MUTATIONS IN CHILDREN WITH CONGENITAL ADRENAL HYPERPLASIA (21-HYDROXYLASE DEFICIENCY)

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1. Introduction

congenital adrenal hyperplasia (CAH) is a group of autosomal recessive disorders resulting from defect in the enzymes involved in the adrenal steroidogenesis pathway leading to compromised cortisol synthesis. Depending on the severity of steroid block, patients can have different alterations in glucocorticoid, mineralocorticoid and sex steroid production (El-Maouche et al., 2017).
Corticotrophin releasing hormone (CRH) and adren ocorticotropic hormone (ACTH) are secreted in exc ess in CAH due to defective cortisol synthesis, which reduces the negative feedback to the brain an d pituitary gland. Although the elevated ACTH can't overcome the block in cortisol production, its trophic influence causes the adrenal glands to en large (Turcu and Auchus, 2015).
The 21-hydroxylase deficiency (21-OHD) is the most frequent enzyme defect in CAH and the most prevalent cause of adrenal insufficiency in pediatrics (Speiser, 2015).
The gene encoding 21-hydroxylase (21-OH) is *CYP21A2* gene. More than 250 genetic variants of *CYP21A2* are capable of causing human disease. The majority of these will result in classical forms of 21-OHD (Witchel, 2017).

Overview on 21-OHD Congenital adrenal hyperplasia and its phenotypes

The 21-OH enzyme converts progesterone to deoxycorticosterone in the biosynthesis of aldosterone and converts 17-hydroxy progesterone (17-OHP) to 11-deoxycortisol in the biosynthesis of cortisol (Therrell et al., 1998, Miller, 2019).
Congenital adrenal hyperplasia due to 21-OHD has been classified into classic form including salt wasting (SW) and simple virilizing (SV) phenotypes, and non-classic (NC) form, although current thinking outlooks *CYP21A2* mutations and the associated phenotypes as a continuum, hence, disease severity is better classified on the basis of residual activity of 21-OH (Witchel, 2018, Claahsen–van der Grinten et al., 2021).
Little or no residual enzyme activity is found in the SW form resulting in both cortisol and aldosterone defects (Held et al., 2020).
Infants with SW form if not appropriately managed will develop dangerous SW crises in the first weeks of life, manifesting by poor feeding, vomiting, dehydration, hypotension, hyponatremia, hyperkalemic metabolic acidosis and shock (Jacobson, 2021).
In the SV form, the gland produces aldosterone somewhat more than that in SW form and consequently patients don't develop SW crisis, but they still have extreme cortisol deficiency and severely elevated androgens. There is residual enzymatic activity of 1% to 5% of normal in those patients (Parsa and New, 2017).
Despite the fact that gonadal development is normal in those patients, excessive levels of androgen production and prenatal exposure to powerful androgens like testosterone and androstenedione during vital stage of sexual development leads to variable grades of female virilization, including clitoromegaly, labioscrotal folds fusion, and urogenital sinus formation (Van Der Straaten et al., 2020).
Except for hyperpigmentation, the external genitalia is normal in affected males (Heather et al., 2015).
In the contrary, Patients with NC form preserve up to 20–30% of the enzyme activity, which is adequate for sufficient cortisol and aldosterone production. Hence, they do not have adrenal insufficiency, and they present at different ages with manifestations of androgen excess (Turcu and Auchus, 2015).

Diagnosis of 21-OHD congenital adrenal hyperplasia

- *Hormonal testing*

The precursor to product ratio is the cornerstone of diagnosis for any enzymatic problem. These ratios are maximized by cosyntropin stimulation, which is crucial in all situations with uncertain baseline data (NEW et al., 1983).
An elevated ACTH, 17-OHP concentration with low cortisol values provides confirmation of the diagnosis of 21-OHD with most patients having random 17-OHP levels more than 5000 ng/dl (Nimkarn et al., 2016).
For those with clinical manifestations indicative of NC form, early morning basal 17-OHP is a useful screening method. A threshold value of 200 ng/dl has 100% sensitivity and 99% specificity for NC form diagnosis in patients with premature adrenarche (Jha and Turcu, 2021).
Retesting is done following cosyntropin stimulation in the case of intermediate screening levels (200–1200 ng/dl). Classic 21-OHD is diagnosed when stimulated 17-OHP level >10,000 ng/dl whereas NC 21-OHD is diagnosed when 17-OHP levels > 1000 ng/dl (Ahmed, 2016).

- *Karyotyping and radiological investigations:*

Chromosomal analysis and pelvic ultrasound (US) are recommended for virilized female infants to confirm an XX karyotype and the presence of a uterus (Raza et al., 2019).
A bone age x-ray (x-ray of left hand) should be done to assess for skeletal maturation advancement.

- *Newborn screening*

Newborn screening for 21-OHD was first applied in 1978 and is now offered in the United States and a

lot of countries (PANG et al., 1977, Pang et al., 1988).

Although it depends on the economic and healthcare status of each country, its crucial role comes from its ability to early diagnose CAH cases and reduce morbidity and mortality for severely affected babies especially for boys who are frequently not diagnosed at birth and experience crises days later (Hird et al., 2014) .

In dried blood spots, first-tier screening measures 17-OHP by an immunoflurometric assay. A 17-OHP value >20,000 ng/dl is suggestive of 21-OHD; although, false-positive results are frequently found in premature and critically ill babies (Coulm et al., 2012).

Recently, liquid chromatography tandem mass spectrometry (LC-MS/MS) allowed precise steroid hormone assessment. The use of LC-MS/MS as the second-tier for mass screening has found to lessen the recall rats (de Hora et al., 2022).

