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# Biomarkers in congenital adrenal hyperplasia

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## Abstract

Monitoring of hormone replacement therapy represents a major challenge in the management of congenital adrenal hyperplasia (CAH). In the absence of clear guidance and standardised monitoring strategies, there is no consensus among clinicians regarding the relevance of various biochemical markers used in practice, leading to wide variability in their application and interpretation. In this review, we summarise the published evidence on biochemical monitoring of CAH. We discuss temporal variations of the most commonly measured biomarkers throughout the day, the interrelationship between different biomarkers, as well as their relationship with different glucocorticoid and mineralocorticoid treatment regimens and clinical outcomes. Our review highlights significant heterogeneity across studies in both aims and methodology. However, we identified key messages for the management of patients with CAH. The approach to hormone replacement therapy should be individualised, based on the individual hormonal profile throughout the day in relation to medication. There are limitations to using 17-hydroxyprogesterone, androstenedione and testosterone, and the role of additional biomarkers such as 11-oxygenated androgens which are more disease specific should be further established. Noninvasive monitoring via salivary and urinary steroid measurements is becoming increasingly available and should be considered, especially in the management of children with CAH. Additionally, this review indicates the need for large scale longitudinal studies analysing the interrelation between different monitoring strategies used in clinical practice and health outcomes in children and adults with CAH.

## KEYWORDS

biomarkers, congenital adrenal hyperplasia, glucocorticoids, hormonal profiles, hormonal replacement

## 1 | INTRODUCTION

The monitoring of corticosteroid replacement represents an ongoing challenge in managing congenital adrenal hyperplasia (CAH), with insufficient doses incurring the risk of adrenal crisis and death, whilst

overtreatment is associated with growth suppression, fertility issues and adverse effects on metabolic, cardiovascular and bone health.<sup>1,2</sup>

A range of hormonal biomarkers in blood, urine or saliva can be employed to gauge the quality of CAH control and guide therapy before problems manifest clinically. However, significant variability

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exists in clinical practice with regards to the use of biomarker profiles, timing of sample collection, laboratory assays used, target ranges, and interpretation of results.<sup>2</sup> Herein, we summarise the evidence published over the last 30 years in relation to biochemical markers of treatment control in patients with CAH.

## 2 | PATHOPHYSIOLOGY

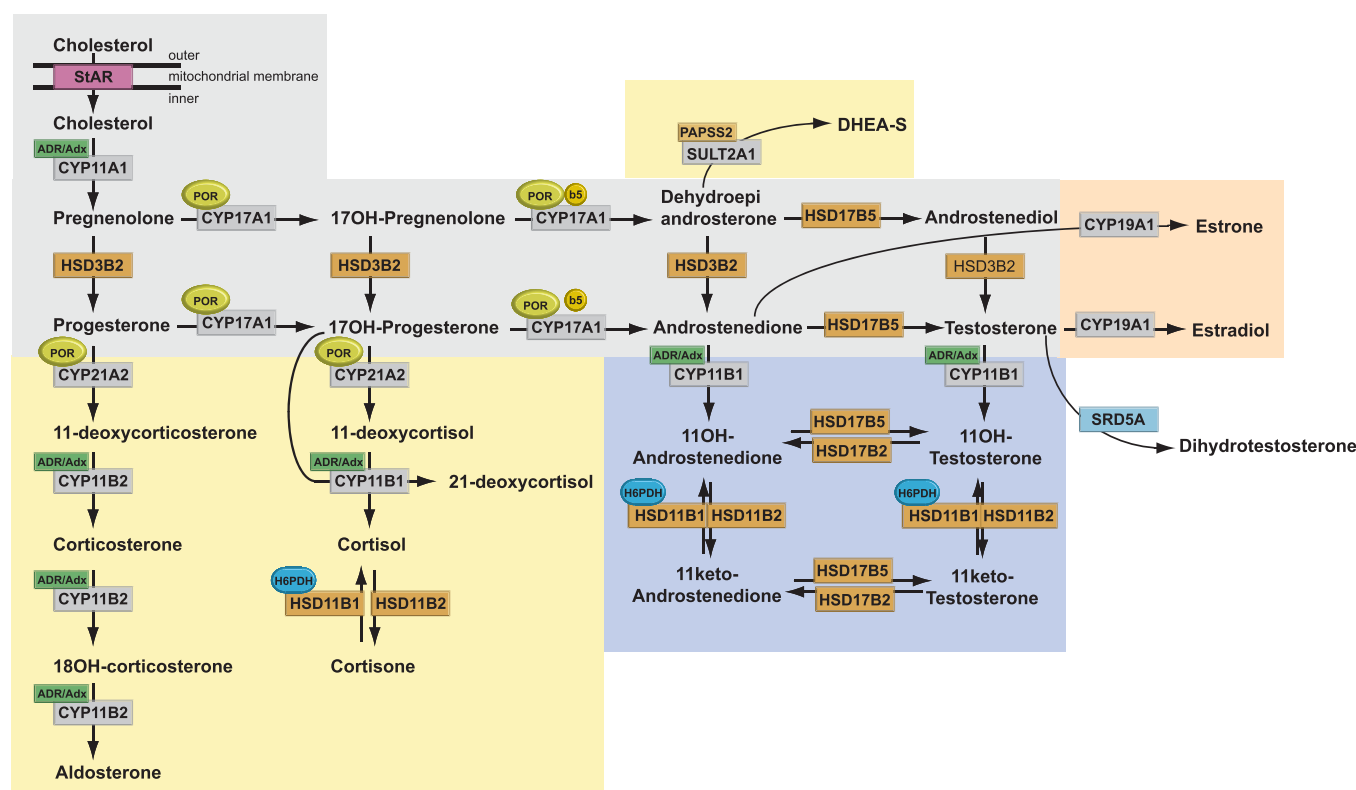
The focus of CAH monitoring for most patients is on adrenal androgens and their precursors. In 95% of cases CAH is caused by impaired function of 21-hydroxylase which converts 17-hydroxyprogesterone (17OHP) into the cortisol precursor 11-deoxycortisol, and progesterone into deoxycorticosterone (Figure 1). Deficiency of 21-hydroxylase results in cortisol and, in two thirds of patients, aldosterone deficiency. The consequent build-up of 17OHP leads to excessive sex steroid hormone synthesis, starting with androstenedione production by 17,20-lyase. Disruption in the steroid pathway at other steps leads to different imbalances, and the second most common form of CAH is 11 $\beta$ -hydroxylase deficiency, impairing the conversion of 11-deoxycortisol into cortisol and 11-deoxycorticosterone (DOC) into

