Detection and management of late-onset 21-hydroxylase deficiency in women with hyperandrogenism

Dépistage et prise en charge du déficit en 21-hydroxylase à révélation tardive chez les femmes avec hyperandrogénie

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1. Frequency of D21OH-NC among women referred for hyperandrogenism or hirsutism

D21OH-NC is one of the most frequent genetic disorders with an estimated 1/1000, although numbers vary substantially across ethnical groups and countries, making this rate a very tentative one indeed [1].

The frequency of D21OH-NC among women presenting with hirsutism or hyperandrogenism was estimated in various ways, mainly by specialized teams belonging to hospitals. Therefore, it might be overestimated compared to the frequency of D21OH-NC in the general population of women with hirsutism, due to biased recruitment. In a 1985 French publication [2], D21OH-NC frequency was an estimated 6% (D21OH-NC cases screened for in a population of 400 women referred for hirsutism). In a more recent American study [3] where 823 patients were referred for hirsutism, only 1.6% of cases were found to have D21OH-NC. This last frequency is close to the 2.2% reported in 2008, about 280 non-selected Spanish patients [4]. And in an Italian retrospective study, 4.3% of the 950 files of women presenting with signs of hyperandrogenism were considered to be D21OH-NC cases [5]. Therefore, it appears that, though the frequency of D21OH-NC in women presenting with hirsutism and/or hyperandrogenism is highly variable, it is most likely under 5%. As stated earlier, this fluctuation is probably due to recruitment biases and differences in screening techniques (threshold values of serum 17-hydroxyprogesterone [17OHP] base levels or peak levels after cosyntropin stimulation test).

2. Screening for D21OH-NC in women presenting with hyperandrogenism

It is currently admitted by the greater community of endocrinologists that diagnosis of D21OH-NC is based on morning plasma 17OHP levels, sampled at best during the first part of the cycle. Testing should be carried out in the absence or at a distance of glucocorticoid treatment, as even ‘substitute’ treatment, and even more when it is pharmacological, inhibits the corticotrophic axis and might cause false negative results. Sam...
ples for 17OHP assay should be drawn in the morning, for after noon, 17OHP levels can be within normal range in women with D21OH-NC due to nycthemeral fluctuations of the adrenal gland secretion.

Some specialists diverge on the threshold of 17OHP that defines D21OH-NC, as they do on whether or not basal levels of the steroid alone suffice to diagnose the condition, and when a 250 μg cosyntropin stimulation test is required.

There are very few studies available to settle the matter, and those that do exist feature small numbers of patients and imperfect methodology. Their findings should therefore be considered with caution. Yet, a few studies have attempted to answer the question directly or indirectly, thus establishing some interesting anchoring points. Among the most significant works is the multicenter trial carried out by Moran et al. [6]: they measured baseline 17OHP levels in 218 non-genotyped patients with highly probable D21OH-NC (the diagnosis was based on 17OHP levels above 10 ng/ml – see below). The main positive point of this study was its international multicenter approach where different assayng techniques were used to measure 17OHP levels, thus making it more representative of routine medical practice. In this trial, 18 patients with D21OH-NC out of 218 (approximately 8%) had morning basal 17OHP levels below 2 ng/ml, which is a threshold value for diagnosing the condition according to some [6]. Six patients (2.7%) even had levels below 1 ng/ml. Another study included a smaller number of patients but was very slick, methodologically speaking: patients were all genotyped, 17OHP levels were measured in the morning during the follicular phase, and explicitly without any steroid intake. Using the same 2 ng/ml threshold, the authors also found 8% of false negatives, i.e. patients with genetically proven D21OH-NC whose 17OHP levels were below the threshold [7].

In a more recent French publication including 161 patients, most of them genotyped, 13 (8%) had 17OHP levels below the 2 ng/ml threshold [8]. Overall, what we find in these publications is that basal 17OHP levels are not a reliable enough criteria to diagnose all D21OH-NC patients, as only a little over 90% of them were accurately diagnosed with the test. On the other hand, it is an interesting test, as long as it is correctly performed – in the morning and at a distance from steroid intake – for it detects the vast majority (90% and beyond) of patients. From this data, we conclude that when seeking to rule out the diagnosis of D21OH-NC, measuring basal 17OHP levels alone does not suffice.

Consequently, we recommend that screening for D21OH-NC be based on basal 17OHP levels, bearing in mind that this single test cannot totally exclude the diagnosis.

