Congenital Adrenal Hyperplasia: The Lancet Seminar Series

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Congenital Adrenal Hyperplasia: The Lancet Seminar Series

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Search Strategy and Selection Criteria:

We searched the Cochrane Library (January 2010-Septmeber 2016), MEDLINE (January 2010-Septmeber 2016), and EMBASE (January 2010-Septmeber 2016). Keywords and controlled vocabulary and their synonyms were used when appropriate. We used variations of the search term "congenital adrenal hyperplasia" in combination with the terms "diagnosis/diagnostics", "genetics", "genomics", "adrenal crisis", "glucocorticoid", "mineralocorticoid", "gene therapy", "quality of life", "well-being", "screening", "metabolomics", "prenatal", "antenatal", "bone mineral density", "tumor", "pregnancy", "treatment/therapy/ therapeutic", "fertility/fecundity", "surgery", "management", "metabolic", "complications". We largely selected publications in the past 5 years, but did not exclude commonly referenced and highly regarded older publications. We also searched the reference lists of articles identified by this search strategy and selected those we judged relevant. Review articles and book chapters are cited to provide readers with more details and more references than this Seminar has room for.

Introduction

In 1865, Luigi De Crecchio, an Italian pathologist, described the life of a male, whose autopsy revealed female internal anatomy and large adrenal glands, representing the first known case of presumed congenital adrenal hyperplasia (CAH)[1] (Figure 1). However, treatment for CAH was not introduced until almost a century later when cortisone was given for what was then known as the adrenogenital syndrome.[17-19]

CAH comprises a group of seven autosomal recessive diseases caused by a defect in any one of the several steps leading to cortisol biosynthesis: 21-hydroxylase (21OH), 11β-hydroxylase (11βOH), 17α-hydroxylase/ 17,20-lyase (17OH), 3β-hydroxysteroid dehydrogenase type 2 (3βHSD2), steroidogenic acute regulatory protein (StAR), P450 cholesterol side-chain cleavage enzyme (SCC), and P450 Oxidoreductase (POR). The pathophysiology and genetics of the various types of CAH have been elucidated in the past 50 years. Multiple hormonal imbalances occur and CAH manifests with a range of severity clinically and biochemically, with or without alterations in glucocorticoid, mineralocorticoid, and sex steroid production. Both severe (classic) and mild (nonclassic) forms have been described.

Over 95% of CAH is due to 21OH deficiency,[20] characterized by impaired cortisol and aldosterone production and androgen excess. Life-saving neonatal screening for classic CAH due to 21OH deficiency was first performed in Alaska in 1977[7] and is currently being carried out worldwide in over 40 countries, including all 50 US states since 2009, though not yet implemented in the UK.[7,20-22] Although all types of classic CAH are rare orphan diseases, the nonclassic form of 21OH deficiency is estimated to be one of the most common autosomal recessive disorders.[23]

Despite these advances, CAH remains one of the most challenging endocrine disorders to diagnose, manage and treat because of the direct and indirect effects on steroidogenic pathways and the rarity of these conditions. Advances in genetics, metabolomics and treatment strategies continue to improve our understanding of these complex diseases and aim to improve patient outcome. Following is an overview of these complex disorders.

Genetics and Pathophysiology of the Various Forms of CAH

All types of CAH are monogenetic and autosomal recessive. Most patients are compound heterozygotes, meaning they have different mutations in the two alleles. The clinical manifestation follows the less severely mutated allele, and, in general, there is good genotype-phenotype correlation.[24,25]

Adrenal steroidogenesis occurs by a series of steps facilitated by the zone specific enzyme expression and different types of CAH interrupt this process at distinct branchpoints. In addition to the classical well-established steroidogenesis pathway, an alternative pathway to active androgen biosynthesis exists (termed "backdoor" pathway),[26,27] which may play a role in the pathophysiology of CAH (Figure 2A). The clinical manifestation of CAH is closely related to the type and severity of the impairment.

21-hydroxylase (21OH) deficiency

The gene for 21OH, *CYP21A2*, is located within the human leukocyte antigen class III region of chromosome 6 (Table 1). *CYP21A2* and a homologous pseudogene*, CYP21A1P*, lie approximately 30 kb apart.   Meiotic recombination events are common in this genomic region

because of the high degree of sequence homology between duplicated genes. Approximately 95% of *CYP21A2* disease causing mutations are *CYP21A1P* derived variants or deletions due to recombination events.[24,35]

Defective 21-hydroxylation results in elevated precursors, most notably 17-hydroxyprogesterone (17OHP), which is used for diagnosis, and decreased glucocorticoid and mineralocorticoid synthesis (Figure 2B). ACTH-stimulated androgen production occurs because there is no block in the pathway leading to adrenal androgens.

Conventionally, classic 21OH deficiency is subclassified into salt-wasting (SW) and simple virilizing (SV) forms, reflecting the severity of aldosterone deficiency. Mutations which completely inactivate *CYP21A2* result in the SW phenotype, which, without neonatal screening, presents in the first 2 weeks of life with a life-threatening adrenal crisis (Table 2).[20] Patients with classic SV CAH have mutations that retain 1-2 % of 21OH activity, and minimal aldosterone production prevents a neonatal crisis. Excess fetal adrenal androgen exposure results in virilization of the external genitalia of 46,XX classic patients (SW and SV) (Figure 3A). Without neonatal screening, SV males are diagnosed as toddlers with signs and symptoms of androgen excess. Postnatal excess androgen leads to premature pubic hair and rapid skeletal growth. Patients with the nonclassic form retain up to 50 percent of enzyme activity and mostly do not have adrenal insufficiency, but may have partial glucocorticoid deficiency, and female genitalia are normal. Patients may present with mild androgen excess or have little or no symptoms. In fact, the term cryptic CAH was coined to define patients with nonclassic CAH who are identified by family genetic studies, but are otherwise asymptomatic.[36]

11-hydroxylase (11βOH) deficiency

CAH due to 11βOH deficiency is due to *CYP11B1* mutations (Table 1). *CYP11B1* functions in the

zona fasciculate to convert 11-deoxycortisol to cortisol and deoxycorticosterone (DOC) to

corticosterone, under ACTH regulation (Figure 2C). The majority of *CYP11B1* mutations

correspond to minimal or absent enzyme activity resulting in a classic phenotype.[37]

Impaired 11-hydroxylation results in decreased corticosterone and cortisol synthesis, with

subsequent increase in ACTH and excess androgens, because of shunting of the pathway

towards androgen production. Normally corticosterone and DOC production by *CYP11B1*

transcription in the adrenal zona fasciculata is minimal, but DOC levels can rise dramatically

under the influence of ACTH.[38] DOC is a weak mineralocorticoid, however elevated DOC

suppresses the renin-angiotensin system and results in extracellular fluid volume expansion,

hypertension, low plasma renin activity and low aldosterone levels, although the ability to

produce aldosterone remains. These effects may not occur in the neonatal period due to the

renal mineralocorticoid resistance that is present in the first few months of life. Clinically,

patients present similar to 21OH deficiency, with signs of androgen excess, but also have

hypertension rather than salt loss (Table 2).[28] A nonclassic form of *CYP11B1* deficiency exists

but is very rare.[39]

17α-hydroxylase/ 17,20-lyase (17OH) deficiency

The *CYP17A1* gene encodes an enzyme that expresses both 17α-OH and 17,20-lyase activities

(Table 1). Due to its location in the steroidogenic pathway, severe mutations impair adrenal and

gonadal sex steroid production (Figure 2D), which causes sexual infantilism and pubertal failure

(Table 2).[31] There is a block in DHEA production, which prevents adrenarche and the development of pubic and axillary hair. *CYP17A1* is expressed in the zona fasciculate and zona reticularis, not in the zona glomerulosa. Therefore, ACTH-mediated steroidogenesis results in elevated DOC and corticosterone. High levels of DOC cause sodium retention, hypertension and hypokalemia, with suppression of aldosterone production. Corticosterone, which has glucocorticoid activity, prevents adrenal crisis, although cortisol production is low or absent. Both 46,XX and 46,XY patients have female external genitalia, and usually present during puberty as females with lack of secondary sexual characteristics, hypergonadotropic hypogonadism and low-renin hypertension (Table 2). Isolated 17,20-lyase- deficiency has been reported[40] but is extremely rare and in truly isolated form is caused by mutations in cytochrome b5 (CYB5A), the co-factor needed by CYP17A1 to exert 17,20 lyase activity.[41] Although phenotype variability occurs, a nonclassic form with subtle clinical manifestations has not been defined.

