with classical forms whether salt wasting (*SW*) or simple virilizing (*SV*), while mild mutations are associated with the non-classical (*NC*) or cryptic forms. (Adapted from Morel *et al.*, 1992.)

population (Stikkelbroeck *et al.*, 2003b). In Finland, there seem to be multiple independent founder CYP231 gene mutations arisen from a single individual, some of which are of local fairly recent origin, while others seem to have been introduced in Finland by ancient European immigrants (Levo and Partanen, 1997; Levo *et al.*, 1999).

De novo deletions and *de novo* apparent conversions have been reported, the latter usually involving the intron 2 nt 656 g mutation and comprising ~1% of 21-hydroxylase-deficient alleles. The allele frequency of *de novo* gene conversion in intron 2 in the general population is estimated 1 in 2×10^4 (White and Speiser, 2000).

Finally, 21-hydroxylase deficiency might be revealed by an incidentaloma (adrenal adenoma) late in life (Ravichandran *et al.*, 1996). Screening of 21-OH mutations in patients with adrenal adenomas indicates a higher frequency of heterozygotes for classic CAH (16%) than in the general population (1–2%), as well as for a manifest 21-OH deficiency (2%) (Baumgartner-Parzer *et al.*, 2002). Chimeric *CYP21P/CYP21* genes have been described, being for instance the consequence of a 26 or 32 kb deletion in the *C4-CYP21* repeat module of *CYP21P*, tenascin A (*XA*), serine/threonine nuclear protein kinase (RP2), and the *C4B* and *CYP21* genes (Lee, 2004).

In brief, several strategies based on PCR-driven amplification with allele-specific oligonucleotides to the *CYP21* gene have been developed. One reaction for PCR amplification of the *CYP21* gene and the chimeric *CYP21P/CYP21* gene using mixed primers in combination with nested PCR and single-strand conformation polymorphism is considered highly efficient and accurate for molecular diagnosis of 21-hydroxylase deficiency. In some laboratories full sequencing of the coding part of the *CYP21* gene and of its promoter is also used (Guidollet-Tardy, 2002).

Association between genotype and phenotype

The results of *in vitro* transfection studies, with some exceptions, confirm the relationship between genotype and phenotype. So far, all mutations causing 21-hydroxylase deficiency apparently result from either a complete deletion of *C4B* and *CYP21P* (a product of unequal crossing-over during meiosis), or gene microconversion events (resulting in the transfer to *CYP21* of mutations normally present in the pseudogene) (Figure 3). Indeed, the study of the *in vitro* activity of P450c21 from mutated genes transfected in COS cells has shown that there were various degrees of severity in the enzyme deficiency, and has led to the conclusion that there are close associations between genotypes and phenotypes.

This has been agreed by several authors (White *et al.*, 1994; Frisch *et al.*, 2002; Grigorescu-Sido *et al.*, 2002; Guidollet-Tardy, 2002; Hughes, 2002), with a few exceptions concerning the intron 2 mutation, or special rearrangements (L'Allemand *et al.*, 2000).

In brief, patients carrying 'severe' mutations which destroy all P450c21 activity (complete absence of the *CYP21* gene, gene conversion, 8 bp deletion or specific point mutations) present the classical SW form of 21-hydroxylase deficiency, whether homozygous or heterozygous for any of these lesions. In these groups, 25% of the mutant alleles carry either deletion mutations or large gene conversions, while 75% have *CYP21* mutations alone, or associated with *C4A* and *CYP21P* gene deletions, with *C4B* and *CYP21* deletions, with *CYP21P* and *C4* duplication, or undefined mutations (Morel and Miller, 1991). In addition, a change of isoleucine to asparagine at codon 172 is the most common cause of the SV form of 21-hydroxylase deficiency.

In contrast, three point mutations (V282L, P453S and P30L) have maintained a partial enzyme activity, and result in NC forms of the disease. The presence of any of these three 'mild' mutations on one allele will determine the clinical expression of the disease. Whether homozygous (mild/mild) or compound heterozygote (severe/mild), the patient will have a NC form of 21-hydroxylase deficiency. However, he/she will be at risk of having a child with classical 21-hydroxylase deficiency if the partner is carrying a severe mutation on one allele, whether he/she is a simple heterozygote, a compound heterozygote or affected with classical CAH) (Figure 4).
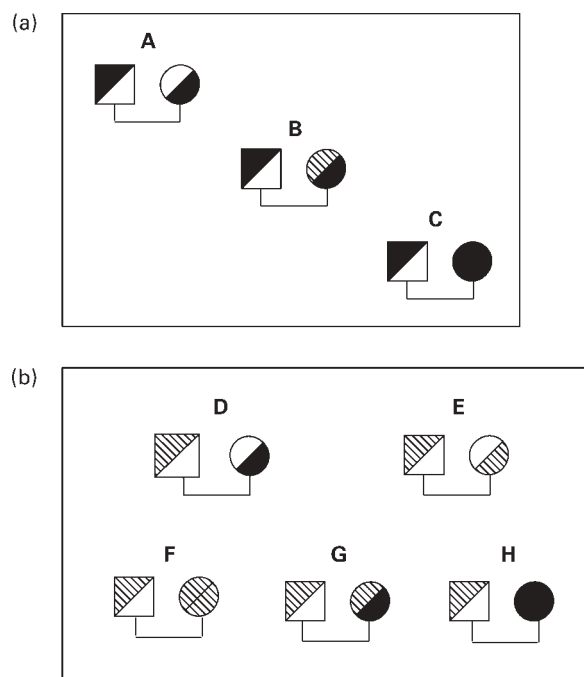
Figure 4. Pedigrees found in congenital adrenal hyperplasia (CAH) families. Severe mutations are illustrated in black, mild mutations are shown by hatched areas. The classical forms of the disease are due to the combination of *severe/severe* mutations, whereas the combination of *mild/severe* mutations is associated with non-classical forms. (**a**) Prediction of the clinical form of CAH in a couple at risk: if a partner is known to have a severe mutation. The couple is at risk of having a child with a classical form of CAH if the other partner has at least one severe mutation (whether heterozygote (**A**), or affected with a non-classical CAH (**B**) or a classical form of the disease (**C**). (**b**) In contrast, when one partner is known to be heterozygote for a mild mutation, there will be no risk of the couple having a child affected with a classical CAH whatever the genotype of the other partner: heterozygote for severe (**D**) or mild mutation (**E**), CAH affected with a non-classical form (**F** or **G**), or even a classical form (**H**) of CAH.

The most common lesion in classical 21-hydroxylase deficiency is the amino acid A → G substitution 13 bp before the end of intron 2, resulting in aberrant splicing of pre-mRNA, which, for hitherto unexplained reasons, does not always correlate with clinical expression. It should also be stressed that genetic diagnosis is more complicated for 21-hydroxylase deficiency than for many other monogenic disorders due to the high variability of the locus. This includes coexistence of two or more mutations on an allele, or the presence of more than one *CYP21/C4* repeat unit on the same chromosome. An interesting case illustrates the difficulties of genotyping in 21-hydroxylase deficiency (Baumgartner-Parzer *et al.*, 2003).

Therefore genotyping must be made with segregation of mutations in families, before genetic counselling, particularly when prenatal diagnosis and treatment are requested. Experienced molecular biologists can now predict not only the risk of a couple having an affected child, but also what would be the clinical form (classical or NC) of the disease. Moreover, genotypes of deletion, gene conversion or severe mutations motivate prenatal treatment of affected female fetuses, whereas genotypes of less severe mutations do not (see examples in Figure 4).

The incidence of *CYP21* mutations in 21-hydroxylase deficiency has been extensively studied in the last 10 years. No significant differences have been observed in Caucasian populations; the most frequent single mutations are the mutation in intron 2 in SW and SV forms, I172N in SV form and V281L in NC form (Figure 3).

From the screening of a large cohort of 21-hydroxylase patients (>1500 families), a similar incidence was observed, except for some less frequent mutations (Guidollet-Tardy, 2002). In particular, the Q318X mutation creating a stop codon was identified in >7% of chromosomes, contrasting with its low incidence in other European studies (for instance, 1:186 chromosomes in Sweden). As the population studied included Caucasians and Arabs, an extensive molecular study of the *CYP21* gene has been done in 25 unrelated Tunisian families with classical 21-OH deficiency: all affected females presented genital masculinization at birth and all have had some degree of clinical salt loss. A high consanguinity was observed. Using a cascade strategy (digestion by restriction enzymes, sequencing), mutations have been identified in all affected chromosomes (Guidollet-Tardy, 2002). Surprisingly, Q318X mutation appears to be the most common (~ 40%) and is somewhat linked with a *CYP21* gene polymorphism. The incidence of other mutations does not differ from that previously described in intron 2 (28%) and I172N (12%). As no mild mutation alone was found, a close association between genotype and phenotype exists. It is the first time that such a large prevalence of rare *CYP21* mutations has been reported in a given ethnic group. If confirmed by other studies including more patients, screening for the Q318X mutation in an Arab population should be considered.

Treatment

Treatment is based on the principle of replacing normal glucocorticoid and mineralocorticoid needs, in the classical forms, and psychological support. Most female patients also need surgical repair of the external genitalia.

Glucocorticoid treatment

Usual treatment includes hydrocortisone and 9α-fludrocortisol, spilt into two or three daily doses. Salt supplementation is recommended in infants and children. At these ages, the preferred glucocorticoid replacement is hydrocortisone, theoretically in a dose of $10-15 \, \text{mg/m}^2$/day, but adjusted to the smallest dose able to normalize growth and physical maturation, and at the same time to maintain hormonal values within acceptable limits. The bioavailability of oral hydrocortisone is high and may result in supraphysiological cortisol concentrations within $1-2 \, \text{h}$ after administration of high doses. As the clearance of hydrocortisone is lower in the evening, and hence the drug more bioavailable after a night-time dose (Charmandari *et al.*, 2001), this suggests that it is not advisable to weight the daily dose higher in the evening. In children and adolescents, the physiological cortisol secretion has been re-estimated at $\sim 6-7 \, \text{mg/m}^2$/day (Kerrigan *et al.*, 1993). However, supraphysiological glucocorticoid doses are required to suppress adrenal androgen adequately in CAH patients. It is important to realise that a single dose of hydrocortisone, being shortlasting, is not capable of controlling adrenocortical secretions over the nycthemeron.