- *Prenatal diagnosis (PND) and intervention:*

The external genitalia of female fetuses become virilized when there is excess androgens present in the intrauterine stage in the classic forms of CAH. These individuals typically live with these virilization symptoms for the rest of their lives, especially in moderate to severe instances. Among other things, the burden of this illness is caused by the requirement for genitoplasty during childhood and gynecological treatments before the initiation of sexual contact (de Castro et al., 2023).

Prenatal dexamethasone (DEX) therapy was first described 40 years ago and is proven to be capable of decreasing degrees of virilization in diseased female infants consequently reducing the necessity for genitoplasty (Bachelot et al., 2017).

Dexamethasone should be initiated by 8 weeks' gestation to be effective, as anatomy of the genitalia is sensitive to dihydrotestosterone (DHT) action at this stage (Witchel and Miller, 2012).

As a result, presumptive treatment should be initiated before the PND can be proven. As only female babies with homozygous or compound heterozygous *CYP21A2* gene mutations should continue to receive medication until term, up to 88% of pregnancies with parents who are carriers for CAH may be treated unnecessarily (only 1 in 8 fetuses is an affected female) (de Castro et al., 2023).

The guidelines of the German Society for Pediatric Endocrinology and Diabetes as well as the American Endocrine Society guidelines, declares that priority is given to preventing unwanted maternal and fetal exposure to DEX and avoiding its potential adverse effects, such as impairment of normal neurological development, above the psychological load brought on by the virilization of the external genitalia. Hence, research involving long-term follow-up should be carried out in countries that permit this kind of therapy, at hospitals that are capable of handling such cases, and with permission from the institutional ethics committee and parental informed agreement (Dörr et al., 2015).

- *Genetic testing:*

Genotyping of *CYP21A2* gene is essential for 21-OHD CAH diagnosis confirmation and to differentiate CAH from other conditions in which there may be overlapping symptoms such as hirsutism, acne, or infertility (e.g., polycystic ovarian syndrome (PCOS) (Trakakis et al., 2011, Bidet et al., 2010, Unluhizarci et al., 2010).

The goal of protecting female embryos with classic 21-OHD from virilization is another crucial role for genetic testing. When compared to female siblings who were not treated in utero, prenatal DEX treatment of the mother bearing an affected female can diminish genital virilization. Nevertheless, prenatal DEX treatment remains debatable (Ilany and Cohen, 2021).

Moreover, genetic analysis is an adjunct to newborn screening as well as newer technologies such as preimplantation genetic diagnosis (PGD) of embryos conceived through in vitro fertilization (Baumgartner-Parzer et al., 2020).

several techniques like PCR-based mutation detection, sequencing, and multiplex ligation-dependent probe amplification (MLPA) may segregate the pathogenic genetic variants and ensure RCCX unit copy number in patients, Moreover, parental genetic analysis isolate the maternal and paternal pathogenic variants and ascertain that mutations are on opposite alleles (trans) (Xu et al., 2013).

Management of 21-OHD congenital adrenal hyperplasia

I-Treatment of classic form

- *Glucocorticoids therapy in pediatric patients*

The goals of treating 21-OHD are to restore adequate levels of GCs and mineralocorticoids, reduce excess adrenal androgen, and promote development and maturation that is equivalent to that of healthy children (Ishii et al., 2022). it is crucial to have an equilibrium between hyper-androgenism and hyper-cortisolism (Speiser et al., 2018).

The European and American guidelines recommend initiating hydrocortisone (HC) treatment at 25 mg/m2 /d maximally. Under normal circumstances, it should start at a dose as low as 10–15 mg/m2 /d, and then decreasing the dose as the androgens reaching the target levels (Creighton et al., 2003, Speiser et al., 2018) .

Hydrocortisone is the used GCs for maintenance treatment in children. Due to its short half-life, HC

has less side effects than long-acting GCs, such as impaired growth, cushignoid features and osteopenia (Paizoni et al., 2020).
Prednisolone and DEX have a growth reducing consequences 15 folds as reported by (Punthakee et al., 2003) and 70–80 fold as reported by (Rivkees and Crawford, 2000) compared to HC. So, they are not allowed to be used as maintenance treatment in children.

- *Mineralocorticoids therapy in pediatric patients*

The European and American guidelines recommend the administration of fludrocortisone (FC) in all patients with classic 21-OHD (Speiser et al., 2018).
The doses of FC and NaCl depend on serum sodium and potassium levels, plasma renin activity or active renin concentration, weight gain, and blood pressure (Ishii et al., 2022).
In a meta-analysis, the group receiving FC therapy had substantially greater adult height SDS (adjusted for parental height) than the group receiving no FC treatment (Muthusamy et al., 2010).

II-Treatment of non-classic form
It is vital to regularly assess the physical examination, height, weight, and bone age in NC 21-OHD patients with hormonal abnormalities but without any symptoms or signs of GCs or mineralocorticoid deficiency to establish the rationale for GCs or mineralocorticoid replacement therapy (Bonfig et al., 2018).
Guidelines do not advocate treating the subclinical NC form similarly to the classic type since no significant research have demonstrated the advantages of doing so (Ishii et al., 2022).

III-Surgical treatment
The goals of female genital surgery are to assure a gender-matched genital look, maintain sexual and reproductive function in maturity, and reduce surgically linked issues including sexual or voiding dysfunction (Ishii et al., 2022).
Surgery is frequently performed beginning at 6 months of age (when replacement treatment is established) until before the emergence of gender labelling (1 year and 6 months to 2 years old) (Wang and Poppas, 2017).
Clitoroplasty and vaginoplasty are executed at the same time in most patients though, vaginoplasty may be performed at puberty in some cases (Almasri et al., 2018).