3β-hydroxysteroid dehydrogenase type 2 (3βHSD2) deficiency

3β-hydroxysteroid dehydrogenase exists in two isoforms, type 1 (3βHSD1) and type 2 (3βHSD2), encoded by *HSD3B1* and *HSD3B2* respectively (Table 1). *HSD3B2* is highly expressed in the adrenals and gonads, while *HSD3B1* is expressed in the placenta and peripheral tissues.

Impaired 3βHSD2 results in decreased aldosterone, cortisol, and androstenedione, with a subsequent increase in renin, ACTH, and DHEA (Figure 2E). DHEA can be converted to testosterone by extraadrenal 3βHSD1. Patients present in infancy with a salt-wasting adrenal crisis, underdeveloped 46,XY genitalia and rarely 46,XX virilization (Table 2).

The hormonal criteria for diagnosing 3βHSD2 deficiency has changed over the past three decades because the initial studies identifying a possible nonclassic form were not based on genetic findings and subsequent genetic studies failed to confirm the diagnosis.[42-44] Nonclassic 3βHSD2 deficiency exists, but is extremely rare.

P450 Oxidoreductase (POR) deficiency

POR plays a key role in the electron transport that occurs in the endoplasmic reticulum and several enzymes, including 17OH, 21OHD, and aromatase, depend on POR for their catalytic activity (Figure 2F). The discovery of POR deficiency in 2004[15,16] provided an explanation for multiple hormonal deficiency initially known as apparent combined 17OH and 21OH deficiency. Lack of placental aromatization of fetal androgens could contribute to the virilization observed in some mothers carrying affected babies. However, the production of androgens via an alternative pathway to the most potent androgen, non-aromatizable 5α-dihydrotestosterone (DHT), may also explain the prenatal virilization of affected females, while postnatally affected individuals invariable display sex steroid deficiency.[16] POR also acts also as an electron donor to cytochrome P450 (CYP) enzymes other than steroidogenic CYP enzymes, explaining POR deficiency-associated changes in drug metabolism[45] and the pathogenesis of the often observed skeletal dysplasia.[46]

Most *POR* mutations retain some enzymatic function; homozygous mutations with complete loss of function may not be viable, as seen in rodent models.[47] Presentation varies from mildly affected adults with amenorrhea and polycystic ovaries (females) or androgen deficiency (males), to severe hormone disturbances causing atypical genitalia in both 46,XX and 46,XY

(Table 1, 2). 46,XX virilization does not progress, and patients postnatally have sex steroid deficiency. Craniosynostosis, radioulnar or radiohumeral synostosis, midface hypoplasia, and other skeletal manifestations resembling the Antley-Bixler syndrome may occur.

Generally, POR deficient patients do not have mineralocorticoid deficiency as impairment of 17-hydroxylase increases mineralocorticoid intermediates, and affected adults may develop hypertension. Patients have variable cortisol response to cosyntropin testing, with most patients requiring either permanent or stress dose glucocorticoid coverage.[32]

Lipoid CAH

Classic lipoid CAH is characterized by deficiency of all steroid hormones and is due to *StAR* mutations (Table 1, Figure 2G). *StAR* regulates the transfer of cholesterol from the outer to the inner mitochondrial membrane, a key step in steroidogenesis initiation. When cholesterol cannot be mobilized, adrenal lipid droplets accumulate and are seen on autopsy, thus the name lipoid CAH. Lipoid CAH is one of rarest forms of CAH, resulting in neonatal crisis, and both 46,XX and 46,XY infants have female external genitalia (Table 2).[48] Later presentation up to one year of age has been described.[49]

The pathogenesis of lipoid CAH is explained by a two-hit model: first, loss of StAR leads to the accumulation of intracellular cholesterol and cholesterol esters, and the second hit arises from destruction of the cellular function by the accumulated products.[48,50,51] This mechanism explains some unusual phenotype. Spontaneous puberty has been described in 46,XX patients, because of minimal ovarian StAR expression.[48] The ovaries are quiescent during fetal life and childhood and therefore toxic accumulation of cholesterol can be delayed until adolescence.

A nonclassic form was first described in 2006[52] associated with mutations that retain approximately 20-30% of StAR activity. Most of these cases were initially misdiagnosed as Addison's disease or isolated familial glucocorticoid deficiency.[53] Nonclassic patients may present as toddlers or as late as adulthood, with insidious onset of glucocorticoid deficiency, hyperpigmentation and high ACTH (but mostly intact mineralocorticoid function). A wide variation in gonadal function has been reported ranging from hypergonadotropic hypogonadism to normal.[48] Similarly, 46,XY nonclassic patients may have normal male genitalia and normal puberty or be born with atypical genitalia.[54]

Cholesterol side-chain cleavage enzyme (SCC) deficiency

P450 cholesterol side chain cleavage enzyme (SCC) is the first and rate-limiting step in the steroidogenic pathway (Figure 2G), encoded by *CYP11A1* (Table 1) and is clinically and biochemically identical to lipoid CAH (Table 2); however patients typically have atrophic adrenals and gonads.[48] Less than 40 cases have been reported.

Similar to nonclassic lipoid CAH, nonclassic SCC deficiency has been described with delayed adrenal insufficiency onset and variable gonadal effect, caused by mutations corresponding to 7-30% of retained enzyme activity.[55-58]

Since StAR and SCC deficiency are similar clinically and biochemically, DNA testing is the only definitive method to distinguish between the two, with StAR deficiency being more common.

Diagnosis

Neonatal screening for 21OH deficiency is performed by measuring 17OHP in dried blood spots on filter paper. Second-tier screening with LC-MS/MS can efficiently measure a panel of steroids; this has been used to successfully diagnose 11βOH deficiency,[59] but the focus of neonatal screening remains the detection of 21OH deficiency. Premature, stressed, or ill infants could have falsely elevated 17OHP; specificity is improved with gestational age stratification.[60] Testing using 21-deoxycortisol, which is elevated in 21OH deficiency, might increase neonatal screening specificity.[61]

If an infant has a positive neonatal screen or is clinically suspicious for CAH (i.e. ambiguous genitalia), confirmatory testing is indicated. Although a basal panel of LC-MS/MS steroids may be diagnostic, diagnosis often requires cosyntropin testing,[20,62] and is based on a characteristic rise in adrenal hormone(s) preceding the enzymatic block (Table 2). It should be kept in mind that 17OHP may be elevated in other types of CAH, such as 11βOH or POR deficiency. An alternative approach is urinary steroid profiling, which illustrates the entire steroid metabolome.[63] Additional testing and genotyping may be needed to confirm the diagnosis.

Screening for 21OH deficiency after infancy relies on measuring early morning (before 8 am) 17OHP. A level above 1,000 ng/dL (30 nmol/L) is diagnostic for 21OH deficiency, although a random level of 10,000 ng/dL (303 nmol/L) or greater is commonly observed in the classic form (Table 2).[20] A level of less than 200 ng/dL (6 nmol/L) usually excludes nonclassic CAH if measured during the follicular phase of a reproductive-age woman.[64] Cosyntropin stimulation testing is often needed for diagnosis of the nonclassic form.

The diagnosis of POR can be best made with a urinary steroid profile, revealing characteristic precursor accumulation that can be captured by steroid ratios[32] while serum steroid analysis

often yields confusing results due to the overlapping effects of combined 17OH and 21OH deficiency. Further criteria for urinary and serum metabolites have been suggested to diagnose POR prenatally or differentiate POR from 21OH deficiency.[65,66]

Management of CAH

Medical Treatment

Glucocorticoid therapy

The mainstay of treatment in the classic forms of CAH is chronic glucocorticoid therapy (Panel). Because of their growth suppressing effect, long-acting glucocorticoids are avoided in children, but sometimes used in adults.[68] The goal is to optimize control of excess hormones, while replacing deficient hormones and avoiding the potential Cushingoid side effects of glucocorticoid therapy. Laboratory results should guide but not define management; clinical evaluation should always be considered.