corticosterone. DOC has mineralocorticoid properties, leading to a different clinical presentation of hypertension alongside glucocorticoid deficiency.<sup>3</sup>

The serum biomarkers most commonly employed in practice to assess CAH treatment are 17OHP, androstenedione and to a lesser extent testosterone.<sup>4</sup> Measurement by Liquid Chromatography Tandem Mass-Spectrometry (LC-MS/MS) is superior to radio-immunoassay, which may overestimate adrenal hormones by 30%–50% due to cross-reactivity.<sup>5</sup> Interpretation of these biomarkers can be challenging due to variation in their concentrations throughout the day, in relation to replacement doses and across ages and sexes.

## 3 | TEMPORAL VARIATION OF BIOMARKERS

Similar to cortisol, its precursor 17OHP and the androgen precursor androstenedione exhibit significant diurnal variation within patients with CAH, peaking at 07:00 AM and reaching a nadir between 01:00 and 03:00 AM. Diurnal variation has also been shown with ACTH,



**FIGURE 1** Adrenal steroid hormone biosynthesis. The grey background corresponds to reactions that take place in the adrenal cortex and the gonads, the blue background to those that take place in the adrenal and peripheral tissues, such as the adipose, adrenal specific processes have a yellow background, the orange background corresponds to reactions that take mainly place in the ovary, while processes that occur in the peripheral tissues are left unshaded. Grey and orange boxes are used to indicate CYP and HSD steroidogenic enzymes, the pink box indicates the steroidogenic acute regulatory protein and the blue one, 5 $\alpha$ -reductase. The small green boxes correspond to adrenodoxin/adrenodoxin reductase, orange box to 3-phosphoadenosine-5-phosphosulfate synthase type 2, yellow ovals to P450 oxidoreductase, orange circles to cytochrome b5 and blue ovals to the coenzyme hexose-6-phosphate dehydrogenase. [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/terms-and-conditions)]

androstosterone, testosterone, progesterone and the more recently characterised adrenal-specific 11-oxygenated androgens.<sup>6–8</sup> However, in female patients with 21-hydroxylase deficiency, intra-class correlation analysis showed good repeatability for 17OHP, androstenedione, and testosterone measured in the same individual on different dates.<sup>9</sup> In healthy children, there is a significant difference in the concentrations of adrenal hormones dependent upon sex, age and pubertal status, with adrenal androgens rising after the age of five and testosterone rising markedly in boys after 10 years of age when testicular production becomes more dominant.<sup>10,11</sup>