IV-Novel treatment strategies
Modified-release oral GCs and continuous GCs delivery systems have been developed in an effort to maximize control of hyper-androgenism in patients with 21-OHD while reducing GCs daily dosage (Merke et al., 2021).
Prior research has shown that continuous subcutaneous hydrocortisone infusion (CSHI), which simulates circadian cortisol secretion and is used to treat patients with 21-OHD that is difficult to control with oral GCs therapy based on the conventional biomarkers 17-OHP and androstenedione, can improve disease control (Nella et al., 2016).
In women with poorly managed classic CAH, abiraterone acetate, an orally active, strong 450c17 inhibitor, corrected pre-dose serum androstenedione levels (Auchus et al., 2014).
In addition to standard treatment, another medical technique involves decreasing the excess androgen in the adrenal glands by utilizing the CRH receptor type 1 antagonist NBI-77860 (Turcu et al., 2016).
Animal studies are being conducted employing intravenous injection of an adenovirus-cyp21a1 vector to induce functional enzyme expression, twenty years after the first report that adenoviral gene therapy temporarily restored enzyme activity in a mouse model of 21-OHD (Prasad and Deswal, 2022).
Theoretically, CAH might be cured using gene therapy targeting a patient's own adrenal stem cells. Cell-based therapeutics and the currently under development gene editing technologies may also be alternatives for disease cure in the future (Ruiz-Babot et al., 2018).

Clinical and social Impact of congenital adrenal hyperplasia:

- *Quality of life in CAH patients*

According to recent research, CAH patients may have worse cognition and quality of life as a result of their chronic condition, adrenal hormone imbalance, excessive exposure to androgens, and prolonged GCs medication (Merke and Auchus, 2020).
Among over 1000 kids with classic CAH, a systematic review and meta-analysis revealed decreased stature for mid-parental heights (-1.03 standard deviations, or 7 cm), although many of these children were diagnosed before newborn screening was implemented and did not benefit from early treatment commencement (Muthusamy et al., 2010).

- *Gonadal dysfunction in CAH patients*

The hypothalamic-pituitary-gonadal axis is impacted by elevated adrenal androgens. Patients who have extended periods of insufficient hormonal regulation possess the risk of developing central precocious puberty (Haddad and Eugster, 2019)
One of the most significant long-term outcomes of CAH in both sexes is gonadal dysfunction, which can manifest as early as adolescence and has a variety of etiologies and clinical implications (Speiser et al., 2018)

Infertility in both men and women as well as hypogonadism are clinical symptoms of gonadal dysfunction (Bouvattier et al., 2015).
In addition to hormonal irregularities, physical and psychological problems in females, homosexuality, and a lack of enthusiasm in having children all may be contributing factors to reduced fertility rates (Daae et al., 2020).
Gonadal dysfunction in males is mostly caused by testicular tumors that can hamper their reproductive ability (Engels et al., 2019).

- *Gender identity in CAH patients*

The majority of 46-XX patients with 21-OHD reared as female at birth reported having a female gender identity, and there was no evidence of a relationship between gender identity and external genitalia virilization scores (Meyer-Bahlburg et al., 2006).
Around 5% of 46-XX patients assigned as females and more than 10% of 46-XX patients assigned as male had gender dysphoria (Dessens et al., 2005).
Physicians should be aware that 21-OHD with 46-XX is a disease of sex development (DSD), and it is crucial to set up a health support system offered by a multidisciplinary team for those patients (Speiser et al., 2018).

Molecular genetics of 21-hydroxylase deficiency CAH

The *CYP21A2* gene is located 30 kb away from its highly similar pseudogene *(CYP21A1P)*, which has around 98% homology, on the short arm of chromosome 6 at locus p21.3 (6p21.3) (Prado et al., 2021).
Both of genes have 10 exons and has an extremely similar genetic homology. Due to many pathogenic variations, minor insertions or deletions, and point pathogenic mutations that prohibit the production of a functional protein, the pseudogene *CYP21A1P* is inactive (White et al., 1986).

- *Types of CYP21A2 mutations*

Gene abnormalities can range from small deletions to point mutations (PMs). The combination of these defects in the two *CYP21A2* alleles results in the clinical phenotype, with the phenotype often determined by the allele that is least affected (Falhammar et al., 2015, Gidlöf et al., 2013).
Recombination and/or gene microconversion events between *CYP21A2* gene and the pseudogene, accounts for around 75% of mutations identified (Higashi et al., 1988, Sinnott et al., 1990, Simonetti et al., 2018).
Gene conversions can be *large* which may result in the formation of inactive chimeric gene that comes from a meiotic recombination event in which the last product is inactive chimeric gene with its 5′ end belonging to *CYP21A1P* and the 3′ end to *CYP21A2* (Pignatelli and Pereira, 2021), or *micro-conversions* with transmission of a single or multiple pseudogene-derived pathogenic variants to the *CYP21A2* gene (Strachan, 1994, Tusie-Luna and White, 1995).

On the other hand, large deletions and large gene conversions extending to about 30kb represent about 20–30% (Simonetti et al., 2018, Carrozza et al., 2021)
Only a few mutations that alter 21-OH function are novel variants independent of the pseudogene. It is anticipated that 1-2% of CAH alleles come from *CYP21A2* de novo germ-line variations (Rabbani et al., 2011, de Carvalho et al., 2016).

Numerous fast techniques to identify those mutations have been developed, including allele-specific oligonucleotide hybridization, Amplification-Refractory Mutation System PCR (ARMS PCR), and ligase chain reaction. These methods have been developed because it has been reported that there are 10 *CYP21A1P*-derived mutations accounting for about 90% of the point/small mutations found in CAH patients. All these techniques should consider the problems in *CYP21A2* -specific amplification owing to the high homology with *CYP21A1P*, that may lead to wrong results and allele dropout phenomena (Espinosa Reyes et al., 2020).