Hydrocortisone is not available in all countries—some have to use cortisone acetate. An exceptional case of failure of treatment

of CAH has been reported, because of concomitant defective 11β-hydroxysteroid dehydrogenase reductase activity (Nordenstrom *et al.*, 1999a). Despite optimal substitution therapy, and compliance, control of classical CAH is often inadequate at puberty, and the problems encountered relate to hypocortisolism and/or hyperandrogenism. Indeed, puberty is associated with alterations in cortisol pharmacokinetics resulting in increased clearance and volume of distribution with no change in half-life (Charmandari *et al.*, 2002a). On the other hand, it has been suggested that at puberty an increased sensitivity of the androgen receptor may explain the poor growth spurt due to a rapidly advanced bone age. However, the observation of a patient presenting with both CAH and AR gene mutation does not support this concept as the sole factor for reducing final height in most CAH patients (Giwercman *et al.*, 2002). Thus it has been proposed to combine morning hydrocortisone with a night-time dose of prednisone in order to suppress the overnight rise in ACTH.

Other long-acting, more potent glucocorticoids, such as prednisone and dexamethasone, have negative effects upon growth, but are more widely used in adult CAH patients because they are more convenient. Adolescent and adult may be treated with prednisone (5–7.5 mg daily in two divided doses, or dexamethasone 0.25–0.5 mg in one or two daily doses). These patients on long-acting steroids should be monitored carefully for signs of Cushing's syndrome (rapid weight gain, hypertension, striae and osteopenia), effect on body mass index (BMI) and blood pressure (Roche *et al.*, 2003). Nowadays, in adulthood lower dose of prednisolone (Punthakee *et al.*, 2003) or dexamethasone (Li *et al.*, 2003) than previously used are recommended.

Mineralocorticoid treatment

In SW patients life-long mineralocorticoid treatment is necessary, but additional salt supplements are needed to maintain plasma sodium concentration and renin in the normal ranges during infancy (0.5–5 nmol/l/day) (Mullis *et al.*, 1990). In our experience, salt supplementation (2–3 g/day) is given from birth to late childhood. We adjust the mineralocorticoid dose necessary to restore normal renin levels (active renin, or plasma renin activity). The only oral mineralocorticoid available is 9α-fluocortisol. The dose is usually 30–75 µg per day, rarely >150 µg in our experience, but some authors apparently use higher doses (up to 400 µg/day) probably because they do not supplement correctly for salt deprivation. In infants, we distribute the total treatment in three divided doses, and we ask the pharmacist to prepare adequate capsules for the parents' convenience and to support compliance. When the treatment is not adequately controlled, we always correct first the mineralocorticoid needs, because this will diminish the need for glucocorticoids. Indeed, when renin is elevated, ACTH shows a tendency to increase, thus larger doses of glucocorticoids are needed. SV patients are also given mineralocorticoids when plasma renin is elevated. On the other hand, NC forms never need mineralocorticoid treatment, and should not be treated systematically by glucocorticoids (Merke and Kabbani, 2001). Treatment should be only symptomatic.

Stress dosing

In case of adrenal crisis, surgery or severe acute disease, treatment must be adapted as detailed in Table II. It is common to hear that NC patients never have a salt crisis. However, throughout the world the community of paediatric endocrinologists has experienced a few cases with tragic consequences, particularly if over-treated.

Dose titration

The biological criteria to optimize treatment are controversial. Some authors use mainly clinical development (growth velocity and bone age). Others measure hormone levels: 17-OHP (in serum or saliva) and/or serum androstenedione and/or testosterone which are believed to be the most sensitive index of biochemical control. We believe that 17-OHP should not return to normal for age levels, but being kept in a reasonable range in the morning before the first therapeutic doses (30–100 nmol/l). Nycthemeral variations should be maintained, and nadir values be <10 nmol/l. Testosterone is also a very useful parameter in females at all ages, and in prepubertal boys, and contrary to 17-OHP should be maintained in the normal range for age. In pubertal females, androstenedione and LH should be monitored because of the risk of developing polycystic ovaries. In

Table II. Treatment to be made in classical forms of congenital adrenal hyperplasia prior to any surgery or anaesthesia

Day before (−1)	Hydrocortisone im (2 mg/kg) i.m. injection
Day of surgery (0)	
Before surgery	Hydrocortisone (cortisol; 2 mg/kg) and Syncortyl (DOC acetate; 2.5 or 5 mg) i.m. injection
During surgery	Perfusion i.v. with glucose solution 10% with NaCl 20% (a total of 0.5 g/kg body weight of sodium chloride in 24 h) containing cortisol hemisuccinate (2.5 mg/kg)
	The volume of perfusion is a maximum of 150 ml/kg/day
After surgery, in the evening	Hydrocortisone (2.5 mg/kg) i.m. injection
Day after surgery (+1)	NaCl (0.5 g/kg) split into two or three doses during the day
	Hydrocortisone (2 to 5 mg/kg) i.m. injection
	Syncortyl (only if necessary according to plasma electrolytes) (2.5 or 5 mg)
Following days (+2 to +4)	Reduce progressively the doses of hydrocortisone, for example if the child is doing well give oral treatment:
	on day +3 = twice the usual dose
	on day +4 = 1.5 times the usual dose
	on day +5 = the usual dose
	Close follow-up from day −1 to day +5 of: plasma electrolytes twice a day, proteins, blood pressure every 4 h, weight, diuresis etc.

pubertal males, testosterone and LH/FSH indicate whether treatment is well controlled, because increased adrenal androgens may suppress the pituitary.

Although dehydroepiandrosterone (DHEA) and its sulphate (DHEAS) are quantitatively the most important adrenal androgens, they are of no use to monitor treatment because they are readily suppressed in treated patients (Brunelli *et al.*, 1995).

Renin, aldosterone and potassium are useful to monitor mineralocorticoid treatment. They should return to normal values for age, but not below, since it is important not to give overdoses to the patients. Blood pressure should also be evaluated routinely.

Surgery

In virilized females, the principal aims of surgery are to reduce clitoris/phallus size, create a vaginal orifice that will allow menstrual flow and intercourse, and to correct the urogenital sinus and vaginal pouch to prevent incontinence. Current thinking and practice are evolving, or even controversial. In the past, the surgeons have undertaken early clitoral recession and labioscrotal reduction, followed by vaginal pull-through at 2–4 years of age. Later on, early clitoroplasty and genitoplasty were recommended, definitely by the age of 18 months and in the hands of experienced paediatric surgeons, a single-stage procedure was performed with encouraging results (Donahoe and Gustafson, 1994; Farkas and Chertin, 2001). The commitment to raise a 46,XX child as a boy is a very rare event, and the surgery requires many steps (Dasgupta *et al.*, 2003)

In recent years, the approach to genital reconstruction has changed, because minor to major vaginal reconstruction is cruelly needed at adolescence (Creighton *et al.*, 2001). An alternative approach is to do partial clitoral reduction in infancy, with vaginoplasty reserved for late adolescence. The decision of whether, when, and what type of genital surgery is desirable needs to be reviewed with the family and/or the patient, experienced surgeons, endocrinologists, and psychologists (Clayton *et al.*, 2002).

Alternative treatment(s), new approaches

During the past 50 years, since the discovery of cortisone therapy as an effective treatment for CAH, many advances have been made in the management of 21-hydroxylase deficiency. 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.

New treatment approaches to classical CAH represent potential solutions to these unresolved issues. At the National Institutes of Health, a long-term randomized clinical trial is investigating a new treatment regimen combining a reduced hydrocortisone dose, an anti-androgen, and an aromatase inhibitor. Peripheral blockade of androgens may also be helpful in the adult CAH woman with polycystic ovarian syndrome (PCOS) (White and Speiser, 2002).

Recently a promising approach was to give CAH children growth hormone treatment with or without GnRH analogues in order to improve final height (New, 2001a).

Other promising new treatment approaches include GnRH agonist-induced pubertal delay with or without growth hormone

therapy, alternative glucocorticoid preparations or dose schedules. New treatment approaches currently under investigation include combination therapy to block androgen action and inhibit estrogen production, and bilateral adrenalectomy in the most severely affected patients (Gunther *et al.*, 1997). Adrenalectomy will not alleviate problems caused by gonadal adrenal rests. Other approaches, which are in a preclinical stage of investigation, include treatment with a corticotrophin-releasing hormone antagonist and gene therapy (Merke *et al.*, 2002). The applicability and success of these new approaches await the results of current research.

Prenatal diagnosis and treatment (**Forest *et al.*, 1993; Forest, 1997; New, 1998**)

Prenatal diagnosis

Prenatal diagnosis of 21-hydroxylase deficiency has always improved at the same time as the advances of endocrinology and genetic fields. By 1975, prenatal diagnosis had been attempted in the second trimester of pregnancy on the basis of raised 17-OHP levels in the amniotic fluid (Forest, 1985; New, 1990). Diagnosis and genetic tracking of CAH were greatly facilitated by its linkage to HLA. However, the molecular approach of 21-hydroxylase deficiency has been an important step in prenatal diagnosis, improving its early timing and safety. Indeed, studies of the *C4-CYP21* gene locus and the *CYP21* mutations simplify the procedures for an early and accurate prenatal diagnosis in the first trimester.

