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Non-classic congenital adrenal hyperplasia caused with common and rare forms: Unresolved issues and implications on clinical management

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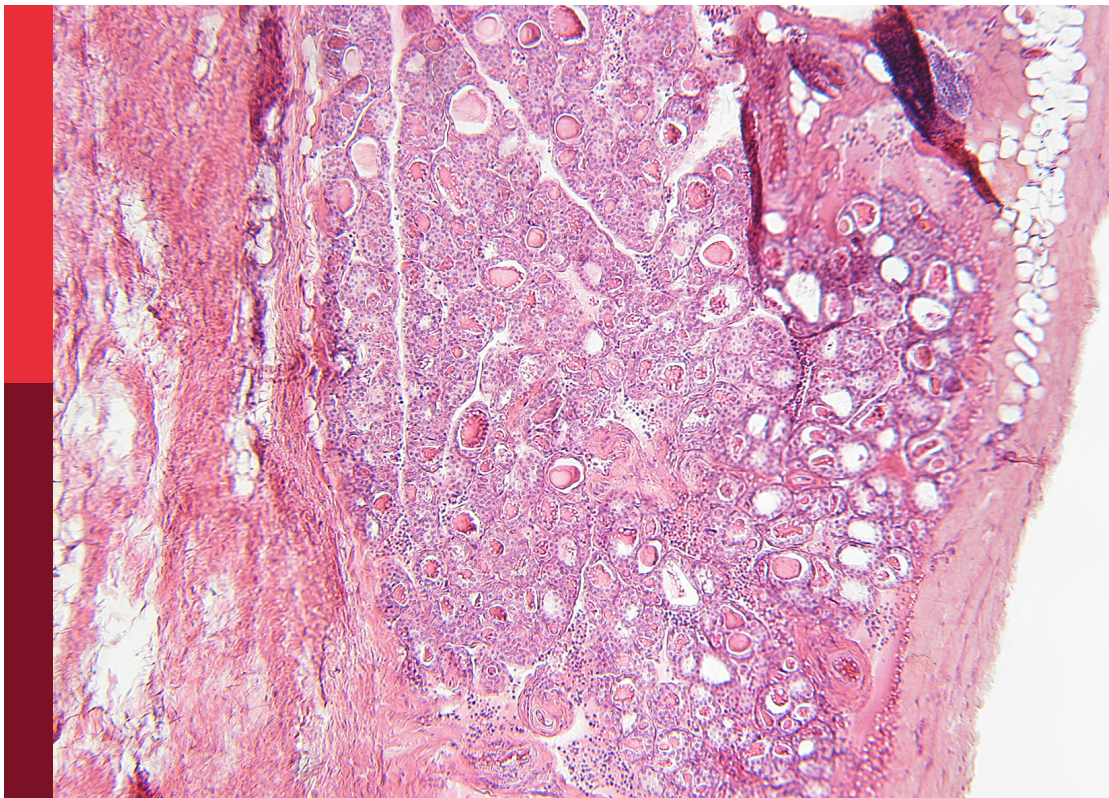

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Non-classic congenital adrenal hyperplasia caused with common and rare forms: Unresolved issues and implications on clinical management

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Editorial: Non-classic congenital adrenal hyperplasia caused with common and rare forms: Unresolved issues and implications on clinical management

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congenital adrenal hyperplasia, non-classical type, diagnosis, reproduction, clinical management

Editorial on the Research Topic

Non-classic congenital adrenal hyperplasia caused with common and rare forms: Unresolved issues and implications on clinical management

The non-classic type of Congenital Adrenal Hyperplasia (NCCAH) is easily to be mis- or undiagnosed. It is characterized mainly by primary amenorrhea/oligomenorrhea/repeated functional ovary cysts/anovulatory cycles/infertility and/or sex hormonal disorders. Multidisciplinary team (MDT) consulting by pediatric, gynecological, or reproductive endocrinologists should be provided to make an etiological diagnosis of various enzyme deficiencies and make a plan of reasonable management.

This Research Topic has focused on diagnosis and differential diagnosis, the characteristics of phenotypes, new genotypes, clinical management, reproduction and assistant reproductive technique (ART) for NCCAH patients. It should be helpful to broaden and widen the understanding about the management of NCCAH patients.

For example, we learn in the review of Adriaansen et al. that the main problem in NCCAH patients is not the inadequate cortisol production, but the increased production of adrenal androgens. Hyperandrogenism can lead to rapid postnatal growth, advanced bone age, and premature pubarche in childhood, as well as acne, hirsutism, menstrual irregularities (in females), and insulin resistance in adulthood. Therefore, NCCAH patients should be carefully evaluated and the advantages and disadvantages of different treatment options used in patients with NCCAH were discussed in this review. In this retrospective observational study of Simon et al., the interaction between muscle-to-fat ratio (MFR) and components of

metabolic syndrome in pediatric subjects with NCCAH were explored. It was founded that children and adolescents with NCCAH have a body composition characterized by an imbalance of MFR, while glucocorticoid therapy does not appear to adversely affect their body composition. Lu et al, reported a rare male case of non-classic Lipoid congenital adrenal hyperplasia (LCAH) caused by mutations in the steroidogenic acute regulatory protein (StAR) and reviewed the literature. It showed that the clinical phenotypes of non-classic LCAH are highly variable. Routine physical examination, laboratory measurement, genetic testing, and, importantly, enzymatic activity assay may facilitate the early diagnosis of non-classic LCAH. Yin et al. reported a rare 17- OHD female patient with normal 46,XX karyotype accompanied by ovarian gonadoblastoma with dysgerminoma. Although the tumorigenesis is common in 46 XY 17OHD patient, but it cannot be ruled out in very rare 46 XX CAH patient. Infertility is always one of the main clinical manifestations of partial female 17-OHD patients. Jiang et al. reported two female infertile patients with partial 17-OHD delivered successfully after assisted reproductive treatment and review the literature. It suggested that the pregnancy potentials of infertile partial 17-OHD women seemed to increase with the adoption of IVF-ET. Considering the sustained elevated P4 level, PPOS is a feasible protocol for them in COH. In the systematic review of Guo et al., a meta-analysis was performed to evaluate the pregnancy complications in CAH women caused by rare enzymatic deficiency excluding 21OHD. The result showed that fertility is possible for these patients but special care was necessary when planning, seeking and during pregnancy.

Author contributions

YL and DY are responsible for initiating and summarizing this Research Topic. QT, YK are guested editor for this Research Topic. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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ACTH-independent Cushing's syndrome due to ectopic endocrinologically functional adrenal tissue caused by a *GNAS* heterozygous mutation: a rare case of McCune–Albright syndrome accompanied by central amenorrhea and hypothyroidism: a case report and literature review

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In a small number of cases, the development of ectopic residual adrenal lesions during embryogenesis causing Cushing's syndrome due to the production of excess cortisol has been reported. A 29-year-old woman was admitted to our hospital for fatigue and recent amenorrhea. Her plasma ACTH was <1.5 pg/mL, and her serum cortisol was 21.4 pg/mL after the 8 mg dexamethasone suppression test, revealing the presence of ACTH-independent Cushing's syndrome; however, her bilateral adrenal glands were atrophied. Abdominal CT revealed a 40-mm round tumor on the right renal hilum and remarkably accumulated ^{131}I-labelled adosterol. CT and bone scintigraphy showed that ^{99m}Tc-methylene diphosphonate had accumulated in her dissymmetric skull at the right-frontoparietal region. The tumor on the right renal hilum was laparoscopically removed. Her cortisol levels rapidly decreased to below the normal range, and glucocorticoids were administered to rescue adrenal insufficiency. The resected tumor was yellowish in appearance and 4.5×3.0×2.8 cm in size. Immunohistochemical staining for SF-1, P450scc, CYP17A, CYP21A, and CYP11B1 indicated that this tumor produced cortisol. Exome sequencing analysis revealed that the *GNAS* heterozygous mutation (c.601C>T, p. Arg201Cys; accession number, NM_000516.5) was found in approximately 20% of the adrenal tumor sample. A mutation of *GNAS*, encoding the Gsα subunit that mediates GPCR signaling, causes the constitutive activation of adenylyl cyclase, resulting in hypersecretion of

hormones regulated by the GPCR. *GNAS* mutation is one of the major genetic causes of cortisol-producing adrenal tumors independent of ACTH secretion. Considering the combination of *GNAS* mutation with one of the typical clinical triad characteristics, fibrous dysplasia of bone, we diagnosed this patient with McCune–Albright syndrome accompanied by ACTH-independent Cushing's syndrome caused by an ectopic residual adrenal tumor due to *GNAS* mutation. This case highlights that *GNAS* involves a previously unknown pathological mechanism in which inhibition of the natural elimination of remnant tissue leads to ectopic endocrine hypersecretion.

KEYWORDS

GNAS mutation, Cushing's syndrome, McCune–Albright syndrome, ectopic adrenal tumor, fibrous dysplasia

Introduction

Cushing's syndrome, characterized by excess endogenous glucocorticoids, is a rare disease, with a prevalence of approximately 0.7-2.4 cases per million people per year. A total of 15%–20% of cases are adrenocorticotropic hormone (ACTH)-independent (1). A unilateral cortisol-producing tumor due to either adenoma or adrenocortical carcinoma is the primary cause (1); an ectopic cortisol-producing tumor in extra-adrenal tissue is an exceedingly uncommon aetiology for hypercortisolism.

McCune–Albright syndrome (MAS), a disease caused by a mutation of *GNAS*, predisposes individuals to hormonal hypersecretion from endocrine organs with G-protein-coupled receptors (GPCRs). However, there are only a few cases of MAS accompanied by ACTH-independent Cushing's syndrome, which was limited in infancy, suggesting that ACTH-independent Cushing's syndrome, especially in adults, is one of the rarest endocrine disorders in MAS (2). Here, we present the first case of ACTH-independent Cushing's syndrome caused by an ectopic extra-adrenal cortisol-producing tumor at the renal hilum concomitant with *GNAS* mutation, which is crucial to the pathogenesis of MAS.

Case description

A 29-year-old woman presenting with a six-month history of amenorrhea and general fatigue concomitant with lid oedema was admitted to our hospital. Spontaneous menstruation had started at the age of 13 years, and she had maintained a regular menstrual cycle since the age of 28. Her breasts, pubic hair, and axillary hair started to grow when she was approximately 13 years old. An annual medical check-up revealed hypertension four years previously, and she was treated with 40 mg amlodipine, 20 mg azilsartan, and 30 mg azosemide. Her legs and eyelids were oedematous approximately nine months before admission, and purpura emerged on the distal extremities. She had no significant medical history, including endocrine dysfunction, bone disease, or intellectual disability.

Her blood pressure was 142/104 mmHg. Her weight was 51.7 kg, her height was 154 cm (body mass index of 21.8 kg/m^2), and her right and left grip strength was 16.8 and 13.3 kg, respectively. She had a typical moon-face, central obesity with petechiae, and thin limbs; however, she had no other cushingoid features, such as red abdominal striae or a buffalo hump. Oedema was observed in the bilateral eyelids and lower extremities; the latter was accompanied by subcutaneous bleeding. Physical examination revealed that the right-frontoparietal region of her cranium was asymmetrically deformed, exhibiting bulging; however, cafe-au-lait spots were not observed.

A laboratory examination showed that most of the pituitary hormones secreted from the anterior lobe were at low levels, and corresponding target hormones, except for cortisol, were decreased: ACTH 3.4 pg/mL, cortisol 26.8 μg/dL; thyroid-stimulating hormone (TSH) 0.09 μIU/mL, free thyroxine (FT_4) 0.65 ng/dL, and free triiodothyronine (FT_3) 1.25 pg/mL; luteinizing hormone (LH) 4.6 mIU/mL, follicle-stimulating hormone (FSH) 6.1 mIU/mL, and E_2 21 pg/mL; growth hormone (GH) 0.1 ng/mL, and insulin-like growth factor 1 (IGF-1) 81 ng/mL; and prolactin (PRL) 16.5 ng/dL. Thyrotropin-releasing hormone (TRH), growth hormone releasing peptide-2 (GHRP-2), and gonadotropin-releasing hormone (GnRH) provocation tests revealed poor responses of TSH and GH and low elevations of PRL, LH, and FSH, indicating that she suffered from central hypopituitarism. Notably, the urinary cortisol level was higher than the normal range (716 μg/day), which was accompanied by hypercortisolaemia with loss of circadian rhythm. The serum cortisol level after the administration of 8 mg dexamethasone was not suppressed (21.4 μg/dL), indicating that hypercortisolaemia was caused by

ACTH-independent Cushing's syndrome. However, abdominal computed tomography (CT) showed that both her adrenal glands were atrophied. No apparent hormonal dysfunction of the other adrenal gland was observed (plasma aldosterone 93 pg/mL, plasma renin activity 1.0 ng/mL/hr, serum dehydroepiandrosterone sulphate (DHEA-S) 180 ng/mL, and urinary concentrations of the catecholamine metabolites metanephrine and normetanephrine 0.05 and 0.14 mg/day, respectively). Coincidentally, an enhanced CT scan showed a 40-mm round tumor on the right renal hilum (Figure 1A); the tumor remarkably accumulated ^{131}I-labelled adosterol but not in the adrenal glands (Figure 1B). According to these findings, we considered that this extra-adrenal tumor ectopically produced cortisol independent of ACTH secretion. In addition, a head CT scan showed dissymmetric bone thickening with a "ground-glass" appearance, which consisted of a cystic or solid mass at right-frontoparietal region of the temporal bone (Figure 1C). Bone scintigraphy showed that ^{99m}Tc-methylene diphosphonate (MDP) accumulated in multiple bones, including skull lesions (Figure 1D), suggesting that these bone features were caused by fibrous dysplasia.

The tumor on the right renal hilum was laparoscopically removed. The resected tumor was yellowish in appearance, 16 g in weight, and 4.5×3.0×2.8 cm in size. Positive immunohistochemical staining for StAR, CYP11A1, CYP17A, HSD3B, CYP21A, CYP11B1, and SF-1 (Figures 2A–F, H) indicated that the tumor was capable of producing cortisol from cholesterol, a source reagent, *via* an authentic enzymatic reaction. In contrast, staining for CYP11B2 and Ki-67 was negative (Figures 2G, I). An exome sequencing analysis using the Illumina platform revealed that a *GNAS* heterozygous mutation (c.601C>T, p. Arg201Cys; accession number, NM_000516.5) was found in approximately 20% of the adrenal tumor sample.

After resection, the patient's cortisol levels rapidly decreased to below the normal range, and glucocorticoids were administered to rescue adrenal insufficiency. Antihypertensive medication was not required to control her blood pressure. Three months after surgery, the Cushingoid features disappeared, she spontaneously resumed menstruation, and levothyroxine was discontinued. A year later, her cortisol levels remained within the normal range without replacement therapy. Finally, we diagnosed her with MAS because the coexisting findings of fibrous dysplasia and ACTH-independent hypercortisolaemia caused by the ectopic tumor on the right renal hilum could be explained by only one cause, *GNAS* mutation.

Discussion

The patient underwent the surgical removal of the extra-adrenal tumor on the right renal hilum, which possessed a

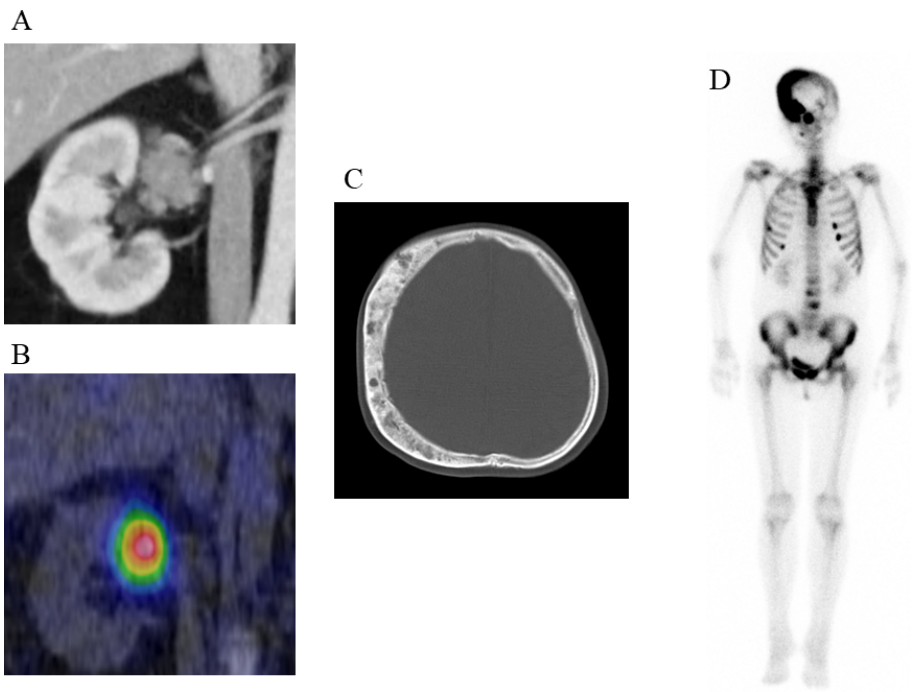

FIGURE 1
Clinical images of the patient **(A)** Enhanced CT scan showing a 40-mm round tumor with a relatively reduced contrast effect in the right renal hilum. **(B)** Remarkable ^{131}I-labelled adosterol accumulation in the tumor but not in the adrenal glands. **(C)** CT scan showing dissymmetric bone thickening with a "ground-glass" appearance, which consisted of cystic or solid mass at right-frontoparietal region of the temporal bone. **(D)** ^{99m}Tc-methylene diphosphonate (MDP) accumulation in the skull lesion.

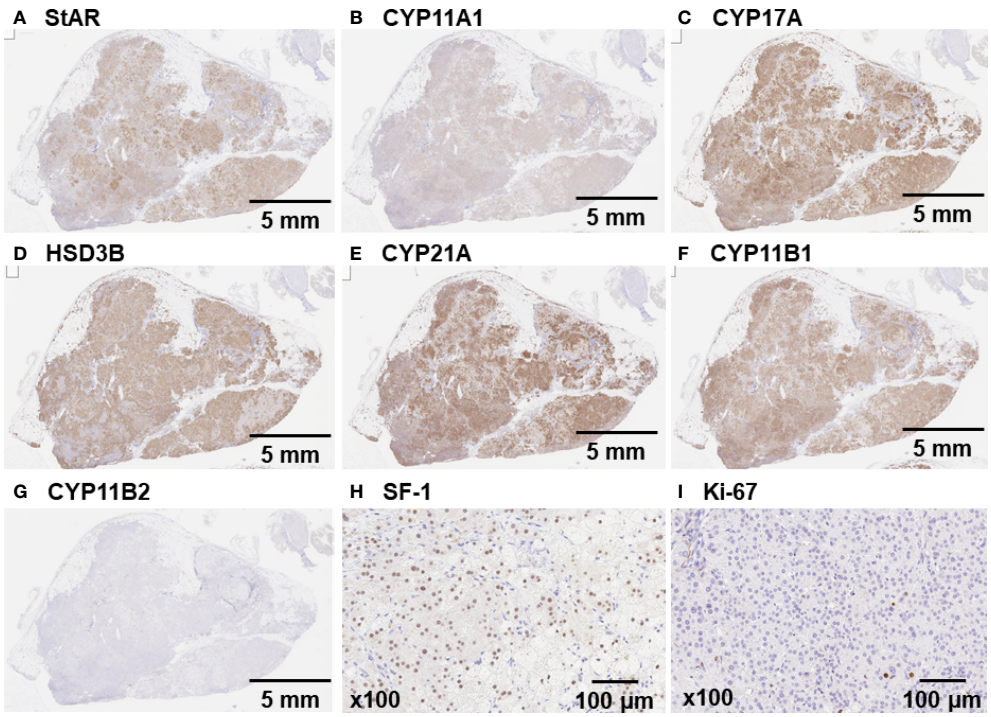

FIGURE 2
Immunohistochemical staining of the tumor on the right renal hilum Immunohistochemical staining confirmed a pituitary adenoma that was markedly positive for **(A)** StAR, **(B)** CYP11A1, **(C)** CYP17A, **(D)** HSD3B, **(E)** CYP21A, **(F)** CYP11B1, and **(H)** SF-1. **(G and I)** Staining for CYP11B2 and Ki-67 was negative.

GNAS heterozygous mutation. Tumor resection induced the normalization of ACTH-independent hypercortisolaemia and the resolution of hypopituitarism, including hypogonadotropic amenorrhea and central hypothyroidism.

One of the unique clinical features in this case was that the tumor on the right renal hilum produced cortisol. Adrenal foetal zone tissue that detaches in the neonatal period and migrates through the urogenital tract can result in residual tissue that forms tumors on the retroperitoneum, testis, broad ligament, ovaries, and inguinal region, including the kidneys, and this residual tissue is considered ectopic adrenal tissue (3, 4). Only 1% of these lesions are observed in adults due to atrophic changes during tissue development, although they are observed in 50% of newborn infants (3), and most cases are asymptomatic (5). However, in our case, the tumor on the right renal hilum possessed all of the adrenal-specific biosynthetic enzymes at each step in the conversion of cholesterol to cortisol, and the gene expression of the tumor is known to be regulated by steroidogenic factor-1:SF-1 (6). In addition, ^{131}I-labelled adosterol accumulated in this tumor. These observations were sufficient to consider that this tumor was an ectopic adrenal gland. Moreover, this ectopic lesion was an endocrinologically functional adrenal gland that was responsible for ACTH-independent Cushing's syndrome because tumor resection induced the resolution of hypercortisolaemia and led to adrenal failure. Previous reports describing ectopic adrenal tumors that secrete adrenocortical hormones are summarized in Table 1 (3, 5, 7–19). Among the fifteen previous cases, these tumors were most commonly found in those aged less than 40 years old (11 cases), and the majority produced cortisol (14 cases) and were classified as adenoma or hyperplasia (10 cases). The most common tumor site was around a kidney, such as the renal hilum (6 cases), and all these tumors were benign. The histopathological characteristics and steroidogenic enzyme profile of ectopic cortisol-producing adrenocortical adenomas are similar to those of monotopic adrenocortical adenomas and those in our case (16). A review of the literature supported our diagnosis of a functional ectopic adrenal tumor at the renal hilum that caused ACTH-independent Cushing's syndrome.

The most crucial and novel finding is that we detected for the first time the *GNAS* mutation, encoding the Gsα subunit that mediates GPCR signalling, from this endocrinologically functional ectopic tumor. *GNAS* mutation causes the constitutive activation of adenylyl cyclase, which activates cAMP-dependent protein kinase A (PKA), leading to the hyperproduction of cortisol through the acceleration of the cAMP response element-binding protein CREB (20). Indeed, the *GNAS* mutation was detected in 16.9% out of 65 cases of

TABLE 1 Reported cases of Cushing's syndrome due to ectopic adrenal tissue.

No.	Publicationyear	Age/ Sex	Size of tumor	Location of tumor	Hormonal levels (before treatment)	Histology	Immuno-stainingfindings	mRNA expression	Genetic mutation	Remarks
1	1963 (7)	40/M	7 cm	inferior pole of the left kidney	plasma 17-OHCS 10.1 μg/dL	hyperplasia	NA	NA	NA	2 years after bilateral adrenalectomy No recurrence (but died of another disease 17 months later)
2	1966 (8)	59/M	7.5 cm	above the upper pole of the right kidney	urinary 17-OHCS 29 mg/day urinary 17-KS 5 mg/day	carcinoma	NA	NA	NA	4 months after bilateral adrenalectomy
3	1969 (9)	26/F	15 cm	upper pole of the left kidney	17-KS 159 mg/day	carcinoma	NA	NA	NA	No recurrence for 15 months
4	1972 (10)	34/F	1.5 cm and 1.2 cm	each para-ovarian area	Cor 15 μg/dL	adrenocortical tissue	NA	NA	NA	After bilateral adrenalectomy and external irradiation of the pituitary gland
5	1981 (11)	23/F	18 cm	liver	Cor 26 μg/dL	(probably) malignant	Cor (+) testosterone (+)	NA	NA	Died of a pulmonary embolism before treatment
6	1985 (12)	21/F	12 cm	liver	Cor 41 μg/dL UFC 1627 μg/day	carcinoma with low malignancy potential (difficult to determine whether this tumor was benign or malignant)	NA	NA	NA	No recurrence for 9 months Resection after ketoconazole therapy
7	1998 (5)	33/F	3.0 cm	adjacent to the left adrenal gland	Cor 14 μg/dL ACTH 6.1 pg/mL UFC 78 μg/day	adenoma	NA	NA	NA	14 years after left adrenalectomy
8	2000 (13)	63/F	3.5 cm	left renal hilum	Cor 25 μg/dL (after 1 mg Dex) ACTH < 1.0 pg/mL UFC 130 μg/day (after 2 mg Dex)	adenoma	NA	NA	NA	No recurrence for 9 months
9	2010 (14)	35/F	3.8 cm	left renal hilum	Cor 25 μg/dL ACTH < 5.0 pg/mL UFC 645 μg/day	adenoma	CYP17A1 (+) CYP21 (+)	CYB11B1 (+) (higher than normal adrenal level)	*PRKAR1A* (-) *PDE8B* (-) *PDE11A* (-)	3 months after bilateral adrenalectomy
10	2012 (15)	38/M	4.0 cm	left renal hilum	Cor 27 μg/dL ACTH < 1.0 pg/mL	adenoma	NA	NA	NA	No recurrence

(Continued)

TABLE 1 Continued

No.	Publicationyear	Age/ Sex	Size of tumor	Location of tumor	Hormonal levels (before treatment)	Histology	Immuno-stainingfindings	mRNA expression	Genetic mutation	Remarks
					UFC 1927 μg/ day					
11	2014 (16)	53/F	1) 3.5 cm 2) 2.7 cm	1, 2) left renal hilum	1) NA 2) Cor 18 μg/dL ACTH < 5.0 pg/mL UFC 159 μg/ day	1, 2) adenoma	1) NA 2) Melan-A (+) HSD3B2 (+) CYP17A1 (+)	1) NA 2) CYP11B1 (+) CYP11B2 (+) CYP17A1 (+) HSD3B2 (+) (similar to adrenal CPA)	1, 2) NA	1) First diagnosis 2) 2 years after first surgery, recurrence (+)
12	2016 (17)	37/F	3.4 cm	right renal sinus	NA (Cor was higher than normal)	adenoma	synaptophysin (+) CD56 (+), vimentin (+) Ki-67 (2%) calretinin (+) inhibin-α (+) chromogranin A (-) CD117 (-), CD10 (-) CK7 (-), EMA (-) CK-pan (-), melan-A (-) pax-8(±)	NA	NA	No recurrence for a month
13	2018 (3)	18/F	3.0 cm	left renal hilum	Cor 21 μg/dL ACTH 1.3 pg/ mL UFC 1824 μg/ day	adenoma	inhibition (+) melan-A (+) synaptophysin (+) vimentin (+) AE1/AE3 (+) HMB45 (±) CD34 (+, angiographic) NSE (-), CgA (-)	NA	NA	No recurrence for 12 months
14	2018 (18)	46/M	3.6 cm	right renal hilum	Cor 37 μg/dL ACTH < 1.0 pg/mL UFC 159 μg/ day	adenoma and myelolipoma metaplasia	KI-67 (3%)	NA	NA	No recurrence for 6 months
15	2018 (19)	21/F	16 cm (CT scan)	liver	Cor 27 μg/dL	carcinoma	CD 56 (+), HEP 1 (+) NSE (+) (liver biopsy)	NA	NA	Referred to another hospital for surgical therapy,
16	our case	29/F	4.0 cm	right renal hilum	Cor 26.8 μg/ dL, ACTH 3.4 pg/mL, UFC 716 μg/day	cor-producing lesion (benign)	StAR (+) CYP11A1 (+) CYP17A (+) HSD3B (+) CYP21A (+) CYP11B1 (+) SF-1 (+) CYP11B2 (-)	NA	*GNAS* (+) c.601C>T, p.Arg201Cys	presence of fibrous dysplasia

NA, not available.
17-OHCS, 17-hydroxycorticosteroid; 17-KS, 17-ketosteroid; Cor, cortisol; UFC, urinary free cortisol; ACTH, adrenocorticotropic hormone; Dex, dexamethasone; CPA, cortisol-producing adrenocortical adenoma.

ACTH-independent Cushing's syndrome (21), suggesting that this mutation is one of the causal molecular pathogeneses for cortisol-producing adrenocortical adenoma. We considered that residual adrenal tissue, which possessed the *GNAS* mutation occurring in early development, autonomously secreted excessive cortisol through an increase in cAMP due to the constitutive activation of Gsα, resulting in ACTH-independent Cushing's syndrome in this patient.

One of the more recognized diseases associated with *GNAS* mutation is MAS. MAS is classically defined as a clinical triad

involving fibrous dysplasia of bone, café-au-lait spots, and precocious puberty (2). MAS is often diagnosed according to the presence of two or more of these typical features. However, it is now recognized that those phenotypes are more complex. The prevalence of major findings, fibrous dysplasia of bone, café-au-lait spots, and precocious puberty (female) were 98%, 66%, and 50%, respectively (22), suggesting that patients with MAS who have typical triad are not frequent. If patients have only fibrous dysplasia, which is the most common feature in MAS (23), identification of *GNAS* mutation by genetic testing is needed to establish the diagnosis (24). This suggests that the detection of this underlying genetic pathogenesis along with MAS-related symptoms is essential in establishing a definitive diagnosis of MAS. The Gsα protein, which is encoded by the *GNAS* gene, is a ubiquitous cellular component. If Gsα mutation occurs in the GPCR signalling pathway of the endocrine system, such as in LH, TSH, GnRH, ACTH, or bone tissue, it causes constant activation of intracellular signal transduction and results in precocious puberty, thyrotoxicosis, ACTH-independent Cushing's syndrome, acromegaly, or FGF23-related hypophosphatemic rickets/osteomalacia (2). Such hypersecretion from endocrine organs with GPCRs is known to be associated with MAS (22). In this case, our patient did not have a café-au-lait spot and did not suffer from precocious puberty. However, we detected ACTH-independent hypercortisolaemia accompanied by *GNAS* gene mutation in the extra-adrenal tumor as well as fibrous dysplasia with typical findings on CT and bone scintigraphy. MAS is the most probable single aetiology that accounts for the observed findings in both the bone and ectopic adrenal glands.

Hypopituitarism, which was observed in this patient, causing conditions such as amenorrhea and hypothyroidism is not a typical clinical feature of MAS. Elevated cortisol is known to act on the hypothalamus and reduce the basal gonadotropins level, resulting in hypogonadotropic hypogonadism. Hypercortisolism suppresses TRH and TSH release, leading to central hypothyroidism. Hypopituitarism in the patient spontaneously recovered after the removal of the renal hilum tumor, indicating that hyposecretion of anterior pituitary hormones, except for ACTH, was caused by secondary hypopituitarism, not primary hypopituitarism.

This is the first report of an ectopic endocrinologically functional adrenal tumor due to a *GNAS* heterozygous mutation causing ACTH-independent Cushing's syndrome. The patient in this case had concomitant typical fibrous dysplasia; thus, we supposed that the clinical symptoms were caused by MAS. *GNAS*-related GPCRs are widely distributed in the body; thus, the clinical symptoms of MAS caused by the somatic mosaic phenotype vary widely. This case highlights that *GNAS* is associated with a previously unknown pathological mechanism in which inhibition of the natural elimination of remnant tissue leads to ectopic endocrine hypersecretion.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Ethics statement

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

KT wrote the first draft of the manuscript. MY contributed to the writing of the manuscript. KT, ST, SI, and TT made contributions to the acquisition of the clinical data. MY and KK made critical revisions. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Getting pregnant with congenital adrenal hyperplasia: Assisted reproduction and pregnancy complications. A systematic review and meta-analysis

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Many patients with congenital adrenal hyperplasia (CAH) refrain from seeking pregnancy, suffer from infertility or worry about pregnancy complications, mainly due to genitalia abnormalities, anovulation, unreceptive endometrium and metabolic disturbances. Despite those challenges, many live births have been reported. In this systematic review, we focused on the key to successful assisted reproduction strategies and the potential pregnancy complications. We did a systematic literature search of Pubmed, Medline and Scopus for articles reporting successful pregnancies in CAH other than 21-hydroxylase deficiency, and found 25 studies reporting 39 pregnancies covering deficiency in steroidogenic acute regulatory protein, 17α-hydroxylase/17,20-lyase, 11β-hydroxylase, P450 oxidoreductase, cytochrome b5 and 3β-hydroxysteroid dehydrogenase. We summarized various clinical manifestations and tailored reproduction strategy for each subtype. Furthermore, a meta-analysis was performed to evaluate the pregnancy complications of CAH patients. A total of 19 cross-sectional or cohort studies involving 1311 pregnancies of classic and non-classic CAH patients were included. Surprisingly, as high as 5.5% (95% CI 2.3%-9.7%) of pregnancies were electively aborted, and the risk was significantly higher in those studies with a larger proportion of classic CAH than those with only non-classical patients (8.43% (4.1%-13.81%) VS 3.75% (1.2%-7.49%)), which called for better family planning. Pooled incidence of miscarriage was 18.2% (13.4%-23.4%) with a relative risk (RR) of 1.86 (1.27-2.72) compared to control. Glucocorticoid treatment in non-classical CAH patients significantly lowered the miscarriage rate when compared to the untreated group (RR 0.25 (0.13-0.47)). CAH patients were also more susceptible to gestational diabetes mellitus, with a prevalence of 7.3% (2.4%-14.1%) and a RR 2.57 (1.29-5.12). However, risks of preeclampsia, preterm birth and small for gestational age were not significantly different. 67.8% (50.8%-86.9%) CAH

patients underwent Cesarean delivery, 3.86 (1.66-8.97) times the risk of the control group. These results showed that fertility is possible for CAH patients but special care was necessary when planning, seeking and during pregnancy.

Systematic Review Registration: PROSPERO https://www.crd.york.ac.uk/PROSPERO/display_record.php?RecordID=342642, CRD42022342642.

KEYWORDS

congenital adrenal hyperplasia (CAH), assisted reproduction technology (ART), pregnancy complication, meta-analysis, systematic review, miscarriage, abortion (induced), glucocorticoid therapy

1 Introduction

Congenital adrenal hyperplasia (CAH) is a family of autosomal recessive diseases caused by defects of enzymes in adrenal steroidogenesis, which is the most frequent monogenic disorder affecting sexual development and fertility (1). The different types and remaining activity of the mutant enzyme lead to a spectrum of clinical presentations, including the salt-wasting form (SW), the simple virilizing form (SV), and the non-classical form (NC). CAH may affect both male and female fertility. The main cause of male infertility in CAH is testicular adrenal rest tumors (TARTs) (2, 3), which are benign, bilateral tumors in rete testis developed under the trophic effects of chronic adrenocorticotropic hormone (ACTH) elevation, compressing the seminiferous tubules (4). In our study, we focused on fertility in female CAH patients. A series of obstacles lie in patients' attempts at pregnancy. Classical CAH women might have malformations of external genital organs such as labial fusion and clitoral hypertrophy, which render sexual intercourse unpleasant or prohibitive even after corrective surgery. While non-classical patients might be asymptomatic during childhood, persistently elevated progestogens could lead to anovulation, unreceptive endometrium, and unfavorable cervical mucus, resulting in irregular menses and infertility (5).

Since the first live birth was achieved by a patient with 21-hydroxylase deficiency (21OHD) in 1956 (6), the fertility rate has greatly improved over the past 60 years. In the 1980s, only half of 80 classical 21OHD women reported adequate vaginal introitus to be heterosexually active, among whom 15 gave birth (7). However, in the 2020s, the fertility rates of simple virilizing (41.8%) and non-classical (40.8%) patients were greatly improved to be comparable to those of the common population (45.8%) in Sweden, although only 8.1% of salt-wasting patients had biological children (8). Fertility treatment for 21OHD patients has been summarized (5). For patients with rarer types of CAH other than 21OHD, recent advances in genetics and assisted reproductive technology (ART) aided their diagnosis and fertility, who presented with a drastically different clinical picture and required tailored fertility treatment.