- *Genotype-phenotype correlation*

There is a wide variety of symptoms since the majority of patients are compound heterozygotes with distinct mutations on each allele, and the individual's phenotype often reflects the remaining enzyme activity of their mutation (New et al., 2013b).
According to the classification (null, A, B, C, D) provided by (Speiser et al., 1992) , the severity of mutations is classified by its residual enzymatic activity: ‘null’ (0% residual function; del/con, G110_8bp, E6 cluster, F306+t, Q318X, R356W). ‘A’ (0–1% residual function; I2G), ‘B’ (1–2% residual function; I172N) or ‘C’ (20–60% residual function; P30L, V281L, P453S).
In the *SW form*, patients have a total loss of function mutations on the two alleles, in the *SV form*, patients have a total loss of function mutation on one allele and the I172N or intron 2 splicing mutation on the other one, whilst in the *NC form* patients might be compound heterozygotes, with one severe and one mild mutations or they might be homozygotes with two mild mutations, they usually have varying mutations on each allele with one of them having the mild missense mutation like V281L (Witchel, 2017).
Generally, there is a good correlation between the genotype and the phenotype with specific genotypes

associated with SW, SV or NC-CAH (Riedl et al., 2019, Wang et al., 2020) *(Figure.1)*.
In a recent study on Portuguese pediatric cohort the global genotype-phenotype correlation was 92.4 % especially in SW and NC-CAH patients (Santos-Silva et al., 2019).
Usually, a better correlation is achieved on SW and NC forms and the genotype can expect the severity of the disease (New et al., 2013a).

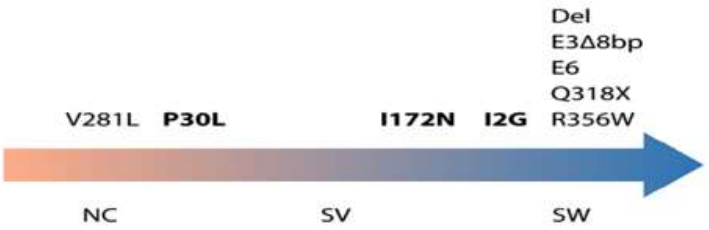

Figure (1) Genotype-phenotype correlation in 21-OHD CAH with the best correlation found in mutations of the SW & NC phenotypes.

Special consideration should be given to SV form, where considerable phenotypic heterogeneity is frequently seen and, occasionally, even in circumstances where the genotype and phenotype are thought to be well correlated (Narasimhan and Khattab, 2019).
Although the correlation between the genotype and phenotype around 80–90 % worldwide (Zhang et al., 2017), the biggest cohort of CAH patients, who includes data from 1507 families with at least one member having CAH, reported about 39% of discordance between genotype and phenotype (New et al., 2013b).
This phenotypic variability could be justified by the existence of alleles carrying more than one pathogenic variant consequently, all types of mutations association might happen (Wedell, 1998).
Another explanation for the genotype-phenotype discordance is not sequencing the entire gene in most studies hence, not having a full delineation of the whole number of mutations (Pignatelli et al., 2019).

2. Conclusion

Given the fact that the clinical presentation represents a continuum of reductions in enzyme activity of which the three levels of severity generally considered and the complicated locus structure with transfer of sequences between *CYP21A2* and its pseudogene, the genetic diagnosis of 21- OHD is not always entirely straightforward and represent merely a systematization to guide and facilitate the clinical practice.

3. References

AHMED, M. A. 2016. *Application of Pharmacometrics to Rare Diseases.* University of Minnesota.

ALMASRI, J., ZAIEM, F., RODRIGUEZ-GUTIERREZ, R., TAMHANE, S. U., IQBAL, A. M., PROKOP, L. J., SPEISER, P. W., BASKIN, L. S., BANCOS, I. & MURAD, M. H. 2018. Genital reconstructive surgery in females with congenital adrenal hyperplasia: a systematic review and meta-analysis. *The Journal of Clinical Endocrinology & Metabolism,* 103**,** 4089-4096.

AUCHUS, R. J., BUSCHUR, E. O., CHANG, A. Y., HAMMER, G. D., RAMM, C., MADRIGAL, D., WANG, G., GONZALEZ, M., XU, X. S. & SMIT, J. W. 2014. Abiraterone acetate to lower androgens in women with classic 21-hydroxylase deficiency. *The Journal of Clinical Endocrinology & Metabolism,* 99**,** 2763-2770.

BACHELOT, A., GROUTHIER, V., COURTILLOT, C., DULON, J. & TOURAINE, P. 2017. MANAGEMENT OF ENDOCRINE DISEASE: Congenital adrenal hyperplasia due to 21-hydroxylase deficiency: update on the management of adult patients and prenatal treatment. *European journal of endocrinology,* 176**,** R167-R181.

BAUMGARTNER-PARZER, S., WITSCH-BAUMGARTNER, M. & HOEPPNER, W. 2020. EMQN best practice guidelines for molecular genetic testing and reporting of 21-hydroxylase deficiency. *European Journal of Human Genetics,* 28**,** 1341-1367.

BIDET, M., BELLANNE-CHANTELOT, C., GALAND-PORTIER, M.-B., GOLMARD, J.-L., TARDY, V., MOREL, Y., CLAUIN, S., COUSSIEU, C., BOUDOU, P. & MOWZOWICZ, I. 2010. Fertility in women with nonclassical congenital adrenal hyperplasia due to 21-hydroxylase deficiency.

The Journal of Clinical Endocrinology & Metabolism, 95**,** 1182-1190.

BONFIG, W., ROEHL, F., RIEDL, S., BRÄMSWIG, J., RICHTER-UNRUH, A., FRICKE-OTTO, S., HÜBNER, A., BETTENDORF, M., SCHÖNAU, E. & DÖRR, H. 2018. Sodium chloride supplementation is not routinely performed in the majority of German and Austrian infants with classic salt-wasting congenital adrenal hyperplasia and has no effect on linear growth and hydrocortisone or fludrocortisone dose. *Hormone Research in Paediatrics*, 89**,** 7-12.