Nowadays, genotyping is made by allele-specific amplification (Theodoropoulou *et al.*, 2001) or full sequencing (Guidollet-Tardy, 2002), but pitfalls may be observed in cases of complex gene conversions and rearrangements between the *CYP21* and *CYP21P*, which pose unique complications for prenatal diagnosis (Mao *et al.*, 2002). Thus, it has been proposed that direct mutation detection should be supported by linkage analysis, whenever possible, in order to provide more comprehensive information for the family (Mao *et al.*, 2002).

Point mutations of the *CYP21* gene could be detected after specific PCR amplification of the functional *CYP21* gene. Study of the *C4-CYP21* gene locus by Southern blot analysis and that of the *CYP21* gene mutations by PCR simplify the procedures for an early and accurate prenatal diagnosis in the first trimester (New *et al.*, 2001). In these circumstances, most families are informative. Moreover, using this direct genetic analysis associated with the possibility of detecting the heterozygotes in a non-related-CAH population, a prenatal diagnosis by molecular methods could be done in a family without a previous CAH affected child.

Nowadays, prenatal diagnosis is usually performed on chorionic villous sampling (CVS). However, if CVS is not available or refused by the parents, amniocentesis can still be performed, and be very helpful.

Genetic counselling

When considering prenatal diagnosis and/or treatment, the first step is to offer genetic counselling. The role of genetic counselling is to give the parents all available information about the disease, to explain to them what are the clinical forms and their consequences, the possibility of prenatal diagnosis and prenatal

treatment. Prediction of the clinical form of the disease in a couple at risk is possible, based on the complete genotype of the future parents (Figure 4).

Genetic counselling is easy within the same kindred, provided there are adequate hormonal and molecular genetic studies in a nuclear family with a previously affected child. Complete genetic analysis is made in the index case, and the lesions identified in the parents (in order to exclude a *de novo* mutation). A clinico-genetic correlation should be made, thus both parents should also have hormonal studies, i.e. measurement of basal or ACTH-stimulated 17-OHP levels, in order to document a NC form of the disease, cryptic or ignored.

Genetic counselling is more difficult when the index case is a parent or a relative, or even when there is no index case. There are increasing and pressing requests for genetic counselling from CAH patients or relatives of a CAH patient, when they desire children. Often the genetic status of the partner is unknown, but the couple want to know what is the risk of having a CAH child, and what would be the clinical expression in case of CAH. Heterozygote detection is not a goal for prenatal diagnosis *per se*, but has to be performed on this occasion.

Although neonatal screening is now available (see below), such studies do not allow the detection of NC forms of 21-hydroxylase deficiency and all the more so that of heterozygotes in a general population. On the other hand, it is not yet conceivable to use molecular genetic studies for population screening of carriers of 21-hydroxylase deficiency. However, adequate hormonal studies can now allow the detection of heterozygotism. Indeed, heterozygote subjects for 21-hydroxylase deficiency have subtle anomalies after ACTH stimulation. As mentioned above, post-ACTH levels of 21-deoxycortisol are significantly elevated in heterozygotes, with little overlap, and the test is discriminating in $\sim 90\%$ of the individuals tested (Figure 5).

Our strategy for detection of heterozygote is currently straightforward. The indication for such studies is when one person of a given couple is known to be heterozygote (or eventually at risk for it). A search for heterozygocity will be made in the partner. The first step is to give the subject a short ACTH test and measure 21-deoxycortisol levels. If the subject is predicted to be heterozygote, molecular genetic studies of her/his *CYP21* genes are made. According to the gene lesions found, one can now predict whether the offspring is at risk for a classical or NC form of CAH, and whether prenatal diagnosis is indicated or not (Figure 6).

Prenatal therapy

Sexual ambiguity is a major complication of the disease, bearing the risk of sex misassignment, requiring difficult reconstructive surgery fraught with risks of functional impairment, and eventually leading to impaired long-term quality of life. This is why prenatal therapy has been proposed for preventing the *in utero* virilization of CAH females. (David and Forest, 1984). The rationale of treatment involves providing sufficient glucocorticoid levels to the fetus to suppress excessive ACTH stimulation. Dexamethasone was chosen because it crosses the placenta and has a long half-life. Protocol was as follows: treatment was proposed to mothers at risk to have a fetus with a classical form of CAH, and who decided to continue pregnancy whether the fetus was affected or not. Treatment had to be be started early ($\geq$ 8th
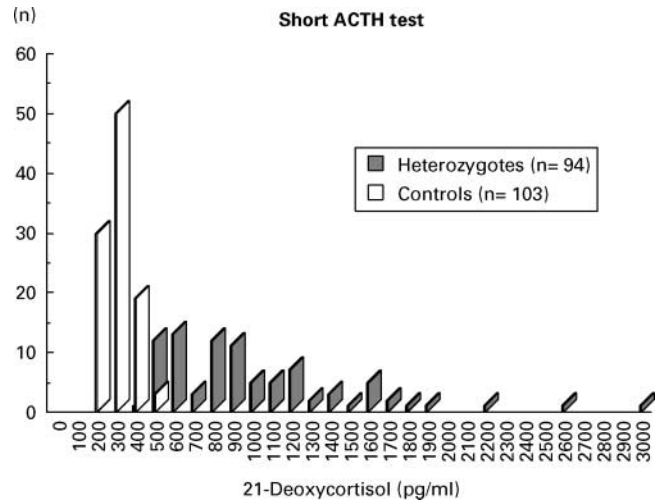

Figure 5. Adrenocorticotrophic hormone-stimulated levels of 21-deoxycortisol. Overlap between controls and heterozygotes is minimal (threshold value 550 pg/ml) (M.G.Forest, unpublished data).
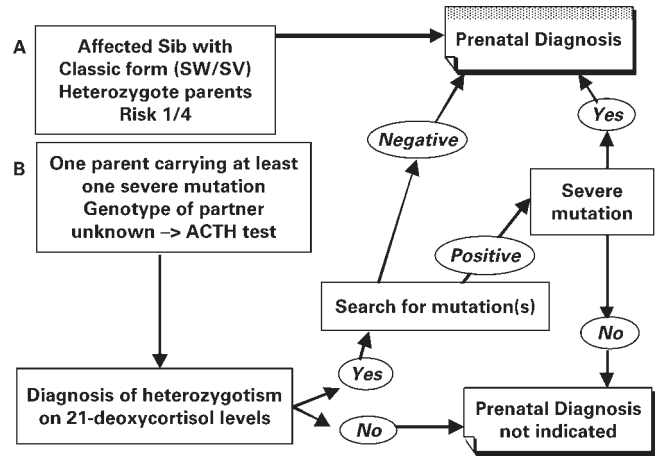

Figure 6. Flow chart of prenatal diagnosis in a couple in whom one partner is known to have a severe mutation in the *CYP21* gene on at least one allele, but nothing known for the other partner.

week of amenorrhoea), i.e. prior to any possible prenatal diagnosis, continued until term only in the case of a CAH female fetus and stopped in all other cases. Thus, seven out of eight fetuses were treated needlessly. Prenatal diagnosis in treated mothers is nowadays performed on CVS, by molecular studies of the 21-hydroxylase genes (Figure 7).

Fetal sexing is part of the prenatal diagnosis. It has been known for at least a decade that fetal cells are found in maternal blood. Fetal sex prediction could be achieved using PCR targeted at the SRY gene by analysing cell-free fetal DNA in maternal serum. Unfortunately, the results reported to date show lack of sensitivity, especially in the first trimester of pregnancy. A new highly sensitive real-time PCR was developed to detect an SRY gene sequence in maternal serum in the first trimester (6–11 weeks pregnancy). No false negative results were observed. Furthermore, no false positive results occurred, although 27 women who carried a female fetus during the current pregnancy had had at
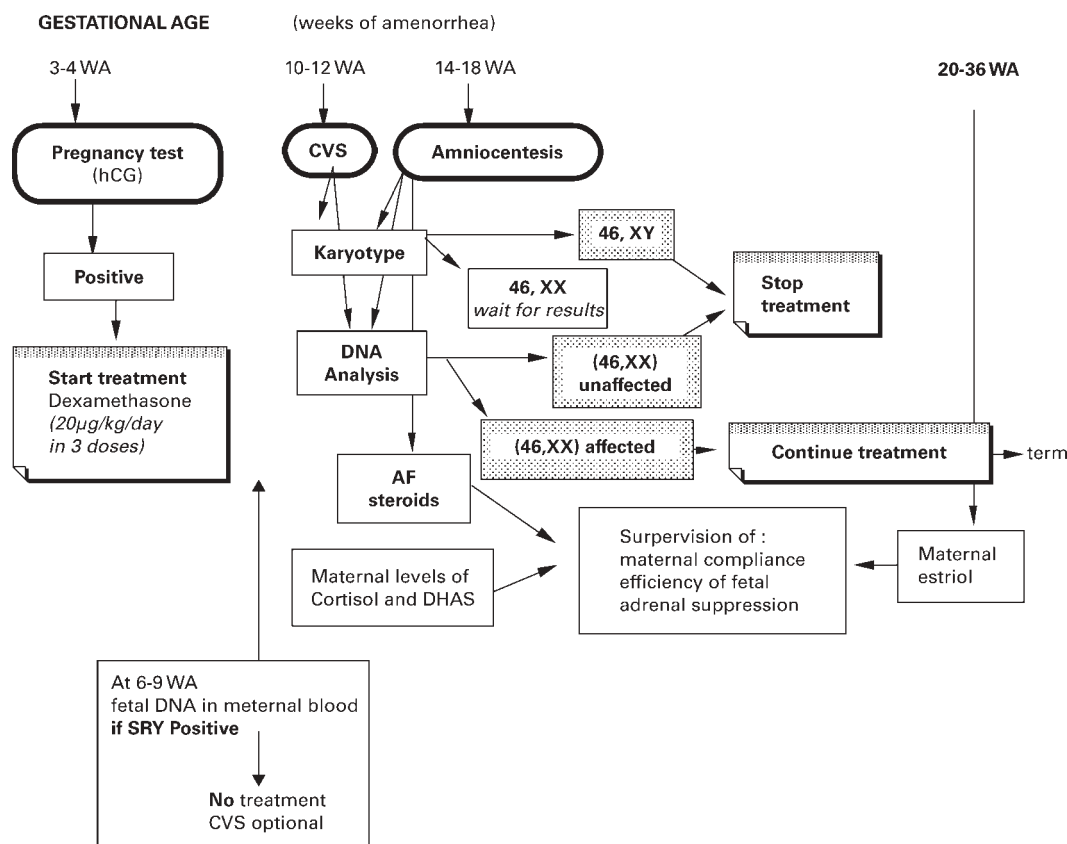
Figure 7. Flow chart of prenatal treatment in a couple at risk for a classic form of 21-hydroxylase deficiency. AF = amniotic fluid.

least one previous male-bearing pregnancy. Thus, the analysis of cell-free fetal DNA in maternal plasma for fetal sex determination would abolish the need for corticosteroid administration and CVS in women with male fetuses at risk for 21-hydroxylase deficiency: then only three out of eight fetuses at risk would have unnecessary treatment (Morel *et al.*, 2003).