In general, higher doses of glucocorticoids are needed to achieve adequate suppression of hormone excess (i.e. androgens in the virilizing forms of CAH, classic 21OH, 11βOH; DOC in 17OH deficiency), whereas lower replacement doses of glucocorticoids should suffice if all steroids are deficient. Lower glucocorticoid doses may also be used in the nonclassic forms of CAH, if treatment is indicated.[69]

For women planning to conceive, a glucocorticoid that does not reach the fetus and is inactivated by placental 11βHSD2, such as prednisone, prednisolone or hydrocortisone, is typically used and continued throughout pregnancy.

Patients with nonclassic CAH are treated according to symptomatology. Children with nonclassic 21OH deficiency should be treated if they have progressive signs and symptoms of virilization with advanced skeletal maturation. Women with nonclassic CAH with signs of androgen excess can often be successfully treated with oral contraceptive, if needed in combination with spironolactone. Glucocorticoid therapy is used for female infertility in nonclassic 21OH deficiency and has been reported to reduce miscarriage rate when taken throughout pregnancy.[70,71]

Mineralocorticoid replacement

Mineralocorticoid, in the form of fludrocortisone, is given to achieve a plasma renin activity in the normal range in the salt-wasting forms of CAH (Panel)[20]. The dose is independent of body size, although higher doses are usually needed during the first 6 months of life due to neonatal physiological mineralocorticoid resistance. Infants also require salt supplementation. Although patients with SV 21OH deficiency have some aldosterone production, relative aldosterone insufficiency exists and fludrocortisone is recommended as it allows for glucocorticoid reduction leading to improved height outcomes.[72]

Glucocorticoid stress dosing

Patients receiving glucocorticoid treatment, including those with the nonclassic form, need to be educated on adrenal crisis prevention and increasing glucocorticoid dose during intercurrent illness (Panel). Intramuscular, subcutaneous, or intravenous hydrocortisone should be given when oral intake is not possible, and stress dose coverage is identical to that recommended in

primary adrenal insufficiency.[67] Patients with classic 21OH deficiency also have epinephrine deficiency, due to abnormal adrenomedullary formation;[73] this places patients at risk for hypoglycemia, especially when fasting, or during acute illness. Adrenomedullary function has not been studied in the rarer forms of CAH.

Patients with nonclassic CAH may have suboptimal cortisol response on stimulation testing (< 18 ug/dl). If asymptomatic, daily glucocorticoid is not indicated, but glucocorticoid stress coverage should be employed during serious illness or major surgery.

Sex steroids

Sex steroid replacement is started around the time of physiological puberty in 17OH deficiency, 3βHSD deficiency, lipoid CAH, SCC deficiency, and POR (as needed). Androgen (males) and estrogen (females), with progestin to induce cyclical bleeding (if uterus present) are advanced slowly to adult regimens (Panel).

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Anti-hypertensive

In both 11 βOH and 17OH deficiency, glucocorticoid therapy is often sufficient to control hypertension by suppressing DOC. However, because high dose glucocorticoid therapy and complete suppression of the HPA axis should be avoided, DOC is not fully suppressed and many patients eventually become hypertensive. A mineralocorticoid receptor antagonist or calcium channel blocker can be used[31,74] (Panel).

Controversial Therapies

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Genital Surgery

Surgery for disorders of sex development (DSD) is a complex issue that has generated much controversy. Historically, surgeons have recommended surgery based on genital appearance and fertility potential. In the past two decades, some advocacy groups and physicians have recommended delaying surgery so that patients can participate in the decision regarding surgical intervention. Conversely, others have expressed concern regarding the lack of outcome data and psychosocial stress of not doing early surgery. Most importantly, the family should always be educated on the pros and cons of having and not having surgery. An interdisciplinary team of specialists is often required to navigate the decision making process.[75]

An international group of experts in CAH appointed by the Endocrine Society to develop Clinical Practice Guidelines for CAH due to 21OH deficiency concluded that surgery be considered for significantly virilized 46,XX CAH patients.[20] Timing of the surgery is beyond the scope of this article but options include a one stage approach with neurovascular-sparing clitoroplasty, labioplasty, and vaginoplasty being done simultaneously in infancy (norm in many countries including US and UK), waiting until puberty for any surgery, and performing labioplasty and clitoroplasty in infancy with vaginoplasty delayed until puberty. Most CAH patients with 21OH deficiency prefer early surgery.[76] Although *in utero* exposure to androgen has been shown to affect behavior with male typical behavior patterns commonly seen in classic 46,XX CAH patients, gender dysphoria is extremely rare and the recommended sex assignment of 46,XX DSD due to CAH is female.[75]

The main challenge of surgery of the 46,XX virilized CAH patient remains the imperfect functional and cosmetic outcomes, including urinary incontinence, vaginal stenosis, and clitoral

pain, all of which can impact psychosocial and sexual well-being.[77] Many of the new surgical approaches have not been present for long enough to assess outcomes. Patients should be referred to a specialist surgeon with DSD management experience.

Surgical reconstruction of 46,XY atypical genital is complex. Chordee repair and surgery for distal hypospadias have high success rates, but proximal hypospadias repair is more challenging with higher complication and reoperation rates. The main complications are urethral stricture, meatal stenosis, urethrocutaneous fistula and glans wings separation.[78]

Early gonadal neoplastic changes were observed histologically as early as 1 year of age in a 46,XY patient with classic lipoid CAH.[79] Gonadectomy is recommended in severely affected 46,XY patients being raised female, although risk of gonadal malignancy is unknown.

Prenatal treatment

For over 30 years, dexamethasone was offered to pregnant women at risk for having a child with classic virilizing CAH, aiming to suppress fetal androgen production and reduce virilization of an affected female. Dexamethasone, unlike hydrocortisone and prednisolone, crosses the placental barrier to the fetus without inactivation. Today, prenatal therapy is controversial, as only 1 of 8 fetuses will be an affected female when both parents are carriers. Long-term effects of *in utero* dexamethasone exposure are unknown with potential effects on the brain, behavior and cognition described.[80-83]

Testing of fetal cells present in maternal circulation is being studied to avoid 46,XY treatment and initiate early treatment in affected 46,XX.[84] Cell-free fetal DNA obtained from the mother's plasma as early as 5 weeks gestation has correctly identified fetal CAH status in 14 families.[85]

Multiple international groups, including medical societies in the U.S. and Europe, have stated that prenatal therapy should only be considered in a research setting with full disclosure of the potential risks and benefits.[20,86] The long-term effects of prenatal dexamethasone exposure requires further study but early non-invasive fetal DNA testing would potentially limit exposure to affected female fetuses.

Bilateral Adrenalectomy

Bilateral adrenalectomy has been successfully used to treat female infertility with uncontrolled hyperandrogenism in 21OH deficiency[87] and uncontrolled hypertension in 11βOH deficiency.[88] Although follow-up of patients who have undergone adrenalectomy is overall positive, patients appear to be at increased risk of adrenal crisis and about one-third develop adrenal rest tissue if glucocorticoid dose is too low.[89] Adrenalectomy should only be considered in patients who have failed all available medical interventions.

Long-term Complications

Glucocorticoid deficiency is characteristic of the severe forms of CAH, and is potentially life-threatening. A study in Sweden of 588 CAH patients compared to a national population-based registry revealed excess mortality in CAH because of adrenal crises,[90] highlighting the importance of this aspect of the disease. A cross-sectional questionnaire-based study of 122 CAH patients found the majority of adrenal crises occurred during infancy, with a second peak around late adolescence, and precipitated mainly by respiratory and gastro-intestinal infections.[91]

All children with CAH receiving glucocorticoid therapy are at risk for growth impairment and short stature. This effect is dose-dependent, thus management is always aimed at treating with the lowest possible effective dose. Alterations in sex steroid exposure can also influence height. Late puberty may occur with the rare types of CAH associated with sex steroid deficiency, and can enhance adult height; vice versa, exposure to excess androgens and estrogens in the virilizing types of CAH can result in early puberty and early epiphyseal fusion (Figure 3B). A meta-analysis of 35 studies of classic 21OH deficiency showed an average final height of 1.38 standard deviations below the population norm.[72] Whereas studies reveal an association between higher doses of hydrocortisone and shorter final height,[92,93] earlier rather than later diagnosis and treatment has been associated with improved height outcomes, emphasizing the importance of prevention of hyperandrogenism.

