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Congenital adrenal hyperplasia: focus on the molecular basis of 21-hydroxylase deficiency

João Gonçalves[1,*], Ana Friães[2] and Luís Moura[3]

Congenital adrenal hyperplasia (CAH) is an autosomal recessive disorder caused by defects in one of several steroidogenic enzymes involved in the synthesis of cortisol from cholesterol in the adrenal glands. More than 90% of cases are caused by 21-hydroxylase deficiency, and the severity of the resulting clinical symptoms varies according to the level of 21-hydroxylase activity. 21-Hydroxylase deficiency is usually caused by mutations in the *CYP21A2* gene, which is located on the RCCX module, a chromosomal region highly prone to genetic recombination events that can result in a wide variety of complex rearrangements, such as gene duplications, gross deletions and gene conversions of variable extensions. Molecular genotyping of *CYP21A2* and the RCCX module has proved useful for a more accurate diagnosis of the disease, and prenatal diagnosis. This article summarises the clinical features of 21-hydroxylase deficiency, explains current understanding of the disease at the molecular level, and highlights recent developments, particularly in diagnosis.

Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive disorders of adrenal steroidogenesis characterised by a complete or partial deficiency of one of the adrenal enzymes necessary for the synthesis of cortisol from cholesterol. The resulting decrease in cortisol or in cortisol and aldosterone production is associated with changes in the levels of sex steroid hormones, which interfere with primary and secondary sexual development from fetal to adult life. Cortisol synthesis is positively regulated by adrenocorticotropic hormone (ACTH) produced by the anterior pituitary, which in turn is controlled by corticotrophin-releasing hormone (CRH)

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secreted by the hypothalamus. Low levels of cortisol synthesis lead to an overproduction of ACTH; this continuously stimulates the adrenal gland (in order to produce more cortisol), giving rise to hyperplasia of the adrenal tissue (Refs 1, 2).

The three main categories of steroid hormones (mineralocorticoids, glucocorticoids and adrenal androgens) are synthesised in the adrenal cortex from cholesterol. The enzymes (and corresponding genes) necessary for the successive steps of steroid hormone biosynthesis are depicted in Figure 1. The different clinical phenotypes of CAH are best understood by a comprehensive analysis of the enzymatic deficiency and the effects resulting from: (1) low levels of hormones with decreased production; (2) accumulation of intermediary hormones upstream of the enzymatic defect; and (3) the physiological actions of the three major steroid hormones.

Aldosterone, the major mineralocorticoid synthesised by the zona glomerulosa of the adrenal cortex, regulates the excretion of electrolytes in the kidney and maintains intravascular volume and blood pressure: it enhances the reabsorption of sodium, accompanied by water and chloride retention, and excretion of potassium ions into the urine (Ref. 3). Aldosterone biosynthesis is regulated

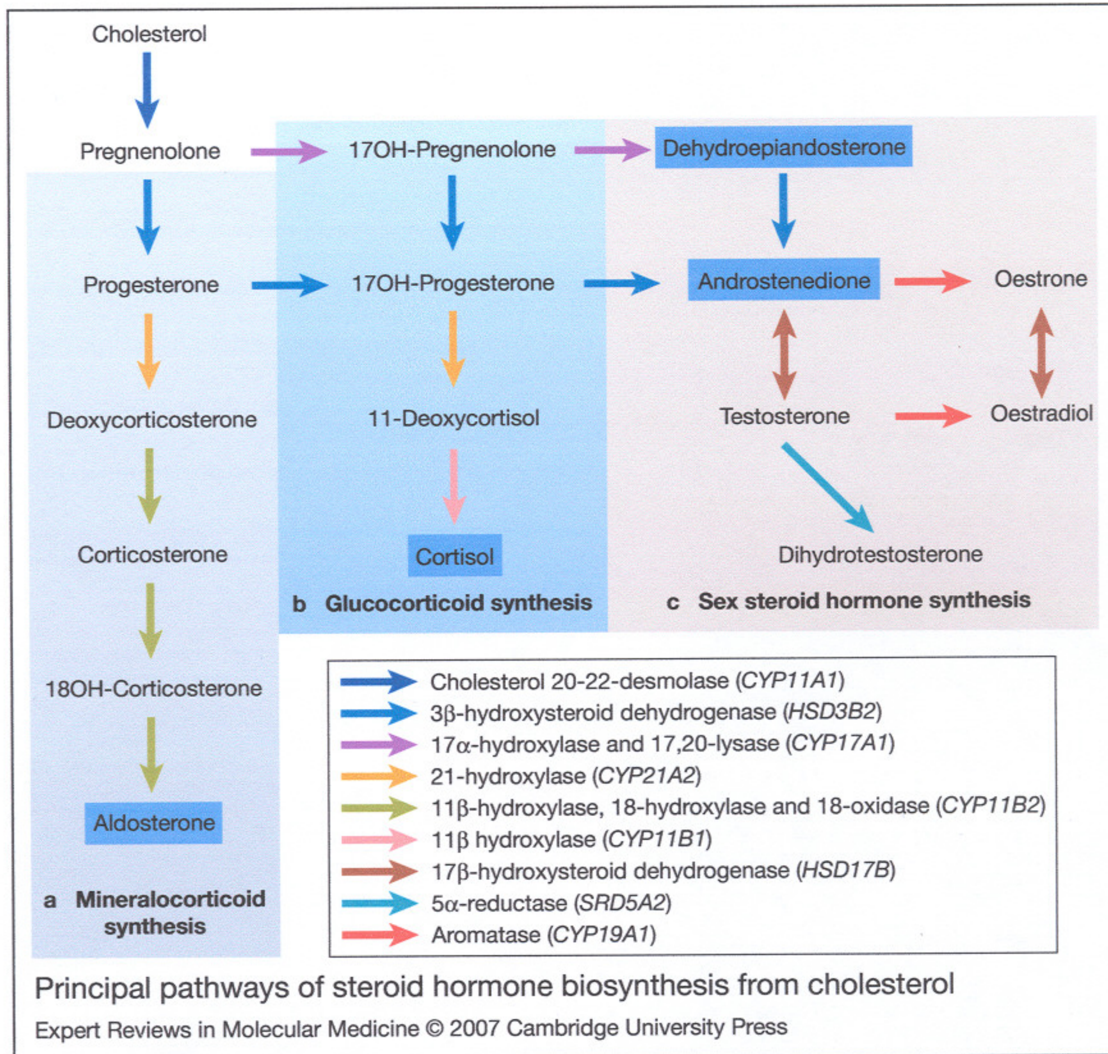

Figure 1. Principal pathways of steroid hormone biosynthesis from cholesterol. *(See next page for legend.)*

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mainly by potassium levels and by the renin–angiotensin system. The renin hormone – produced by the kidney in response to hypotension, lowered intravascular volume, or hyperkalaemia – cleaves angiotensinogen (produced by the liver) to angiotensin I. This peptide, under the action of angiotensin-converting enzyme (mainly present in the pulmonary endothelium), is converted to angiotensin II. Angiotensin II stimulates the zona glomerolosa to increase the biosynthesis of aldosterone (Ref. 4).

Cortisol, the principal glucocorticoid, is produced by the adrenal zona fasciculata. Cortisol has multiple and important functions in most of the metabolic pathways: it plays a key role in blood sugar maintenance, promoting gluconeogenesis during periods of physical or psychological stress, regulates blood pressure and homeostasis, and supports cardiovascular and immunological functions. In addition, it was recently demonstrated for the first time that during early fetal life, between 8 and 10 weeks post-conception, cortisol synthesised by human adrenal cortex is able to down-regulate androgen biosynthesis by suppression of the fetal hypothalamic–pituitary–adrenal axis. This negative feedback mechanism, occurring during a critical period of primary sexual differentiation, provides transient physiological conditions that promote the normal differentiation of female external genitalia (Ref. 5).

Dehydroepiandrosterone and androstenedione are the adrenal androgens produced mainly by the adrenal zona reticularis. In normal physiological conditions, these two sex steroids (which are weak androgens) have no significant function in primary sexual differentiation during early male and female fetal life. However, during this period of life, some of the enzymatic defects that impair cortisol biosynthesis lead to accumulation of sex hormone precursors, which are converted to high doses of dehydroepiandrosterone and androstenedione and subsequently metabolised to testosterone and dihydrotestosterone, mainly in peripheral tissues. These two more potent androgens induce virilisation of the external genitalia in females, causing, at birth, various degrees of sexual ambiguity, as discussed below (Refs 6, 7, 8).

Figure 1. Principal pathways of steroid hormone biosynthesis from cholesterol. (*Legend; see previous page for figure.*) Major products of adrenal steroidogenesis are indicated with a blue box. Enzymatic activities catalysing each bioconversion (coloured arrows) are shown in the key below, where genes encoding each enzyme are given in italics (*CYP* gene symbols show that the corresponding enzyme is a member of the cytochrome P450 superfamily). The steroidogenic acute regulatory protein (STAR) promotes cholesterol transport to the inner mitochondrial membrane (not shown) (Ref. 140). The first step of steroidogenesis is the conversion of cholesterol to pregnenolone; this requires three reactions (20α-hydroxylation, 22-hydroxylation, and cleavage of cholesterol side chain), which are all catalysed by cholesterol 20-22-desmolase (Ref. 88). (a) Mineralocorticoid synthesis. The biosynthesis of mineralocorticoids (in the zona glomerulosa), requires the action of 3β-hydroxysteroid dehydrogenase, which converts pregnenolone to progesterone. This is subsequently converted to deoxycorticosterone by 21-hydroxylase. The last enzymatic steps that culminate in aldosterone biosynthesis are performed by a single enzyme – aldosterone synthase (encoded by *CYP11B2* gene) – which has three enzymatic activities: 11β-hydroxylation of deoxycorticosterone, 18-hydroxylation and 18-oxidation of corticosterone. (b) Glucocorticoid synthesis. To produce the glucocorticoid cortisol (in the zona fasciculata), the *CYP17A1*-encoded enzyme catalyses the bioconversion of pregnenolone to 17-hydroxypregnenolone. 3β-hydroxysteroid dehydrogenase uses 17-hydroxypregnenolone as a substrate, converting it to 17-hydroxyprogesterone, which is also produced by the 17α-hydroxylation of progesterone. 21-hydroxylase mediates the bioconversion of 17-hydroxyprogesterone to 11-deoxycortisol, which is converted into cortisol by the action of 11β-hydroxylase. (c) Sex steroid hormone synthesis. The production of adrenal androgens results from the conversion of 17-hydroxypregnenolone to dehydroepiandrosterone by the CYP17A1 enzyme as a result of its 17,20-lyase activity, and dehydroepiandrosterone is subsequently converted by 3β-hydroxysteroid dehydrogenase to androstenedione. The latter is also converted by the CYP17A1 enzyme from 17-hydroxyprogesterone (Ref. 7). There are several isozymes with 17β-hydroxysteroid dehydrogenase activity. While type 5 isozyme HSD17B5 (encoded by *AKR1C3*) converts androstenedione into testosterone in the adrenal gland during early fetal life (Ref. 5), other isozymes, like HSD17B3 and HSD17B1, are responsible for testosterone biosynthesis in the testes and ovaries. During puberty, aromatase converts androstenedione and testosterone to oestrone and oestradiol, respectively. Testosterone is metabolised to dihydrotestosterone by 5α-reductase, predominantly in genital skin and in other androgen target tissues.