#### 4 | RELATIONSHIP BETWEEN BIOMARKERS AND EXOGENOUS GLUCOCORTICOID REPLACEMENT

Pharmacokinetic studies of synthetic hydrocortisone in children with CAH have shown significant interindividual variation in cortisol metabolism.<sup>12–15</sup> A morning dose of hydrocortisone has a sharp and significant effect on 17OHP which drops by 90% of the pre-dose concentration within 3 h after administration, and androstenedione by 70% within 4 h.<sup>16</sup> A study in adults with CAH has shown a longer time for adrenocortical markers to reach a minimum with different preparations, with trough concentrations 4–5 h post-prednisolone and 10 h following dexamethasone,<sup>8</sup> highlighting the importance of interpreting adrenal steroids in relation to both the timing of administration and preparation of glucocorticoids. Whilst higher doses of hydrocortisone are associated with higher serum cortisol, there was significant variability in its absorption and metabolism between different patients. Exploring hormonal profiles within individual patients therefore has the potential to improve CAH control; however, profiling is expensive and laborious.<sup>12,14,16,17</sup> Moreover, 4-h 17OHP profiles were not found to predict hyperandrogenism, indicating that they need to be interpreted in association with other biomarkers.<sup>18</sup>

Whilst 11-oxygenated androgens exhibit a circadian pattern in CAH that is influenced by the GC replacement regime, their fluctuation is slower and more modest compared to that of 17OHP. Both 11-hydroxytestosterone (11OHT) and 11-ketotestosterone (11KT) differed by less than 50% between peak and trough, which may allow for more flexibility in the timing of monitoring blood tests.<sup>6</sup> There is ongoing work to establish normative ranges and to gain more insight into the interdependency of 11-oxygenated androgens with medication, clinical outcomes and other biomarkers of control.<sup>19–21</sup>

Adrenal steroid biomarkers are elevated in CAH patients on lower doses of GCs. A dose-dependent effect on morning fasted 17OHP, androstenedione and testosterone has been shown in those using 15 versus 25 mg/m<sup>2</sup> per day hydrocortisone.<sup>15</sup> However, growth was suppressed in patients on higher doses, leading to the conclusion that normalisation of 17OHP in CAH is inappropriate, and indicative of overtreatment. A tighter range of 13.5–17.8 mg/m<sup>2</sup> per day studied in 24 patients in the first 3 years of life failed to find a relationship between dose and 17OHP or androstenedione

concentrations. This may be partially attributed to the use of cortisone acetate in this study, which requires hepatic conversion to become active and has 20% reduced bioactivity compared to hydrocortisone.<sup>22</sup> Alongside adjunctive treatment, elevated markers are found at lower hydrocortisone doses; a cross-over study of 12 prepubertal patients comparing 12.9 to 7.9 mg/m<sup>2</sup>/day hydrocortisone alongside flutamide and testolactone showed a rise in 17OHP with the lower hydrocortisone dose.<sup>23</sup> Following 28 CAH patients on the same regime for 2 years showed elevated 17OHP, androstenedione, DHEA, DHEAS, and testosterone associated with lower hydrocortisone doses. 17OHP was also found to discriminate between standard and medium-release GC treatment, being significantly lower in the latter.<sup>24</sup>

A reverse circadian replacement regime, where the highest GC doses are administered in the evening, is still favoured by some clinicians,<sup>25</sup> despite evidence of association with metabolic complications including insulin resistance.<sup>26</sup> Measurement of serum 17OHP has failed to discriminate between circadian versus reverse-circadian regimes,<sup>27,28</sup> possibly due to its wide variability with the time of day and GC administration. An Italian study with 57 children with CAH comparing the benefits of monitoring serum biomarkers before or after the morning replacement dose concluded that using pre-medication measurements improved final height.<sup>29</sup> However, the practicalities of measuring blood samples in everyday practice before medication administered very early in the morning must be considered. International consensus guidelines, therefore, recommend blood biomarkers to be measured at consistent times relative to medication schedule and time of day.<sup>30</sup>

#### 5 | THE RELATIONSHIP BETWEEN BLOOD BIOMARKERS AND CLINICAL OUTCOMES IN CAH

The high prevalence of adverse health outcomes in CAH, including abnormal growth, weight gain, cardiovascular and metabolic disease, infertility, in the context of biochemical markers of poor control, was demonstrated by large cohort studies in adults and children.<sup>31–35</sup> Elevated biomarkers of disease control have been associated with detrimental height,<sup>17,36–39</sup> body mass index (BMI), menstrual irregularities,<sup>31,33</sup> bone mineral density (BMD)<sup>40,41</sup> and testicular adrenal rest tumours (TARTs).<sup>42</sup> The relationship between biomarkers and clinical outcomes was statistically insignificant in several small studies.<sup>40–47</sup> However, this may relate to variable time collection in relation to the GC dose and dichotomising continuous into groups based on different arbitrary thresholds, rendering the interpretation of effect sizes across studies difficult. The analysis of international data from the I-CAH/DSD registry (*n* = 345) showed large variability in 17OHP and androstenedione between different centres and no correlation between these biomarkers and weight standard deviation score (SDS).<sup>48</sup>