Cardiovascular disease risk factors commonly coexist with CAH. In a cross-sectional UK study of classic 21OH deficient patients, over half of women were obese; and one-third had insulin resistance.[94] In a Swedish cohort of 588 CAH patients, there was a higher frequency of hypertension, hyperlipidemia, diabetes, and venous thromboembolism compared to controls.[95]

Long term glucocorticoid exposure, particularly higher doses for achievement of tight control, is a main risk factor for compromising bone health. Studies over the past two decades show evidence of lower BMD in patients with 21OH deficiency as compared to controls, with the prevalence of low BMD (osteoporosis or osteopenia) ranging from 37 to 81%,[94,96-98] with some studies reporting increased fracture rate.[96,99]

Both males and females with the hypogonadal forms of CAH suffer from infertility. However, successful pregnancy has been achieved in one woman with classic lipoid CAH with clomiphene

citrate stimulation followed by progesterone supplementation.[100] *In vitro* fertilization and

transfer of cryopreserved embryos has successfully resulted in a live birth in lipoid CAH and

17OH deficiency.[101,102] Patients with POR, *StAR*, and *CYP17A1* mutations may also have ovarian

cysts and ovarian cyst torsion.[103-105]

In the virilizing forms of CAH, excess adrenal sex steroids can lead to hypogonadotropic

hypogonadism[106,107] and increased progesterone can interfere with endometrial

implantation.[108,109] This may resolve with optimizing glucocorticoid management and

suppression of follicular phase progesterone enhances the likelihood of ovulation and

subsequent conception.[110,111]

A main cause of male infertility in classic 21OH and 11βOH deficiency is adrenal rest tissue.

Adrenal rest is thought to arise from aberrant cells of adrenocortical origin that migrate during

fetal development along with the gonad after the adrenal and gonadal cells separate from the

urogenital ridge. Adrenal rest is most commonly found in the rete testis (Figure 3C) and has

been described in the ovaries and broad ligament.[112,113] Testicular adrenal rest tissue (TART)

causes obstructive azoospermia and deficient spermatogenesis. Low inhibin B levels reflect

declining Sertoli cell function and can be used to monitor.[106]   The prevalence of TART is

between 44-94% of men[98,107,114,115] and 21-33% of boys[98,116] with classic 21OH, and has also

been reported in 11βOH and HSD3B2 deficiency,[117-119] and rarely in nonclassic CAH.[114] TART

shrinkage and reversal of infertility are possible with glucocorticoid therapy;[120] however the

response is variable, because non-reversible fibrotic changes may occur over time.[121]

When unsuccessful, other modalities, such as intra-cytoplasmic sperm injection, could be

considered.[122] Testis-preserving surgery with TART resection has not restored fertility,[123] but

can be done for orchialgia; simultaneous successful intraoperative sperm retrieval was recently reported.[124]

Future Directions

Alternative Androgen Synthesis Pathways

The quest for new and improved biomarkers of disease severity or treatment response in CAH has included exploration of alternative androgen synthesis pathways. The so-called "backdoor" pathway, leads to the synthesis of DHT, without DHEA, androstenedione and testosterone as intermediates, originating directly from 17-hydroxyprogesterone (Figure 2A). This pathway has been implicated in the normal development of male genitalia[54] and the prenatal virilization of affected females.[16] Accumulation of 17-hydroxyprogesterone, as observed in 21OH and POR deficiency, increases the substrate flow to the "backdoor" pathway and subsequent studies have described increased alternative pathway metabolite excretion in patients with CAH due to POR [125] and 21OHD deficiency.[126]

A further alternative androgen synthesis pathway involves the generation of 11-oxygenated C19 steroids in the adrenal cortex via CYP11B1 activity (Figure 2A),[127] including, 11-ketotestosterone and 11-keto-DHT, androgens that bind and activate the androgen receptor.[128,129] 11-oxygenated C19 steroids have been shown to be increased in CAH due to 2OH deficiency [130,131] and exaggerated activity of both backdoor and 11-oxygenated C19 pathways persists in treated patients, even if the classic androgen pathway activity is downregulated.[130]

Insights into these novel steroid markers will help to improve monitoring tools and define treatment targets.

Genetic Advances

Genetic studies of CAH have provided insight into the pathophysiology and subtle clinical aspects of the disease. Initially described in 1989,[132] the *TNXB* gene which encodes the tenascin-X protein, a glycoprotein expressed in connective tissue, and its highly homologous pseudogene *TNXA*, flank *CYP21A2* and its pseudogene *CYP21A1P*, respectively. Chimeric genes which impair both the *CYP21A2* and *TNXB* genes were found to explain an unusual observed phenotype of a connective tissue dysplasia consistent with hypermobility-type Ehlers Danlos syndrome in patients with 21OH deficiency.[133,134] This novel syndrome, CAH-X, was prevalent in 8.5 % of a cohort of 246 unrelated patients with 21OH deficiency.[135]

Apart from CAH genes, other genes may modify steroid action, salt balance or androgen sensitivity and affect phenotype.[136,137,138]

Genotyping is essential in confirming the carrier state, and is useful for genetic counseling or establishing the diagnosis of a patient who cannot undergo accurate hormonal testing due to glucocorticoid therapy. Genotyping may one day predict future outcomes and be efficacious in screening programs.[139]

Novel Therapies

Most of the adverse outcomes in patients with CAH are attributable to hormonal imbalances or treatment related comorbidities. New and improved therapies are being developed that target

different aspects of the pathophysiology of CAH (Figure 4) and are being studied in classic 21OH deficiency.

One approach is to replace cortisol in a physiological manner. Circadian cortisol replacement might achieve improved ACTH control and thus adrenal steroid secretion. A modified-release oral hydrocortisone preparation, Chronocort®, was successful in achieving lower androgen levels while decreasing the hydrocortisone equivalent dose using a twice-daily regimen in a Phase 2 study of 16 patients with classic 21OH deficiency.[140] A phase 3 study is currently underway (NCT02716818). Continuous subcutaneous hydrocortisone infusion via an insulin pump mimicking cortisol circadian rhythm, has similarly shown adequate ACTH suppression with lower total hydrocortisone dose compared to conventional treatment,[139-142] and showed improved quality-of-life and fatigue in 8 patients with classic 21-OH deficiency.[142] Long-standing comorbidities, such as insulin resistance and TART, remained mostly unchanged by 6 months, suggesting that early intervention is key and other approaches may be needed to treat well established comorbidities.

As ACTH is the primary driver for excess steroid accumulation, strategies for reducing ACTH are under way. A phase I, proof-of-principle study with a corticotropin-releasing factor type 1 receptor antagonist, lowered morning ACTH or 17OHP in 6 of 8 females with classic 21-OH deficiency after a single dose.[143] Future, multi-dose trials are needed.

Pharmacologic blockade or inhibition of sex steroid synthesis in prepubertal children or women receiving sex hormone replacement would allow for lower dose glucocorticoid replacement in the virilizing forms of CAH. This was studied in 28 prepubertal children with classic 21OH deficiency using an anti-androgen and aromatase-inhibitor in combination with

lower dose hydrocortisone and fludrocortisone, and was successful in normalizing growth over 2 years.[144] Pharmacologic inhibition of sex steroid synthesis was also tested in adult women with CAH receiving gonadal hormone replacement in a phase I, 6-day, dose-escalation study of Abiraterone acetate, a potent CYP17A1 inhibitor.[145] Androstenedione normalized in 5 of 6 women when abiraterone was added to physiologic doses of glucocorticoid and fludrocortisone, showing promising results. Pharmacologic inhibition of sex steroid synthesis is also being studied with ATR-101 (NCT02804178), an inhibitor of acyl-coenzyme A: cholesterol-O-acyltransferase 1, in a phase II study of classic 21-OH deficiency.[146]

Adrenal enzyme inhibitors with adrenolytic properties may be useful in the treatment of CAH. Mitotane, inhibits CYP11B1 and CYP11A1, and is adrenolytic with longer term use. Mitotane was successfully used to shrink TART and restore fertility in a 29-year-old male with classic 21OH deficiency.[147] However, due to multiple toxicities of mitotane, the development of alternative adrenolytic therapies is needed.