Although many pregnancies went uneventful, clinicians and patients worried about the risk of pregnancy complications due to significantly higher incidence of obesity, hypertension, and insulin resistance before pregnancy and corticoid supplementation during pregnancy (9). Several studies have reported an increased risk of gestational diabetes mellitus (GDM), small for gestational age (SGA) (10), and cesarean delivery (8), while others report uneventful pregnancies. Also, some studies have recommended glucocorticoid use in the non-classical type of CAH to lower the miscarriage rate (11, 12), but the study by Eyal et al. (13) suggested that glucocorticoid treatment made no difference.

In this systematic review and meta-analysis, we aimed to summarize the ART use in female patients with rare types of CAH based on case reports of successful live births. Furthermore, we performed a series of meta-analyses to evaluate the prevalence of pregnancy complications in CAH, including miscarriage, elective abortion, GDM, preeclampsia, preterm birth, SGA, and cesarean delivery. We then calculated the relative risk of pregnancy complications in CAH patients compared to the general population. We further compared the effect of glucocorticoid treatment on preventing miscarriage in the non-classical type of CAH.

2 Methods

2.1 Search strategy and selection criteria

PubMed, Medline, Scopus, and forward and backward citations were searched to identify studies between database inception and 1 June 2022. Search terms are listed in Appendix 1, and the language was restricted to English. A total of 723 titles and abstracts were screened after the removal of duplicates (Figure 1). For pregnancies in CAH other than 21OHD, the inclusion criteria were case reports

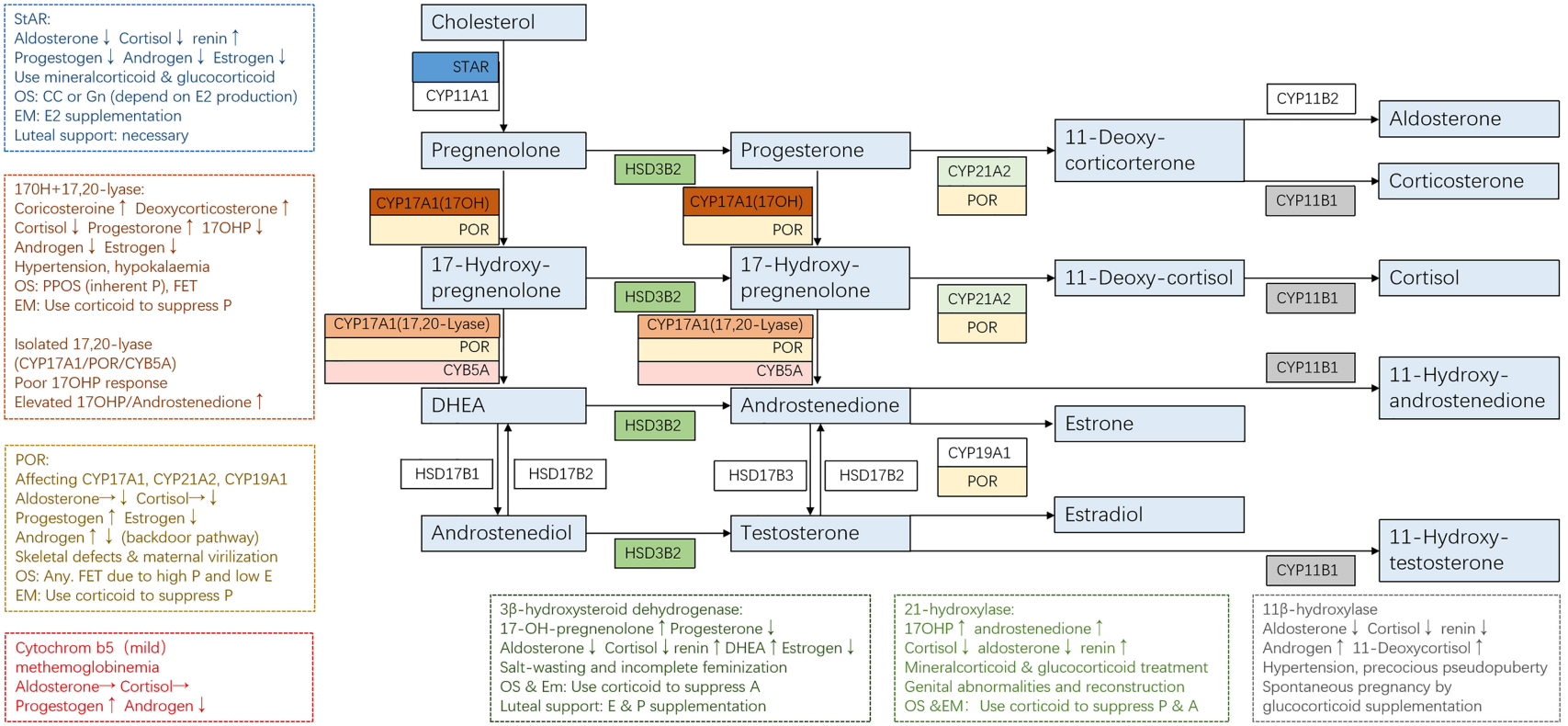

FIGURE 1
PRISMA flowchart of literature search and selection. PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses.

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with clinical pregnancy achieved, and four studies reporting attempts without success (14–18) and one written in French (19) were excluded. For pregnancy complications in CAH, the inclusion criteria were cross-sectional, case–control, or cohort in design with pregnancy outcomes reported (n = 21). One paper without detailed pregnancy outcomes (20) and five case series with less than 10 pregnancies (21–25) were excluded for fear that sampling bias would be dramatic considering the occurrence of common complications. This study followed Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (26) with a checklist in Appendix 2 and was registered on https://www.crd.york.ac.uk/PROSPERO/display_record.php?RecordID=342642 PROSPERO (CRD42022342642).

2.2 Quality assessment and data extraction

The criteria for risk of bias assessment were adapted from the Hoy tool (27), with a maximum score of 8 for prevalence studies and a maximum score of 10 for prevalence and risk studies. Quality assessment (Appendix 3) and data extraction were independently done by two reviewers and cross-checked. Discrepancies were resolved through discussion with the other authors. For studies reporting pregnancy complications, the primary outcomes of interest were miscarriage, elective abortion, GDM, preeclampsia, preterm birth, SGA, and C-section. When calculating the rate of miscarriage and elective abortion, the denominator was total clinical pregnancies. However, the rates of other complications were calculated within ongoing pregnancies. Incomplete follow-up of the patients was excluded from the relevant analyses.

2.3 Data analysis

Given that there are 0% and 100% in the prevalence of pregnancy complications, prevalence rates were calculated from raw proportions after the Freeman–Tukey double-arcsine transformation (28), and the Shapiro test showed normal distribution. The inverse variance method was used for pooling based on a random-effects model (29). When a control group without adrenal insufficiency was provided, a crude odds ratio (OR) and 95% CI were calculated using the Mantel–Haenszel method based on a random-effects model. If there was a cell count of zero, 0.5 is added to each cell frequency to correct for continuity. I^2 was used to estimate heterogeneity, and an I^2 value > 50% indicated significant heterogeneity. Subgroup analyses were performed according to non-classical or assorted types of patients. Egger's test was used to assess potential publication bias whenever the number of studies was sufficient, with $p < 0.1$ indicating significance. Analyses and forest plots were done with R (4.2.0).

3 Results

3.1 Assisted reproductive technology for various types of congenital adrenal hyperplasia

The clinical manifestation of CAH might vary significantly depending on the mutations and adherence to treatment. For some non-classical patients, spontaneous pregnancy could be achieved simply by optimizing glucocorticoid and mineralocorticoid therapy (13). On the contrary, classical patients usually present a tricky situation where genetic diagnosis helped to give us a clear understanding of their underlying pathophysiology and was essential to developing an appropriate therapeutic strategy for better follicular, endometrium, and corpus luteum development (Figure 2 and Table 1).

3.1.1 Steroidogenic acute regulatory protein

Steroidogenic acute regulatory protein (StAR) accounts for about 86% of the transfer of cholesterol from the outer to inner mitochondrial membrane, where it is converted to pregnenolone after the cleavage of the side chain by P450 side-chain cleavage enzyme (P450scc, encoded by gene *CYP11A1*) (30). This is the initial and rate-limiting step in steroidogenesis. Therefore, mutated StAR impedes steroidogenesis and accumulates cholesterol, causing lipoid CAH, which is the most severe form of steroidogenesis and is characterized by the near absence of all steroids, high basal ACTH, and plasma renin activity, and grossly enlarged adrenals stacked with cholesterol and cholesterol esters (31).

StAR is expressed in the gonads and adrenal glands but not in the placenta, so affected 46,XX individuals will manifest at birth with glucocorticoid and mineralocorticoid deficiency and puberty sex steroid production problems. Although the steroidogenic pathway is affected, germ cell migration and maturation are theoretically normal. According to the reported cases (32–35), female lipoid CAH patients with glucocorticoid and mineralocorticoid substitution enter puberty normally because a low level of estrogen produced by the StAR-independent pathway is enough to support secondary sex characters and menarche. However, the higher demands for estrogen necessary for early follicular development, the positive feedback of luteinizing hormone (LH) surge, and endometrium growth cannot be met. Anovulation, high LH/follicle-stimulating hormone (LH/FSH) ratio, and ovarian cysts may resemble polycystic ovary syndrome (PCOS), but low testosterone should raise attention. Ovulation induction is

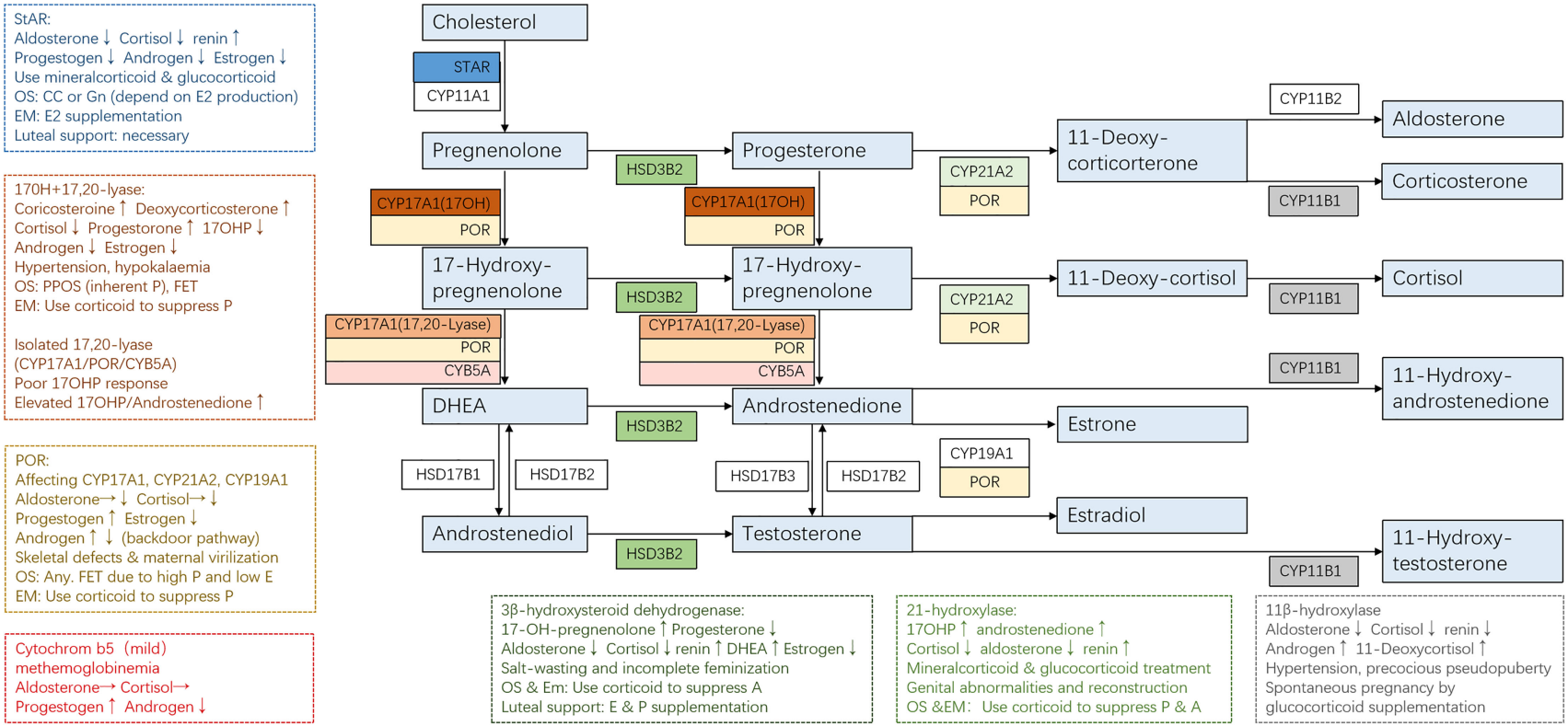

FIGURE 2
Adrenal steroidogenesis and ART use in different CAH subtypes. Clinical manifestations and corresponding protocols are summarized from case reports of pregnancies and are listed in dashed borders, but the actual situation varies from person to person. OS, ovarian stimulation; CC, clomiphene; Gn, gonadotropin; PPOS, progestin-primed ovarian stimulation; EM, endometrium preparation; FET, frozen embryo transfer.

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TABLE 1 Pregnancies in CAH other than 21-hydroxylase deficiency.

Gene	Author	Year	Mutation	Type	External genitalia	Menses	Other	Ovarian stimulation	Trigger	Embryo transfer	Corticoid	Endometrial preparation	Luteal phase	Pregnancy complications
StAR	Khoury	2009	Homo p.L275P	C	Female	Regular, without ovulation	/	CC	No	/	No	No	No	Miscarriage at 6 weeks
							/	CC	No	/	No	No	P	Quadruple pregnancy with 1 naturally lost at 7 weeks and 1 feticide at 8 weeks; gestational hypertension; preterm birth
							/	CC	No	/	No	No	P	Preterm birth
	Sertedaki	2009	Homo p.K236delfs*43	C	Female	Irregular	Ovarian cyst	Luteal phase GnRH-a	hCG	Fresh	PDS	E2	E2+P	Husband -/K236delfs*43, chorionic villus biopsy after PGT, C-section
	Albarel	2016	Homo p.T240Sfs*81	C	Female	Amenorrhea	Obese	GnRH-a	hCG	FET	HCT+FC	E2	E2+P	Miscarriage at 8 weeks
								/	/	FET	HCT+FC	E2	E2+P	No
	Hatabu	2019	p.Q258*/ p.R272C	NC	Female	Regular	/	No	No	/	No	No	No	C-section
							/	No	No	/	No	No	No	C-section
			p.Q258*/ p.M225T	C	Female	Irregular	Ovarian cyst	CC	No	/	NR	No	No	C-section
CYP17A1 (17OH+ 17, 20-lyase)	Ben-Nun	1995	NR	C	Infantile	Amenorrhea	BP↑, K↓	Donated oocyte	/	FET	DXM	Estradiol implants	E2+P	Twin pregnancy, HELLP syndrome, C-section, preterm birth, only one survived
	Levran	2003	NR	NC	Female	Irregular	/	GnRHa	hCG	FET	DXM	GnRHa-HRT	E2+P	Triplet live birth
	Bianchi	2016	p.W406R/ p.P428L	NC	Female	Amenorrhea	BP↑	GnRHa	hCG	FET	DXM	GnRHa-HRT	E2+P	C-section at 30 weeks due to acute fetal distress and a true umbilical knot
	Yang	2017	Homo p.V236G	C	Infantile	Amenorrhea	BP↑, ovarian cyst	Inherent P +HMG	hCG	FET	DXM	E2	E2+P	HELLP syndrome, C-section, preterm birth
	Falhammar	2018	Homo exon 1-6 deletion	C	Female	Amenorrhea	BP↑, K↓, obese	NR	NR	NR	PDS	NR	NR	C-section
	Kitajima	2018	Homo p.S54del	NC	Female	Amenorrhea	BP↑, ovarian cyst	GnRHa	hCG	FET	DXM	E2	E2+P	No
										FET	DXM	E2	E2+P	Massive intrapartum hemorrhage due to placenta accreta
	Xu	2022	Homo p. R496C	NC	Female	Amenorrhea	BP↑	PPOS	hCG	FET	DXM	GnRHa-HRT	E2+P	No
			p. I332T/p. D487_F489del	NC	Female	Irregular	Ovarian cyst	inherent P+CC +HMG	hCG+ GnRH-a	FET	DXM	E2	E2+P	Cleft lip and palate, termination
CYP17A1 (17, 20-lyase)	Blumenfeld	2021	Homo p.E305G	NC	Female	Irregular	Ovarian cyst	GnRHa	hCG	FET	PRED	GnRHa-HRT	E2+P	Miscarriage
								/	/	FET	PRED	GnRHa-HRT	E2+P	No

(Continued)

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TABLE 1 Continued

Gene	Author	Year	Mutation	Type	External genitalia	Menses	Other	Ovarian stimulation	Trigger	Embryo transfer	Corticoid	Endometrial preparation	Luteal phase	Pregnancy complications
POR	Song	2018	Homo p.Y326D	NC	Female, vaginal atrasia	Irregular	Unicornuate uterus, ovarian cyst	LE+HMG	NR	FET	DXM	E2	E2+P	No
	Papadakis	2020	c.1249-1G>C/ c.1324C>T	NC	Female	Irregular	Ovarian cyst	GnRH-a	hCG	FET	HCT	NR	E2+P	Twin pregnancy
			p.Gln609*/ p.W620S	NC	Female	Irregular	Ovarian cyst	GnRH-antagonist	hCG	FET	DXM	NR	E2+P	Preeclampsia, C-section
	Zhang	2020	IVS14-1G>C/ p.V603_Q606del	NC	Aberrant	Irregular	Ovarian cyst	luteal phase GnRH-a	hCG	FET	PDS	E2	E2+P	Twin pregnancy, chronic hypertension in pregnancy, preterm, C-section
	Pan	2021	p.R457H/ p.P399_E401del	NC	Female	Amenorrhea	Ovarian cyst, mild skeletal malformation	GnRH-a	hCG	FET	DXM	E2	E2+P	Twin pregnancy, C-section
CYB5A	Leung	2020	Homo Y35*	NC	Female	Regular	Methemoglobinemia	No	No	/	No	No	No	No
HSD3B2	Rojansky	1991	NR	NC	Female	Irregular	Hirsutism, obese	HMG	hCG	FET	DXM	CC	NR	No
CYP11B1	Toaff	1975	NR	NC	Female	Regular	/	No	No	/	No	No	No	No
								No	No	/	DXM	No	No	No
	Simm	2007	DS+2/p.G444D	C	Aberrant	Irregular	Insulin resistance	CC	NR	/	DXM	NR	NR	Pregnancy-induced hypertension
	Parajes	2010	Homo p.P159L	NC	Female	Regular	Hirsutism	No	No	/	PDS	No	No	4 uncomplicated pregnancies
	Menabo	2014	p.R143W/ p.A306V	NC	Female	NR	Hirsutism	No	No	/	PDS	No	No	NR
	Mooij	2015	Homo p.R143W	NC	Female	Irregular	Hirsutism	NR	NR	/	PDS	NR	NR	Twin pregnancy, miscarriage at 17 weeks
								NR	NR	/	PDS	NR	NR	4 uncomplicated pregnancies
	Zacharieva	2019	p.D480Tfs*2/ p.V316M	NC	Female	Regular	Hirsutism, BP↑	No	No	/	DXM	No	No	Chronic hypertension, C-section
								No	No	/	DXM	No	No	Elective abortion
	Krishnan	2021	NR	C	Aberrant	Regular	Hirsutism, BP↑	No	No	/	PDS	No	No	Preeclampsia, C-section, preterm birth

NC, non-classical; C, classical; NR, not reported; CC, clomiphene, LE, letrozole; HCT, hydrocortisone; PDS, prednisolone; DXM, dexamethasone; FC, fludrocortisone.
*, nonsense mutation.

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necessary when the patient has irregular menses. A human chorionic gonadotropin (hCG) stimulation or a clomiphene test might be performed to see the capacity for estrogen production, with spontaneous puberty and regular menses being signs of responsiveness. If the patient is responsive, clomiphene might be used to induce ovulation. If the patient failed to produce enough estradiol for endometrium proliferation after clomiphene (CC), extra estrogen administration is beneficial, and ART is recommended. Progesterone supplementation is necessary for luteal support, and spontaneous abortion occurred in one patient without progesterone supplementation. Luteal support should be sustained until placental function takes over.

Unlike StAR, P450scc is present in all steroidogenic tissues including the placenta. Considering that progesterone produced by the placenta was necessary for preventing miscarriage since the second trimester, few fetuses with P450scc mutations reached term gestation. Most reported cases were in 46,XY patients with complete sex reversal with adrenal insufficiency (36). Non-classic P450scc deficiency resembles non-classic lipoid CAH in terms of hormonal presentations. However, all patients with P450scc deficiency have been reported to have normal-sized or small adrenals, in contrast to the massive adrenal enlargement in lipoid CAH. No pregnancy has been achieved in patients with P450scc deficiency.

3.1.2 CYP17A1

17α-Hydroxylase (17OH) and 17,20-lyase (17,20-desmolase) are considered two separate functions of the same enzyme P450c17 encoded by gene *CYP17A1*, the function of which depends on the local factors (37, 38). Therefore, mutations in CYP17A1 could cause three different forms of enzymatic deficiency: 1) combined deficiencies of the two functions, which is the most common form, 2) isolated 17OH deficiency, and 3) isolated 17,20-lyase deficiency. Patients with double deficiency (39–45) suffer from hypertension and impaired glucocorticoid production. Low estradiol levels might lead to infantile genitalia. High levels of progesterone inhibit the GnRH/LH pulse frequency and result in amenorrhea, unreceptive endometrium, and ovarian cysts. To date, no spontaneous pregnancy has been reported in women with 17OHD. Live birth has only been achieved by ART in 17OHD due to embryo–endometrium asynchrony under high progesterone. As for ovarian stimulation, the inherently high progesterone levels render the protocol a progestin-primed one in essence. Frozen embryo transfer or the "freeze all" strategy is a great advantage in the high P situation. Corticoid administration rather than the gonadotropin-releasing hormone agonist (GnRHa) is the key to suppressing P and ensuring endometrium proliferation and the proper timing for embryo implantation.

It is noteworthy that the activities of both 17α-hydroxylase and 17,20-lyase are dependent on the availability of cytochrome P450 oxidoreductase (POR), which is the obligatory electron transfer flavoprotein. Other flavoproteins can partially substitute POR for the 17-hydroxylase activity but not the 17,20-lyase activity, so 17,20-lyase is particularly vulnerable to the abundance and function of POR (46). In addition, the optimal 17,20-lyase reaction requires the facilitation of cofactor protein cytochrome b5, which stimulates the rate of the reaction to over 10-fold (47). Therefore, isolated 17,20-lyase deficiency is a syndrome, which may be caused by specific mutations in the CYP17A1 (p.R347H, p.R347C, p.R358Q, and p.E305G), POR (p.G539R), and CYB5A (p.W27X and p.H44L) (14). Isolated 17,20-lyase deficiency is characteristic of an elevated ratio of 17OHP to androstenedione and showed low cortisol levels under the stimulation of ACTH or 17OHP. Only one pregnancy has been reported in isolated 17,20-lyase deficiency (38), demonstrating persistent progesterone and low estrogen. The patient experienced three failed *in vitro* fertilization (IVF) cycles and retrieved 37 oocytes using the long GnRHa protocol for the fourth time. Due to the high serum progesterone concentration, all embryos were cryopreserved. Hormone replacement therapy was used to prepare the endometrium due to inherently low estrogen levels, along with prednisone 30 mg/day. Live birth was achieved after two cycles of embryo transfer.

3.1.3 POR

The P450 oxidoreductase (encoded by *POR* gene) transfers electrons from reduced nicotinamide adenine dinucleotide phosphate (NADPH) to all P450 enzymes, including P450c17 (17OH/17, 20-lyase), P450c21 (21-OH), and P450aro (aromatase). POR deficiency diverts steroids into the "backdoor pathway" of dihydrotestosterone biosynthesis. The extent to which various enzymes are affected depends on the specific mutations of *POR* gene, resulting in high clinical variability. Phenotypes of female patients include high levels of P (100%), pregnenolone (100%), 17OHP (96%), corticosterone (83%) and deoxycorticosterone (70%), adrenal insufficiency after ACTH stimulation (78%), skeletal malformations (84%), and ovarian cysts (39%) (48). Given that POR was expressed in the placenta, reduced activity of placental aromatase might lead to intrauterine androgen excess causing virilized genitalia in affected female individuals (78%) and maternal virilization during pregnancy (21%). Clinical manifestations might resemble both 21OHD (abnormal genitalia) and 17OHD (elevated progesterone levels and low estradiol levels). The unreceptive endometrium under high P rendered ART application and frozen embryo transfer mandatory. Ovarian stimulation protocol did not seem to affect oocyte quality despite the significantly low E2 levels. Just like 17OHD, frozen embryo transfer and corticoid supplementation to suppress P

during endometrium preparation were consistently utilized by all patients (49–52).

3.1.4 CYB5A

Cytochrome b5 serves as an allosteric cofactor favoring 17,20-lyase reaction. CYB5A mutation leads to an isolated and partial 17,20-lyase deficiency. An important feature in diagnosis is normal cortisol response but absent or blunted 17OHP response after ACTH stimulation. Patients manifest methemoglobinemia, with normal sexual development, regular menses, and spontaneous pregnancy (44).

3.1.5 HSD3B2

Type II 3β-hydroxysteroid dehydrogenase deficiency (3βHSDIID) impaired both adrenal and gonadal steroidogenesis. Patients have excess production of androgen precursors, which are converted to active androgens in the peripheral tissues by the normal type I 3βHSD. Clinical presentation might vary, ranging from severe neonatal salt-wasting with normal external genitalia and regular menses to complete dependence on estradiol therapy to undergo complete feminization and menses (53). Only one pregnancy has been reported with HSD3B2 mutation (54). The patient had normal genitalia and signs of hirsutism and obesity. She presented with increased 17-OH-pregnenolone and DHEAS with normal electrolytes and blood pressure. With dexamethasone treatment, ovarian stimulation with HMG and hCG went smoothly, and the pregnancy was uneventful after frozen embryo transfer, resulting in the delivery of a healthy full-term female infant.

3.1.6 CYP11B1

11β-hydroxylase (CYP11B1) converts 11-deoxy-cortisol to cortisol and converts androstenedione and testosterone to their 11-hydroxy forms. Therefore, 11OHD results in hyperandrogenism, glucocorticoid deficiency, and hyporeninemic hypertension due to elevated mineralocorticoid precursors. Nevertheless, the degree of hyperandrogenism did not correlate with the extent of mineralocorticoid excess. Hyperandrogenism in 11OHD may present with precocious pseudopuberty, characterized by accelerated growth during childhood and reduced final height. Androgen excess may also suppress later stages of follicular development and impair endometrial receptivity, despite that some individuals may have regular menses. Correct diagnosis of non-classical 11OHD was essential because immediate restoration of fertility and rapid normalization of the blood pressure could be achieved after the initiation of corticosteroid therapy (19, 55–61).

3.2 Pregnancy complications of congenital adrenal hyperplasia patients

Fourteen cross-sectional and five cohort studies were included (7, 8, 10–13, 62–74), reporting outcomes of 1,311 pregnancies of CAH patients. The study characteristics of included studies are listed in Table 2. The mean pregnancy age ranges from 23 to 31.8 years, and the mean body mass index ranges from 21.3 to 26.9 kg/m^2. Twelve studies consisted of mixed types of CAH, while seven studies focused on the non-classical type. Prevalence and relative risk of pregnancy complications are summarized in Figures 3 and 4, respectively. Sixteen studies reported miscarriage rate, rendering a pooled prevalence of 18.2% (95% CI 13.4%–23.4%) with a medium heterogeneity. Subgroup analysis did not show a significant difference in miscarriage rate between non-classical type and assorted type. Two studies provided the relative risk of miscarriage compared to the general population, and the pooled relative risk was 1.86 (1.27–2.72). The miscarriage rate of the glucocorticoid treatment group is significantly lowered than that of the untreated group in the non-classical type of CAH patients (RR 0.25 (0.13–0.47)), as shown in Figure 5. Another major reason for early termination of pregnancy is elective abortion, accounting for as high as 5.5% (2.3%–9.7%) of CAH pregnancies. Subgroup analysis revealed that the rate of elective abortion among studies of non-classical type was significantly lower than in studies of assorted types (3.75% (1.2%–7.49%) vs 8.43% (4.1%–13.81%), p = 0.026).

Within ongoing pregnancies, CAH patients were more susceptible to gestational diabetes mellitus, with a prevalence of 7.3% (2.4%–14.1%) and an RR of 2.57 (1.29–5.12). However, risks of preeclampsia, preterm birth, and small gestational age were not significantly different, with a prevalence of 2.5% (1.1%–4.4%), 5.7% (2.6%–9.7%), and 10.1% (3.2%–19.6%), respectively. Alarmingly, 67.8% (50.8%–86.9%) of CAH patients underwent cesarean delivery, 3.86 (1.66–8.97) times the risk of the control group. Despite the significant heterogeneity, bias assessment showed asymmetry in the funnel plot (Egger's test p = 0.40). Subgroup analysis according to the subtype of CAH was not appropriate for preeclampsia, GDM, SGA, and C-section with only 1 or 0 study focusing on the non-classical type, and subgroup analysis for preterm birth showed no significant difference.

4 Discussion

In this review, we systematically summarized the pregnancy case reports of CAH other than 21OHD and elaborated the customized fertility treatment for each subtype. To the best of our knowledge, there has been no such systematic review despite several reviews in this aspect (5, 53, 75, 76). In addition, we calculated the pooled prevalence and relative risk of pregnancy complications in CAH

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TABLE 2 Characteristics of studies included in the meta-analysis.

Study	Year	Study design	Country	N of pregnant patients (CAH subtypes)	N of pregnancies (CAH subtypes)	Age (years)	BMI (kg/m^2)	Pair of twins	Corticoid usage	Control group
Hirschberg et al.	2021	Cohort	Sweden	61 (26 SV + 8 SW + 16 NC + 11?)	108 (NR)	28.1 (4.9)	23.3 (3.5)	0	NR	Age-matched controls
Hagenfeldt et al.	2008	Cohort	Sweden	16 (9 SV + 2 SW + 3 NC + 2?)	31 (19 SV + 3 SW + 3 NC + 4?)	30	NR	0	Yes	Age-matched controls
Badeghiesh et al.	2020	Cohort	USA	NR	299 (NR)	23.1% > 35	7.7% obese	8	NR	General population
Remde et al.	2016	Cohort	Germany	12 (5 SV + 2 SW + 5 NC)	25 (6 SV + 3 SW + 16 NC)	NR	24	1	Yes	Autoimmune adrenalitis
Bothou et al.	2020	Cohort	8 countries	NR	32 (NR)	31.8 (6.1)	25.7 (4.6)	0	Yes	Addison disease or secondary adrenal insufficiency
Yu et al.	2012	Cross-sectional	China	8 (5 SV + 3 NC)	12 (6 SV + 6 NC)	31.3 (3.3)	NR	0	Yes	
Casteras et al.	2009	Cross-sectional	UK	21 (13 SV +8 SW)	34 (20 SV + 14 SW)	27.3 (5.4)	26.9 (6.1)	0	Yes	
Hoepffner et al.	2004	Cross-sectional	Germany	9 (4 SV + 5 SW)	11 (5 SV + 6 SW)	26.1 (3.3)	26.6 (3.9)	0	Yes	
Krone et al.	2001	Cross-sectional	Germany	18 (12 SV + 1 SW + 5 NC)	36 (24 SV + 3 SW + 9 NC)	27.9 (5.2)	23.7 (3.2)	0	Yes	
Jääskeläinen et al.	2000	Cross-sectional	Finland	9 (8 SV + 1 SW)	13 (12 SV + 1 SW)	30.6 (2.9)	25	0	Yes	
Mulaikal et al.	1987	Cross-sectional	USA	16 (15 SV + 1 SW)	26 (25 SV + 1 SW)	NR	NR	0	Yes	
Klingensmith et al.	1977	Cross-sectional	USA	10 (8 SV + 2 SW)	15 (13 SV + 2 SW)	26.3	NR	0	Yes	
Pan et al.	2021	Cross-sectional	China	NR	19 NC	29.9 (2.9)	22.1 (2.9)	0	Yes	
Jiang et al.	2019	Cross-sectional	China	20 NC	27 NC	30.8 (3.7)	21.3 (2.3)	0	Yes	
Kulshreshtha et al.	2008	Cross-sectional	India	5 NC	13 NC	23.0 (3.5)	NR	0	Yes	
Eyal et al.	2017	Cross-sectional	USA	72 NC	183 NC	30.7 (4.9)	24.4 (4.6)	5	Miscarriage: 6/43 of usage group vs 31/124 of non-usage group	
Bidet et al.	2010	Cross-sectional	France	85 NC	187 NC	26.7 (8.9)	24.0 (4.6)	3	Miscarriage: 5/77 of usage group vs 29/110 of non-usage group	
Moran et al.	2006	Cross-sectional	9 countries	104 NC	206 NC	29.7 (9.7)	NR	4	Miscarriage: 4/65 of usage group vs 35/138 of non-usage group	
Feldman et al.	1992	Cross-sectional	France	20 NC	37 NC	24.6 (5.2)	NR	0	Miscarriage: 0/19 of usage group vs 6/18 of non-usage group	

CAH type is categorized as the salt-wasting form (SW), the simple virilizing form (SV), and the non-classic form (NC).
?, unknown; NR, not reported.

patients for the first time, which responded to the worries of CAH patients and controversies of researchers.