The mode of action of dexamethasone is still debated, because it is not certain that the hypothalamic–pituitary–adrenal axis is operating at the onset of treatment. It has been suggested that the action is direct on the adrenal secretion itself. However, this hypothesis is not supported by recent *in vitro* experiments (Dardis and Miller, 2003).

When the mother is CAH-affected, the treatment protocol represents a somewhat different situation because she is having a substitution treatment, mostly consisting of glucocorticoids (hydrocortisone or dexamethasone) and mineralocorticoids. It is important to determine the risk for the fetus in the couple. If the couple is found to be at risk for the classic form, then maternal treatment is adapted or changed to the current protocol of prenatal treatment (total daily dose of 20 μg/kg body weight of dexamethasone, split into two or three doses), with no change in mineralocorticoids. However, if the fetus is only at risk for a NC form, maternal treatment need not be modified.

Satisfactory results of prenatal treatment rely on early initiation of treatment and a divided (i.e. twice daily or three times daily) daily dose. The effective dose was investigated, and appeared to be 20 μg/kg of maternal weight per day (Forest, 1997). The occurrence of variable side-effects in the mothers,

severe in a few, suggests that the minimal effective dose should be used and that unnecessary treatment should be discontinued as early as possible. It is also important to remember that successful prenatal therapy depends upon parental motivation and effective collaboration between paediatricians, gynaecologists, biologists and geneticists (Forest, 1997). The accumulated European (Ritzen, 1998; Forest and Dörr, 2003) and American (Carlson *et al.*, 1999; New, 2001b) experiences show that the benefits of dexamethasone treatment clearly outweigh the risks and can help to allay anxiety and encourage future pregnancies in these families. The present state of art indicates that prenatal therapy is effective in significantly reducing or even eliminating virilization of CAH females, sparing the children the consequences of genital surgery, sex misassignment, and gender confusion (Carlson *et al.*, 1999).

However, both the physical and psychological development of these children and the possibility of long-term adverse effects in the mothers need to be further evaluated (Forest *et al.*, 1998; Lajic *et al.*, 1998).

In view of these and other concerns, the prenatal treatment of CAH remains an experimental therapy and, hence, must only be done with fully informed consent in controlled prospective trials approved by human experimentation committees at centres that see enough of these patients to collect meaningful data.

Although no somatic teratological side-effects have been found to date, animal experiments in the rat have suggested that dexamethasone may have adverse effects of glucocorticoids on brain, in particular the hippocampus, and might be responsible

for hypertension later in life (Seckl and Miller, 1997). However, recent studies in the sheep show no deleterious effect of dexamethasone therapy in early pregnancy (Dodic *et al.*, 2003). The question whether there are long-term side-effects in unaffected human infants who nowadays submitted to very short prenatal treatment is more difficult to answer. A preliminary study was unable to document any adverse effects of early prenatal dexamethasone treatment in the doses recommended for the treatment of pregnancies at risk for CAH on motor and cognitive development (Meyer-Bahlburg *et al.*, 2004a) or other features (Kay *et al.*, 2000). At the moment such questions cannot be answered because the CAH and unaffected subjects should be followed for ≥20 years. This is why it is believed that a registry should be open for this treatment.

The main benefit of prenatal therapy is to ameliorate potentially genital ambiguity in affected female subjects, which it does. It is important that prenatal treatments would be in the hands of very specialized teams made of clinicians, biologists and geneticists. The risks of unnecessarily treating unaffected pregnancies, which now seem small, may not be fully elucidated for many years. Prenatal treatment must be done under careful, centralized and ideally long-term medical supervision.

Are there other beneficial effects of prenatal treatment? We believe so. In CAH-affected females, prenatal treatment not only prevents sexual ambiguity, but also may prevent the consequences of the *in utero* exposure to androgens, which might be deleterious for brain programming and induce PCOS. In both sexes, prenatal treatment prevents adrenal hypertrophy. Thus, the postnatal treatment with hydrocortisone is easier and more rapid to adjust at smaller dosages.

Long-term outcome

Survival

In severe forms of 21-hydroxylase deficiency, adrenal insufficiency may lead to a life-threatening event. A SW crisis during the first weeks of life may be the first sign of the disease in boys without a family history. In populations where there is no neonatal screening, affected females are ≥30% in excess (Murtaza *et al.*, 1980), which means that many boys died without being diagnosed.

The risk of glucocorticoid and mineralocorticoid deficiency is also high in treated patients exposed to infection, trauma, surgery or other significant stress that a healthy subject would cope with by increasing his cortisol secretion (Miller, 1994). A significant number of deaths due to insufficient substitution have been reported (Swerdlow *et al.*, 1998). Otherwise limited complications occur and survival of well-treated patients is expected.

Adult height

In all reports, adult height has remained below target height (Muirhead *et al.*, 2002). This is due to the use of supraphysiological levels of glucocorticoids necessary to control adrenal androgen excess, leading to hypercortisolism (Miller, 1994). The detrimental effect may result from different mechanisms, suppression of growth hormone secretion, or direct action on bone growth (Adler and Rosen, 1994). Besides, there is a risk of osteoporosis, although this is debated (de Almeida Freire *et al.*,

2003; Christiansen *et al.*, 2004). In early childhood, BMI correlates negatively with adult height (Yu and Grant, 1995).

As pointed out in the past by the group of Knorr in Munich, and recently documented in CAH patients in the first year of life and between the ages of 8 and 14 years, there is a dose-dependent negative effect of glucocorticoids on linear growth (Stikkelbroeck *et al.*, 2003d). Indeed, growth is inhibited by excessive glucocorticoid treatment and this negative effect is dose-dependent in the first year of life (Manoli *et al.*, 2002). Thus, in early childhood excessive metabolic control may be more harmful for linear growth than under-substitution with glucocorticoids (Girgis and Winter, 1997). Early diagnosed CAH patients who received lower cortisol equivalent doses during the first year of life reached a better final height (Balsamo *et al.*, 2003). Some height is also lost during puberty (Manoli *et al.*, 2002). Hence, monitoring treatment over the first 2 years and during puberty is critical for the height outcome of these patients (New *et al.*, 1989).

In boys, excessive prepubertal androgen exposure due to uncontrolled CAH is associated with a reduction in adult somatic height but it does not routinely result in micropenis (Levy and Husmann, 1996).

Fertility

Fertility is classically reported as low in CAH female patients. Several factors have been suggested to contribute to this impaired fertility: adrenal overproduction of androgens and progestins (17-OHP and progesterone), ovarian hyperandrogenism, POCS, ovarian adrenal rest tumours, neuroendocrine factors, genital surgery, and psychological factors such as delayed psychosexual development, reduced sexual activity and low maternal feelings. Improving endocrine, surgical and psychological management could contribute to improving fertility chances in these patients (Stikkelbroeck *et al.*, 2003a). Moreover, a recent report demonstrates that when adequately treated with a combination of glucocorticoids and mineralocorticoids, sexually active patients with the classic phenotype of CAH can become pregnant (Hoepffner *et al.*, 2004). This further emphasizes the importance of mineralocorticoid replacement therapy.

The prevalence of testicular adrenal rest tumours in postpubertal CAH patients might be higher than previously reported, being 1:10 (Stikkelbroeck *et al.*, 2003c). These benign tumours are often related to sub-optimal or even lack of glucocorticoid substitution, and usually, but not always, disappear with optimized therapy with dexamethasone (Benvenga *et al.*, 1999). These adrenal rests have been shown to lead to temporary infertility, but it is not certain if they cause permanent testicular failure (Cutfield *et al.*, 1983). Ultrasonography should be the method of first choice for detection and follow-up of these lesions (Stikkelbroeck *et al.*, 2003c). The presence of adrenal rests within the testes of adult males with classic CAH are more frequent in the SW form, and are associated with a higher risk for infertility (Cabrera *et al.*, 2001).

A single case of ovarian tumour identical to the testicular tumour of the adrenogenital syndrome has been reported in a CAH female under treatment for 35 years (Al-Ahmadie *et al.*, 2001). Characteristic clinical and radiological findings help to differentiate this tissue from other tumours.

Effect of prenatal androgens on different dimorphisms

Prenatal androgen does not influence the development of human spatial abilities (Hines *et al.*, 2003), nor hand preferences and language lateralization, two manifestations of neural asymmetry (Mathews *et al.*, 2004).

By contrast, gender-typed behaviours and interest are influenced by prenatal androgens, CAH girls being more interested in masculine toys and having greater preference for masculine careers (Servin *et al.*, 2003).

