

Non-classic congenital adrenal hyperplasia due to 21-hydroxylase deficiency revisited: an update with a special focus on adolescent and adult women

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BACKGROUND: Non-classic congenital hyperplasia (NCAH) due to 21-hydroxylase deficiency is a common autosomal recessive disorder characterized by androgen excess.

OBJECTIVE AND RATIONALE: We conducted a systematic review and critical assessment of the available evidence pertaining to the epidemiology, pathophysiology, diagnosis and management of NCAH. A meta-analysis of epidemiological data was also performed.

SEARCH METHODS: Peer-reviewed studies evaluating NCAH published up to October 2016 were reviewed. Multiple databases were searched including MEDLINE, EMBASE, Cochrane, ERIC, EBSCO, dissertation abstracts, and current contents.

OUTCOMES: The worldwide prevalence of NCAH amongst women presenting with signs and symptoms of androgen excess is 4.2% (95% confidence interval: 3.2–5.4%). The clinical consequences of NCAH expand from infancy, i.e. accelerated growth, to adolescence and adulthood, i.e. premature pubarche, cutaneous symptoms and oligo-ovulation in a polycystic ovary syndrome (PCOS)-like clinical picture. The diagnosis of NCAH relies on serum 17-hydroxyprogesterone (17-OHP) concentrations. A basal 17-OHP concentration ≥ 2 ng/ml (6 nmol/l) should be used for screening if more appropriate in-house cut-off values are not available. Definitive diagnosis requires a 17-OHP concentration ≥ 10 ng/ml (30 nmol/l), either basally or after cosyntropin-stimulation. Molecular genetic analysis of the *CYP21A2* gene, which is responsible for 21-hydroxylase activity, may be used for confirmation purposes and should be offered to all patients with NCAH along with genetic counseling because these patients frequently carry alleles that may result in classic CAH, the more severe form of the disease, in their progeny. Treatment must be individualized. Glucocorticoid replacement therapy may benefit pediatric patients with accelerated growth or advanced bone age or adult women seeking fertility, whereas adequate control of menstrual irregularity, hirsutism and other cutaneous symptoms is best served by the use of oral contraceptive pills and/or anti-androgens. Some women may need ovulation induction or assisted reproductive technology to achieve pregnancy. Patients with NCAH have a higher risk of miscarriage and may benefit from glucocorticoid treatment during pregnancy.

WIDER IMPLICATIONS: Evidence-based diagnostic and treatment strategies are essential for the proper management of women with NCAH, especially considering that these patients may need different therapeutic strategies at different stages during their follow-up and that appropriate genetic counseling may prevent the occurrence of CAH in their children.

Key words: 17-hydroxyprogesterone / 21-hydroxylase deficiency / androgen excess / hirsutism / hyperandrogenism / miscarriage / non-classic congenital adrenal hyperplasia / polycystic ovary syndrome / pregnancy / premature pubarche

Introduction

A number of guidelines on congenital adrenal hyperplasia have been published; most have primarily focused primarily on classic congenital adrenal hyperplasia (CAH). Yet, in many ways non-classic adrenal hyperplasia (NCAH) differs significantly from CAH not only because of its later and dissimilar clinical presentation, but also because of the need for distinct considerations regarding therapy. This review is exclusively focused on NCAH and represents the integrated view of a special expert committee appointed by the Androgen Excess & PCOS (AE-PCOS) Society, Inc., with expertise in diverse fields including pediatric, adult and reproductive endocrinology.

Methods

Panel

The AE-PCOS Board appointed a panel of experts on NCAH, selected from those researchers who had authored many original articles in the field, to review all evidence available about NCAH. Panel members and Board Directors constituted the Writing Committee. Prior to efforts on this review, selected members of the AE-PCOS committee declared all interests and activities potentially resulting in conflicts of interest. One senior member had to quit the committee because she was contemporaneously chairing a committee preparing guidelines for a different scientific society. All the other members of the committee declared no conflicts of interest because of similar activities or financial interests.

Data selection and statistical analysis

Peer-reviewed studies evaluating NCAH published up to October 2016 were reviewed. Multiple databases were searched including MEDLINE, EMBASE, Cochrane, ERIC, EBSCO, dissertation abstracts and current contents. This review focused on the epidemiology, pathophysiology, diagnosis and treatment of the disease. Some studies were eliminated because the data were either not related to the focus of the systematic review, insufficient for epidemiological analysis or reported in previous publications. All data sources were analyzed while recognizing positive publication bias.

A meta-analysis was performed to obtain pooled prevalence estimates on NCAH prevalence in subjects with signs and symptoms of androgen excess. The terms of the MEDLINE search for this meta-analysis were: (prevalence OR frequency OR frequencies OR prevalences) AND (non classic OR non-classic OR late-onset OR late onset) AND (adrenal hyperplasia OR 21-hydroxylase OR 21 α -hydroxylase OR CYP21 OR CYP21A2). The search was completed by examining the references listed in the articles identified. After study identification, screening, selection and inclusion, a random-effects model was applied considering the heterogeneity of the studies in terms of age, race, ethnicity and hyperandrogenic symptoms of the populations being described. Double arcsine transformations were applied to stabilize the variance (Doi et al., 2015). The forest plot in Fig. 1 depicts the pooled prevalence estimate as a diamond, with the lateral points indicating confidence intervals. The left hand column included study identifiers and the right-hand columns included plots and corresponding numerical information for the prevalence found in each of these studies, with squares and horizontal lines representing confidence

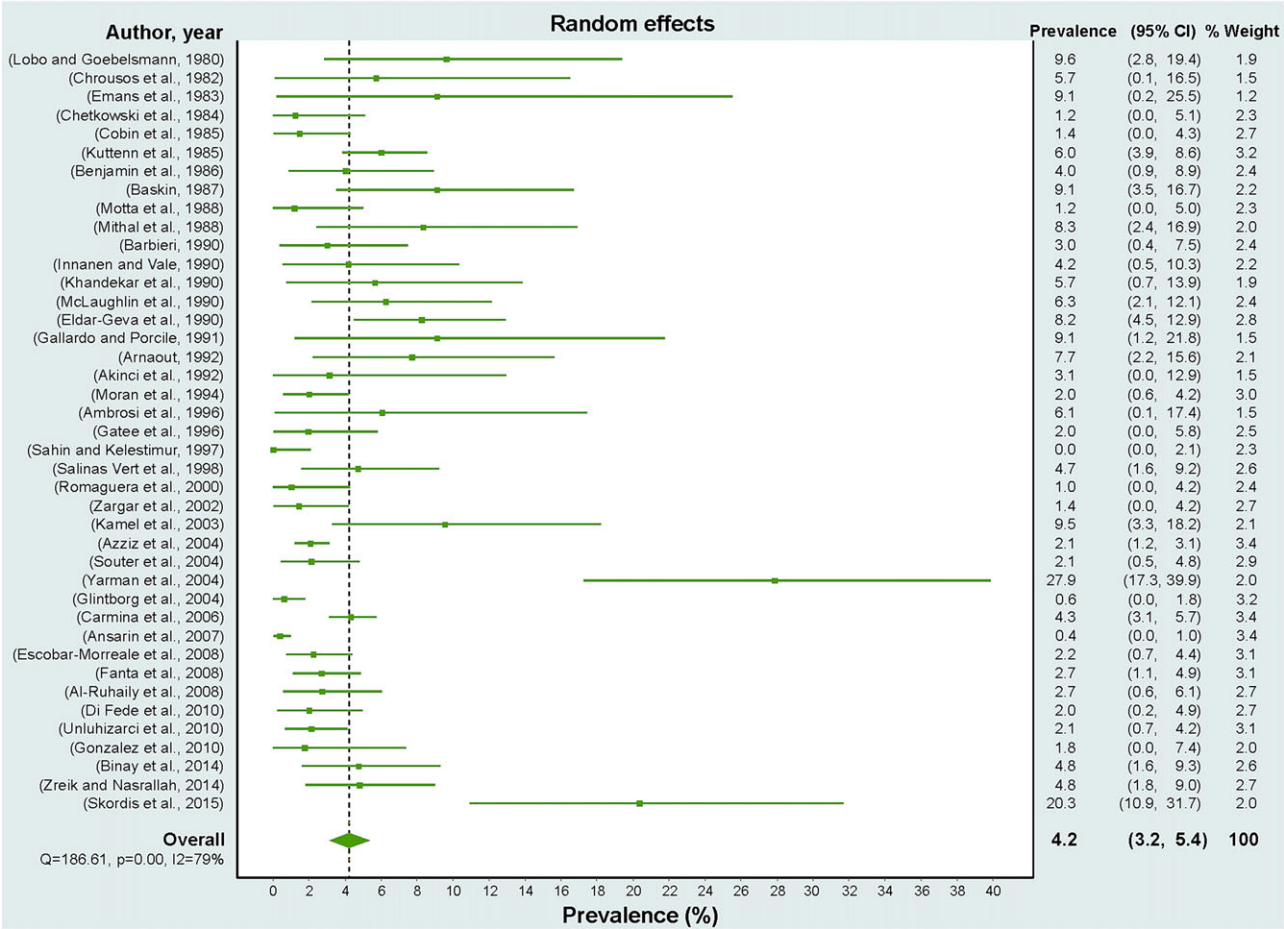


Figure 1 Meta-analysis of the prevalence (%) of non-classic congenital adrenal hyperplasia (NCAH) among patients presenting with signs and symptoms of androgen excess. The terms of the MEDLINE search were (prevalence OR frequency OR frequencies OR prevalences) AND (non classic OR non-classic OR late-onset OR late onset) AND (adrenal hyperplasia OR 21-hydroxylase OR 21 α -hydroxylase OR CYP21 OR CYP21A2). This was completed by a hand search of the references lists of the articles identified. The overall characteristic of the studies included in the meta-analysis are summarized in Table 1. PRIMA flow-chart of the search and funnel plot of the studies included in the meta-analysis are included in Fig. 2. Data were submitted to a random effects model using MetaXL 3.0 software (Doi et al., 2015).

intervals. Publication bias was assessed by a funnel plot representing the double arcsine transformation of the prevalence against the standard error (Sterne and Egger, 2001). MetaXL 3.0 software was used for the meta-analysis (Doi et al., 2015). The overall characteristics of the studies included in the meta-analysis are summarized in Table 1. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (Moher et al., 2009) recommended flow-chart and funnel plot of the studies included in the meta-analysis are depicted in Fig. 2.

Process

Each chapter of this review was prepared by at least two investigators and then reviewed by all authors of the committee. Analyzed papers included individual studies, systematic reviews, hand searches and abstracts. Criteria for inclusion/exclusion of the published papers related to each chapter were agreed upon by at least two reviewers in each area and arbitrated by a third when necessary. Levels of evidence were

assessed and graded from A to D (www.nhmrc.gov.au/publications/synopses/cp65syn.htm). A or B level was considered necessary for recommendations regarding diagnosis and therapy of NCAH patients. The final manuscript was reviewed and approved by the AE-PCOS Society Board. Institutional Review Board approval was not obtained because the study reviewed publicly available medical literature.

Epidemiology of NCAH

Hyperandrogenic non-classic congenital adrenal hyperplasia (NCAH) can be associated with three distinct enzyme defects: (a) defects of 21-hydroxylase (21-OH) activity, catalyzed by cytochrome P450c21A2, encoded by CYP21A2; (b) defects of 11 β -hydroxylase (11-OH) activity, catalyzed by cytochrome P450c11, encoded by CYP11B1; and (c) defects of 3 β -hydroxysteroid dehydrogenase (3 β -HSD) activity, catalyzed by type II 3 β -HSD, encoded by HSD3B2.

Table 1 Prevalence of non-classic congenital adrenal hyperplasia (NCAH) in series of women presenting with signs and symptoms of androgen excess.

References	Country	Sex	Age	Complaint	Series size	NCAH	
					(n)	(n)	(%)
Lobo and Goebelsmann (1980)	United States	Female	Premenopausal	Hirsutism and oligomenorrhea	52	5	9.6
Chrousos <i>et al.</i> (1982)	United States	Female	Premenopausal	Hirsutism	35	2	5.7
Emans <i>et al.</i> (1983)	United States	Female	Adolescent	Androgen excess	22	2	9.1
Chetkowski <i>et al.</i> (1984)	United States	Female	Premenopausal	Hirsutism	83	1	1.2
Cobin <i>et al.</i> (1985)	United States	Female	Premenopausal	PCOS and hirsutism	139	2	1.4
Kuttann <i>et al.</i> (1985)	France	Female	Premenopausal	Hirsutism	400	24	6.0
Benjamin <i>et al.</i> (1986)	United States	Female	Premenopausal	PCOS	100	4	4.0
Baskin (1987)	United States	Female	Adolescents and adults	Hirsutism and/or amenorrhea	77	7	9.1
Motta <i>et al.</i> (1988)	Italy	Female	Adolescents and adults	Hirsutism	85	1	1.2
Mithal <i>et al.</i> (1988)	India	Female	Premenopausal	Hirsutism	60	5	8.3
Barbieri (1990)	United States	Female	Adult	Clinical hyperandrogenism	100	3	3.0
Innanen and Vale (1990)	Canada	Female	Premenopausal	Mild to moderate hirsutism	72	3	4.2
Khandekar <i>et al.</i> (1990)	India	Female	Premenopausal	Hirsutism	53	3	5.6
McLaughlin <i>et al.</i> (1990)	Ireland	Female	Premenopausal	Clinical hyperandrogenism	96	6	6.3
Eldar-Geva <i>et al.</i> (1990)	Israel	Female	Premenopausal	Clinical hyperandrogenism	170	14	8.3
Gallardo and Porcile (1991)	Chile	Female	Premenopausal	Hirsutism	33	3	9.1
Arnaout (1992)	France	Female	Premenopausal	Hirsutism	65	5	7.7
Akinci <i>et al.</i> (1992)	Turkey	Female	Adolescent	Hirsutism	32	1	3.1
Moran <i>et al.</i> (1994)	Mexico	Female	Premenopausal	Hirsutism	250	5	2.0
Ambrosi <i>et al.</i> (1996)	Italy	Female	Premenopausal	Hirsutism	33	2	6.1
Gatee <i>et al.</i> (1996)	United Arab Emirates	Female	Premenopausal	Hirsutism	102	2	1.9
Sahin and Kelestimur (1997)	Turkey	Female	Premenopausal	PCOS	83	0	0.0
Salinas Vert <i>et al.</i> (1998)	Spain	Female	Premenopausal	Hirsutism	127	6	4.7
Romaguera <i>et al.</i> (2000)	Puerto Rico	Female	Premenopausal	Hirsutism	100	1	1.0
Zargar <i>et al.</i> (2002)	India	Female	Pre- and postmenopausal	Hirsutism	142	2	1.4
Kamel <i>et al.</i> (2003)	Turkey	Female	Premenopausal	Hirsutism	63	6	9.5
Azziz <i>et al.</i> (2004)	United States	Female	Premenopausal	Clinical hyperandrogenism	873	18	2.1
Souter <i>et al.</i> (2004)	United States	Female	Premenopausal	Minimal unwanted hair growth	188	4	2.1
Glintborg <i>et al.</i> (2004)	Denmark	Female	Premenopausal	Hirsutism	340	2	0.6
Yarman <i>et al.</i> (2004)	Turkey	Female	Premenopausal	Hirsutism and polycystic ovaries	61	17	27.9
Carmina <i>et al.</i> (2006)	Italy	Female	Premenopausal	Clinical hyperandrogenism	950	41	4.3
Ansarin <i>et al.</i> (2007)	Iran	Female	Premenopausal	Hirsutism	790	3	0.4
Escobar-Morreale <i>et al.</i> (2008)	Spain	Female	Premenopausal	Clinical hyperandrogenism	270	6	2.2
Fanta <i>et al.</i> (2008)	Czech Republic	Female	Premenopausal	Clinical and biochemical hyperandrogenism	298	8	2.7
Al-Ruhaily <i>et al.</i> (2008)	Arabia	Female	Premenopausal	Hirsutism	148	4	2.7
Di Fede <i>et al.</i> (2010)	Italy	Female	Premenopausal	Mild hirsutism	152	3	2.0
Unluhizarci <i>et al.</i> (2010)	Turkey	Female	Premenopausal	Hirsutism, hyperandrogenism	285	6	2.1
Gonzalez <i>et al.</i> (2010)	United States	2:1 Males	Adolescent	Alopecia	57	1	1.8
Binay <i>et al.</i> (2014)	Turkey	Female	Premenopausal	Hirsutism and hyperandrogenemia	126	6	4.8
Zreik and Nasrallah (2014)	Lebanon	Female	Adolescents and adults	Hirsutism	146	7	4.6
Skordis <i>et al.</i> (2015)	Cyprus	Female	Children	Premature pubarche	59	12	20.3