Overall, the fertility rate of CAH patients has been greatly improved, from the common 21OHD to other rarer subtypes. This is a result of various factors, including the earlier diagnosis and better adherence to treatment; improved understanding of how estrogen, androgen, and progesterone affect ovulation and endometrium; and the wider application of ART. A cohort study showed that 14.7% of CAH women had children without ART and 2.4% with ART (20). There are several situations where ART use has prominent merits. The first and most common indication is anovulation, which may be secondary to high progesterone (as in 21OHD, 17OHD, and POR deficiency), high androgen (as in 11OHD), or low estrogen (as in StAR deficiency). These abnormalities disrupt the hypothalamus–pituitary–ovarian axis, leading to impaired follicular development and diminished LH surge. Some patients may return to regular ovulation after corticoid treatment, and ovulation induction is needed when ovulation fails to be restored. The second and most important indication is the

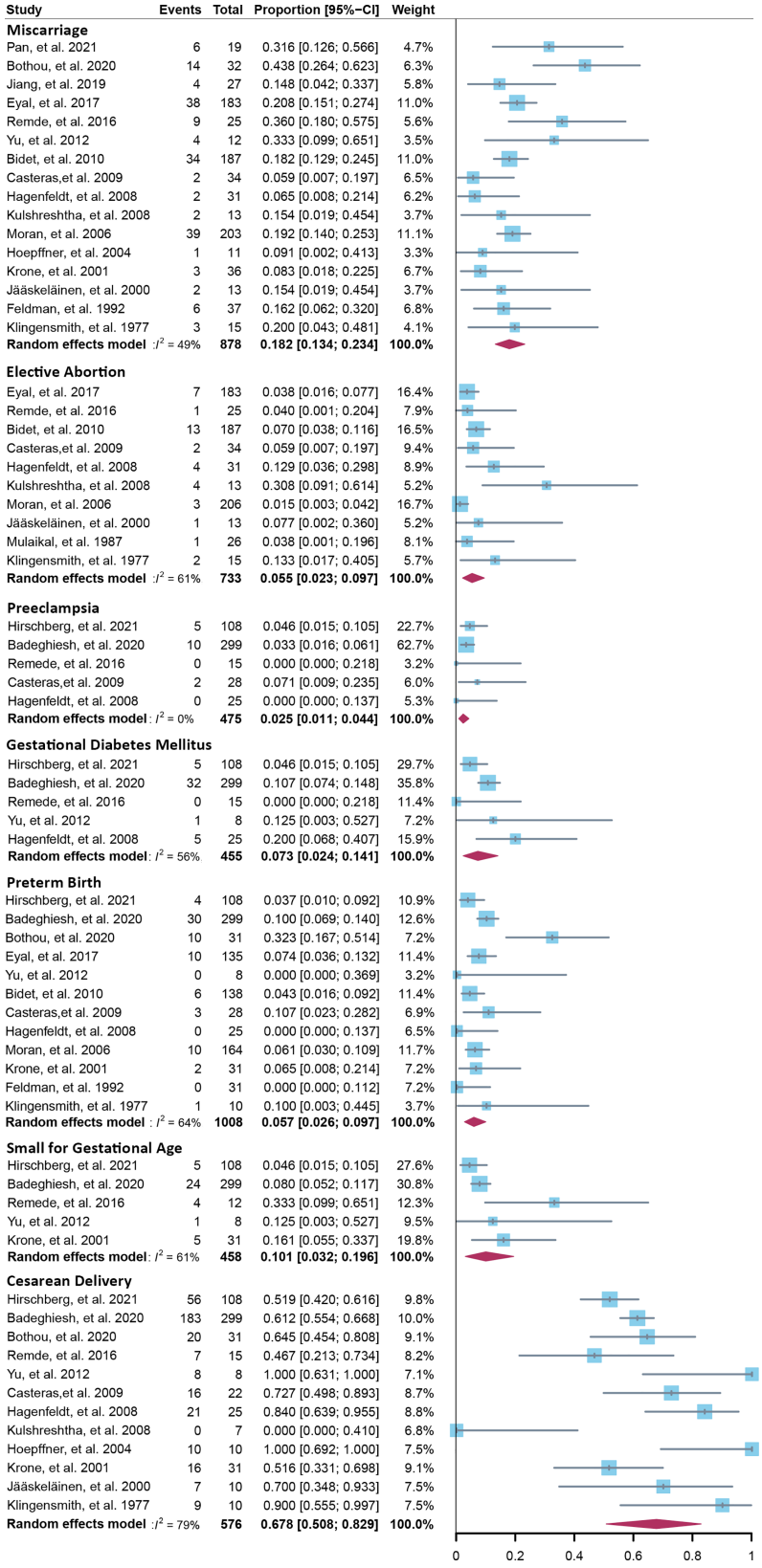

FIGURE 3
Prevalence of pregnancy complications in CAH patients. CAH, congenital adrenal hyperplasia.

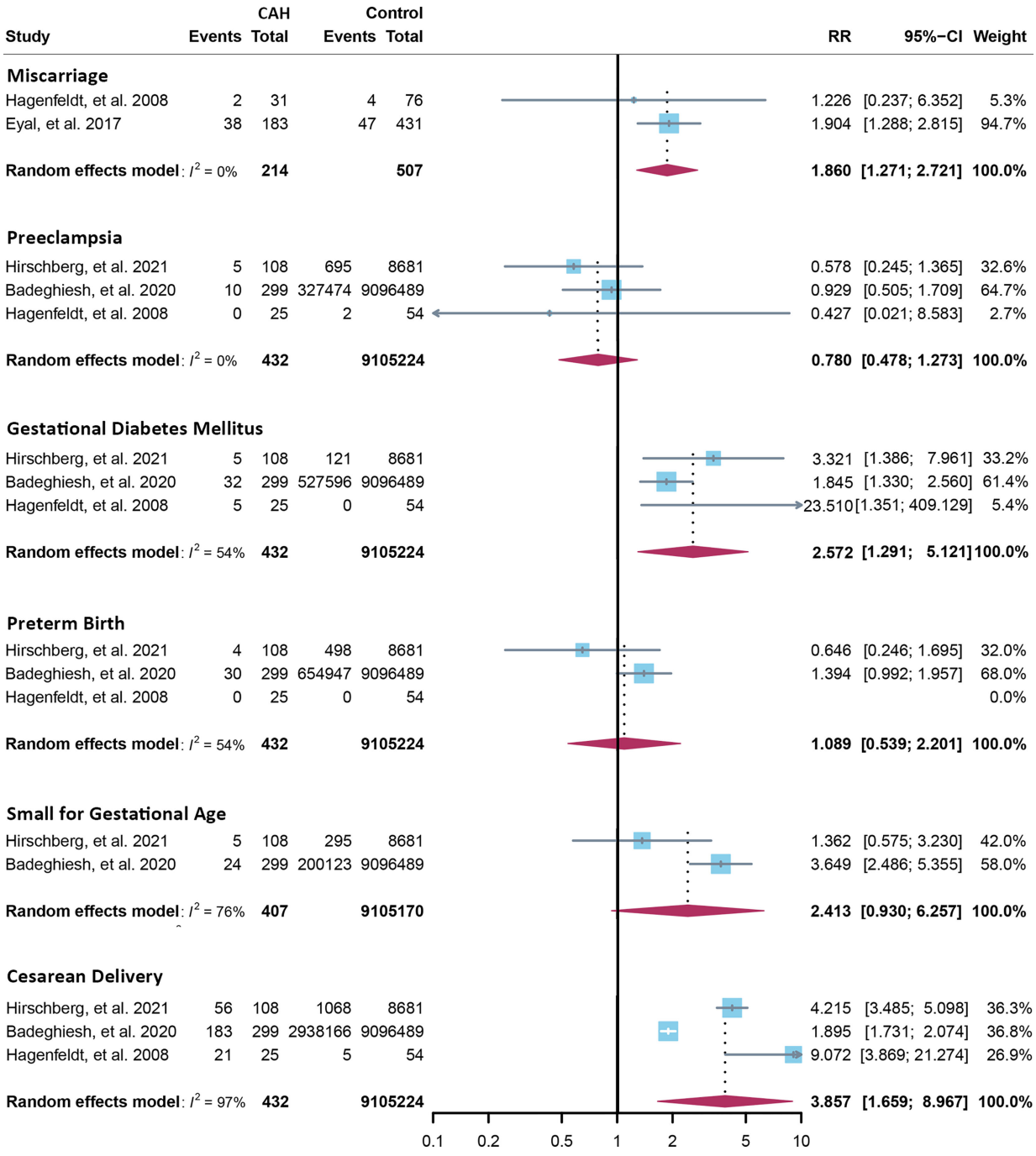

FIGURE 4
Relative risk of pregnancy complications in CAH. CAH, congenital adrenal hyperplasia.

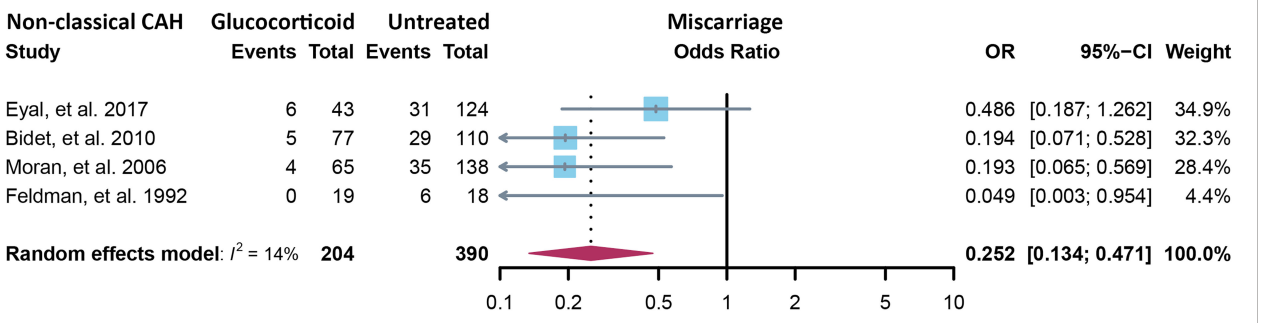

FIGURE 5
Relative risk of miscarriage in the non-classical form of CAH between glucocorticoid treatment and non-treatment. CAH, congenital adrenal hyperplasia.

detrimental effect of high progesterone on endometrium receptivity. When selecting the protocol for ovarian stimulation under high progesterone, the long GnRHa protocol was a popular choice in the hope of lowering progesterone, but the effect was somewhat limited. In fact, progesterone during the follicular phase did not affect oocyte quality and helped to prevent premature LH surges (77). With the strategy of frozen-embryo transfer, we could circumvent the adverse effect of progesterone on the endometrium. In endometrium preparation, the key lies in the suppression of endogenous progesterone to below 0.45 ng/ml by use of glucocorticoid (and mineralocorticoid when necessary) (72). The third indication is preimplantation genetic testing. Because of the autosomal recessive nature of CAH, genotyping the partner is recommended before pregnancy. If the husband was heterozygous for the same gene, preimplantation testing and thus ART were advisable (33).

The miscarriage rate of 18.2% was significantly elevated in CAH patients, as compared to 11.8% in the women receiving ART treatment (78) and 15.3% for the total population (79), but the reason remains unclear. About 48% of early pregnancy loss was due to chromosomal abnormalities, and advanced maternal age was an important determinant (80). In our study, the mean pregnancy age of all included studies was below 35, so advanced maternal age was not our prime suspect. No testing of the chorionic villi was ever reported in miscarriage cases of CAH patients, and it may be a direction for future research. We subgrouped the results of miscarriage rate by body mass index (BMI) (average BMI ≥25 or <25) and type of CAH (non-classical or assorted) and found no significant difference between subgroups. However, glucocorticoid treatment in the non-classical type of CAH patients significantly lowered the miscarriage rate (RR 0.25 (0.13–0.47)). Therefore, proper glucocorticoid treatment might be the key. Another possible reason for the increased miscarriage rate is insufficient luteal support, which is not uncommon in CAH patients (81).

Unexpectedly, the elective abortion rate reached 5.5% among CAH patients, which was higher than the global rate of 3.9% (82). The elective abortion rate was significantly higher in those studies with a larger proportion of classic CAH than those with only non-classical patients, which indicated that the severity of the disease was the main cause of abortion. On the one hand, patients with classical CAH were usually under the impression of infertility, so birth control might be overlooked, which results in unintended pregnancies. On the other hand, women with severe CAH were more disadvantaged in education, employment, and marital status, which might explain the increased abortion rate (83).

Women with CAH are expected to be more vulnerable to gestational diabetes mellitus, because of the increased prevalence of obesity, insulin resistance, hyperglycemia, and corticoid treatments (9, 84). Our results showed that the risk of gestational diabetes was elevated [RR 2.67 (1.29–5.12)]. However, the absolute prevalence of GDM in CAH patients was 7.3%, which is comparable to 7.49% of singleton pregnancies of natural conception and 8.47% of singleton pregnancies after ART (85). The discrepancy of these results may lie in the small number of studies (n = 3) and CAH patients (n = 432) included in the study of relative risk. As for risk factors of GDM, the proportion of overweight and obesity raised our concern, as evidenced in Table 2 that the average BMI in four studies reached or exceeded 25 kg/m^2. However, among five studies that reported GDM prevalence, only two reported BMI, so further analyses of how BMI affects the GDM rate among CAH patients were not allowed. Since the age-adjusted risk of GDM increased with increasing BMI category among all ethnic groups (86), we recommended a better control of BMI before pregnancy for CAH patients. In addition, one study also proposed that keeping BMI below 23.36 kg/m would improve the pregnancy rate of embryo transfer among non-classical 21OHD women (72).

Twin pregnancies were important risk factors for all pregnancy complications. The rate of multiple gestations was high for rare types of CAH (as illustrated in Table 1) but was moderate for 21OHD patients (as shown in Table 2). We postulated that more follicles were stimulated or more embryos were transferred to increase the opportunity of

pregnancy in rare types of CAH, the decision of which should be prudent to improve pregnancy outcome.

Alarmingly but not surprisingly, the rate of cesarean section nearly quadrupled in CAH women. This was mostly due to small maternal pelvis, vaginal stenosis, and fear of vaginal tear at parturition and sometimes due to severe hypertensive disorders during pregnancy. Interestingly, three single-center studies reported either 0% or 100% C-section rate among classical and non-classical 21OHD patients (65, 68, 70), which reflected, to some extent, how the tendency of the clinicians might affect the choice of mode of delivery.

There are several limitations to this study. First, the estimates of pregnancy complications were limited to 21OHD, since other subtypes were too rare. However, different subtypes and different mutations in the same gene could have distinct manifestations and thus various risks of pregnancy complications. Second, the number of studies included in the calculation of relative risk was limited, and the validity of the results was therefore impaired. Future studies are called for, especially cross-sectional census or multicenter studies. Third, the summary of the rare types of CAH is susceptible to selection bias, due to the fact that women with milder deficiencies are easier to get pregnant. Fourth, when applying these results to assess individual risks in the clinical setting, other factors needed to be taken into consideration, such as age, ethnicity and previous pregnancy history, which are not discussed in this research.

5 Conclusions

In our study, we summarized the clinical manifestations and considerations of ART use in rare types of CAH. As the diagnosis and treatment are improving, fertility issues should be fully addressed with all types of CAH patients. Women should be aware of their fertility possibilities and accessible fertility treatment. If they are reluctant to or not appropriate for pregnancy, information on contraception should be provided to decrease the elective abortion rate, especially for the more severe types of CAH patients. If women have fertility desire, fertility treatment could be designed according to their mutations and clinical manifestations. Multiple gestations should be avoided by reducing multiple follicular developments during ovulation induction or the number of embryos transferred. Better control of BMI may be beneficial to embryo implantation and the prevention of GDM. For the non-classical type of CAH, glucocorticoid treatment is recommended to prevent miscarriage. Should miscarriage happen, a diagnostic workup is necessary. Overall, by establishing the prevalence and relative risk of pregnancy complications in CAH patients, we made the initial step toward prevention. Future studies are urgently needed to address whether different types of CAH affect the risk of pregnancy complications and to find out other interventions that are beneficial to pregnancy outcomes.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Author contributions

XG and JS designed the study. XG, YZ and YY performed literature searches, study selection, data extraction, and quality assessment. LZ, and KU did the data analyses. XG wrote the initial draft of the manuscript, MJ and BJ contributed to the writing of discussion. JS coordinated the study and make revisions of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.982953/full#supplementary-material

APPENDIX 1
Literature Search Strategy.

APPENDIX 2
PRISMA checklist.

APPENDIX 3
Quality Assessment of included studies.

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Reproductive endocrine characteristics and *in vitro* fertilization treatment of female patients with partial 17α-hydroxylase deficiency: Two pedigree investigations and a literature review

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Background: 17α-hydroxylase/17, 20-lyase deficiency (17-OHD) is caused by the mutations of the *CYP17A1* gene. The classical phenotype of 17-OHD includes hypertension, hypokalemia, and abnormal sexual development, with partial 17-OHD typically less severe than the complete deficiency. Infertility is always one of the main clinical manifestations of partial 17-OHD. However, to date, the pregnancy potentials of partial 17-OHD female patients have rarely been investigated, and few live-birth cases have been reported among them. Moreover, the reproductive endocrine characteristics of partial 17-OHD female patients have not been completely clarified and the treatment skills of *in vitro* fertilization and embryo transfer (IVF-ET) have not been well summarized yet.

Methods: Two Chinese infertile female patients clinically diagnosed as partial 17-OHD were enrolled and their pedigree investigations were performed. Hormones were determined to depict the endocrine conditions of partial 17-OHD female patients. The adrenocorticotropic hormone (ACTH) stimulation test was performed to evaluate the functions of the adrenal cortex. Genotype analysis was conducted by next-generation sequencing (NGS) and Sanger sequencing was used to verify the results. IVF-ET was performed for the treatment of their infertility. Specifically, the progestin-primed ovarian stimulation (PPOS) protocol was chosen for the controlled ovarian hyperstimulation (COH) cycles, and the hormone replacement treatment (HRT) protocol was adopted for the endometrial preparation in frozen–thawed embryo transfer (FET) cycles.

Results: Hormone assays revealed a reduced estradiol (E2) and testosterone (T) level, and an elevated progesterone (P4) level. The classic ACTH stimulating test

evidenced a suboptimal response of cortisol to ACTH. Genotype analysis demonstrated that the proband1 carried two variants: c.1459_1467del (p.Asp487_Phe489del)^{het} and c.995T>C (p.Ile332Thr)^{het}. The proband2 was found to be a homozygote with the mutation of c.1358T>A (p.Phe453Ser)^{hom}. The two female patients both succeeded in pregnancy and delivery of healthy babies through IVF-ET, with the usage of PPOS, HRT, and low-dose glucocorticoids.

Conclusions: Partial 17-OHD female patients manifested menstrual cycle disorders and infertility clinically; displayed high P4 and low E2 and T; showed sparse pubic hair in physical examinations; and revealed multiple ovarian cysts in ultrasonic visualization. Moreover, the pregnancy potentials of infertile partial 17-OHD women seemed to increase with the adoption of IVF-ET. Considering the sustained elevated P4 level, PPOS is a feasible protocol for them in COH.

KEYWORDS

17-OHD, infertility, PPOS, IVF-ET, reproductive endocrinology

Introduction

Congenital adrenal hyperplasia (CAH) encompasses a series of autosomal recessive disorders, characterized by enzymatic defects in the synthesis of cortisol (1). Among them, 17α-hydroxylase/17, 20-lyase deficiency (17-OHD) is considered the rarest one, with an estimated prevalence of 1 in 50,000–100,000, accounting for about 1% of CAH cases (2, 3).

This condition is caused by mutations within the cytochrome P450 family 17 subfamily A member 1 (*CYP17A1*) gene, located on chromosome 10q24-q25 (4). The encoded P450c17 includes the activities of both 17α-hydroxylase and 17, 20-lyase (5). The 17α-hydroxylase is a key enzyme required for the synthesis of cortisol and the 17, 20-lyase reaction is essential for the production of sex steroids (6). Therefore, *CYP17A1* mutations will lead to failure in cortisol and sexual hormone synthesis as well as high adrenocorticotropic hormone (ACTH) in plasma.

Clinically, the classic presentation of 17-OHD includes hypertension, hypokalemia, and abnormal sexual development (7). Disruption of sexual development affects men and women differently. In men, the deficiency causes feminization of external genitalia, and in women, it causes primary amenorrhea (gonadal dysplasia) (8). The severity of the features is variable (9). Two types of the condition are recognized: complete 17-OHD, which is more intensive, and partial 17-OHD, which is typically less severe but much rarer than the complete deficiency (10).

The diagnosis of 17-OHD is based on a comprehensive overview of clinical manifestation, serum hormone levels, and gene sequencing. However, the clinical and biochemical presentations of this disorder remain highly variable, and 10%–15% of patients are normotensive at diagnosis, which increases the difficulty of an accurate preliminary diagnosis (11). Adolescent girls usually seek help for delayed puberty (12). Women in the fertile stage usually seek help for primary infertility.

In fact, infertility is always a disturbing problem accompanying partial 17-OHD patients (13). Assisted reproductive techniques (ART) have been tried in women, but mostly without any success (13). The mechanism of infertility in 17-OHD female patients may be attributed to their abnormal hormones, with high progesterone and low estrogen levels inhibiting follicular and endometrial growth. ART can bypass a part of these defects, and some reports on successful live births of 17-OHD female patients through *in vitro* fertilization (IVF) support this theory (14–20).

Herein, we report two cases of very rare 46, XX partial 17-OHD who underwent IVF in our center using progesterone-primed ovarian stimulation (PPOS) and get a successful live birth. Meanwhile, we performed the pedigree analysis of these two probands to strengthen the association between genotypes and phenotypes in female patients with partial 17-OHD. Furthermore, combined with the literature review, we summarize the reproductive endocrine characteristics of 17-OHD women and the management skills in their IVF treatments.

Materials and methods

Basic information

The proband of the first family was a 29-year-old patient, who came to our assisted reproduction department after 3 years

of primary infertility. The patient's menarche was at the age of 16 and then she had oligomenorrhea for 13 years with cycles of 5 days/30–90 days. She suffered from recurrent bilateral ovarian cysts and had undergone two laparoscopic procedures to remove them. One was right ovarian cystectomy in October 2017, the other was left ovarian cystectomy in June 2018, and both of the postoperative pathological findings showed follicular cysts. The hysterosalpingography (HSG) in 2017 showed that her fallopian tube was obstructed on both sides, so when she received the second laparoscopic surgery in 2018, she underwent salpingoplasty and hydrotubation at the same time, after which her bilateral fallopian tube became partially obstructed. The parameters of her husband's semen examination in our center were all within the normal range. She had received two cycles of ovarian stimulation treatment with clomiphene citrate in 2018 before coming to our center, during which the dominant follicle and ovulation were observed by transvaginal ultrasound (TVS), but she was unable to conceive.

The proband of the second family was a 31-year-old woman who had been infertile for 4 years. The patient had her menarche when she was 15 years old and her menstrual cycle was irregular, ranging from 25 days to 35 days. Each of her menstrual period lasted for 8 days while the menstrual flow was scanty. She underwent HSG in 2019 and the result showed that her bilateral fallopian tube was partially obstructed. Her husband's semen test result displayed good density and motility.

The other family members in the pedigrees were referred to our department because they required clinical evaluation. Here, the patients underwent detailed examinations, including blood pressure measurements at rest, height and weight assessments, relevant blood tests (including G-banded karyotyping), and pelvic ultrasound and CT scanning.

Determinations of hormones

Serum follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), progesterone (P4), testosterone (T), prolactin, double hydrogen testosterone (DHT), and anti-mullerian hormone (AMH) levels were determined by chemiluminescent immunoassays. 17-hydroxyprogesterone (17-OHP), adrenocorticotropic hormone (ACTH), cortisol, and thyroid-stimulating hormone (TSH) were measured using radioimmunoassay at three different time points within 1 day (8 a.m., 4 p.m. and 12 a.m., respectively). The ACTH stimulation test was performed to evaluate the function of the adrenal cortex. In detail, Cosyntropin 25u (SPH No.1 Biochemical & Pharmaceutical, China) was injected intravenously, and blood samples were collected at 0, 30, and 60 min after the injections.

Pedigree investigation and DNA sequencing

We conducted the pedigree surveys on the probands by interviewing the patients and their husbands and obtaining the patients' family histories. The blood samples of these two families were collected. We intended to collect as many blood samples as possible from the family members and made the probands mobilize their entire families involved in the testing, but whether to accept the DNA sequencing in the end depended on the wishes of their family members. Genomic deoxyribonucleic acid (DNA) was isolated from the peripheral blood leukocytes of each family member. The entire coding region of the patients and their family members, including the exon–intron boundaries, was analyzed using next-generation sequencing (NGS). Variants that had a frequency <1% and affected amino acid coding or splice sites that were nonsynonymous changes were retained and further evaluated. Sanger sequencing was used to verify the results. The gene sequencing results were analyzed using Chromas software and compared with the corresponding sequences from the UCSC and NCBI databases to determine abnormalities in the *CYP17A1* gene.

In vitro fertilization and embryo transfer treatment

Since the two probands both visited our center for infertility, we started our treatment on them immediately after the clinical diagnosis was confirmed. In consideration of the unique reproductive characteristics of 17-OHD, together with their tubal factors, we decided to undertake *in vitro* fertilization and embryo transfer (IVF-ET) treatment. The PPOS protocol was adopted in the controlled ovarian hyperstimulation (COH) cycle in both of the patients. Specifically, as displayed in Figure 1, the patient was administered human menopausal gonadotropin (HMG, Anhui Fengyuan Pharmaceutical Co.) 150 IU/day initially. At the same time, patients were given Clomiphene citrate (Fertilan; Codal-Synto Ltd., France) 50 mg/day. When at least one dominant follicle reached 20 mm in diameter or three dominant follicles reached 18 mm in diameter, hCG 2000 IU (Lizhu Pharmaceutical Trading Co.) was used in combination with triptorelin 0.1 mg (Decapeptyl, Ferring Pharmaceuticals) to trigger ovulation. TVS-guided oocyte retrieval was performed around 36 h after the trigger. Day 3 embryos from IVF/ICSI treatment were graded according to the Cummins' criteria, while the Gardner and Schoolcraft system was used to grade blastocysts. All of the viable embryos were frozen by vitrification, and hormone replacement treatment (HRT) was applied to endometrial preparation in the frozen–thawed embryo transfer (FET) cycle, in which dexamethasone was prescribed 0.75 mg/day before embryo transfer and hydrocortisone (10 mg at 8 a.m., 5 mg at 4 p.m.) was used after embryo transfer. From

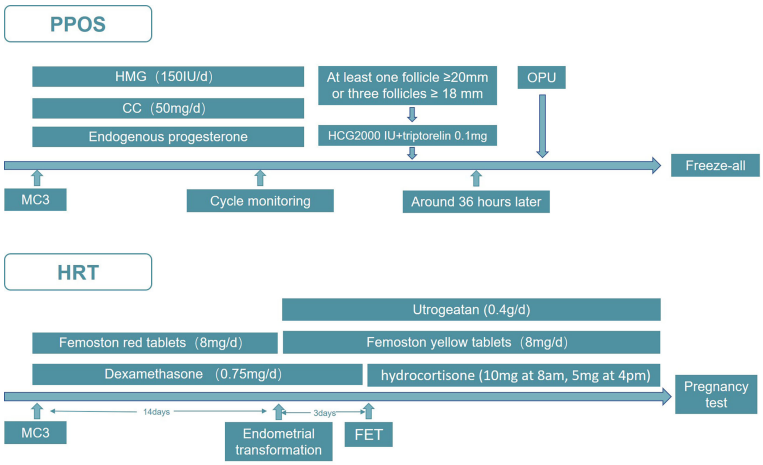

FIGURE 1
The PPOS and HRT protocol used in IVF-ET.

menstrual cycle day 3 (MC3) onwards, oral E2 (Femoston red tablets; Solvay Pharmaceuticals B.V.) 8 mg/day was given, with Femoston (yellow tablets; Solvay Pharmaceuticals B.V.) 8 mg/day and Utrogeatan 0.4 g/day starting the endometrial transformation. On the third day after endometrial transformation, embryos of good quality were transferred. The luteal-phase support continued until 12 weeks of the gestational week and the hydrocortisone was maintained throughout the pregnancy.

Results

Physical examination and auxiliary examinations

The results of physical examination and auxiliary examinations are detailed in Table 1. Proband 1 showed a height of 168 cm, a body weight of 67 kg, and a body mass index (BMI) of 19.1 kg/m^2. Her blood pressure was 108/64 mmHg. Her breast development was in Tanner stage III and there was no pubic or axillary hair (Tanner stage I). Proband 2 presented with a height of 157 cm, a body weight of 55 kg, and a BMI of 22.31 kg/m^2. Her blood pressure was 115/70 mmHg. Her Tanner stage assessments were Tanner stage III and Tanner stage I, based on the growth of breast and pubic hair, respectively. The external genitalia of the two patients were phenotypically female. Both of their pelvic ultrasounds revealed multiple ovarian cysts, together with a small uterus. Enhanced CT scanning of Proband 1 showed that the left adrenal junction was slightly thickened, about 10 mm wide, while the right adrenal gland presented with normal morphology and dimensions (Figure 2). The enhanced CT scanning of Proband 2 was normal. In addition, their chromosome karyotypes were both 46, XX.

TABLE 1 Physical examination and auxiliary examinations of the patients.

	Proband 1	Proband 2
Height (cm)	168	157
Body weight (kg)	67	55
BMI (kg/m^2)	19.1	22.31
Blood pressure (mmHg)	108/64	115/70
Tanner stage assessment (breast)	III	III
Tanner stage assessment (pubic hair)	I	I
Pelvic ultrasonography	Multiple ovarian cysts and a small uterus	Multiple ovarian cysts and a small uterus
Adrenal enhanced CT	The left adrenal junction was slightly thickened	Normal
Karyotypes	46, XX	46, XX

BMI, body mass index.

Hormonal measurements

Laboratory investigations disclosed the following common features (detailed values and reference ranges shown in Table 2): the serum FSH and LH were almost within the normal range, with the FSH level of proband 1 exceeding the normal upper limit; the E2 level was reduced, and the serum DHT and T were around the lower limit of the normal range, while the P4 was high (the assay was performed on menstrual cycle day 3 in follicular phase). The AMH was 1.43 ng/ml and 6.70 ng/ml, respectively. The patients also presented with normal blood potassium and sodium, as well as TSH. Furthermore, in the luteal phase, the patients took another three blood tests at 8 a.m., 4 p.m., and 0 a.m., respectively. The results showed increased ACTH, decreased 17-OHP and relatively normal level of cortisol, for all of the time points. The classic ACTH stimulating test evidenced a suboptimal response of cortisol to ACTH, while the dehydroepiandrosterone sulfate (DHEA-S) level maintained quite low (Table 3). Of note, if cortisol did not reach two times the basal value after ACTH stimulation, we considered it a blunt response and the patients may suffer from adrenal insufficiency (21).

CYP17A1 gene sequencing and pedigree analysis

We conducted pedigree surveys on the probands. Two kinds of pathogenic mutations were found in family 1 and another kind of mutation was detected in family 2 in the *CYP17A1* gene. The Sanger sequencing was performed to verify the mutations. The phenotypes and genotypes of the probands and their family members are detailed in Table 4.

In family 1 (Figure 3), the proband (III4) carried two variants: the missing mutation c.1459_1467del (p.Asp487_Phe489del)^{het} and the base substitution mutation c.995T>C (p.lle332Thr)^{het}. Her elder sister (III2) harbored two identical genetic mutations, with similar clinical symptoms including infertility for 10 years, menstrual disorders (abnormal uterine bleeding history), recurrent multiple ovarian cysts, and an absence of pubic and axillary hair. These two variants were verified in their preceding generation. Their father (II3) carried c.1459_1467del^{het}, without any special clinical manifestations. Although their mother's genotype was unknown because she had passed away several years ago, we discovered her aunt (II2, mother's elder sister) with the mutation of c.995T>C (p.lle332Thr)^{het}. Her aunt also had no pubic or axillary hair, but she had regular menstrual periods. In the meantime, the patient's other aunt (II6, mother's younger sister) and her elder female cousin (III8) were proved to be wild type after gene sequencing. This aunt showed no pubic and axillary hair likewise, while her two grown daughters (III8&III10) were normal referring to body hair. Furthermore, the patient's mother (II4) and maternal grandmother (I2) were absent of pubic or axillary hair, despite unknown genotypes.

In family 2 (Figure 4), the proband (IV2) was found to be a homozygote genetically with the mutation of c.1358T>A (p.Phe453Ser)^{hom}. Her family had a history of consanguineous marriage, namely, her grandmother (II2) and maternal grandfather were brothers (II3) and sisters. Therefore, many members of her family all carried this disease-causing gene mutation (II2, II3, III1, III2, and IV3). However, because they were all heterozygotes [c.1358T>A (p.Phe453Ser)^{het}], their clinical phenotypes were all normal. The younger brother of the proband was also a carrier of this mutation and already had a son.

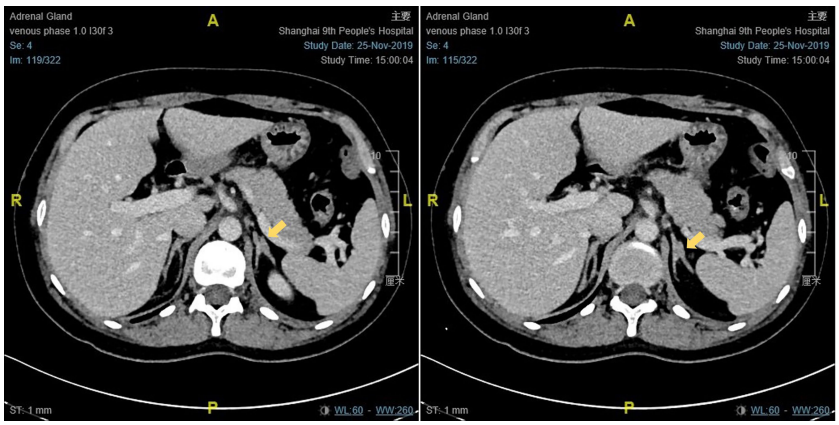

FIGURE 2
The enhanced CT scanning result of proband 1.

TABLE 2 Laboratory hormone profiles of the patients.

	Proband 1	Proband 2	Reference range
FSH (mIU/ml)	10.44 (H)	6.59 (N)	Follicular phase:3.03-8.08
LH (mIU/ml)	3.28 (N)	4.52 (N)	Follicular phase:1.80-11.78
E2 (pg/ml)	10 (L)	27 (L)	Follicular phase:21-251
P4 (ng/ml)	4.20 (H)	2.10 (H)	Follicular phase:<0.3
T (ng/ml)	0.13 (N)	0.11 (N)	0.11-0.57
prolactin (ng/ml)	14.47 (N)	17.21 (N)	Non pregnant: 2.80-29.20
DHT (pg/ml)	15.60 (L)	14.90 (L)	15.6-142
AMH (ng/ml)	1.43 (N)	6.70 (N)	0.17-7.37
Blood potassium (mmol/l)	4.06 (N)	4.12 (N)	3.5-5.1
Blood sodium (mmol/l)	142 (N)	140 (N)	135-145
TSH (uU/ml)	3.18 (N)	2.68 (N)	0.35-4.94
ACTH 8 a.m. (pg/ml)	192 (H)	167 (H)	12-46
ACTH 4 p.m. (pg/ml)	38.9 (H)	43.2 (H)	6-23
ACTH 12 a.m. (pg/ml)	15.3 (H)	13.9 (H)	/
Cortisol 8 a.m. (ug/dl)	14.5 (N)	16.7 (N)	6.7-22.6
Cortisol 4 p.m. (ug/dl)	5.69 (N)	7.12 (N)	3.35-11.3
Cortisol 12 a.m. (ug/dl)	1.41 (N)	1.97 (N)	0-5.62

FSH, follicle-stimulating hormone; LH, luteinizing hormone; E2, estradiol; P4, progesterone; T, testosterone; DHT, double hydrogen testosterone; AMH, anti-mullerian hormone; TSH, thyroid-stimulating hormone; ACTH, adrenocorticotropic hormone.

COH and FET cycle outcomes

We performed two controlled ovarian stimulation cycles (COH) and two FET cycles of proband 1. In the first COH cycle, the antral follicle count (AFC) was 3. A total of seven oocytes were achieved from 10 punctured follicles. Among them, six oocytes were in metaphase II (MII) and underwent intracytoplasmic sperm injection (ICSI) for fertilization. Five oocytes were fertilized and cleavaged, and three of them developed into high-quality embryos according to Cummins's criteria (8CII*3). In the second COH cycle, the AFC was 4. Five follicles were punctured, three oocytes were derived (MII*3), three oocytes were fertilized through ICSI and cleavaged, and finally, three top-quality embryos were harvested (10CI, 8CI, and 7CII). In the FET cycle, two embryos of good quality were transferred (10CI and 7CII), resulting in an intrauterine singleton pregnancy. The patient chose to terminate the pregnancy due to ultrasonographic evidence of cleft lip and palate in the 23rd week of gestation, despite being properly informed about the risks of continuing the pregnancy versus ending it. In the case of the aborted fetus, whole-exon sequencing revealed heterozygous *CYP17A1* and *SLC12A3* mutations, which did not appear to be related to the phenotype of the fetus. The patient underwent the second FET cycle with another two top-quality embryos (8CII and 8CII). An intrauterine singleton pregnancy was successfully achieved again (Supplementary Figure 1 shows the ultrasound images 4 weeks/6 weeks after embryo transfer). The pregnancy was uneventful

TABLE 3 ACTH stimulating test results of the patients.