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Table 1. Summary of the various enzymatic defects that cause congenital adrenal hyperplasia

Defective enzyme/protein	Gene (location)	Biochemical consequences	Major clinical features	Major hormonal alterations	Prevalence	Refs
STAR	*STAR* (8p11.2)	Cholesterol transport to the inner mitochondrial membrane is blocked: severe impairment of all adrenal and gonadal steroid synthesis; cellular accumulation of cholesterol and esters, causing congenital lipoid adrenal hyperplasia (CLAH)	Hyponatraemia, hyperkalaemia, hypovolaemia, failure to thrive, vomiting, hypoglycaemia, lethargy. 46,XX: spontaneous puberty may occur, with menstrual cycles often anovulatory. 46,XY: phenotypically female; never enter puberty. Individuals with mild CLAH and normal male genitalia have been described	↓ 17-Hydroxyprogesterone ↓ Aldosterone ↓ Progesterone ↓ Androstenedione ↓ DHEA ↓ Cortisol ↓ Testosterone ↑ Renin activity ↑ ACTH Null response to ACTH or hCG	Rare in Europe, but accounts for 5% of CAH cases in Japan and Korea	78, 79, 80, 81, 82, 83, 84, 85, 86, 87
Cholesterol 20-22-desmolase (CYP11A1, P450Scc)	*CYP11A1* (15q23- q24)	Cholesterol side chain cleavage (first and rate-limiting step of steroidogenesis) is blocked: impairment of all steroidogenesis	Usually incompatible with term gestations because of low levels of progesterone production by placenta; adrenal insufficiency; rare cases with clinical manifestations of CLAH or with complete sex reversal	↓ 17-Hydroxyprogesterone ↓ Aldosterone ↓ Progesterone ↓ Androstenedione ↓ DHEA ↓ Cortisol ↓ Testosterone ↑ Renin activity ↑ ACTH	Rare	88, 89, 90, 91, 92, 93
3β-hydroxysteroid dehydrogenase type 2 (HSD3B2)	*HSD3B2* (1p13.1)	3β-Dehydrogenation of pregnenolone, 17-hydroxypregnenolone and DHEA is blocked: abnormal adrenal and gonadal steroidogenesis	**Salt-wasting.** Crisis during the first months of life. 46,XY: ambiguous external genitalia or hypospadias. 46,XX: possible clitoromegaly with or without hyperpigmentation **Non-salt-wasting.** 46,XY: ambiguous external genitalia. 46,XX: possible ambiguous genitalia, premature pubarche, hirsutism, primary or secondary amenorrhoea **Nonclassical (rare).** Premature pubarche; hyperandrogenism in women after puberty	↑ 17-Hydroxypregnenolone ↑ Pregnenolone ↓ Aldosterone ↑ DHEA ↓ Cortisol ↓ Testosterone – 46,XY ↑ Testosterone – 46,XX ↓ Renin activity ↑ ACTH	<10% of CAH cases	94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105

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Table 1. Summary of the various enzymatic defects that cause congenital adrenal hyperplasia *(continued)*

Defective enzyme/protein	Gene (location)	Biochemical consequences	Major clinical features	Major hormonal alterations	Prevalence	Refs
17α-hydroxylase/ 17,20-lyase (CYP17A1)	*CYP17A1* (10q24-q25)	17α-Hydroxylase + 17,20-lyase deficiency: impairment of biosynthesis of glucocorticoids, androgens and oestrogens; accumulation of deoxycorticosterone and corticosterone	17α-Hydroxylase + 17,20-lyase deficiency: hypokalaemia; hypertension. 46,XY: born with ambiguous genitalia or complete female phenotype; hypertension. 46,XX: delayed puberty, primary amenorrhoea; persistently elevated gonadotropins; hypertension	17α-Hydroxylase + 17,20-lyase deficiency: ↓ 17-Hydroxyprogesterone ↓ Aldosterone ↑ Deoxycorticosterone ↑ Corticosterone ↓ DHEA ↓ Cortisol ↑ ACTH ↓ Gonadal and adrenal sex steroids	≤1% of CAH cases; 17,20-lyase deficiency alone has an extremely low incidence	106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122
		17,20-Lyase deficiency: impairment of biosynthesis of DHEA, androstenedione, testosterone and oestradiol	17,20-Lyase deficiency: absence of pubertal development. 46,XY: pseudohermaphroditism. 46,XX: absence of pubertal development, primary or secondary amenorrhoea	17,20-lyase deficiency: ↓ Androstenedione ↑ LH ↑ FSH		
11β-Hydroxylase (CYP11B1)	*CYP11B1* (8q21)	11β-Hydroxylation of 11-deoxycortisol is blocked: impairment of biosynthesis of cortisol and corticosterone; accumulation of 11-deoxycortisol, deoxycorticosterone and adrenal androgens	Hypertension with or without hypokalaemia; rapid somatic growth; bone age acceleration; short adult stature. 46,XY: early puberty; spermatogenesis may be impaired. 46,XX: born with variable degrees of external genitalia masculinisation; premature adenarche; amenorrhoea; hirsutism Mild form: androgen excess signs in female children; hirsutism and oligomenorrhoea in adult females; absence of hypertension	↑ 17-Hydroxyprogesterone or normal ↑ Androstenedione ↑ DHEA ↓ Cortisol ↑ 11-Deoxycortisol ↑ Deoxycorticosterone ↑ ACTH Mild forms: 11-Deoxycortisol and deoxycorticosterone may be normal (ACTH stimulation is required)	5–8% of CAH cases; 1/50 000–1/10 0000 births in general population	123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134

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Table 1. Summary of the various enzymatic defects that cause congenital adrenal hyperplasia *(continued)*

Defective enzyme/protein	Gene (location)	Biochemical consequences	Major clinical features	Major hormonal alterations	Prevalence	Refs
P450 (cytochrome) oxidoreductase	*POR* (7q11.2)	Impaired electron transfer from NADPH to steroidogenic type II P450 enzymes (CYP17A1, CYP21A2 and CYP19A1), leading to partial combined deficiencies of 17α-hydroxylase/17,20-lyase and 21-hydroxylase activities: defective adrenal and gonadal steroidogenesis	Most patients present genital malformations, disordered steroidogenesis and congenital malformations characterised by craniofacial dysmorphism and skeletal features usually diagnosed as Antley–Bixler syndrome (ABS). Patients without ABS and with ambiguous genitalia or disordered steroidogenesis have also been described. 46,XY: undermasculinisation at birth and defective pubertal development. 46,XX: variable virilisation of external genitalia and poor pubertal development	↑ 17-Hydroxyprogesterone ↑ Progesterone ↑ Pregnenolone ↑17-OH Pregnenolone ↑17-OH Progesterone ↑ Deoxycorticosterone or normal ↑ Corticosterone ↓ DHEA or normal ↓ Androstenedione or normal ↑ ACTH or normal ↓ Cortisol after ACTH stimulation ↓ Oestradiol ↓ Testosterone or normal in 46,XY	Rare	9, 10, 11, 12, 135, 136, 137, 138
21-hydroxylase (CYP21A2)	*CYP21A2* (6p21.3)	21-Hydroxylation of progesterone and 17-hydroxyprogesterone is blocked: impairment of biosynthesis of cortisol and, in most severe cases, aldosterone; accumulation of progesterone and 17-hydroxyprogesterone; increased secretion of adrenal androgens	**Salt-wasting form**: hyponatraemia, hyperkalaemia, hyperreninaemia, lethargy, poor feeding, vomiting, failure to thrive. 46,XX: born with variable degrees of external genitalia virilisation **Simple virilising form**: premature pubarche, rapid linear growth and advanced bone age with short adult stature, impaired fertility. 46,XY: progressive penile enlargement, small testes. 46,XX: born with variable degrees of external genitalia virilisation; hirsutism, male-pattern baldness, cystic ovaries, amenorrhoea **Nonclassical form**: precocious puberty, accelerated growth, short adult stature, cystic acne, impaired fertility. 46,XX: hirsutism, temporal baldness, delayed menarche, polycystic ovaries, menstrual irregularities.	**Salt-wasting form**: ↑ 17-hydroxyprogesterone (before and after stimulation with ACTH) ↑ Progesterone ↑ Androstenedione ↑ DHEA ↑ Testosterone **Simple virilising form**: ↑ 17-hydroxyprogesterone (before and after stimulation with ACTH) ↑ Progesterone ↑ Androstenedione ↑ Testosterone **Nonclassical form:** ↑ 17-hydroxyprogesterone (before and after stimulation with ACTH)	90–95% of CAH cases. **Classical:** 1/15 000 births in general population **Nonclassical:** 1/100 births in general population	13, 14, 17, 18, 19, 20, 21, 139

Abbreviations: ACTH, adrenocorticotropic hormone; CAH, congenital adrenal hyperplasia; DHEA, dehydroepiandrosterone; FSH, follicle-stimulating hormone; hCG, human chorionic gonadotropin; LH, luteinising hormone.

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The enzymatic deficiencies that most frequently cause CAH are defects in 21-hydroxylase, 11β-hydroxylase, 3β-hydroxysteroid dehydrogenase, and less frequently 17α-hydroxylase/17,20-lyase, cholesterol 20-22-desmolase and the transporter protein StAR (steroidogenic acute regulatory protein). In addition, a new form of CAH was recently described, characterised by deficiencies in both 21-hydroxylase and 17α-hydroxylase/17,20-lyase activities as a result of mutations in the electron donor enzyme P450 oxidoreductase (POR) (Refs 9, 10). Most patients with POR deficiency have skeletal malformations, genital anomalies in both sexes and abnormal steroid profiles (Refs 10, 11, 12). The clinical and molecular features of all these enzymatic defects are summarised in Table 1. For deficiency in 21-hydroxylase, the main subject of this paper, a more detailed review is presented in the next sections.

Clinical features of 21-hydroxylase deficiency

21-hydroxylase deficiency (21-OHD) is the most common cause of CAH, accounting for 90–95% of all cases. Patients with 21-OHD have a deficiency in cortisol biosynthesis, which in most of the severe cases is also accompanied by aldosterone deficiency. The decreased adrenal secretion of cortisol gives rise, because of the absence of negative feedback to the hypothalamus and pituitary, to an increased secretion of CRH and ACTH. The steroid precursors prior to this enzymatic deficiency (progesterone and 17-hydroxyprogesterone) are accumulated and shunted through the adrenal androgen biosynthetic pathway. The increased secretion of adrenal androgens from the eighth week of gestation, and the resulting production of high levels of testosterone and dihydrotestosterone, particularly affects sexual differentiation in females, and causes advanced somatic development in both sexes during childhood (Ref. 13). Clinically, although there is a continuous spectrum of phenotypic manifestations associated with this disease, it has been useful to divide 21-OHD into three forms: salt-wasting classical 21-OHD, simple virilising classical 21-OHD, and nonclassical 21-OHD.