BOUVATTIER, C., ESTERLE, L., RENOULT-PIERRE, P., DE LA PERRIÈRE, A. B., ILLOUZ, F., KERLAN, V., PASCAL-VIGNERON, V., DRUI, D., CHRISTIN-MAITRE, S. & GALLAND, F. 2015. Clinical outcome, hormonal status, gonadotrope axis, and testicular function in 219 adult men born with classic 21-hydroxylase deficiency. A French national survey. *The Journal of Clinical Endocrinology & Metabolism*, 100**,** 2303-2313.

CARROZZA, C., FOCA, L., DE PAOLIS, E. & CONCOLINO, P. 2021. Genes and Pseudogenes: Complexity of the RCCX Locus and Disease. *Frontiers in Endocrinology***,** 941.

CLAAHSEN–VAN DER GRINTEN, H. L., SPEISER, P. W., AHMED, S. F., ARLT, W., AUCHUS, R. J., FALHAMMAR, H., FLÜCK, C. E., GUASTI, L., HUEBNER, A. & KORTMANN, B. 2021. Congenital adrenal hyperplasia–current insights in pathophysiology, diagnostics and management. *Endocrine reviews*.

COULM, B., COSTE, J., TARDY, V., ECOSSE, E., ROUSSEY, M., MOREL, Y., CAREL, J.-C. & GROUP, D. S. 2012. Efficiency of neonatal screening for congenital adrenal hyperplasia due to 21-hydroxylase deficiency in children born in mainland France between 1996 and 2003. *Archives of pediatrics & adolescent medicine*, 166**,** 113-120.

CREIGHTON, S., RANSLEY, P., DUFFY, P., WILCOX, D., MUSHTAQ, I., CUCKOW, P., WOODHOUSE, C., MINTO, C., CROUCH, N. & STANHOPE, R. 2003. Regarding the consensus statement on 21-hydroxylase deficiency from the Lawson Wilkins Pediatric Endocrine Society and the European Society for Paediatric Endocrinology. *The Journal of Clinical Endocrinology & Metabolism*, 88**,** 3454-3456.

DAAE, E., FERAGEN, K. B., WAEHRE, A., NERMOEN, I. & FALHAMMAR, H. 2020. Sexual orientation in individuals with congenital adrenal hyperplasia: a systematic review. *Frontiers in Behavioral Neuroscience***,** 38.

DE CARVALHO, D. F., MIRANDA, M. C., GOMES, L. G., MADUREIRA, G., MARCONDES, J., BILLERBECK, A., RODRIGUES, A. S., PRESTI, P. F., KUPERMAN, H. & DAMIANI, D. 2016. Molecular CYP21A2 diagnosis in 480 Brazilian patients with congenital adrenal hyperplasia before newborn screening introduction. *European Journal of Endocrinology*, 175**,** 107-116.

DE CASTRO, M., MARTINS, C. S. & ANTONINI, S. R. 2023. Prenatal dexamethasone treatment of congenital adrenal hyperplasia: are we any closer to considering it safe? *The Journal of Clinical Endocrinology & Metabolism*, 108**,** e9-e10.

DE HORA, M. R., HEATHER, N. L., WEBSTER, D. R., ALBERT, B. B. & HOFMAN, P. L. 2022. Evaluation of a New Laboratory Protocol for Newborn Screening for Congenital Adrenal Hyperplasia in New Zealand. *International Journal of Neonatal Screening*, 8**,** 56.

DESSENS, A. B., SLIJPER, F. M. & DROP, S. L. 2005. Gender dysphoria and gender change in chromosomal females with congenital adrenal hyperplasia. *Arch Sex Behav*, 34**,** 389-97.

DÖRR, H., BINDER, G., REISCH, N., GEMBRUCH, U., OPPELT, P., WIEACKER, P. & KRATZSCH, J. 2015. Experts' Opinion on the Prenatal Therapy of Congenital Adrenal Hyperplasia (CAH) Due to 21-Hydroxylase Deficiency–Guideline of DGKED in cooperation with DGGG (S1-Level, AWMF Registry No. 174/013, July 2015). *Geburtshilfe und Frauenheilkunde*, 75**,** 1232-1238.

EL-MAOUCHE, D., ARLT, W. & MERKE, D. P. 2017. Congenital adrenal hyperplasia. *The Lancet*, 390**,** 2194-2210.

ENGELS, M., SPAN, P. N., VAN HERWAARDEN, A. E., SWEEP, F. C., STIKKELBROECK, N. M. & CLAAHSEN-VAN DER GRINTEN, H. L. 2019. Testicular adrenal rest tumors: current insights on prevalence, characteristics, origin, and treatment. *Endocrine Reviews*, 40**,** 973-987.

ESPINOSA REYES, T. M., COLLAZO MESA, T., LANTIGUA CRUZ, P. A., AGRAMONTE MACHADO, A., DOMÍNGUEZ ALONSO, E. & FALHAMMAR, H. 2020. Molecular diagnosis of patients with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *BMC Endocrine Disorders*, 20**,** 1-8.

FALHAMMAR, H., WEDELL, A. & NORDENSTRÖM, A. 2015. Biochemical and genetic diagnosis of 21-hydroxylase deficiency. *Endocrine*, 50**,** 306-14.

GIDLÖF, S., FALHAMMAR, H., THILÉN, A., VON DÖBELN, U., RITZÉN, M., WEDELL, A. & NORDENSTRÖM, A. 2013. One hundred

years of congenital adrenal hyperplasia in Sweden: a retrospective, population-based cohort study. *Lancet Diabetes Endocrinol,* 1**,** 35-42.