The left-hand digit ratio development is not influenced by *in utero* exposure to androgens (Buck *et al.*, 2003). The results are consistent with the idea that behavioural masculinization in girls with CAH results from high levels of androgens during fetal development and not in postnatal life (Berenbaum *et al.*, 2000). For others, prenatal androgen exposure reduces the ratio of the length of the second digit (2D) to the length of the fourth digit (4D). Normally, the 2D:4D ratio is greater in women than in men. Thus, prenatal androgen exposure plays a role in the establishment of the sex difference in human finger length patterns (Brown *et al.*, 2002).

Finally, prenatal glucocorticoid deficiency with resulting alterations in the regulation of the hypothalamic–pituitary–adrenal axis, sex steroid excess, or some combination of the latter, preferentially affect the growth and development of the amygdala, a structure with major functional implications that warrant further exploration (Merke *et al.*, 2003).

Psychosexual development

Prenatal androgenization affects gender-related behaviour but not gender identity (Meyer-Bahlburg *et al.*, 2004b). Both physical and behavioural masculinization are related to each other and to genotype, indicating that behavioural masculinization is a consequence of prenatal androgen exposure (Hall *et al.*, 2004).

To address questions about sex assignment in children with ambiguous genitalia, gender identity was studied in girls with CAH in relation to the characteristics of the disease and treatment, particularly genital appearance and surgery. Gender identity in girls with CAH was not related to the degree of genital virilization or age at which genital reconstructive surgery was done. Thus, moderate androgen excess early in development appears to produce a small increase in the risk of atypical gender identity, but this risk cannot be predicted from genital virilization (Berenbaum and Bailey, 2003).

Levels of CRH are elevated in patients with depression and anxiety and are expected to be elevated in CAH patients. It is unknown whether patients with 21-hydroxylase deficiency have an increased incidence of these psychiatric disorders.

Sexual life

CAH females have highly abnormal results for sensation in the clitoris. Genital surgery may disrupt sensory input. Sexual function also appears to be impaired and this may relate to the compromised sensitivity and restricted introitus. The possibility that women with CAH have deficient clitoral sensation *ab initio* cannot be excluded. These striking findings must be evaluated further in the light of the controversy about the issue of genital surgery in children with CAH (Crouch *et al.*, 2004).

Women with the SV form reported greater satisfaction and fewer concerns regarding their psychosexual and surgical outcome than women with the SW form.

Other

The presence of prostatic glandular tissue in females with 21-hydroxylase deficiency has been documented. These patients may be at risk not only for malignancies seen in genotypic females but also for prostate cancer (Winters *et al.*, 1996).

Transitional care

To meet the needs of the young woman with CAH, it is important that the transition from paediatric to adult care be a process of parallel consultations over several years, always involving an experienced gynaecological endocrinologist. Questions about menstruation, sexuality, fertility and the possible necessity of complementary surgery are always important issues that need to be discussed (Hagenfeldt, 2004).

CAH is a lifelong disorder, which poses management problems that are age- and sex-specific. The condition merits an organized, multi-disciplinary transitional care format similar to the kind that is now well established for Turner's syndrome in many centres. In the eyes of the paediatrician, achieving optimal growth is the primary target of CAH management during infancy and childhood. Fixation on this objective can be to the detriment of the patient, because it may result in failure to appreciate the significance of metabolic disturbances that occur in later childhood, particularly in females, and which may be the progenitor of chronic problems such as obesity, insulin resistance and infertility in adult life. Similarly, the care of the adult patient with CAH comprises more than just prescribing steroid replacement for primary adrenal insufficiency. The transition period between childhood and adulthood is an opportune time for reviewing the various management options and to assess the efficacy of steroid replacement, to consider alternative novel treatment modalities and to apply a checklist to the multi-faceted aspects of the medical, surgical and psychological needs of the patient (Hughes, 2004).

Neonatal screening (**Forest *et al.*, 1998; Brosnan *et al.*, 1999**)

This is made feasible by a simple technique: measurement of 17-OHP levels on dried blood samples. Early diagnosis of CAH can be lifesaving. With the advent of newborn screening programmes, fewer cases are missed. Because false positive results occur, especially in premature and low birthweight babies, infants with borderline elevations, although requiring follow-up, are often considered normal. Given the heterogeneity of phenotypes of CAH, less severe forms, especially in males, could result in marginally abnormal laboratory results early in life, with possible adverse effects later in life. Moreover, a recent report (Gudmundsson *et al.*, 1999) describing a newborn female who, despite severe virilization, had only a borderline elevation in 17-OHP on newborn screening as well as the initial confirmatory testing, emphasizes the broad range of 17-OHP levels in CAH, the lack of correlation of these levels with clinical phenotype and the importance of the timing of both screening and confirmatory tests. Due to the complexity of interpreting these tests, an experienced paediatric endocrinologist should control any screening programme for CAH.

Some authors are rather enthusiastic for neonatal screening (Pang, 1997). More than 7.5×10^6 newborns have been screened for 21-hydroxylase deficiency worldwide. As a result of such efforts, neonatal screening for CAH has proven to be highly reliable and has benefited countless numbers of affected newborns with classical 21-hydroxylase deficiency by contributing to the early diagnosis of the disorder. Even in developing countries this programme is seriously envisioned (Chu et al., 2002) because of its simplicity and relatively low cost.

The screening process, however, is less reliable among low birthweight or preterm infants, and recent studies show that newly established normative reference levels based on birthweight or gestational age may minimize false positive rates and improve the efficacy of newborn screening for CAH, particularly in low birthweight newborns. In Switzerland it is considered highly reliable (Steigert et al., 2002), as well as in France. Furthermore, hormonal screening allows earlier treatment.

Recent experience in which the adjustment of 17-OHP threshold values to both age and birthweight (Olgemoller et al., 2003), and/or improved specificity of the method used (mass spectrometry) (Lacey et al., 2004) shows that the predictive value might be markedly improved. It is possible to identify the vast majority of classical cases of CAH in affected females without a neonatal mass screening programme. However, a significant number of boys with the SV form would be missed, whereas both SW boys and girls are diagnosed clinically (Torok et al., 2003a). Neonatal screening for CAH can be falsely negative in the event of neonatal dexamethasone treatment, illustrating the importance of a medical history (Rohrer et al., 2003).

Finally, neonatal screening must be confirmed by genotyping (Nordenstrom et al., 1999b). However, whereas molecular genetic diagnosis is a valuable tool, it cannot replace clinical acumen and hormonal assays (Speiser, 2001).

The value of screening of neonates for CAH is not universally accepted. Procedures for screening are recommended, however, in order to provide a structure for the testing and ultimately bring together data that will allow the screening to be judged beneficial or to be dismissed as no better than clinical recognition of the disease state.

Conclusions

The management of children and adolescents with CAH remains difficult. To assess the current European practice in diagnosis and management of CAH, an ESPE (European Society for Paediatric Endocrinology) survey was circulated in 2000/2001 (Riepe et al., 2002). The questionnaire was answered by 34% of ESPE members, representing 125 institutions, which cared for 6553 CAH patients. Paediatric endocrinologists, surgeons, gynaecologists, geneticists, and psychologists were involved in the immediate care of the CAH neonate and his family. Forty-four per cent of centres took part in neonatal screening programmes. In families at risk, prenatal dexamethasone therapy was started at a median gestational age of 6 weeks in a median dose of 20 µg/kg/day. Fifty-three per cent reported maternal adverse events, 8% observed adverse fetal events. Regarding feminizing surgery, 33% reported simultaneous clitoris reduction and vaginoplasty during infancy. However, clitoridectomy was still reported by 13% of centres, and vaginal dilatations have been performed by 27%. Although 71% of female CAH patients presented with psychosexual problems, only 17% undertook routine psychodiagnosis and counselling.

Hydrocortisone was the substance used for the treatment of CAH during growth in 84%; the median dose (mg/m^2/day) was 17.5 in infants, 15 in children and adolescents, and 13.75 in adults. The glucocorticoid dose was increased 2–6-fold during intercurrent stress. Mineralocorticoid was administered in cases of clinically manifest SW and of elevated plasma renin activity, to decrease high glucocorticoid doses, or according to genotype. All participating ESPE members felt the need for further improvement in prenatal diagnosis and treatment, compliance during puberty, screening programmes, psychological aspects, and corrective surgery.

References

Adler RA and Rosen CJ (1994) Glucocorticoids and osteoporosis. Endocrinol Metab Clin North Am 23,641–654.

Al-Ahmadie HA, Stanek J, Liu J, Mangu PN, Niemann T and Young RH (2001) Ovarian 'tumor' of the adrenogenital syndrome: the first reported case. Am J Surg Pathol 25,1443–1450.

Bachega TA, Billerbeck AE, Marcondes JA, Madureira G, Arnhold IJ and Mendonca BB (2000) Influence of different genotypes on 17-hydroxy-progesterone levels in patients with nonclassical congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Clin Endocrinol (Oxf) 52, 601–607.

Balsamo A, Cicognani A, Baldazzi L, Barbaro M, Baronio F, Gennari M, Bal M, Cassio A, Kontaxaki K and Cacciari E (2003) CYP21 genotype, adult height, and pubertal development in 55 patients treated for 21-hydroxylase deficiency. J Clin Endocrinol Metab 88,5680.

Baumgartner-Parzer SM, Pauschenwein S, Waldhausl W, Polzler K, Nowotny P and Vierhapper H (2002) Increased prevalence of heterozygous 21-OH germline mutations in patients with adrenal incidentalomas. Clin Endocrinol (Oxf) 56,811–816.

Baumgartner-Parzer SM, Nowotny P, Waldhausl W and Vierhapper H (2003) A rare duplicated 21-hydroxylase haplotype and a de novo mutation: a family analysis. J Clin Endocrinol Metab 88,2794–2796.

Benvenga S, Smedile G, Lo Giudice F and Trimarchi F (1999) Testicular adrenal rests: evidence for luteinizing hormone receptors and for distinct types of testicular nodules differing for their autonomization. Eur J Endocrinol 141,231–237.