	Hormones	Before ATCH stimulating test	30min after ATCH stimulating test	60min after ATCH stimulating test
Proband 1	P4 (ng/ml)	3.20	3.50	3.60
	AD (ng/ml)	<0.30	<0.30	<0.30
	DHEA-S (ug/dl)	<15.00	<15.00	<15.00
	Cortisol (ug/dl)	1.45	15.2	15.5
	17-OHP (ug/dl)	0.57	0.59	0.62
Proband 2	P4 (ng/ml)	2.10	2.30	2.30
	AD (ng/ml)	<0.30	<0.30	<0.30
	DHEA-S (ug/dl)	<15.00	<15.00	<15.00
	Cortisol (ug/dl)	16.7	17.6	17.9
	17-OHP (ug/dl)	0.62	0.69	0.67

ACTH, adrenocorticotropic hormone; P4, progesterone; AD, androstenedione; DHEA-S, sulfated dehydroepiandrosterone; 17OHP, 17-hydroxyprogesterone.

TABLE 4 The phenotypes and genotypes of the patient's family members.

Family 1	Phenotype	Genotype	Mutation type
Proband (III2)	Menstrual disorder, infertility, no pubic or axillary hair	c.1459_1467del/c.995T>C	Compound heterozygote
Father (II1)	Normal	c.1459_1467del/wt	Heterozygote
Mother (II2)	No pubic or axillary hair	Unknown	Unknown
Aunt (II3) (mother's elder sister)	No pubic or axillary hair	c.995T>C/wt	Heterozygote
Aunt (II4) (mother's younger sister)	No pubic or axillary hair	wt/wt	WT
Elder sister (III1)	Menstrual disorder, infertility, no pubic or axillary hair	c.1459_1467del/c.995T>C	Compound heterozygote
Elder female cousin (IV3)	Normal	wt/wt	WT
Family 2	Phenotype	Genotype	Mutation type
Proband (IV2)	Menstrual disorder, infertility, no pubic, or axillary hair	c.1358T>A/c.1358T>A	Homozygote
Father (III1)	Normal	c.1358T>A/wt	Heterozygote
Mother (III2)	Normal	c.1358T>A/wt	Heterozygote
Younger brother (IV3)	Normal	c.1358T>A/wt	Heterozygote
Grandmother (II2)	Normal	c.1358T>A/wt	Heterozygote
Maternal grandfather (II3)	Normal	c.1358T>A/wt	Heterozygote

without any pregnancy complications. Finally, a healthy male baby was delivered in January 2022, with a birth weight of 3,100 g and a birth length of 48 cm.

For proband 2, we performed one COH cycle and one FET cycle, which also resulted in a live birth. To be specific, in the COH cycle, 19 antral follicles were observed on MC3. After ovarian stimulation, 17 follicles were punctured and 10 oocytes were collected. Among them, seven oocytes were in the MII phase and received ICSI for insemination, which developed into four top-quality embryos for cryopreservation (8CII*2, 7CII, and 9CII). In the subsequent FET cycle, we chose two of the embryos for transfer (8CII*2). A smooth intrauterine singleton pregnancy was obtained, and finally, a healthy male baby was delivered in October 2021, with a birth weight of 3,300 g and a birth length of 50 cm.

Discussion

Congenital adrenal hyperplasia is an autosomal recessive disorder due to a defect in any of the enzymes involved in the process of steroidogenesis. The most common cause of CAH is 21-hydroxylase deficiency (21-OHD) (22). Meanwhile, 17-OHD is rarely seen and accounts for only approximately 1% of all CAH cases. This is a complex disease that can be classified mainly into "complete" and "partial" type. Of the two types, partial deficiency is much rarer, with certain degrees of estrogenic and androgenic functions, which reduce the severity of the situation (23).

By means of pedigree survey and gene sequencing, we detected two mutations in family 1 and another mutation in family 2. Both proband 1 and her sister were compound

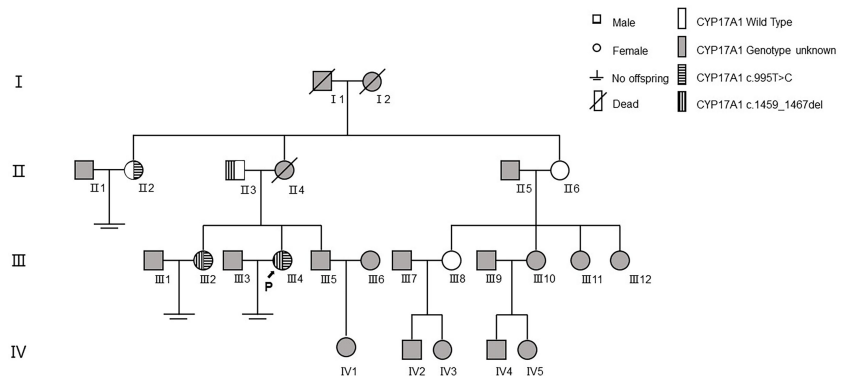

FIGURE 3
The family tree of proband 1.

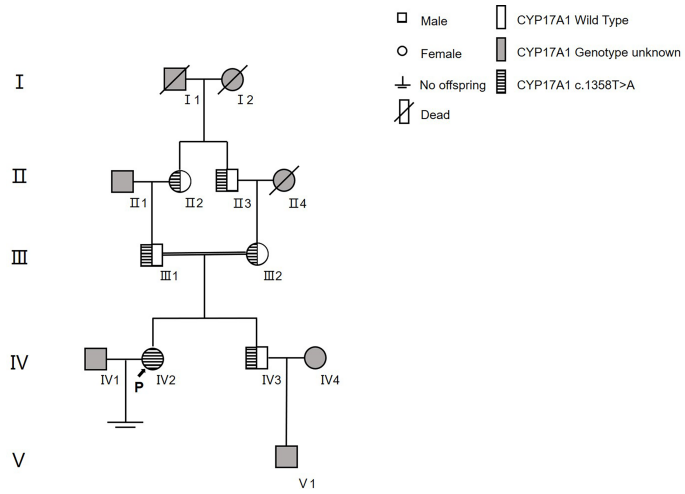

FIGURE 4
The family tree of proband 2.

heterozygotes with c.1459_1467del (p.Asp487_Phe489del)^{het} and t c.995T>C (p.lle332Thr)^{het} in family 1, and the proband 2 was a homozygote with c.1358T>A (p.Phe453Ser)^{hom} in family 2. To date, more than 100 mutations in exons and introns have been considered pathogenic or disease-causing mutations in 17α-OHD according to the ClinVar and HGMD databases.

For the part of clinical manifestations and physical examinations, the patients presented with oligomenorrhea, infertility, absence of pubic or axillary hair, and undeveloped breast, which were highly consistent with the characteristics mentioned in other cases (24, 25). Unlike complete 17-OHD women, the patient was not featured by primary amenorrhea or hypertension. Instead, they suffered from a series of problems involved in the reproductive systems, the difference of which was suggestive of a milder defect in partial 17-OHD.

The first heterozygous variant c.1459_1467del (p.Asp487_Phe489del) is located in Exon 8, which has been found homozygous or compound heterozygous in many patients leading to 17-OHD (21, 26–29) and co-segregated with disease in two Chinese families (30, 31). *In vitro* functional tests showed that the cells transfected with this variant had no 17α-hydroxylase activity or 17,20 lyase activity (26). This variant is included in HGMD and ClinVar as a "pathogenic variant" (ClinVar: 631622). According to the gnomAD database, the allele frequency of the mutation in the population database is currently less than 0.01% (9/274506). The allele frequency in the East Asian population is highest (0.05%), without homozygotes.

The second heterozygous mutation c.995T>C (p.Ile332Thr) is located in Exon 6. The mutation is in a compound hybrid form in a 17-OHD patient. *In vitro* functional studies have shown that the mutant protein still has a portion of 17α-hydroxylase activity (about 10%–25%) and a part of 17,20 lyase activity (about 10%) (32). This mutation is included in HGMD as a "pathogenic mutation" but not included in ClinVar. The gnomAD database shows that the allele frequency of this mutation in the population is currently less than 0.01% (5/282,842). The allele frequency is highest in the Latin population (<0.01%), and there is also no homozygote.

The third homozygous mutation c.1358T>A (p.Phe453Ser) is located in Exon 8 and has been reported to cause partial 17-OHD in a Chinese woman (28). It was revealed that the 17α-hydroxylase activity of this mutant protein was reduced to 29% of that of the wild type. This variant is included in HGMD and ClinVar as a "pathogenic variant", but not included in the gnomAD database. The ClinVar database shows that the allele frequency of this mutation in the population is less than 0.01% (3/264,690).

Although pedigree investigation helped confirm the diagnosis through gene sequencing, still there was a confusing phenotype in family 1. The proband's two aunts both presented with an absence of pubic or axillary hair, even though their genotypes were heterozygote (c.995T>C/wt) and wt/wt. The paradox was that the phenotype was mostly a character of 17-OHD, while the genotype was a symbol of normal 17α-hydroxylase activity and 17,20 lyase activity theoretically. This could be partially explained by family traits since hair thickness was individualized, and in this family, sparse hair might be idiopathic. Another possible explanation was that there was another gene mutation apart from *CYP17A1* related to lack of hair, such as *EPS8L3* accounting for hypotrichosis 5 (33).

Women diagnosed with 17-OHD have tried IVF-ET in the past few decades, but they have mostly failed (13). However, there are still some reports of successful live birth of this kind of patient through IVF-ET. Based on a comprehensive consultation

of literature associated with IVF-ET treatments on 17-OHD women, we listed the following cases in chronological order (Table 5): a 33-year-old woman with 17-OHD and IVF-donated oocytes resulted in a live birth in 2003 (17); in the same year, an infertile 17-OHD woman achieved pregnancy and delivered three healthy babies with the help of IVF using her own oocytes (14); a successful live birth in a 26-year-old woman with IVF was documented in 2016 (20); two consecutive live births of a 24-year-old 17OHD woman followed (19); another successful live birth in a 26-year-old woman with IVF using was recorded in 2018 (18); Blumenfeld et al. described the achievement of the first successful pregnancy and delivery in a patient with 17,20-lyase deficiency (16); the first two Chinese cases of partial 17-OHD conceived and had a live birth through PPOS protocol (15). Notably, one of the cases in the last literature is just the proband 1 in our report (15). However, since the first report of this case did not include a complete pedigree survey and it was not centered on the patient's reproductive endocrine characteristics, we paid more attention to her infertility and IVF-ET treatment in this report and described this case in detail again (the detailed treatment records are shown in Supplementary Tables). Moreover, we summarized the following reproductive-related commonalities in infertile partial 17-OHD women from our two cases together with these literature reviews.

The first distinctive reproductive endocrine feature shared by partial 17-OHD female patients was increased P4, which was usually clinically manifested as irregular menstruation and infertility. When coping with such a situation in IVF, we chose the PPOS protocol for ovarian stimulation in our cases. This protocol was originally proposed in 2015 (34) and had been proved to effectively prevent premature LH surges with progesterone supplementation in the early follicular phase, while achieving high-quality embryos and satisfactory pregnancy rates (35, 36). It had also been demonstrated that PPOS was a safe protocol without adverse effects on offspring (37). Therefore, this protocol had been widely adopted and included in the ESHRE Guideline in 2020 (38). Given the increased progesterone of partial 17-OHD women, PPOS seemed to be a suitable COH protocol, in which we happened to utilize endogenous progesterone to suppress LH surges instead of exogenous additions. Furthermore, it is unnecessary to strictly suppress progesterone and keep it within the normal range throughout the COH process. Moreover, compared to the GnRH-agonist protocol applied in the previous cases, PPOS presents the advantages of a simpler procedure, shorter stimulation duration, as well as a less financial investment.

Meanwhile, to address the impact of increased progesterone on endometrial preparation during the embryo transfer cycles, a freeze-all strategy was taken. All viable embryos were frozen by vitrification and FET was performed using the HRT protocol subsequently, which is in agreement with the regimen applied in the former cases. Generally, corticosteroid was prescribed to keep progesterone at relatively low levels before endometrium transformation. In our case, we used dexamethasone 0.75 mg/day from the last menstrual cycle to the day before embryo

TABLE 5 Previous literature of live births achieved by 17-OHD women undergoing IVF-ET.

Year	Author	Origin	Mutation	COH protocol	FET cycle	Diminish P4 production	Gestational weeks	Neonatal outcomes
2003	Ben-Nun I et al. (17)	Israel	NA	NA (donated oocytes)	HRT	Dexamethasone	25+4	A surviving male twin[a] (weight 883 g)
2003	Levran D et al. (14)	Israel	NA	HMG	HRT	Long-acting GnRH-a + Dexamethasone	NA	Triplet live birth
2016	Bianchi PH et al. (20)	Brazil	p.W406R/P428L	Long GnRH-a protocol	HRT	Long-acting GnRH-a + Dexamethasone	30+4	Male (weight 1,945 g)
2018	Kitajima M et al. (19)	Japan	p.S54del/S54del	Short GnRH-a protocol	HRT	Dexamethasone	NA	Male (weight 3,980 g)
				Short GnRH-a protocol	HRT	Dexamethasone	39	Male (weight 3,972 g)
2018	Falhammar H et al. (18)	Sweden	NA (homozygote)	NA	NA	Prednisolone	37	Female (weight 3,290 g)
2021	Blumenfeld Z et al. (16)	Israel	p.E305G/E305G	long GnRH-a protocol	HRT	Long-acting GnRH-a + Prednisolone	41+2	Female (weight 3,650 g)
2022	Xu Y et al. (15)	China	p.I332T/ D487_F489del	PPOS	HRT	Dexamethasone→Hydrocortisone	38	Male (weight NA)
		China	p.R496C/R496C	PPOS	HRT	Long-acting GnRH-a + Dexamethasone→prednisone	38+6	Female (weight NA)

[a]One of the twins dying within minutes of delivery.
17-OHD, 17α-hydroxylase/17, 20-lyase deficiency; IVF-ET, in vitro fertilization and embryo transfer; COH, controlled ovarian hyperstimulation; FET, frozen-thawed embryo transfer; P4, progesterone; NA, not applicable; HRT, hormone replacement treatment; GnRH-a, gonadotropin releasing hormone agonist; ACTH, adrenocorticotropic hormone.

transfer, and after embryo transfer, hydrocortisone (10 mg at 8 a.m., 5 mg at 4 p.m.) was used ever since through the gestation. However, in the previous two cases in which the patient had a live birth, dexamethasone was used throughout the pregnancy without change. It should be noticed that, unlike hydrocortisone and prednisolone, dexamethasone crosses the placental barrier to the fetus without inactivation (39). In our preceding study on 21-OHD patients undergoing IVF, we recommend stopping the administration of dexamethasone after embryo transfer to avoid possible adverse influences on the fetus (40).

The second noticeable reproductive endocrine characteristic of partial 17-OHD women was decreased E2. In the previous cases, very low estradiol had also been noticed (19, 20). Centered on this abnormal sexual hormone, the following problems may arise for the reproductive system and COH process.

First of all, weakened negative feedback of estrogen to the pituitary gland may result in slightly increased FSH and LH levels, which make the patients more prone to develop ovarian cysts. However, considering the high P4 levels, the feedback weakening and the increases in gonadotropins might not be so obvious. What we could observe is usually a slightly elevated FSH level relative to the ovarian reserve. In our report, the cases all had relatively high FSH levels as well as a history of ovarian cyst operation before they came to our hospital. Since ovarian surgery might cause injury to the ovarian function, the ovarian reserve of our patient (proband 1) was relatively low with an AMH value of 1.43 ng/ml and an AFC of 3. However, the case reported in 2016 documented an AFC of 32, despite the limited number of dominant follicles (20); the women recorded in 2018 presented with hyper-response with 21 oocytes retrieved (19), although both of the cases had a lack of ovarian surgery histories. It reminded clinical physicians that when confronted with ovarian cysts in partial 17-OHD women, which seems to be a sticky condition requiring sophisticated and accurate treatment, they need to take a comprehensive diagnosis, strictly obey operation indications, and prudently manipulate, to avoid further damage to fertility. Next, the extremely low estradiol level of partial 17-OHD women made it difficult for physicians to evaluate the follicle development situations as well as the appropriate timing for triggering in the COH process. Therefore, we could only use follicular diameter rather than estradiol level as the indicator to make adjustments to the drug dosage in COH and determine when to perform oocyte retrieval. Moreover, it is perceived by some that complete estradiol deficiency causes follicular developmental arrest (13). Therefore, a small dose of estradiol was applied to COH in one of the previous cases. However, evidence also shows that favorable oocyte maturation and embryo development can occur in a low estradiol environment (41). Given that all of the cases produced high-quality embryos resulting in a live birth, even if without the external supplementation of estrogen, it appeared that reduced estradiol had no adverse effect on the development and maturation of follicles in partial 17OHD women, or on the embryo developmental potentials.

The third silent trait of reproductive endocrinology in partial 17-OHD women was decreased T, which often presented with sparse hair clinically. Since it is in the female population, low testosterone had very little effect on reproductive system development and fertility. Nevertheless, this feature can still inspire clinicians that if no pubic hair or sparse pubic hair is noticed during the gynecological examination, it is necessary to pay attention to the growth of other body hair and not miss the diagnosis of 17-OHD.

An intriguing phenomenon is that the fallopian tubes of the two patients were all partially obstructed. Meanwhile, the infertile woman in family 1 (III2) also had infertility, with her fallopian tubes partially obstructed too, although her husband had oligospermia. However, since previous reports did not mention the results of tubal patency, we could not reach a conclusion that fallopian tube obstruction was a common finding based on the two cases. Instead, we thought of it as a chance result.

Moreover, the laboratory assays also demonstrated reduced 17-OHP and elevated ACTH, with the ACTH stimulating test revealing an inadequate response, explaining the thickened adrenal on CT scanning. These observations were in accordance with those in previous literature (10, 24, 25, 42). However, the potassium concentration and sodium concentration were normal in our results, while most of the cases documented declining potassium in the blood (23–25, 42).

Furthermore, it should not be neglected that endometrial cavity fluid (ECF) appeared repeatedly in our patients in both COH cycles and FET cycles. This phenomenon had not been referred to in any previous literature. As it was commonly recognized that ECF has a negative influence on implantation and pregnancy, it was a troublesome problem that need to be solved. Fortunately, the ECF disappeared spontaneously the day before embryo transfer in the return visit in our cases. Otherwise, we might perform aspiration of uterine effusion once we confirmed the existence of ECF. The causative reasons for ECF were not completely understood and were speculative to be a tubal factor, polycystic ovarian disease, subclinical uterine infections, and so on (43, 44). For our patients, the formation causes of ECF also remained unknown, possibly relevant to 17-OHD itself due to the consistency of the manifestations of our cases.

Conclusion

In conclusion, we are among the first to report the live births of infertile women diagnosed with partial 17-OHD, who underwent IVF-ET using the PPOS protocol. Meanwhile, we conducted two pedigree investigations to further clarify the

genotypes and phenotypes of these patients. Considering the sustained elevated P4 level, PPOS is a feasible regimen for 17-OHD patients in COH, while it is also convenient, efficient, and economic. Furthermore, we made a systematic review of the cases of infertile 17-OHD women who achieved pregnancy and live birth through IVF-ET. Taken together, we depicted and concluded the reproductive characteristics of partial 17-OHD women clinically and biochemically. In summary, clinical manifestations include menstrual cycle disorders and infertility; endocrine hormone examination displays high progesterone, low estrogen, and low testosterone; physical examination shows sparse pubic hair; and ultrasound examination reveals multiple ovarian cysts. Based on these features, it is vital to make an early but accurate diagnosis in women of this disorder to help them obtain reproductive success.

Data availability statement

The data presented in the study are deposited in the Genebank repository, accession number ON815636-ON815639.

Author contributions

YK, YW, and JQ contributed to the conception and design of the study. SJ and YX analyzed the data and drafted the manuscript. All authors participated critical discussion and reviewed the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.970190/full#supplementary-material

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Body composition in children and adolescents with non-classic congenital adrenal hyperplasia and the risk for components of metabolic syndrome: An observational study

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Background: Treated or untreated non-classic congenital adrenal hyperplasia (NCCAH) diagnosed in childhood could pose an increased risk of obesity and metabolic derangements in adolescence and early adulthood. We aimed to explore the interaction between muscle-to-fat ratio (MFR) and components of metabolic syndrome in pediatric subjects with NCCAH.

Methods: This retrospective observational study was conducted in the Tel Aviv Medical Center from January 2018 to January 2022. The study group comprised 75 subjects (26 males) with NCCAH (61 hydrocortisone-treated [21 males] and 14 untreated [5 males]) and 134 healthy sex- and age-matched subjects (41 males) with normal puberty served as controls. Body composition was measured by bioelectrical impedance analysis (BIA) and muscle-to-fat ratio (MFR) z-scores were calculated. Stepwise linear regression models were applied to evaluate explanatory variables for MFR z-scores, blood pressure percentiles, lipid profiles, and glucose metabolism.

Results: The median age [interquartile range] was 7.5 years [5.3, 8.8] at NCCAH diagnosis and 12.3 years [8.9, 15.4] at BIA. The median cumulative hydrocortisone dose was 7620 mg/m^2 [2547, 12903]. Subjects with NCCAH had higher mean BMI z-scores and lower median MFR z-scores compared to controls [(0.47 ± 0.97 vs. -0.19 ± 1.04, p<0.001) and (-0.74 [-1.06, -0.14] vs.-0.37 [-0.99, 0.15], p=0.045), respectively]. The linear regression models

dependent variables and their explanatory variables were: MFR z-score (R^2 = 0.253, p<0.001) - socioeconomic position index (β=0.348, p=0.003), birthweight z-score (β=-0.258, p=0.013), and duration of hydrocortisone treatment in years (β=0.048, p=0.023); systolic blood pressure percentile (R^2 = 0.166, p<0.001) - MFR z-score (β=-9.75, p<0.001); TG/HDL ratio (R^2 = 0.116, p=0.024) - MFR z-score (β=-0.300, p=0.024). No significant variables were found for glucose.

Conclusions: Children and adolescents with NCCAH have a body composition characterized by an imbalance between muscle and fat tissues, which may place them at increased risk for early-onset cardiometabolic derangements. It is reassuring that glucocorticoid therapy aimed to alleviate androgen overproduction does not appear to adversely affect their body composition.

KEYWORDS

bioelectrical impedance analysis (BIA), body composition, children and adolescents, fat percentage, metabolic syndrome (MetS) components, muscle-to-fat ratio (MFR), non-classic congenital adrenal hyperplasia (NCCAH)

Introduction

Non-classic congenital adrenal hyperplasia (NCCAH) is a group of enzymatic disorders characterized by a mild defect in cortisol biosynthesis. The most common form of NCCAH is caused by variations in the *CYP21A2*, the gene encoding for the 21-hydroxylase enzyme (1–3). Symptoms are variable and depend upon the extent and duration of postnatal hyperandrogenism. Individuals may be asymptomatic or display premature adrenarche and pubarche, accelerated linear growth with bone age advancement and compromised adult height, central precocious puberty, acne, hirsutism, menstrual disorders, and infertility (4). Glucocorticoid therapy is not always indicated but rather reserved and tailored for symptomatic cases of hyperandrogenism, in an attempt to alleviate androgen overproduction (5). The therapeutic spectrum of glucocorticoids is narrow, and the supraphysiological doses that are often needed to control the hyperandrogenism may have adverse metabolic implications, such as weight gain, increased blood pressure, hyperglycemia, and hyperlipidemia (6). Alternatively, sustained hyperandrogenism (in untreated/undertreated cases) may also affect body composition and metabolism. Chronic androgen excess has been reported in association with increased visceral adiposity and insulin resistance and their metabolic consequences (7).

There is scarce knowledge about the metabolic consequences of treatment with glucocorticoids and/or hyperandrogenism in pediatric patients with NCCAH. One early study reported an increased rate of obesity and impaired insulin sensitivity [as assessed by Homeostatic Model Assessment for Insulin Resistance (HOMA-IR)] in adults but not in children with NCCAH (8). Hyperinsulinemia and insulin insensitivity associated with hyperandrogenism were reported in untreated NCCAH women (9), and adolescents with NCCAH were found to be at a higher risk of increased artery intima-media thickness in an even earlier study (10). Contrarily, a recent study reported that patients with NCCAH diagnosed in childhood, whether treated or untreated, were not found to be at increased risk for overweight, obesity, or metabolic derangements in adolescence and early adulthood (11).

An increased rate of obesity and a sedentary lifestyle have resulted in a marked increase in the prevalence of cardiovascular disease (CVD) risk factors (hypertension, altered glucose metabolism, dyslipidemia, and abdominal obesity) in adulthood (12–17) and in adolescence (18–24). Our Pediatric Endocrine Unit implemented bioelectrical impedance analysis (BIA) of body composition in January 2018 as part of the routine assessment of patients referred for endocrine consultation (25). We subsequently reported the predictive value of muscle-to-fat ratio (MFR) z-scores in assessing CVD risk factors in youth with overweight and obesity (26). There are, however, limited data on obesity and cardiometabolic derangements among pediatric patients with NCCAH (27). In this study, we explored the interaction between body composition parameters and CVD risk factors in children and adolescents with NCCAH, with special focus upon the contributory role of hyperandrogenism and steroid therapy.

Methods

Study population

This real-life observational study comprised of pediatric subjects (5-18 years of age) whose body composition assessment was routinely monitored at our endocrine unit in a tertiary medical center extended from January 2018 to January 2022. Both the NCCAH patient and healthy control groups were recruited from our endocrine unit. The BIA database was queried to generate a list of patients with the diagnosis of "NCCAH" and "observation of growth". The electronic medical records of suitable children and adolescents were reviewed, and those who fulfilled the inclusion criteria were included in the analysis. The study group comprised 75 subjects (26 males) with NCCAH (61 hydrocortisone-treated and 14 untreated), and 134 healthy sex- and age- matched subjects (41 males) with normal puberty served as their controls. NCCAH was defined on the basis of an adrenocorticotropic hormone (ACTH)-stimulated 17-hydroxyprogesterone (17-OHP) serum level of >40 nmol/L according to published guidelines (5, 28). Fifty-six of the 75 individuals with NCCAH underwent molecular analysis. The healthy controls were defined as having normal stature (10^{th}< height percentile< 90^{th}) and normal timing of puberty and pubertal progression. Subjects with medical conditions which could lead to fluid retention (e.g., renal failure, congestive heart failure), severe underweight [body mass index (BMI) z-score ≤ -2.0], metabolic bone disease, malignancies, or genetic syndromes, or those who were using medications which could affect body composition were excluded from the study. The study population flowchart is presented in Figure 1.

Clinical evaluation

The study protocol was approved by our medical center's Institutional Review Board which waived informed parental consent. The data were handled in accordance with the principles of good clinical practice. The routine clinical evaluation of patients at endocrine referral includes a comprehensive medical interview. Female patients were questioned about their menstrual cycle (whether menses were present or absent and whether the cycle was regular or irregular), as well as their use of oral contraceptives. Anthropometric assessment of height [by means of a commercial Harpenden stadiometer (Holtain Ltd., Crosswell, United Kingdom)] and weight in light clothing (by BIA), as well as measurement of blood pressure (BP) were carried out at the first visit and repeated at each follow-up visit. Blood pressure was measured by a registered pediatric nurse who used the Welch Allyn Vital Signs Monitor VSM 300 (Welch Allyn, Inc., Beaverton, OR, USA) and chose the appropriate size cuff. The BP measurement was repeated up to three times with intervals of 5 minutes

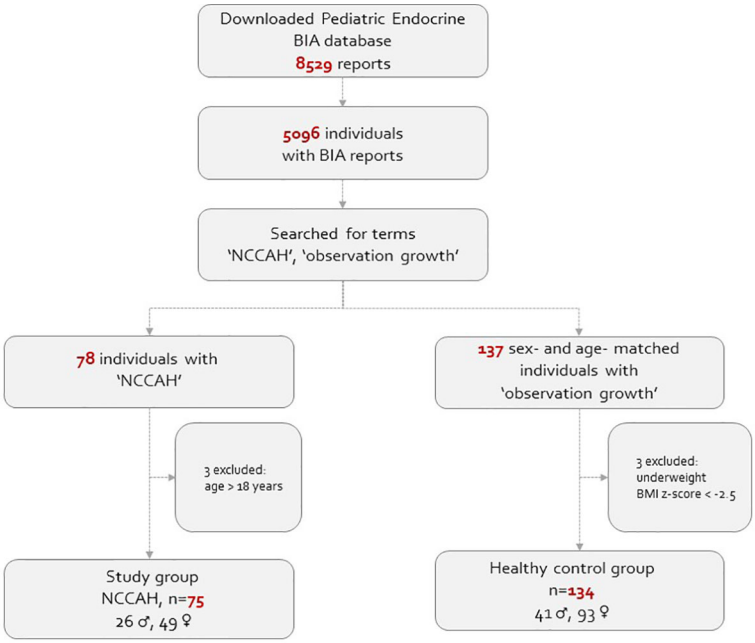

FIGURE 1
The study population flowchart.

between measurements when the BP values were abnormal. The attending parent's height was measured at first visit, and BIA weight assessment was offered as well. The height of an absent parent was provided by the available one.

The study participant's physical examination included pubertal staging and a search for clinical signs indicative of hyperandrogenism, hypercortisolism, and insulin resistance. The decision to initiate glucocorticoids for NCCAH patients was at the discretion of the pediatric endocrinologist who was monitoring the patient. Therapy was reserved for symptomatic patients (children with premature and/or rapid progression of pubarche or bone age advancement and adolescent girls with hyperandrogenism). Titration of the glucocorticoid dose was according to a composite of growth trajectory, weight gain, clinical signs of hyperandrogenism, a hormonal profile designed to maintain 17OHP levels slightly above the upper limit of the normal, and androstenedione and testosterone levels at sex, age, and Tanner stage-appropriate levels, and bone age advancement. Gonadotropin-releasing hormone analogue (GnRHa) treatment was considered for individuals with central precocious puberty. The combination of GnRHa and growth hormone (GH) therapy was considered in individuals with compromised adult height prediction (5).

Body composition analysis

Body composition was measured by BIA [Tanita Body-Composition Analyzer (Tanita MC-780 MA) and GMON Professional Software], which has been clinically proven to be accurate and reliable and to provide highly reproducible results (29). The BIA measurements were performed in the morning (from 8:00 AM until 11:00AM) during the routine clinic visit, preferably with the subject in a fasting state and not after strenuous physical activity. The BIA measures whole body as well as segmental analysis (trunk, upper, and lower limbs) of fat and muscle. The report includes the following data: body weight (kilograms, 0.1 kg increments), fat percentage (FATP, whole body, 0.1% increments), truncal fat percentage (TFATP, 0.1% increments), fat mass (kilograms, 0.1 kg increments), muscle mass (whole body, 0.1 kg increments), total body water percentage (TBW, 0.1% increments), and estimated basal metabolic rate (BMR, expressed in kcal, 1 kcal increments). Calculated variables included: appendicular skeletal muscle mass (ASMM = the sum of muscle mass of four limbs) and MFR [ASMM (kg)/fat mass (kg)]. The z-scores for MFR were calculated according to BIA pediatric reference curves (30).

Biochemical analysis

The hormonal evaluation included 17-OHP and cortisol levels at referral, baseline and following intravenous administration of 0.25 mg Synacthen (Novartis, New York, NY, USA), with a 17OHP measurement at baseline and 30 and 60 min after ACTH injection (31). The test was conducted in the early follicular phase of the menstrual cycle in post-menarcheal girls. Follow-up basal androgen levels were measured every 4-6 months or earlier when dose adjustment was required. Hormonal analyses in serum were performed with commercial kits in the endocrine laboratory of our hospital. Cortisol was measured using the Coat-A-Count radioimmunoassay (Diagnostics Products Corporation, Los Angeles, CA, USA), testosterone level was determined by electrochemiluminescence (Rosh, Cobas E 601), 17OHP was measured with the direct quantitative enzyme immunoassay (DBC, Diagnostic Biochem Canada Inc), and the androstenedione level was determined by chemiluminescence using the Immulite 2000 Xpi immunoassay system (Siemens) (32).

Annual fasting measurements of glucose levels and lipid profile tests were performed as part of the routine standard of care. Serum glucose was measured with the glucose oxidase colorimetric method (Hitachi 917 automated analyzer, Roche Diagnostics, Mannheim, Germany), and serum total cholesterol (TC), triglycerides (TG), and high-density lipoprotein (HDL) levels were measured with the enzymatic colorimetric method (Hitachi 904 automated analyzer, Roche Diagnostics). Low-density lipoprotein (LDL) was calculated according to the Friedewald formula: $\mathrm{LDL} = \mathrm{TC} - \mathrm{HDL} - \frac{\mathrm{TG}}{5}$. Fasting plasma TG concentrations of >110 mg/dL were considered elevated, and an HDL <40 mg/dL was considered low (18). Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) was calculated as follows: $\frac{\text{fasting insulin IU/mL X fasting glucose mg/dL}}{405}$ (33). Levels in peri-pubertal children (<11.5 years) were compared to values in European children and categorized as elevated when they were at the $\geq 90^{th}$ percentile, while levels ≥1.9 were considered elevated in youths (34).

Data collection

All hospital medical records are electronic, with access to the individual's health maintenance organization laboratory data. Sociodemographic characteristics, ethnicity, perinatal history, medical conditions, medications and family history of cardiovascular disease risk factors were retrieved from the medical files. Clinical data, anthropometric measurements, vital signs, pubertal staging, and laboratory evaluation were extracted at the time points of BIA assessments.

Definition of study variables

Socioeconomic position

The SEP by home address [SEP cluster and SEP index] was analyzed based on the Israel Central Bureau of Statistics'

Characterization and Classification of Statistical Areas within Municipalities and Local Councils by the Socio-Economic Level of the Population (35). Residential SEP cluster, which was based on locality of residence, was coded on a 1-10 scale and grouped into low (1–4), medium (5–7), and high (8–10) categories. The SEP index is an adjusted calculation of 14 variables that measure social and economic levels in the domains of demographics, education, standard of living, and employment, ranging from the lowest (-2.797) to the highest (+2.590).

Clinical characteristics

BMI was calculated as weight in kilograms divided by height in meters squared. The patient's height, weight, and BMI values were converted to sex- and age-specific standard deviation scores (z-scores) according to the CDC 2000 growth charts (36). Weight status was defined according to BMI z-scores as follows: underweight as BMI percentile $\leq 5^{th}$ percentile (z-score ≤-1.65), overweight as BMI percentile $\geq 85^{th}$ and $<95^{th}$ percentiles (1.04 ≤ z-score <1.65), and obesity as BMI percentile $\geq 95^{th}$ percentile (z-score ≥1.65) (37, 38).

Birth weight z-scores were calculated by PediTools Electronic Growth Chart Calculators based on the Fenton growth chart for preterm infants (39). Appropriate birth weight for gestational age (AGA) was defined as corrected birth weight z-scores between -1.645 to 1.645, small for gestational age (SGA) as birth weight z-scores <-1.645, and large for gestational age (LGA) as birth weight z-scores >1.645. The height, weight, and BMI values were converted to sex- and age-specific z-scores according to the CDC 2000 growth charts (36). SBP and DBP percentiles were calculated by means of an online age-based pediatric BP calculator (40).

Pubertal stages were graded according to Tanner scores for testicular volume in boys and for breast development in girls. Onset of puberty was defined as genitalia Tanner stage 2 with a testicular volume >3 mL in boys and appearance of breast buds in girls, with or without sexual hair. The subject was considered fully pubertal when pubertal signs corresponded to Tanner stage 5 (41, 42).

Glucocorticoid exposure

The glucocorticoid dosage was expressed as hydrocortisone in mg per body surface area (mg/m^2). All available daily doses of treatment divided by the corresponding body surface area were summed to obtain annual cumulative doses of hydrocortisone. Each patient's total cumulative hydrocortisone dose was determined by compiling these annual cumulative hydrocortisone doses (43).