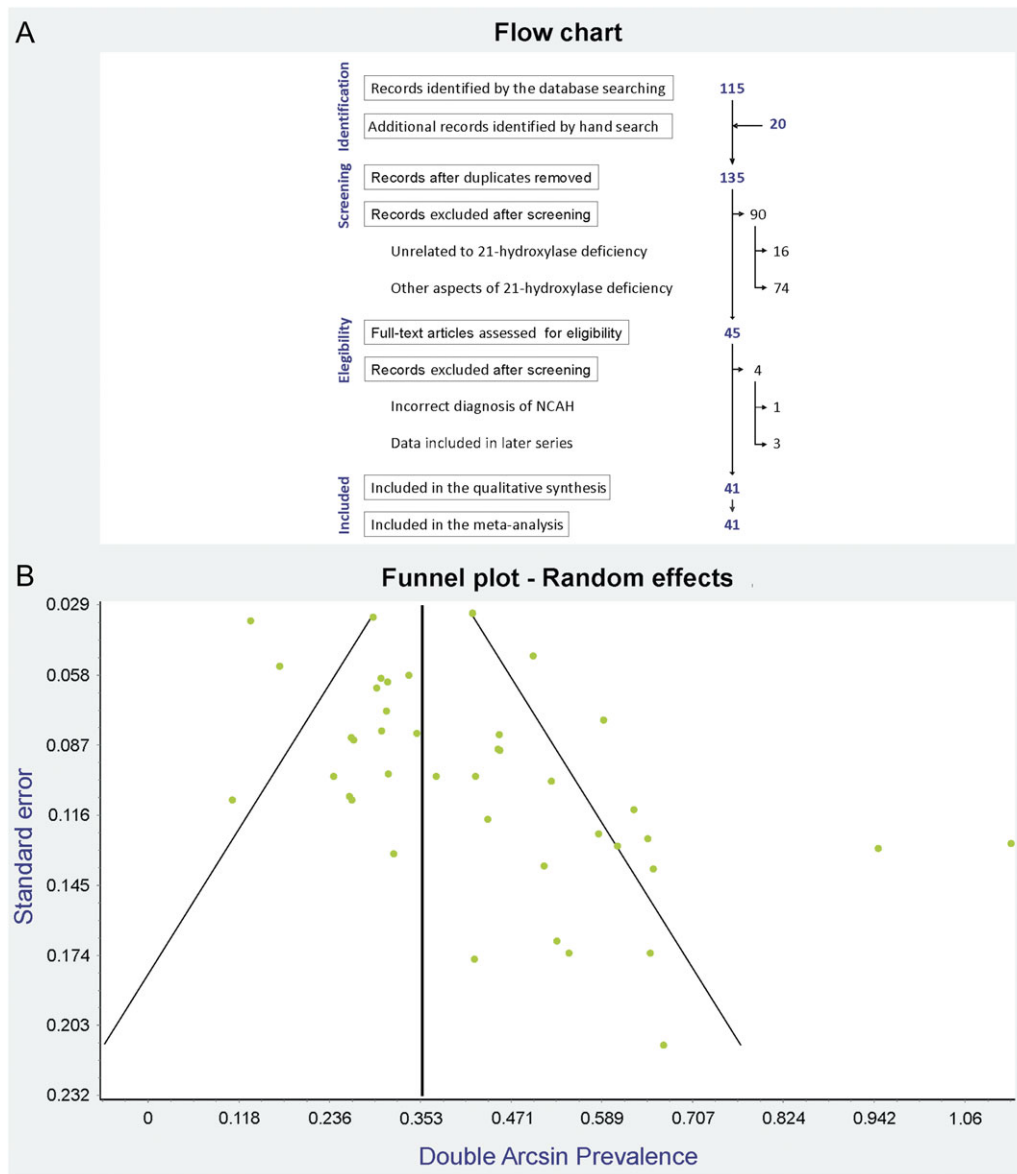


Figure 2 PRISMA flow-chart (A) and funnel plot (B) of the studies included in the meta-analysis of the prevalence of NCAH among patients presenting with signs and symptoms of androgen excess. For the calculations of standard errors and of double arcsine in the funnel plot, prevalences were introduced as per unit.

The vast majority of NCAH patients seeking medical attention have the 21-OH deficient type associated with *CYP21A2* mutations (Azziz and Zacur, 1989) (Level of Evidence: A). In contrast, NCAH due to mutations of *CYP11B1* and *HSD3B2* are extremely rare (Azziz et al., 1991; Joehrer et al., 1997; Lutfallah et al., 2002; Moran et al., 1999). Thus, when referring to NCAH, we will be solely referring to the 21-OH deficiency variety unless otherwise noted.

There are at least three approaches for determining the prevalence of NCAH. The first is based on the prevalence of carriers or genetic markers in a population. Speiser and colleagues (Speiser et al., 1985) used this approach 30 years ago when they evaluated HLA-B genotype

data in families containing multiple family members affected by NCAH together with the results of quantitative hormonal tests. These investigators estimated that the prevalence of NCAH was far more common than the classic form of 21-OH deficiency, with a prevalence of 3.7% (or 4:100) in Ashkenazi Jews, 1.9% in Hispanics, and 0.1% (or 1:1000) in the diverse Caucasian population (Speiser et al., 1985).

Another approach to understanding the prevalence of NCAH can arise from population screening for the disorder. Unfortunately, neonatal screening is relatively insensitive for identifying children affected with NCAH, and consequently population-based based data are still lacking.

Because the exclusion of NCAH is mandatory as part of the scheme to diagnose PCOS (Azziz *et al.*, 2006, 2009), a large amount of data on the prevalence of NCAH among hyperandrogenic women is available (Table I). In North America, a prevalence of NCAH of 1–2% in populations of White and Hispanic hyperandrogenic patients has been found (Chetkowski *et al.*, 1984; Moran *et al.*, 1994; Romaguera *et al.*, 2000; Sanchez *et al.*, 2001). In contrast, studies in Spain, France, Italy and Canada have yielded frequencies between 3% and 6% (Kutten *et al.*, 1985; Motta *et al.*, 1988; Innanen and Vale, 1990; Escobar-Morreale *et al.*, 2008), and other studies in the Middle East and India have demonstrated prevalences of 5–10% (Mithal *et al.*, 1988; Eldar-Geva *et al.*, 1990; Khandekar *et al.*, 1990; Arnaout, 1992; Zreik and Nasrallah, 2014). Similar to the classic form of the disorder, NCAH in Black patients is relatively uncommon (Moran *et al.*, 2000) (Level of Evidence: C).

Using these data, and understanding that hyperandrogenism affects ~10% of the female population (Azziz *et al.*, 2006, 2009), it may be estimated that 21-OH deficient NCAH affects between 1:1000/2000 (e.g. in Anglo-Saxons) and 1:100 (e.g. in Ashkenazi Jewish and certain Middle Eastern and Indian sub-continent) women in the general population, and between 1% and 10% of hyperandrogenic women depending on the ethnicity of the population being studied (Level of Evidence: C).

A much more precise estimate of worldwide prevalence of NCAH may be obtained by a meta-analysis of the large number of studies published in hyperandrogenic women. Here we report the results of a meta-analysis of all studies published to date and assessing the prevalence of NCAH among women presenting with symptoms of androgen excess. The worldwide prevalence of NCAH among hyperandrogenic women was 4.2% (95% confidence interval: 3.2–5.4%) (Table I, Figs 1 and 2) and appears to be higher than that suspected using only North American data (Level of Evidence: A).

Genetics of NCAH

NCAH is an autosomal recessive disorder due to mutations in the 21-hydroxylase (*CYP21A2*) gene resulting in a 30–50% reduction in the activity of the enzyme. This gene is mapped to a complex genetic region at chromosome 6p21.3 where it lies in close proximity to a highly homologous pseudogene, *CYP21A1P*. *CYP21A2* and *CYP21A1P* are arranged in tandem repeats with the *C4A* and *C4B* genes, which encode complement component 4. The tenascin (*TNX*) and serine threonine nuclear protein kinase (*RP*) genes are also located in this region. These four genes, *RP*, *C4*, *CYP21* and *TNX*, form a unit or module known as *RCCX*. Most alleles carry two *RCCX* modules such that one has *CYP21A2* and the other has *CYP21A1P*.

To date, over 200 *CYP21A2* mutations have been reported (<http://www.hgmd.cf.ac.uk>; <http://www.cypalleles.ki.se>). Yet, despite the large number of reported mutations, approximately 10 mutations account for the majority of affected alleles. The complexity of this locus leads to nonallelic homologous recombination resulting into gene conversion events in which the functional gene acquires deleterious *CYP21A1P* sequences. Unequal crossover and misalignment during meiosis generate duplications and deletions of the *RCCX* modules. Haplotypes with three or four *RCCX* modules have been described (Parajes *et al.*, 2008). Another example of misalignment is a *CYP21A1P/CYP21A2*

chimera in which a portion of the *CYP21A1P* gene is fused to a portion of the *CYP21A2* gene (Chen *et al.*, 2012). Rarely, CAH can be associated with uniparental disomy (Parker *et al.*, 2006). The de-novo mutation rate is ~1%.

Most NCAH individuals are compound heterozygotes showing different mutations on each allele (Tables II and III). The majority of patients carry one mutation causing severe enzyme deficiency in one allele and one mutation encoding a mild defect in the other. The NCAH phenotype roughly reflects the enzymatic activity encoded by the milder mutation. Approximately 25–50% of individuals with NCAH are reported to have mild mutations on both alleles (Speiser *et al.*, 2000; Bidet *et al.*, 2009; Livadas *et al.*, 2015). Mild mutations associated with NCAH include P30L, V281L, P453S, and R339H among others. Although the P30L mutation is typically associated with the NCAH patient phenotype, *in-vitro* functional studies indicate that P30L causes greater loss of function than the V281L and P453S mutations. Not surprisingly, the P30L mutation has also been identified in individuals with simple-virilizing CAH reflecting a greater degree of androgen excess (Barbaro *et al.*, 2015) (Level of evidence: A).

More recently, mutations located in non-coding genomic regions have been detected in patients with NCAH. For example, the promoter of the pseudogene, *CYP21A1P*, has 20% of the transcriptional activity of the *CYP21A2* promoter due to the presence of specific nucleotide variants, –126C > T, –113G > A, –110T > C, and –103A > G. Microconversions involving this region decrease *CYP21A2* gene transcription and are associated with NCAH. Additional mutations in non-coding regions of *CYP21A2* associated with NCAH include a variant in the steroidogenic factor-1 binding site located in the 5'-untranslated regulatory region and a variant located in the 3'-untranslated region (Araujo *et al.*, 2007) (Level of evidence: B).

As would be anticipated, specific *CYP21A2* mutations occur more frequently among particular ethnic groups. The mild V281L mutation associated with NCAH is the most common affected allele among Ashkenazi Jews. In other ethnic groups, severe mutations associated with CAH dominate the picture, with a large deletion being the most common mutation among Native Americans (40%) and Anglo-Saxons (28%) and the intron 2 splicing mutation being the only mutation reported for the Yupik Eskimos and being among the most common affected alleles in Iranians and Native Americans (Wilson *et al.*, 2007) (Level of evidence: B).

Using computational analyses, mutations associated with classic CAH have been found to disrupt essential enzyme functions such as membrane anchoring or substrate binding (Pallan *et al.*, 2015). Several mechanisms have been proposed for mutations associated with NCAH. Mutations associated with NCAH can be located in hydrophobic clusters, which abolish the hydrophobicity and destabilize the local environment. For the V281L mutation, the increase in chain length leads to a steric disruption, which minimally impairs enzyme activity (Haider *et al.*, 2013). Other mutations associated with NCAH may disrupt interactions with the P450 oxidoreductase protein interfering with electron transfer. Mutations that alter hydrogen bonding can destabilize the tertiary protein structure leading decreased enzyme activity (New *et al.*, 2013) (Level of evidence: B).

In general, *CYP21A2* mutations on both alleles will be identified when cosyntropin-stimulated 17-OHP concentrations are greater than 15 ng/ml (45 nmol/l). However, some individuals with diagnostic genotypes have adrenocorticotrophic hormone (ACTH)-stimulated 17-OHP values

Table II CYP21A2 mutations associated with NCAH due to 21-hydroxylase deficiency when present in homozygosis or double heterozygosity.