HADDAD, N. G. & EUGSTER, E. A. 2019. Peripheral precocious puberty including congenital adrenal hyperplasia: causes, consequences, management and outcomes. *Best Practice & Research Clinical Endocrinology & Metabolism,* 33**,** 101273.

HEATHER, N. L., SENEVIRATNE, S. N., WEBSTER, D., DERRAIK, J. G., JEFFERIES, C., CARLL, J., JIANG, Y., CUTFIELD, W. S. & HOFMAN, P. L. 2015. Newborn screening for congenital adrenal hyperplasia in New Zealand, 1994–2013. *The Journal of Clinical Endocrinology & Metabolism,* 100**,** 1002-1008.

HELD, P. K., BIRD, I. M. & HEATHER, N. L. 2020. Newborn screening for congenital adrenal hyperplasia: Review of factors affecting screening accuracy. *International journal of neonatal screening,* 6**,** 67.

HIGASHI, Y., TANAE, A., INOUE, H., HIROMASA, T. & FUJII-KURIYAMA, Y. 1988. Aberrant splicing and missense mutations cause steroid 21-hydroxylase [P-450 (C21)] deficiency in humans: possible gene conversion products. *Proceedings of the National Academy of Sciences,* 85**,** 7486-7490.

HIRD, B. E., TETLOW, L., TOBI, S., PATEL, L. & CLAYTON, P. E. 2014. No evidence of an increase in early infant mortality from congenital adrenal hyperplasia in the absence of screening. *Archives of disease in childhood,* 99**,** 158-164.

ILANY, J. & COHEN, O. 2021. Assessing the risk of having a child with classic 21-hydroxylase deficiency: a new paradigm. *Trends Endocrinol Metab,* 32**,** 423-432.

ISHII, T., KASHIMADA, K., AMANO, N., TAKASAWA, K., NAKAMURA-UTSUNOMIYA, A., YATSUGA, S., MUKAI, T., IDA, S., ISOBE, M. & FUKUSHI, M. 2022. Clinical guidelines for the diagnosis and treatment of 21-hydroxylase deficiency (2021 revision). *Clinical Pediatric Endocrinology*.

JACOBSON, J. D. 2021. Endocrine emergencies. *Biochemical and Molecular Basis of Pediatric Disease.* Elsevier.

JHA, S. & TURCU, A. F. 2021. Nonclassic Congenital Adrenal Hyperplasia: What Do Endocrinologists Need to Know? *Endocrinol Metab Clin North Am,* 50**,** 151-165.

MERKE, D. P. & AUCHUS, R. J. 2020. Congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *New England Journal of Medicine,* 383**,** 1248-1261.

MERKE, D. P., MALLAPPA, A., ARLT, W., BRAC DE LA PERRIERE, A., LINDÉN HIRSCHBERG, A., JUUL, A., NEWELL-PRICE, J., PERRY, C. G., PRETE, A. & REES, D. A. 2021. Modified-release hydrocortisone in congenital adrenal hyperplasia. *The Journal of Clinical Endocrinology & Metabolism,* 106**,** e2063-e2077.

MEYER-BAHLBURG, H. F., DOLEZAL, C., BAKER, S. W., EHRHARDT, A. A. & NEW, M. I. 2006. Gender development in women with congenital adrenal hyperplasia as a function of disorder severity. *Arch Sex Behav,* 35**,** 667-84.

MILLER, W. L. 2019. Congenital adrenal hyperplasia: time to replace 17OHP with 21-deoxycortisol. *Hormone research in paediatrics,* 91**,** 416-420.

MUTHUSAMY, K., ELAMIN, M. B., SMUSHKIN, G., MURAD, M. H., LAMPROPULOS, J. F., ELAMIN, K. B., ABU ELNOUR, N. O., GALLEGOS-OROZCO, J. F., FATOURECHI, M. M. & AGRWAL, N. 2010. Adult height in patients with congenital adrenal hyperplasia: a systematic review and metaanalysis. *The Journal of Clinical Endocrinology & Metabolism,* 95**,** 4161-4172.

NARASIMHAN, M. L. & KHATTAB, A. 2019. Genetics of congenital adrenal hyperplasia and genotype-phenotype correlation. *Fertil Steril,* 111**,** 24-29.

NELLA, A. A., MALLAPPA, A., PERRITT, A. F., GOUNDEN, V., KUMAR, P., SINAII, N., DALEY, L.-A., LING, A., LIU, C.-Y. & SOLDIN, S. J. 2016. A phase 2 study of continuous subcutaneous hydrocortisone infusion in adults with congenital adrenal hyperplasia. *The Journal of Clinical Endocrinology & Metabolism,* 101**,** 4690-4698.

NEW, M. I., ABRAHAM, M., GONZALEZ, B., DUMIC, M., RAZZAGHY-AZAR, M., CHITAYAT, D., SUN, L., ZAIDI, M., WILSON, R. C. & YUEN, T. 2013a. Genotype-phenotype correlation in 1,507 families with congenital adrenal hyperplasia owing to 21-hydroxylase deficiency. *Proc Natl Acad Sci U S A,* 110**,** 2611-6.

NEW, M. I., ABRAHAM, M., GONZALEZ, B., DUMIC, M., RAZZAGHY-AZAR, M., CHITAYAT, D., SUN, L., ZAIDI, M., WILSON, R. C. & YUEN, T. 2013b. Genotype–phenotype correlation in 1,507 families with congenital adrenal hyperplasia owing to 21-hydroxylase deficiency. *Proceedings of the National Academy of Sciences,* 110**,** 2611-2616.

NEW, M. I., LORENZEN, F., LERNER, A. J., KOHN, B., OBERFIELD, S. E., POLLACK, M. S., DUPONT, B., STONER, E., LEVY, D. J. & PANG, S. 1983. Genotyping steroid 21-hydroxylase deficiency: hormonal reference

data. *The Journal of Clinical Endocrinology & Metabolism,* 57**,** 320-326.