Ten studies have reported height parameters in relation to typical hormonal markers; four of them failed to detect any association but were limited by sample sizes of 28 or less.<sup>40,43–45</sup>

A larger study involving 50 infants with CAH found inconsistency between the follow-up 17OHP measurements and growth velocity.<sup>49</sup> A negative correlation was found between final adult height and androstenedione and testosterone in 54 pre-pubertal patients,<sup>36</sup> whereas 17OHP correlated positively with height SDS and height velocity in 22 children under 10 years of age.<sup>17</sup> Studies involving post-pubertal subjects reported higher concentrations of 17OHP associated with reduced height SDS,<sup>37</sup> reduced predicted height in groups with higher average 17OHP,<sup>38</sup> and indicated that children who could not undergo regular biomarker measurement achieved reduced final adult height.<sup>39</sup> This is consistent with excess adrenal androgens reflected by higher biomarker concentrations causing accelerated skeletal maturation before puberty to the detriment of final height.<sup>50</sup>

Bone maturation or BMD has been investigated in relation to biomarkers in CAH in two paediatric and three adult studies. Increasingly advanced bone age was shown in children with consistently raised 17OHP compared to those whose 17OHP was suppressed.<sup>40,41</sup> A small study of 13 women aged 20–29 years with CAH showed no association between BMD and morning serum androgens.<sup>46</sup> Similar results were reported by a study of 26 adult females, which did however find reduced DHEA and DHEAS in patients with osteopenia.<sup>51</sup> The androstenedione to testosterone ratio, used to ascertain how much testosterone derives from the adrenal in comparison to the gonads, when measured in 16 years olds with CAH correlated positively with BMD later in adulthood.<sup>52</sup> No association was found for 17OHP and testosterone with BMD in 33 children with CAH.<sup>53</sup>

A sensitive marker of adrenarche, DHEAS has been found to have limited value in monitoring CAH, as it was shown to be absent or suppressed even during long periods of adequate control as judged by morning serum 17OHP and testosterone.<sup>54</sup> Following puberty, high BMI, irregular menstrual cycles and hyperandrogenism have all been linked with high concentrations of serum 17OHP and androstenedione.<sup>33</sup> Ovulation has only been assessed in a very small cohort of eight women split into poor and good control by their 17OHP, where a difference in the number of anovulatory cycles was not detected.<sup>55</sup> The novel markers 11-ketotestosterone (11KT) and 11-hydroxyandrostenedione (11OHA4) may have utility in assessing fertility in adults with CAH, where they have been shown to be raised in females with secondary amenorrhoea and males with hypogonadotropic hypogonadism.<sup>56</sup>

Adrenal nodules in CAH have been linked with disease control. Within a cohort of 26 patients, adrenal nodules were more common before initiating treatment, and more likely to regress in patients with poor biochemical control after their treatment was optimised and control improved.<sup>57</sup> The 11-oxygenated C19 androgens have also been shown to be raised in a cohort of seven patients with TARTs in comparison to 32 without TARTs, supporting the potential for these markers to be used to monitor CAH.<sup>42</sup> A subsequent study conducted by the same group showed that TARTs also produce 11-oxygenated C19 androgens of which 11-hydroxytestosterone is predominant.<sup>58</sup> A recent cohort study involving older patients

compared 11 men with TARTs and 21 without and failed to find a difference in 11-ketotestosterone or 11-hydroxyandrostenedione, although the lack of power from the small numbers involved must be considered.<sup>56</sup>

In a small cohort ( $n = 36$ ) a relationship was found between the reduced adrenomedullary function and the occurrence of acute sickness in infants with 21-hydroxylase deficiency, suggesting that epinephrine concentrations could be an independent predictor of illness incidence.<sup>59</sup>