CAH is a monogenic disease, therefore gene therapy, with the use of cell-based and gene-editing technologies, may be able to restore the defective steroidogenesis.[148] Adrenal transplantation with novel technology using bovine adrenocortical cells has been successful in animal models of adrenal insufficiency.[149] Future technological and genetic advances may enable us to one day cure CAH.

Conclusion

CAH is a group of rare diseases that if left undiagnosed and untreated would result in high morbidity and mortality. The identification of alternative adrenal biomarkers has provided

insight into the origin and synthesis of steroid production and has the potential to alter disease management. Decades of progress in understanding the genetics and pathophysiology of the various forms of CAH have led to a recent explosion in the investigation of new and improved therapies which promise to improve patient outcome.

Competing Interest Statement

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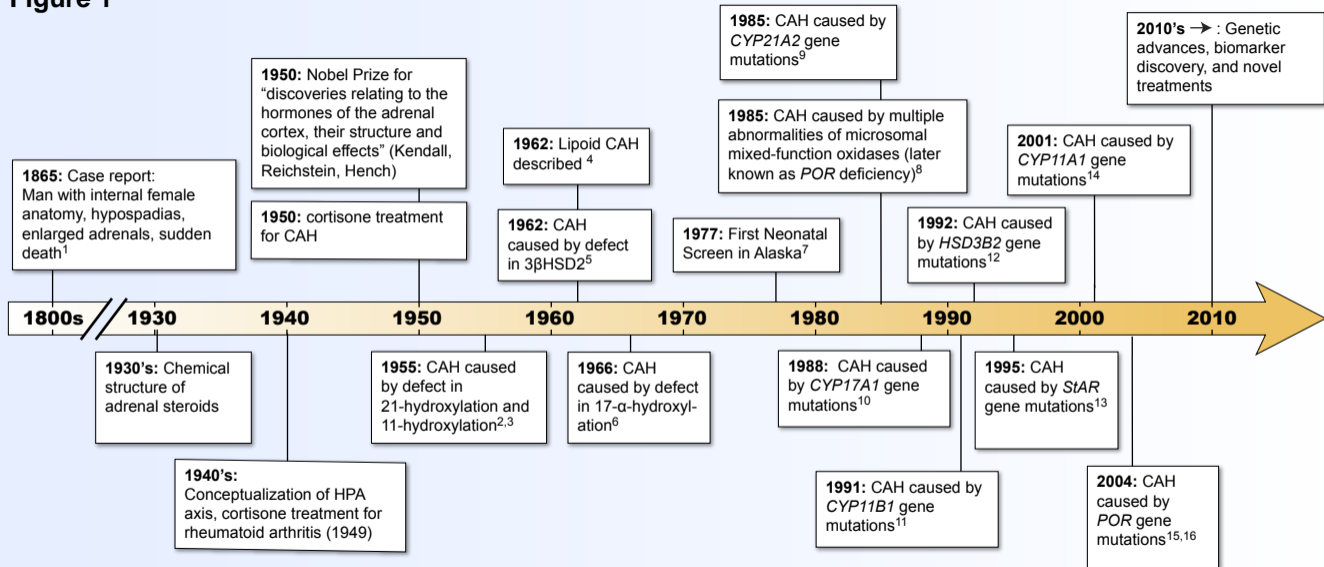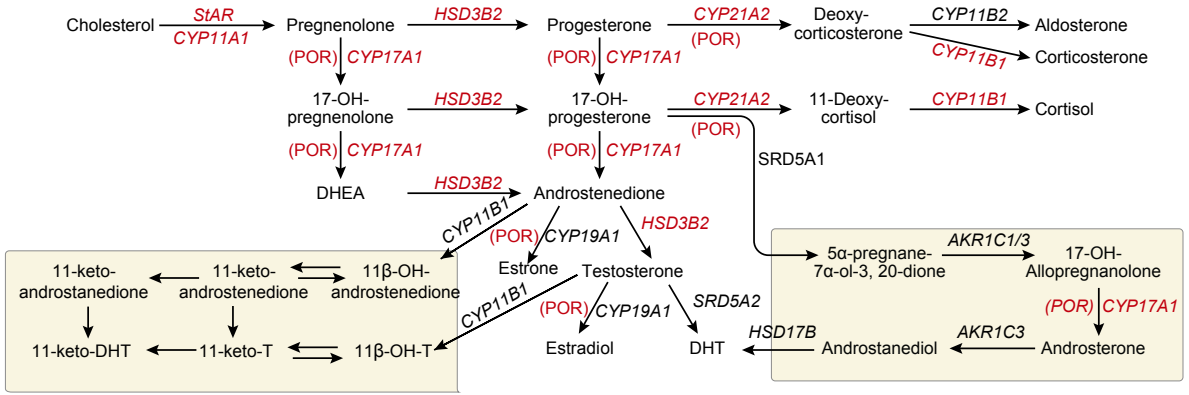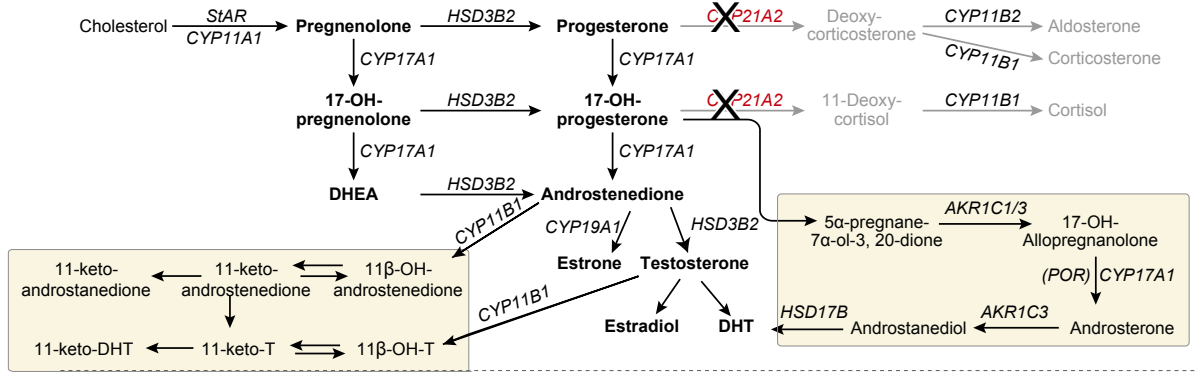
Figure 1

1865: Case report: Man with internal female anatomy, hypospadias, enlarged adrenals, sudden death[1]

1930's: Chemical structure of adrenal steroids

1940's: Conceptualization of HPA axis, cortisone treatment for rheumatoid arthritis (1949)

1950: Nobel Prize for "discoveries relating to the hormones of the adrenal cortex, their structure and biological effects" (Kendall, Reichstein, Hench)

1950: cortisone treatment for CAH

1955: CAH caused by defect in 21-hydroxylation and 11-hydroxylation[2,3]

1962: Lipoid CAH described[4]

1962: CAH caused by defect in 3βHSD2[5]

1966: CAH caused by defect in 17-α-hydroxyl-ation[6]

1977: First Neonatal Screen in Alaska[7]

1985: CAH caused by CYP21A2 gene mutations[9]

1985: CAH caused by multiple abnormalities of microsomal mixed-function oxidases (later known as POR deficiency)[8]

1988: CAH caused by CYP17A1 gene mutations[10]

1991: CAH caused by CYP11B1 gene mutations[11]

1992: CAH caused by HSD3B2 gene mutations[12]

1995: CAH caused by StAR gene mutations[13]

2001: CAH caused by CYP11A1 gene mutations[14]

2004: CAH caused by POR gene mutations[15,16]