Classical 21-OHD

Classical 21-OHD occurs in two major forms: salt-wasting and simple virilising. Patients with renal salt-wasting account for three-quarters of classical 21-OHD cases. In addition to having a severe cortisol deficiency, they do not synthesise enough aldosterone and therefore are not able to maintain sodium homeostasis. Severely affected patients usually present between the first and fourth weeks of age with hyponatraemia, hyperkalaemia, hyperreninaemia, hypovolaemic shock and hypoglycaemia. Other symptoms are also common, such as lethargy, poor feeding, vomiting and failure to thrive. In the female fetus, androgen excess causes variable degrees of external genitalia virilisation, and consequently newborn females have genital ambiguity or male-resembling external genitalia with bilateral cryptorchidism, which may result in errors of sex assignment at birth. In the male fetus, the testicular androgens normally produced at high levels induce the normal masculinisation of the external genitalia, and the excess of adrenal androgens usually has no effect until childhood, although sometimes a macrogenitossomia at birth is observed. By contrast to the situation in affected newborn females, where the presence of genital ambiguity contributes to the diagnosis of severe 21-OHD, the normal external genitalia in affected newborn males do not alert paediatricians to the diagnosis of a severe form of CAH before the onset of a salt-wasting crisis. This critical health condition can lead to death if appropriate medical assistance is not available (Refs 13, 14).

The simple virilising form of 21-OHD occurs in around a quarter of the cases of classical 21-OHD. In this form of CAH, as 21-hydroxylase activity is residual but not complete, and because the adrenal gland is stimulated by the renin–angiotensin system, adequate levels of aldosterone are still produced, allowing normal sodium homeostasis. Adrenal glands under continuous stimulation by increased levels of ACTH may synthesise approximately normal levels of cortisol, although cortisol precursors are still accumulated and consequently directed into the biosynthetic pathway of adrenocortical androgens. As mentioned above, the peripheral conversion of high levels of dehydroepiandrosterone and androstenedione into testosterone and dihydrotestosterone results in external genitalia masculinisation in the 46,XX fetus, and females are consequently born with variable degrees of external genitalia virilisation,

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ranging from mild hypertrophy of the clitoris and partial fusion of the labioscrotal folds to a phallic urethra and a complete fusion of the labioscrotal folds. It is important to emphasise that for children with external genitalia ambiguity or with normal male genitalia without palpable testes, the possibility of 21-OHD should be considered (among the different causes of genital ambiguity, 21-OHD is the most frequent). In the 46,XY fetus, the adrenal excess of androgen precursors has no effect on the external genitalia masculinisation. Later in life, untreated children of both sexes with simple virilising 21-OHD develop signs of androgen excess that usually include premature appearance of pubic hair, followed by development of axillary hair, acne, facial hair, rapid linear growth and advanced bone age, although, due to premature epiphyseal fusion, patients usually have a short adult stature. After childhood, untreated females do not undergo normal puberty and in most cases present clitoral enlargement, hirsutism, male-pattern baldness, cystic ovaries, and amenorrhoea or irregular menses, and consequently primary infertility or reduced fertility. Prepubertal males usually have progressive penile enlargement, small testes and later in life invariably show seriously impaired fertility (Ref. 13).

The phenotypic variability in degree of external genitalia ambiguity in females with classical 21-OHD might reflect differences in the efficiency of conversion of the adrenal androgens into the two more potent androgens, as well as the androgen receptor gene expression level, the affinity of the androgen receptor for its ligands (testosterone and dihydrotestosterone), and the efficiency of androgen receptor transactivation on target androgen-regulated genes (Ref. 15).

Nonclassical 21-OHD

The nonclassical form of 21-OHD is associated with less severe symptoms of hyperandrogenisation. This is due to a mild deficiency of 21-hydroxylase and to slightly elevated levels of 21-hydroxylase adrenal steroid precursors. Aldosterone biosynthesis is not disturbed in patients with nonclassical 21-OHD. The clinical manifestations of mild androgen excess are variable. Females are usually born with normal external genitalia (sometimes a mild clitoromegaly is observed), and both males and females may show signs of androgen excess at any time after postnatal development. Children may present with early pubic hair, precocious puberty, and accelerated growth associated with advanced bone age. Females may show one or more of the following symptoms: hirsutism, temporal baldness, delayed menarche, polycystic ovaries and menstrual irregularities. Adult males and females may have cystic acne, impaired fertility, and short stature due to premature epiphyseal fusion. In addition, asymptomatic patients may be identified and diagnosed during the familial screening of one affected individual (Ref. 16).

Prevalence of CAH arising from 21-OHD

Neonatal screening for classical CAH arising from 21-OHD, established mainly in Caucasian populations, found an overall prevalence of approximately 1/15 000 live births, ranging from 1/10 000 to 1/16 000 in the USA, and from 1/10 000 to 1/25 000 in European countries (Refs 17, 18, 19). The highest prevalence occurs in two geographically isolated populations: 1/280 among Yupik Eskimos (Alaska) and 1/2100 in the island of La Reunion (Indian Ocean) (Ref. 17).

The milder, nonclassical form of 21-OHD is much more common, with a prevalence of 1/100 in the general population, although a higher ethnic-specific incidence is found among Ashkenazi Jews (1/27), Hispanics (1/40) and Yugoslavs (1/60) (Ref. 20).

Hormonal diagnosis of 21-OHD

The biochemical diagnosis of 21-OHD cannot be made through direct measurements of the enzymatic activity because 21-hydroxylase is essentially expressed only in the adrenal cortex. Instead, diagnosis of 21-OHD is based on elevated baseline and ACTH-stimulated levels of serum 17-hydroxyprogesterone, the main substrate of 21-hydroxylase, measured by immunoassay. In addition to elevated basal levels of 17-hydroxyprogesterone (usually higher than 10 000 ng/dl), patients with classical 21-OHD also secrete progesterone, dehydroepiandrosterone, androstenedione and testosterone in excess. Patients with the salt-wasting form show the highest 17-hydroxyprogesterone levels

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of >20 000 ng/dl, while patients with the simple virilising form usually have 17-hydroxyprogesterone concentrations of 10 000–20 000 ng/dl (Ref. 13); however, some patients with these two clinical forms present 17-hydroxyprogesterone levels that overlap, and a cut-off cannot be established. The nonclassical 21-OHD patients have lower 17-hydroxyprogesterone after ACTH stimulation (1000–10 000 ng/dl). Basal serum 17-hydroxyprogesterone levels in most of the latter patients are not distinguishable from heterozygotes for classical or nonclassical 21-OHD. The large data obtained from neonatal screening of CAH demonstrated that preterm and stressed newborns have higher 17-hydroxyprogesterone levels in serum than babies born at term, generating false-positive results. In order to avoid these false-positive results, cut-off values were established based on gestational age (in Japan and Europe) or on birth weight (in the USA). The neonatal screening is also not efficient in the identification of newborns with the nonclassical form of the disease. It is important to recognise that other steroidogenic deficiencies, such as 11β-hydroxylase deficiency and 3β-hydroxysteroid dehydrogenase deficiency type 2, may be misdiagnosed as 21-OHD. In these cases, it is important to perform a complete adrenocortical hormone profile and evaluate ratios of precursors versus products after ACTH stimulation (Refs 13, 19).

Molecular features of 21-OHD

The *CYP21A2* locus

Human adrenal 21-hydroxylase is encoded by the *CYP21A2* gene, also previously called *CYP21* and *CYP21B*, which is located on 6p21.3 within class III *HLA*, ~30 kb from a pseudogene (*CYP21A1P*; known also as *CYP21P* and *CYP21A*). These two genes share about 98% homology in their ten exons and about 96% in the introns, but *CYP21A1P* is inactive because of the presence of several deleterious mutations (Refs 21, 22). The gene and the pseudogene are included in what is called the RCCX region (Fig. 2), which, in the most common alleles, has a bimodular form (RCCX–RCCX), composed of two sets of four genes arranged in tandem: *RP1–C4A–CYP21A1P–TNXA–RP2–C4B–CYP21A2–TNXB* (Ref. 23). The *C4A* and *C4B* genes code for the fourth component of serum complement, *RP1* encodes a putative nuclear protein similar to DNA helicase, and *RP2* is a truncated, nonfunctional copy of *RP1*. *TNXB* encodes a putative extracellular matrix protein (tenascin X) and overlaps the *CYP21A2* gene on the opposite DNA strand. Likewise, *TNXA* is a truncated copy of *TNXB* and overlaps *CYP21A1P*, also on the opposite DNA strand. Monomodular and trimodular alleles resulting from genetic recombination events have also been described (Ref. 24).

Mutations causing 21-OHD

In October 2006, the Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/ac/index.php) had a total of 103 entries for *CYP21A2* mutations: 87 point mutations or small deletions or insertions, seven splicing mutations, and nine gross deletions, duplications or gene conversions.

As a result of its structure of repeated genes arranged in tandem, the RCCX region is prone to a high frequency of recombination events, which can lead to (1) unequal crossing-overs during meiosis, resulting in a wide variety of arrangements depending on the breakpoints, such as gene duplications and gross gene deletions encompassing the *C4* and *CYP21A2* genes (Refs 25, 26), or to (2) large or short gene conversions in which the *CYP21A1P* mutations, which drastically impair its transcription and prevent the production of a functional enzyme, are transferred to *CYP21A2* (Fig. 3). Gross deletions encompassing variable portions of *C4A*, *CYP21A1P*, *TNXA*, *RP2*, *C4B*, *CYP21A2* and *TNXB* produce different types of chimaeras in the RCCX modules. The resulting alleles may have different extents of the *CYP21A1P* sequence in the 5′ portion of the 21-hydroxylase gene attached to the 3′ portion of *CYP21A2* (*CYP21A1P–CYP21A2* chimaeras), or a 5′ portion of the *TNXA* gene attached to the 3′ portion of *TNXB* (*TNXA–TNXB* chimaeras) (Ref. 27). In both cases, the resulting allele does not have a functional *CYP21A2* gene. The *CYP21A1P–CYP21A2* chimaeras do not lead to the production of active 21-hydroxylase not only because of the deleterious mutations of the *CYP21A1P* in the 5′ portion, but also because the promoter sequence of the pseudogene has only 20% of the transcriptional activity of the *CYP21A2* promoter (Ref. 28). The *TNXA–TNXB*

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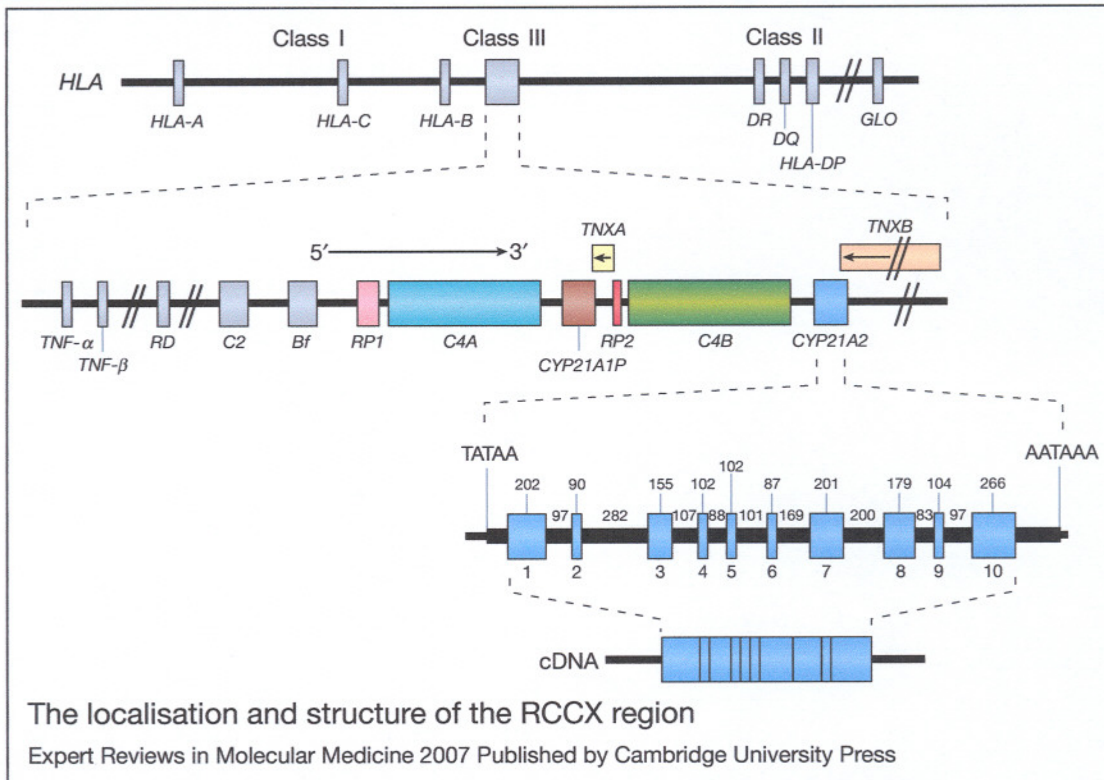