Statistical analyses

The data were analyzed with the Statistical Package for the Social Sciences software version 27 (SPSS Inc., Chicago, IL). Violin plots were created using R-studio 4.2 software with the ggplot2 package. Repeated measurements of BMI and MFR z-scores and BP percentiles taken between diagnosis and the last clinic visit served for the calculation of the mean intrapersonal and SD for each parameter. All statistical tests were two-sided. The Shapiro-Wilk test was applied to assess the normality of continuous data. The data are expressed as means ± standard deviations (SDs) for normally distributed variables and median and interquartile range [IQR] for skewed distribution. Pearson's chi-square test was performed to compare the distribution of categorical variables between the NCCAH group and the control group. An independent sample t-test or an independent sample Mann-Whitney was performed to compare between two groups (NCCAH vs healthy controls) for continuous variables with normal or skewed distribution, as appropriate. Linear regression models using the stepwise approach were applied to assess the association between body composition parameters (MFR z-score and BMI z-score) and metabolic syndrome components (BP, TG/HDL ratio, and glucose levels) in the NCCAH group. The variables entered into the models included: sex, SEP index, family history of obesity, age (at diagnosis and at BIA), perinatal characteristics (gestational age and birthweight z-scores), hydrocortisone exposure (mean dose, treatment duration, and cumulative dose) and MFR z-scores (for metabolic syndrome components). A p value ≤0.05 was considered significant.

Results

The NCCAH group comprised 75 subjects (27 [34.7%] males, median age at first BIA 11.2 years [IQR 8.2, 14.7]). Fifty-six of them (74.7%) had available genetic information in their clinical files, revealing that 27 (48.2%) were homozygous for the V281L variation and 22 (39.3%) were compound heterozygous for one mild and one severe variation.

The sociodemographic characteristics of the NCCAH group and their controls are presented in Table 1. Ethnic distribution of the NCCAH group revealed that 46.6% were Jews of Ashkenazi origin, 15% were Sephardic Jews, 37% were mixed Ashkenazi/Sephardic Jews, and 1.4% were of mixed Jewish/non-Jewish origin. The SEP of subjects with NCCAH was above average (the median SEP cluster was 8 [IQR 7, 9], and the median SEP index was 1.278 [IQR 0.773, 1.751]), but it was significantly lower compared to the healthy control group ($p = 0.009$). There were no significant group differences in sex, age, ethnic distribution, marital status, or number of children in the family.

The pregnancy and perinatal characteristics of the NCCAH group and their controls are presented in Table 2. Most of the pregnancies in the NCCAH group were singletons (89.3%), and most followed spontaneous conception (80%). Assisted reproduction was required in 20% of the pregnancies, and it consisted of pharmacological ovulation induction in 14.3% and

in vitro fertilization in 5.7%. *In utero* exposure to gestational diabetes mellitus (GDM) was reported in 1.4% NCCAH pregnancies, exposure to steroids in 17.2%, and exposure to both levothyroxine and aspirin in 1.4%. Delivery was spontaneous and vaginal in most cases (77.1%), elective C-section (10%), and emergency C-section or vacuum extraction (12.9%). Most infants were born at term (77.8%) and appropriate for gestational age (82.9%), with a median birth weight of 3060 grams and normal median adjusted birth weight z-scores (-0.385 [IQR -0.853, 0.230]); 19.4% were born pre-term and 11.4% were born SGA. Comparative analysis of the NCCAH study group and healthy control group revealed a similar rate of spontaneous conception with a significantly different distribution of assisted forms of reproduction (p = 0.004), lower rates of GDM (p = 0.027), a different distribution of maternal medications (p < 0.001) and a higher proportion of preterm births (p = 0.035). There were no significant group differences in the number of fetuses, mode of delivery, gestational age, or and birth weight parameters.

The median age of the NCCAH study group at diagnosis was 7.5 years [IQR 5.3, 8.8], and most of them were prepubertal at diagnosis (n = 55, 73.3% in Tanner 1 gonadarche and n = 33, 44% in Tanner 1 adrenarche), with a minority in full puberty (n = 4, 5.3%). The reasons for endocrine referral in descending order of frequency were premature adrenarche (n = 37, 49.3%), a parent and/or sibling with NCCAH (n = 22, 29.3%), bone age advancement (n = 8, 10.7%), central precocious puberty (n = 3, 4%), short stature (n = 3, 4%), and hirsutism (n = 2, 2.7%). Anthropometric measurements (mean ± SD) at NCCAH diagnosis revealed a height z-score of 0.30 ± 1.09, a weight z-score of 0.4 ± 1.05, and a BMI z-score of 0.37 ± 1.11, with a median bone age advancement of 15 months. Most of the NCCAH group (80%, 21 boys and 40 girls) received glucocorticoid therapy starting at a median age of 7.8 years

TABLE 1 Sociodemographic characteristics of the NCCAH group and their healthy controls.

	NCCAH	Healthy controls	*p*-value
Number	75	134	
Sex, *n (%)*			
Males	26 (34.7)	41 (30.6)	0.545
Females	49 (65.3)	93 (69.4)	
Age at first BIA assessment			
Age, years, median [IQR]	11.2 [8.2, 14.7]	11.4 [8.6, 14.6]	0.453
Ethnicity, *n (%)*			
Ashkenazi Jew	34 (46.6)	46 (34.3)	0.384
Sephardic Jew	11 (15)	25 (18.7)	
Ashkenazi/Sephardic Jew	27 (37)	60 (44.8)	
Jew/non-Jew	1 (1.4)	3 (2.2)	
Socioeconomic position (SEP)			
SEP cluster categories, *n (%)*			
Low (1-4)	2 (2.7)	4 (3)	**0.009**
Medium (5-7)	28 (37.3)	24 (18)	
High (8-10)	45 (60)	107 (79)	
Cluster, median [IQR]	8 [7, 9]	8 [8, 9]	0.060
Index, median [IQR]	1.278 [0.773, 1.751]	1.470 [1.083, 1.903]	0.072
Household, *n (%)*			
Two-parent	71 (96)	121 (94.5)	0.403
Single-parent by choice	0 (0)	2 (1.6)	
Divorced	1 (1.3)	4 (3.1)	
Widow	2 (2.7)	1 (0.8)	
Children in the family			
Number	3 [2, 3]	2 [2, 3]	0.392
Birth order	2 [1, 2]	1 [1, 2]	0.435

Data are expressed as number and (percent) or median [interquartile range]. Chi-squared tests were performed to compare categorical variables between groups, and the Mann-Whitney test was performed to compare linear variables with skewed distribution. A p-value of ≤0.05 was considered significant. Bold indicates significant. Socioeconomic position (SEP) by cluster of localities of residence ranged from 1 to 10, with 1 being the lowest rating and 10 the highest. The SEP index is an adjusted calculation of 14 variables that measure social and economic levels in the domains of demographics, education, standard of living, and employment (range from the lowest -2.797 to the highest 2.590).
Data not documented in medical records: ethnicity 2 NCCAH, SEP 2 healthy controls, household 7 (1 NCCAH, 6 healthy controls), number of children in the family 15 (8 NCCAH, 7 healthy controls).
NCCAH, non-classic congenital adrenal hyperplasia; n, number; NCCAH, non-classic congenital adrenal hyperplasia; BIA, bioelectrical impedance analysis.

TABLE 2 Pregnancy and perinatal characteristics of the NCCAH group and their healthy controls.

	NCCAH	Healthy controls	*p*-value
Method of conception, *n (%)*			
Spontaneous	56 (80)	103 (83.1)	**0.004**
Ovulation induction	10 (14.3)	2 (1.6)	
IUI	0 (0)	4 (3.2)	
IVF biological parents	4 (5.7)	10 (8.1)	
IVF sperm donation	0 (0)	3 (2.4)	
IVF egg donation	0 (0)	2 (1.6)	
Maternal conditions, *n (%)*			
Gestational diabetes mellitus	1 (1.4)	12 (9.7)	**0.027**
Exposure to medications, *n (%)*			
No exposure	56 (80)	114 (92)	**<0.001**
Glucocorticoids	12 (17.2)	1 (0.8)	
Levothyroxine	1 (1.4)	6 (4.8)	
Insulin	0 (0)	3 (2.4)	
Metformin	0 (0)	0 (0)	
Aspirin	1 (1.4)	0 (0)	
Fetus, *n (%)*			
Singleton	67 (89.3)	113 (91.1)	0.676
Twin	8 (10.7)	11 (8.9)	
Mode of delivery, *n (%)*			
Spontaneous vaginal	54 (77.1)	97 (79.5)	0.860
Induction	0 (0)	1 (0.8)	
Vacuum extraction	2 (2.9)	2 (1.6)	
Elective C-section	7 (10)	13 (10.7)	
Urgent C-section	7 (10)	9 (7.4)	
Gestational age (GA)			
GA, weeks	39 [38, 40]	39 [38.4, 40]	0.205
Preterm,<37 weeks	14 (19.4)	13 (10.6)	**0.035**
Term, 38-42 weeks	56 (77.8)	110 (89.4)	
Postterm, ≥ 42 weeks	2 (2.8)	0 (0)	
Birth parameters			
Birth weight, *grams*	3060 [2700, 3375]	3000 [2700, 3300]	0.930
Birth weight, *z-score*	-0.385 [-0.853,0.230]	-0.550 [-1.030, 0.130]	0.285
Birth weight categories, *n (%)*			
SGA	8 (11.4)	13 (11.5)	0.783
AGA	58 (82.9)	96 (85)	
LGA	4 (5.7)	4 (3.5)	

Data are expressed as number and (percent) or median [interquartile range]. Chi squared tests were performed to compare categorical variables between groups, and the Mann-Whitney was performed to compare linear variables with skewed distribution. A p-value of ≤0.05 was considered significant. Bold indicates significant. Data not documented in medical records: conception method in 15 (5 NCCAH, 10 healthy controls), gestational diabetes mellitus, and medications administered during pregnancy in 15 (5 NCCAH and 10 healthy controls), number of fetuses in 10 healthy controls, mode of delivery in 17 (5 NCCAH and 12 healthy controls), gestational age in 16 (3 NCCAH and 13 healthy controls), birth weight in 25 (4 NCCAH and 21 healthy controls).
NCCAH, non-classic congenital adrenal hyperplasia; GDM, gestational diabetes mellitus; SGA, small for gestational age; AGA, appropriate for gestational age; LGA, large for gestational age.

and ceasing at a median age of 13.9 years; 14 children (5 boys and 9 girls) were treatment-naïve at the time of the BIA. The median duration of therapy was 36.5 months [IQR 10.5, 68.9], and the median dose was 6.59 mg/m^2 with a lifetime cumulative glucocorticoid dose of 7,620 mg/m^2 [IQR 2,547, 12,903].

At last BIA assessment, the median age of the NCCAH group was 12.3 years [IQR 8.9, 15.4], 19 (25.3%) were overweight/obese (BMI z-score ≥1.036), among them 10 (13.3%) were obese (BMI z-score ≥1.645). The median number of hours spent per week engaging in physical activity

did not differ between groups (NCCAH = 3 [IQR 1, 5] vs controls = 3 [IQR 1, 4]), p = 0.978). Comparative analyses of the clinical and metabolic characteristics of the NCCAH cohort and their healthy controls at last BIA assessment are presented in Table 3.

Fifteen (20%) patients with NCCAH had documented comorbid conditions, the most common of which was ADHD in 10 individuals (13.3%, of whom four were treated with stimulant medications), Hashimoto hypothyroidism in 2 (2.7%, well-controlled with levothyroxine), celiac disease in 1 (1.3%, seronegative under gluten-free diet), developmental delay in 1 (1.3%, treated with risperidone), and anxiety disorder in 1 (1.3%, treated with selective serotonin reuptake inhibitors). Fourteen patients with NCCAH had documented dietary vitamin supplementation: 11 (14.7%) received vitamin D, 2 (2.7%) received vitamin B12, and 1 (1.3%) was treated with an iron supplement. The mean (± SD) or median [IQR] serum chemistry levels in the NCCAH group were: glucose 83 mg/dL (81, 91), total cholesterol 160.6 ± 26.3 mg/dL, LDL-c 87.7 ± 22.2 mg/dL, HDL-c 56.4 ± 12.2 mg/dL, triglycerides 69.5 mg/dL [50.25, 94.5], non-HDL-c 101.3 mg/dL [83.9, 119.8], and TG : HDL ratio 1.26 [0.89, 1.83].

Age at gonadarche in the NCCAH group was similar to that of the healthy controls and age at adrenarche was significantly younger than the healthy controls [(9.8 ± 1.6 years vs. 10.1 ± 1.6 years, p = 0.419) and 8.6 ± 1.9 years vs. 10.3 ± 1.6 years, p< 0.001), respectively]. Among the adolescent girls, no differences were found in median age at menarche between the NCCAH and healthy control groups (12.8 years [IQR 12.1, 13.8] and 12.8 years [IQR 12, 13], p = 0.709). Post-menarcheal females (22 NCCAH patients and 27 controls) reported similar rates of regular menses in ~73% (72.7% NCCAH patients and 74.1% controls), while irregular menses were reported in 9.1% of the NCCAH patients and 22.2% of the controls. Use of oral contraceptives was reported in 18.2% of the NCCAH patients and 3.7% of the healthy controls. During the course of follow-up, 10 (4 boys) pubertal subjects with NCCAH received GnRHa therapy: 6 (2 boys) were treated with GnRHa alone for the indication of precocious puberty and 4 were treated with a combination of GnRHa and GH due to predicted short stature in adulthood. All 10 subjects were off GnRHa and GnRHa/GH treatment at the time of BIA assessment.

The body composition of the NCCAH group was characterized by higher fat and muscle mass compared to the control group. The fat mass was 10.4 kg [IQR 7.15, 16.35] vs 8.2 kg [IQR 5.8, 12.68] (p = 0.005), and the muscle mass was 13.3 kg [IQR 9.15, 17.05] vs 10.8 kg [IQR 7.5, 14.9] (p=0.017). Comparative analyses and graphical depiction of the median of average MFR z-scores, BMI z-scores, and BP percentiles (systolic and diastolic) during repeated visits of the two groups are presented in Figures 2A–D. The NCCAH patients were characterized by significantly higher BMI z-scores (p = 0.001) and lower MFR z-scores (p = 0.045) than their controls, without significant differences in BP percentiles. Graphic visualization of

TABLE 3 Clinical and metabolic characteristics of NCCAH and their healthy controls at last BIA assessment.

	NCCAH	Healthy controls	*p*-value
Age, years	12.3 [8.9, 15.4]	11.5 [8.7, 14.6]	0.526
Anthropometrics			
Height, z-score	-0.01 ± 0.94	-0.36 ± 0.79	**0.004**
Weight, z-score	0.40 ± 0.99	-0.31 ± 1.00	**<0.001**
Body mass index, z-score	0.47 ± 0.97	-0.19 ± 1.04	**<0.001**
Blood pressure, percentiles			
Systolic BP	72.0 [56.0, 83.5]	73 [49.3, 85.8]	0.885
Diastolic BP	67.0 [50.0, 77.0]	60.5 [45.0, 79.0]	0.285
Body composition parameters			
Fat percentage	24.2 [20.8, 29.5]	22.7 [19.4, 25.9]	**0.041**
Truncal fat percentage	18.7 [15.55, 23.95]	17.1 [14.43, 21.23]	**0.039**
Muscle-to-fat ratio, z-score	-0.78 [-1.09, -0.11]	-0.35 [-0.94, 0.15]	**0.029**
Laboratory evaluation			
Glucose, mg/dL	83 [81,91]	85 [81, 89]	0.734
Cholesterol, mg/dL	160.6 ± 26.3	165.5 ± 26.4	0.339
LDL-c, mg/dL	87.1 [71.2, 104.7]	91.9 [80.6, 106.0]	0.190
HDL-c, mg/dL	54 [47, 64]	54 [49, 61]	0.832
Triglycerides, mg/dL	69.5 [50.3, 94.5]	67 [51, 97]	0.920
Non-HDL-c, mg/dL	101.3 [83.9, 119.8]	107 [96, 119.4]	0.213
TG : HDL-c ratio	1.26 [0.89, 1.83]	1.16 [0.94, 1.94]	0.904

Data are expressed as mean and standard deviation or median [interquartile range]. A p value of ≤0.05 was considered significant. Bold indicates significant.
BP, blood pressure; LDL-c Low density lipoprotein cholesterol; HDL-c High density lipoprotein cholesterol; TG, triglycerides

the distributions of BMI z-scores, MFR z-scores, and BP values showed that the two groups had a dissimilar center, spread, and distribution of these clinical parameters (Figure 2). Subgroup analysis by pubertal status revealed no significant differences in MFR z-scores in both the NCCAH group and the healthy controls.

Stepwise linear regression models were applied to evaluate explanatory variables for MFR z-scores, BP percentiles, lipid profiles, and glucose metabolism values in the NCCAH group, (Table 4). The final model for higher MFR z-scores ($R^2 = 0.253$, $p < 0.001$) included higher SEP index ($p = 0.003$), lower birthweight z-scores ($p = 0.013$), and longer duration of hydrocortisone treatment ($p = 0.023$). The final model for higher systolic BP percentile ($R^2 = 0.166$, $p < 0.001$) included lower MFR z-scores ($p < 0.001$). The final model for higher TG/HDL ratio ($R^2 = 0.116$, $p = 0.024$) included lower MFR z-scores ($p = 0.024$). No significant variables were found for glucose levels.

Discussion

In this observational study children and adolescents with NCCAH did not have a greater rate of overweight and obesity than their healthy sex- and age-matched controls. They did, however, have an unfavorable body composition, with an imbalance between muscle and adipose tissue. Factors, such as a lower SEP and higher birthweight z-scores adversely affected their body composition while the duration of hydrocortisone therapy was found to be beneficial.

Our results revealed that youth with NCCAH had a similar prevalence of overweight/obesity compared to the general Israeli pediatric population (44). These results are in line with a recent Israeli study on the prevalence of overweight/obesity in adolescents and young adults with NCCAH (11). Most studies report on an increased BMI in children and adults with classic and non-classic CAH (8, 45–51). Of note, the focus of those studies was on the classic form of CAH, while the NCCAH form was under-represented. A Swedish study that compared patients with 21-hydroxylase deficiency who were listed in national population registries as having either salt-wasting, simple virilizing, or NCCAH found that obesity was consistently increased in all subgroups and most pronounced in patients with NCCAH (52). Although our subjects with NCCAH did not have an increased rate of obesity, they did have higher BMI z-scores on average compared to their healthy controls.

Interestingly, the body composition of our NCCAH subjects was characterized by a low MFR z-score due to higher fat mass, indicating that their muscle mass was relatively low compared to their fat mass, thus placing them at risk for sarcopenic obesity

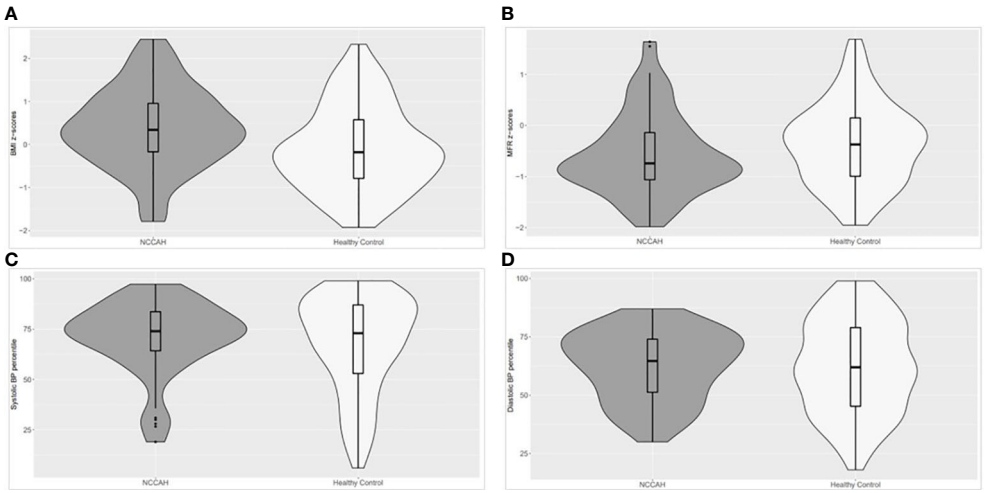

FIGURE 2
Violin plot (distributions of numeric data using probability density curves) depicting the distribution of the median of average BMI z-scores, MFR z-scores, and BP percentiles (systolic and diastolic) in the NCCAH study group and their healthy controls. NCCAH patients are presented in dark grey and healthy controls in white. The violin includes a box plot and summarizes five values: the minimum, first quartile, median, third quartile, and maximum, enabling simultaneous visualization of multiple distributions for comparison. The width describes how frequently that value occurs in the data set: wider regions of the density plot indicate that the value occurs more frequently, and narrower regions indicate that the value occurs less frequently. **(A)** The NCCAH study group had significantly higher BMI z-scores than their healthy controls (median [IQR]: 0.34 [-0.17, 0,96] and -0.18 [-0.79, 0.58], respectively, $p = 0.001$). **(B)** The NCCAH group had significantly lower MFR z-scores than their healthy controls (median [IQR]: -0.74 [-1.06, -0.14] and -0.37 [-0.99, 0.15], respectively, $p = 0.045$). **(C)** The NCCAH study group had similar systolic BP percentiles as their healthy controls (median [IQR]: 74 [64.3, 83.7] and 73 [53.0, 87.0], respectively, NS). **(D)** The NCCAH group had similar diastolic BP percentiles as their healthy controls (median [IQR]: 64.7 [51.3, 74.0] and 62 [42.3, 79.0], respectively, NS).

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TABLE 4 Stepwise linear regression models in the NCCAH study group.

MFR z-scores					
				95% confidence interval	
	R^2	SE	*p* value	lower	upper
Model	0.253		**<0.001**		
Constants	β				
SEP index	0.348	0.114	**0.003**	0.121	0.575
Birthweight z-score	-0.258	0.101	**0.013**	-0.460	-0.055
Duration of hydrocortisone treatment, *years*	0.048	0.002	**0.023**	0.001	0.007
Systolic BP percentiles					
				95% confidence interval	
	R^2	SE	*p* value	lower	upper
Model	0.166		**<0.001**		
Constants	β				
MFR z-score	-9.751	2.668	**<0.001**	-15.077	-4.425
TG : HDL-c ratios					
				95% confidence interval	
	R^2	SE	*p* value	lower	upper
Model	0.116		**0.024**		
Constants	β				
MFR z-score	-0.300	0.128	**0.024**	-0.558	-0.042

Stepwise linear regression models were applied to evaluate explanatory variables for MFR z-scores, systolic BP percentiles, and TG : HDL-c ratios. Variables entered into the models: sex, SEP index, family history of obesity, age (at diagnosis and at BIA), perinatal characteristics (gestational age, birthweight z-scores), and hydrocortisone exposure (mean dose, treatment duration, cumulative dose); MFR z-scores were also included for SBP percentiles and TG : HDL-c ratios. Girls compromised the reference group for sex, and lack of family history of obesity compromised the reference group. A p-value of ≤0.05 was considered significant. Bold indicates significant.
NCCAH, non-classic congenital adrenal hyperplasia; MFR, muscle to fat ratio; SEP, Socioeconomic position; BP, blood pressure; TG, triglycerides; HDL-c High density lipoprotein cholesterol.

(53). Sex differences in body composition are primarily attributable to the level and action of sex steroid hormones that drive the dimorphisms during pubertal development. Sexual dimorphism in human body composition is evident from fetal life, but becomes more pronounced during puberty. At birth, males have a similar fat mass as females but are longer in stature and have greater lean mass (54, 55). Adult males having greater muscle mass (56), larger and stronger bones (57), and reduced limb fat, with a similar degree of central abdominal fat. Females have a more peripheral distribution of fat in early adulthood (58). These differences mandate a sex-adjusted interpretation of the body composition measurement. The comparison between boys and girls with NCCAH in our study revealed similar unfavorable sex- and age-adjusted body composition parameters in both sexes. There are scarce data on body composition and the role of hyperandrogenism in subjects with NCCAH. A recent study on 30 adults (5 males) with NCCAH that assessed body composition by means of BIA reported similar lean and fat mass in comparison to healthy controls (59). Another study that assessed body composition with DEXA in 12 children and adolescents with NCCAH reported higher lean body mass adjusted for sex, age, height, and pubertal status (46). Those authors concluded that the greater lean body mass and parameters of insulin resistance in children with NCCAH most likely reflect the adverse metabolic effects of prolonged postnatal androgen excess (46). Our findings on body composition are not comparable to those of previous reports since we utilized a composite score of both muscle and fat components. The interaction between circulating androgens and body composition parameters is complex: hyperandrogenism may contribute to increased muscle mass, but contrarily, it may lead to increased regional adiposity (60). While we can offer no solid explanation for our findings, we speculate that the phenotypic spectrum of subjects with NCCAH and their ability to accumulate muscle mass are also affected by androgen receptor sensitivity (61). Variable androgen receptor sensitivity may modulate the emergence of premature adrenarche in children with NCCAH and determine their clinical manifestations (61).

Glucocorticoid therapy in subjects with NCCAH is reserved and tailored for symptomatic cases of hyperandrogenism (5). Clinicians are aware of the possible adverse metabolic implications of glucocorticoid therapy (6) and the fine balance required to avoid sustained hyperandrogenism or glucocorticoid overtreatment (7). It is encouraging that prolonged glucocorticoid exposure, with substantial cumulative doses, apparently did not adversely affect the body composition of our NCCAH patients. Moreover, duration of glucocorticoid exposure was found to harbor a protective effect on the balance between muscle and fat tissue. Early diagnosis of

NCCAH and initiation of glucocorticoid therapy in a timely manner has been shown to have a beneficial effect on adult height (62). Of note, the mean daily dose of hydrocortisone in our group was low (6.59 mg/m^2) compared to that of Eyal et al.'s multicenter study conducted in Israeli patients with NCCAH (12.8 ± 4.0 mg/m^2) (62). Our observations support the accumulating evidence that glucocorticoid therapy aimed to alleviate androgen overproduction is advantageous.

MFR was found to be an indicator for metabolic syndrome and its components in adults (63–65). We had earlier demonstrated the predictive value of MFR z-scores in assessing CVD risk factors in youth with overweight and obesity (26), and its association with hypertension in underweight/overweight children (25). The current study expands upon the clinical implications of MFR assessment in children, namely, that a lower MFR z-score is associated with both a higher systolic BP and a higher atherogenic dyslipidemia index (TG : HDL-c ratio) in young patients with NCCAH, possibly predicting future development of metabolic syndrome in their adult life (66).

Both classic and non-classic CAH was associated with established excess cardiovascular and metabolic morbidity in a large Swedish cohort (52). However, the mechanism remains unknown since glucocorticoid dose and duration as well as other clinical characteristics were not assessed (52). In addition, previous studies usually report on the corticosteroid dosage at the time of the study while the cumulative dose during the entire treatment period remains obscure. Of note, our patients with NCCAH did not have higher rates of elevated BP or dyslipidemic lipid profiles. Further prospective studies on larger cohorts are warranted to explore the link between the diagnosis of NCCAH, the level of hyperandrogenism, glucocorticoid exposure, and excess cardiovascular and metabolic morbidity.

Early onset of metabolic complications may also stem from other factors that are unrelated to NCCAH pathophysiology. Our NCCAH group was characterized by higher rates of prematurity compared to healthy controls. Preterm birth has been strongly associated with metabolic syndrome components and cardiovascular disease in adult life (67). Of note, gestational age did not emerge as an explanatory factor in the models predicting metabolic syndrome components in our patient population.

Another key factor in determining metabolic risk is adversative socioeconomic circumstances (68). In our study, a lower SEP index was identified as significant predictor for unfavorable body composition, leading us to speculate that socioeconomic circumstances may affect the risk for metabolic derangements in our patients with NCCAH throughout life. This finding should be taken into consideration in clinical management, and the appropriate social services should be consulted.

The main limitation of this study is its retrospective design which does not allow us to establish causality between exposure to hydrocortisone and metabolic outcomes. Hydrocortisone doses were calculated according to the parent-reported doses documented in the medical files, thereby lacking data on drug accountability and introducing information bias. In addition, our study did not include questionnaires for evaluating nutritional, behavioral, and psychological aspects that may affect weight status and body composition. Of note, we recently reported that health-related quality of life was not adversely affected by NCCAH among adequately treated children and adolescents (69) Although our tertiary care center serves all sectors of the Israeli population, including patients of various ethnic origins and SEP from both urban and rural areas, this study may be prone to selection bias and may not be representative of the Israeli population with NCCAH. The main strength of the present study lies in the relatively long follow-up of a NCCAH cohort attending a single tertiary medical center and which underwent a comprehensive uniform evaluation of anthropometric, body composition, BP and pubertal status measurements by the same trained medical personnel. The use of healthy controls, and the calculation of sex- and age-adjusted z-scores/percentiles allows for comparisons between subjects and more robust interpretation of the data. To the best of our knowledge, this is the first study to explore MFR z-scores and cumulative hydrocortisone doses in a pediatric population with NCCAH.

Conclusion

In conclusion, our findings suggest that youth with NCCAH have a body composition characterized by an imbalance between muscle and fat tissue, placing them at an increased risk for early-onset cardiometabolic derangements. Implementation of BIA as a part of routine assessment may assist in the identification of cardiometabolic risk factors in youth with NCCAH, thus enabling the physician to apply interventions to those in need of risk reduction. It is reassuring that low-dose glucocorticoid therapy in pediatric patients with NCCAH aimed to alleviate androgen overproduction does not appear to adversely affect body composition.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Tel Aviv Sourasky Medical Center IRB. Written informed consent from the participants' legal guardian/next of

kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

Author contributions

ABS designed the study, gathered, analyzed and interpreted the data, wrote the first draft, revised the final manuscript and decided on submission. AB conceived and designed the study, analyzed and interpreted the data, contributed to the first draft and revised the manuscript incorporating contributions from coauthors, and decided on submission. AS-B conceived and designed the study, contributed to the data used in this study, reviewed and edited the manuscript, and contributed to the discussion. MY-G assisted in statistical analysis, interpreted the data of the study and contributed to the discussion. AU, ASD, AA, AO, OE and NW contributed to the data used in this study, reviewed and edited the manuscript, and contributed to the discussion. YL conceived and designed the study, analyzed and interpreted the data, critically revised the manuscript incorporating contributions from coauthors and decided on submission. All authors have read and approved the final manuscript. YL is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Dexamethasone application for *in vitro* fertilisation in non-classic 17-hydroxylase/17,20-lyase-deficient women

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Context: High progesterone levels in the follicular stage interfere with the implantation window, causing infertility in women with 17-hydroxylase/17,20-lyase deficiency (17OHD). Dexamethasone can restore cortisol deficiency and suppress inappropriate mineralocorticoid secretion to control hypertension in 17OHD patients, but poses risks to the foetus if administered during pregnancy.

Objective: We prospectively explored a rational glucocorticoid use protocol for assistive reproduction in a woman with non-classic 17OHD that reduced glucocorticoid side effects.

Method: In this study, the treatment protocol for this 17OHD patient included the following steps. First, the appropriate type and dose of glucocorticoid for endogenous progesterone suppression was determined. Then, glucocorticoid was discontinued to increase endogenous progesterone levels for ovarian stimulation. Next, dexamethasone plus GnRHa were used to reduce progesterone levels in frozen embryos for transfer. Once pregnancy was confirmed, dexamethasone was discontinued until delivery.

Results: Dexamethasone, but not hydrocortisone, reduced progesterone levels in the 17OHD woman. After endogenous progesterone-primed ovarian stimulation, 11 oocytes were retrieved. Seven oocytes were 2PN fertilised and four day-3 and two day-5 embryos were cryopreserved. After administering dexamethasone plus gonadotropin-releasing hormone agonist (GnRHa) to reduce progesterone levels to normal, hormone replacement therapy was administered until the endometrial width reached 9 mm. Exogenous progesterone (60 mg/day) was used for endometrial preparation. Two thawed embryos were transferred on day 4. Dexamethasone was continued until pregnancy confirmation on the 13th day post-transfer. Two healthy boys, weighing 2100 and 2000 g, were delivered at 36 weeks' gestation.

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Conclusion: Rational use of dexamethasone synchronised embryonic development with the endometrial implantation window, while not using in post-implantation avoided its side effects and promoted healthy live births in women non-classic 17OHD undergoing *in vitro* fertilisation.

KEYWORDS

17-hydroxylase/17,20-lyase deficiency, dexamethasone, infertility, *in vitro* fertilization, pregnancy

Introduction

17α-hydroxylase/17,20-lyase (P450c17) is a cytochrome P450 enzyme that is expressed mainly in the adrenal cortex and gonads and plays a role in both 17α-hydroxylase and 17,20-lyase activity in steroid hormone synthesis (Figure 1). The P450c17 is encoded by *CYP17A1* (NM-000102), located on 10q24.3. The *CYP17A1* mutations can cause 17OHD and differences in *CYP17A1* mutations determine clinical manifestations and hormone level variations. Deficiency in 17α-hydroxylase/17,20-lyase (17OHD) was first reported by Biglieri et al. in 1966 (1). Low cortisol levels in 17OHD lead to ACTH hypersecretion and in absence of P450c17, its substrates (deoxycorticosterone, 18-deoxycorticosterone, and progesterone) accumulate, with concomitant product (cortisol, androgen, and oestrogen) reduction (2).

As such, 17OHD is clinically divided into classic and non-classic types. The classic type of 17OHD is characterised by hypertension, hypokalaemia, primary amenorrhoea, and sexual infantilism, whereas non-classic cases may only show infertility (3). Elevated progesterone levels are a key characteristic of 17OHD in both classic and non-classic types (4). High progesterone levels in the follicular stage interfere with the implantation window, resulting in infertility among women with 17OHD (5, 6). The follicular development in females with classic 17OHD is arrested because of the lack of ovarian oestrogen synthesis (7). However, non-classic 17OHD patients can still conceive, because residual enzyme activity allows synthesis of low levels of oestrogen. Therefore, assisting such patients in achieving pregnancy is a clinically important. Because of the rarity of such cases and the complexity of the condition, only a few patients with non-classic17OHD have obtained live births by *in vitro* fertilisation (IVF) and frozen transfer (8–11). In the IVF process of 17OHD infertile women, glucocorticoids were routinely used to reduce elevated blood progesterone (8–12), but long-term use of glucocorticoids during pregnancy can increase the risk of side

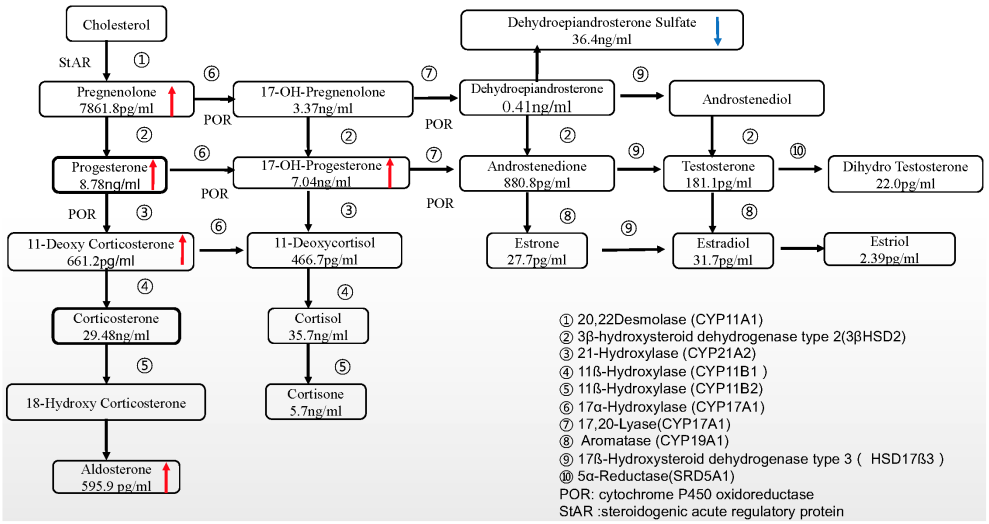

FIGURE 1
Profiles of steroid hormones detected by mass spectrometry.

effects of mothers and fetuses, and even cause fetal malformations (12–14).