Berenbaum SA and Bailey JM (2003) Effects on gender identity of prenatal androgens and genital appearance: evidence from girls with congenital adrenal hyperplasia. J Clin Endocrinol Metab 88, 1102–1106.

Berenbaum SA, Duck SC and Bryk K (2000) Behavioral effects of prenatal versus postnatal androgen excess in children with 21-hydroxylase-deficient congenital adrenal hyperplasia. J Clin Endocrinol Metab 85, 727–733.

Beyenburg S, Watzka M, Clusmann H, Blümcke I, Bidlingmaier G, Elger CE and Stoffel-Wagner B (2001) Messenger RNA of steroid 21-hydroxylase (CYP21) is expressed in the human hippocampus. Neurosci Lett 308,111–114.

Billerbeck AE, Mendonca BB, Pinto EM, Madureira G, Arnhold IJ and Bachega TA (2002) Three novel mutations in CYP21 gene in Brazilian patients with the classical form of 21-hydroxylase deficiency due to a founder effect. J Clin Endocrinol Metab 87,4314–4317.

Bristow J, Tee MK, Gitelman SE, Mellon SH and Miller WL (1993) Tenascin-X: a novel extracellular matrix protein encoded by the human XB gene overlapping P450c21B. J Cell Biol 122,265–278.

Brosnan PG, Brosnan CA, Kemp SF, Domek DB, Jelley DH, Blackett PR and Riley WJ (1999) Effect of newborn screening for congenital adrenal hyperplasia. Arch Pediatr Adolesc Med 153,1272–1278.

Brown WM, Hines M, Fane BA and Breedlove SM (2002) Masculinized finger length patterns in human males and females with congenital adrenal hyperplasia. Horm Behav 42,380–386.

Brunelli VL, Chiumello G, David M and Forest MG (1995) Adrenarche does not occur in treated patients with congenital adrenal hyperplasia resulting from 21-hydroxylase deficiency. Clin Endocrinol (Oxf) 42,461–466.

Buck JJ, Williams RM, Hughes IA and Acerini CL (2003) In-utero androgen exposure and 2nd to 4th digit length ratio—comparisons between healthy controls and females with classical congenital adrenal hyperplasia. Hum Reprod 18,976–979.

Cabrera MS, Vogiatzi MG and New MI (2001) Long term outcome in adult males with classic congenital adrenal hyperplasia. J Clin Endocrinol Metab 86,3070–3078.

Carlson AD, Obeid JS, Kanellopoulou N, Wilson RC and New MI (1999) Congenital adrenal hyperplasia: update on prenatal diagnosis and treatment. J Steroid Biochem Mol Biol 69,19–29.

Charmandari E, Johnston A, Brook CG and Hindmarsh PC (2001) Bioavailability of oral hydrocortisone in patients with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. J Endocrinol 169,65–70.

Charmandari E, Brook CG and Hindmarsh PC (2002a) Why is management of patients with classical congenital adrenal hyperplasia more difficult at puberty? Arch Dis Child 86,266–269.

Charmandari E, Eisenhofer G, Mehlinger SL, Carlson A, Wesley R, Keil MF, Chrousos GP, New MI and Merke DP (2002b) Adrenomedullary function may predict phenotype and genotype in classic 21-hydroxylase deficiency. J Clin Endocrinol Metab 87,3029–3030.

Christiansen P, Molgaard C and Muller J (2004) Normal bone mineral content in young adults with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Horm Res 61,133–136.

Chu SY, Tsai WY, Chen LH, Wei ML, Chien YH and Hwu WL (2002) Neonatal screening for congenital adrenal hyperplasia in Taiwan: a pilot study. J Formos Med Assoc 101,691–694.

Clayton PE, Miller WL, Oberfield SE, Ritzen EM, Sippell WG and Speiser PW (2002) Consensus statement on 21-hydroxylase deficiency from the European Society for Paediatric Endocrinology and the Lawson Wilkins Pediatric Endocrine Society. Horm Res 58,188–195.

Creighton SM, Minto CL and Steele SJ (2001) Objective cosmetic and anatomical outcomes at adolescence of feminising surgery for ambiguous genitalia done in childhood. Lancet 358(9276),124–125.

Crouch NS, Minto CL, Laio LM, Woodhouse CR and Creighton SM (2004) Genital sensation after feminizing genitoplasty for congenital adrenal hyperplasia: a pilot study. Br J Urol Int 93,135–138.

Cutfield RG, Bateman JM and Odell WD (1983) Infertility caused by bilateral testicular masses secondary to congenital adrenal hyperplasia (21-hydroxylase deficiency). Fertil Steril 40,809–814.

Dardis A and Miller WL (2003) Dexamethasone does not exert direct intracellular feedback on steroidogenesis in human adrenal NCI-H295A cells. J Endocrinol 179,131–142.

Dasgupta R, Schnitzer JJ, Hendren WH and Donahoe PK (2003) Congenital adrenal hyperplasia: surgical considerations required to repair a 46,XX patient raised as a boy. J Pediatr Surg 38,1269–1273.

David M and Forest MG (1984) Prenatal treatment of congenital adrenal hyperplasia resulting from 21-hydroxylase deficiency. J Pediatr 105, 799–803.

de Almeida Freire PO, de Lemos-Marini SH, Maciel-Guerra AT, Morcillo AM, Matias Baptista MT, de Mello MP and Guerra GJ (2003) Classical congenital adrenal hyperplasia due to 21-hydroxylase deficiency: a cross-sectional study of factors involved in bone mineral density. J Bone Miner Metab 21,396–401.

Dodic M, Tersteeg M, Jefferies A, Wintour EM and Moritz K (2003) Prolonged low-dose dexamethasone treatment, in early gestation, does not alter blood pressure or renal function in adult sheep. J Endocrinol 179, 275–280.

Donahoe PK and Gustafson ML (1994) Early one-stage surgical reconstruction of the extremely high vagina in patients with congenital adrenal hyperplasia. J Pediatr Surg 29,352–358.

Farkas A and Chertin B (2001) Feminizing genitoplasty in patients with 46XX congenital adrenal hyperplasia. J Pediatr Endocrinol Metab 14, 13–22.

Forest MG (1985) Pitfalls in prenatal diagnosis of 21-hydroxylase deficiency by amniotic fluid steroid analysis? A six years experience in 102 pregnancies at risk. Ann NY Acad Sci 458,130–147.

Forest MG (1992) Adrenal function tests. In Ranke MB (ed) Functional endocrinologic diagnostics in children and adolescents. J & J Verlag, Mannheim, pp 248–274.

Forest MG (1996) Peripubertal hyperandrogenism. Minerva Pediatr 48, 179–191.

Forest MG (1997) Prenatal diagnosis, treatment, and outcome in infants with congenital adrenal hyperplasia. Curr Opin Endocrinol Diabet 4, 209–217.

Forest MG and Dörr HG (2003) Prenatal therapy in congenital adrenal hyperplasia due to 21-hydoxylase deficiency: Retrospective follw-up study of 253 treated pregnancies in 215 families. Endocrinologist 13,252–259.

Forest MG and Morel Y (1992) Formes non classiques dites 'tardives' du déficit en 21-hydroxylase: diagnostic différentiel avec les hétérozygotes et conseil génétique. Rev Franç Endocrinol Clin 33,47–61.

Forest MG, David M and Morel Y (1993) Prenatal diagnosis and treatment of 21-hydroxylase deficiency. J Steroid Biochem Mol Biol 45,75–82.

Forest MG, Morel Y and David M (1998) Prenatal treatment of congenital adrenal hyperplasia. Trends Endocrinol Metab 9,284–289.

Frisch H, Waldhauser F, Lebl J, Solyom J, Hargitai G, Kovacs J, Pribilincova Z, Krzisnik C, Battelino T and MEWPE-CAH Study Group (2002) Congenital adrenal hyperplasia: lessons from a multinational study. Horm Res 57 (Suppl),95–1001.

Girgis R and Winter JS (1997) The effects of glucocorticoid replacement therapy on growth, bone mineral density, and bone turnover markers in children with congenital adrenal hyperplasia. J Clin Endocrinol Metab 82,3926–3929.

Gitelman SE, Bristow J and Miller WL (1992) Mechanisms and consequences of the duplication of the human C4/P450c21/gene locus. Mol Cell Biol 12,2124–2134.

Giwercman YL, Nordenskjold A, Ritzen EM, Nilsson KO, Ivarsson SA, Grandell U and Wedell A (2002) An androgen receptor gene mutation (E653K) in a family with congenital adrenal hyperplasia due to steroid 21-hydroxylase deficiency as well as in partial androgen insensitivity. J Clin Endocrinol Metab 87,2623–2628.

Grigorescu-Sido A, Schulze E, Grigorescu-Sido P, Heinrich U, Nistor T and Duncea I (2002) Mutational analysis and genotype-phenotype correlation in patients with classic 21-hydroxylase deficiency from transylvania (north-west Romania). J Pediatr Endocrinol Metab 15,1505–1514.

Gudmundsson K, Majzoub JA, Bradwin G, Mandel S and Rifai N (1999) Virilising 21-hydroxylase deficiency: timing of newborn screening and confirmatory tests can be crucial. J Pediatr Endocrinol Metab 12, 895–901.

Guidollet-Tardy V (2002) Le déficit en 21-hydroxylase Mise au point de l'exploration moléculaire de 1538 patients et 188 hétérozygotes; Corrélations génotype-phénotype. PhD Thesis, University Lyon I, 256pp.

Gunther DF, Bukowski TP, Ritzen EM, Wedell A and Van Wyk JJ (1997) Prophylactic adrenalectomy of a three-year-old girl with congenital adrenal hyperplasia: pre- and postoperative studies. J Clin Endocrinol Metab 82,3324–3327.

Hagenfeldt KB (2004) Congenital adrenal hyperplasia due to 21-hydroxylase deficiency—the adult woman. Growth Horm IGF Res 14 (Suppl A), 67–71.