Figure 2. The localisation and structure of the RCCX region. *RP1*, *C4A*, *C4B*, *CYP21A2 and TNXB* are functional genes; *CYP21A1P*, *TNXA* and *RP2* are nonfunctional pseudogenes. *TNXA* and *TNXB* are encoded on the opposite DNA strand. The sizes (bp) of the *CYP21A2* exons (blue boxes) are indicated above them; the sizes of the introns are indicated between the exons; the numbering of the exons is indicated beneath the boxes. The black solid line represents the untranslated region of the gene and the slashes indicate the gene or DNA sequence is longer than shown. Abbreviations: *C2*, complement component 2; *C4A* and *C4AB*, complement component 4A and 4B; *CYP21A1P*, cytochrome P450, family 21, subfamily A, polypeptide 1 pseudogene; *CYP21A2*, cytochrome P450, family 21, subfamily A, polypeptide 2; *HLA*, human leukocyte antigen; *RD*, also known as *RDBP* (RNA-binding protein); *RP1* and *RP2*, also known as *STK19* and *STK19P*, respectively ('serine/threonine kinase 19 gene and pseudogene'); *TNF*, tumour necrosis factor; *TNXA*, tenascin XA pseudogene; *TNXB*, tenascin XB. The top half of the figure, showing the position of the RCCX region relative to neighbouring genes on chromosome 6, is adapted from Ref. 7: Donohoue, P.A., Parker, K. and Migeon, C.J. (1995) Congenital adrenal hyperplasia. In The Metabolic and Molecular Bases of Inherited Disease (7th edn) (Scriver, C.R. et al., eds), pp. 2929-2966, McGraw Hill, New York.

chimaeras are formed as a result of a gross deletion that includes the *CYP21A2* gene (Ref. 27).

Some authors have reported alleles with duplications of the *CYP21A2* gene (Refs 29, 30, 31, 32). This may complicate mutation analysis: one of the duplicated copies might have mutations that are detected by the traditional methods but the other one might be a functional copy, rendering this allele perfectly active. Different extents of gene conversions comprising several *CYP21A2* exons have also been reported – namely gene conversions from exon 1 to 7, from exon 1 to 3, from exon 3 to 7, from exon 3 to 8, from exon 4 to 7, and from exon 5 to 8 (Refs 29, 33, 34, 35).

Apart from gene deletions and large gene conversions, there are eight mutations reported with a higher frequency in the *CYP21A2* gene (Fig. 4): g.89C > T (p.P30L), g.655A/C > G (I2 splicing), g.707_714delGAGACTAC (p.G110_Y112delfs), g.999T > A (p.I172 N),

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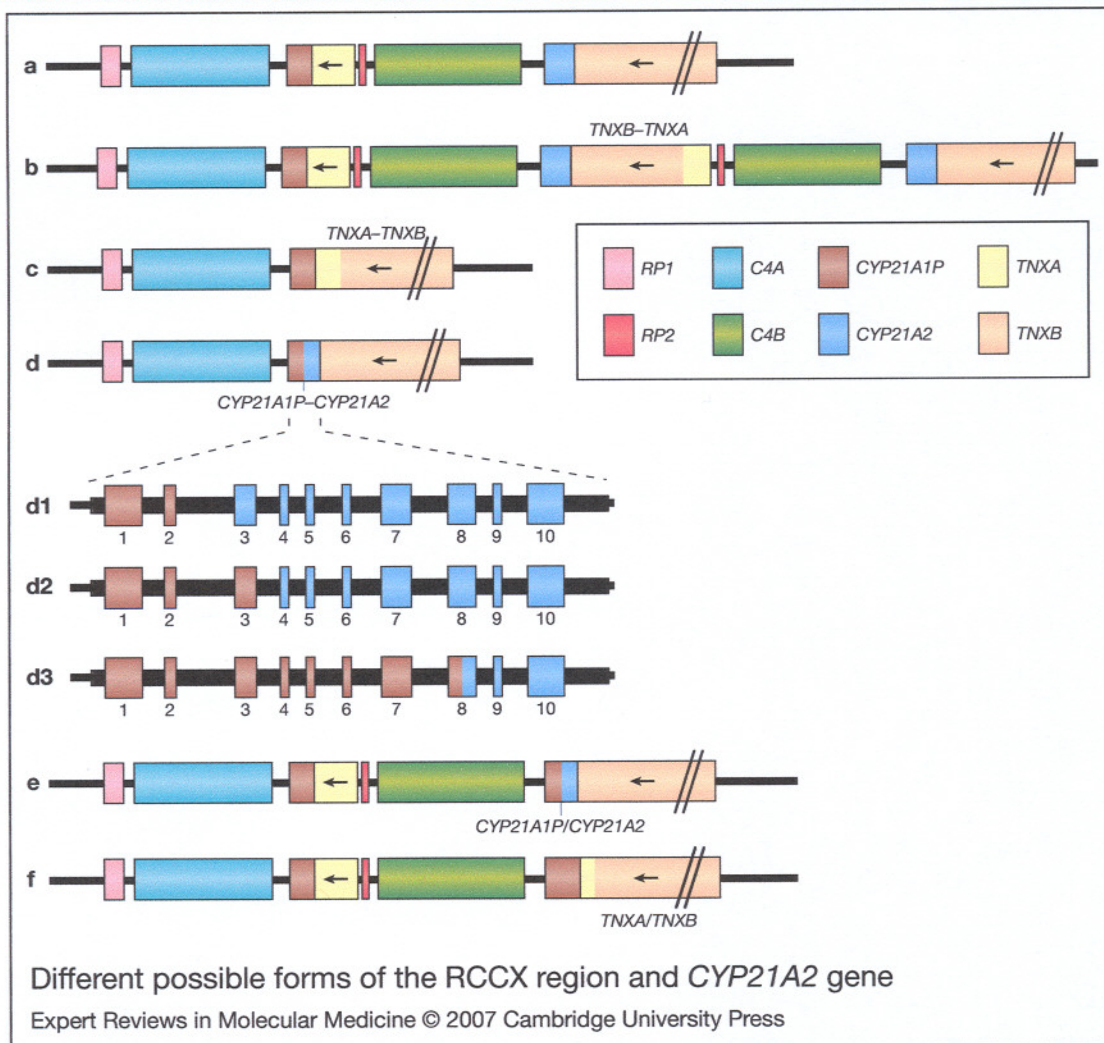

Figure 3. Different possible forms of the RCCX region and *CYP21A2* gene. (a) Standard bimodular form of the RCCX region; (b) trimodular form of the RCCX region, with a duplication of *RP2*, *C4B*, *CYP21A2* and *TNXB*, resulting in a chimaeric *TNXB–TNXA* gene; (c) monomodular form of the RCCX region arising from a deletion in the region *TNXA–RP2–C4B–CYP21A2–TNXB*, resulting in a chimaeric *TNXA–TNXB* gene; (d) monomodular form of the RCCX region arising from a deletion in the region *CYP21A1P–TNXA–RP2–C4B–CYP21A2*, resulting in a chimaeric *CYP21A1P–CYP21A2* gene; (d1–3) three of the distinct *CYP21A1P–CYP21A2* chimaeras described by Lee (Ref. 27); (e) partial gene conversion of *CYP21A2* to *CYP21A1P*; (f) complete *CYP21A2* gene conversion to *CYP21A1P* and partial conversion of *TNXB* to *TNXA*. Abbreviations: see Figure 2 legend.

g.1683G > T (p.V281L), g.1994C > T (p.Q318X), g.2108C > T (p.R356W) and g.2578C > T (p.P453S) (Ref. 13). Except for the last one, all the other seven mutations are present in *CYP21A1P* and are presumed to have been transferred to *CYP21A2* by short gene conversions. The mutation g.2578C > T (p.P453S) has been suggested to be occasionally present in the pseudogene as a polymorphism, and transferred to *CYP21A2* by gene conversion events just like the other most frequent point mutations (Ref. 36). Three of these mutations are associated with the nonclassical form of 21-OHD, one is typically related to simple

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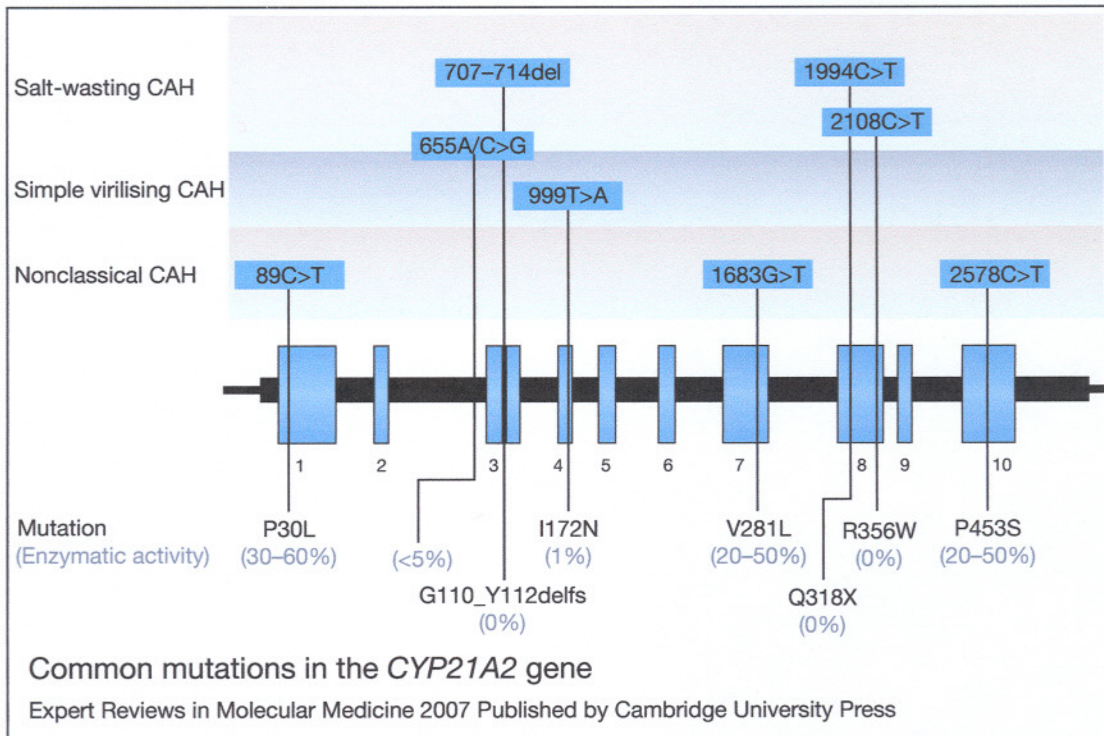

Figure 4. Common mutations in the *CYP21A2* gene. The schematic indicates the localisation of the eight most frequent *CYP21A2* mutations in most populations, and the corresponding forms of congenital adrenal hyperplasia (CAH). The changes in amino acid sequence are given below the exons (except for 655A/C > G, which affects splicing), and enzymatic activity (based on in vitro studies; Ref. 13) resulting from each mutation compared with the normal 21-hydroxylase activity is indicated in parentheses. Figure adapted, with permission from Perrin White (University of Texas Southwestern Medical Center, Dallas, TX, USA) and The Endocrine Society, from Ref. 13: White, P.C. and Speiser, P.W. (2000) Congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Endocr Rev 21(3), 245-291 (Copyright 2000, the Endocrine Society).

virilising classical 21-OHD, and three to salt-wasting classical 21-OHD. The mutation g.655A/C > G has been reported both in salt-wasting and in simple virilising cases. This mutation activates cryptic splicing receptor sequences causing the incorrect processing of almost all the mRNA. A small amount of normally spliced mRNA can be detected in cell cultures; therefore, in the absence of other mutations, small quantities of functional enzyme can be produced, thus preventing the salt-wasting crisis (Refs 37, 38).