Nucleotide changes*	Protein	Exon	Clinical phenotype	Activity <i>in vitro</i> [†]	References
g.89C>T	p.P30L	1	NCAH/SV	60%	Tusie-Luna et al. (1991)
g.140A>G	p.Y47C	1	NCAH	–	Tardy and Morel (2007)
g.185A>T	p.H62L	1	NCAH	45%	Ezquieta et al. (2002), Pinto et al. (2003), Soardi et al. (2008)
g.734A>G	p.H119R	3	NCAH	32%	Capoluongo et al. (2008b, d), Concolino et al. (2009a)
g.739A>C	p.K121Q	3	NCAH/SV	14%	Riepe et al. (2008)
g.749G>A	p.R124H	3	NCAH	–	Usui et al. (2004)
g.772C>T	p.R132C	3	NCAH	35%	Minutolo et al. (2011), Taboas et al. (2014)
g.817T>C	p.C147R	3	NCAH/SV	–	Robins et al. (2006)
g.929C>T	p.R149C	4	NCAH	36	Minutolo et al. (2011), Taboas et al. (2014)
g.929G>C	p.R149P	4	NCAH	23%	Chu et al. (2014)
g.981T>C	p.L166P	4	NCAH	–	Robins et al. (2006)
g.987C>A	p.T168N	4	NCAH	–	Vrzalova et al. (2010)
g.996T>A	p.I171N	4	NCAH	–	Barbaro et al. (2006)
g.1153T>A	p.I194N	5	NCAH	33%	Capoluongo et al. (2008c), Concolino et al. (2009a)
g.1343C>T	p.R224W	6	NCAH	52%	Concolino et al. (2007, 2009b)
g.1362T>C	p.I230T	6	NCAH	63%	Tardy et al. (2010)
g.1380A>G	p.R233G	6	NCAH	8%	Robins et al. (2006), Barbaro et al. (2015)
g.1588T>C	p.V249A	7	NCAH	–	Concolino et al. (2010)
g.1683G>T	p.V281L	7	NCAH	50%	Speiser et al. (1988), Tusie-Luna et al. (1990), Barbat et al. (1995)
g.1689A>C	p.M283L	7	NCAH	–	Ezquieta et al. (2002)
g.1689A>G	p.M283V	7	NCAH	16%	Minutolo et al. (2011), Taboas et al. (2014)
g.1744C>A	p.S301Y	7	NCAH	–	Stikkelbroeck et al. (2003)
g.1752G>A	p.V304M	7	NCAH	46%	Lajic et al. (2002)
g.1981C>A	p.L317M	7	NCAH	–	Deneux et al. (2001)
g.1981C>G	p.L317V	7	NCAH	–	Bojunga et al. (2005)
g.2012A>G	p.D322G	8	NCAH	18%	Loidi et al. (2006), Bleicken et al. (2009), Minutolo et al. (2011)
g.2058G>A	p.R339H	8	NCAH	50%	Helmberg et al. (1992)
g.2063C>T	p.R341W	8	NCAH	5%	Barbaro et al. (2015)
g.2064G>C	p.R341P	8	NCAH/SV	0.7%	Pinto et al. (2003), Barbaro et al. (2006)
g.2138C>T	p.R366C	8	NCAH	37%	Robins et al. (2006), Barbaro et al. (2015)
g.2147C>T	p.R369W	8	NCAH/SV	46%	Tardy et al. (2010)
g.2286C>G	p.N387K	9	NCAH	–	Wasniewska et al. (2009)
g.2296G>A	p.A391T	9	NCAH	38%	Robins et al. (2006, 2007)
g.2344G>A	p.D407N	9	NCAH	73%	Capoluongo et al. (2008a), Concolino et al. (2009b)
g.2512G>A	p.E431K	10	NCAH	–	Dain et al. (2006), Minutolo et al. (2011)
g.2524C>T	p.R435C	10	NCAH	–	Deneux et al. (2001)
g.2578C>T	p.P453S		NCAH	50–68%	Helmberg et al. (1992), Owerbach et al. (1992), Nikoshkov et al. (1997)
g.2597C>A	p.P459H	10	NCAH	–	Wang et al. (2007)
g.2640G>T	p.M473I	10	NCAH	85%	Robins et al. (2006), Barbaro et al. (2015)
g.2657G>T	p.R479L	10	NCAH	–	Zeng et al. (2004)
g.2665C>T	p.P482S	10	NCAH	70%	Balsamo et al. (2000), Barbaro et al. (2004)
g.2669G>C	p.R483P	10	NCAH	–	Wedell and Luthman (1993), Nikoshkov et al. (1998)
g.2669G>A	p.R483Q	10	NCAH	1.1%	Stikkelbroeck et al. (2003), Robins et al. (2007)

Updated from <http://www.cypalleles.ki.se/cyp21.htm> (last accessed Nov 1 2016) and Haider et al. (2013).

NCAH, non-classic congenital adrenal hyperplasia; SV, simple virilizing classic congenital adrenal hyperplasia.

*Numbering starts from A in the initiation codon. [†]Using 17-OHP as substrate.

Table III CYP21A2 genotypes associated with NCAH due to 21-hydroxylase deficiency in different series.

Country (n)	Multi-center (34)	France (161)	Argentina (159)	United States (43)	United States (545)	Brazil (114)	Greece (280)
References	Speiser et al. (2000)	Bidet et al. (2009)	Marino et al. (2011)	Finkelstein et al. (2012)	New et al. (2013)	Moura-Massari et al. (2013)	Livadas et al. (2015)
V281L/V281L	20.6	25	43.4	27.9	38.5	47.5	38.1
V281L/Intron 2	17.6	17.7	15.1	7.0	19.8	16.2	20.6
V281L/LG	5.9	9.7	8.2	11.6	17.4	11	7.8
V281L/R356W	2.9	2.4	1.9	2.3	2.4	5.1	1.4
V281L/I172N	11.8	5.6	6.9	5.0	5.7		12.1
V281L/Q318X	5.9	5.6	3.8	7.0	3.7		5.0
V281L/exon 3	2.9	0.8	0.6		1.0	1.0	8.5
V281L/P453S		7.2	5.0	2.3		7.1	23.7
V281L/P30L		1.6	1.3		1.3		18.0
V281L/intron 2, V281L			1.9	2.3		1.0	1.4
V281L, R483fs/LG						1.0	
V281L/intron 2, Q318X			0.6				
V281L/Intron 2, R356W						1.0	
V281L,P453S/P453S		0.8					
V281L/W22X							2.1
V281L/R483P		2.4					1.4
V281L/5'gene conversion		3.2					
V281L/W19X		0.8					
V281L/exon 6			1.3		1.3		
V281L/L307fs	2.9	0.8					
V281L/S460_P465del		0.8					
V281L/R408C		0.8					
V281L/P482S				2.3			3.6
V281L/R426P				2.3			
V281L/complex rearrangement	5.9	0.8					
V281L/I172N,V281L		0.8					
P453S/P453S							2.9
P453S/W22X							0.07
P453S/intron 2	2.9	0.8	0.6				4.3
P453S/exon 3							2.1
P453S/I172N				2.3			1.4
P453S/LG			1.3				0.01
P453S/exon 6		0.8					
P453S/R356W	2.9						
P453S/Q318X	2.9						
P453S/Q318X, R356W		0.8					
P453S/P482S							2.2
P30L/P30L					0.4		5.8
P30L/I172N		0.8		2.3	1.3		5.0
P30L/intron 2		0.8		2.3	2.8		7.8

Continued

Table III *Continued*

Country (n)	Multi-center (34)	France (161)	Argentina (159)	United States (43)	United States (545)	Brazil (114)	Greece (280)
References	Speiser et al. (2000)	Bidet et al. (2009)	Marino et al. (2011)	Finkelstein et al. (2012)	New et al. (2013)	Moura-Massari et al. (2013)	Livadas et al. (2015)
P30L/V281L,R483Q		0.8					
P30L/R356W					0.4		0.07
P30L/Q318X					0.4		0.01
P30L/P453S	2.9						5.8
P30L/exon 6					0.2		
P30L/intron 2			1.2				5.0
P30L/exon 3					0.4		5.0
P30L/LG				2.3	2.3		3.5
P30L/L307fs							0.07
P30L + other mutations	8.8						
I172N/P482S							2.8
Intron 2/P482S							0.01
Intron 2/promoter							0.01
Intron 2/I172N				2.3			
Intron 2/LG						1.0	
I172N/I172N				2.3			
R356W/P482S							0.07
R435C/Q318X		0.8					
I172N, R356W, P453S*	2.9						

Data are frequencies (%).

Abbreviations: LG, large deletion; intron 2, IVS2-13A/C>G (splicing mutation); exon 3, 8-bp deletion (G110Efs); exon 6, I236N + V237E + M239K; L307fs, T insertion in exon 7;

*, alleles were not fully segregated.

between 10 and 15 ng/ml (30–45 nmol/l). In some instances, molecular genetic analysis may be necessary to confirm the diagnosis of NCAH (Ambroziak et al., 2016).

Pathophysiology of NCAH

The pathophysiology of classic 21-hydroxylase deficiency is well known (Merke, 2015). More severe forms of 21-hydroxylase deficiency (salt-wasting and simple virilizing CAH) are the consequence of the impairment of cortisol biosynthesis with accumulation of steroid intermediates (mainly 17-OHP). The resulting reduction of circulating cortisol leads to increased ACTH production and to hyperstimulation of adrenal androgen pathway. The excessive ACTH stimulation also determines the hypertrophy of fasciculata and reticularis zones, leading to adrenal hyperplasia and to adrenocortical nodularity in some cases (Falhammar and Torpy, 2016) (Level of evidence: A).

In contrast, the pathophysiology of non-classic 21-hydroxylase deficiency may be more complex (Fig. 3). In fact, while a few patients present with increased ACTH and have the same pathophysiological mechanisms of CAH (Chrousos et al., 1982), in the majority of the affected subjects with NCAH, ACTH production is normal (Azziz et al., 1994). Cortisol response to ACTH may be normal or slightly

impaired but some patients with NCAH may have an over-responsive glucocorticoid response to ACTH stimulation (Carmina et al., 1984; Carmina and Lobo, 1990; Azziz et al., 1994; Carmina, 1995). However, adrenal androgen secretion and its response to ACTH are increased in NCAH (Carmina et al., 1984; Carmina and Lobo, 1990; Azziz et al., 1994; Carmina, 1995). Interestingly, in these patients, DHEAS serum levels are generally normal while androstenedione, testosterone and DHT are elevated but similar to the levels found in PCOS patients (Carmina et al., 1984; Carmina and Lobo, 1990; Levin et al., 1991; Azziz et al., 1994; Carmina, 1995) (Level of evidence: A).

It has been difficult to understand how increased adrenal androgen secretion may be maintained in the absence of increased ACTH. It has been suggested that in NCAH, excessive adrenal androgen secretion mainly results from altered enzyme kinetics due to CYP21A2 missense mutations (Witchel and Azziz, 2010). The mutated enzyme is transcribed and translated into a protein, but the enzyme protein is less efficient than the wild type. The net result is an increased precursor to product ratio, independent of ACTH levels. This concept is important in treatment because normalization of 17OHP levels may lead to excessive glucocorticoid administration.

Other mechanisms may contribute to hyperandrogenism of NCAH patients including ovarian dysfunction and peripheral synthesis of androgens from steroid precursors (Fig. 3). Polycystic ovarian

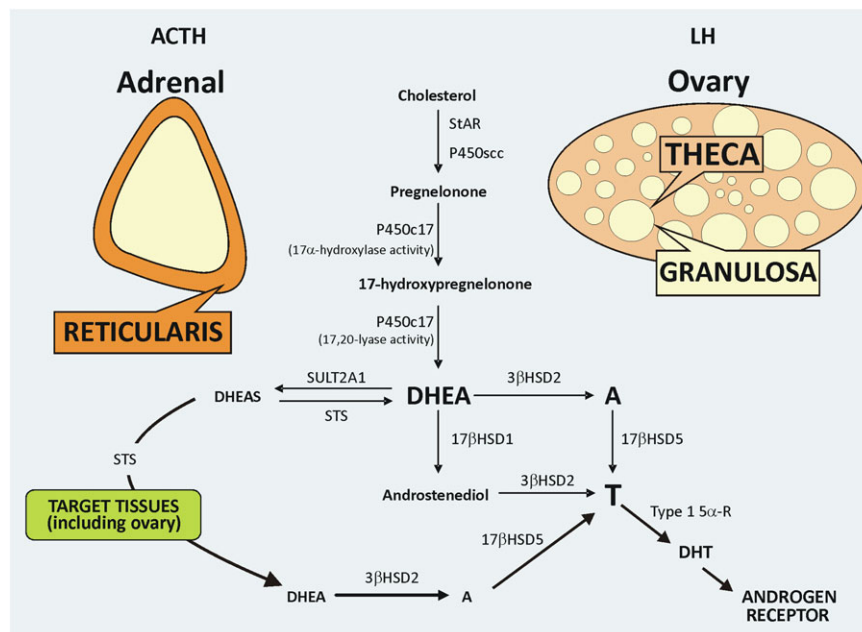


Figure 3 Mechanisms possibly contributing to androgen excess in NCAH. 3βHSD2, 3β-hydroxysteroid dehydrogenase type 2; 5α-R, 5α-reductase type 1; 17βHSD5, 17β-hydroxysteroid dehydrogenase type V; A, androstenedione; ACTH, adrenocorticotropin; DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone-sulfate; DHT, dihydrotestosterone; HPA, hypothalamic–pituitary–adrenal; HPO, hypothalamic–pituitary–ovary; P450c17, 17α-hydroxylase/17,20-lyase; P450ssc, cholesterol side chain cleavage enzyme; StAR, steroidogenic acute regulatory protein; STS, steroid sulfatase; SULT2A1, sulfotransferase; T testosterone.

morphology is a frequent finding in women with NCAH (Levin *et al.*, 1991; Carmina and Lobo, 1994; Carmina, 1995; Pall *et al.*, 2010; Witchel and Azziz, 2010) and ovarian dysfunction may contribute to androgen excess in these women (Level of evidence: B), as demonstrated by the improvement of clinical and biochemical hyperandrogenism after ovarian suppression (Carmina and Lobo, 1994). The exaggerated adrenal secretion of progesterone and androgens might disrupt the hypothalamic–pituitary–ovarian axis favouring rapid GnRH pulse frequency and LH hypersecretion contributing to ovarian androgen excess (Blank *et al.*, 2007). Chronic androgen excess, and glucocorticoid administration around puberty in certain cases, may favor abdominal visceral adiposity, insulin resistance and its metabolic consequences, and exacerbate adrenal and ovarian androgen production (Escobar-Morreale and San Millan, 2007; Kim and Merke, 2009).

In addition, the role of a backdoor pathway in the hyperandrogenism of NCAH has been suggested (Auchus, 2004). Both progesterone and 17-OHP may be converted into more potent androgens such as dihydrotestosterone (DHT) by the backdoor pathway (Kamrath *et al.*, 2012) (Fig. 3). This pathway involves 5αpregnane-3α-, 17α-diol-20-one (PdIol) synthesis after the 5α- and 3α reduction of 17-OHP by 5α-R type I and reductive 3αHSD type I–4 enzymes (AKRIC). Then, PdIol is cleaved to androsterone by the 17α-hydroxylase and 17, 20-lyase activities of P450c17, and androstenediol is synthesized from the latter by type 3/6 17β-hydroxysteroid dehydrogenase. Finally, 3α-hydroxysteroid dehydrogenase can oxidize androstenediol to DHT in target tissues (Kamrath *et al.*, 2012; Turcu *et al.*, 2014) (Level of evidence: B).

In summary, hyperandrogenism in NCAH results from (Fig. 3):

- (1) Adrenal hyperactivity depending on altered enzyme kinetics without increased ACTH circulating levels.
- (2) Increased peripheral conversion to androgens of circulating excessive levels of steroid metabolites.
- (3) Increased ovarian androgen secretion determined by the appearance of a secondary PCOS-like phenotype in NCAH patients.

Clinical presentation of NCAH

Clinical presentation of NCAH during childhood and adolescence

Most children with NCAH are asymptomatic in the prepubertal years and exhibit normal external genitalia at birth and throughout early childhood. Premature pubarche may be the first clinical presentation with onset reported as early as 6 months of age (Level of evidence: A). In one study including 25 females younger than 10 years-old, 92% had premature pubarche as presentation of NCAH (Moran *et al.*, 2000). In another study, as many as 60% of children with NCAH presented with premature adrenarche (Kohn *et al.*, 1982). In fact, the prevalence of NCAH in children with premature adrenarche has been reported to vary from 5% to 20% (Dacou-Voutetakis and Dracopoulou, 1999).

Children with NCAH may demonstrate rapid linear growth, bone age advancement and tall stature (Oberfield *et al.*, 2011). Short stature has also been reported in subjects with NCAH, especially when

glucocorticoid therapy was begun prior to the onset of puberty (Speiser et al., 2010). In a cohort of 30 adult NCAH patients, 7% had a predicted adult height standard deviation score (SDS) of -2.0 or less (Finkelstein et al., 2012). Among 141 women with NCAH studied by Livadas and colleagues (Livadas et al., 2015), only 1 (0.7%) demonstrated short stature (defined as height less than the third percentile for sex and age using local population standards). Overall, while some NCAH patients may be at risk for short stature, this risk appears to be relatively small and most children reach a relatively normal final height for family standards (Speiser et al., 2010) (Level of evidence: B). This likely is due to the fact that these children have not been exposed to prolonged suppressive effects of glucocorticoid treatment on linear growth (Weintrob et al., 1997).