Hall CM, Jones JA, Meyer-Bahlburg HF, Dolezal C, Coleman M, Foster P, Price DA and Clayton PE (2004) Behavioral and physical masculinization are related to genotype in girls with congenital adrenal hyperplasia. J Clin Endocrinol Metab 89,419–424.

Hines M, Fane BA, Pasterski VL, Mathews GA, Conway GS and Brook C (2003) Spatial abilities following prenatal androgen abnormality: targeting and mental rotations performance in individuals with congenital adrenal hyperplasia. Psychoneuroendocrinology 28,1010–1026.

Hoepffner W, Schulze E, Bennek J, Keller E and Willgerodt H (2004) Pregnancies in patients with congenital adrenal hyperplasia with complete or almost complete impairment of 21-hydroxylase activity. Fertil Steril 81,1314–1321.

Hughes I (2002) Congenital adrenal hyperplasia: phenotype and genotype. J Pediatr Endocrinol Metab 15,1329–1340.

Hughes IA (2004) Congenital adrenal hyperplasia: transitional care. Growth Horm IGF Res 14 (Suppl A), 60–66.

Ibanez L, Bonnin MR, Zampolli M, Prat N, Alia PJ and Navarro MA (1995) Usefulness of an ACTH test in the diagnosis of nonclassical 21-hydroxylase deficiency among children presenting with premature pubarche. Horm Res 44,51–56.

Kamel N, Tonyukuk V, Emral R, Corapcioglu D, Bastemir M and Gullu S (2003) The prevalence of late onset congenital adrenal hyperplasia in hirsute women from Central Anatolia. Endocr J 50,815–823.

Kay HH, Bird IM, Coe CL and Dudley DJ (2000) Antenatal steroid treatment and adverse fetal effects: what is the evidence? J Soc Gynecol Invest 7,269–278.

Kayes-Wandover KM and White PC (2000) Steroidogenic enzyme gene expression in the human heart. J Clin Endocrinol Metab 85,2519–2525.

Kerrigan JR, Veldhuis JD, Leyo SA, Iranmanesh A and Rogol AD (1993) Estimation of daily cortisol production and clearance rates in normal pubertal males by deconvolution analysis. J Clin Endocrinol Metab 76, 1505–1510.

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Kharrat M, Tardy V, M'Rad R, Maazoul F, Jemaa LB, Refai M, Morel Y and Chaabouni H (2004) Molecular genetic analysis of Tunisian patients with a classic form of 21-hydroxylase deficiency: identification of four novel mutations and high prevalence of Q318X mutation. J Clin Endocrinol Metab 89,368–374.

Kohn B, Levine LS, Pollack MS, Pang S, Lorenzen F, Levy D, Lerner AJ, Rondanini GF, Dupont B and New MI (1982) Late-onset steroid 21-hydroxylase deficiency: a variant of classical congenital adrenal hyperplasia. J Clin Endocrinol Metab 55,817–827.

Koppens PF, Hoogenboezem T, Drop SL, de Muinck-Keizer-Schrama SM and Degenhart HJ (1998) Aldosterone production despite absence or defectiveness of the CYP21 genes in two patients with salt-losing congenital adrenal hyperplasia caused by steroid 21-hydroxylase deficiency. Clin Endocrinol (Oxf) 49,815–822.

L'Allemand D, Tardy V, Gruters A, Schnabel D, Krude H and Morel Y (2000) How a patient homozygous for a 30-kb deletion of the C4-CYP 21 genomic region can have a nonclassic form of 21-hydroxylase deficiency. J Clin Endocrinol Metab 85,4562–4567.

Lacey JM, Minutti CZ, Magera MJ, Tauscher AL, Casetta B, McCann M, Lymp J, Hahn SH, Rinaldo P and Matern D (2004) Improved specificity of newborn screening for congenital adrenal hyperplasia by second-tier steroid profiling using tandem mass spectrometry. Clin Chem 50, 467–468.

Lajic S, Wedell A, Bui TH, Ritzen EM and Holst M (1998) Long-term somatic follow-up of prenatally treated children with congenital adrenal hyperplasia. J Clin Endocrinol Metab 83,3872–3880.

Lee HH (2004) The chimeric CYP21P/CYP21 gene and 21-hydroxylase deficiency. J Hum Genet 49,65–72.

Levo A and Partanen J (1997) Mutation-haplotype analysis of steroid 21-hydroxylase (CYP21) deficiency in Finland. Implications for the population history of defective alleles. Hum Genet 99,488–497.

Levo A, Jaaskelainen J, Sistonen P, Siren MK, Voutilainen R and Partanen J (1999) Tracing past population migrations: genealogy of steroid 21-hydroxylase (CYP21) gene mutations in Finland. Eur J Hum Genet 7, 188–196.

Levy JB and Husmann DA (1996) Congenital adrenal hyperplasia: is there an effect on penile growth? J Urol 156,780–782.

Li HY, Dahir KM and Blevins LSJ (2003) Treatment of adult patients with congenital adrenal hyperplasia due to 21-hydroxylase deficiency: a clinical practice audit. Endocr Pract 9,347–352.

Manoli I, Kanaka-Gantenbein C, Voutetakis A, Maniati-Christidi M and Dacou-Voutetakis C (2002) Early growth, pubertal development, body mass index and final height of patients with congenital adrenal hyperplasia: factors influencing the outcome. Clin Endocrinol (Oxf) 57,669–676.

Mao R, Nelson L, Kates R, Miller CE, Donaldson DL, Tang W and Ward K (2002) Prenatal diagnosis of 21-hydroxylase deficiency caused by gene conversion and rearrangements: pitfalls and molecular diagnostic solutions. Prenat Diagn 22,1171–1176.

Mathews GA, Fane BA, Pasterski VL, Conway GS, Brook C and Hines M (2004) Androgenic influences on neural asymmetry: handedness and language lateralization in individuals with congenital adrenal hyperplasia. Psychoneuroendocrinology 29,810–822.

Merke D and Kabbani M (2001) Congenital adrenal hyperplasia: epidemiology, management and practical drug treatment. Paediatr Drugs 3, 599–611.

Merke DP, Chrousos GP, Eisenhofer G, Weise M, Keil MF, Rogol AD, Van Wyk JJ and Bornstein SR (2000) Adrenomedullary dysplasia and hypofunction in patients with classic 21-hydroxylase deficiency. N Engl J Med 343,1362–1368.

Merke DP, Bornstein SR, Avila NA and Chrousos GP (2002) NIH conference Future directions in the study and management of congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Ann Intern Med 136,320–334.

Merke DP, Fields JD, Keil MF, Vaituzis AC, Chrousos GP and Giedd JN (2003) Children with classic congenital adrenal hyperplasia have decreased amygdala volume: potential prenatal and postnatal hormonal effects. J Clin Endocrinol Metab 88, 1760–1765.

Meyer-Bahlburg HF, Dolezal C, Baker SW, Carlson AD, Obeid JS and New MI (2004a) Cognitive and motor development of children with and without congenital adrenal hyperplasia after early-prenatal dexamethasone. J Clin Endocrinol Metab 89,610–614.

Meyer-Bahlburg HF, Dolezal C, Baker SW, Carlson AD, Obeid JS and New MI (2004b) Prenatal androgenization affects gender-related behavior but not gender identity in 5–12-year-old girls with congenital adrenal hyperplasia. Arch Sex Behav 33,97–107.

Migeon CJ, Rosenwaks Z, Lee PA, Urban MD and Bias WB (1980) The attenuated form of congenital adrenal hyperplasia as an allelic form of 21-hydroxylase deficiency. J Clin Endocrinol Metab 51,647–649.

Miller WL (1994) Clinical review 54: Genetics, diagnosis, and management of 21-hydroxylase deficiency. J Clin Endocrinol Metab 78,241–246.

Morel Y and Miller WL (1991) Clinical and molecular genetics of congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Adv Hum Genet 20,1–68.

Morel Y, André J, Uring-Lambert B, Hauptmann G, Bétuel H, Tosi M, Forest MG, David M, Bertrand J and Miller WL (1989) Rearrangements and point mutations of P450c21 genes are distinguished by five restriction endonuclease haplotypes identified by a new probing strategy in 57 families with congenital adrenal hyperplasia. J Clin Invest 83,527–536.

Morel Y, Murena M, Nicolino M and Forest MG (1992) Molecular genetics of the congenital adrenal hyperplasia due to 21-hydroxylase deficiency. In Saez JM, Brownie AC, Capponi A, and Chambaz EM (eds) Cellular and Molecular Biology of the Adrenal Cortex, Vol 222. Colloque INSERM/John Libbey Eurotext Ltd, Paris, pp 123–136.

Morel Y, Tardy V, Costa JM, Forest MG and David M (2003) Déficit en 21-hydroxylase: nouvelles démarches déduites des études moléculaires. Ann Endocrinol (Paris) 64,456–470.

Muirhead S, Sellers EA and Guyda H (2002) Indicators of adult height outcome in classical 21-hydroxylase deficiency congenital adrenal hyperplasia. J Pediatr 141,247–252.

Mullis PE, Hindmarsh PC and Brook CG (1990) Sodium chloride supplement at diagnosis and during infancy in children with salt-losing 21-hydroxylase deficiency. Eur J Pediatr 150,22–25.

Murtaza L, Sibert JR, Hughes I and Balfour IC (1980) Congenital adrenal hyperplasia—a clinical and genetic survey. Are we detecting male salt-losers? Arch Dis Child 55,622–625.

New MI (1990) Prenatal diagnosis and treatment of adrenogenital syndrome (Steroid 21-hydroxylase deficiency). Dev Pharmacol Ther 15,200–210.

New MI (1998) Diagnosis and management of congenital adrenal hyperplasia. Annu Rev Med 49,311–328.

New MI (2001a) Factors determining final height in congenital adrenal hyperplasia. J Pediatr Endocrinol Metab 14,933–937.