NIMKARN, S., GANGISHETTI, P. K., YAU, M. & NEW, M. I. 2016. 21-hydroxylase-deficient congenital adrenal hyperplasia.

PAIZONI, L., AUER, M. K., SCHMIDT, H., HÜBNER, A., BIDLINGMAIER, M. & REISCH, N. 2020. Effect of androgen excess and glucocorticoid exposure on metabolic risk profiles in patients with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *The Journal of Steroid Biochemistry and Molecular Biology,* 197**,** 105540.

PANG, S., HOTCHKISS, J., DRASH, A. L., LEVINE, L. S. & NEW, M. I. 1977. Microfilter paper method for 17α-hydroxyprogesterone radioimmunoassay: its application for rapid screening for congenital adrenal hyperplasia. *The Journal of Clinical Endocrinology & Metabolism,* 45**,** 1003-1008.

PANG, S., WALLACE, M. A., HOFMAN, L., THULINE, H. C., DORCHE, C., LYON, I. C., DOBBINS, R. H., KLING, S., FUJIEDA, K. & SUWA, S. 1988. Worldwide experience in newborn screening for classical congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Pediatrics,* 81**,** 866-874.

PARSA, A. A. & NEW, M. I. 2017. Steroid 21-hydroxylase deficiency in congenital adrenal hyperplasia. *The Journal of steroid biochemistry and molecular biology,* 165**,** 2-11.

PIGNATELLI, D., CARVALHO, B. L., PALMEIRO, A., BARROS, A., GUERREIRO, S. G. & MACUT, D. 2019. The complexities in genotyping of congenital adrenal hyperplasia: 21-hydroxylase deficiency. *Frontiers in Endocrinology,* 10**,** 432.

PIGNATELLI, D. & PEREIRA, S. S. 2021. Congenital Adrenal Hyperplasia Due to 21-Hydroxylase Deficiency: Genetic Characterization and the Genotype–Phenotype Correlation. *In:* ERTORER, M. E. (ed.) *Fertility and Reproductive Outcomes in Different Forms of Congenital Adrenal Hyperplasia.* Cham: Springer International Publishing.

PRADO, M. J., SINGH, S., LIGABUE-BRAUN, R., MENEGHETTI, B., RISPOLI, T., KOPACEK, C., MONTEIRO, K., ZAHA, A., ROSSETTI, M. R. & PANDEY, A. V. 2021. Characterization of Mutations Causing CYP21A2 Deficiency in Brazilian and Portuguese Populations.

PRASAD, R. & DESWAL, S. 2022. New Horizons: Molecular Basis and Novel Therapeutics in Congenital Adrenal Hyperplasia. *Indian Journal of Clinical Biochemistry,* 37**,** 1-2.

PUNTHAKEE, Z., LEGAULT, L. & POLYCHRONAKOS, C. 2003. Prednisolone in the treatment of adrenal insufficiency: a re-evaluation of relative potency. *The Journal of pediatrics,* 143**,** 402-405.

RABBANI, B., MAHDIEH, N., ASHTIANI, M.-T. H., AKBARI, M.-T. & RABBANI, A. 2011. Molecular diagnosis of congenital adrenal hyperplasia in Iran: focusing on CYP21A2 gene. *Iranian Journal of Pediatrics,* 21**,** 139.

RAZA, J., ZAIDI, S. Z. & WARNE, G. L. 2019. Management of disorders of sex development–With a focus on development of the child and adolescent through the pubertal years. *Best Practice & Research Clinical Endocrinology & Metabolism,* 33**,** 101297.

RIEDL, S., RÖHL, F.-W., BONFIG, W., BRÄMSWIG, J., RICHTER-UNRUH, A., FRICKE-OTTO, S., BETTENDORF, M., RIEPE, F., KRIEGSHÄUSER, G. & SCHOENAU, E. 2019. Genotype/phenotype correlations in 538 congenital adrenal hyperplasia patients from Germany and Austria: discordances in milder genotypes and in screened versus prescreening patients. *Endocrine connections,* 8**,** 86-94.

RIVKEES, S. A. & CRAWFORD, J. D. 2000. Dexamethasone treatment of virilizing congenital adrenal hyperplasia: the ability to achieve normal growth. *Pediatrics,* 106**,** 767-773.

RUIZ-BABOT, G., BALYURA, M., HADJIDEMETRIOU, I., AJODHA, S. J., TAYLOR, D. R., GHATAORE, L., TAYLOR, N. F., SCHUBERT, U., ZIEGLER, C. G. & STORR, H. L. 2018. Modeling congenital adrenal hyperplasia and testing interventions for adrenal insufficiency using donor-specific reprogrammed cells. *Cell Reports,* 22**,** 1236-1249.

SANTOS-SILVA, R., CARDOSO, R., LOPES, L., FONSECA, M., ESPADA, F., SAMPAIO, L., BRANDÃO, C., ANTUNES, A., BRAGANÇA, G. & COELHO, R. 2019. CYP21A2 gene pathogenic variants: a multicenter study on genotype–phenotype correlation from a Portuguese pediatric cohort. *Hormone research in paediatrics,* 91**,** 33-45.

SIMONETTI, L., BRUQUE, C. D., FERNÁNDEZ, C. S., BENAVIDES-MORI, B., DELEA, M., KOLOMENSKI, J. E., ESPECHE, L. D., BUZZALINO, N. D., NADRA, A. D. & DAIN, L. 2018. CYP21A2 mutation update: Comprehensive analysis of databases and published genetic variants. *Human mutation,* 39**,** 5-22.

SINNOTT, P., COLLIER, S., COSTIGAN, C., DYER, P. A., HARRIS, R. & STRACHAN, T. 1990. Genesis by meiotic unequal crossover of a de novo deletion that contributes to steroid 21-

hydroxylase deficiency. *Proceedings of the National Academy of Sciences,* 87**,** 2107-2111.