2010's → : Genetic advances, biomarker discovery, and novel treatments
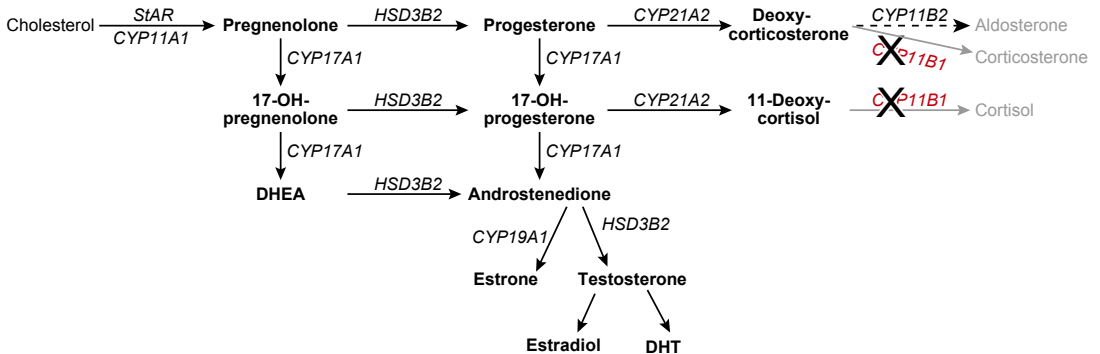
1800s // 1930 1940 1950 1960 1970 1980 1990 2000 2010
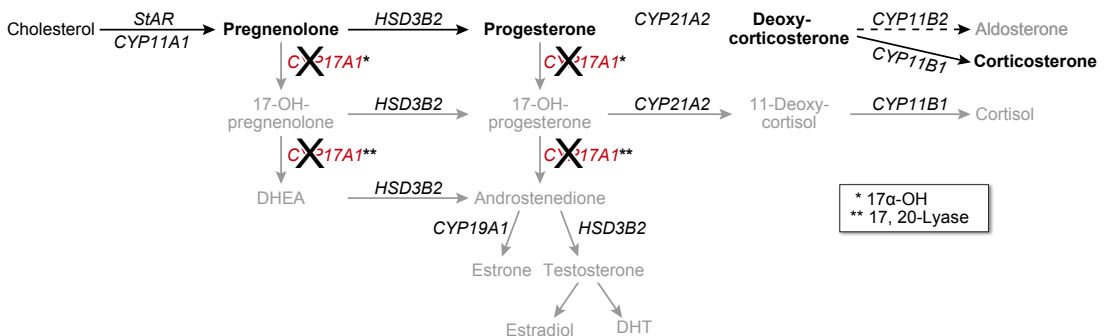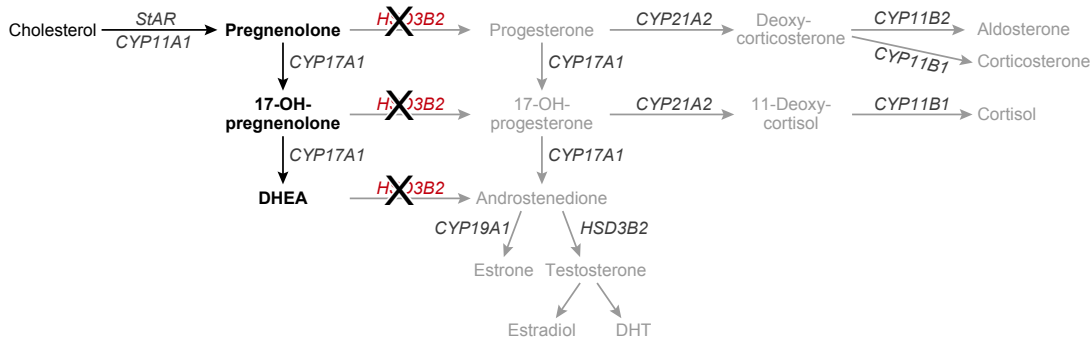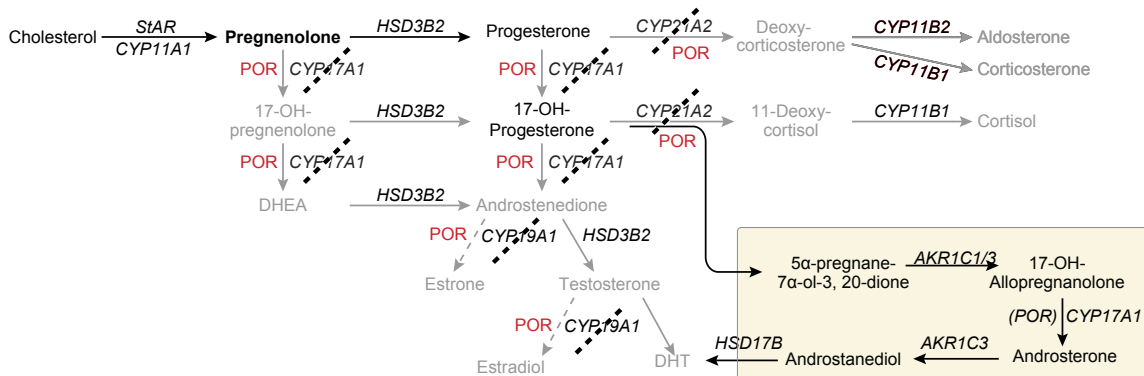
Figure 2

A Adrenal Hormone Synthesis and Alternative Pathways

B 21-hydroxylase deficiency

C 11β-hydroxylase deficiency

D 17-hydroxylase deficiency

E 3βHSD2 deficiency

F POR deficiency

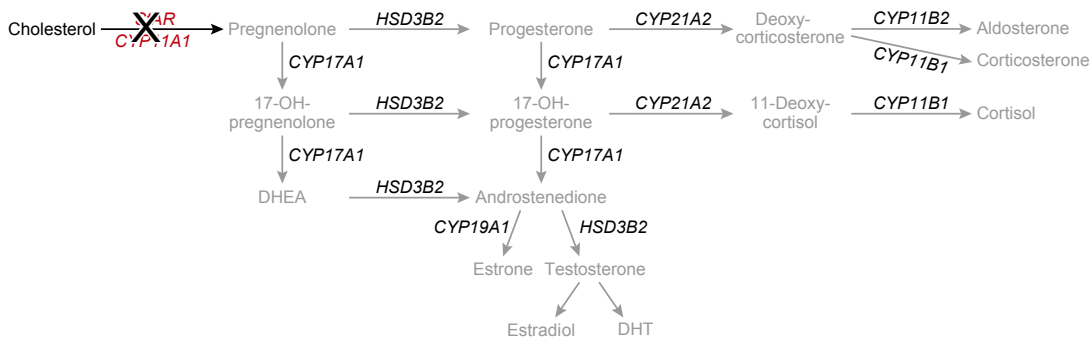**G** Lipoid congenital adrenal hyperplasia or P450 cholesterol side chain cleavage