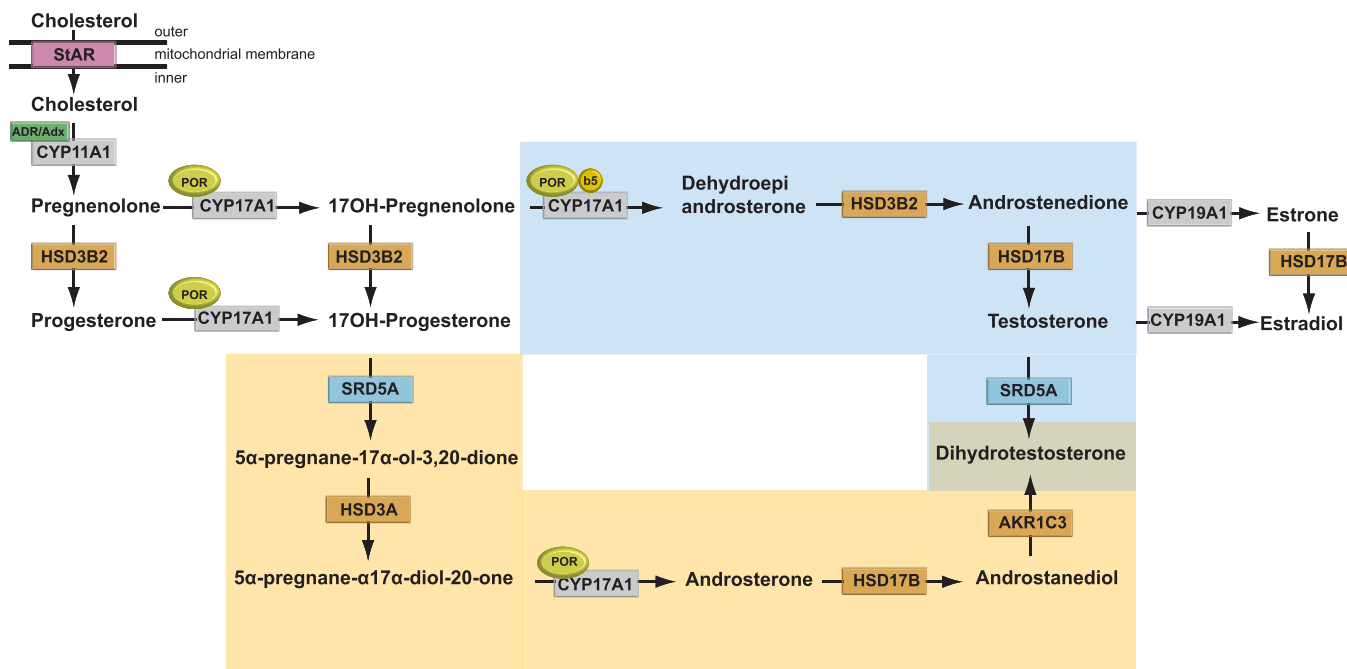
## 6 | THE RELATIONSHIP BETWEEN BIOMARKERS

The commonly used markers 17OHP and androstenedione have been shown to correlate strongly in patients with CAH.<sup>48</sup> Concordance was strongest in the morning when point readings of androstenedione correlated with 17OHP to produce an  $r^2$  of .81,<sup>60</sup> and area under the curve of a full 24 h of 17OHP readings correlated with 8:00 AM androstenedione to produce an  $r^2$  of .66 in poorly controlled patients.<sup>61</sup> Whilst androstenedione varies diurnally to a lesser degree than 17OHP, their change from baseline in patients with CAH was proportional ( $r^2 = .46$ ).<sup>16</sup>

Testosterone was found to correlate with 17OHP, when corrected for age and sex, but to a lesser degree than androstenedione. In 42 girls with CAH spanning pubertal stages, an  $r^2$  of .31 was reported,<sup>62</sup> with a lower correlation found in a mixed sex cohort.<sup>6</sup> Another study reported positive correlation with 11-oxygenated androgens seen before puberty, while in boys with Tanner stage five 11-ketotestosterone or 11-hydroxyandrostenedione correlated negatively with testosterone, indicating a shift from adrenal to gonadal androgen dominance following puberty, a process that is suppressed in CAH by the adrenal hyperandrogenism.<sup>42</sup> The positive correlation between 11-oxygenated androgens and 17OHP and androstenedione, as well as ACTH, is stronger than with testosterone.<sup>42,63–65</sup>

The association between adrenal androgens and cortisol is straightforward in healthy controls where single point measurements of serum cortisol correlate with 17OHP, androstenedione, testosterone, 11-hydroxyandrostenedione and 11-ketotestosterone.<sup>16,51</sup> This association was not present in a cohort of 78 children with CAH treated with oral hydrocortisone.<sup>64</sup> Similarly, in 34 children with CAH on oral hydrocortisone no relationship was found between cortisol and 17OHP or androstenedione when comparing absolute blood concentrations or area under the curve over 6 h.<sup>16</sup> Longer 24-h profiling of cortisol and 17OHP in 19 patients who were poorly controlled, analysed how correlation varies with time lag, and found cortisol to correlate closest with 17OHP measured 6 h and 40 min beforehand, the time lag varying between night and day.<sup>61</sup> This is consistent with the fundamental difference between healthy children in which these biomarkers are used to produce cortisol, whereas in CAH these biomarkers rise as an indication of cortisol deficiency.