New MI (2001b) Prenatal treatment of congenital adrenal hyperplasia. The United States experience. Endocrinol Metab Clin North Am 30,1–13.

New MI, Lorenzen F, Lerner AJ, Kohn B, Oberfield SE, Pollack MS, Dupont B, Stoner E, Levy DJ, Pang S et al. (1983) Genotyping steroid 21-hydroxylase deficiency: hormonal reference data. J Clin Endocrinol Metab 57,320–326.

New MI, Gertner JM, Speiser PW and Del Balzo P (1989) Growth and final height in classical and nonclassical 21-hydroxylase deficiency. J Endocrinol Invest 12,91–95.

New MI, Carlson A, Obeid J, Marshall I, Cabrera MS, Goseco A, Lin-Su K, Putnam AS, Wei JQ and Wilson RC (2001) Prenatal diagnosis for congenital adrenal hyperplasia in 532 pregnancies. J Clin Endocrinol Metab 86,5651–5657.

Nordenstrom A, Marcus C, Axelson M, Wedell A and Ritzen EM (1999a) Failure of cortisone acetate treatment in congenital adrenal hyperplasia because of defective 11beta-hydroxysteroid dehydrogenase reductase activity. J Clin Endocrinol Metab 84,1210–1213.

Nordenstrom A, Thilen A, Hagenfeldt L, Larsson A and Wedell A (1999b) Genotyping is a valuable diagnostic complement to neonatal screening for congenital adrenal hyperplasia due to steroid 21-hydroxylase deficiency. J Clin Endocrinol Metab 84,1505–1509.

Olgemoller B, Roscher AA, Liebl B and Fingerhut R (2003) Screening for congenital adrenal hyperplasia: adjustment of 17-hydroxyprogesterone cut-off values to both age and birth weight markedly improves the predictive value. J Clin Endocrinol Metab 88,5790–5794.

Pang S (1997) Current status of neonatal screening for congenital adrenal hyperplasia. Curr Opin Pediatr 9,407–412.

Pang S and Clark A (1993) Congenital adrenal hyperplasia due to 21-hydroxylase deficiency: newborn screening and its relationship to the diagnosis and treatment of the disorder. Screening 2,105–139.

Prader A and Gurtner HP (1955) Das Syndrom des Pseudohermaphrotismus masculinus bei kongenitaler Nebennierenindenhyperplasie ohne androgenüberproduktion (adrenaler Pseudohermaphroditismus masculinus). Helv Pædiatr Acta 10,397–411.

Punthakee Z, Legault L and Polychronakos C (2003) Prednisolone in the treatment of adrenal insufficiency: a re-evaluation of relative potency. J Pediatr 143,402–405.

Ravichandran R, Lafferty F, McGinniss MJ and Taylor HC (1996) Congenital adrenal hyperplasia presenting as massive adrenal incidentalomas in

the sixth decade of life: report of two patients with 21-hydroxylase deficiency. J Clin Endocrinol Metab 81,1776–1779.

Riepe FG, Krone N, Viemann M, Partsch CJ and Sippell WG (2002) Management of congenital adrenal hyperplasia: results of the ESPE questionnaire. Horm Res 58,196–205.

Ritzen EM (1998) Prenatal treatment of congenital adrenal hyperplasia: a commentary. Trends Endocrinol Metab 9,293–295.

Roche EF, Charmandari E, Dattani MT and Hindmarsh PC (2003) Blood pressure in children and adolescents with congenital adrenal hyperplasia (21-hydroxylase deficiency): a preliminary report. Clin Endocrinol (Oxf) 58,589–596.

Rogoff D, Gomez-Sanchez CE, Foecking MF, Wortsman J and Slominski A (2001) Steroidogenesis in the human skin: 21-hydroxylation in cultured keratinocytes. J Steroid Biochem Mol Biol 78,77–81.

Rohrer TR, Gassmann KF, Pavel ME and Dorr HG (2003) Pitfall of newborn screening for congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Biol Neonate 83,65–68.

Seckl JR and Miller WL (1997) How safe is long-term prenatal glucocorticoid treatment? J Am Med Assoc 277,1077–1079.

Servin A, Nordenstrom A, Larsson A and Bohlin G (2003) Prenatal androgens and gender-typed behavior: a study of girls with mild and severe forms of congenital adrenal hyperplasia. Dev Psychol 39,440–450.

Shackleton CH (1976) Congenital adrenal hyperplasia caused by defect in steroid 21-hydroxylase. Establishment of definitive urinary steroid excretion pattern during first weeks of life. Clin Chim Acta 67,287–298.

Sherman SL, Aston CE, Morton NE, Speiser PW and New MI (1988) A segregation and linkage study of classical and nonclassical 21-hydroxylase deficiency. Am J Hum Genet 42,830–838.

Slominski A, Hermak G and Mihm M (1996) ACTH receptor, CYP11A1, CYP17, and CYP21A2 genes are expressed in skin. J Clin Endocrinol Metab 81,2746–2749.

Speiser PW (2001) Molecular diagnosis of CYP21 mutations in congenital adrenal hyperplasia: implications for genetic counseling. Am J Pharmacogenom 1,101–110.

Speiser PW, Agdere L, Ueshiba H, White PC and New MI (1991) Aldosterone synthesis in salt-wasting congenital adrenal hyperplasia with complete absence of adrenal 21-hydroxylase. N Engl J Med 324, 145–149.

Steigert M, Schoenle EJ, Biason-Lauber A and Torresani T (2002) High reliability of neonatal screening for congenital adrenal hyperplasia in Switzerland. J Clin Endocrinol Metab 87,106–110.

Stikkelbroeck NM, Hermus AR, Braat DD and Otten BJ (2003a) Fertility in women with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Obstet Gynecol Surv 58,275–284.

Stikkelbroeck NM, Hoefsloot LH, de Wijs IJ, Otten BJ, Hermus AR and Sistermans EA (2003b) CYP21 gene mutation analysis in 198 patients with 21-hydroxylase deficiency in The Netherlands: six novel mutations and a specific cluster of four mutations. J Clin Endocrinol Metab 88,3852–3859.

Stikkelbroeck NM, Suliman HM, Otten BJ, Hermus AR, Blickman JG and Jager GJ (2003c) Testicular adrenal rest tumours in postpubertal males with congenital adrenal hyperplasia: sonographic and MR features. Eur Radiol 13,1597–1603.

Stikkelbroeck NM, Van't Hof-Grootenboer BA, Hermus AR, Otten BJ and Van't Hof MA (2003d) Growth inhibition by glucocorticoid treatment in

salt wasting 21-hydroxylase deficiency: in early infancy and (pre)puberty. J Clin Endocrinol Metab 88,3525–3530.

Stoner E, Dimartino-Nardi J, Kuhnle U, Levine LS, Oberfield SE and New MI (1986) Is salt-wasting in congenital adrenal hyperplasia due to the same gene as the fasciculata defect? Clin Endocrinol (Oxf) 24,9–20.

Swerdlow AJ, Higgins CD, Brook CG, Dunger DB, Hindmarsh PC, Price DA and Savage MO (1998) Mortality in patients with congenital adrenal hyperplasia: a cohort study. J Pediatr 133,516–520.

Theodoropoulou M, Barta C, Szoke M, Guttman A, Staub M, Niederland T, Solyom J, Fekete G and Sasvari-Szekely M (2001) Prenatal diagnosis of steroid 21-hydroxylase deficiency by allele-specific amplification. Fetal Diagn Ther 16,237–240.

Torok D, Eckhardt G and Solyom J (2003a) Twenty years experience in rapid identification of congenital adrenal hyperplasia in Hungary. Eur J Pediatr 162,844–849.

Torok D, Halasz Z, Garami M, Homoki J, Fekete G and Solyom J (2003b) Limited value of serum steroid measurements in identification of mild form of 21-hydroxylase deficiency. Exp Clin Endocrinol Diabetes 111,27–32.

Wedell A (1998) an update on the molecular genetics of congenital adrenal hyperplasia: diagnostic and therapeutic aspects. J Pediatr Endocrinol Metab 11,581–589.

Wedell A and Luthman H (1993) Steroid 21-hydroxylase (P450c21): a new allele and spread of mutations through the pseudogene. Hum Genet 91,236–240.

Weise M, Mehlinger SL, Drinkard B, Rawson E, Charmandari E, Hiroi M, Eisenhofer G, Yanovski JA, Chrousos GP and Merke DP (2004) Patients with classic congenital adrenal hyperplasia have decreased epinephrine reserve and defective glucose elevation in response to high-intensity exercise. J Clin Endocrinol Metab 89,591–597.

White PC and Speiser PW (2000) Congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Endocr Rev 21,245–291.

White PC and Speiser PW (2002) Long-term consequences of childhood-onset congenital adrenal hyperplasia. Best Pract Res Clin Endocrinol Metab 16,273–288.

White PC, Tusie Luna MT, New MI and Speiser PW (1994) Mutations in steroid 21-hydroxylase(CYP21). Hum Mut 3,373–378.

Winters JL, Chapman PH, Powell DE, Banks ER, Allen WR and Wood DP, Jr (1996) Female pseudohermaphroditism due to congenital adrenal hyperplasia complicated by adenocarcinoma of the prostate and clear cell carcinoma of the endometrium. Am J Clin Pathol 106,660–664.

Wudy SA, Hartmann M and Svoboda M (2000) Determination of 17-hydroxyprogesterone in plasma by stable isotope dilution/benchtop liquid chromatography–tandem mass spectrometry. Horm Res 53, 68–71.

Yu AC and Grant DB (1995) Adult height in women with early-treated congenital adrenal hyperplasia (21-hydroxylase type): relation to body mass index in earlier childhood. Acta Paediatr 84,899–903.

Zhou Z, Agarwal VR, Dixit N, White P and Speiser PW (1997) Steroid 21-hydroxylase expression and activity in human lymphocytes. Mol Cell Endocrinol 127,11–18.