Female adolescents may present with severe acne, hirsutism, and even alopecia (Moran et al., 2000; New, 2006) (Level of evidence: B). Moreover, clitoromegaly can be found in 11% of adolescents (Moran et al., 2000). In a study of 220 adolescents, menstrual irregularities (56%) or even primary amenorrhea (9%) were the presenting sign of NCAH (Moran et al., 2000). The long-term outcome of these findings will be discussed later with respect to fertility in this population.

In male adolescents, gynecomastia may be the presenting symptom. Wasniewska et al. (2008) reported two boys presenting with either prepubertal or pubertal gynecomastia. In both boys, gynecomastia completely regressed 5–8 months after the institution of glucocorticoid replacement therapy. They suggested that NCAH due to 21-hydroxylase deficiency should be kept in mind in the differential diagnosis of either prepubertal or pubertal gynecomastia (Wasniewska et al., 2008).

Limited data exist in patients with NCAH regarding obesity, metabolic syndrome, and exercise intolerance. It has been suggested that children with classic CAH have increased fat mass (Speiser et al., 1992). The BMI distribution in German patients with CAH, although not NCAH patients, was skewed towards larger figures when compared with appropriate controls (Volkl et al., 2006). Similarly, increased abdominal adiposity was noted in adolescents and young adults with classic CAH due to 21-hydroxylase deficiency with increased visceral to subcutaneous adipose tissue ratios when compared with matched controls (Kim et al., 2015). Finally, adolescents with congenital adrenal hyperplasia exhibit impaired exercise performance and an enhanced systolic blood pressure response to exercise that appears to be related to glucocorticoid therapy (Marra et al., 2015). Extrapolation of these findings to patients with NCAH must be considered cautiously.

Clinical presentation of NCAH in adult women

Clinical symptoms in adult women with 21-hydroxylase deficient NCAH encompass two principal areas: those due to hypothalamic–pituitary–ovarian dysfunction and those due to dermatologic signs of hyperandrogenism. Other clinical symptoms in adulthood may include metabolic dysfunction and anatomic defects of the adrenal cortex.

Adult women with NCAH generally present with hirsutism, acne and/or androgenic alopecia. In various studies, the prevalence of hirsutism ranged from 60% to 80% (Bidet et al., 2009; Moran et al., 2000; Pall et al., 2010; Finkelstein et al., 2012), acne was found in approximately one-third of cases (Moran et al., 2000), and alopecia was seen in 2–8% (Moran et al., 2000; Livadas et al., 2015) (Level of evidence: A). Clitoromegaly has been reported in 6–20% of adult women with NCAH (Moran et al., 2000).

Over 30–50% of patients with NCAH show overt ovulatory and menstrual dysfunction (Moran et al., 2000; Livadas et al., 2015) (Level of evidence: B). Polycystic ovarian morphology (PCOM) may also be present. In one study, enlarged ovaries were found in 44% of 25 adult women with NCAH and PCOM was referred (but the number of follicles was not measured) in 80% of these patients (Carmina, 1995). In another study, PCOM was found in only 24% of NCAH patients (Pall et al., 2010).

Even though adrenal hyperplasia and adenomas have been reported in patients with NCAH, the actual prevalence of such pathology remains largely unknown (Azziz and Kenney, 1991). However, the finding of a small increase in the prevalence of genotypically verified CAH, including NCAH, in patients presenting with adrenal incidentalomas in a recent meta-analysis further suggests an association between both disorders (Falhammar and Torpy, 2016) (Level of evidence: B). Accordingly, patients with adenomas identified incidentally should be first evaluated for NCAH/CAH before any attempt of surgical treatment (Jaresch et al., 1992). Tumor size may improve with corticosteroid therapy (Kiedrowicz et al., 2015) (Level of evidence: D). Whether or not adrenal incidentalomas in patients with NCAH require follow-up is still unclear, even though in the authors' clinical experience progression to malignancy or to syndromes of hormone hypersecretion are very unlikely (Level of evidence: C). In a series of parents of patients with classic CAH, 10/249 (4%) were found to have cryptic/asymptomatic NCAH and one female patient was found to have a small adrenal myelolipoma (Nandagopal et al., 2011).

Some investigators have reported that adult males and females with NCAH demonstrate evidence of mild insulin resistance (Bayraktar et al., 2004), although others have been unable to confirm these findings (Moran et al., 2000). One recent study reported that adult patients with NCAH demonstrated an increased risk of metabolic and cardiovascular morbidities (Falhammar et al., 2015). Not only were obesity and type 2 diabetes much more common in the NCAH patients than in the general population, but the odds ratio for cardiovascular disease (mainly for stroke) was also elevated (2.9). However, the number of NCAH patients ($n = 75$) was relatively small and the number of events were too few to accurately assess the actual prevalence of cardiovascular events in adult subjects with NCAH. Nevertheless, and even though these preliminary data need confirmation in larger follow-up studies and in other populations, it appears reasonable and safe to extend to NCAH subjects the current recommendations for the assessment and management of cardiometabolic dysfunction also observed in PCOS (Wild et al., 2010).

Fertility and reproductive outcome in women with NCAH

Despite the adrenal and ovarian androgen excess, the majority of women with NCAH will conceive spontaneously (Feldman et al., 1992; Moran et al., 2006) (Level of evidence: B). Nonetheless, in one study, the rate of a singleton live birth was higher in NCAH women diagnosed and treated for their disorder prior to conceiving than in those patients who conceived spontaneously (86% vs. 69%, respectively); there were no differences in the rate of ectopic pregnancy, preterm birth, stillbirths, twins or multiple pregnancies (Moran et al., 2006). Spontaneous miscarriages have been reported in 25% of pregnancies occurring before the diagnosis of NCAH was made, a figure that decreases to 10% in women in whom diagnosis was performed

and treatment was started before pregnancy (Moran *et al.*, 2006; Bidet *et al.*, 2010), suggesting that prompt diagnosis and therapy may reduce the rate of pregnancy loss in NCAH (Level of evidence: B).

Between 10% and 30% of NCAH women of reproductive age complain of infertility (Moran *et al.*, 2000, 2006; Bidet *et al.*, 2010) (Level of evidence: B). Anovulation is the main cause of subfertility in NCAH women, which is most evident by the positive outcome in response to ovulation induction (Reichman *et al.*, 2014; Lekarev *et al.*, 2015). However, persistent elevated progesterone concentrations (due to excess circulating levels of progesterone and 17-OHP of adrenal origin) may also result in an unfavorable cervical mucus and a persistent decidualized or hypo- or atrophic endometrium (Reichman *et al.*, 2014).

Clinical presentation of NCAH in adult males

Data regarding adult male patients with NCAH due to 21-hydroxylase deficiency are extremely limited. Only a few papers including case reports and small series are available in the literature. It would be expected that NCAH occurs equally in men and women, since it is an autosomal recessive disease. But among the 440 patients with NCAH patients, only 96 were males (New, 2006). Accordingly, the great majority of male patients are asymptomatic and most are identified during genetic screening carried out for purposes of genetic counseling. Only a few seek medical advice because of prepubertal or pubertal manifestations (Witchel, 2013).

Testicular adrenal rest tumors (TARTs) are the most important cause of infertility in male patients with CAH due to 21-hydroxylase deficiency and are more frequent in the salt-wasting form (Cabrera *et al.*, 2001). While TART may present also in males with NCAH due to 21-hydroxylase deficiency (Chrousos *et al.*, 1981; Falhammar *et al.*, 2012; Nandagopal *et al.*, 2011), most data suggest that TART are quite uncommon in these patients (Pinkas *et al.*, 2010). Hence, the routine measurement of 17-OHP in the evaluation of male infertility is not recommended (Level of evidence: B).

Diagnosis of NCAH

Measurement of basal and cosyntropin-stimulated 17-hydroxyprogesterone concentrations

The clinical picture does not permit a definite diagnosis of NCAH (Table IV, Level of evidence: A). Moreover, the NCAH phenotype may

be highly variable even within a family sharing the same CYP21A2 genotype (Bidet *et al.*, 2009). Hence, the diagnosis of NCAH relies mostly on the finding of serum 17-OHP concentrations above 10 ng/ml (30 nmol/l) either in basal conditions or after cosyntropin-stimulation (New *et al.*, 1983; Dewailly *et al.*, 1986; Azziz and Zacur, 1989) (Level of evidence: A).

Screening is conducted by measuring this steroid precursor early in the morning during the follicular phase of the menstrual cycle. A basal 17OHP level cut-off value of 2 ng/ml (6 nmol/l) is recommended for screening, although ideally the reference value should be established at each laboratory since lower figures must be valid based on specific methodology used for 17-OHP determinations (Escobar-Morreale *et al.*, 2008) (Table IV). An increased basal 17-OHP result less than 10 ng/ml (30 nmol/l) must be confirmed by the finding of 17OHP above 10 ng/ml (30 nmol/l) after stimulation of adrenal function by an intravenous bolus of cosyntropin (Azziz *et al.*, 1994; Trapp and Oberfield, 2012).

The cosyntropin-stimulation test should be performed in the morning during the follicular phase of the menstrual cycle. 17-OHP concentrations are measured 30 and/or 60 min after a 250 µg intravenous bolus of cosyntropin. The 10 ng/ml (30 nmol/l) cut-off value corresponds approximately to 3-fold the upper normal limit of 17-OHP concentrations observed in normal women after cosyntropin-stimulation. If available, CYP21A2 genotyping should follow the biochemical diagnosis of NCAH, not only for diagnostic confirmation but also for detecting compound heterozygosity for severe alleles that may result in CAH cases in the progeny of these patients (Table IV). Of note, normal basal and ACTH-stimulated 17-OHP responses do not exclude a carrier status for mild or severe CYP21A2 mutations (Armengaud *et al.*, 2009) (Table IV).

For children presenting with premature pubarche, consideration of the diagnosis of NCAH is warranted when elevated basal 17-OHP, androstenedione, and testosterone concentrations are elevated and/or bone age is advanced. It has been suggested that a cut-off value of basal 17-OHP > 2 ng/ml is adequately sensitive and specific to identify prepubertal children with NCAH (Armengaud *et al.*, 2009).

The effectiveness of this diagnostic approach in adolescents and adults is supported by several studies (Azziz *et al.*, 1999; Bidet *et al.*, 2009; Nandagopal *et al.*, 2011). However, the election of a cut-off basal 17-OHP value for the screening of 21-hydroxylase deficient NCAH is matter of debate. In a study by Azziz *et al.* (1999), eight healthy controls, 20 patients with genetically confirmed NCAH and 284 patients with hyperandrogenism were investigated with the aim

Table IV Evidence based recommendations for the diagnosis of NCAH (Level of evidence A or B).

- (1) We recommend screening for NCAH due to 21-hydroxylase deficiency in all patients presenting with signs and symptoms of androgen excess except in specific ethnic populations in which the prevalence of this disorder is negligible.
- (2) We recommend the clinical diagnosis of NCAH due to 21-hydroxylase deficiency to require a circulating 17-OHP concentration above 10 ng/ml (30 nmol/l) either basally or after cosyntropin-stimulation.
- (3) We recommend CYP21A2 genotyping in patients presenting with circulating 17-OHP concentration above 10 ng/ml (30 nmol/l) with the aim of confirming the diagnosis and identifying severe alleles that may increase the risk of CAH in the offspring of NCAH patients.
- (4) We suggest establishing local assay-specific 17-OHP cut-off values for the screening of NCAH due to 21-hydroxylase deficiency. If these are not available, conducting cosyntropin-stimulation in patients presenting with basal 17-OHP above 2 ng/ml (6 nmol/l) would identify most patients with the disorder.
- (5) We recommend against making any conclusion about the CYP21A2 carrier status of any person based on the results of a cosyntropin-stimulation test.

of establishing the cut-off value of basal 17-OHP concentrations for the screening of NCAH due to 21-hydroxylase deficiency. A cosyntropin-stimulated 17-OHP level above 10 ng/ml was used as the criterion for the diagnosis. They suggested that a basal 17-OHP level of 2 ng/ml should be used to maximize detection of NCAH.

However, Escobar-Morreale *et al.* (2008) found that the most appropriate cut-off value for the diagnosis of NCAH due to 21-hydroxylase deficiency was a basal follicular phase 17-OHP above 1.7 ng/ml (5.1 nmol/l), which had 100% sensitivity and 88.6% specificity, in a series of 270 consecutive women with hyperandrogenism submitted to cosyntropin-stimulation, of whom six had NCAH confirmed by *CYP21A2* molecular genetic testing. The authors suggested that the cut-off value should be established for every laboratory, or, if this is not possible, the cut-off value should be lowered to 1.7 ng/ml (5.1 nmol/l) from 2 ng/ml (6 nmol/l) as the upper limit of the normal range. Of note, these suggestions are reinforced by the finding of basal plasma 17-OHP levels below 2 ng/ml (6 nmol/l) in 13 of the 161 NCAH patients reported by Bidet *et al.* (2009).

Unluhizarci *et al.* (2010) investigated the prevalence of NCAH due to 21-hydroxylase deficiency in 285 Turkish women with hirsutism or hyperandrogenism in a nationwide study. When cosyntropin-stimulated 17-OHP levels exceeding 10 ng/ml (30 nmol/l) was accepted as hormonal criterion, 2.1% of the women had NCAH. The diagnosis was confirmed by *CYP21A2* genotyping in all patients. Because the lowest 17-OHP level in NCAH women in that study was 2.2 ng/ml (6.7 nmol/l), the 2 ng/ml (6 nmol/l) cut-off value appeared to be adequate in this setting.

In a multi-center study including 220 women with NCAH due to 21-hydroxylase deficiency, defined by basal and/or cosyntropin-stimulated 17-OHP concentrations above 10 ng/ml (30 nmol/l), the median ACTH-stimulated 17-OHP level was 37 ng/ml (113 nmol/l) yet 10% of the patients had basal 17-OHP values below 2 ng/ml (6 nmol/l) (Moran *et al.*, 2000). According to this study, 10% of NCAH patients would be undiagnosed if the cut-off for basal 17-OHP level was established at a 2 ng/ml (6 nmol/l) concentration.

In conclusion, we suggest using a 2 ng/ml (6 nmol/l) cut-off value of basal 17OHP concentrations for the screening of NCAH in cases where in-house cut-off values are not available (Table IV, Level of evidence: A).

Genetic aspects of diagnosis

The *CYP21A2* locus is quite complicated, precluding its molecular genetic analysis as the first line diagnostic test (Level of evidence: A). However, it is essential for genetic counseling since many patients with NCAH carry a severe allele that might result in CAH in their progeny (Table IV, Level of evidence: A).

Since one allele can carry multiple mutations, merely identifying two *CYP21A2* mutations in the absence of diagnostic hormone test results is insufficient to confirm the diagnosis of CAH. Multiple genetic testing strategies such as PCR-based mutation detection methods, DNA sequencing, and multiplex ligation-dependent probe amplification may be needed to accurately ascertain the mutations in an affected individual. In some instances, it is necessary to confirm that mutations are on opposite alleles. To segregate the specific alleles, *CYP21A2* genetic analyses can be obtained from parents to discriminate the specific maternal and paternal mutations.

During a study that involved comprehensive genetic testing of 145 unrelated patients with CAH, 10/249 (4%) parents were found to have genotype analyses diagnostic for NCAH (Nandagopal *et al.*, 2011). All 10 parents were compound heterozygotes for a severe mutation on one allele and a mild mutation on their other allele; their affected children had inherited the severe mutation. These parents were largely asymptomatic, but ~50% of the mothers reported subfertility.

Genetic analysis can be a useful adjunct to newborn screening for family studies, and to accurately distinguish between heterozygous mutation carriers and affected individuals (Ambroziak *et al.*, 2016). Importantly, the caveats for molecular diagnosis include the complexity of this genetic locus, identification of multiple mutations on a single allele, detection of different *CYP21A2* mutations within a family, and cryptic NCAH in other family members (Level of evidence: B).