Figure 3B

2 to 20 years: Girls
Stature-for-age and Weight-for-age percentiles

Figure 4

Figure 1-4 Legends

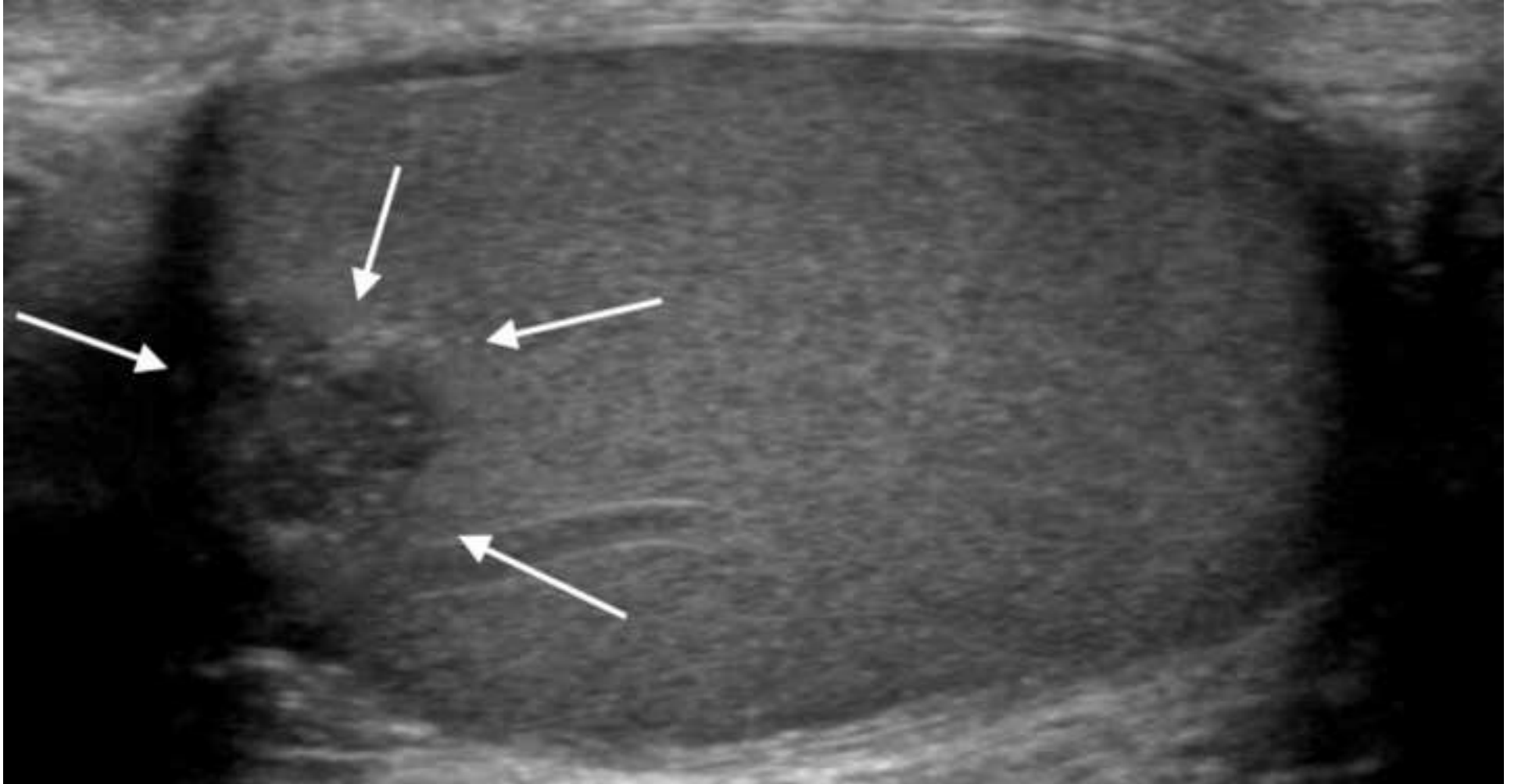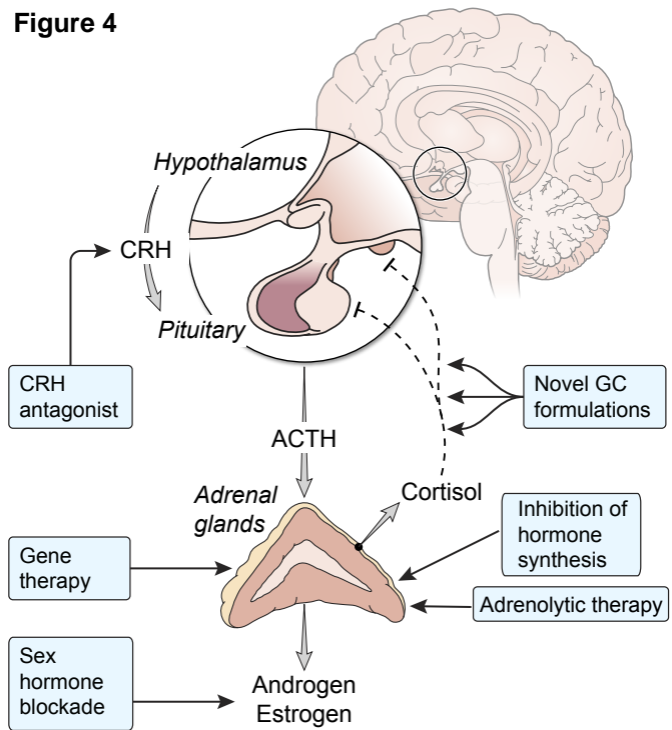**Figure 1: Timeline denoting important discoveries in adrenal steroidogenesis, treatment landmarks and gene discovery of congenital adrenal hyperplasia**

Figure 2: Adrenal steroidogenesis pathways
The CAH-causing genes are depicted in red. Panel A shows the classical steroidogenesis pathway and alternative pathways leading to androgen production in the light yellow boxes. Panels B through G show the various forms of CAH and the impact of the specific impairment on the adrenal steroidogenic pathway. Light grey denotes deficient hormones in low levels due to the preceding block in steroid production. Dashed arrows denote indirect suppression of the subsequent hormone. Dashed lines across enzymes denote apparent enzyme deficiency. DHT = dihydrotestosterone; T= Testosterone.

Figure 3: Adverse outcomes in congenital adrenal hyperplasia. Panel A shows atypical genitalia with clitoromegaly and posterior labial fusion of a 46,XX infant with 21-hydroxylase deficiency. Panel B is the growth chart of a female with classic 21-hydroxylase deficiency who experienced early puberty and early epiphyseal fusion of her bones due to excess adrenal sex steroids and obesity due to excess glucocorticoid therapy. Both likely contributed to her adult short stature. A right sided, lobulated echopenic focus measuring 2.7 x 1.0 x 1.1 cm consistent with testicular adrenal rest tissue is shown in Panel C.

Figure 4: Novel and emerging treatments in the management of congenital adrenal hyperplasia targeting various aspects of the hypothalamic-pituitary-adrenal (HPA) axis and steroid production.

Table 1

Table 1- Genetic Causes and Clinical Features of the Various Forms of Congenital Adrenal Hyperplasia

Type of CAH	21-hydroxylase deficiency	11β-hydroxylase deficiency	17α-hydroxylase/ 17,20-lyase deficiency	3β-hydroxy-steroid dehydrogenase type 2 deficiency	P450 Oxidoreductase deficiency	Lipoid adrenal hyperplasia	Cholesterol side chain cleavage enzyme deficiency
Genetic Cause (Affected Gene, OMIM#)	*CYP21A2* #201910	*CYP11B1,* #202010	*CYP17A1,* #202110	*HSD3B2,* #201810	*POR,* #201750	*StAR,* #600617	*CYP11A1,* #118485
Incidence	Classic: 1:10,000 to 1:20,000[22] NC: 1:1,000[23]	1:100,000[28] Caucasians, 1:6,000[29] Moroccan Jews* NC: unknown	1:50,000[30] Increased frequency in Brazil*[30,31]	Rare	Rare, 130 cases from 11 countries reported[32]	Rare, mostly Japanese, Korean, and Palestinian populations[33]	Rare, < 30 patients, mostly from Eastern Turkey[34]
Affected Organs	Adrenal	Adrenal	Adrenal, gonads	Adrenal, gonads	Adrenal, gonads, liver, skeletal	Adrenal, gonads	Adrenal, gonads
DSD	Classic: 46,XX NC: No	Classic: 46,XX NC: No	46,XY	Classic: 46,XY, 46, XX (rare) NC: No	46,XX, 46,XY (variable)	46,XY NC: 46,XY (variable)	46,XY NC: 46,XY (variable)
Salt-wasting	Classic: Yes NC: No	No	No	Yes	No	Classic: yes NC: Minimal to none	Classic: yes NC: Minimal to none
Hypertension	No	Yes NC: variable	Yes	No	Yes	No	No
Postnatal Virilization	Classic: yes NC: yes	Classic: Yes NC: Yes	No	Classic: 46,XX NC: 46,XX	No	No	No
Sex Steroid	No	No	Yes	Classic: yes	Yes	Yes	Yes

Deficiency				NC: no		NC: variable	NC: variable
Other					± Skeletal malformations ± Maternal virilization		

*Due to presence of a founder mutation

CAH=congenital adrenal hyperplasia; DSD=Disorder of Sex Development; NC=nonclassic, POR= P450 Oxidoreductase; StAR= Steroidogenic acute regulatory protein.