DHEA is frequently suppressed in CAH and appears to be unrelated to 17OHP or androstenedione,<sup>8,66</sup> and consequently



**FIGURE 2** Diagram of the “alternative backdoor” pathway to dihydrotestosterone. The blue background corresponds to reactions that take place as part of the “classic” pathway and the orange background corresponds to the “alternative” pathway. Grey and orange boxes are used to indicate CYP and HSD steroidogenic enzymes, the pink box indicates the steroidogenic acute regulatory protein and the blue one, 5 $\alpha$ -reductase. The small green boxes correspond to adrenodoxin/adrenodoxin reductase, orange box to 3-phosphoadenosine-5-phosphosulfate synthase type 2, yellow ovals to P450 oxidoreductase, orange circles to cytochrome b5. [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1111/ce.14960)]

DHEA or DHEAS are not recommended as markers of CAH control.<sup>2</sup> Alternative biomarkers include 21-deoxycortisol, suggested to be superior to 17OHP in both monitoring of CAH and screening,<sup>67</sup> and correlating with 17OHP in pre-dose blood samples.<sup>5</sup> Further markers correlating strongly with 17OHP include 16OHP, progesterone, 3- $\alpha$ -androstenediol glucuronide (3a-AG),<sup>6,42,68–70</sup> Androsterone and urinary 5 $\beta$ -pregnane-3 $\alpha$ ,20 $\alpha$ -diol (pdIol), produced by the “alternative backdoor” pathway (Figure 2) that leads to dihydrotestosterone without requiring the intermediaries of androstenedione or testosterone, correlated with the traditional serum markers androstenedione and 17OHP in adults with CAH.<sup>8</sup> Leptin, secreted by the white adipose tissue, is known to have important roles in human metabolism by regulating food intake and energy expenditure, and may be of use in CAH. Leptin correlated positively with the pre-dose cortisol concentration and negatively with testosterone in patients with CAH.<sup>71</sup> Along with the 11-oxygenated androgens, these markers warrant further investigation, but they cannot be currently employed in clinical practice in the absence of disease specific target ranges.

## 7 | DRIED BLOOD SPOT

Measurement of biomarkers, most commonly 17OHP, from dry blood-spot samples on filter paper presents the advantages of requiring less blood, reducing the emotional trauma associated with the blood collection and being easier for testing patients at home.

However, they require multiple blood tests on the same day. This collection is therefore cheaper and more convenient in the determination of daily profiles. While some heterogeneity in methodology and results exists among the studies that explored the relationship between blood spot 17OHP and serum biomarkers,<sup>15,72–77</sup> the correlation was always strong with  $r^2$  between .76 and .96, and strongest when samples were collected before the morning GC dose. Diurnal fluctuations of 17OHP resemble those seen in blood samples.<sup>73,74,77–79</sup> On the other hand, blood-spot androstenedione relates poorly to serum measurements, agreement in categorising the marker as low, medium or high being achieved only 59% of the time.<sup>15</sup>

Dried blood spot 17OHP correlates with urinary steroid profiles in patients with CAH, in particular the urinary pregnanetriol/tetrahydrocortisone ratio.<sup>79</sup> A link was also established between height velocity and the biochemical control established from dried blood spot 17OHP.<sup>78</sup> While these results are promising, the use of blood spot measurements in monitoring CAH is subject to technical availability and not widely applicable.

## 8 | SALIVARY BIOMARKERS

The development of novel assay technologies brought forth the prospect of establishing non-invasive monitoring strategies for CAH, including measuring steroid hormones in saliva, which has been in use in some centres for over 40 years.<sup>80</sup> Both 17OHP and



androstenedione correlate well between saliva and serum, this relationship is the strongest for 17OHP when measured by LC-MS/MS, with an  $r^2$  up to .97.<sup>64,81–83</sup> Whilst serum and salivary cortisol correlate well in healthy individuals, concentrations do not correlate as well in those with CAH.<sup>64,84</sup>

The kinetics of cortisol and 17OHP in saliva after a morning dose of hydrocortisone follow similar patterns to those observed in blood.<sup>83,85</sup> One study comparing multiple salivary measurements and serum measurements of 17OHP in 23 patients found salivary measurements to be more sensitive in detecting improved disease control in comparison to serum measurements. Nonetheless, interpreting a normal 07:00 AM single timepoint measurement of 17OHP in saliva failed to detect over 50% of patients that warranted treatment adjustment upon review of their daily profile,<sup>86</sup> evidencing the potential difficulty in relying on single timepoint measurements.