SPEISER, P. W. 2015. Congenital adrenal hyperplasia. *F1000Research,* 4.

SPEISER, P. W., ARLT, W., AUCHUS, R. J., BASKIN, L. S., CONWAY, G. S., MERKE, D. P., MEYER-BAHLBURG, H. F., MILLER, W. L., MURAD, M. H. & OBERFIELD, S. E. 2018. Congenital adrenal hyperplasia due to steroid 21-hydroxylase deficiency: an Endocrine Society clinical practice guideline. *The Journal of Clinical Endocrinology & Metabolism,* 103**,** 4043-4088.

SPEISER, P. W., DUPONT, J., ZHU, D., SERRAT, J., BUEGELEISEN, M., TUSIE-LUNA, M. T., LESSER, M., NEW, M. I. & WHITE, P. C. 1992. Disease expression and molecular genotype in congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *The Journal of clinical investigation,* 90**,** 584-595.

STRACHAN, T. 1994. Molecular pathology of 21-hydroxylase deficiency. *Journal of inherited metabolic disease,* 17**,** 430-441.

THERRELL, B. L., BERENBAUM, S. A., MANTER-KAPANKE, V., SIMMANK, J., KORMAN, K., PRENTICE, L., GONZALEZ, J. & GUNN, S. 1998. Results of screening 1.9 million Texas newborns for 21-hydroxylase-deficient congenital adrenal hyperplasia. *Pediatrics,* 101**,** 583-590.

TRAKAKIS, E., DRACOPOULOU-VABOULI, M., DACOU-VOUTETAKIS, C., BASIOS, G., CHRELIAS, C. & KASSANOS, D. 2011. Infertility reversed by glucocorticoids and full-term pregnancy in a couple with previously undiagnosed nonclassic congenital adrenal hyperplasia. *Fertility and sterility,* 96**,** 1048-1050.

TURCU, A. F. & AUCHUS, R. J. 2015. The next 150 years of congenital adrenal hyperplasia. *The Journal of steroid biochemistry and molecular biology,* 153**,** 63-71.

TURCU, A. F., SPENCER-SEGAL, J. L., FARBER, R. H., LUO, R., GRIGORIADIS, D. E., RAMM, C. A., MADRIGAL, D., MUTH, T., O'BRIEN, C. F. & AUCHUS, R. J. 2016. Single-dose study of a corticotropin-releasing factor receptor-1 antagonist in women with 21-hydroxylase deficiency. *The Journal of Clinical Endocrinology & Metabolism,* 101**,** 1174-1180.

TUSIE-LUNA, M.-T. & WHITE, P. C. 1995. Gene conversions and unequal crossovers between CYP21 (steroid 21-hydroxylase gene) and CYP21P involve different mechanisms. *Proceedings of the National Academy of Sciences,* 92**,** 10796-10800.

UNLUHIZARCI, K., KULA, M., DUNDAR, M., TANRIVERDI, F., ISRAEL, S., COLAK, R., DOKMETAS, H. S., ATMACA, H., BAHCECI, M. & BALCI, M. K. 2010. The prevalence of non-classic adrenal hyperplasia among Turkish women with hyperandrogenism. *Gynecological Endocrinology,* 26**,** 139-143.

VAN DER STRAATEN, S., SPRINGER, A., ZECIC, A., HEBENSTREIT, D., TONNHOFER, U., GAWLIK, A., BAUMERT, M., SZELIGA, K., DEBULPAEP, S. & DESLOOVERE, A. 2020. The external genitalia score (EGS): a European multicenter validation study. *The Journal of Clinical Endocrinology & Metabolism,* 105**,** e222-e230.

WANG, L. C. & POPPAS, D. P. 2017. Surgical outcomes and complications of reconstructive surgery in the female congenital adrenal hyperplasia patient: What every endocrinologist should know. *The Journal of Steroid Biochemistry and Molecular Biology,* 165**,** 137-144.

WANG, X., WANG, Y., MA, D., ZHANG, Z., LI, Y., YANG, P., SUN, Y. & JIANG, T. 2020. Neonatal Screening and genotype-phenotype correlation of 21-hydroxylase deficiency in the Chinese population. *Frontiers in genetics,* 11**,** 1851.

WEDELL, A. 1998. An update on the molecular genetics of congenital adrenal hyperplasia: diagnostic and therapeutic aspects. *Journal of Pediatric Endocrinology and Metabolism,* 11**,** 581-590.

WHITE, P. C., NEW, M. I. & DUPONT, B. 1986. Structure of human steroid 21-hydroxylase genes. *Proc Natl Acad Sci U S A,* 83**,** 5111-5.

WITCHEL, S. F. 2017. Congenital adrenal hyperplasia. *Journal of pediatric and adolescent gynecology,* 30**,** 520-534.

WITCHEL, S. F. 2018. Genetics and Pathophysiology of Congenital Adrenal Hyperplasia. *Adrenal Disorders.* Springer.

WITCHEL, S. F. & MILLER, W. L. 2012. Prenatal treatment of congenital adrenal hyperplasia-not standard of care. *J Genet Couns,* 21**,** 615-24.

XU, Z., CHEN, W., MERKE, D. P. & MCDONNELL, N. B. 2013. Comprehensive mutation analysis of the CYP21A2 gene: an efficient multistep approach to the molecular diagnosis of congenital adrenal hyperplasia. *The Journal of molecular diagnostics,* 15**,** 745-753.

ZHANG, B., LU, L. & LU, Z. 2017. Molecular diagnosis of Chinese patients with 21-hydroxylase deficiency and analysis of genotype–phenotype correlations. *Journal of International Medical Research,* 45**,** 481-492.