Table 2

Table 2 – Clinical Presentation and Biochemical Findings

Type/ Enzyme	Clinical Presentation	Hormonal Profile	Cosyntropin stimulation testing*	Other testing
21-hydroxylase deficiency	Classic: atypical genitalia (46,XX), neonatal salt-wasting (75%), virilization < 4 year old (46,XY) NC: Precocious pubarche, hirsutism, oligomenorrhea/ amenorrhea, female infertility	↑: 17OHP, ↑21-deoxycortisol, androstenedione, renin ↓: cortisol, aldosterone	17OHP > 1,000 ng/dL (30 nmol/L) (several times higher for classic)	NC: Early morning follicular phase 17OHP< 200 ng/dL (< 6 nmol/L) usually excludes NC CAH
11β-hydroxylase deficiency	Classic: atypical genitalia (46,XX), virilization < 4 year old (46,XY), hypertension, hypokalemia NC: Precocious pubarche, hirsutism, oligomenorrhea/ amenorrhea, female infertility, ± hypertension	↑: DOC, 11-deoxycortisol, androstenedione, 17OHP (mild) ↓: Cortisol, aldosterone, corticosterone, renin	11-deoxycortisol > 3 times the upper limit of normal (several times higher for classic)	
17α-hydroxylase/17,20-lyase deficiency	Adolescent female with absence of 2' sexual characteristics,	↑: DOC, corticosterone (>4000ng/dL, 115 nmol/L), progesterone	Poor response of 17OHpregn and 17OHP	

	hypertension, hypokalemia.	↓: cortisol, aldo, 17OH-pregn, 17OHP, renin DHEA, androstenedione	Elevated ratio of: DOC/cortisol, corticosterone/sex steroids	
3β-hydroxysteroid dehydrogenase type 2 deficiency	atypical genitalia (46,XX: rare, mild; 46,XY), neonatal salt-wasting NC: Precocious pubarche, hirsutism, oligomenorrhea/ amenorrhea (46,XX); atypical genitalia (46,XY: mild)	↑: 17OH-pregn, DHEA, renin ↓: Cortisol, aldosterone, progesterone, 17OHP, androstenedione, DOC, 11-deoxycortisol	17OHpregn > 5000 ng/dL (150 nmol/L) Elevated Ratios pregnenolone / progesterone, 17OHpregn /17OHP	Poor testosterone response hCG stimulation in infancy
P450 Oxidoreductase deficiency	Atypical genitalia, ± skeletal manifestation (Antley-Bixler), ± maternal virilization	↑: pregnenolone, progesterone, 17OHP, DOC, corticosterone, ↓: DHEA, androstenedione Variable (normal or low): cortisol, aldosterone.	Variable 17OHP response, variable cortisol response (often inadequate)	Urine steroid metabolite profile shows characteristic diagnostic profile
Lipoid adrenal hyperplasia or SCC enzyme deficiency	Classic: Female genitalia, neonatal salt-wasting NC: Adrenal insufficiency (2 years- adulthood), variable gonadal function, variable genitalia (46,XY: mild)	↑: renin ↓: All steroids NC: variable	Minimal to no response NC: variable response, ↓cortisol common	Classic: Minimal response hCG stimulation Genetic testing needed to differentiate lipoid CAH and SCC

				deficiency

*Administration of standard dose of 250 mcg cosyntropin (in very low birth weight infants the dose may be reduced to 0.125mg), concomitant measurement of 17OHP, cortisol, DOC, 11-DOC, 17-OH-pregnenelone, DHEA, and androstenedione at baseline and 60 minutes distinguishes 21OH deficiency from other rarer forms of CAH.

NC=nonclassic. 17OHP=17-hydroxyprogesterone. DHEA=dehydroepiandrosterone. DOC=deoxycorticosterone. 17OHpregn=17-hydroxypregnenolone. hCG=human chorionic gonadotropin. SCC=side chain cleavage.

Panel

Panel: Management of Congenital Adrenal Hyperplasia

Glucocorticoid Replacement
- Needed in classic forms of CAH, variable use in NC forms and PORD
- Children: hydrocortisone (8-15 mg/m^2/day) divided into 3 doses, using the lowest dose allowing normal growth while controlling adrenal steroids
- Adolescents and adults: hydrocortisone 2-3 times daily or longer-acting[20,67] glucocorticoids, such as prednisone (5-7.5 mg/day; 1-2 times daily), prednisolone (3-7 mg/day; 1-2 times daily) or dexamethasone (0.25-0.5 mg/day; once daily)
- Monitor for over-replacement: weight gain, central obesity, striae, stunted growth (children), declining bone mineral density
- Monitor for under-replacement: weight loss, fatigue, hyperandrogenism (21OH and 11βOH deficiency), hypertension (11βOH, 17OH deficiency and in adult POR deficiency patients)
- In women, monitor for cycle regulatory and, if appropriate, anovulation
- In males, monitor for testicular adrenal rest tissue (TART) employing testicular ultrasound from adolescence onward; if positive, then offer sperm count and motility assessment and counsel re the possibility of cryopreservation of semen

Stress Dosing
- Needed if patient receiving glucocorticoid therapy or cortisol response to cosyntropin stimulation <18 µg/dl (500 nmol/L)
- Double or triple glucocorticoid during intercurrent illness (fever, gastrointestinal illness), surgery or trauma
- Intramuscular or subcutaneous hydrocortisone if unable to take oral glucocorticoid (home regimen). Children 50 mg/m2; adults 100 mg IV bolus followed by 200 mg over 24hours (hospital regimen)[67]

Mineralocorticoid Replacement
- Needed in salt-wasting forms of CAH
- Fludrocortisone 50-300 mcg daily to achieve a plasma renin activity in the mid-normal range
- First 6-12 months of life: sodium chloride 1-2 g (17-34 mEq) daily, divided and given with feeds
- Monitor for over-replacement: hypertension, oedema, and suppressed plasma renin activity
- Monitor for under-replacement: salt-craving, orthostatic hypotension, elevated plasma renin activity
- Encourage salt intake during hot weather and conditions that promote excessive sweating. Consider seasonal adjustment of fludrocortisone dose in countries with very hot summers.

Sex Steroid Replacement
- Needed in CAH forms that result in sex steroid deficiency
- For pubertal females, oral estradiol (0.5 mg per day advanced to 1-2 mg per day); or transdermal (25 mcg per day advanced to 75-100 mcg per day) over 2-3 years; progesterone,

added following 2 years of estrogen monotherapy or when breakthrough bleeding occurs, 100-200 mg per day, or medroxyprogesterone acetate 5-10 mg per day, or norethindrone acetate 2.5-5 mg per day, for 5-10 days, in women with intact uterus[31]

- For pubertal males, intramuscular testosterone (50 mg per monthly titrated to about 200 every 2 weeks) or transdermal testosterone (titrated to 25-100g per day)[31]

Anti-hypertensive treatment
- Needed if glucocorticoid unsuccessful in treating hypertension in 11βOH and 17OH deficiency
- spironolactone 50-200 mg per day in 1 or 2 divided doses or eplerenone 50-100 mg per day
- Calcium-channel blockers, such as amlodipine, 2.5-10 mg per day, may be used

Anti-androgen treatment
- Oral contraceptives ± spironolactone to control hirsutism, amenorrhea in nonclassic 21OH and 11βOH deficiency

Infertility
- Initiate glucocorticoid for nonclassic forms 21OH and 11βOH deficiency
- Optimize glucocorticoid therapy with suppression of follicular phase progesterone (females) and

shrinkage TART (males) for 21OH and 11βOH deficiency
- Clomiphene citrate stimulation with progesterone supplementation for hypogonadal forms CAH
- Consider In vitro fertilization (females), intra-cytoplasmic sperm injection (males)

Pregnancy
- If on glucocorticoid therapy, hydrocortisone, prednisone, or prednisolone can be used, dexamethasone should be avoided
- Increase glucocorticoid dose by 20-40%, particularly during third trimester[67]
- Stress dosing for labor and delivery

Additional Monitoring Requirements
- Clinical evaluation frequently in first year of life, every 4-6 months for growing child and yearly for adults
- Patients on glucocorticoid replacement should wear emergency bracelet/card, receive sick day rule education, and carry emergency hydrocortisone kit
- Screening for psychological and sexual health issues and late-onset complications of genital surgery, if indicated
- Age-appropriate vitamin D and calcium intake and bone mineral density screening during early adulthood
- Orthopaedic management may be needed for PORD

CAH=congenital adrenal hyperplasia. NC=nonclassic. PORD= P450 Oxidoreductase deficiency. 21OH=12-hydroxylase. 11βOH=11β-hydroxylase. 17OH=17-hydroxylase. TART=testicular adrenal rest tissue.

Web Appendix

Click here to download Web Appendix: CAH Seminar_Summaries_Nov29.docx