Salivary androstenedione and 17OHP measured pre-dose in CAH significantly increase during puberty, rising at an earlier age in girls than boys. The proportions also change; whilst before puberty, a patient with normal androstenedione can have 17OHP three times the upper reference limit, this may increase to six times during puberty.<sup>80</sup> In 39 CAH patients monitored throughout childhood, salivary androstenedione correlated negatively with final height, adjusted for parental height and appropriate covariates including dose.<sup>87</sup> Androstenedione in this case was more effective than 17OHP in predicting height deficit, suggesting that the two markers complement one another, high androstenedione being an effective marker of undertreatment, while low 17OHP effectively indicating overtreatment.

As in serum, the 11-oxygenated C19 steroids including 11OHA4 and 11KT show strong potential to be used as biomarkers of CAH control in saliva. Correlation has been shown between their salivary and blood concentrations, as well as between them and the traditional markers 17OHP, androstenedione and testosterone.<sup>64</sup> A study using five timepoint measurements showed the diurnal variations in salivary 17OHP and androstenedione, as well as 11OHA4 and 11KT,<sup>88</sup> with evening measurements being most comparable between CAH patients and controls. This highlights again the importance of interpreting the results appropriately in relation to time of day and administration of GC doses.

## 9 | URINARY BIOMARKERS

Another alternative to blood hormonal measurements is offered by urinary steroid profiles, which allow relatively convenient whole day hormone analysis through 24-h collection, and the comfort of non-invasive sample collection. Measuring urinary cortisol metabolites can differentiate high versus low dose regimes, and thus has the potential to evaluate compliance.<sup>23,89</sup> Pregnanetriol, the main urinary metabolite of 17OHP, correlates well with morning serum 17OHP before GC dose ( $r^2 = .76-.91$ ), although the strength of correlation falls in samples taken 2 or 4 h after the dose.<sup>90,91</sup> The related pregnanetriol-3-glucuronide (PT3G), corrected for creatinine, has shown again the

importance of not normalising 17OHP in CAH, as well as accounting for age with these markers as levels differ significantly throughout childhood. Urine PT3G below the 50th centile of normative values was associated with poor growth consistent with overtreatment, including cases that would have been missed by monitoring only serum 17OHP.<sup>92</sup>

Assessing composite measures from urinary markers improves their utility. Increased tetrahydrocortisone (cortisone metabolite) in relation to tetrahydrocortisol (cortisol metabolite) reflects lower 11-beta-hydroxysteroid dehydrogenase activity, a higher ratio associated with poor control.<sup>93</sup> Employing sums of urinary metabolites adjusted for age and transformed to SDSs provides the most insight from urinary measurements, and further emphasized the clinical potential of 11-oxygenated androgens which are raised in CAH, in contrast to adrenal androgens androsterone and DHEA, which are suppressed.<sup>65</sup> The same research group analysed urinary steroid profiles defining four urinary metabolotypes in CAH to assess treatment efficacy. The latter subset of patients, characterised as treatment failure, have high androgens despite high cortisol metabolites, likely due to poor chronic compliance with short term improved compliance during urinary sampling.<sup>94</sup>

The increase in alternative pathways to androgen biosynthesis that occur in CAH results in excess excretion of the typical markers 11OH-androsterone and 3a,5a-17-hydroxypregnanolone in the urine.<sup>8,95</sup> Measurement of over 500 urinary steroid metabolite profiles in a cohort of 150 children treated with hydrocortisone and fludrocortisone showed suppression of adrenarche in treated children, with low androgen metabolites but high 11-hydroxyandrosterone, showing that 11-oxygenated androgens represent the main source of hyperandrogenism in CAH.<sup>96</sup>

One practical caveat of using urinary steroid profiles relates to the required 24-h collection time period and the relatively high costs of these assays. Whilst routine use of urinary steroid profiles may be difficult in clinical practice, the research evidence supports their value in providing comprehensive information that may be used in monitoring disease control and adjusting the GC regime in the future.

## 10 | BIOMARKERS OF MINERALOCORTICOID REPLACEMENT

Aldosterone deficiency requiring replacement is needed in 75% of patients with classic CAH.<sup>97</sup> However, even patients who synthesise sufficient aldosterone to prevent an adrenal crisis can benefit from mineralocorticoid treatment and achieve adrenal suppression with lower GC doses.<sup>98</sup> The recommended replacement dose is an absolute 50–200 µg/day fludrocortisone,<sup>30</sup> resulting in higher relative doses in infants and young children.<sup>99</sup> Median absolute doses deemed necessary in infants and children have also been shown to decrease from a median of 200 µg/day in children under 6 months, to 125 µg/day between 19 and 24 months of age, attributed to the partial aldosterone resistance that occurs in neonates.<sup>100</sup> However, international guidance concedes that the

optimum dose of fludrocortisone has not been critically studied.<sup>30</sup> High mineralocorticoid doses are associated with hypertension in children,<sup>101</sup> and registry data has shown higher relative blood pressures in children with CAH younger than 8 years.<sup>102</sup> Further work is therefore required to define the optimal dosing, and in the meantime, it is crucial that replacement doses are reviewed and monitored regularly.