We conducted this prospective case study to explore a rational glucocorticoid use protocol for IVF in women with 17OHD, while reducing the side effects of dexamethasone in foetuses and mothers with non-classic 17OHD-related infertility.

Methods and results

Case characteristics

A 28-year-old infertile woman with non-classic 17OHD presented with main complaints of irregular menstruation and primary infertility. She was diagnosed with non-classic 17OHD using steroid hormone profiling (Figure 1), adrenocorticotrophic hormone stimulation test, and genetic testing. The patient exhibited relatively normal secondary female phenotypes. Clinical history revealed that menarche occurred at the age of 14 years, and menstruation cycles varied between 2 and 3 months and lasted 3-4 days. Appearance of spontaneous cysts on ovaries was noted intermittently. The adrenal magnetic resonance imaging results, blood pressure, and plasma potassium levels were normal. The basic maternal progesterone levels in the follicular phase (reference range: 0.31–1.52 ng/mL) are listed in Table 1. The low dose dexamethasone test (0.5 mg, every 6 hours for 2 days) showed marked cortisol inhibition and normal progesterone levels. Genetic testing revealed two mutations in *CYP17A1*, including c.932_939 del TTAAATGG (chr10-104592780–104592787 mat) and c.403T >C (chr10-104595044 pat). c.403T>C(p.F135L) variant was not reported in HGMD and ClinVar databases. The population frequency of c.403T>C variants was not found in gnomAD, Exac and 1000genomes databases(PM2-supporting). Through the analysis of NGS data of the family, c.932_939delTTAAATGG variant was inherited from the mother, while c.403T>C was inherited from the father, which is consistent with the in trans variants' transmission in the autosomal recessive pattern of inheritance for 17OHD(PM3). Multiple lines of computational evidence(SIFT, PROVEAN、Polyphen-2_HDIV and Polyphen-2_HVAR) supported a deleterious effect on the gene(PP3). According to ACMG classification, c.932_939delTTAAATGG was classified as pathogenic, while c.403T>C was classified as a variant of uncertain significance.

Treatment protocol

Due to the asynchronous development of follicles and the endometrium, we performed IVF. The treatment protocol included the following four steps. First, we determined the appropriate type and dosage of glucocorticoids for endogenous progesterone inhibition. Second, we stopped using glucocorticoids to increase endogenous progesterone levels for progesterone-primed ovarian stimulation (PPOS). Third, after ovulation induction and egg retrieval, dexamethasone plus GnRHa were used to reduce progesterone levels for frozen embryo transfer. Fourth, we discontinued dexamethasone administration once pregnancy was confirmed, until delivery.

Determination of the appropriate glucocorticoid type and dosage for endogenous progesterone inhibition

In this case study, use of hydrocortisone (Xinyi Pharmaceutical Co., Ltd, Shanghai, China) to reduce

TABLE 1 Hormone levels of the patient.

	Units	Reference values	Baseline values	Hydrocortisone			Dexamethasone	
				Before treatment	10 mg bid for 14 days	10 mg tid for 14 days	Before treatment	0.375mg Qd for 14 days
FSH	mIU/ml	3.85–8.78	5.87					
LH	mIU/ml	2.12–10.89	7.86					
E	pg/ml	27–122	32					
P	ng/ml	0.31–1.52	5.11	2.39	4.13	4.26	5.01	0.5
T	ng/ml	0.1–0.75	0.27	0.06	0.33	0.02		<0.01
PRL	ng/ml	3.34–26.72	9.58					
Cortisol	μg/ml	4.4–19.9	15.31					
ACTH	pg/ml	7.2–63.3	43.23					
K	mmol/L	3.5–5.3			3.57	3.86		
Glu	mmol/L	3.61–6.11			6.21	5.54		
17OHP	ng/ml	0.1–0.8	6.2					

FSH, follicle-stimulating hormone; LH, luteinising hormone; E, oestrogen; P, progesterone; T, testosterone; PRL, prolactin, ACTH, adrenocorticotropic hormone; Glu, glutamate; 17OHP, 17-hydroxyprogesterone; bid, twice a day; tid, three times a day.

progesterone levels was first tested because of its mild side effects on foetuses. However, the progesterone level did not reduce successfully, even after increasing the drug dose to 30 mg/day. Hydrocortisone was discontinued when the patient developed Cushing's symptoms (facial rounding and weight gain). Two months later, the medication was switched to dexamethasone (Lisheng Pharmaceutical Co. Ltd. Tianjin, China), starting with a low dose (0.375 mg/day at bedtime). After 14 days, her progesterone level dropped to 0.5 ng/ml, without obvious adverse reactions. We chose dexamethasone 0.375 mg daily to control the patient's pre-transfer progesterone level. The hormone changes are listed in Table 1.

Use of endogenous progesterone to block the luteinising hormone surge without glucocorticoid application

After the dose and type of glucocorticoid were determined, dexamethasone administration was stopped. This resulted in an increase in endogenous progesterone (5.07 ng/ml) in the follicular phase. Human menopausal gonadotropin (hMG; Menopur, Ferring Pharmaceuticals, Saint-Prex, Switzerland) and urofollitropin for injection (uFSH, Livzon Pharmaceutical Group, Inc., Zhuhai, China) were used for 13 days of controlled ovarian stimulation. The hormone levels achieved were listed in Table 2. This treatment resulted in two dominant follicles, approximately 18 mm in size. We used 250 μg of Ovitrelle (Merck, Darmstadt, Germany) to induce oocyte maturation. Thirty-six hours later 11 oocytes were retrieved. Seven oocytes underwent normal 2PN fertilisation, of which four day-3 embryos and two day-5 embryos were cryopreserved.

Dexamethasone plus GnRHa reduce endogenous progesterone for hormone replacement therapy

After the first menstrual cycle post-retrieval, we used long-acting GnRHa—triptorelin acetate (Dophereline, Ipsen, Paris, France) 3.75 mg once, followed by 0.375 mg dexamethasone daily to reduce endogenous progesterone, as hormone replacement therapy prior to frozen embryo transfer. One month later, the patient's progesterone level had reduced to 0.04 ng/ml. Oral oestradiol was used until the endometrial width reached 9 mm. During this period, the blood progesterone level was 0.21 ng/ml. Therefore, exogenous progesterone (60 mg/day) was administered to prepare the endometrium. On the 4th day of progesterone supplementation, two thawed embryos were transferred.

Discontinuation of dexamethasone from pregnancy confirmation until delivery

Thirteen days after embryo transfer, when serum human chorionic gonadotropin (hCG) was 864.25 IU/ml, dexamethasone use was discontinued. By 28 days after IVF, transvaginal ultrasound examination detected viable pregnancy of twins in the uterus. During early pregnancy, as the fasting blood glucose level was slightly elevated, insulin was used to control the blood glucose to the target level. The patient's blood pressure remained normal during pregnancy. The ultrasound results throughout pregnancy were normal. In the 36th week of pregnancy, two healthy baby boys were delivered, weighing 2100 g and 2000 g. We have been following the postnatal development of the twins, and their motor and language developments were normal so far (15 month old). The introduction of the treatment process are listed in Figure 2.

Discussion

This report highlights the rational use of glucocorticoid, particularly dexamethasone rather than hydrocortisone, in women with 17OHD undergoing IVF. First, this process includes the use of endogenous high progesterone levels to promote ovulation without the use of glucocorticoids. Second, we used dexamethasone to reduce endogenous progesterone levels for hormone replacement therapy before transfer of frozen embryos. Glucocorticoid use was discontinued from pregnancy confirmation to delivery. This approach facilitated the birth of healthy twin boys to an infertile woman with non-classic 17OHD.

High progesterone levels disturb the embryo implantation window. Thus, progesterone levels need to be reduced during embryo transfer (5). However, high progesterone levels are not a

TABLE 2 Hormone levels during controlled ovarian hyperstimulation.

	D2	D6	D8	D11	D12	D13	D14
FSH (mIU/ml)	5.6	8.13	8.76	8.55	12.63	15.98	17.22
LH (mIU/ml)	9.11	4.58	2.76	1.92	1.47	1.33	1.34
E2 (pg/ml)	43	275	663	1114	1187	1395	2186
P (ng/ml)	5.07	3.15	4.17	3.34	3.57	4.02	5.47

D, The menstrual cycle day in controlled ovarian stimulation; FSH, follicle-stimulating hormone; LH, luteinising hormone; E2, oestradiol; P, progesterone.

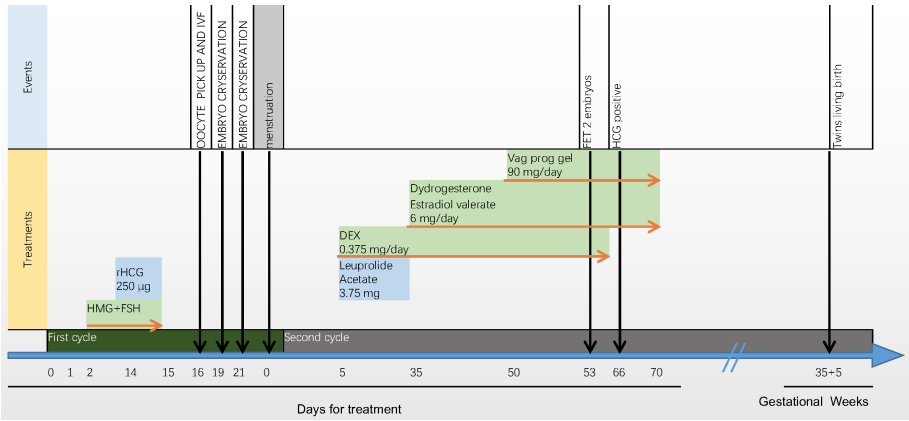

FIGURE 2
Introduction of the treatment process. The oestradiol concentration moderately increased from 43 pg/ml to 2186 pg/ml, and the progesterone concentration remained relatively constant from 3.15 ng/ml to 5.47 ng/ml. After triggering by human chorionic gonadotrophin (hCG), 11 oocytes were retrieved, of which seven were fertilised. Four day-3 and two day-5 embryos were cryopreserved. Leuprolide acetate (3.75 mg daily) and a low dose of dexamethasone (0.375 mg daily) were administered in the first cycle after ovum pick-up (OPU). One month later, 14 days after oestradiol valerate administration, the endometrial width reached 9 mm. Exogenous progesterone (60 mg daily for 3 days) was added, and frozen–thawed embryo transfer was performed. After 3 days, two embryos (8/II/1 and 7/II/1) were transferred.

barrier to controlled ovarian hyperstimulation. Previous studies have shown that ovarian stimulation could be performed under the blockade of endogenous or exogenous progesterone to suppress early onset LH peaks (15). Progesterone levels higher than 2 ng/ml before oestrone administration are sufficient to inhibit the positive action of oestrogen on LH release (16–18). In this study, we stopped applying glucocorticoid pre-stimulation to allow return of progesterone to high levels to perform the PPOS protocol. The protocol presented in this study resulted in relatively high oestrogen levels and qualified embryos. We propose that this is an appropriate controlled ovarian stimulation strategy for women with 17OHD. Although Xu et al. used endogenous progesterone in controlled ovarian stimulation (19), the patient had a high progesterone level of > 30 ng/ml. Our case study reports detailed information on various hormone levels, and conditions suitable for endogenous progesterone stimulation, the data on which is lacking.

In 17OHD cases, elevated progesterone is very common in controlled ovarian stimulation, despite the use of dexamethasone (8, 9, 11, 20). This may be due to the deficiency of 17OH in the developing ovarian follicles. Embryo cryopreservation is the better protocol. In all previous reports of embryo transfer in 17OHD cases, frozen embryo transfer was used for all live births (9, 10, 12, 19). We used a hormone replacement cycle for frozen embryo transfer, and a regimen of lowering endogenous progesterone with dexamethasone plus GnRHa before the transfer. The GnRHa was used to inhibit follicular development and avoid high progesterone levels due to 17OHD in the ovary.

Azziz et al. reported that there were altered enzyme kinetics and overactivation of the renin–angiotensin–aldosterone axis in 21OHD cases (21). Increasing fludrocortisone dosage in patients with increased plasma renin activity and 17a-hydroxyprogesterone concentration resulted in a better control of congenital adrenal hyperplasia hormone disorders (22). In 17OHD, over-secretion of corticosterone, 11-deoxycorticosterone (DOC), 18-hydroxy-DOC, 18-hydroxycorticosterone, and 19-norDOC leads to low-renin hypertension and hypokalaemia (23). Irrespective of whether hypertension is present, glucocorticoids have been used in 17OHD women undergoing IVF, from egg retrieval to delivery, in previous studies (9, 10, 12, 19). In this study, we found that dexamethasone, and not hydrocortisone, effectively reduced the patient's high progesterone levels. We deduced that hydrocortisone has mineralocorticoid activity, which further inhibits renin–angiotensin, decreases CYP11B2 activity, and leads to increased progesterone accumulation. Another reason may be the longer half-life of dexamethasone and its strong inhibitory effect on the hypothalamus–pituitary–adrenal gland axis.

Dexamethasone can also cause adverse effects during IVF. Ben-Nun et al. reported an IVF pregnancy was terminated due to uncontrolled hypertension at 25 weeks (12). The long-term use of dexamethasone can also cause hypertension and hyperglycemia (24). Foetal malformations can also arise due to dexamethasone use (14). To reduce dexamethasone-related side effects, Xu et al. used dexamethasone before frozen embryo transfer and changed the glucocorticoid to prednisone after embryo transfer. However, the first pregnancy was terminated because the foetus had cleft lip and palate (19). Therefore, we stopped glucocorticoid administration once pregnancy was detected to promote pregnancy and avoid the side effects of administration on foetus. Our patient did not have hypertension or hypokalaemia. We speculate that glucocorticoids are not necessary to maintain pregnancy.

Our case study is not without limitations. Our report is based on a single case and further research with robust study designs are crucial to reinforce our observations.

Conclusion

In this 17OHD infertile case, use of dexamethasone for IVF reduced glucocorticoid-associated side effects and promoted healthy live births. Glucocorticoids were only used to reduce progesterone levels in the artificial endometrial scheme and were not used during controlled ovary stimulation and post-implantation pregnancy. Our study provides a referable proof for patients with non-classical 17OHD who report basic progesterone level > 2 ng/ml and do not present with hypertension or hypokalaemia. However, owing to the low incidence of 17OHD, more cases are needed for further validation.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the ethics committee of Peking university, first hospital. The patients/participants provided their written informed consent to participate in this study.

Author contributions

X-LY, T-TZ designed the research. X-LY, T-TZ, JS, QX, Y-RK, SW and YX conducted the research. X-LY, T-TZ wrote the article and X-LY had primary responsibility for final content. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Clinical characteristics of a male child with non-classic lipoid congenital adrenal hyperplasia and literature review

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Background: Lipoid congenital adrenal hyperplasia (LCAH) is a rare and severe disorder that is caused by mutations in the steroidogenic acute regulatory protein (StAR). Non-classic LCAH is defined as late-onset glucocorticoid deficiency and even complete male external genitalia in 46,XY individuals. However, to date, few cases of non-classic LCAH have been reported.

Methods: It was attempted to describe the clinical characteristics of a male child with complete male external genitalia in terms of age of onset, adrenal function, and biochemical indicators. Previously reported cases were also reviewed to investigate the relationship of age of onset with enzymatic activity in non-classic LCAH.

Results: The patient with complete male external genitalia was diagnosed with non-classic LCAH, in which the reason for his referral to a local hospital at the of age 1.25 years was progressive skin hyperpigmentation, and plasma adrenocorticotropic hormone (ACTH) level was elevated to higher than 1,250 pg/ml. The compound heterozygous mutations c.772C>T/c.562C>T in *STAR* gene were identified *via* genetic testing. The literature review resulted in identification of 47 patients with non-classic LCAH from 36 families. The mutational analysis showed that c.562C>T mutation was prevalent in patients with non-classic LCAH, accounting for 37.2% of the total mutant alleles, which could reflect the founder effect on the non-classic LCAH population. In total, 28 46,XY patients were reported, including 22 (78.5%) cases with complete male external genitalia and six (21.5%) cases with different degrees of hypospadias.

Conclusion: The clinical phenotypes of non-classic LCAH are highly variable. Routine physical examination, laboratory measurement, genetic testing, and, importantly, enzymatic activity assay may facilitate the early diagnosis of non-classic LCAH. The age of primary adrenal insufficiency (PAI) onset may not be a diagnostic basis for non-classic LCAH, and enzymatic activity assay determination may be more effective.

KEYWORDS

lipoid congenital adrenal hyperplasia, non-classic LCAH, steroidogenic acute regulatory protein, enzymatic activity, phenotype and genotype

Introduction

Lipoid congenital adrenal hyperplasia (LCAH) is a rare and severe disorder that is caused by mutations in the steroidogenic acute regulatory protein (StAR) (1). StAR is a cytoplasmic enzyme with 285 amino acids encoded by the *STAR* gene located on chromosome 8p11.2 and consists of seven exons. It plays an indispensable role in the delivery of cholesterol from the outer to the inner mitochondrial membrane in the initial steps of steroid synthesis (2). Patients with LCAH are typically characterized by lipoid accumulation in the adrenal glands, adrenal insufficiency in infants, and female external genitalia regardless of karyotype. Regarding several cases caused by *STAR* gene mutations, LCAH is divided into classic LCAH and non-classic LCAH according to sex reversal and age of onset (3). Non-classic LCAH is defined as late-onset glucocorticoid deficiency and even complete male external genitalia in XY individuals. The present study aimed to report the clinical and molecular characterization of a Chinese male child with complete male external genitalia who was diagnosed with non-classic LCAH. Previously reported cases were also reviewed to enhance the understanding of non-classic LCAH.

Methods

Patient

In the present study, the patient has undergone extensive physical and laboratory examination for the evaluation of primary adrenal sufficiency. Collection of his clinical features and laboratory data was attempted when he was initially admitted to a hospital for adrenal insufficiency. Routine examination included testing of liver and kidney functions and measurement of the levels of serum insulin-like growth factor-1 (IGF-1) and insulin-like growth factor-1-binding protein 3 (IGFBP3). Furthermore, assessment of renin-angiotensin-aldosterone system (RAAS) function and gonadal function, measurement of plasma adrenocorticotropic hormone (ACTH) and cortisol levels, and ACTH stimulation test were performed. Bone age was assessed using the Greulich–Pyle (G-P) and Tanner–Whitehouse 3 (TW3) methods. Clinical data on the first and follow-up visits were collected, including birth history, history of growth and development, physical examination, laboratory data, and radiological data.

Molecular and enzymatic activity assays

Genomic DNA was extracted from the peripheral blood using the DNA extraction kit (Qiagen Hilden, Germany). Primers were designed according to the *STAR* gene sequence from GenBank. The *STAR* gene was amplified using polymerase chain reaction (PCR). The enzymatic activity of four mutants of the *STAR* gene was herein investigated, including some mutations that had been functionally analyzed previously or merely reported. Non-steroidogenic COS-7 cells (CRL-1651; http://www.atcc.org) were cultured in Dulbecco's modified Eagle's medium (DMEM; Life Technologies Corp., Carlsbad, CA, USA) supplemented with 12% fetal bovine serum and 1% antibiotics (50 units/ml penicillin and 50 μg/ml streptomycin) at 37°C in an incubator (5% CO_2 with humidity of 95%). Cells were seeded into 12-well plates and transfected using Lipofectamine 2000 reagent (Invitrogen Inc., Carlsbad, CA, USA) with 1 μg of empty plasmid, wild-type *STAR*, or mutant *STAR* constructs together with 1 μg of F2 plasmid, which were kindly provided by Walter L. Miller, UCSF, San Francisco, USA (4), at a confluence of approximately 80%. After 48 h of transfection, the culture supernatants were collected and the pregnenolone synthesis from endogenous cholesterol or 22(R)-hydroxycholesterol was assayed with an ELISA kit (ALPCO Diagnostics Inc., Salem, NH, USA). The empty plasmid served as a negative control to measure StAR-independent steroidogenesis. In the positive control, soluble hydroxysterol 22(R)-hydroxycholesterol (5 μg/ml; Sigma-Aldrich Chemie GmbH, Munich, Germany) was added. It is widely

accepted that 22(R)-hydroxycholesterol can directly enter the mitochondria for the synthesis of steroid hormones without the contribution of enzymes. Therefore, the positive control reflects the maximum production potential of various enzymes in the cells to catalyze the production of pregnenolone. All experiments were carried out in triplicate, and each experiment was repeated three times. In the positive well, cells were incubated with 3 µl 22R-hydroxycholesterol (22R-OH) as the physiological source of cholesterol pregnenolone that was synthesized from endogenous cholesterol or 22(R)-hydroxycholesterol.

Literature review

"Congenital lipoid adrenal hyperplasia" was searched in the PubMed and Wanfang databases to retrieve English and Chinese published articles. A total of 106 articles were published until September 2021, in which the diagnosis of non-classic LCAH was confirmed by genotyping, and participants' clinical characteristics were described.

Statistical analysis

All data were analyzed using Microsoft Excel software.

Results

Case presentation

The Chinese male child was born at a gestational age of 37 weeks with complete male external genitalia (descended testes, micropenis without hypospadias). His birth weight was 3.34 kg, and his birth length was 50 cm. He had no history of adrenal insufficiency, and the only reason for his referral to a local hospital at the age of 1.25 years was progressive skin hyperpigmentation. Laboratory examination revealed plasma ACTH of more than 1,250 pg/ml (normal range, 7–65 pg/ml), cortisol 8 a.m. was 1.36 µg/dl (normal range, 6.7–22.6 µg/dl), cortisol 4 p.m. was 0.97 µg/dl (normal range, 6.7–22.6 µg/dl), and testosterone level was <0.1 ng/ml, and he was therefore diagnosed with primary adrenal insufficiency (PAI). Hydrocortisone was given as treatment for PAI. At the age of 43 months, he was referred to our department for the elevated ACTH level and a micropenis. His height and weight were 111 cm [+2.9 standard deviation (SD)] and 24 kg (+3 SD), respectively; his blood pressure was 86/56 mmHg. Examination showed generalized hyperpigmentation of the skin. His stretched penile length was 1.5 cm, testicular volume was 3 ml, and Tanner stage of PH1 was recorded. Laboratory examination revealed that the levels of sodium and potassium were 135 (range, 130–147) and 4.22 (range, 3.5–5.1) mmol/L, and no hyperkalemia and hyponatremia were detected. Moreover, the following laboratory data were collected: plasma ACTH level of 1,744 (normal range, 12–78) pg/ml, basal 17-hydroxyprogesterone (17-OHP) level of 0.07 (normal range, 0.07–1.53) ng/ml, cortisol level of 0.2 (normal range, 6.7–22.6) µg/dl, plasma RAAS activity of 9.99 (normal range, 0.1–5.5) ng/ml/h, aldosterone (Ald) level of 0.1 (normal range, 29.4–161.5) pg/ml, angiotensin II level of 134.22 (normal range, 18–103) pg/ml, and testosterone level of 0.01 (normal range, 1.75–7.81) ng/ml. The ACTH stimulation test showed no changes in the levels of cortisol and 17-OHP (Table 1). Chromosome analysis revealed a 46,XY karyotype. Luteinizing hormone-releasing hormone (LHRH) stimulation test evaluated the hypothalamic-pituitary-gonadal (HPG) axis (Table 2), and the result indicated that the HPG axis was not activated. The ultrasound revealed that the left testis was 21 * 11 * 8 mm^3 and the right testis was 17 * 11 * 10 mm^3, and the adrenal ultrasound displayed normal data without enlargement.

Mutational analysis and enzymatic activity assay

The results of exome sequencing revealed compound heterozygous *STAR* mutation for c.772C>T (p.Q258X) and c.562C>T (p.R188C). Pedigrees of his family with *STAR* gene mutations and Sanger sequencing confirmation of compound heterozygous mutations analyzed by the direct DNA sequencing are shown in Figure 1. The activity of StAR was assessed, and it was found that the wild-type mutation had 0.35%/10.47% activity (Figure 2).

Literature review

All English and Chinese published articles on non-classic LCAH published until September 2021 and indexed in the PubMed and Wanfang databases were retrieved. In total, 14 mutations in 47 patients from 36 families were found to be associated with non-classic LCAH (Table 3) (3–17). As shown in Table 3, 46,XY and 46,XX patients accounted for 59.6% (28/47) and 40.4% (19/47), respectively. Moreover, 22 male non-classic LCAH

TABLE 1 The results of ACTH stimulation test.

	0 min	30 min	60 min
Progesterone(0.14-2.06 ng/ml)	0.01	0.01	0.01
17-OHP(0.07-1.53 ng/ml)	0.07	0.03	0.04
Cortisol(6.7–22.6 µg/dl)	0.2	0.41	0.22
Dehydroepiandrosterone sulfate (5-57 µg/dl)	2.9	3.1	2.9
Androstenedione(0.15-3.10 ng/ml)	0.15	0.11	0.05
Testosterone (1.75-7.81 ng/ml)	0.01	0.01	0.01
Ald(29.4-161.5 pg/ml)	0.1	0.1	0.1

17-OHP, 17-hydroxyprogesterone; ACTH, adrenocorticotropic hormone; Ald, aldosterone.

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TABLE 2 The results of LHRH stimulation test.

	0 min	30 min	60 min	90 min
Luteinizing hormone (IU/L)	0.3	3.6	2.8	1.5
Follicle-stimulating hormone (IU/L)	0.6	3.7	4.4	3.9

LHRH, luteinizing hormone-releasing hormone.

patients with complete male external genitalia (78.5%) and six cases of hypospadias with different degrees (21.5%) were identified. Figure 3 shows the diagram of the *STAR* gene indicating the location of mutations (including enzymatic activity) identified in patients with non-classic LCAH. These mutations included 14 missense mutations without nonsense, frameshift, and splice-site mutations. Moreover, c.562C>T,STAR-R188C was detected as the most common mutation of *STAR* gene in non-classic LCAH patients, accounting for 37.2%, including 14 homozygous mutations and one compound heterozygous mutation. Enzymatic activity assay showed that c.562C>T,STAR-R188C mutation had reserved 13.6% (3) and 12.8% (4) activity, sufficient to maintain a virilizing phenotype. The other less common mutations included STAR-E123K (2%), STAR-T164K (1%), STAR-L178Q (1%), STAR-V187M (3%), STAR-R192C (7%), STAR-A218V (5%), STAR-G221S (4%), STAR-G221D (2%), STAR-M255T (2%), STAR-L260P (1%), STAR-F267S (1%), STAR-R272C (4%), and STAR-L275P (1%). Enzymatic activity of <10% was found in two patients, including one 46,XY patient with compound heterozygous mutations p.L260P and p.F267S who was born with a micropenis, third-degree hypospadias, severe chordee, a hypoplastic scrotum, and palpable testes, with enzymatic activities of 1.8% and 9.5%; the other case was a 46,XX girl with homozygous G221D mutation who first presented at the age of 11 months with adrenal crisis including vomiting that led to the diagnosis of congenital adrenal hyperplasia (CAH), and an enzymatic activity of 6.3% was recorded.

Discussion

1. Clinical and molecular characterization of the patient

LCAH is a rare and fatal disease caused by loss of functional mutations of StAR. To date, more than 190 patients with LCAH caused by 70 *STAR* mutations have been reported (17). Patients with classic LCAH were typically characterized by lipoid accumulation in the adrenal glands, adrenal insufficiency in infants, and female external genitalia, regardless of karyotype. Some patients with *STAR* mutations developed hyperpigmentation and PAI after infancy, especially 46,XY patients presented with male external genitalia, and they were classified as non-classic LCAH (3). In the present case report, the patient had a micropenis with normal testes and hyperpigmentation at the age of 7 months. Laboratory investigation revealed a high ACTH level and low levels of cortisol and testosterone, and the patient was diagnosed with PAI with signs of mineralocorticoid and glucocorticoid deficiency and adrenal androgen deficiency. Based on the presence of a micropenis and the later onset of hyperpigmentation, along with failed ACTH stimulation test and XY karyotype, we suspected the diagnosis of mutations of *NR0B1*, *CYP11A1*, and *STAR* genes or familial glucocorticoid deficiency (FGD) (*MC2R*, *MRAP*, *NNT*, and *AAAS*). Gene sequencing is the most appropriate diagnostic method to differentiate these diseases. Ishii et al. (9) described non-classic LCAH as either Quigley grade 1 in XY karyotype, no episode of salt losing or requirement of fludrocortisone, or onset of PAI at 1 year or older, while the onset age of PAI is overlapped between non-classic LCAH and classic LCAH (18). In the present study, it was attempted to report a boy with complete male external genitalia who was diagnosed with non-classic LCAH. He had enzymatic activity of greater than 10%, and one girl was diagnosed with classic LCAH with the same

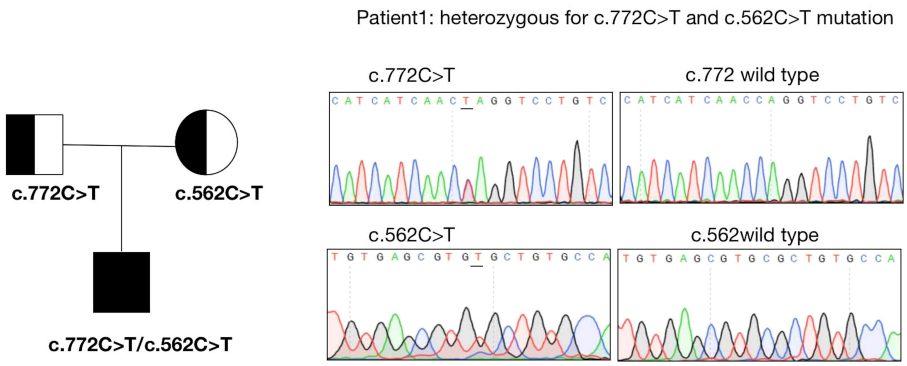

FIGURE 1
Sanger sequencing confirmation of the patient.

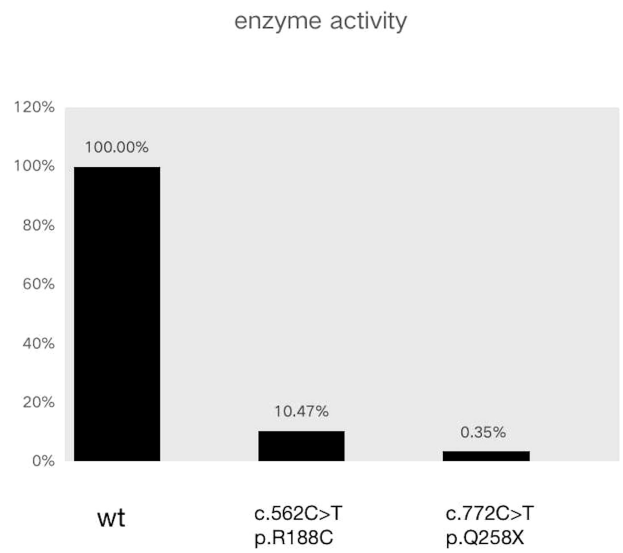

FIGURE 2
Enzyme activity assay to detect the pregnenolone concentration in this patient.

mutation as this patient's, whereas the onset age of PAI was before 1 year old. A previous study demonstrated that the age of onset of non-classic LCAH was after the age of 2 years (2). Therefore, consideration is essential to indicate whether the onset age of PAI is the basic clue for the diagnosis of non-classic LCAH, and the literature review was therefore performed.

2. Phenotype and genotype of non-classic lipoid congenital adrenal hyperplasia

Baker et al. (3) in 2006 first reported that LCAH patients with 46,XY karyotype can present with complete male external genitalia, and 14 mutations in 47 patients from 36 families have been identified to cause non-classic LCAH. All previously reported cases were also reviewed to investigate the genotype–phenotype relationship. The mutational analysis showed that c.562C>T(p.R188C) mutation was prevalent in patients with non-classic LCAH, accounting for 37.2% of the total mutant alleles, which could reflect the founder effect on the non-classic LCAH population. It was revealed that the same mutation had different clinical manifestations. We also found that 17 reported patients with c.562C>T homozygous mutation had different phenotypes: among 11 patients with 46,XY karyotype, nine had complete male external genitalia while two had glandular hypospadias, with the onset age of PAI from birth to 48 years; six patients with 46,XX karyotype had different onset ages of PAI (0–17 years). In our previous study (17), patient F34 (46,XY) had complete male external genitalia and with the compound heterozygous variants (c.367G>A/c.465+2T>A,p.Glu123Lys/unknown), in which the enzymatic activity assay revealed that STAR-Glu123Lys was reserved by 26.15%, and the high residual activity could rescue the steroidogenesis capacity of fetal Leydig cells because he had developed male external genitalia although he had first presented at the age of 6 months with adrenal insufficiency after receiving the first dose of vaccine. Patient F35 shared the same mutation as patient F34, while they were from different families, and she had first presented at the age of 9 months with adrenal insufficiency. Patient F36 was diagnosed at the age of 33 months when he presented with pneumonia, and routine investigation showed hyponatremia without hyperkalemia. This case had c.562C>T and c.772C>T mutations, and the residual enzymatic activity could also rescue the steroidogenesis capacity of fetal Leydig cells because he had developed male external genitalia in the presence of a micropenis, and assessment of RAAS revealed a high renin level and a low Ald level without hyponatremia and hyperkalemia; thus, he was diagnosed with non-classic LCAH. Moreover, no significant correlation between genotype and phenotype of non-classic LCAH was identified.

3. 46,XY male patients with non-classic lipoid congenital adrenal hyperplasia

It is widely accepted that the current classification criteria for non-classic LCAH include late age of onset (generally defined as onset after age of 2–4 years) and external genital development that is consistent with the chromosomal karyotype (2). The majority of LCAH patients presented with female external genitalia, while few cases were reported with male external

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TABLE 3 Fourteen mutations in 47 patients with non-classic LCAH from 36 families.