In Fig. 4, an algorithm for the diagnosis of NCAH is shown.

Treatment of NCAH

Prepubertal and adolescent patients

Treatment with glucocorticoids for NCAH should be considered only for pre- and peripubertal children who have inappropriately early onset or rapid progression of pubarche or bone age (Table V, Level of evidence: B). Routine treatment with GnRH analogs and GH are not recommended and should be considered experimental (Trapp and Oberfield, 2012). In adolescents and young adults with NCAH, treatment is reserved for those who demonstrate important or clinically significant hyperandrogenism. However, recent data suggest that replacement with glucocorticoids should be considered when major surgery or trauma in childhood or even when 'fatigue' occurs, particularly in those NCAH patients with a decreased cortisol response to cosyntropin. A cortisol cut-off value of 18 µg/dl (497 nmol/l), below which glucocorticoid therapy may be considered, has been recommended in children and adolescents (Stoupa *et al.*, 2015) (Level of evidence: C).

Adult women

In Fig. 5, an algorithm for the treatment of NCAH is shown.

Treatment of hyperandrogenism

Theoretically adrenal androgen excess could be treated either by suppressing ACTH production with glucocorticoids and therefore lowering the excessive androgen production by the adrenal glands or by blocking the effects of androgens on their receptors with anti-androgens (Escobar-Morreale *et al.*, 2012). However, in adult women with NCAH, reduction of circulating androgens may also be obtained by blocking ovarian androgen secretion by the use of estrogen-progestin combination preparations or GnRH agonists (Carmina and Lobo, 1994; Trapp and Oberfield, 2012). Most available data suggest that, in these patients, both peripheral androgen blockade or ovarian androgen suppression are more effective than glucocorticoids in reducing circulating androgens (Frank-Raue *et al.*, 1990; Spritzer *et al.*, 1990) (Table V, Level of evidence: B). One controlled randomized trial on a series of 30 patients compared the effectiveness of cyproterone acetate (CPA) versus hydrocortisone (Spritzer *et al.*, 1990). Based on the hirsutism score, CPA was more effective than hydrocortisone (hirsutism improved in 54% and 24% patients, respectively). In another randomized trial

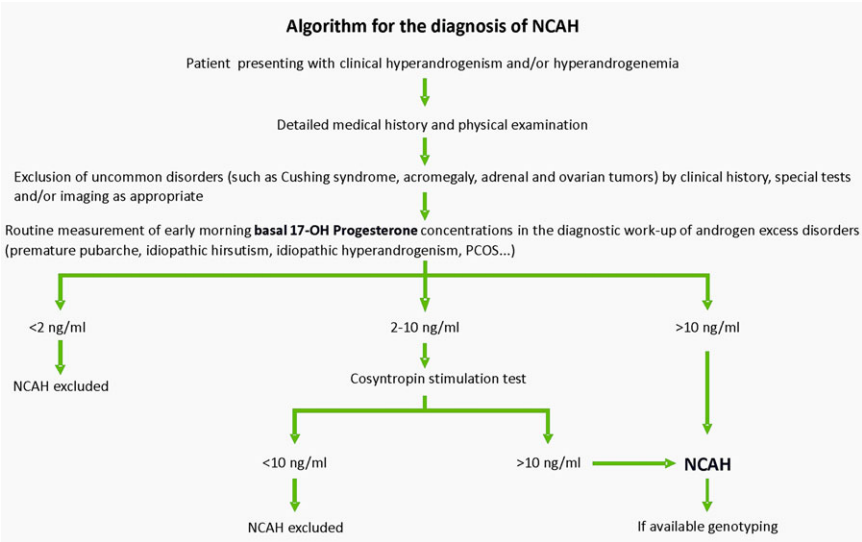


Figure 4 Algorithm for the diagnosis of NCAH. 17-OH progesterone, 17-hydroxyprogesterone.

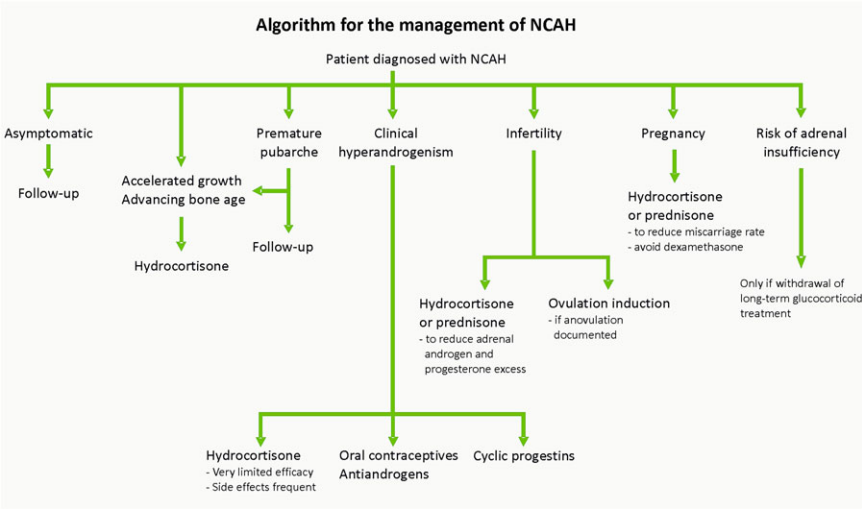


Figure 5 Algorithm for the treatment of NCAH.

including 28 patients, CPA associated with ethinylestradiol was found to be superior to dexamethasone (hirsutism improved in 66% and 31% patients, respectively) (Frank-Raue *et al.*, 1990). In countries where CPA is not available, spironolactone could be used as an alternative androgen blocking drug. Although the anti-mineralocorticoid action of spironolactone could be a concern when treating patients with NCAH, to date this hypothetical risk has not been documented. A concern regarding the use of spironolactone, or for that matter any anti-androgen in a female experiencing unprotected coitus, would be the risk of impaired virilization of the external genitalia of a male fetus in the event that an unanticipated pregnancy occurred. Generally, when

fertility is not an immediate concern, estrogen–progestin combination preparations represent the most commonly used treatment of adult women with NCAH (Escobar-Morreale *et al.*, 2012).

Management of infertility in NCAH

NCAH adult women who have not conceived spontaneously and who demonstrate overt or subclinical ovulatory dysfunction may benefit from glucocorticoids or from ovulation induction (Table V, Level of evidence: B).

Several studies, mostly retrospective and including small series, suggest that glucocorticoids improve fertility probably by lowering adrenal

Table V Evidence based recommendations for the treatment of NCAH (Level of evidence A or B).

- (1) In children or adolescents with NCAH, treatment with glucocorticoids should be considered only for those who have inappropriately early onset of pubarche or rapid progression of bone age.
- (2) In adult women with NCAH, ovarian androgen suppression or peripheral androgen blockade are more effective than glucocorticoids for reducing circulating androgens and their effects.
- (3) Adult women with NCAH who have not conceived spontaneously and who demonstrate overt or subclinical ovulatory dysfunction may benefit from glucocorticoids or from ovulation induction.

androgen and progesterone excess that impair ovulation and endometrial receptiveness, respectively. In a recent study of 38 patients who presented with oligo-amenorrhea before treatment, 27 achieved regular menstrual cycles with hydrocortisone treatment (average dose: 17.5 ± 7.5 mg/day) (Bidet et al., 2010). Among the 11 patients presenting with amenorrhea before treatment, only three still had amenorrhea after glucocorticoid-only treatment. Plasma testosterone and androstenedione concentrations decreased significantly in all patients.

When hydrocortisone therapy does not lead to normal ovarian function, ovulation induction with clomiphene citrate or exogenous gonadotropins, or assisted reproductive techniques, should be considered following the usual protocol for these treatments (Table V, Level of evidence: B).

Management of pregnancy in NCAH

There are no clear guidelines concerning treatment of NCAH during pregnancy. Cross-sectional data suggest that early pregnancy losses might be lower when patients are on glucocorticoid therapy and these data support continuing corticosteroids during pregnancy (Trapp and Oberfield, 2012).

Feldman et al. (1992) reported 6 miscarriages in 18 pregnancies (33%) occurring in 10 NCAH patients before diagnosis, and none among pregnancies occurring during hydrocortisone treatment. Moran et al. (2006) observed a markedly lower rate of miscarriage after diagnosis compared to prior to diagnosis among 101 NCAH patients and 203 pregnancies (6.2% vs. 25.4%, respectively). However, in this study, the respective benefits of glucocorticoids and ovulation induction were not clearly established. Bidet et al. (2010) observed that the risk of miscarriage in the absence of glucocorticoid treatment was significantly higher than with treatment (odds ratio: 4.5, 95% confidence interval: 1.4–14.2). Although these studies suggest that glucocorticoid replacement reduces the miscarriage risk, most of the data about this issue were obtained from infertile anovulatory women, which may have biased the results (Level of evidence: C).

Glucocorticoid replacement is usually maintained at a hydrocortisone dose of 20–25 mg/day or a prednisone dose of 2.5–5 mg/day, but no study so far has prospectively addressed the benefits of this practice to prevent early pregnancy loss and/or improve pregnancy outcome in NCAH. Of note, the selection of glucocorticoid is important since dexamethasone crosses the fetoplacental barrier and may have a long-term detrimental impact on fetal intellectual development (Harris and Seckl, 2011; Wallenstein et al., 2016) (Level of evidence: D). Therefore, dexamethasone should be avoided during pregnancy in favor of replacement doses of glucocorticoids such as hydrocortisone and prednisone, which are easily metabolized by placental 11 beta-hydroxysteroid dehydrogenase type 2.

Cortisol-binding globulin concentrations increase in pregnancy, and as hydrocortisone administered exogenously is bound by this globulin (Jung et al., 2011), hypothetically the dose of hydrocortisone should be increased in pregnant patients with NCAH. However, in actual practice there are no biochemical markers and few clinical markers that should be followed in pregnancy, and doses are rarely adjusted.

Risk of adrenal insufficiency

As previously reported, there is limited evidence that some patients with NCAH who present with reduced cortisol response to ACTH need glucocorticoid supplementation when undergoing major surgery or if trauma occurs (Stoupa et al., 2015). There are no data demonstrating the benefits of prophylactic supraphysiological loading with glucocorticoids prior to surgery or other elective procedures on the quality of life, on potential morbidity, or on mortality rates, nor have potential secondary effects been investigated (Young et al., 2010). Therefore, the risk/benefit ratio has not been established and routine systematic hydrocortisone loading for elective procedures in NCAH patients cannot be recommended (Level of evidence: D).

A review of the literature identified only a few cases of adults with NCAH who suffered acute adrenal insufficiency (Young et al., 2010; Falhammar et al., 2014; Reichman et al., 2014). However, two cases of fatal adrenal crisis have been reported in young NCAH patients (Falhammar et al. 2014), although these unfortunate events probably resulted from the discontinuation of long-term chronic glucocorticoid treatment. Therefore, in patients who have been on long-term glucocorticoid replacement and whose treatment is stopped abruptly, the risk of acute adrenal insufficiency may be substantial, and possibly even higher than for other subjects being treated with prolonged glucocorticoid therapy, because of the combination of corticotroph inertia and a relative inefficiency in cortisol secretion.

There is no consensus regarding the recommendation that NCAH patients carry a medical alert identification, available in various forms such as wallet cards, bracelets, necklaces, etc., stating that the patient has NCAH and may be at risk for adrenal insufficiency, and that systematic glucocorticoid treatment should be considered prior to surgery or if acute trauma or acute physical stress occurs. Nevertheless, patients on chronic glucocorticoid therapy may need to carry with them medical alert identification.

Future perspectives on the diagnosis and treatment of NCAH

Most of the studies addressing the biochemical diagnosis of NCAH due to 21-hydroxylase deficiency have relied on direct immunoassays

such as radioimmunoassay or time-resolved fluorescence assay to measure circulating 17-OHP concentrations. As happens with any immunoassay measuring steroid hormones at the nanomolar range, 17-OHP immunoassays suffer from reliability issues because of the lack of sensitivity, specificity and of matrix effects, often overestimating true steroid values (Meier *et al.*, 2004; Rauh, 2009). Such issues may contribute to the discrepancies in the 17-OHP cut-off values suggested for screening of NCAH described above. Hopefully, the use of liquid chromatography coupled with mass spectrometry (LC/MS) assays will improve the accurate measurement of 17-OHP concentrations and also provide the analysis of steroid panels that may discriminate 21-hydroxylase deficiency from other conditions (Turcu *et al.*, 2015).

Regarding treatment, new drugs that simulate physiologic glucocorticoid secretion or provide ease of delivery are under investigation at present, but have been studied primarily in CAH patients. Most recently evaluated is Chronocort®, a modified release formulation of hydrocortisone (Mallappa *et al.*, 2015). Corticotropin-releasing factor receptor antagonists are being investigated in adult females with CAH (Auchus *et al.*, 2015) and subcutaneous hydrocortisone therapy has been tried in a limited number of individuals with CAH who have a rapid clearance of cortisol or those who cannot tolerate oral glucocorticoid therapy (Nella *et al.*, 2016). However, more important than finding new glucocorticoid-like drugs is to determine whether glucocorticoid treatment, aside from its use in patients presenting with accelerated growth, cortisol insufficiency, or fertility issues, is truly necessary in most NCAH patients. In fact, most of the chronic problems developing in NCAH patients (increased risk of obesity, type 2 diabetes and cardiovascular disease) might be related more closely to prolonged glucocorticoid use than to the disorder itself. The pathophysiology of NCAH is quite different from that of CAH and long-term glucocorticoid treatment should be reserved only for a few select patients.

There is also preliminary data suggesting that abiraterone acetate, a steroidal P450c17 inhibitor used in prostate cancer, may be a useful therapeutic agent in adult women with CAH (Auchus *et al.*, 2014). On the contrary, no data are available in patients with CAH regarding the possible use of steroid sulfatase inhibitors. Finally, neurokinin-3 receptor antagonists are being investigated in women with PCOS with the aim of reducing LH pulse frequency and LH and T levels (George *et al.*, 2016). Whether these drugs may be useful also in NCAH is unclear at present.

Authors' roles

E.C. organized the study and participated in the collection of literature data, manuscript writing and critical discussion. He read and approved the final manuscript. D.D. participated in the collection of literature data, manuscript writing and critical discussion. He read and approved the final manuscript. H.F.E.-M. conducted the meta-analysis of the prevalence of NCAH among women with androgen excess and participated in the collection of literature data, manuscript writing and critical discussion. He read and approved the final manuscript. F.K. participated in the collection of literature data, manuscript writing and critical discussion. He read and approved the final manuscript. C.M. participated in the collection of literature data, manuscript writing and critical discussion. He read and approved the final manuscript.

S.O. participated in the collection of literature data, manuscript writing and critical discussion. She read and approved the final manuscript. S.W. participated in the collection of literature data, manuscript writing and critical discussion. She read and approved the final manuscript. R.A. participated in the collection of literature data, manuscript writing and critical discussion. He read and approved the final manuscript.

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Conflict of interest

The authors have no competing interests to declare.