Molecular diagnosis of 21-OHD

As discussed above, 21-OHD is diagnosed biochemically by measuring levels of 17-hydroxyprogesterone after stimulation with ACTH, but this method does not differentiate clearly between heterozygous carriers and normal individuals (Ref. 39). Therefore, the molecular diagnosis of 21-hydroxylase by detection of mutations in the *CYP21A2* gene is of major importance to complement the biochemical diagnosis.

The direct screening of *CYP21A2* mutations requires two steps. The first one is the isolation of the *CYP21A2* gene from the pseudogene, since the latter is inactive and has most of the mutations of interest. Currently, this step is usually performed by PCR with primers specific for *CYP21A2*, which do not allow the concomitant amplification of *CYP21A1P* (Refs 40, 41). The product of this first step is used as template DNA for detection of mutations in the second step. The screening of the most common mutations is regularly

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performed by PCR-based and/or sequencing techniques. For example, allele-specific PCR using pairs of primers specific for the detection of each of the eight most frequent mutations has been widely used (Ref. 42), but, more recently, multiplex mini-sequencing has allowed the concomitant screening of all the frequent mutations by single-base extension using a specific primer for each mutation and fluorophore-labelled ddNTPs, followed by separation of the fragments by capillary electrophoresis using an automated sequencing apparatus (Refs 29, 43, 44). When the mutations detected by these methods do not explain the phenotype exhibited by the patient, novel mutations can be searched for by screening the *CYP21A2* exons by SSCP (single-strand conformation polymorphism) analysis, by DHPLC (denaturing high-performance liquid chromatography) analysis, or by direct sequencing of the several exons and noncoding regions of *CYP21A2* (Refs 45, 46, 47).

All the above-mentioned techniques fail, however, to detect gross gene deletions and duplications, as well as large gene conversions, which have a significant frequency in most populations (Ref. 13). These abnormalities are best characterised if family members are included, and by using laborious Southern-blotting methodology with the appropriate probes for *CYP21* and for *C4* genes. Traditionally, cDNA probes are used, but Southern blotting using genomic DNA *CYP21A2* probes has also been described (Ref. 48). The restriction enzymes most commonly used are *Taq*I and *Bgl*II, although different endonucleases have been utilised to characterise complex rearrangements and extra copies of *C4* and *CYP21A2* (Refs 48, 49). In addition, real-time quantitative PCR has been recently used to rapidly detect *CYP21A2* deletions/conversions (Ref. 50). However, this method is critically dependent on an accurate DNA quantification and showed some discrepancies when compared with Southern-blotting results, particularly when discriminating between two and three gene copies. Moreover, it does not allow the distinction between gene deletions and gene conversions or different combinations of alleles with distinct abnormalities. A semiquantitative strategy based on a two-step PCR and *Taq*I digestion has also been proposed (Ref. 51), and showed a very good concordance with Southern blotting, but the interpretation of the results might occasionally be difficult and misleading, since it relies on visual quantification (on agarose gels) of PCR/digestion products, which are dependent on PCR yields. For a rapid and easier detection of chimaeric genes and *CYP21A2* deletions, Lee and co-workers have recently developed a simpler method based on extended PCR followed by *Taq*I restriction (Ref. 23); however, this does not differentiate total gene conversions from *CYP21A2* complete deletions, nor does it detect gene duplications. Thus, although these methods are very useful for diagnostic purposes because they provide a rapid, easy and economic means of detecting deleterious gene abnormalities resulting from recombination events, the results are often ambiguous and in those cases Southern-blotting analysis is still needed for confirmation and further characterisation of the RCCX modules.

Whenever a new genetic alteration is identified in either the coding sequence, intronic sequences or promoter region of the *CYP21A2* gene, it is important to evaluate and predict its consequences for 21-hydroxylase activity at different levels: (1) the nature of the amino acid alteration, associated structural alterations and enzymatic or functional consequences; (2) possible alterations of the normal splicing sites (elimination or creation of new splicing sites); and (3) impairment of gene expression. It is of major importance to perform structural and functional studies in vitro, for a better prediction of the CAH phenotype associated with the mutation (Refs 52, 53, 54).

Frequency of common *CYP21A2* mutations

Rearrangements of the RCCX module resulting in *CYP21A2* deletions or large gene conversions are one of the most common causes of 21-OHD worldwide, accounting for over 20% of defective *CYP21A2* alleles in most populations (Refs 13, 55). There are, however, some countries with frequencies under 10% for these types of mutations – namely Egypt, Mexico and Portugal (Refs 29, 55, 56). The mutation g.655A/C > G is by far the most common severe point mutation in the great majority of the published studies, with frequencies usually above 25%. However, there are some exceptions: in Austria, Finland, China and Iran (Refs 33, 57, 58, 59) g.999T > A

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(p.I172 N) was more common; in Portugal both these mutations were detected with the same frequency (Ref. 29); and in Tunisia, the most frequent mutation was g.1994C > T (p.Q318X) (Ref. 35). Among nonclassical 21-OHD alleles, the most common mutation is usually either g.1683G > T (p.V281L) or g.89C > T (p.P30L) (Refs 29, 55, 59, 60, 61). As Kotaska et al. (Ref. 55) concluded, the overall frequency of mutation g.1683G > T (p.V281L) is higher in South Europe and North Africa, where the nonclassical form of the disease has a higher prevalence. However, the highest frequency of this mutation is registered among the Ashkenazi Jews (Jews of Eastern European origin), with a prevalence of more than 10% in the general population (Ref. 13).

Genotype–phenotype correlations

CYP21A2 mutations can be predicted to cause a certain phenotype – salt-wasting, simple virilising or nonclassical – on the basis of the reduction of the enzymatic activity they produce. However, most CAH patients are actually compound heterozygous for two or more mutations. Since 21-OHD is an autosomal recessive disease, the phenotype of the patient should reflect the mutation that is predicted to cause a less severe impairment of the enzymatic activity. This approach to predicting phenotype has been shown to be correct in 80% to ~100% of cases for null mutations and patients with salt-wasting 21-OHD, and for the p.V281L mutation and patients with nonclassical 21-OHD (Refs 13, 29, 62). Slight deviations to this correlation occur in the forms of the disease with intermediate severity and with mutations such as p.I172N, g.655A/C > G or p.P30L, which in vivo may produce variable 21-hydroxylase activity. Sometimes it is difficult to differentiate between the simple virilising and nonclassical forms in male patients, because their symptoms of androgen excess are usually not detected at birth, so they may be incorrectly assigned as nonclassical 21-OHD patients. Otherwise, some of the causes that contribute to discrepancies between the predicted and the patient's phenotype may be the association of mutations of different severities in the same or in distinct alleles, the existence of mutations that impair *CYP21A2* gene expression, and the presence of alterations in other genes, which may disturb 21-hydroxylase function or expression. It has also been proposed that some alterations usually considered to be polymorphisms, such as p.K102R and p.S268T, might have a synergistic effect when present in the same allele, resulting in a decreased 21-hydroxylase activity (Ref. 59). However, deviations to the predicted genotype–phenotype might also be caused by an incorrect genotyping. Studies based on the screening of known mutations fail to detect novel mutations, which might also affect significantly the enzymatic activity or expression. Moreover, the occurrence of allele drop-out, in which a certain allele is preferentially amplified by PCR, has been reported (Refs 63, 64, 65). Patients might then be incorrectly genotyped as homozygotes for a certain mutation, when they are in fact heterozygotes or hemizygotes.

Genetic counselling and prenatal diagnosis

Some of the above-mentioned genotyping limitations can be minimised by the study of the *CYP21A2* gene of both parents of the patient, thus confirming if the detected mutations are in opposite alleles of the proband. Furthermore, this additional study provides data useful for genetic counselling of the patient and family, as well as for prenatal diagnosis in future pregnancies.

Pregnancies at risk for a child affected with classical CAH are subjected to prenatal therapy with dexamethasone, which must begin in the early first trimester in order to efficiently prevent female genital ambiguity. However, there is consensus that glucocorticoid therapy should be used with caution because of the associated risk of potential adverse effects both to the mother (such as weight gain, hyperglycaemia, cutaneous striae, irritability, gastrointestinal intolerance, and increased blood pressure) and the fetus (congenital malformations, intrauterine growth retardation, mood fluctuations and shyness) – although none of these risks has been proved to be directly associated with the treatment, with some studies reporting the same frequency of fetal malformations in pregnancies subjected to dexamethasone treatment and in the general population (Refs 13, 66). It is therefore desirable to perform prenatal diagnosis as early as possible, since only affected females will suffer from genitalia virilisation. If the fetus is male or does not carry *CYP21A2* mutations in both

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alleles, dexamethasone therapy can be immediately suspended, saving both mother and fetus from unnecessary glucocorticoid exposure (Refs 13, 66).

In pregnancies at risk for a child affected with classical CAH, noninvasive early fetal sex determination from maternal plasma is now possible (Refs 67, 68, 69), although conventional methodologies are still currently performed. Cells obtained by chorionic villus sampling (at 10–12 weeks of gestation) or by amniocentesis (at 14–16 weeks) can be cultured and used for sex determination by PCR amplification of the *SRY* (sex-determining region of the Y chromosome; no cell culture required in this case), fluorescence in situ hybridisation (FISH) or conventional karyotyping, as well as for searching for the *CYP21A2* mutations previously identified in the parents. As a quality-control measure in prenatal diagnosis, fetal DNA should be retested using informative tandem repeats (VNTRs or STRs) to ensure lack of maternal contamination, while simultaneously allowing paternity confirmation. Preimplantation genetic diagnosis (Refs 70, 71) has also been used for the diagnosis of 21-OHD (Ref. 65); however, despite the great advantage of the very early diagnosis, this methodology is currently carried out only in specialised centres (Ref. 72).