		Gender	Nucleotide change	aa change	Age	Residual activity	Reference
F1	46, XX	F	c.559G>A/c.559G>A	p.V187M	4y	21.6%	Baker et al., 2006 (3)
F2-1	46, XY	M	c.562C>T/c.562C>T	p.R188C	2.2y	13.6%	Baker et al., 2006 (3)
F2-2	46, XY	M	c.562C>T/c.562C>T	p.R188C	2.8y	13.6%	Baker et al., 2006 (3)
F3-1	46, XY	M	c.577C>T/c.577C>T	p.R192C	5y	50%	Metherell et al., 2009 (5)
F3-2	46, XX	F	c.577C>T/c.577C>T	p.R192C	?	50%	Metherell et al., 2009 (5)
F3-3	46, XX	F	c.577C>T/c.577C>T	p.R192C	?	50%	Metherell et al., 2009 (5)
F4-1	46, XX	F	c.562C>T/c.562C>T	p.R188C	2y	13.6%	Metherell et al., 2009 (5)
F4-2	46, XY	M	c.562C>T/c.562C>T	p.R188C	58y	13.6%	Metherell et al., 2009 (5)
F5-1	46, XY	M	c.562C>T/c.562C>T	p.R188C	6y	13.6%	Metherell et al., 2009 (5)
F5-2	46, XY	M	c.562C>T/c.562C>T	p.R188C	?	13.6%	Metherell et al., 2009 (5)
F6-1	46, XX	M	c.562C>T/c.562C>T	p.R188C	1.5y	13.6%	Metherell et al., 2009 (5)
F6-2	46, XX	M	c.562C>T/c.562C>T	p.R188C	<1y	13.6%	Metherell et al., 2009 (5)
F6-3	46, XY	F	c.562C>T/c.562C>T	p.R188C	<1y	13.6%	Metherell et al., 2009 (5)
F7	46, XY	glandular hypospadias	c.562C>T/c.562C>T	p.R188C	3y	13.6%	Metherell et al., 2009 (5)
F8	46, XY	hypospadias	c.562C>T/c.562C>T	p.R188C	4y	12.8%	Sahakitrungruang et al., 2010 (6)
F9	46, XY	Ambiguous genitalia	c.6732T>C/c.6753T>C	p.L260P/p.F267S	0	1.8%/9.5%	Sahakitrungruang et al., 2010 (6)
F10	46, XY	Ambiguous genitalia	c.562C>T/c.749C>A	p.R188C/p.W250X	3.5m	12.8%	Sahakitrungruang et al., 2010 (6)
F11	46, XX	F	c.5780G>A/c.5780G>A	p.G221D	12y	6.3%	Sahakitrungruang et al., 2010 (6)
F12-1	46, XX	F	c.661G>A/c.125-126insG	p.G221S/ p.T44H_S46X	10m	30-50%	Flück et al., 2011 (7)
F12-2	46, XY	M	c.661G>A/c.125-126insG	p.G221S/ p.T44H_S46X	14m	30-50%	Flück et al., 2011 (7)
F13	46, XY	?	/	p.M225T/p.Q258X	10m	43%	Nakae et al., 1997 (8)
F14	46, XY	M	c.950T>C/c.577C>T	p./L275P/p.R192C	2m	24%/50%	Bose et al., 1996 (4)
F15	46, XX	F	c.653C>T/c.950T>C	p.A218V/p.L275P	35d	20%	Bose et al., 1996 (4)
F16	46, XY	M	c.653C>T/c.772C>T	p.A218V/p.Q258X	1m	20%/0	Nakae et al., 1997 (8)
F17-1	46, XY	M	c.653C>T/c.772C>T	p.A218V/p.Q258X	9d	20%/0	Nakae et al., 1997 (8)
F17-2	46, XX	F	c.653C>T/c.772C>T	p.A218V/p.Q258X	35d	20%/0	Nakae et al., 1997 (8)
F18	46, XX	F	c.653C>T/c.772C>T	p.A218V/p.Q258X	13d	20%/0	Nakae et al., 1997 (8)

(Continued)

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TABLE 3 Continued

		Gender	Nucleotide change	aa change	Age	Residual activity	Reference
F19	46, XY	M	c.815G>A/c.772C>T	p.R272C/p.Q258X	5y	35%/0	Ishii et al., 2020 (9)
F20	46, XY	M	c.815G>A/c.772C>T	p.R272C/p.Q258X	4y	35%/0	Ishii et al., 2020 (9)
F21	46, XY	M	c.64_177.del/c.64_177.del	p.G22_L59del	0	0	Ishii et al., 2020 (9)
F22	46, XY	M	c.661G>A/c.653C>T	p.G221S/p.A218V	17m	30-50%/20%	Bae, et al., 2020 (10)
F23-1	46, XX	F	c.553T>A/c.737A>G	p.L178Q/p.D246F	2y	?	Luo et al., 2020 (11)
F23-2	46, XY	M	c.553T>A/c.737A>G	p.L178Q/p.D246F	2y	?	Luo et al., 2020 (11)
F24	46, XX	F	c.562C>T/c.562C>T	p.R188C	14m	12.8%	Burget et al., 2018 (12)
F25	46, XX	F	c.562C>T/c.562C>T	p.R188C	3y	12.8%	Tsai et al., 2016 (13)
F26-1	46, XY	M	c.562C>T/c.562C>T	p.R188C	6y	12.8%	Tsai et al., 2016 (13)
F26-2	46, XY	M	c.562C>T/c.562C>T	p.R188C	9y	12.8%	Tsai et al., 2016 (13)
F27	46, XY	M	c.562C>T/c.562C>T	p.R188C	4y	12.8%	Tsai et al., 2016 (13)
F28	46, XX	F	c.562C>T/c.562C>T	p.R188C	17y	12.8%	Tsai et al., 2016 (13)
F29	46, XX	F	c.815G>A/c.772C>T	p.R272C/p.Q258X	29m	35%/0	Kang et al., 2017 (14)
F30	46, XY	Ambiguous genitalia	Exon1and >50 bp upstream of promoter		0	?	Piya et al., 2017 (15)
F31	46, XY	M	c.815G>A/c.772C>T	p.R272C/p.Q258X	18m	35%/0	Liang et al., 2021 (16)
F32	46, XY	Ambiguous genitalia	c.491C>A/c.707_708delAGinsCTT	p.T164K/ p.K236Tfs*47	2.5m	?/<1% (4)	Liang et al., 2021 (16)
F33	46, XY	M	c.306+3_c.306+6delAAGT/661G>A	Intron3-4 fs/p.G221S	13Y	0/17.19%	Zhang et al., 2021 (17)
F34	46, XY	M	c.367G>A/c.465+2T>A	p.E123K/ Intron 4-5	6m	26.15%	Zhang et al., 2021 (17)
F35	46, XX	F	c.367G>A/c.465+2T>A	p.E123K/ Intron 4-5	9m	26.15%	Zhang et al., 2021 (17)
F36	46, XX	F	c.229C>T/c.559G>A	p.Q77X/ p.V187M	2.75y	<1%/20%	Zhang et al., 2021 (17)

d, days; m, months; y, years; F, female; M, male; LCAH, lipoid congenital adrenal hyperplasia.

genitalia that were diagnosed as non-classic LCAH. A high residual activity could rescue the steroidogenesis capacity of fetal Leydig cells because these patients develop male external genitalia. The clinical phenotype is related to the residual enzymatic activity, in which the enzymatic activity >10%–20% is considered as non-classic LCAH and the enzymatic activity <10% is considered as classic LCAH (9). The literature review resulted in the identification of 28 46,XY patients, of whom 22 cases had complete male external genitalia (78.5%), their residual enzymatic activity was not more than 20%, even was <10%, and the onset age of PAI was 0–58 years; there were six cases with different degrees of hypospadias (21.5%), in which their residual enzymatic activity was ≥10% and even >20%. Therefore, a higher residual enzymatic activity can maintain the formation of male external genitalia, while it does not necessarily indicate that the onset age of PAI is late, and it could be affected by several factors, such as stress, temperature, sodium intake, and StAR-independent steroid pathways. It is noteworthy that 46,XY patients can be diagnosed before the age of 1 year, and the onset age of the same mutation is also different in classic and

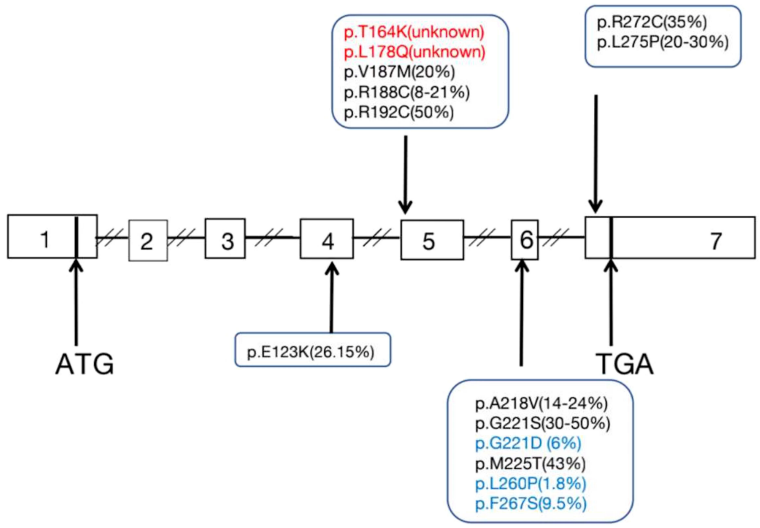

FIGURE 3
Diagram of the STAR gene showing the location of mutations (Including enzyme activity) identified in patients with non-classic LCAH.

non-classic cases. Thus, the onset age can be used as one of the diagnostic indicators of non-classic LCAH, but not the only one.

The clinical phenotypes of non-classic LCAH are highly variable. Routine physical examination, laboratory measurement, genetic testing, and, importantly, enzymatic activity assay may facilitate the early diagnosis of non-classic LCAH. Further study is required to verify the findings of the present case report.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving human participants were reviewed and approved by Ruijin Hospital Ethics Committee, Shanghai JiaoTong University School of Medicine. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

WL, TZ, and LZ as co-first authors wrote and translated this article. XW, JW, LY, YX, ZD, WW, and SS collected all the clinical information of these patients. RH, GN, CL, and XM are the corresponding authors who edited and reviewed this manuscript and approved the version to be published.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Congenital adrenal hyperplasia due to P450 oxidoreductase deficiency

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Objective: To raise awareness of Cytochrome P450 Oxidoreductase Deficiency (PORD, a rare form of congenital adrenal hyperplasia (CAH), through a case of pregnant woman with virilization symptoms.

Case description: A 30-year-old Chinese woman was referred to hospital after 7 years of presenting signs of virilization, including voice deepening, acromegaly, hirsutism, clitoromegaly, and acne. These symptoms appeared since her third gestation. Her second birth died 9 hours after birth and had signs of clitoris hypertrophy. Her third born was a son who presented with flat nose, radius and humerus bone malformation, and small penis at birth. Panel of POR-related genetic tests revealed that the patient carried c.1370 G>A (p.R457H), which is a POR heterozygous gene, while her husband carried a POR heterozygous gene as well, c.1379 C>A (p.S460Y). Two heterozygous mutations of the POR were found in her son: c.1370 G>A and c.1379 C>A. In PORD, c.1370 G>A (p.R457H) was reported as a susceptible gene, while c.1379 C>A (p.S460Y) has not been reported as responsible for the disease so far.

Discussion and literature review: PORD is a rare form of CAH and caused by POR gene mutations. Most PORD patients are identified and diagnosed in pediatrics department. Internal medicine and obstetrics physicians are unfamiliar with the disease. As clinical manifestations are diverse, PORD could be easy to miss or to be misdiagnosed. Typical clinical manifestation includes adrenal insufficiency-related symptoms, such as bone malformations and sexual development disorders. PORD is diagnosed through genetic testing. Investigations of steroid metabolic products in urine through gas chromatography-mass spectrometry or liquid chromatography-mass spectrometry are also helpful for the diagnosis, but neither of them are widely available in China. In this case, the patient had a history of infertility, and her third child was born with congenital defect and carried a PORD-related gene. In general clinical practice, if a pregnant woman presents with abnormal virilization symptoms, CAH possibilities should be considered, including rare causes such as PORD.

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Conclusion: PORD is a rare autosomal recessive genetic disease. We summarised the clinical characteristics and genotypes that were previously reported in the Chinese population and identified a novel mutation.

KEYWORDS

congenital adrenal hyperplasia, cytochrome P450 oxidoreductase deficiency, *POR* mutation, c.1379 C>A (p.S460Y) variant, maternal hyperandrogenism

1 Introduction

Cytochrome P450 oxidoreductase deficiency (PORD) is a relatively rare autosomal recessive genetic disease, which is a rare subtype of congenital adrenal hyperplasia (CAH). Mutations in *POR* affect cytochrome P450 oxidoreductase, an enzyme required for the normal functioning of more than 50 enzymes in the cytochrome P450 family. Thus, *POR* mutations cause a decrease in the activities of various enzymes, leading to disorders of steroid hormone synthesis and a series of clinical symptoms, including abnormal genital development, characteristic skeletal deformities, maternal virilisation during pregnancy, and abnormal secretion of steroid hormones. PORD can be easily missed and misdiagnosed because of the diverse biochemical and clinical manifestations of each subtype of CAH, and because significantly different clinical characteristics result from the different gene mutations. Only approximately 100 cases of PORD have been reported worldwide (1-5), some of which have been temporarily misdiagnosed as CYP17A1 deficiency, CYP19A1 deficiency, or CYP21A2 deficiency. Therefore, in addition to common aetiologies, rare aetiologies should also be considered in the diagnosis of CAH, including PORD.

We here report the case of a mother with virilisation during pregnancy, who gave birth to two children with PORD successively, after delayed diagnosis and treatment. Her son was found to have a previously unreported mutation, c.1379 C>A in *POR* (p.S460Y). Here, we analysed the gene mutation type in the context of their clinical characteristics, and also summarised and analysed the clinical characteristics of 20 Chinese PORD patients previously reported to facilitate a deeper understanding of the disease.In addition, we have identified an unreported mutation locus in the Chinese population, which adds to the genetic diagnosis of PORD in the Chinese population.

2 Case description

2.1 Case 1 (The mother, carrier of POR heterozygous gene)

The index case was a 30-year-old female who was admitted to the Endocrinology Department of our hospital in May 2020 due to development of a deep voice and enlarged hands and feet that had developed 7 years ago. She signed an informed consent form after admission.

The patient began presenting signs of irilization since the 16th week of her second pregnancy 7 years before admission. She developed voice deepening, facial acne, mandibular prognathism, enlarged hands and feet (with an increase in shoe size from 39 to 42), clitoromegaly and a significant increase in pubic and armpit hair, and other abnormal symptoms. At that time, the patient delivered a full-term baby girl (see Case 2) by caesarean section, who died within hours of birth. After the delivery, the irilization manifestations of the patient significantly diminished compared to earlier, but the deep voice, large hands and feet, and mandibular prognathism were not significantly relieved. No abnormality in sex hormones was found in tests performed 42 days after delivery. The patient had a third pregnancy 2 years before presenting to our institution, and no noticeable signs of irilization emerged during that pregnancy. She delivered a full-term baby boy with PORD (Case 3).

The patient had no significant change in body weight over the past 7 years. She had undergone resection of benign nodules on the left thyroid lobe 12 years earlier. She denied a history of administration of glucocorticoids and had no history of exposure to radioactive substances. Her menstruation cycles were regular and occurred approximately every 30 days, and the age of menarche was 14 years.

She had a reproductive history of gravida 4 (2 miscarriages and 2 full-term births), para 2 (G4P2). One boy is still alive. She had an induced abortion in the first trimester 8 years ago when she had her first pregnancy, because she took medicine by mistake on account of abdominal pain. At term with her second pregnancy 7 years prior to presentation, she gave birth to a baby girl with clitoromegaly, who died within a few hours after birth. Later, 2 years before presentation, with her third pregnancy, she gave birth to a full-term baby boy who was later diagnosed with PORD by genetic testing. She underwent an induced abortion in the first trimester 1 year before presentation, when she was pregnant a fourth time, because this pregnancy occurred near the time of her last childbirth.

Her spouse was in good health. Neither her parents, nor the patient and her spouse, were consanguineous. Other than her

son who was diagnosed with PORD, there was no history of similar disease in her family.

2.1.1 Physical examination

At presentation, her blood pressure was 116/82 mmHg and her pulse was 89 beats per minute. Her height was 165 cm, and her weight was 83.7 kg, yielding a body mass index of 30.74 kg/m^2. Her waist circumference was 101 cm, and hip circumference was 112 cm, with a waist-to-hip ratio of 0.90. She had systemic obesity, and no abnormalities in the heart, lungs, and abdomen. No skin atrophy, acne, and purple abdominal striae were found. She had a deep male voice, but no Adam's apple or beard. Her hair distribution was normal, but not significantly long, and thick hair was found. There was no noticeable increase in pubic hair or armpit hair. The Tanner stages of pubic hair and breasts were PH4 and B4, respectively. The clitoris was enlarged. The external genitalia showed stage I irilization according to Prader staging. No apparent abnormality was found in other parts of the body.

2.1.2 Laboratory and auxiliary examination

No abnormality was found in the blood cortisol, adrenocorticotropin (ACTH), sex hormones, androgen classification, and thyroid function of the patient. Detailed biochemical examination results see Table 1.

On ultrasound, the uterus was slightly enlarged. The nature of the hypoechoic area (10 mm × 6 mm × 8 mm) in the right ovary and of the cystic hypoechoic area (14 mm × 10 mm × 8 mm) in the left ovary remained to be examined. She had undergone a partial left thyroidectomy, and sonographic images of the thyroid manifested diffuse involvement. A mixed nodule (about 6 mm × 4 mm × 6 mm) was seen in the middle of the right thyroid lobe (ACR-TIRADS 2). Plain and contrasted enhanced computed tomography of the adrenals showed no bilateral adrenal abnormalities. Fiberoptic laryngoscopy indicated chronic pharyngitis and possible laryngopharyngeal reflux.

2.1.2.1 Diagnosis of the patient

Upon genetic analysis, the patient was found to be a heterozygous carrier of mutation in *POR* (1370C>A heterozygote, normal phenotype). She was diagnosed with obesity, a right thyroid nodule, post-partial thyroidectomy (left lobe), a possible ovarian cyst, and chronic pharyngitis.

2.1.2.2 Treatment and follow-up

The patient was advised to lose weight through lifestyle intervention and to undergo genetic counselling with prenatal diagnosis during subsequent pregnancies.

2.2 Case 2 (The daughter of Case 1, suspected with PORD)

Case 2 was the daughter of Case 1 and was a child with suspected PORD. This second child was a baby girl born at full term by caesarean section (in 2013). At birth, she was found to have abnormal signs of clitoromegaly, a protruding forehead, and a flat-bridged nose. She died within 9 hours of birth.

2.3 Case 3 (The son of Case 1, diagnosed with PORD)

Case 3 was the son of Case 1 and was a child with PORD. This third child was born full-term by caesarean section (May 2018). At birth, he was found to have abnormal signs: a short penis, chordee (urine discharge from the glans penis during urination), a slightly protruding forehead, a flat-bridged nose, and flexion of the upper limbs that could not be straightened. His biochemical examination results see Table 1. X-ray images indicated bilateral radiohumeral synostosis, and congenital developmental deformity was considered. Chromosomal examination revealed a 46 XY karyotype.

2.4 POR genetic testing

The peripheral blood of Case 1(the mother), her spouse, and Case 3(her son) were collected for DNA analysis. All coding exons and adjacent sequences of *POR* were detected (Guangzhou Jiajian Medical Testing Co., Ltd., Guangzhou, China). The genetic test results are shown in Table 2. High-throughput Next Generation Sequencing (NGS) data analysis found that *POR* in Case 3 included a compound heterozygous missense mutation at 2 loci: c.262G>A in exon 4 and c.1609G>A in exon 13. C.262G>A. The former heterozygous missense mutation leads to changes of glycine at position 88 to serine (p.Gly88Ser, G88S), while the latter heterozygous missense mutation results in a change of glycine at position 537 to serine (p.Gly537Ser, G537S). The results from subsequent Sanger sequencing of the corresponding sites in the parents indicated that the nucleotide variation c.262G>A in exon 4 of *POR* of the patient was inherited from the mother, and the nucleotide variation c.1609G>A in exon 13 was inherited from the father. This was consistent with the NGS results. The mother was heterozygous for *POR* c.1370 G>A (p.R457H), and her spouse was heterozygous for *POR* c.1379 C>A (p.S460Y).

The c.1370 G>A (p.R457H) variant has been reported as a pathogenic variant in multiple PORD-related clinical cases, while the c.1379 C>A (p.S460Y) variant has not previously been reported in clinical cases. This variant is "likely pathogenic" according to the American College of Medical Genetics and Genomics (ACMG) variant classification guidelines (PMID: 25741868) (6).

REVEL, Polyphen2, MutationTaster, and other computer prediction software predicted a deleterious variant (REVEL score: 0.959) (Figure 1).Meanwhile, the variant has been included in the ClinVar database as a pathogenic/probably pathogenic variant

TABLE 1 Biochmeical examination results of case 1 and case 3.

	Case 1 (The mother)			Case 3 (The child)							
	Results	Reference range	Unit	At age of 5months	Reference range	Unit	Before treatment*	After 1 month of treatment*	After 3 months of treatment*	Reference range	Unit
Blood ACTH (08:00)	46	0-46	pg/mL	**12**.00	0–10.12	pmol/L	340.39	/	**112.9**	118.0-618.0	nmol/L
Serum Cortisol (08:00)	502.3	118.60-618.0	nmol/L	340.39	118.6–618	nmol/L	**12.00**	**26.40**	1.27	0-10.12	pmol/L
Testosterone	0.69	0.35-2.60	nmol/L	**0.96**	0–0.7	nmol/L	/	/	/	/	/
Progesterone	0.44	0.31-1.52	μg/L	/	/	/	/	/	/	/	/
Prolactin	11.96	3.34–26.72	μg/L	10.99	2.1–17.7	ng/ml	/	10.42	/	2.1-17.7	nmol/L
Estradiol	34	19.86–148.13	ng/L	**108.00**	0–73	pmol/L	/	**85.43**	/	0-73.4	pmol/L
Follicle stimulating hormone	8.38	3.85–8.78	IU/L	**3.14**	0.26–3.0	IU/L	/	1.55	/	0.26-3.0	IU/L
Luteinizing hormone	2.19	2.12–10.89	IU/L	**2.64**	0.02–0.3	IU/L	/	**1.12**	/	0.02-0.3	IU/L
Dehydroepiandrosterone sulfate	212.6	98.80–340.0	μg/dl	**0.97**	2.17–15.2	μmol/L	/	/	/	/	/
17-hydroxyprogesterone (17OHP)	1.21	0.1-0.8	ng/ml	**7.2**	< 14.0	nmol/L	/	/	/	/	/
Dihydrotestosterone (DHT)	219.3	24.0–368.0	pg/ml	/	/	/	/	/	/	/	/
Androstenedione	2.55	1.22–8.73	nmol/L	**<1.05**	1.0–11.5	nmol/L	/	/	/	/	/
Anti-Müllerian hormone	1.03	2.80–6.30	ng/ml	/	/	/	/	/	/	/	/
Sex hormone-binding globulin	64.39	32.4–128.0	nmol/L	/	/	/	/	/	/	/	/
24-h urinary 17-ketosteroid excretion	12.9	6–25.0	mg/24 h	/	/	/	/	/	/	/	/
24-h urinary 17-hydroxysteroid excre	tion 2.8	2.0–10.0	mg/24 h	/	/	/	/	/	/	/	/
AST	16	15-40	U/L	/	/	/	/	/	/	/	/
ALT	10	Sep-50	U/L	/	/	/	/	/	/	/	/
Total Protein	**64.2**	65.0-85.0	g/L	/	/	/	/	/	/	/	/
Albumin	**38.3**	40.0-55.0	g/L	/	/	/	/	/	/	/	/
Total bilirubin	5.8	3.4-22.2	μmol/L	/	/	/	/	/	/	/	/
Direct bilirubin	1.3	0.0-3.4		/	/	/	/	/	/	/	/
γ-GGT	11	Oct-60	U/L	/	/	/	/	/	/	/	/

(Continued)

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TABLE 1 Continued

Case 1 (The mother)				Case 3 (The child)							
	Results	Reference range	Unit	At age of 5months	Reference range	Unit	Before treatment*	After 1 month of treatment*	After 3 months of treatment*	Reference range	Unit
ALP	54	45-125	U/L	/	/	/	/	/	/	/	/
Creatinine	52	44-133	μmol/L	/	/	/	/	/	/	/	/
Uric Acid	300	120-452	μmol/L	/	/	/	/	/	/	/	/
Glucose	5.3	3.9-5.6	mmol/L	/	/	/	**6.30**	/	**7.6**	4.1-5.9	mmol/L
CO2CP	25	22-31	mmol/L	/	/	/	/	/	/	/	/
TG	0.82	0.31-2.30	mmol/L	/	/	/	/	/	/	/	/
CHOL	4.12	2.90-6.00	mmol/L	/	/	/	/	/	/	/	/
HDL-C	1.4	0.80-1.96	mmol/L	/	/	/	/	/	/	/	/
LDL-C	2.46	1.30-3.60	mmol/L	/	/	/	/	/	/	/	/
Blood Sodium	3.84	3.5-5.3	mmol/L	/	/	/	**136.8**	/	138.0	138.0-144.0	mmol/L
Blood Potassium	141.4	137-147	mmol/L	/	/	/	4.54	/	3.68	3.4-5.7	mmol/L

Case 2 lacked the relevant data because she died after birth.

*The affected child was administered hydrocortisone acetate in doses from 1.6 mg tid, and the dose was adjusted according to the patient's condition. During the paediatric follow-up, the levels of blood ACTH and sex hormones returned to normal.

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TABLE 2 Genetic test results of Case 1 (the mother), Case 3 (her son) and her spouse.

Gene	OMIM number	HG19 position	Transcript	Nucleotide and amino acid variations	Zygote	Class of ACMG[a] mutation	Inheritance
POR	124015	chr7: 75614497	NM-000941	c.1370G>A (p.R457H)	Heterozygote	Pathogenic	Mother (Heterozygotes)
POR	124015	chr7: 75614506	NM-000941	c.**1379C>A** (**p.S460Y**)	Heterozygote	Likely Pathogenic	Father (Heterozygotes)

Test Results

Gene	Chromosomes Location[c]	Reference serials	cDNA level variations	Protein level variations	Zygote	Origin of mutation	Genetic modality	Class of ACMG mutation	Associated disease
POR	chr7: 75614497	NM_001395413.1	c.1361G>A	p.Arg454His	Heterozygote	Mother	AR[b]	Pathogenic	Antley-Bixler Syndrome accompanied by genital abnormalities and steroidogenic disorders
POR	chr7: 75614506	NM_001395413.1	c.1370C>A	p.Ser457Try	Heterozygote	Father	AR	Likely Pathogenic	

Remarks: a:ACMG:American College of Medical Genetics and Genomics . b:AR:Autosomal recessive.
c:Chromosomes Location:Human reference genome GRCH37/hg19.
NGS data revealed that the patient carries a compound heterozygous variant of POR gene on chromosome 17, one of which is NM_001395413.1: c.1361G>A: p.Arg454His, heterozygous and inherited from the mother. This indicates that the variation from G to A at nucleotide 1361 of the gene coding region results in a variation of protein translation from arginine to histidine at amino acid position 454. And this variant is not included in the 1000 genomes, gnomAD and other databases.

(Variation ID: 16907). The variant has also been identified in Antley-Bixler Syndrome patients (OMIM# 201750) with genital abnormalities and steroidogenesis disorders and has been reported in several publications (7–10) (PMID: 16470797, 20101697, 15793702, 15483095, etc.). Notably, this variant is considered pathogenic according to the ACMG guidelines (2015 edition). Another variant is NM_001395413.1: c.1370C>A: p.Ser454Try, which encompasses a variation in the gene coding region of the nucleotide at position 1370 from C to A, resulting in a change in protein translation from serine to tyrosine at amino acid position 457.

2.4.1 Treatment and follow-up

The affected child was administered hydrocortisone acetate in doses from 1.6 mg tid, and the dose was adjusted according to the patient's condition. During the paediatric follow-up, the levels of blood ACTH and sex hormones returned to normal.

3 Discussion

CAH is a group of autosomal recessive genetic diseases caused by defective enzyme functions due to mutations in genes encoding essential enzymes for steroid hormone synthesis, which eventually results in steroid hormone synthesis disorders. The most common cause of CAH is 21-hydroxylase deficiency (21-OHD) (accounting for more than 90% of the causes), followed by 11β-hydroxylase deficiency (11β-OHD) (accounting for 5–8%). 17-Alpha hydroxylase or 17, 20-lyase deficiency and 3 beta-hydroxydehydrogenase deficiency are less common, accounting for approximately 1% each. Other types of CAH, including PORD, are even rarer. Since the first report in 1985, only about 100 PORD patients have been reported worldwide (1, 11).

Cytochrome P450 oxidoreductase (POR) is a flavoprotein that acts as an electron transporter in the synthesis of various steroid hormones and participates in many physiological reactions of the body (1). Mutations in *POR* can cause impaired activity of the POR enzyme and decreased activities of steroidogenic enzymes in the P450 enzyme system (cytochrome P450 monooxygenases [CYP], including CYP17A1, CYP21A2, and CYP19A1 [aromatase] and other enzymes), eventually giving rise to abnormal secretion of sex hormones and glucocorticoids. Typical clinical manifestations are hermaphroditism at birth, characteristic skeletal developmental malformations, maternal virilisation, and abnormal steroid secretion, without deficiency in mineralocorticoids. However, the clinical manifestations are diverse, and the disease is easy to miss or be misdiagnosed, because the degree of impairment of POR enzyme activity is directly related to the clinical phenotype.

Case 1 (The mother of the PORD foetus) in this study was a phenotypically normal PORD gene carrier with a history of adverse pregnancy, who gave birth to two children (one living) with PORD. The patient was asymptomatic before pregnancy and was only experiencing virilisation in the second trimester (pregnant with a female foetus), and symptoms of virilisation were relieved after delivery.

Pathophysiology process are different during pregnancy of a female foetus and a male foetus and this results in overproduction

FIGURE 1
Polyphen2 prediction report & Prediction of protein conservation.

of androgen and lack of androgen respectively. During pregnancy with a female PORD foetus (with a 46 XX kartoytypoe in general), the reason of virilisation was considered to be caused by excessive androgen elevation through "backdoor bathway". The underlying mechanisms are mentioned below.

Under normal physiological conditions, POR is involved in the synthesis of cortisol and sex hormones. It catalyses the conversion of progesterone to deoxycortisol and 17-hydroxyprogesterone to 11-deoxycorticosterone in the zona reticularis and zona fasciculata of the adrenal cortex, respectively.

During pregnancy with a female PORD foetus (with a 46 XX karyotype in general), there is an overproduction of androgen. The conversion of androgen precursors to oestrogens in the body is abnormal due to the decreased activity of various enzymes, such as CYP17A1, CYP21A2, and CYP19A1 (aromatase) in the female foetus; thus, the levels of 17-alpha hydroxyprogesterone synthesised by the foetal adrenals are significantly increased. A large accumulation of 17-alpha-hydroxyprogesterone activates an alternative pathway for DHT synthesis, which can be converted to 5α-pregnan-3α, 17α-diol-20-one, and androsterone *via* this pathway, ultimately resulting in an overproduction of DHT. This pathway does not form part of the conventional androgen production pathway and is known as the "backdoor pathway" (Figure 2). The highly active DHT enters the mother's body *via* the placenta, which can lead to significant virilisation of the mother during pregnancy, possibly accompanied by the excretion of androgen metabolites. The "backdoor pathway" of DHT synthesis is closed as soon as the foetus is born (12). Thus, virilisation during pregnancy can gradually be diminished after childbirth. In addition, our patient's second, female child also had PORD, and the death within a few hours of birth was presumably attributed to a life-threatening adrenal crisis in the child. This affected baby girl was born virilised. Clitoromegaly occurs in children with 46 XX under the action of high concentrations of DHT. It has been reported that mildly affected female patients have isolated clitoromegaly, while, in severe cases, the clitoris resembles the scrotum (12).

In contrast, during pregnancy with a male foetus(with a 46 XY karyotype in general), there is a lack of androgens synthesised

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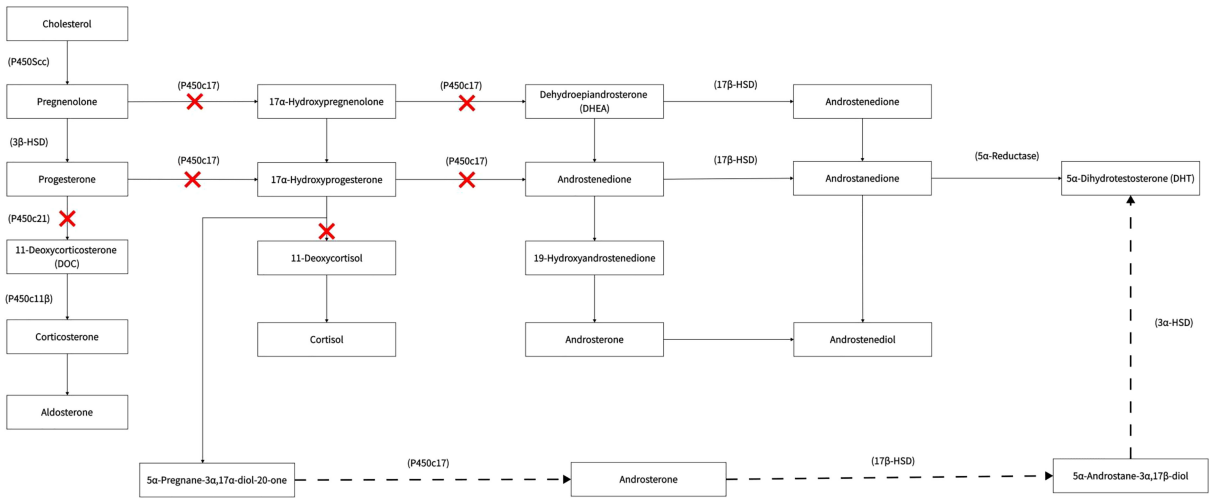

FIGURE 2
Backdoor pathway.

during the embryonic period. In male foetus, POR deficiency affects the activity of 17, 20-lyase, resulting in a significant decrease in the synthesis of androgens, such as the downstream hormones dehydroepiandrosterone, androstenedione, and testosterone. The quantity of androgens received by the mother during pregnancy was reduced accordingly and explained why the mother is not presenting symptoms of virilisation during pregnancy. Due to the lack of male hormones, the baby boy was born with a small penis, congenital chordee, and other reduced masculinity signs. Those with mild symptoms may present with blurred external genitalia and pseudohermaphroditism, and those with severe symptoms may have severe hypospadias or female genitalia.

Furthermore, patients may also have characteristic craniofacial deformities and synostosis in addition to hermaphroditism. The two affected babies in this study had skeletal deformities, such as a slightly protruding forehead and a flat-bridged nose at birth, and the male child also had characteristic deformities, such as synostosis. It has been reported in the literature that about 90% of patients with POR deficiency may have different degrees of craniofacial deformity or specific manifestations, such as synostosis of the long bones of the extremities. Other manifestations include midface hypoplasia, characterised by low-set ears and a pear-shaped nose, craniosynostosis, arachnodactyly, crooked fingers and toes, radiohumeral synostosis, and other synostoses (also known as Antley–Bixler syndrome [ABS]). More severely affected children present with femoral bowing, neonatal fractures, and choanal atresia (4). It is currently believed that skeletal deformities are related to the impaired activity of cholesterol synthesis enzymes in chondrocytes (2). Both lanosterol 14-α-demethylase (CYP51A1) and squalene monooxidase (SQMG) are essential enzymes involved in cholesterol synthesis in bones (11), and defects in their functions lead to impaired cholesterol synthesis in chondrocytes, which can cause defective cell differentiation and increased apoptosis. This is the main cause of skeletal deformities.

The typical clinical manifestations of PORD are ambiguous sex and characteristic skeletal deformities. Male neonates exhibit hypomasculinisation, while female infants exhibit excessive virilisation. Second, patients may present with craniofacial deformities and synostoses, resembling ABS. Moreover, symptoms such as maternal virilisation during pregnancy and sex hormone synthesis disorder in adulthood, which leads to delayed puberty, are also common.

In terms of diagnosis, gas chromatography-mass spectrometry or liquid chromatography-mass spectrometry detection of residual enzyme activity caused by POR deficiency is currently regarded as the gold standard for PORD diagnosis (13, 14). Typical biochemical changes are increased steroid metabolites, such as pregnenolone and progesterone, and 17-OHP metabolites and decreased steroid metabolites, including androgen metabolites. This technique has high specificity and sensitivity and is helpful for the diagnosis and identification of other types of CAH. It allows early detection of increased excretion of pregnenolone and progesterone metabolites in some heterozygous parents. However, at present, only a few laboratories are set up to conduct screening; therefore, screening is challenging (13, 15). The alternative is the chemiluminescence technique, which is frequently used in clinics to detect cortisol and sex steroids but there are limitations on accuracy (15). It is important to note that the hallmark steroids 17OHD and 21OHD may be present in different combinations, which may result in misdiagnosis.