References

- Akinci A, Yordam N, Ersoy F, Ulusahin N, Oguz H. The incidence of non-classical 21-hydroxylase deficiency in hirsute adolescent girls. *Gynecol Endocrinol* 1992;**6**: 99–106.
- Al-Ruhaily AD, Malabu UH, Sulimani RA. Hirsutism in Saudi females of reproductive age: a hospital-based study. *Ann Saudi Med* 2008;**28**:28–32.
- Ambrosi B, Re T, Passini E, Bochicchio D, Ferrario R. Value of buserelin testing in the evaluation of hirsute women. *J Endocrinol Invest* 1996;**19**:204–209.
- Ambrosiak U, Kepczynska-Nyk A, Kurylowicz A, Malunowicz EM, Wojcicka A, Miskiewicz P, Macech M. The diagnosis of nonclass congenital adrenal hyperplasia due to 21-hydroxylase deficiency, based on serum basal or post-ACTH stimulation 17-hydroxyprogesterone, can lead to false-positive diagnosis. *Clin Endocrinol (Oxf)* 2016;**84**:23–29.
- Ansarin H, Aziz-Jalali MH, Rasi A, Soltani-Arabshahi R. Clinical presentation and etiologic factors of hirsutism in premenopausal Iranian women. *Arch Iran Med* 2007;**10**:7–13.
- Araujo RS, Mendonca BB, Barbosa AS, Lin CJ, Marcondes JA, Billerbeck AE, Bachega TA. Microconversion between CYP21A2 and CYP21A1P promoter regions causes the nonclassical form of 21-hydroxylase deficiency. *J Clin Endocrinol Metab* 2007;**92**:4028–4034.
- Armengaud JB, Charkaluk ML, Trivin C, Tardy V, Breart G, Brauner R, Chalumeau M. Precocious pubarche: distinguishing late-onset congenital adrenal hyperplasia from premature adrenarche. *J Clin Endocrinol Metab* 2009;**94**:2835–2840.
- Arnaout MA. Late-onset congenital adrenal hyperplasia in women with hirsutism. *Eur J Clin Invest* 1992;**22**:651–658.
- Auchus RJ. The backdoor pathway to dihydrotestosterone. *Trends Endocrinol Metab* 2004;**15**:432–438.
- Auchus RJ, Buschur EO, Chang AY, Hammer GD, Ramm C, Madrigal D, Wang G, Gonzalez M, Xu XS, Smit JW *et al.* Abiraterone acetate to lower androgens in women with classic 21-hydroxylase deficiency. *J Clin Endocrinol Metab* 2014;**99**: 2763–2770.
- Auchus RJ, Turcu AF, Spencer-Segal JL, Farber RH, Luo R, Grigoriadis DE, O'Brien CF. A pharmacokinetic and biomarker study of the corticotropin-releasing factor receptor antagonist NBI-77860 in adult females with classic, 21-hydroxylase deficiency, congenital adrenal hyperplasia (CAH). *Endocrine Society's 97th Annual Meeting and Expo*. The Endocrine Society, San Diego, CA, 2015.
- Azziz R, Boots LR, Parker CR Jr., Bradley Jr., Zacur HA. 11 beta-hydroxylase deficiency in hyperandrogenism. *Fertil Steril* 1991;**55**:733–741.
- Azziz R, Carmina E, Dewailly D, Diamanti-Kandaraki E, Escobar-Morreale HF, Futterweit W, Janssen OE, Legro RS, Norman RJ, Taylor AE *et al.* Position statement: criteria for defining polycystic ovary syndrome as a predominantly hyperandrogenic syndrome: an Androgen Excess Society guideline. *J Clin Endocrinol Metab* 2006;**91**:4237–4245.

- Azziz R, Carmina E, Dewailly D, Diamanti-Kandarakis E, Escobar-Morreale HF, Futterweit W, Janssen OE, Legro RS, Norman RJ, Taylor AE et al. The Androgen Excess and PCOS Society criteria for the polycystic ovary syndrome: the complete task force report. *Fertil Steril* 2009;**91**:456–488.
- Azziz R, Dewailly D, Owerbach D. Clinical review 56: nonclassic adrenal hyperplasia: current concepts. *J Clin Endocrinol Metab* 1994;**78**:810–815.
- Azziz R, Hincapie LA, Knochenhauer ES, Dewailly D, Fox L, Boots LR. Screening for 21-hydroxylase-deficient nonclassic adrenal hyperplasia among hyperandrogenic women: a prospective study. *Fertil Steril* 1999;**72**:915–925.
- Azziz R, Kenney PJ. Magnetic resonance imaging of the adrenal gland in women with late-onset adrenal hyperplasia. *Fertil Steril* 1991;**56**:142–144.
- Azziz R, Sanchez LA, Knochenhauer ES, Moran C, Lazenby J, Stephens KC, Taylor K, Boots LR. Androgen excess in women: experience with over 1000 consecutive patients. *J Clin Endocrinol Metab* 2004;**89**:453–462.
- Azziz R, Zacur HA. 21-Hydroxylase deficiency in female hyperandrogenism: screening and diagnosis. *J Clin Endocrinol Metab* 1989;**69**:577–584.
- Balsamo A, Cacciari E, Baldazzi L, Tartaglia L, Cassio A, Mantovani V, Piazzzi S, Cicognani A, Pirazzoli P, Mainetti B et al. CYP21 analysis and phenotype/genotype relationship in the screened population of the Italian Emilia-Romagna region. *Clin Endocrinol (Oxf)* 2000;**53**:117–125.
- Barbaro M, Baldazzi L, Balsamo A, Lajic S, Robins T, Barp L, Pirazzoli P, Cacciari E, Cicognani A, Wedell A. Functional studies of two novel and two rare mutations in the 21-hydroxylase gene. *J Mol Med (Berl)* 2006;**84**:521–528.
- Barbaro M, Lajic S, Baldazzi L, Balsamo A, Pirazzoli P, Cicognani A, Wedell A, Cacciari E. Functional analysis of two recurrent amino acid substitutions in the CYP21 gene from Italian patients with congenital adrenal hyperplasia. *J Clin Endocrinol Metab* 2004;**89**:2402–2407.
- Barbaro M, Soardi FC, Ostberg LJ, Persson B, de Mello MP, Wedell A, Lajic S. In vitro functional studies of rare CYP21A2 mutations and establishment of an activity gradient for nonclassic mutations improve phenotype predictions in congenital adrenal hyperplasia. *Clin Endocrinol (Oxf)* 2015;**82**:37–44.
- Barbat B, Bogyo A, Raux-Demay MC, Kuttent F, Boue J, Simon-Bouy B, Serre JL, Mornet E. Screening of CYP21 gene mutations in 129 French patients affected by steroid 21-hydroxylase deficiency. *Hum Mutat* 1995;**5**:126–130.
- Barbieri RL. Hyperandrogenic disorders. *Clin Obstet Gynecol* 1990;**33**:640–654.
- Baskin HJ. Screening for late-onset congenital adrenal hyperplasia in hirsutism or amenorrhea. *Arch Intern Med* 1987;**147**:847–848.
- Bayraktar F, Dereli D, Ozgen AG, Yilmaz C. Plasma homocysteine levels in polycystic ovary syndrome and congenital adrenal hyperplasia. *Endocr J* 2004;**51**:601–608.
- Benjamin F, Deutsch S, Saperstein H, Seltzer VL. Prevalence of and markers for the attenuated form of congenital adrenal hyperplasia and hyperprolactinemia masquerading as polycystic ovarian disease. *Fertil Steril* 1986;**46**:215–221.
- Bidet M, Bellanne-Chantelot C, Galand-Portier MB, Golmard JL, Tardy V, Morel Y, Clauin S, Coussieu C, Boudou P, Mowzowicz I et al. Fertility in women with non-classical congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *J Clin Endocrinol Metab* 2010;**95**:1182–1190.
- Bidet M, Bellanne-Chantelot C, Galand-Portier MB, Tardy V, Billaud L, Laborde K, Coussieu C, Morel Y, Vaury C, Golmard JL et al. Clinical and molecular characterization of a cohort of 161 unrelated women with nonclassical congenital adrenal hyperplasia due to 21-hydroxylase deficiency and 330 family members. *J Clin Endocrinol Metab* 2009;**94**:1570–1578.
- Binay C, Simsek E, Cilingir O, Yuksel Z, Kutlay O, Artan S. Prevalence of nonclassic congenital adrenal hyperplasia in Turkish children presenting with premature pubarche, hirsutism, or oligomenorrhoea. *Int J Endocrinol* 2014;**2014**:768506.
- Blank SK, McCartney CR, Helm KD, Marshall JC. Neuroendocrine effects of androgens in adult polycystic ovary syndrome and female puberty. *Semin Reprod Med* 2007;**25**:352–359.
- Bleicken C, Loidi L, Dhir V, Parajes S, Quinteiro C, Dominguez F, Grotzinger J, Sippell WG, Riepe FG, Arlt W et al. Functional characterization of three CYP21A2 sequence variants (p.A265V, p.W302S, p.D322G) employing a yeast co-expression system. *Hum Mutat* 2009;**30**:E443–E450.
- Bojunga J, Welsch C, Antes I, Albrecht M, Lengauer T, Zeuzem S. Structural and functional analysis of a novel mutation of CYP21B in a heterozygote carrier of 21-hydroxylase deficiency. *Hum Genet* 2005;**117**:558–564.
- Cabrera MS, Vogiatzi MG, New MI. Long term outcome in adult males with classic congenital adrenal hyperplasia. *J Clin Endocrinol Metab* 2001;**86**:3070–3078.
- Capoluongo E, Concolino P, Carrozza C, Santonocito C, Zuppi C. Gene symbol: CYP21A2. Disease: adrenal hyperplasia. *Hum Genet* 2008b;**123**:550.
- Capoluongo E, Concolino P, Minucci A, Giardina B. Gene symbol: CYP21A2. Disease: adrenal hyperplasia. *Hum Genet* 2008c;**123**:550.
- Capoluongo E, Concolino P, Vendittelli F, Zuppi C. Gene symbol: CYP21A2. Disease: adrenal hyperplasia. *Hum Genet* 2008a;**123**:549–550.
- Capoluongo E, Concolino P, Vendittelli F, Zuppi C. Gene symbol: CYP21A2. Disease: non-classic 21-Hydroxylase deficiency. *Hum Genet* 2008d;**123**:553.
- Carmina E. Pathogenesis and treatment of hirsutism in late-onset congenital adrenal hyperplasia. *Reprod Med Rev* 1995;**4**:179–187.
- Carmina E, Gagliano AM, Rosato F, Maggiore M, Janni A. The endocrine pattern of late onset adrenal hyperplasia (21-hydroxylase deficiency). *J Endocrinol Invest* 1984;**7**:89–92.
- Carmina E, Lobo RA. Pituitary-adrenal responses to corticotropin-releasing factor in late onset 21-hydroxylase deficiency. *Fertil Steril* 1990;**54**:79–83.
- Carmina E, Lobo RA. Ovarian suppression reduces clinical and endocrine expression of late-onset congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Fertil Steril* 1994;**62**:738–743.
- Carmina E, Rosato F, Janni A, Rizzo M, Longo RA. Extensive clinical experience: relative prevalence of different androgen excess disorders in 950 women referred because of clinical hyperandrogenism. *J Clin Endocrinol Metab* 2006;**91**:2–6.
- Cobin RH, Futterweit W, Fiedler RP, Thornton JC. Adrenocorticotrophic hormone testing in idiopathic hirsutism and polycystic ovarian disease: a test of limited usefulness. *Fertil Steril* 1985;**44**:224–226.
- Concolino P, Mello E, Zuppi C, Capoluongo E. Molecular diagnosis of congenital adrenal hyperplasia due to 21-hydroxylase deficiency: an update of new CYP21A2 mutations. *Clin Chem Lab Med* 2010;**48**:1057–1062.
- Concolino P, Santonocito C, Minucci A, Carrozza C, Zuppi C, Capoluongo E, Giardina B. Novel human pathological mutations. Gene symbol: CYP21A2. Disease: non-classic 21-hydroxylase deficiency. *Hum Genet* 2007;**122**:559.
- Concolino P, Vendittelli F, Mello E, Carelli Alinovi C, Minucci A, Carrozza C, Santini SA, Zuppi C, Capoluongo E. Two novel CYP21A2 missense mutations in Italian patients with 21-hydroxylase deficiency: identification and functional characterisation. *IUBMB Life* 2009a;**61**:229–235.
- Concolino P, Vendittelli F, Mello E, Minucci A, Carrozza C, Rossodivita A, Giardina B, Zuppi C, Capoluongo E. Functional analysis of two rare CYP21A2 mutations detected in Italian patients with a mild form of congenital adrenal hyperplasia. *Clin Endocrinol (Oxf)* 2009b;**71**:470–476.
- Chen W, Xu Z, Sullivan A, Finkelstein GP, Van Ryzin C, Merke DP, McDonnell NB. Junction site analysis of chimeric CYP21A1P/CYP21A2 genes in 21-hydroxylase deficiency. *Clin Chem* 2012;**58**:421–430.
- Chetkowski RJ, DeFazio J, Shamonki I, Judd HL, Chang RJ. The incidence of late-onset congenital adrenal hyperplasia due to 21-hydroxylase deficiency among hirsute women. *J Clin Endocrinol Metab* 1984;**58**:595–598.
- Chrousos GP, Loriaux DL, Mann DL, Cutler GB Jr. Late-onset 21-hydroxylase deficiency mimicking idiopathic hirsutism or polycystic ovarian disease. *Ann Intern Med* 1982;**96**:143–148.
- Chrousos GP, Loriaux DL, Sherins RJ, Cutler GB Jr. Unilateral testicular enlargement resulting from inapparent 21-hydroxylase deficiency. *J Urol* 1981;**126**:127–128.
- Chu X, Ding H, Cui G, Xu Y, Wang DW, He Y. Functional consequences of a novel point mutation in the CYP21A2 gene identified in a Chinese Han patient with nonclassic 21-hydroxylase deficiency. *Clin Endocrinol (Oxf)* 2014;**80**:927–928.
- Dacou-Voutetakis C, Dracopoulou M. High incidence of molecular defects of the CYP21 gene in patients with premature adrenarche. *J Clin Endocrinol Metab* 1999;**84**:1570–1574.
- Dain L, Minutolo C, Buzzalino N, Belli S, Oneto A, Charreau E, Alba L. Gene symbol: CYP21A2. Disease: adrenal hyperplasia, CYP21. *Hum Genet* 2006;**119**:363.
- Deneux C, Tardy V, Dib A, Mornet E, Billaud L, Charron D, Morel Y, Kuttent F. Phenotype-genotype correlation in 56 women with nonclassical congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *J Clin Endocrinol Metab* 2001;**86**:207–213.
- Dewailly D, Vantyghem-Haudiquet MC, Sainsard C, Buvat J, Cappoen JP, Ardaens K, Racadot A, Lefebvre J, Fossati P. Clinical and biological phenotypes in late-onset 21-hydroxylase deficiency. *J Clin Endocrinol Metab* 1986;**63**:418–423.