It is advisable to confirm all prenatal diagnoses soon after birth because of the possibility of errors, including false-negative diagnosis. It is important to bear in mind that de novo mutations occur in the *CYP21A2* gene with a considerable frequency of 1–1.5%, which can complicate molecular prenatal diagnosis of 21-OHD: the fetus might have a mutation that does not exist in the parents and that might not be screened on a first approach (Ref. 73).

Research in progress and outstanding research questions

Intense research over the past three decades in human molecular biology associated with human endocrinology has produced a plethora of discoveries that encompass the identification of genes coding for enzymes, transcription factors, hormones and receptors, physiological functions of endocrine hormones and receptors, and the characterisation of the molecular pathology of numerous endocrinological diseases, including nearly all forms of CAH. In addition, with this knowledge it has been possible to offer prenatal diagnosis and genetic counselling to couples at risk of having an affected baby with the most severe forms of CAH. However, the prediction of phenotype based on genotype may fail in some situations, namely in the ones associated with mutations of intermediate severity, as occurs with some mutations identified in the deficiencies described above, especially those associated with variable expressivity. This absence of genotype–phenotype correlation is in part due to the incomplete understanding of gene expression regulation, which is dependent on multiple transcription factors (some of which are still unidentified) that interact with each other and with regulatory sequences. In addition, there are also rare forms of CAH and many cases of hyperandrogenic females for whom the molecular defect is unknown. For these issues, it is expected that in the near future new technologies like proteomics, tandem mass spectrometry, microchip arrays and ultra-rapid DNA genotyping and sequencing will contribute to the identification of new disease-associated genes and new biomarkers. After elucidating the physiological functions of these with the use of functional studies and animal models, it is expected that the data will help exclude the false-positive results frequently obtained with the conventional immunoassays used in neonatal screening programmes of CAH, quickly identify the molecular defects of all the CAH forms, and contribute to better genetic counselling and patient treatment.

In the future, with the new technologies of genome and proteome analysis, it will be possible to simultaneously evaluate multiple genes or gene products directly associated with CAH and involved in the same or in multiple physiological pathways. The integration of these data obtained from a large number of patients with pharmacogenomic studies and eventually with gene therapy will have a great impact in individual and global health care (Refs 74, 75).

At present, there is considerable expectation that gene therapy and autograft transplantation of genetically modified adrenal cortical stem cells or multipotent mesenchymal bone marrow cells may achieve a definitive cure for patients with CAH or with other endocrine pathologies

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(Ref. 76). Also currently under research are microRNA molecules that specifically regulate gene expression at the post-transcriptional level (Ref. 77): these microRNAs might be applied in the treatment of hyperandrogenism by regulating specifically the expression of genes directly involved in adrenal androgen biosynthesis, such as *CYP17A1*.

All these subjects are currently under intensive research, so that future experts will have the opportunity to comprehensively, efficiently and simultaneously integrate the evaluation of multiple physiological systems of each patient and select innovative, individualised, curative therapeutics.

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References

1 Itoi, K., Seasholtz, A.F. and Watson, S.J. (1998) Cellular and extracellular regulatory mechanisms of hypothalamic corticotropin-releasing hormone neurons. Endocr J 45, 13-33

2 Waterman, M.R. and Bischof, L.J. (1997) Cytochromes P450 12: diversity of ACTH (cAMP)-dependent transcription of bovine steroid hydroxylase genes. Faseb J 11, 419-427

3 Williams, J.S. and Williams, G.H. (2003) 50th anniversary of aldosterone. J Clin Endocrinol Metab 88, 2364-2372

4 Migeon, C.J. and Donohoue, P.A. (1994) Adrenal disorders. In Wilkins: The Diagnosis and Treatment of Endocrine Disorders in Childhood and Adolescence (4th edn), (Kappy, M.S., Blizzard, R.M. and Migeon, C.J., eds), pp. 717–856, Charles C. Thomas, Springfield

5 Goto, M. et al. (2006) In humans, early cortisol biosynthesis provides a mechanism to safeguard female sexual development. J Clin Invest 116, 953-960

6 White, P.C. (2006) Ontogeny of adrenal steroid biosynthesis: why girls will be girls. J Clin Invest 116, 872-874

7 Donohoue, P.A., Parker, K. and Migeon, C.J. (1995) Congenital adrenal hyperplasia. In The Metabolic and Molecular Bases of Inherited Disease (Scriver, C.R. et al., eds), pp. 2929-2966, McGraw Hill, New York

8 Moore, K.L. and Persaud, T.V.N. (1993) The urogenital system. In The Developing Human - Clinically Oriented Embryology (5th edn), pp. 265–303, W.B. Saunders, Philadelphia

9 Fluck, C.E. et al. (2004) Mutant P450 oxidoreductase causes disordered steroidogenesis with and without Antley-Bixler syndrome. Nat Genet 36, 228-230

10 Fluck, C.E. and Miller, W.L. (2006) P450 oxidoreductase deficiency: a new form of congenital adrenal hyperplasia. Curr Opin Pediatr 18, 435-441

11 Fukami, M. et al. (2005) Cytochrome P450 oxidoreductase gene mutations and Antley-Bixler syndrome with abnormal genitalia and/or impaired steroidogenesis: molecular and clinical studies in 10 patients. J Clin Endocrinol Metab 90, 414-426

12 Huang, N. et al. (2005) Diversity and function of mutations in p450 oxidoreductase in patients with Antley-Bixler syndrome and disordered steroidogenesis. Am J Hum Genet 76, 729-749

13 White, P.C. and Speiser, P.W. (2000) Congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Endocr Rev 21, 245-291

14 Kovacs, J. et al. (2001) Lessons from 30 years of clinical diagnosis and treatment of congenital adrenal hyperplasia in five middle European countries. J Clin Endocrinol Metab 86, 2958-2964

15 Brinkmann, A.O. (2001) Molecular basis of androgen insensitivity. Mol Cell Endocrinol 179, 105-109

16 New, M.I. (2006) Extensive clinical experience: nonclassical 21-hydroxylase deficiency. J Clin Endocrinol Metab 91, 4205-4214 (Erratum in: J Clin Endocrinol Metab 2007, 92, 142)

17 Pang, S.Y. et al. (1988) Worldwide experience in newborn screening for classical congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Pediatrics 81, 866-874

18 Therrell, B.L., Jr. et al. (1998) Results of screening 1.9 million Texas newborns for 21-hydroxylase-deficient congenital adrenal hyperplasia. Pediatrics 101, 583-590

19 van der Kamp, H.J. and Wit, J.M. (2004) Neonatal screening for congenital adrenal hyperplasia. Eur J Endocrinol 151 (Suppl 3), U71-75

20 Speiser, P.W. et al. (1985) High frequency of nonclassical steroid 21-hydroxylase deficiency. Am J Hum Genet 37, 650-667

Accession information: DOI: 10.1017/S1462399407000300; Vol. 9; Issue 11; April 2007
© 2007 Cambridge University Press

21 White, P.C. et al. (1985) Two genes encoding steroid 21-hydroxylase are located near the genes encoding the fourth component of complement in man. Proc Natl Acad Sci U S A 82, 1089-1093

22 Higashi, Y. et al. (1986) Complete nucleotide sequence of two steroid 21-hydroxylase genes tandemly arranged in human chromosome: a *pseudogene and a genuine gene.* Proc Natl Acad Sci U S A 83, 2841-2845

23 Lee, H.H., Lee, Y.J. and Lin, C.Y. (2004) PCR-based detection of the CYP21 deletion and TNXA/TNXB hybrid in the RCCX module. Genomics 83, 944-950

24 Blanchong, C.A. et al. (2000) Deficiencies of human complement component C4A and C4B and heterozygosity in length variants of RP-C4-CYP21-TNX (RCCX) modules in caucasians. The load of RCCX genetic diversity on major histocompatibility complex-associated disease. J Exp Med 191, 2183-2196

25 White, P.C., New, M.I. and Dupont, B. (1984) HLA-linked congenital adrenal hyperplasia results from a defective gene encoding a cytochrome P-450 specific for steroid 21-hydroxylation. Proc Natl Acad Sci U S A 81, 7505-7509

26 Werkmeister, J.W. et al. (1986) Frequent deletion and duplication of the steroid 21-hydroxylase genes. Am J Hum Genet 39, 461-469

27 Lee, H.H. (2005) Chimeric CYP21P/CYP21 and TNXA/TNXB genes in the RCCX module. Mol Genet Metab 84, 4-8

28 Chang, S.F. and Chung, B.C. (1995) Difference in transcriptional activity of two homologous CYP21A genes. Mol Endocrinol 9, 1330-1336

29 Friaes, A. et al. (2006) CYP21A2 mutations in Portuguese patients with congenital adrenal hyperplasia: identification of two novel mutations and characterization of four different partial gene conversions. Mol Genet Metab 88, 58-65

30 Koppens, P.F., Hoogenboezem, T. and Degenhart, H.J. (2002) Duplication of the CYP21A2 gene complicates mutation analysis of steroid 21-hydroxylase deficiency: characteristics of three unusual haplotypes. Hum Genet 111, 405-410

31 Haglund-Stengler, B. et al. (1991) Haplotypes of the steroid 21-hydroxylase gene region encoding mild steroid 21-hydroxylase deficiency. Proc Natl Acad Sci U S A 88, 8352-8356

32 Wedell, A., Stengler, B. and Luthman, H. (1994) Characterization of mutations on the rare duplicated C4/CYP21 haplotype in steroid 21-hydroxylase deficiency. Hum Genet 94, 50-54

33 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

34 Dolzan, V. et al. (1999) Adrenal 21-hydroxylase gene mutations in Slovenian hyperandrogenic women: evaluation of corticotrophin stimulation and HLA polymorphisms in screening for carrier status. Eur J Endocrinol 141, 132-139

35 Kharrat, M. et al. (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

36 Owerbach, D. et al. (1992) Pro-453 to Ser mutation in CYP21 is associated with nonclassic steroid 21-hydroxylase deficiency. Mol Endocrinol 6, 1211-1215

37 Higashi, Y. et al. (1988) Aberrant splicing and missense mutations cause steroid 21-hydroxylase [P-450(C21)] deficiency in humans: possible gene conversion products. Proc Natl Acad Sci U S A 85, 7486-7490

38 Lee, H.H. and Chang, S.F. (2001) Multiple transcripts of the CYP21 gene are generated by the mutation of the splicing donor site in intron 2 from GT to AT in 21-hydroxylase deficiency. J Endocrinol 171, 397-402

39 Honour, J.W. and Rumsby, G. (1993) Problems in diagnosis and management of congenital adrenal hyperplasia due to 21-hydroxylase deficiency. J Steroid Biochem Mol Biol 45, 69-74

40 Lee, H.H. et al. (2000) Analysis of the chimeric CYP21P/CYP21 gene in steroid 21-hydroxylase deficiency. Clin Chem 46, 606-611

41 Owerbach, D., Crawford, Y.M. and Draznin, M.B. (1990) Direct analysis of CYP21B genes in 21-hydroxylase deficiency using polymerase chain reaction amplification. Mol Endocrinol 4, 125-131

42 Wedell, A. and Luthman, H. (1993) Steroid 21-hydroxylase deficiency: two additional mutations in salt-wasting disease and rapid screening of disease-causing mutations. Hum Mol Genet 2, 499-504

43 Krone, N. et al. (2002) Multiplex minisequencing of the 21-hydroxylase gene as a rapid strategy to confirm congenital adrenal hyperplasia. Clin Chem 48, 818-825

44 Keen-Kim, D. et al. (2005) Validation and clinical application of a locus-specific polymerase chain reaction- and minisequencing-based assay for