The most commonly used biomarkers for mineralocorticoid replacement in CAH are electrolytes, plasma renin concentration (PRC) or plasma renin activity (PRA).<sup>4</sup> Unfortunately, the use of PRC and PRA is challenging due to the variability in assays used and sample processing, as well as the influence of dietary salt intake, body position and physical activity before sample collection.<sup>4</sup> In children aged 5–12 years with simple virilising 21OHD<sup>103</sup> it was demonstrated that 200 µg/m<sup>2</sup>/day fludrocortisone reduces PRC, and other studies in adults<sup>104</sup> and children with CAH<sup>105</sup> have shown high doses of fludrocortisone >250 µg/m<sup>2</sup> per day decreasing PRA. A study involving 33 children with CAH showed a negative correlation between blood pressure and PRA which was significantly lower in hypertensive patients across multiple time points from 3 months to 4 years of age.<sup>105</sup>

Multiple regression analysis on a large cohort of over 200 patients with salt wasting CAH showed that PRC did not vary with the fludrocortisone dose, while sodium correlated negatively and potassium positively with the fludrocortisone dose within multivariate analysis.<sup>106</sup> These findings are similar to those reported in patients with autoimmune primary adrenal insufficiency on mineralocorticoid replacement.<sup>107</sup> The association between PRC or PRA and blood pressure in other smaller studies of CAH has been reported to be weak, inconsistent or absent.<sup>104,108–110</sup> These findings do not necessarily suggest that monitoring renin is inappropriate, being possibly linked to the inter-patient variability in the mineralocorticoid replacement required. This would also explain the heterogeneity in results from studies into blood pressure, with several small studies reporting normal blood pressure,<sup>110,111</sup> some studies finding elevated systolic or diastolic readings,<sup>101,112–116</sup> and others reporting that high blood pressure is a transient problem in early childhood.<sup>102,105</sup> Of note, a larger cohort in the United States including 224 CAH patients showed an association between hypertension and suppressed plasma renin activity in paediatric patients.<sup>31</sup>

An alternative biomarker explored in relation to monitoring blood pressure or fluid and electrolyte homeostasis in CAH is serum atrial natriuretic peptide (ANP).<sup>117</sup> It was found that ANP correlated positively with blood pressure in CAH<sup>108</sup> and is lower in patients with PRA > 5 ng/mL/h.<sup>104</sup> Additionally, the effect of fludrocortisone on ANP was demonstrated by two studies<sup>104,108</sup> showing a negative relationship with the mineralocorticoid dose. However, ANP has a very short half-life of 1 min with rapid renal clearance and must be measured within 2 h of collection at room temperature which challenges its use in clinical practice. The more stable MR-proANP that gives more reliable results therefore warrants investigation in CAH.<sup>118</sup>

## 11 | CONCLUSIONS

The evidence informing the use of different biochemical markers for monitoring CAH control is diverse in both aims and methodology, making it difficult to derive precise guidance for clinical practice. Nonetheless, there are some key messages to be considered when managing patients with CAH. Of note, no data on the superiority of a specific monitoring strategy in relation to outcomes exists and is urgently warranted. The approach to hormone replacement therapy should be individualised, ideally based on the patient's hormonal profile throughout the day in relation to medication. When monitoring GC replacement, one-time measurements of biochemical markers have low reliability in assessing disease control, especially if not collected in the morning before the first GC dose. There are significant limitations to the use of 17OHP, androstenedione and testosterone, and further insight should be sought into members of the 11-oxygenated androgens which are more disease specific. Furthermore, non-invasive monitoring via salivary and urinary steroid measurements is becoming increasingly available and should be considered, especially in the management of certain groups such as children with CAH or patients with clinical evidence of hyperandrogenism. The effectiveness of PRC and PRA as biomarkers of mineralocorticoid replacement is limited and should always be interpreted in the context of the patient's blood pressure and clinical picture. Overall, this review indicates that further evidence is needed focused particularly on the interrelation between different monitoring strategies used in clinical practice and health outcomes in children and adults with CAH. The power of international registries to collect rich real-world data on rare conditions should be harnessed to support large multi-centre studies analysing repeated biomarker measurements to inform optimal monitoring and treatment for those living with CAH.

## CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

## DATA AVAILABILITY STATEMENT

The characteristics and summary of the studies included in the review are provided as supporting information.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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