- Di Fede G, Mansueto P, Pepe I, Rini GB, Carmina E. High prevalence of polycystic ovary syndrome in women with mild hirsutism and no other significant clinical symptoms. *Fertil Steril* 2010;**94**:194–197.
- Doi SA, Barendregt JJ, Khan S, Thalib L, Williams GM. Advances in the meta-analysis of heterogeneous clinical trials II: the quality effects model. *Contemp Clin Trials* 2015;**45**:123–129.
- Eldar-Geva T, Hurwitz A, Vecsei P, Palti Z, Milwidsky A, Rosler A. Secondary biosynthetic defects in women with late-onset congenital adrenal hyperplasia. *N Engl J Med* 1990;**323**:855–863.
- Emans SJ, Grace E, Fleischnick E, Mansfield MJ, Crigler JF Jr.. Detection of late-onset 21-hydroxylase deficiency congenital adrenal hyperplasia in adolescents. *Pediatrics* 1983;**72**:690–695.
- Escobar-Morreale HF, Carmina E, Dewailly D, Gambineri A, Kelestimur F, Moghetti P, Pugeat M, Qiao J, Wijayarathne CN, Witchel SF et al. Epidemiology, diagnosis and management of hirsutism: a consensus statement by the Androgen Excess and Polycystic Ovary Syndrome Society. *Hum Reprod Update* 2012;**18**:146–170.
- Escobar-Morreale HF, San Millan JL. Abdominal adiposity and the polycystic ovary syndrome. *Trends Endocrinol Metab* 2007;**18**:266–272.
- Escobar-Morreale HF, Sanchon R, San Millan JL. A prospective study of the prevalence of nonclassical congenital adrenal hyperplasia among women presenting with hyperandrogenic symptoms and signs. *J Clin Endocrinol Metab* 2008;**93**:527–533.
- Ezquieta B, Cueva E, Varela J, Oliver A, Fernandez J, Jariego C. Non-classical 21-hydroxylase deficiency in children: association of adrenocorticotrophic hormone-stimulated 17-hydroxyprogesterone with the risk of compound heterozygosity with severe mutations. *Acta Paediatr* 2002;**91**:892–898.
- Falhammar H, Frisen L, Hirschberg AL, Norrby C, Almqvist C, Nordenskjold A, Nordenstrom A. Increased cardiovascular and metabolic morbidity in patients with 21-hydroxylase deficiency: a Swedish population-based national cohort study. *J Clin Endocrinol Metab* 2015;**100**:3520–3528.
- Falhammar H, Frisen L, Norrby C, Hirschberg AL, Almqvist C, Nordenskjold A, Nordenstrom A. Increased mortality in patients with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *J Clin Endocrinol Metab* 2014;**99**:E2715–E2721.
- Falhammar H, Nystrom HF, Ekstrom U, Granberg S, Wedell A, Thoren M. Fertility, sexuality and testicular adrenal rest tumors in adult males with congenital adrenal hyperplasia. *Eur J Endocrinol* 2012;**166**:441–449.
- Falhammar H, Torpy DJ. Congenital adrenal hyperplasia due to 21-hydroxylase deficiency presenting as adrenal incidentaloma: a systematic review and meta-analysis. *Endocr Pract* 2016;**22**:736–752.
- Fanta M, Cibula D, Vrbikova J. Prevalence of nonclassic adrenal hyperplasia (NCAH) in hyperandrogenic women. *Gynecol Endocrinol* 2008;**24**:154–157.
- Feldman S, Billaud L, Thalabard JC, Raux-Demay MC, Mowszowicz I, Kuttann F, Mauvais-Jarvis P. Fertility in women with late-onset adrenal hyperplasia due to 21-hydroxylase deficiency. *J Clin Endocrinol Metab* 1992;**74**:635–639.
- Finkelstein GP, Kim MS, Sinaii N, Nishitani M, Van Ryzin C, Hill SC, Reynolds JC, Hanna RM, Merke DP. Clinical characteristics of a cohort of 244 patients with congenital adrenal hyperplasia. *J Clin Endocrinol Metab* 2012;**97**:4429–4438.
- Frank-Raue K, Junga G, Raue F, Vecsei P, Ziegler R. Therapy of hirsutism in females with adrenal enzyme defects of steroid hormone biosynthesis: comparison of dexamethasone with cyproterone acetate. *Klin Wochenschr* 1990;**68**:597–601.
- Gallardo E, Porcile A. Hirsutism in Chilean women: importance of 21-hydroxylase congenital deficiency of late onset. *Rev Med Chil* 1991;**119**:147–151.
- Gatee OB, Al Attia HM, Salama IA. Hirsutism in the United Arab Emirates: a hospital study. *Postgrad Med J* 1996;**72**:168–171.
- George JT, Kakkar R, Marshall J, Scott ML, Finkelman RD, Ho TW, Veldhuis J, Skorupskaite K, Anderson RA, McIntosh S et al. Neurokinin B receptor antagonism in women with polycystic ovary syndrome: a randomized, placebo-controlled trial. *J Clin Endocrinol Metab* 2016;**101**:4313–4321.
- Glintborg D, Henriksen JE, Andersen M, Hagen C, Hangaard J, Rasmussen PE, Schousboe K, Hermann AP. Prevalence of endocrine diseases and abnormal glucose tolerance tests in 340 Caucasian premenopausal women with hirsutism as the referral diagnosis. *Fertil Steril* 2004;**82**:1570–1579.
- Gonzalez ME, Cantatore-Francis J, Orlov SJ. Androgenetic alopecia in the paediatric population: a retrospective review of 57 patients. *Br J Dermatol* 2010;**163**:378–385.
- Haider S, Islam B, D'Atri V, Sgobba M, Poojari C, Sun L, Yuen T, Zaidi M, New MI. Structure-phenotype correlations of human CYP21A2 mutations in congenital adrenal hyperplasia. *Proc Natl Acad Sci USA* 2013;**110**:2605–2610.
- Harris A, Seckl J. Glucocorticoids, prenatal stress and the programming of disease. *Horm Behav* 2011;**59**:279–289.
- Helmberg A, Tusie-Luna MT, Tabarelli M, Kofler R, White PC. R339H and P453S: CYP21 mutations associated with nonclassic steroid 21-hydroxylase deficiency that are not apparent gene conversions. *Mol Endocrinol* 1992;**6**:1318–1322.
- Innanen VT, Vale JM. Assessment of the one hour adrenocorticotrophic hormone test in the diagnosis of attenuated 21-hydroxylase deficiency. *J Clin Pathol* 1990;**43**:493–495.
- Jaresch S, Kornely E, Kley HK, Schlaghecke R. Adrenal incidentaloma and patients with homozygous or heterozygous congenital adrenal hyperplasia. *J Clin Endocrinol Metab* 1992;**74**:685–689.
- Joehrer K, Geley S, Strasser-Wozak EM, Azziz R, Wollmann HA, Schmitt K, Kofler R, White PC. CYP11B1 mutations causing non-classic adrenal hyperplasia due to 11 beta-hydroxylase deficiency. *Hum Mol Genet* 1997;**6**:1829–1834.
- Jung C, Ho JT, Torpy DJ, Rogers A, Doogue M, Lewis JG, Czajko RJ, Inder WJ. A longitudinal study of plasma and urinary cortisol in pregnancy and postpartum. *J Clin Endocrinol Metab* 2011;**96**:1533–1540.
- Kamel N, Tonyukuk V, Emral R, Corapcioglu D, Bastemir M, Gullu S. The prevalence of late onset congenital adrenal hyperplasia in hirsute women from Central Anatolia. *Endocr J* 2003;**50**:815–823.
- Kamrath C, Hochberg Z, Hartmann MF, Remer T, Wudy SA. Increased activation of the alternative 'backdoor' pathway in patients with 21-hydroxylase deficiency: evidence from urinary steroid hormone analysis. *J Clin Endocrinol Metab* 2012;**97**:E367–E375.
- Khandekar S, Lata V, Dash RJ. Screening for late onset congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Indian J Med Res* 1990;**92**:79–82.
- Kiedrowicz B, Binczak-Kuleta A, Lubikowski J, Koziol M, Andrysiak-Mamos E, Ostanek-Panka M, Ciechanowicz A, Syrenicz A. Prevalence and clinical outcome of CYP21A2 gene mutations in patients with nonfunctional adrenal incidentalomas. *Horm Metab Res* 2015;**47**:662–667.
- Kim MS, Merke DP. Cardiovascular disease risk in adult women with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Semin Reprod Med* 2009;**27**:316–321.
- Kim MS, Ryabets-Lienhard A, Dao-Tran A, Mittelman SD, Gilsanz V, Schragr SM, Geffner ME. Increased abdominal adiposity in adolescents and young adults with classical congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *J Clin Endocrinol Metab* 2015;**100**:E1153–E1159.
- Kohn B, Levine LS, Pollack MS, Pang S, Lorenzen F, Levy D, Lerner AJ, Rondonini GF, Dupont B, New MI. Late-onset steroid 21-hydroxylase deficiency: a variant of classical congenital adrenal hyperplasia. *J Clin Endocrinol Metab* 1982;**55**:817–827.
- Kuttann F, Couillin P, Girard F, Billaud L, Vincens M, Boucekkin C, Thalabard JC, Maudelonde T, Spritzer P, Mowszowicz I et al. Late-onset adrenal hyperplasia in hirsutism. *N Engl J Med* 1985;**313**:224–231.
- Lajic S, Clauin S, Robins T, Vexiau P, Blanche H, Bellanne-Chantelot C, Wedell A. Novel mutations in CYP21 detected in individuals with hyperandrogenism. *J Clin Endocrinol Metab* 2002;**87**:2824–2829.
- Lekarev O, Lin-Su K, Vogiatzi MG. Infertility and reproductive function in patients with congenital adrenal hyperplasia: pathophysiology, advances in management, and recent outcomes. *Endocrinol Metab Clin North Am* 2015;**44**:705–722.
- Levin JH, Carmina E, Lobo RA. Is the inappropriate gonadotropin secretion of patients with polycystic ovary syndrome similar to that of patients with adult-onset congenital adrenal hyperplasia? *Fertil Steril* 1991;**56**:635–640.
- Livadas S, Dracopoulou M, Dastamani A, Sertedaki A, Maniati-Christidi M, Magiakou AM, Kanaka-Gantenbein C, Chrousos GP, Dacou-Voutetakis C. The spectrum of clinical, hormonal and molecular findings in 280 individuals with non-classical congenital adrenal hyperplasia caused by mutations of the CYP21A2 gene. *Clin Endocrinol (Oxf)* 2015;**82**:543–549.
- Lobo RA, Goebelsmann U. Adult manifestation of congenital adrenal hyperplasia due to incomplete 21-hydroxylase deficiency mimicking polycystic ovarian disease. *Am J Obstet Gynecol* 1980;**138**:720–726.
- Loidi L, Quinteiro C, Parajes S, Barreiro J, Leston DG, Cabezas-Agricola JM, Sueiro AM, Araujo-Vilar D, Catro-Feijoo L, Costas J et al. High variability in CYP21A2 mutated alleles in Spanish 21-hydroxylase deficiency patients, six novel mutations and a founder effect. *Clin Endocrinol (Oxf)* 2006;**64**:330–336.