Accession information: DOI: 10.1017/S1462399407000300; Vol. 9; Issue 11; April 2007
© 2007 Cambridge University Press

congenital adrenal hyperplasia (21-hydroxylase deficiency). J Mol Diagn 7, 236-246

45 Orita, M. et al. (1989) Detection of polymorphisms of human DNA by gel electrophoresis as single-strand conformation polymorphisms. Proc Natl Acad Sci U S A 86, 2766-2770

46 Abrams, E.S., Murdaugh, S.E. and Lerman, L.S. (1990) Comprehensive detection of single base changes in human genomic DNA using denaturing gradient gel electrophoresis and a GC clamp. Genomics 7, 463-475

47 Bobba, A. et al. (1997) Characterisation of CAH alleles with non-radioactive DNA single strand conformation polymorphism analysis of the CYP21 gene. J Med Genet 34, 223-228

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

49 Lee, H.H. et al. (2003) Duplication of 111 bases in exon 1 of the CYP21 gene is combined with deletion of CYP21P-C4B genes in steroid 21-hydroxylase deficiency. Mol Genet Metab 79, 214-220

50 Olney, R.C. et al. (2002) Using real-time, quantitative PCR for rapid genotyping of the steroid 21-hydroxylase gene in a north Florida population. J Clin Endocrinol Metab 87, 735-741

51 Tukel, T. et al. (2003) A novel semiquantitative polymerase chain reaction/enzyme digestion-based method for detection of large scale deletions/conversions of the CYP21 gene and mutation screening in Turkish families with 21-hydroxylase deficiency. J Clin Endocrinol Metab 88, 5893-5897

52 Krone, N. et al. (2005) The residue E351 is essential for the activity of human 21-hydroxylase: evidence from a naturally occurring novel point mutation compared with artificial mutants generated by single amino acid substitutions. J Mol Med 83, 561-568

53 Grischuk, Y. et al. (2006) Four novel missense mutations in the CYP21A2 gene detected in Russian patients suffering from the classical form of congenital adrenal hyperplasia: identification, functional characterization, and structural analysis. J Clin Endocrinol Metab 91, 4976-4980

54 Barbaro, M. et al. (2006) Functional studies of two novel and two rare mutations in the 21-hydroxylase gene. J Mol Med 84, 521-528

55 Kotaska, K., Lisa, L. and Prusa, R. (2003) Common CYP21 gene mutations in Czech patients and statistical analysis of worldwide mutation distribution. Cent Eur J Public Health 11, 124-128

56 Ordonez-Sanchez, M.L. et al. (1998) Molecular genetic analysis of patients carrying steroid 21-hydroxylase deficiency in the Mexican population: identification of possible new mutations and high prevalence of apparent germ-line mutations. Hum Genet 102, 170-177

57 Ko, T.M. et al. (1998) Congenital adrenal hyperplasia. Molecular characterization. J Reprod Med 43, 379-386

58 Vakili, R. et al. (2005) Molecular analysis of the CYP21 gene and prenatal diagnosis in families with 21-hydroxylase deficiency in northeastern Iran. Horm Res 63, 119-124

59 Dolzan, V. et al. (2005) Mutational spectrum of steroid 21-hydroxylase and the genotype-phenotype association in Middle European patients with congenital adrenal hyperplasia. Eur J Endocrinol 153, 99-106

60 Asanuma, A. et al. (1999) Molecular analysis of Japanese patients with steroid 21-hydroxylase deficiency. J Hum Genet 44, 312-317

61 Mathur, R. et al. (2001) Molecular characterization of mutations in Indian children with congenital adrenal hyperplasia due to steroid 21-hydroxylase deficiency. J Pediatr Endocrinol Metab 14, 27-35

62 Stikkelbroeck, N.M. et al. (2003) 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

63 Day, D.J. et al. (1995) Detection of steroid 21-hydroxylase alleles using gene-specific PCR and a multiplexed ligation detection reaction. Genomics 29, 152-162

64 Day, D.J. et al. (1996) Identification of non-amplifying CYP21 genes when using PCR-based diagnosis of 21-hydroxylase deficiency in congenital adrenal hyperplasia (CAH) affected pedigrees. Hum Mol Genet 5, 2039-2048

65 Van de Velde, H. et al. (1999) Fluorescent PCR and automated fragment analysis in preimplantation genetic diagnosis for 21-hydroxylase deficiency in congenital adrenal hyperplasia. Mol Hum Reprod 5, 691-696

66 Lajic, S. et al. (2004) Prenatal treatment of congenital adrenal hyperplasia. Eur J Endocrinol 151 (Suppl 3), U63-69

67 Avent, N.D. and Chitty, L.S. (2006) Non-invasive diagnosis of fetal sex; utilisation of free fetal DNA in maternal plasma and ultrasound. Prenat Diagn 26, 598-603

68 Bartha, J.L., Finning, K. and Soothill, P.W. (2003) Fetal sex determination from maternal blood at

Accession information: DOI: 10.1017/S1462399407000300; Vol. 9; Issue 11; April 2007
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6 weeks of gestation when at risk for 21-hydroxylase deficiency. Obstet Gynecol 101, 1135-1136

69 Hyett, J.A. et al. (2005) Reduction in diagnostic and therapeutic interventions by non-invasive determination of fetal sex in early pregnancy. Prenat Diagn 25, 1111-1116

70 Sermon, K. (2002) Current concepts in preimplantation genetic diagnosis (PGD): a molecular biologist's view. Hum Reprod Update 8, 11-20

71 Renwick, P. and Ogilvie, C.M. (2007) Preimplantation genetic diagnosis for monogenic diseases: overview and emerging issues. Expert Rev Mol Diagn 7, 33-43

72 Thornhill, A.R. et al. (2005) ESHRE PGD Consortium 'Best practice guidelines for clinical preimplantation genetic diagnosis (PGD) and preimplantation genetic screening (PGS)'. Hum Reprod 20, 35-48

73 Mao, R. et al. (2005) The implication of de novo 21-hydroxylase mutation in clinical and prenatal molecular diagnoses. Genet Test 9, 121-125

74 Hsueh, A.J., Bouchard, P. and Ben-Shlomo, I. (2005) Hormonology: a genomic perspective on hormonal research. J Endocrinol 187, 333-338

75 Merke, D.P. et al. (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

76 Yanase, T. et al. (2006) Differentiation and regeneration of adrenal tissues: an initial step toward regeneration therapy for steroid insufficiency. Endocr J 53, 449-459

77 Cuellar, T.L. and McManus, M.T. (2005) MicroRNAs and endocrine biology. J Endocrinol 187, 327-332

78 Bose, H.S. et al. (1996) The pathophysiology and genetics of congenital lipoid adrenal hyperplasia. International Congenital Lipoid Adrenal Hyperplasia Consortium. N Engl J Med 335, 1870-1878

79 Bose, H.S., Pescovitz, O.H. and Miller, W.L. (1997) Spontaneous feminization in a 46,XX female patient with congenital lipoid adrenal hyperplasia due to a homozygous frameshift mutation in the steroidogenic acute regulatory protein. J Clin Endocrinol Metab 82, 1511-1515

80 Fujieda, K. et al. (1997) Spontaneous puberty in 46,XX subjects with congenital lipoid adrenal hyperplasia. Ovarian steroidogenesis is spared to some extent despite inactivating mutations in the steroidogenic acute regulatory protein (StAR) gene. J Clin Invest 99, 1265-1271

81 Lin, D. et al. (1995) Role of steroidogenic acute regulatory protein in adrenal and gonadal steroidogenesis. Science 267, 1828-1831

82 Nakae, J. et al. (1997) Analysis of the steroidogenic acute regulatory protein (StAR) gene in Japanese patients with congenital lipoid adrenal hyperplasia. Hum Mol Genet 6, 571-576

83 Fluck, C.E. et al. (2005) A novel mutation L260P of the steroidogenic acute regulatory protein gene in three unrelated patients of Swiss ancestry with congenital lipoid adrenal hyperplasia. J Clin Endocrinol Metab 90, 5304-5308

84 Chen, X. et al. (2005) A genetic isolate of congenital lipoid adrenal hyperplasia with atypical clinical findings. J Clin Endocrinol Metab 90, 835-840

85 Baker, B.Y. et al. (2006) Nonclassic congenital lipoid adrenal hyperplasia: a new disorder of the steroidogenic acute regulatory protein with very late presentation and normal male genitalia. J Clin Endocrinol Metab 91, 4781-4785

86 Yoo, H.W. and Kim, G.H. (1998) Molecular and clinical characterization of Korean patients with congenital lipoid adrenal hyperplasia. J Pediatr Endocrinol Metab 11, 707-711

87 Stocco, D.M. (2002) Clinical disorders associated with abnormal cholesterol transport: mutations in the steroidogenic acute regulatory protein. Mol Cell Endocrinol 191, 19-25

88 Miller, W.L. (1987) Structure of genes encoding steroidogenic enzymes. J Steroid Biochem 27, 759-766

89 Miller, W.L. (1998) Why nobody has P450scc (20,22 desmoslase) deficiency. J Clin Endocrinol Metab 83, 1399-1400

90 Tajima, T. et al. (2001) Heterozygous mutation in the cholesterol side chain cleavage enzyme (p450scc) gene in a patient with 46,XY sex reversal and adrenal insufficiency. J Clin Endocrinol Metab 86, 3820-3825

91 Katsumata, N. et al. (2002) Compound heterozygous mutations in the cholesterol side-chain cleavage enzyme gene (CYP11A) cause congenital adrenal insufficiency in humans. J Clin Endocrinol Metab 87, 3808-3813

92 Hiort, O. et al. (2005) Homozygous disruption of P450 side-chain cleavage (CYP11A1) is associated with prematurity, complete 46,XY sex reversal, and severe adrenal failure. J Clin Endocrinol Metab 90, 538-541

Accession information: DOI: 10.1017/S1462399407000300; Vol. 9; Issue 11; April 2007
© 2007 Cambridge University Press

93 al Kandari, H. et al. (2006) Homozygous mutation of P450 side-chain cleavage enzyme gene (CYP11A1) in 46,XY patient with adrenal insufficiency, complete sex reversal, and agenesis of corpus callosum. J Clin Endocrinol Metab 91, 2821-2826

94 Berube, D. et al. (1989) Assignment of the human 3 beta-hydroxysteroid dehydrogenase gene (HSDB3) to the p13 band of chromosome 1. Cytogenet Cell Genet 52, 199-200

95 Rheaume, E. et al. (1991) Structure and expression of a new complementary DNA encoding the almost exclusive 3 beta-hydroxysteroid dehydrogenase/delta 5-delta 4-isomerase in human adrenals and gonads. Mol Endocrinol 5, 1147-1157

96 Gingras, S. et al. (1999) Induction of 3beta-hydroxysteroid dehydrogenase/delta5-delta4 isomerase type 1 gene transcription in human breast cancer cell lines and in normal mammary epithelial cells by interleukin-4 and interleukin-13. Mol Endocrinol 13, 66-81

97 Simard, J. et al. (2005) Molecular biology of the 3beta-hydroxysteroid dehydrogenase/delta5-delta4 isomerase gene family. Endocr Rev 26, 525-582

98 Lutfallah, C. et al. (2002) Newly proposed hormonal criteria via genotypic proof for type II 3beta-hydroxysteroid dehydrogenase deficiency. J Clin Endocrinol Metab 87, 2611-2622