3. Diagnostic accuracy of peak 17OHP after cosyntropin stimulation test

To enhance screening efficiency, peak 17OHP, after 250 μg cosyntropin stimulation, has been measured for over two decades [9]. This dynamic test is not routinely carried out in non-specialized laboratories or by non-endocrinologists, and usually requires referral to specialized, hospital, or endocrine department laboratories. Second to genotyping, peak 17OHP level is the most efficient test to diagnose D21OH-NC: among the 550 women with genetically confirmed D21OH-NC that were tested for hyperandrogenism, Morel and Tardy [20] showed that nearly 100% had peak 17OHP levels above 10 ng/ml, regardless of the type of mutation. Because of this very accurate cut-off value, many teams throughout the world are now routinely using the test [1–5]. The specificity of the test is slightly reduced by a few healthy patients carrying usually severe heterozygous mutations that have peak 17OHP levels above the cut-off value [8,10].

But this minor defect can easily be corrected by sequencing the CYP21A2 gene. Therefore, we recommend measuring peak 17OHP levels after 250 μg cosyntropin stimulation to diagnose or to exclude D21OH-NC.

4. Should D21OH-NC be systematically screened for in women presenting with hyperandrogenism?

Considering what was said above, a positive answer to this question would imply testing every patient referred for hyperandrogenism with cosyntropin stimulation. There is currently no consensus among specialists regarding systematic screening of the population of women with signs of hyperandrogenism, given the lack of clear-cut evidence for the medical benefit and cost-effectiveness of such a policy. Opponents argue that besides the complexity of the test, its availability is limited, its cost is high and diagnosing the condition in excess has potential negative effects on quality of life, which are not necessarily compensated by clear medical benefit. The widely accepted compromise consists in systematically proposing measurement of basal 17OHP levels (in the morning, during the follicular phase, at a distance from glucocorticoid intake) to screen for D21OH-NC, as the procedure detects over 90% of cases and is routinely available.

5. D21OH-NC and risk of adrenal insufficiency: should all patients receive long-term glucocorticoid replacement therapy?

Literature regarding this question is scarce, leaving much space for further investigation with specific trials designed to address the subject. There are, however, a few studies of small populations on the matter: two studies including a total of 30 patients assessed the adrenal function with a corticotropin releasing hormone (CRH) test [11,12]. They showed that on average, the adrenal cortical function is preserved but that some patients might have slightly lowered cortisol response to the test. A French team, investigating a group of 17 women, found just the opposite, namely that in some cases response to cosyntropin testing was very low [13]. However, in this publication, there was no mention of previous steroid treatment, which prevents one from drawing any clear conclusion.

There was no study to unequivocally demonstrate that D21OH-NC patients were at a higher risk of acute adrenal insufficiency. There is no data demonstrating the benefit of treatment on the quality of life, on potential morbidity or on mortality rates, nor have potential secondary effects been investigated. Therefore, the risk/benefit ratio cannot, as of today, be established. In the meantime, we cannot recommend systematic steroid treatment, in particular, hydrocortisone treatment, in patients...
with D21OH-NC. The only exception tolerated by the community of experts concerns patients having – whether rightly or wrongly received – supraphysiological doses of glucocorticoids that might cause a corticotrophic deficit, i.e., adrenal insufficiency due to adrenocorticotrophic hormone (ACTH) deficit.

Other matters about which there is no consensus yet, for the same reasons, include carrying a card stating that the patient has adrenal insufficiency and systematic glucocorticoid treatment prior to surgery or acute physical stress.

6. Treatment of hyperandrogenism in D21OH-NC

Theoretically, there are two ways of treating hyperandrogenism: either by slowing the excessive production of androgens by the adrenal gland with glucocorticoids or by blocking the effects of androgens on their receptors with antiandrogens [14]. As was the case in the last paragraph, the problem lies in the lack of literature assessing the treatment of hirsutism and hyperandrogenism in patients with D21OH-NC [14]. Two controlled randomized trials compared the effectiveness of cyproterone acetate (CPA) vs hydrocortisone. In the first trial, on a series of 30 patients, CPA was more effective (symptoms improved in 54% of patients) than hydrocortisone (symptoms improved in 24% of patients) based on the Ferriman and Gallway score [15]. In the second randomized trial including 28 patients with hirsutism, CPA associated with Ethinyl Estradiol (EE) was compared to dexamethasone (symptoms improved in 31% of patients) [16].

Based on these trials, we do not recommend the long-term treatment with glucocorticoids of D21OH-NC patients with hirsutism. CPA seems to be the best first intention treatment when there is no contraindication. The comparable efficiency of CPA and antiandrogens – such as spironolactone or flutamide – in other populations of women with hirsutism (see concerning paragraph) indicates that they might be interesting to use in D21OH-NC patients with hirsutism, though their effect has not specifically been tested on this particular population.