- Lutfallah C, Wang W, Mason JI, Chang YT, Haider A, Rich B, Castro-Magana M, Copeland KC, David R, Pang S. Newly proposed hormonal criteria via genotypic proof for type II 3beta-hydroxysteroid dehydrogenase deficiency. *J Clin Endocrinol Metab* 2002;**87**:2611–2622.
- Mallappa A, Sinaii N, Kumar P, Whitaker MJ, Daley LA, Digweed D, Eckland DJ, Van Ryzin C, Nieman LK, Arlt W et al. A phase 2 study of Chronocort, a modified-release formulation of hydrocortisone, in the treatment of adults with classic congenital adrenal hyperplasia. *J Clin Endocrinol Metab* 2015;**100**:1137–1145.
- Marino R, Ramirez P, Galeano J, Perez Garrido N, Rocco C, Ciccio M, Warman DM, Guercio G, Chaler E, Maceiras M et al. Steroid 21-hydroxylase gene mutational spectrum in 454 Argentinean patients: genotype-phenotype correlation in a large cohort of patients with congenital adrenal hyperplasia. *Clin Endocrinol (Oxf)* 2011;**75**:427–435.
- Marra AM, Improda N, Capalbo D, Salzano A, Arcopinto M, De Paulis A, Alessio M, Lenzi A, Isidori AM, Cittadini A et al. Cardiovascular abnormalities and impaired exercise performance in adolescents with congenital adrenal hyperplasia. *J Clin Endocrinol Metab* 2015;**100**:644–652.
- McLaughlin B, Barrett P, Finch T, Devlin JG. Late onset adrenal hyperplasia in a group of Irish females who presented with hirsutism, irregular menses and/or cystic acne. *Clin Endocrinol (Oxf)* 1990;**32**:57–64.
- Meier U, Schnabel C, Kunz D, Driesch R, Gressner AM. Comparison of three commercial assays for the measurement of 17alpha-hydroxyprogesterone (17alpha-OHPR): limitations of the quality control system. *Clin Chem Lab Med* 2004;**42**:450–454.
- Merke DP Genetics and clinical presentation of classic congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *UpToDate Endocrinol Metab*, 2015. 18 March 2015, last updated.
- Minutolo C, Nadra AD, Fernandez C, Taboas M, Buzzalino N, Casali B, Belli S, Charreau EH, Alba L, Dain L. Structure-based analysis of five novel disease-causing mutations in 21-hydroxylase-deficient patients. *PLoS One* 2011;**6**:e15899.
- Mithal A, Ammini AC, Godbole MM, Khurana ML, Raj D, Karmarkar MG, Ahuja MM. Late-onset adrenal hyperplasia in north Indian hirsute women. *Horm Res* 1988;**30**:1–4.
- Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med* 2009;**6**:e1000097.
- Moran C, Azziz R, Carmina E, Dewailly D, Fruzzetti F, Ibanez L, Knochenhauer ES, Marcondes JA, Mendonca BB, Pignatelli D et al. 21-Hydroxylase-deficient non-classic adrenal hyperplasia is a progressive disorder: a multicenter study. *Am J Obstet Gynecol* 2000;**183**:1468–1474.
- Moran C, Azziz R, Weintrob N, Witchel SF, Rohmer V, Dewailly D, Marcondes JA, Pugeat M, Speiser PW, Pignatelli D et al. Reproductive outcome of women with 21-hydroxylase-deficient nonclassic adrenal hyperplasia. *J Clin Endocrinol Metab* 2006;**91**:3451–3456.
- Moran C, Potter HD, Reyna R, Boots LR, Azziz R. Prevalence of 3beta-hydroxysteroid dehydrogenase-deficient nonclassic adrenal hyperplasia in hyperandrogenic women with adrenal androgen excess. *Am J Obstet Gynecol* 1999;**181**:596–600.
- Moran C, Tapia MC, Hernandez E, Vazquez G, Garcia-Hernandez E, Bermudez JA. Etiological review of hirsutism in 250 patients. *Arch Med Res* 1994;**25**:311–314.
- Motta P, Catania A, Airaghi L, Mangone I, Cantalamessa L, Zanussi C. Prevalence of late-onset adrenal hyperplasia in postmenarchal hirsutism. *J Endocrinol Invest* 1988;**11**:675–678.
- Moura-Massari VO, Bugano DD, Marcondes JA, Gomes LG, Mendonca BB, Bachega TA. CYP21A2 genotypes do not predict the severity of hyperandrogenic manifestations in the nonclassical form of congenital adrenal hyperplasia. *Horm Metab Res* 2013;**45**:301–307.
- Nandagopal R, Sinaii N, Avila NA, Van Ryzin C, Chen W, Finkelstein GP, Mehta SP, McDonnell NB, Merke DP. Phenotypic profiling of parents with cryptic non-classic congenital adrenal hyperplasia: findings in 145 unrelated families. *Eur J Endocrinol* 2011;**164**:977–984.
- Nella AA, Mallappa A, Perritt AF, Gounden V, Kumar P, Sinaii N, Daley LA, Ling A, Liu CY, Soldin SJ et al. A phase 2 study of continuous subcutaneous hydrocortisone infusion in adults with congenital adrenal hyperplasia. *J Clin Endocrinol Metab* 2016;**101**:4690–4698.
- New MI. Extensive clinical experience: nonclassical 21-hydroxylase deficiency. *J Clin Endocrinol Metab* 2006;**91**:4205–4214.
- New MI, Abraham M, Gonzalez B, Dumic M, Razzaghy-Azar M, Chitayat D, Sun L, Zaidi M, Wilson RC, Yuen T. Genotype-phenotype correlation in 1,507 families with congenital adrenal hyperplasia owing to 21-hydroxylase deficiency. *Proc Natl Acad Sci USA* 2013;**110**:2611–2616.
- New MI, Lorenzen F, Lerner AJ, Kohn B, Oberfield SE, Pollack MS, Dupont B, Stoner E, Levy DJ, Pang S et al. Genotyping steroid 21-hydroxylase deficiency: hormonal reference data. *J Clin Endocrinol Metab* 1983;**57**:320–326.
- Nikoshkov A, Lajic S, Holst M, Wedell A, Luthman H. Synergistic effect of partially inactivating mutations in steroid 21-hydroxylase deficiency. *J Clin Endocrinol Metab* 1997;**82**:194–199.
- Nikoshkov A, Lajic S, Vlamis-Gardikas A, Tranebjærg L, Holst M, Wedell A, Luthman H. Naturally occurring mutants of human steroid 21-hydroxylase (P450c21) pinpoint residues important for enzyme activity and stability. *J Biol Chem* 1998;**273**:6163–6165.
- Oberfield SE, Sopher AB, Gerken AT. Approach to the girl with early onset of pubic hair. *J Clin Endocrinol Metab* 2011;**96**:1610–1622.
- Owerbach D, Sherman L, Ballard AL, Azziz R. Pro-453 to Ser mutation in CYP21 is associated with nonclassic steroid 21-hydroxylase deficiency. *Mol Endocrinol* 1992;**6**:1211–1215.
- Pall M, Azziz R, Beires J, Pignatelli D. The phenotype of hirsute women: a comparison of polycystic ovary syndrome and 21-hydroxylase-deficient nonclassic adrenal hyperplasia. *Fertil Steril* 2010;**94**:684–689.
- Pallan PS, Lei L, Wang C, Waterman MR, Guengerich FP, Egli M. Research resource: correlating human cytochrome P450 21A2 crystal structure and phenotypes of mutations in congenital adrenal hyperplasia. *Mol Endocrinol* 2015;**29**:1375–1384.
- Parajes S, Quinteiro C, Dominguez F, Loidi L. High frequency of copy number variations and sequence variants at CYP21A2 locus: implication for the genetic diagnosis of 21-hydroxylase deficiency. *PLoS One* 2008;**3**:e2138.
- Parker EA, Hovanes K, Germak J, Porter F, Merke DP. Maternal 21-hydroxylase deficiency and uniparental isodisomy of chromosome 6 and X results in a child with 21-hydroxylase deficiency and Klinefelter syndrome. *Am J Med Genet A* 2006;**140**:2236–2240.
- Pinkas H, Fuchs S, Klipper-Aurbach Y, Zvulunov A, Raanani H, Mimouni G, Fisch B, Weintrob N. Non-classical 21-hydroxylase deficiency: prevalence in males with unexplained abnormal sperm analysis. *Fertil Steril* 2010;**93**:1887–1891.
- Pinto G, Tardy V, Trivin C, Thalassinou C, Lortat-Jacob S, Nihoul-Fekete C, Morel Y, Brauner R. Follow-up of 68 children with congenital adrenal hyperplasia due to 21-hydroxylase deficiency: relevance of genotype for management. *J Clin Endocrinol Metab* 2003;**88**:2624–2633.
- Rauh M. Steroid measurement with LC-MS/MS in pediatric endocrinology. *Mol Cell Endocrinol* 2009;**301**:272–281.
- Reichman DE, White PC, New MI, Rosenwaks Z. Fertility in patients with congenital adrenal hyperplasia. *Fertil Steril* 2014;**101**:301–309.
- Riepe FG, Hiort O, Grotzinger J, Sippell WG, Krone N, Holterhus PM. Functional and structural consequences of a novel point mutation in the CYP21A2 gene causing congenital adrenal hyperplasia: potential relevance of helix C for P450 oxidoreductase-21-hydroxylase interaction. *J Clin Endocrinol Metab* 2008;**93**:2891–2895.
- Robins T, Bellanne-Chantelot C, Barbaro M, Cabrol S, Wedell A, Lajic S. Characterization of novel missense mutations in CYP21 causing congenital adrenal hyperplasia. *J Mol Med (Berl)* 2007;**85**:247–255.
- Robins T, Carlsson J, Sunnerhagen M, Wedell A, Persson B. Molecular model of human CYP21 based on mammalian CYP2C5: structural features correlate with clinical severity of mutations causing congenital adrenal hyperplasia. *Mol Endocrinol* 2006;**20**:2946–2964.
- Romaguera J, Moran C, Diaz-Montes TP, Hines GA, Cruz RI, Azziz R. Prevalence of 21-hydroxylase-deficient nonclassic adrenal hyperplasia and insulin resistance among hirsute women from Puerto Rico. *Fertil Steril* 2000;**74**:59–62.
- Sahin Y, Kelestimur F. The frequency of late-onset 21-hydroxylase and 11 beta-hydroxylase deficiency in women with polycystic ovary syndrome. *Eur J Endocrinol* 1997;**137**:670–674.
- Salinas Vert I, Audi Parera L, Granada Ybern ML, Lucas Martin A, Pizarro Lozano E, Foz Sala M, Sanmarti Sala A. Defects of adrenal steroidogenesis in patients with hirsutism. *Med Clin (Barc)* 1998;**110**:171–176.
- Sanchez LA, Knochenhauer ES, Gatlin R, Moran C, Azziz R. Differential diagnosis of clinically evident hyperandrogenism: experience with over 1000 consecutive patients. The Annual Meeting of the American Society for Reproductive Medicine, Orlando, FL, October 20–25, 2001 (Abstract O-294). *Fertil Steril* 2001;**76**:S111.

- Skordis N, Shammas C, Phedonos AA, Kyriakou A, Tumba M, Neocleous V, Phylactou LA. Genetic defects of the CYP21A2 gene in girls with premature adrenarche. *J Endocrinol Invest* 2015;**38**:535–539.
- Soardi FC, Barbaro M, Lau IF, Lemos-Marini SH, Baptista MT, Guerra-Junior G, Wedell A, Lajic S, de Mello MP. Inhibition of CYP21A2 enzyme activity caused by novel missense mutations identified in Brazilian and Scandinavian patients. *J Clin Endocrinol Metab* 2008;**93**:2416–2420.
- Souter I, Sanchez LA, Perez M, Bartolucci AA, Azziz R. The prevalence of androgen excess among patients with minimal unwanted hair growth. *Am J Obstet Gynecol* 2004;**191**:1914–1920.
- Speiser PW, Azziz R, Baskin LS, Ghizzoni L, Hensle TW, Merke DP, Meyer-Bahlburg HF, Miller WL, Montori VM, Oberfield SE et al. Congenital adrenal hyperplasia due to steroid 21-hydroxylase deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab* 2010;**95**:4133–4160.
- Speiser PW, Dupont B, Rubinstein P, Piazza A, Kastelan A, New MI. High frequency of nonclassical steroid 21-hydroxylase deficiency. *Am J Hum Genet* 1985;**37**:650–667.
- Speiser PW, Knochenhauer ES, Dewailly D, Fruzzetti F, Marcondes JA, Azziz R. A multicenter study of women with nonclassical congenital adrenal hyperplasia: relationship between genotype and phenotype. *Mol Genet Metab* 2000;**71**:527–534.
- Speiser PW, New MI, White PC. Molecular genetic analysis of nonclassic steroid 21-hydroxylase deficiency associated with HLA-B14,DR1. *N Engl J Med* 1988;**319**:19–23.
- Speiser PW, Serrat J, New MI, Gertner JM. Insulin insensitivity in adrenal hyperplasia due to nonclassical steroid 21-hydroxylase deficiency. *J Clin Endocrinol Metab* 1992;**75**:1421–1424.
- Spritzer P, Billaud L, Thalabard JC, Birman P, Mowszowicz I, Raux-Demay MC, Clair F, Kuttent F, Mauvais-Jarvis P. Cyproterone acetate versus hydrocortisone treatment in late-onset adrenal hyperplasia. *J Clin Endocrinol Metab* 1990;**70**:642–646.
- Sterne JA, Egger M. Funnel plots for detecting bias in meta-analysis: guidelines on choice of axis. *J Clin Epidemiol* 2001;**54**:1046–1055.
- Stikkelbroeck NM, Hoefsloot LH, de Wijs IJ, Otten BJ, Hermus AR, Stermans EA. 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* 2003;**88**:3852–3859.
- Stoupa A, Gonzalez-Briceno L, Pinto G, Samara-Boustani D, Thalassinou C, Flechtner I, Beltrand J, Bidet M, Simon A, Piketty M et al. Inadequate cortisol response to the tetracosactide (Synacthen(R)) test in non-classic congenital adrenal hyperplasia: an exception to the rule? *Horm Res Paediatr* 2015;**83**:262–267.
- Taboas M, Gomez Acuna L, Scaia MF, Bruque CD, Buzzalino N, Stivel M, Ceballos NR, Dain L. Functional studies of p.R132C, p.R149C, p.M283V, p.E431K, and a novel c.652-2A>G mutations of the CYP21A2 gene. *PLoS One* 2014;**9**:e92181.
- Tardy V, Menassa R, Sulmont V, Lienhardt-Roussie A, Lecointre C, Brauner R, David M, Morel Y. Phenotype-genotype correlations of 13 rare CYP21A2 mutations detected in 46 patients affected with 21-hydroxylase deficiency and in one carrier. *J Clin Endocrinol Metab* 2010;**95**:1288–1300.
- Tardy VT, Morel Y. Gene symbol: CYP21A2. *Hum Genet* 2007;**121**:293–294.
- Trapp CM, Oberfield SE. Recommendations for treatment of nonclassic congenital adrenal hyperplasia (NCCAH): an update. *Steroids* 2012;**77**:342–346.
- Turcu A, Smith JM, Auchus R, Rainey WE. Adrenal androgens and androgen precursors-definition, synthesis, regulation and physiologic actions. *Compr Physiol* 2014;**4**:1369–1381.
- Turcu AF, Rege J, Chomic R, Liu J, Nishimoto HK, Else T, Moraitis AG, Palapattu GS, Rainey WE, Auchus RJ. Profiles of 21-carbon steroids in 21-hydroxylase deficiency. *J Clin Endocrinol Metab* 2015;**100**:2283–2290.
- Tusie-Luna MT, Speiser PW, Dumic M, New MI, White PC. A mutation (Pro-30 to Leu) in CYP21 represents a potential nonclassic steroid 21-hydroxylase deficiency allele. *Mol Endocrinol* 1991;**5**:685–692.
- Tusie-Luna MT, Traktman P, White PC. Determination of functional effects of mutations in the steroid 21-hydroxylase gene (CYP21) using recombinant vaccinia virus. *J Biol Chem* 1990;**265**:20916–20922.
- Unluhizarci K, Kula M, Dundar M, Tanriverdi F, Israel S, Colak R, Dokmetas HS, Atmaca H, Bahceci M, Balci MK et al. The prevalence of non-classic adrenal hyperplasia among Turkish women with hyperandrogenism. *Gynecol Endocrinol* 2010;**26**:139–143.
- Usui T, Nishisho K, Kaji M, Ikuno N, Yorifuji T, Yasuda T, Kuzuya H, Shimatsu A. Three novel mutations in Japanese patients with 21-hydroxylase deficiency. *Horm Res* 2004;**61**:126–132.
- Volkl TM, Simm D, Beier C, Dorr HG. Obesity among children and adolescents with classic congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Pediatrics* 2006;**117**:e98–e105.
- Vrzalova Z, Hrubá Z, St'ahlova Hrabincova E, Pouchla S, Votava F, Kolouskova S, Fajkusova L. Identification of CYP21A2 mutant alleles in Czech patients with 21-hydroxylase deficiency. *Int J Mol Med* 2010;**26**:595–603.
- Wallensteen L, Zimmermann M, Thomsen Sandberg M, Gezelius A, Nordenstrom A, Hirvikoski T, Lajic S. Sex-dimorphic effects of prenatal treatment with dexamethasone. *J Clin Endocrinol Metab* 2016;**101**:3838–3846.
- Wang H, Jiang L, Wang PP, Zhou HB, Wang JL, Song LL. Mutation analysis in families with 21-hydroxylase deficiency. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi* 2007;**24**:681–684.
- Wasniewska M, Mirabelli S, Baldazzi L, Salzano G, Messina MF, Lombardo F, Valenzise M, Arrigo T, De Luca F. Novel mutation of CYP21A2 gene (N387K) affecting a non-conserved amino acid residue in exon 9. *J Endocrinol Invest* 2009;**32**:633.
- Wasniewska M, Raiola G, Galati MC, Salzano G, Rulli I, Zirilli G, De Luca F. Non-classical 21-hydroxylase deficiency in boys with prepubertal or pubertal gynecomastia. *Eur J Pediatr* 2008;**167**:1083–1084.
- Wedell A, Luthman H. Steroid 21-hydroxylase (P450c21): a new allele and spread of mutations through the pseudogene. *Hum Genet* 1993;**91**:236–240.
- Weintrob N, Dickerman Z, Sprecher E, Galatzer A, Pertzalan A. Non-classical 21-hydroxylase deficiency in infancy and childhood: the effect of time of initiation of therapy on puberty and final height. *Eur J Endocrinol* 1997;**136**:188–195.
- Wild RA, Carmina E, Diamanti-Kandarakis E, Dokras A, Escobar-Morreale HF, Futterweit W, Lobo R, Norman RJ, Talbott E, Dumesic DA. Assessment of cardiovascular risk and prevention of cardiovascular disease in women with the polycystic ovary syndrome: a consensus statement by the Androgen Excess and Polycystic Ovary Syndrome (AE-PCOS) Society. *J Clin Endocrinol Metab* 2010;**95**:2038–2049.
- Wilson RC, Nimkarn S, Dumic M, Obeid J, Azar MR, Najmabadi H, Saffari F, New MI. Ethnic-specific distribution of mutations in 716 patients with congenital adrenal hyperplasia owing to 21-hydroxylase deficiency. *Mol Genet Metab* 2007;**90**:414–421.
- Witchel SF. Non-classic congenital adrenal hyperplasia. *Steroids* 2013;**78**:747–750.
- Witchel SF, Azziz R. Nonclassic congenital adrenal hyperplasia. *Int J Pediatr Endocrinol* 2010;**2010**:625105.
- Yarman S, Dursun A, Oguz F, Alagol F. The prevalence, molecular analysis and HLA typing of late-onset 21-hydroxylase deficiency in Turkish woman with hirsutism and polycystic ovary. *Endocr J* 2004;**51**:31–36.
- Young J, Tardy V, de la Perriere AB, Bachelot A, Morel Y. Detection and management of late-onset 21-hydroxylase deficiency in women with hyperandrogenism. *Ann Endocrinol (Paris)* 2010;**71**:14–18.
- Zargar AH, Wani AI, Masoodi SR, Laway BA, Bashir MI, Salahuddin M. Epidemiologic and etiologic aspects of hirsutism in Kashmiri women in the Indian subcontinent. *Fertil Steril* 2002;**77**:674–678.
- Zeng X, Witchel SF, Dobrowolski SF, Moulder PV, Jarvik JW, Telmer CA. Detection and assignment of CYP21 mutations using peptide mass signature genotyping. *Mol Genet Metab* 2004;**82**:38–47.
- Zreik RS, Nasrallah MP. The prevalence of endocrinopathies among Lebanese women presenting with hirsutism to an endocrine clinic. *J Med Liban* 2014;**62**:27–32.