99 Johannsen, T.H. et al. (2005) Delayed diagnosis of congenital adrenal hyperplasia with salt wasting due to type II 3beta-hydroxysteroid dehydrogenase deficiency. J Clin Endocrinol Metab 90, 2076-2080

100 Mermejo, L.M. et al. (2005) Refining hormonal diagnosis of type II 3beta-hydroxysteroid dehydrogenase deficiency in patients with premature pubarche and hirsutism based on HSD3B2 genotyping. J Clin Endocrinol Metab 90, 1287-1293

101 Mendonca, B.B. et al. (1987) Male pseudohermaphroditism due to nonsalt-losing 3 beta-hydroxysteroid dehydrogenase deficiency: gender role change and absence of gynecomastia at puberty. J Steroid Biochem 28, 669-675

102 Mendonca, B.B. et al. (1994) Mutation in 3beta-hydroxysteroid dehydrogenase type II associated with pseudohermaphroditism in males and premature pubarche or cryptic expression in females. J Mol Endocrinol 12, 119-122

103 Moisan, A.M. et al. (1999) New insight into the molecular basis of 3beta-hydroxysteroid dehydrogenase deficiency: identification of eight mutations in the HSD3B2 gene eleven patients from seven new families and comparison of the functional properties of twenty-five mutant enzymes. J Clin Endocrinol Metab 84, 4410-4425

104 Zhang, L. et al. (2000) Characterization of two novel homozygous missense mutations involving codon 6 and 259 of type II 3beta-hydroxysteroid dehydrogenase (3betaHSD) gene causing, respectively, nonsalt-wasting and salt-wasting 3betaHSD deficiency disorder. J Clin Endocrinol Metab 85, 1678-1685

105 Marui, S. et al. (2000) Mutations in the type II 3beta-hydroxysteroid dehydrogenase (HSD3B2) gene can cause premature pubarche in girls. Clin Endocrinol 52, 67-75

106 Sparkes, R.S., Klisak, I. and Miller, W.L. (1991) Regional mapping of genes encoding human steroidogenic enzymes: P450scc to 15q23-q24, adrenodoxin to 11q22; adrenodoxin reductase to 17q24-q25; and P450c17 to 10q24-q25. DNA Cell Biol 10, 359-365

107 Nakajin, S. et al. (1981) Microsomal cytochrome P-450 from neonatal pig testis: two enzymatic activities (17 alpha-hydroxylase and c17,20-lyase) associated with one protein. Biochemistry 20, 4037-4042

108 Lee-Robichaud, P. et al. (1995) Modulation of the activity of human 17 alpha-hydroxylase-17,20-lyase (CYP17) by cytochrome b5: endocrinological and mechanistic implications. Biochem J 308, 901-908

109 Fluck, C.E., Miller, W.L. and Auchus, R.J. (2003) The 17,20-lyase activity of cytochrome p450c17 from human fetal testis favors the delta5 steroidogenic pathway. J Clin Endocrinol Metab 88, 3762-3766

110 Yanase, T. (1995) 17 alpha-Hydroxylase/17,20-lyase defects. J Steroid Biochem Mol Biol 53, 153-157

111 Geller, D.H. et al. (1997) The genetic and functional basis of isolated 17,20-lyase deficiency. Nat Genet 17, 201-205

112 Van Den Akker, E.L. et al. (2002) Differential inhibition of 17alpha-hydroxylase and 17,20-lyase activities by three novel missense CYP17 mutations identified in patients with P450c17 deficiency. J Clin Endocrinol Metab 87, 5714-5721

113 Sherbet, D.P. et al. (2003) CYP17 mutation E305G causes isolated 17,20-lyase deficiency by selectively altering substrate binding. J Biol Chem 278, 48563-48569

Accession information: DOI: 10.1017/S1462399407000300; Vol. 9; Issue 11; April 2007
© 2007 Cambridge University Press

114 ten Kate-Booij, M.J. et al. (2004) Deficiency of 17,20-lyase causing giant ovarian cysts in a girl and a female phenotype in her 46,XY sister: case report. Hum Reprod 19, 456-459

115 Martin, R.M. et al. (2003) P450c17 deficiency in Brazilian patients: biochemical diagnosis through progesterone levels confirmed by CYP17 genotyping. J Clin Endocrinol Metab 88, 5739-5746

116 Costa-Santos, M., Kater, C.E. and Auchus, R.J. (2004) Two prevalent CYP17 mutations and genotype-phenotype correlations in 24 Brazilian patients with 17-hydroxylase deficiency. J Clin Endocrinol Metab 89, 49-60

117 Patocs, A. et al. (2005) Novel mutation of the CYP17 gene in two unrelated patients with combined 17alpha-hydroxylase/17,20-lyase deficiency: demonstration of absent enzyme activity by expressing the mutant CYP17 gene and by three-dimensional modeling. J Steroid Biochem Mol Biol 97, 257-265

118 Mussig, K. et al. (2005) 17alpha-hydroxylase/17,20-lyase deficiency caused by a novel homozygous mutation (Y27Stop) in the cytochrome CYP17 gene. J Clin Endocrinol Metab 90, 4362-4365

119 Brooke, A.M. et al. (2006) A novel point mutation in P450c17 (CYP17) causing combined 17alpha-hydroxylase/17,20-lyase deficiency. J Clin Endocrinol Metab 91, 2428-2431

120 Lee, L.S. et al. (2006) A novel compound heterozygous mutation of K494_V495 deletion plus R496L and D487_F489 deletion in extreme C-terminus of cytochrome P450c17 causes 17alpha-hydroxylase deficiency. Mol Cell Endocrinol 249, 16-20

121 Ergun-Longmire, B. et al. (2006) Two novel mutations found in a patient with 17alpha-hydroxylase enzyme deficiency. J Clin Endocrinol Metab 91, 4179-4182

122 Auchus, R.J. (2001) The genetics, pathophysiology, and management of human deficiencies of P450c17. Endocrinol Metab Clin North Am 30, 101-119

123 White, P.C., Curnow, K.M. and Pascoe, L. (1994) Disorders of steroid 11 beta-hydroxylase isozymes. Endocr Rev 15, 421-438

124 Rosler, A., Leiberman, E. and Cohen, T. (1992) High frequency of congenital adrenal hyperplasia (classic 11 beta-hydroxylase deficiency) among Jews from Morocco. Am J Med Genet 42, 827-834

125 Paperna, T. et al. (2005) Mutations in CYP11B1 and congenital adrenal hyperplasia in Moroccan Jews. J Clin Endocrinol Metab 90, 5463-5465

126 Chua, S.C. et al. (1987) Cloning of cDNA encoding steroid 11beta-hydroxylase (P450c11). Proc Natl Acad Sci U S A 84, 7193-7197

127 Tonetto-Fernandes, V. et al. (2006) Serum 21-Deoxycortisol, 17-Hydroxyprogesterone, and 11-deoxycortisol in classic congenital adrenal hyperplasia: clinical and hormonal correlations and identification of patients with 11beta-hydroxylase deficiency among a large group with alleged 21-hydroxylase deficiency. J Clin Endocrinol Metab 91, 2179-2184

128 Zhu, Y.S. et al. (2003) Mutations in CYP11B1 gene: phenotype-genotype correlations. Am J Med Genet A 122, 193-200

129 Grigorescu Sido, A. et al. (2005) 21-Hydroxylase and 11beta-hydroxylase mutations in Romanian patients with classic congenital adrenal hyperplasia. J Clin Endocrinol Metab 90, 5769-5773

130 Lee, H.H. et al. (2005) Novel missense mutations, GCC [Ala306]- > GTC [Val] and ACG [Thr318]- > CCG [Pro], in the CYP11B1 gene cause steroid 11beta-hydroxylase deficiency in the Chinese. Clin Endocrinol 62, 418-422

131 Krone, N. et al. (2005) Congenital adrenal hyperplasia due to 11-hydroxylase deficiency: functional characterization of two novel point mutations and a three-base pair deletion in the CYP11B1 gene. J Clin Endocrinol Metab 90, 3724-3730

132 Krone, N. et al. (2006) Analyzing the functional and structural consequences of two point mutations (P94L and A368D) in the CYP11B1 gene causing congenital adrenal hyperplasia resulting from 11-hydroxylase deficiency. J Clin Endocrinol Metab 91, 2682-2688

133 Joehrer, K. et al. (1997) CYP11B1 mutations causing non-classic adrenal hyperplasia due to 11 beta-hydroxylase deficiency. Hum Mol Genet 6, 1829-1834

134 Clark, P.A. (2000) Nonclassic 11beta-hydroxylase deficiency: report of two patients and review. J Pediatr Endocrinol Metab 13, 105-109

135 Arlt, W. et al. (2004) Congenital adrenal hyperplasia caused by mutant P450 oxidoreductase and human androgen synthesis: analytical study. Lancet 363, 2128-2135

Accession information: DOI: 10.1017/S1462399407000300; Vol. 9; Issue 11; April 2007
© 2007 Cambridge University Press

136 Pandey, A.V. et al. (2004) P450 oxidoreductase deficiency: a new disorder of steroidogenesis affecting all microsomal P450 enzymes. Endocr Res 30, 881-888

137 Adachi, M. et al. (2004) Compound heterozygous mutations of cytochrome P450 oxidoreductase gene (POR) in two patients with Antley-Bixler syndrome. Am J Med Genet A 128, 333-339

138 Williamson, L. et al. (2006) Linking Antley-Bixler syndrome and congenital adrenal hyperplasia: a novel case of P450 oxidoreductase deficiency. Am J Med Genet A 140, 1797-1803

139 New, M.I. (2006) Extensive clinical experience: nonclassical 21-hydroxylase deficiency. J Clin Endocrinol Metab 91, 4205-4214

140 Miller, W.L. (2007) StAR search – what we know about how the steroidogenic acute regulatory protein mediates mitochondrial cholesterol import. Mol Endocrinol 21, 589-601

Further reading, resources and contacts

CYP21A2 allele nomenclature, mutations and associated phenotypes are summarised at:

http://www.cypalleles.ki.se/cyp21.htm

The Online Mendelian Inheritance in Man website provides a register of human genes and genetic disorders:

http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM

The CARES foundation website (focusing on CAH) and the GeneTests website (focusing on genetic screening) provide information on treatment, research, newborn screening and many links to scientific societies and support groups:

http://caresfoundation.org/links.html

http://www.genetests.org/

The Johns Hopkins Children's Center site about CAH has useful information about the disease, especially for patients and their families:

http://www.hopkinschildrens.org/specialties/categorypages/cah

The CLAN organisation help patients and families with CAH in developing countries access medication:

http://www.cahclan.org

The Human Gene Mutation Database contains useful data about mutations in the *CYP21A2* gene:

http://www.hgmd.cf.ac.uk/ac/gene.php?gene=CYP21A2

A general overview of the multiple forms of CAH, genetics, enzymatic defects and the history of this disease can be found at:

http://en.wikipedia.org/wiki/Congenital_adrenal_hyperplasia

Features associated with this article

Figures

Figure 1. Principal pathways of steroid hormone biosynthesis from cholesterol.
Figure 2. The localisation and structure of the RCCX region.
Figure 3. Different possible forms of the RCCX region and *CYP21A2* gene.
Figure 4. Common mutations in the *CYP21A2* gene.

Table

Table 1. Summary of the various enzymatic defects that cause congenital adrenal hyperplasia.

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