7. D21OH-NC and fertility

In addition to hyperandrogenism, D21OH-NC patients are exposed to a higher risk of infertility [17]. It appears to be multifactorial in origin: in some patients, anovulation is due to progestins (progesterone and 17OHP) secreted in excess by the adrenal gland, possibly accentuated by chronic hyperandrogenism. In other patients, abnormal endometrial development (‘microprostegiative pill’ effect) has been noted [18]. Also recorded among these women was their increased risk for miscarriage [17,19]. But infertility was relative, since 138 (68%) out of 203 pregnancies were initiated before D21OH-NC was diagnosed and treated in an international retrospective study [17]. Likewise, in an older study by Feldman et al., spontaneous pregnancies reached 50% before diagnosis [19]. But in both papers, the number of miscarriages decreased after diagnosis and hydrocortisone treatment initiated.

It seems therefore that this treatment might increase fertility and positive outcome of pregnancy in D21OH-NC patients.

We therefore recommend hydrocortisone treatment in women who wish to initiate a pregnancy in an attempt to increase fertility and decrease risks of miscarriage.

8. Indications of 21-hydroxylase gene molecular analysis in patients with D21OH-NC and genetic counselling for women who desire child bearing

The importance of molecular testing in D21OH-NC patients has long been stressed by a leading French team in this field [20–22]. In one of their unpublished molecular studies on a population of about 800 D21OH-NC patients have shown that nearly 60% of patients carried a severe mutation of one allele, with a 50% risk of transmitting the mutated allele to the child to be born. This very high percentage was confirmed in another study published in 2009 [8].

The theoretical risk for a D21OH-NC woman with a severe mutation on one allele to bear a child with the severe, classical form of D21OH is 1/240 (0.4%), before partner genotyping. But in practice, the risk seems even higher, based on a multicenter retrospective study, which collected data on 203 pregnancies. In this study, four children with the severe classical form of D21OH were born to D21OH-NC patients, which was six times the expected rate (2.5% vs 0.4%) [9,20–22]. Moreover, if a severe mutation is identified in the father, the couple will be at major risk (1/4 i.e. 25%) of bearing a child with a severe classical form of D21OH [20–22].

Based on these data, we issue two recommendations: any woman diagnosed with D21OH-NC should undergo molecular analysis of the gene coding for 21-hydroxylase (CYP21A2); as soon as the condition is diagnosed, she should be informed of her risk of bearing a child with a severe, classical form of D21OH and of the necessity of testing her asymptomatic partner for a severe mutation in the same gene. This investigation should be completed with that of the propositus’ parents (molecular analysis of the CYP21A2 gene) to ensure the reliability of results in the patient (see below).

Testing of the partner to find out whether he is heterozygous for a severe mutation (theoretical risk of 1/50 in France) is at best planned once the diagnosis has been confirmed in the propositus. It is usually carried out in two stages:

- biochemical screening by measuring 21-deoxycortisol (21DF) levels after cosyntropin stimulation (at 0’, 60’ and 90’) [20–24];
- sequencing of the CYP21 gene if the peak level is above 550 pg/ml [20–24].

In case of an emergency, such as an unplanned pregnancy, direct sequencing of the CYP21A2 can be scheduled for the father.
9. Relevance of genetic testing for relatives of D21OH-NC patients

Genetic testing of relatives of D21OH-NC patients is crucial, as it guarantees that the genetic tests run on the patient herself are accurate [24]. Tests identify the parental origin of the mutation and ensure that adequate genetic counselling can be provided. It is all the more important if the patient is homozygous for a moderately severe mutation: in this case, current techniques (apart from Multiplex Ligation Probe Amplification [MLPA] techniques still being assessed) cannot differentiate a homozygous state (moderately severe mutation in both alleles) from a hemizygous state (moderate mutation on one allele but large deletion on the second).

Testing the patient’s relatives also provides the benefit of detecting asymptomatic patients who might have the same risk as the D21OH-NC patient of transmitting the severe condition to descendents.

Relatives concerned include siblings, but also second-degree relatives such as cousins. As soon as a severe mutation is discovered in a family, the information should be widely distributed among relatives so as to avoid emergency situations such as pregnancy.

10. Conclusions

Hyperandrogenism is a common reason for referral in endocrinology, gynecology and dermatology. In a non-negligible percentage of patients, a genetic autosomal recessive condition called D21OH-NC causes the symptoms. The simple measure of basal 17OHP levels can detect the great majority of patients affected with D21OH-NC, but to diagnose the condition with certainty, a measure of plasma 17OHP levels after cosyntrpin stimulation should be obtained. Although there is no high level evidence, antiandrogens seem superior to glucocorticoids to treat signs of hyperandrogenism. Fertility, although there is no high level evidence, antiandrogens seem superior to glucocorticoids to treat signs of hyperandrogenism. Fertility, although there is no high level evidence, antiandrogens seem superior to glucocorticoids to treat signs of hyperandrogenism. Fertility, although there is no high level evidence, antiandrogens seem superior to glucocorticoids to treat signs of hyperandrogenism. 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