In terms of molecular genetics, genetic testing is helpful for diagnosis and differential diagnosis, particularly in patients with

atypical clinical manifestations or in those who cannot be diagnosed by biochemical tests. PORD is an autosomal recessive disorder, and most patients have compound heterozygous mutations in *POR*. The genetic polymorphisms in *POR* have significant racial and individual differences. About 200 *POR* mutations and single nucleotide polymorphisms (SNPs) have been reported to date. Among the types of missense mutations, A287P is the most common *POR* mutation in Caucasians, R457H is highly prevalent in Japanese population (16), while A503V is also common in *POR*, with a prevalence of about 27% in the general population (17).

We also reviewed the clinical data of 20 previously reported Chinese patients with PORD (17–26) (12 female patients) (Table 3). After exclusion of patients with missing data, we found that 19 of 19 included patients had abnormal secretion of steroid hormones, 18/19 patients had external genital deformities, 8/19 patients had skeletal deformities, and 10/16 patients had maternal virilisation. Among the 12 pubertal patients, 6/12 cases had delayed puberty, 8/12 cases had delayed growth, and 8/12 cases had ovarian cysts. Understanding of these clinical characteristics can improve awareness of PORD as well as the etiological differentiation of CAH. From the above summary, it can be seen that PORD patients mainly present with varying degrees of abnormal steroid hormone secretion and external genital malformations at birth. And they may habe growth and developmental delays in adulthood, which could be misdiagnosed as other subtypes of CAH. PORD should be considered if patients also have skeletal malformations or virilisation symptoms during pregnancy.

Among the 20 reported PORD cases in China, 8 cases (8/20) had a mutation at p.R457H, 3 cases (3/7) had a homozygous mutation, and 4 cases (4/7) were compound heterozygote for this mutation, with exlusion of 1 case with missing data. This suggests that p.R457H is a hot-spot mutation in the Chinese population, which is similar to the results reported in other countries. The affected child in our case-series was compound heterozygous for mutations in *POR*: the c.1370 G>A (p.R457H) variant from the mother has been reported as a pathogenic variant in multiple PORD cases, while the c.1379 C>A(p.S460Y) variant from the father has not been reported to date. This variant is "likely pathogenic" according to the American College of Medical Genetics guideline for variant classification (PMID: 25741868). Current bioinformatics analysis suggests that it is necessary to investigate the relationship between the novel mutation site and changes in enzyme function and activity further, to confirm that this genetic change leads to changes in enzyme activity.

In terms of treatment, multidisciplinary cooperative management is required for PORD. High-risk populations for PORD should be identified and screened (27). The patient in this study demonstrated virilisation during pregnancy, but the clinician's lack of awareness of the disease led to a family tragedy. If timely detection and prenatal diagnosis were implemented, adverse outcomes could be avoided. Careful attention should be paid to the following populations: those with a family history of CAH or PORD; those with maternal virilisation during pregnancy; those with hermaphroditism or skeletal deformity after birth; and those with delayed puberty development. Tests for related hormones and metabolites can be performed in these populations. Genetic testing is helpful for early diagnosis and differential diagnosis of PORD. In a large number of asymptomatic patients (homozygous or compound heterozygotes for autosomal recessive inheritance) and PORD gene carriers (heterozygotes for autosomal recessive inheritance), the disease is more likely to be missed. Typically, mothers with PORD foetuses have low serum estriol levels, which may be detected during the triple antenatal screening test. Subsequent maternal urinalysis may reveal characteristic manifestations of aberrant steroid precursors, which can facilitate a prenatal diagnosis (13, 28). It is necessary to inform mothers that, once virilisation occurs during pregnancy, it should be dealt with as soon as possible. Preconception health education and genetic counselling are required for patients with confirmed PORD or in the above-mentioned high-risk populations. Patients with confirmed PORD need individualised guidance for better natal and prenatal care. It is recommended that spouses should undergo genetic testing to screen for heterozygous cases before conception, or genetic diagnosis should be performed before embryo implantation. Prenatal genetic screening or amniotic fluid cell testing under ultrasonic should be performed during pregnancy for early identification of foetuses with disease genes and for managing the corresponding risks. For neonates born with hermaphroditism or characteristic skeletal deformities, chromosomal examinations are required to determine the genetic sex. Genetic testing is helpful in diagnosing PORD and distinguishing it from other types of CAH. Adrenal gland (blood ACTH, cortisol, electrolytes, and acid–base balance) and gonadal function should be evaluated in affected children, and the detection items that cannot be assessed but that have important diagnostic value (such as 17-hydroxyprogesterone) should be tested elsewhere before treatment. Timely diagnosis and treatment can avoid severe dehydration, electrolyte imbalance, and adrenal cortical crisis, and thus reduce mortality. The patient(i.e. the mother) should be informed of the need for long-term follow-up after birth, with re-examination of 17-hydroxyprogesterone in 2 weeks. Continued increase in blood 17-hydroxyprogesterone concentration is an important diagnostic indicator of 21-OHD. For patients diagnosed in puberty, ACTH stimulation test should be used to determine the degree of glucocorticoid deficiency. Glucocorticoids should be supplemented as appropriate, and drugs should be administered to improve and restore the patient's secondary sexual characteristics in puberty. If necessary, orthopaedic treatment is needed.

In conclusion, PORD is a group of autosomal recessive genetic disorders. Case 1(the mother) presented signs of virilisation during pregnancy in female foetus, and gave born to a male infant with PORD and a female infant with suspected PORD, and did not receive a timely and precise diagnosis.

TABLE 3 Summary of gene results and clinical characteristics of 20 Chinese patients with PORD.

Case	Karyotype	POR mutation		Exon	Zygote	Inheritance	Clinical features					Bone deformity	Pubertal failure
		Nucleotide changes	Amino acid changes				Steroidgenesis disorders	Genital abnormalties	Delay of puberty	Maternal virilization	Polycystic ovary /infertility		
Case 1	46, XX	c.1370G>A	p.R457H	11	compound	–	Yes	Yes	Yes	Yes	Yes	Yes	No
		c.1493G>C	p.R498P	11	heterozygotes	–							
Case2	46, XX	c.1370G>A	p.R457H	11	heterozygotes	Father	–	–	–	Yes	–	–	–
Case3	46, XX	–	p.R457H	–	–	–	Yes	Yes	Yes	–	Yes	Yes	Yes
Case4	46, XX	c.1370G>A	–	–	heterozygotes	Father	Yes	Yes	–	–	–	Yes	–
		c.917T>G	–	–	–	–							
Case5	46, XY	c.262G>A	p.G88S	2	heterozygotes	Mother	Yes	Yes	Yes	Yes	–	Yes	Yes
		c.1370G >A	p.R457H	11	heterozygotes	Father							
Case6	46, XX	c.1370G>A	p.R457H	11	homozygotes	Father(heterozygous) Mother(heterozygous)	Yes	Yes	Yes	No	Yes	No	No
Case7	46, XX	c.744C>G	p.Y248T	8	heterozygotes	Mother (heterozygous)	Yes	Yes	Yes	Yes	Yes	Yes	Yes
		c.1370G>A	p.R457H	12	heterozygotes	Father (heterozygous)							
Case8	46, XX	c.1370G>A	p.R457H	11	homozygotes	–	Yes	Yes	No	No	Yes	No	Yes
Case9	46, XX	c.667C >T	p.R223X	–	heterozygotes	–	Yes	No	No	–	Yes	No	No
		c.1820A>G	p.Y607C	–	heterozygotes	–							
Case10	46, XX	c.1370G>A	p.R457H	12	homozygotes	Father (heterozygous) Mother (heterozygous)	Yes	Yes	No	Yes	–	Yes	No
Case11	46, XY	c.1820A>G	p.Y607C	14	heterozygotes	Father(heterozygous)	Yes	Yes	No	No	–	No	No
		c.957-958delTG	–	9	heterozygotes	Mother(heterozygous)							
Case12	46, XY	c.919G>T	–	–	heterozygotes	Mother(heterozygous)	Yes	Yes	–	No	No	No	–
		c.1615G>A	–	–	heterozygotes	Father(heterozygous)							
Case13	46, XX	c.1370G>A	p.R457H	–	–	–	Yes	–	Yes	Yes	Yes	Yes	Yes
		c.744C>G	p.Y248X										
Case14	46, XY	c.1370G>A	p.R457H	–	–	–	Yes	Yes	–	Yes	–	Yes	Yes
		c.744C>G	p.Y248X										
Case15	46, XY	c.1370G>A	p.R457H	–	–	–	Yes	Yes	–	Yes	–	Yes	Yes
		c.1660C>T	p.R554X										
Case16	46, XY	c.1370G>A	p.R457H	–	–	–	Yes	Yes	–	No	–	No	–
		c.1820A>G	p.Y607C										

(Continued)

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TABLE 3 Continued

Case	Karyotype	POR mutation		Exon	Zygote	Inheritance	Clinical features					Bone deformity	Pubertal failure
		Nucleotide changes	Amino acid changes				Steroidgenesis disorders	Genital abnormalties	Delay of puberty	Maternal virilization	Polycystic ovary /infertility		
Case17	46, XY	c.1370G>A	p.R457H	–	–	–	Yes	Yes	–	No	–	Yes	–
		c.629A>G	p.D210G										
Case18	46, XX	c.1370G>A	p.R457H	–	–	–	Yes	Yes	Yes	Yes	Yes	Yes	–
		c.517-19_517-	10delGGCCC TGTGinsC										
Case19	46, XY	c.1370G>A	p.R457H	–	–	–	Yes	Yes	–	Yes	–	Yes	–
		c.517-19_517-	10delGGCCC TGTGinsC										
Case20	46, XX	c.1370G>A	p.R457H	–	–	–	Yes	Yes	–	–	–	Yes	–
		c.1370G>A	p.R457H										

* The (-) symbol indicates the absence of Data.

Case 1 (2008, Peking union medical college hospital), case 2 (patient's mother, 2013, Peking union medical college hospital), case 3 (2014, Peking union medical college) ,case 4 (2016, children's hospital affiliated to Shanghai Jiao tong university), case 5 (2017, Peking union medical college), case 6 (2017, the first affiliated hospital of China medical university), Case 7 (2018, the first affiliated hospital of air force military medical university), case 8 (2018, Ruijin hospital), case 9 (2018, Ruijin hospital), case 10 (2019, children's hospital of Chongqing medical university), case 11 (2019 ,children's hospital of Chongqing medical university), case 12 (2018, Medical Genetics Research Center, School of Life Sciences, South University), Case 12-20 (Beijing Children's Hospital, Capital Medical University in 2019) (the first 7 cases were from Beijing Children's Hospital, and the last case was from Children's Hospital of Zhengzhou Medical University).

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Through this case report and by reviewing other cases that have been reported in China, we hope to help physicians to understand the rare disease and avoid misdiagnosis. Attention should be paid to the existence of PORD during differential diagnosis of CAH. *POR* mutation can result in various clinical manifestations, including pseudohermaphroditism at birth, skeletal deformity, maternal hyperandrogenism during pregnancy, and adrenocortical insufficiency. Currently, the diagnosis is mainly based on clinical manifestations, abnormal secretion of steroid hormones, and genetic testing. For clinically suspected patients in whom confirmation by biochemical diagnosis is difficult, genetic analysis is recommended. Among the known mutations, POR p.R457H is a hot-spot mutation in the Chinese population. The variant c.1379 C>A (p.S460Y), identified in this study, is a novel mutation in the Chinese population, which enriches the mutation spectrum in *POR* in the Chinese population. The clinical management of PORD requires multidisciplinary cooperation. Prenatal diagnosis, based on the genotypes of probands and their parents, should be provided for families in which subsequent pregnancies are expected.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

Author contributions

Manuscript writing, contributed to conception and design of study: JZ. Literature review and sections of manuscript: KW, YoH. Statistical analysis and organizing database: YiH, YiL. Experimental support: SWu, XP. Contributed to conception and design of study: YaL, LY. All authors contributed to manuscript revision, read, and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Challenges in treatment of patients with non-classic congenital adrenal hyperplasia

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Congenital adrenal hyperplasia (CAH) due to 21α-hydroxylase deficiency (21OHD) or 11β-hydroxylase deficiency (11OHD) are congenital conditions with affected adrenal steroidogenesis. Patients with classic 21OHD and 11OHD have a (nearly) complete enzyme deficiency resulting in impaired cortisol synthesis. Elevated precursor steroids are shunted into the unaffected adrenal androgen synthesis pathway leading to elevated adrenal androgen concentrations in these patients. Classic patients are treated with glucocorticoid substitution to compensate for the low cortisol levels and to decrease elevated adrenal androgens levels *via* negative feedback on the pituitary gland. On the contrary, non-classic CAH (NCCAH) patients have more residual enzymatic activity and do generally not suffer from clinically relevant glucocorticoid deficiency. However, these patients may develop symptoms due to elevated adrenal androgen levels, which are most often less elevated compared to classic patients. Although glucocorticoid treatment can lower adrenal androgen production, the supraphysiological dosages also may have a negative impact on the cardiovascular system and bone health. Therefore, the benefit of glucocorticoid treatment is questionable. An individualized treatment plan is desirable as patients can present with various symptoms or may be asymptomatic. In this review, we discuss the advantages and disadvantages of different treatment options used in patients with NCCAH due to 21OHD and 11OHD.

KEYWORDS

Non-classic congenital adrenal hyperplasia (NCCAH), 21-hydroxylase deficiency (21OHD), 11-hydroxylase deficiency (11OHD), treatment options, glucocorticoid treatment

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Introduction

Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive disorders with affected adrenal steroidogenesis leading to impaired cortisol synthesis. Consequently, the production of adrenocorticotropic hormone (ACTH) is increased due to reduced negative feedback on the pituitary gland. In most cases, CAH is caused by a deficiency of 21α-hydroxylase (21OHD) (1). In more rare cases, CAH is due to a deficiency of other enzymes such as 11β-hydroxylase, 17α-hydroxylase, or 3β-hydroxysteroid dehydrogenase (2). The specific hallmark of 21OHD and 11OHD is impaired production of cortisol and an elevation of the adrenal androgen concentration. In patients with 21OHD, the conversion of 17-hydroxyprogesterone (17-OHP) to 11-deoxycortisol is impaired, resulting in elevated levels of 17-OHP that is metabolized into 21-deoxycortisol and adrenal androgens (Figure 1) (3). In patients with 11OHD, the conversion of 11-deoxycortisol to cortisol is impaired resulting in increased levels of 11-deoxycortisol and adrenal androgens (Figure 1).

In this review, we focus on the description of 21OHD with a short separate section about 11OHD.

The severity of the disease and clinical presentation of 21OHD depends on the residual enzymatic activity (Figure 2) (4). Although the clinical spectrum is a gradual scale, 21OHD is historically classified into three groups. In the classic salt-wasting (SW) form, there is a (near) complete loss of enzymatic activity (< 1%) leading to a complete deficiency of both cortisol and aldosterone. The aldosterone deficiency in SW patients leads to neonatal salt loss which might be fatal if not recognized and treated. A residual enzymatic activity of 1 – 2%, referred to as the classic simple virilizing (SV) form, is needed to produce sufficient aldosterone and prevent salt wasting (3). Patients with a residual 21α-hydroxylase activity of 20-50% have a less severe phenotype and are grouped as non-classic CAH (NCCAH) (5).

In many countries, patients with classic 21OHD are diagnosed early in life by neonatal screening programs incorporating the measurement of 17-OHP concentrations in dried blood spots. However, in NCCAH patients, basal 17-OHP levels are generally not markedly elevated. CAH can also be diagnosed by the quantification of elevated adrenal steroids in serum taken before and after ACTH stimulation. In addition, genotyping can be performed to confirm the diagnosis in suspected cases.

There is generally sufficient cortisol and aldosterone production during basal conditions, although suboptimal cortisol levels have been described in some NCCAH patients after a stimulation test (6–9). The estimated prevalence of NCCAH is 1 in 200-1,000 in the Caucasian population (10, 11) with a higher prevalence in specific ethnic groups such as Ashkenazi Jews (12).

Due to the enzyme deficiency in both classic CAH and NCCAH patients, the precursor steroids before the enzymatic block increase and are partially shunted into the unaffected adrenal androgen synthesis pathway leading to a variable degree of increased adrenal androgen levels. In the SW and SV form, prenatal androgen concentrations are higher possibly leading to virilization of the external genitals in girls, already *in utero*.

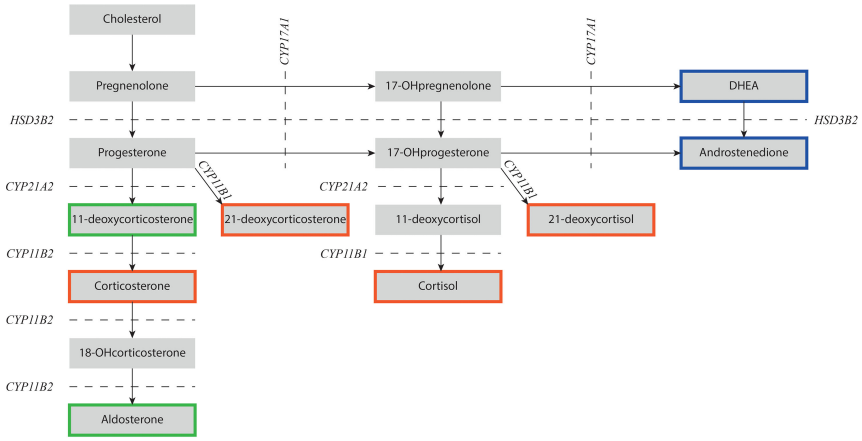

FIGURE 1
Schematic overview of adrenal steroidogenesis. NCCAH patients with 21OHD have impaired 21α-hydroxylase activity (*CYP21A2*) and NCCAH patients with 11OHD have impaired 11β-hydroxylase activity (*CYP11B1*). Precursor steroids prior to the enzymatic block increase and are shunted into androstenedione that can be converted into testosterone and dihydrotestosterone in the gonads. Steroids depicted in a red box have mainly glucocorticoid activity, steroids in a green box have mainly mineralocorticoid activity, and steroids in a blue box have mainly androgen activity. DHEA, dehydroepiandrosterone; *CYP11B1*, 11β-hydroxylase; *CYP11B2*, aldosterone synthase; *CYP17A1*, 17α-hydroxylase/17,20-lyase; *CYP21A2*, 21α-hydroxylase; *HSD3B2*, 3β-hydroxysteroid dehydrogenase type 2; OH-, hydroxy-.

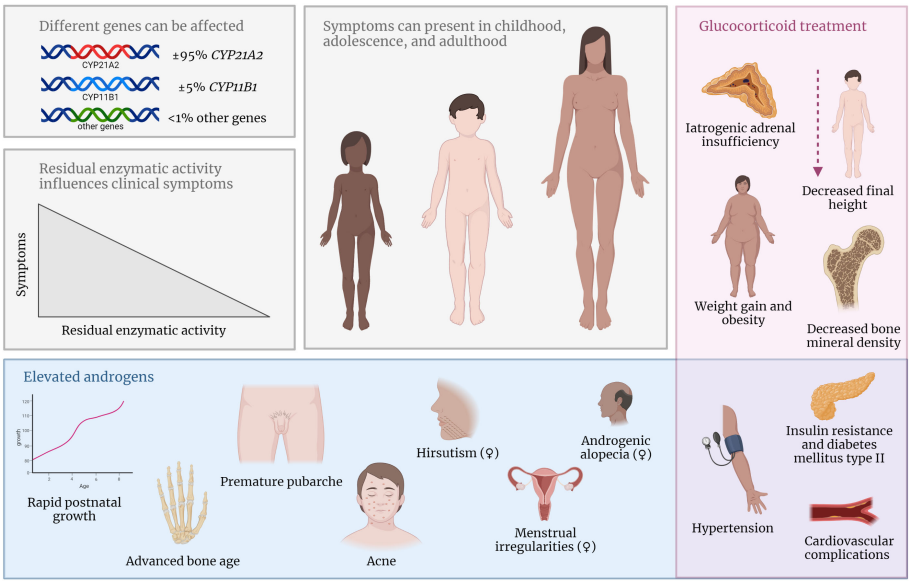

FIGURE 2
In NCCAH, different gene mutations can lead to decreased activity of the effected enzyme. Symptoms can present during childhood, adolescence, and adulthood and are influenced by the residual enzymatic activity. Elevated androgen concentrations can lead to various clinical symptoms (blue box). Side effects of supraphysiological glucocorticoid treatment are common (red box). Symptoms in the purple box occur due to elevated androgens or glucocorticoid treatment.

In NCCAH patients, androgen levels are lower compared to classic patients, and symptoms of hyperandrogenism present later in life. NCCAH patients may present with mild symptoms such as premature pubarche, accelerated bone age during childhood, acne in both boys and girls, and irregular menstruation and/or hirsutism in women (Figure 2). However, many patients are asymptomatic, especially adult males, because testicular androgen production of adult males surpasses adrenal androgen production (13). The clinical phenotype of female NCCAH patients might resemble the phenotype of women with polycystic ovarian syndrome, therefore, it can be challenging to distinguish between these diseases (14).

There is a gradual scale in 21OHD phenotypes and there are no specific cut-off values between classic and non-classic 21OHD. In general, treatment guidelines for classic CAH patients recommend glucocorticoid treatment to substitute for low serum cortisol concentrations and to suppress elevated ACTH to lower adrenal androgen synthesis. However, for NCCAH patients, general guidelines for monitoring and treatment are scarce, and the evidence for recommendations in guidelines is low (11, 15–17).

In this review, we will discuss the clinical aspects of NCCAH patients in childhood, adolescence and adulthood. We will first focus on patients with 21OHD and discuss the effect of treatment on different clinical outcome measures. Thereafter, we will shortly discuss patients with 11-hydroxylase deficiency (11OHD).

Cortisol production in NCCAH

Basal cortisol production

In contrast to classic 21OHD patients in which cortisol production is significantly impaired, most patients with non-classic 21OHD have basal cortisol levels within normal reference ranges when measured by immunoassay (7–9, 18). It should, however, be noticed that immunoassays are not free of cross-reactivity to precursor steroids that are generally increased in (NC)CAH patients leading to a possible overestimation of the cortisol concentration. Therefore, liquid chromatography tandem mass spectrometry (LC-MS/MS) should be used, which is currently the gold standard for quantifying steroid hormones, especially in patients with defects in the steroidogenesis (11).

Oriolo et al. (18) measured cortisol in NCCAH patients by LC-MS/MS in fasting serum taken between 8.00 and 8.30 am. Interestingly, they reported mean (± SD) cortisol levels (383 nmol/L ± 183) that were not significantly different compared to heterozygous carriers of 21α-hydroxylase mutations or women with polycystic ovarian syndrome. In addition, Ueland et al. (19) reported normal cortisol levels (median 346 nmol/L, range 140 – 771) in their cohort of NCCAH patients and heterozygous carriers. Even the lowest reported cortisol level (140 nmol/L) in this cohort of eight patients was within the range of the control group (108 – 653 nmol/L) (20). The absence of

biochemical cortisol deficiency is supported by two studies that reported normal ACTH levels in NCCAH patients (7, 21), indicating sufficient negative feedback from cortisol on the pituitary gland.

These findings are in line with the clinical observations that manifestations of cortisol deficiency are uncommon in untreated NCCAH patients (7, 8). Stoupa et al. (8) reported that 33 out of 35 NCCAH patients (94.3%) showed no signs of hypocortisolism under basal conditions. The remaining two patients (5.7%) suffered from fatigue. These two patients were sisters, and both carried the same genetic defects, namely a V281L mutation and a large gene conversion, from which the latter is a severe mutation.

As most NCCAH patients do not have decreased cortisol production, suppletion with glucocorticoids is not recommended and might even be potentially harmful as glucocorticoids will suppress the pituitary-adrenal axis with consequently the need to use stress dosing in situations of illness. El-Maouche et al. (22) reported Addisonian crises in NCCAH patients receiving hydrocortisone treatment. In addition, Oliveira et al. (23) reported that 15% of their treated NCCAH patients did have at least one episode of acute adrenal insufficiency, while none of their untreated NCCAH patients did.

In conclusion, NCCAH patients have normal basal cortisol production. General treatment with glucocorticoids is not recommended and may even lead to iatrogenic adrenal insufficiency with a risk of developing an Addisonian crisis (Figure 2).

Cortisol production during periods of physical stress

During physical stress, such as sick days or surgery, ACTH levels increase leading to increased production of cortisol, which is necessary to modulate the immune response and maintain adequate blood pressure (24, 25).

An ACTH stimulation test, in which cortisol and other adrenal steroids are measured after ACTH administration, is currently the gold standard to assess cortisol production in a clinical setting. A suboptimal cortisol response is reported in about 21-60% of NCCAH patients (6–9, 26). Nandagopal et al. (7) and Stoupa et al. (8) reported no significant correlation between the cortisol response to synthetic ACTH and the genotype of the patients.

Nonetheless, symptoms of acute adrenal insufficiency during periods of illness are seldomly reported in untreated NCCAH patients (6–9). Bidet et al. (6) described a cohort of 161 untreated NCCAH women (age range 13 – 52 years old) of whom two (1.2%) experienced signs of acute adrenal insufficiency. One patient had high fever for several days due to pyelonephritis and the other patient suffered from repeated vomiting in the first trimester of pregnancy. Both patients survived without the administration of hydrocortisone treatment. Nandagopal et al. (7) described three out of eight NCCAH patients (aged 27, 49, and 66) with a suboptimal cortisol response after ACTH stimulation but without signs of acute adrenal insufficiency during physical stress in their life, even though two of them underwent surgeries, such as Cesarean section, cholecystectomy, and bilateral ankle surgery. In addition, Karachaliou et al. (9) described a cohort of 31 pediatric NCCAH patients of whom seven (21.2%) had impaired cortisol levels after ACTH stimulation. None of these patients experienced signs of acute adrenal insufficiency during their life. These authors question the necessity of stress dosing in NCCAH patients, even in periods of severe stress. However, large cohort studies are necessary to confirm this.

The lack of symptoms, even in patients with a suboptimal cortisol response after ACTH, might be explained by several hypothetical mechanisms:

Firstly, several other steroid hormones are able to bind and activate the glucocorticoid receptor. The ligand binding domain of the glucocorticoid and mineralocorticoid receptors are structurally very similar and cross-reactivity of different adrenal steroids has been demonstrated before (27). Actually, corticosterone, an intermediate in the aldosterone synthesis pathway, is known to have glucocorticoid effects (28) and is the most important glucocorticoid in birds (29) and rodents (30). Specific hydroxyl groups at positions 11, 17, and 21 enhance binding affinity with the glucocorticoid receptor and subsequent glucocorticoid activity (31). Cortisol has a hydroxyl group at these three positions and is known as the endogenous steroid hormone with the greatest affinity for the glucocorticoid receptor in humans. However, other steroids with only two out of three hydroxyl groups can also have a glucocorticoid effect. In 21OHD, 17-OHP and 21-deoxycortisol are elevated (Figure 1). This latter steroid hormone has a hydroxyl group at positions 11 and 17 and Engels et al. (32) reported that 21-deoxycortisol can bind, translocate, and activate the glucocorticoid receptor *in vitro* with a relative potency of 49% compared to cortisol. In addition, 21-deoxycorticosterone, an 11-hydroxylated progesterone molecule, can activate the receptor with a relative potency of 23% compared to cortisol. During periods of physical stress, ACTH increases to stimulate the adrenal cortex. Due to the enzyme deficiency, steroids prior to the enzymatic block, such as 21-deoxycortisol (33) and 21-deoxycorticosterone (34), increase. These precursor steroids may partially compensate for the insufficient cortisol response found in some NCCAH patients and thereby prevent signs of cortisol deficiency.

Secondly, the total cortisol concentration that is generally measured might give an inaccurate reflection of the biological glucocorticoid activity. The total cortisol concentration consists of the cortisol bound to proteins (± 90%), mostly corticosteroid binding globulin, and unbound (i.e., free) cortisol. Only free cortisol activates the glucocorticoid receptor (35–39). It has been

reported that other steroid hormones, that are increased in 21OHD patients, such as testosterone, progesterone, 17-OHP, and 21-deoxycortisol, can influence the binding of cortisol to corticosteroid binding globulin (40–44), possibly increasing free cortisol levels in NCCAH patients.

Lastly, polymorphisms in the glucocorticoid receptor have been associated with differences in sensitivity (45), possibly increasing the sensitivity in some NCCAH patients. Further research is necessary to better understand individual differences in glucocorticoid sensitivity and how we can implement this in routine clinical CAH care.

In conclusion, a suboptimal cortisol response after ACTH stimulation is reported in up to 60% of untreated NCCAH but symptoms of an Addison crisis are not often reported. Therefore, current guidelines recommend stress dosing in those patients with a suboptimal ACTH test only in severe stress situations.

Growth

Untreated and poorly treated children with classic 21OHD show accelerated growth, advanced bone age with early epiphyseal fusion, and subsequently reduced final height due to overproduction of adrenal androgens (46, 47). The effects of adrenal androgens on (increased) bone maturation become already relevant in the second year of life (48, 49). On the long term, these androgens lead to premature epiphyseal closure resulting in reduced final height.

In contrast, in untreated NCCAH patients, reported final heights are within the normal range in most studies (6, 50–52), suggesting that the elevated androgens in these patients do not lead to significant growth reduction. However, one study by New et al. (53) reported a final height below the target height in untreated NCCAH patients. Mutation analyses were not performed in this study, so it is unclear whether differences in genotype could explain the discrepancy between the studies. Einaudi et al. (54) reported that untreated pediatric NCCAH patients with one of the two affected *CYP21A2* mutations classified as moderate or severe (e.g., Q318X, IVS2, or R356W) showed a higher height SDS compared to patients with two mutations classified as mild (e.g., V281L, P453S, or P30L). This is probably due to the higher levels of androgens in the former group leading to growth acceleration during childhood (Figure 2).

To avoid the unwanted effects of adrenal androgens on growth, children with CAH are treated with glucocorticoids to restore the negative feedback on the pituitary gland and consequently decrease the overproduction of adrenal androgens. Mostly, supraphysiological dosages of glucocorticoids are necessary to reach this goal (55), especially during puberty, because cortisol pharmacokinetics changes in this period resulting in increased cortisol clearance (56). The estimated endogenous cortisol production is 5.3 – 7.4 mg/m^2/day (57–60), while recommended dosages for pediatric CAH patients are 10 – 15 mg/m^2/day. Unfortunately, supraphysiological glucocorticoid dosages may suppress growth in children as well and cause additional weight gain in children and adults (Figure 2), especially when long-acting glucocorticoids like prednisone or dexamethasone are used (61–64). Therefore, finding a balance between over- and undertreatment in CAH patients is often challenging and the prescription of long-acting glucocorticoids should be avoided in children (65).

There is no clear evidence that final height is significantly decreased in NCCAH patients receiving glucocorticoid treatment during childhood (47, 51, 66). Wasniewska et al. (51) reported no significant difference in final height between treated and untreated patients. In contrast, Eyal et al. (52) reported reduced final height in NCCAH patients after receiving hydrocortisone compared to untreated patients. However, the group that received treatment during childhood was diagnosed earlier than the untreated group. In addition, there was a significant difference in genotype between the two groups, the occurrence of two mild mutations was respectively 70% in the treated group versus 89% in the untreated group. Furthermore, also heterozygous carriers were included in this study, possibly causing an underestimation of the effect of elevated androgens and glucocorticoids on final height. Weintrob et al. (67) found that the age of initiation of glucocorticoid treatment may also influence final height: NCCAH patients in whom treatment was started at least one year before the onset of puberty had a better height outcome compared to patients who started treatment after the onset of puberty. However, in the group of patients who started treatment after the first signs of puberty, three out of eight had precocious puberty, which might result in compromised final height and may have influenced the results of this study.

Thus, phenotypic heterogeneity, as well as differences in patient population and treatment regimens makes the interpretation and comparison of the results less reliable.

Clinically, growth acceleration is often small in untreated children with NCCAH (68). Bone age can be used as an additional clinical parameter besides monitoring growth velocity to evaluate the effect of adrenal androgens on growth (54, 68). A progressive bone age acceleration may be an indication to start glucocorticoid treatment, but careful counseling about the advantages and disadvantages of this treatment should be offered to patients and parents including the need to use stress dosing. Glucocorticoid treatment can be discontinued when final height is reached. Discontinuation of glucocorticoid treatment in NCCAH patients decreases the risk of long-term complications such as iatrogenic Cushing syndrome with excessive weight gain (69).

In conclusion, negative effects of adrenal androgens in untreated pediatric NCCAH patients are generally mild but patients with more advanced bone age acceleration are

described. Therefore, yearly follow-up of growth and bone age is recommended. Glucocorticoid treatment should only be initiated in children with NCCAH after careful counseling of patients and parents and should be discontinued after reaching the final height.

Puberty

Premature pubarche, defined as the presence of pubic hair before the age of 8 years in girls and 9 years in boys (70), is the most common symptom of androgen excess in prepubertal children with NCCAH with an incidence of 55-92% (71–73). Chronically elevated adrenal androgens can also increase the GnRH pulse frequency in GnRH neurons (74) and can, thereby, potentially activate the pituitary-gonadal axis leading to earlier onset of puberty (71, 75–77). Puberty onset in NCCAH patients is earlier compared to the average population (47, 67) but, in general, within the physiological range. The age of puberty onset is related to the genotype; compound heterozygous patients with one severe mutation (e.g., Q318X, I2 splice, or I172N) and one mild mutation (either V281L or P30L) tend to have an earlier onset of puberty compared to patients with two mild mutations (78). However, true central precocious puberty, defined as activation of the hypothalamus pituitary gonadal axis leading to breast development before the age of 8 years in girls or a testicular volume ≥4 mL before the age of 9 years in boys, is only seen in about 4-5% of untreated NCCAH patients (71). Therefore, hydrocortisone treatment is not recommended to prevent central precocious puberty.

In boys, a testes volume of ≥4 ml indicates an activation of the gonadal axis with increased production of gonadal testosterone (79). During puberty, testicular androgen production greatly overshoots the adrenal androgens production in boys and enduring suppression of androgens seems unfavorable. Therefore, Merke et al. (69) recommend discontinuation of glucocorticoid treatment at a testicular volume of 8-10 mL (Tanner stage 3) when glucocorticoid treatment is used to prevent early pubertal development.

In NCCAH girls treated with glucocorticoids, the age of onset of puberty and menarche is reported as normal (23, 47, 54, 66, 67). One recent study (23) reported a significantly earlier age of menarche in NCCAH patients compared to classic patients but results are hard to compare as all classic patients were treated with glucocorticoids, while 13% of the NCCAH patients were untreated. However, the median age of menarche was still in the normal range in both classic and NCCAH patients. Einaudi et al. (54) described a correlation between age of menarche and severity of the mutation between subtypes of NCCAH, but the median age of menarche was within the normal range for all subtypes. Approximately half of the female NCCAH patients suffer from oligomenorrhoea (72) due to increased production of adrenal androgens, which are aromatized to estrogens, and elevated adrenal progesterone production, both leading to suppression of the hypothalamic-pituitary-gonadal axis (80). Menstrual regularity can be achieved by oral contraceptives with antiandrogenic effects (81).

In conclusion, premature pubarche is a common symptom in NCCAH patients but central precocious puberty is rare and hydrocortisone treatment to prevent precocious puberty is not recommended. If central precocious puberty occurs, hydrocortisone treatment can be started to improve final height, but only after careful counseling.

Transition into adult care

Patients with NCCAH in adolescence and adulthood have reached their final height and completed their pubertal development. Therefore, other treatment goals are important during these stages. Before transition into adult care it is important to assure regular monitoring, education about the disease and long-term follow up (82). Therewith, patients have sufficient information about the disease and the treatment to be self-dependent if necessary. In the next paragraphs, we will discuss the different clinical parameters that should be considered during adolescence and adulthood.

Bone health

Low (83–98), normal (99–105), and high (106) bone mineral densities (BMD) are reported in studies in which both classic and NCCAH patients were included. However, knowledge about bone health in NCCAH patients is scarce because most studies do not present data of NCCAH patients separately (84, 92, 93, 96, 97, 102). It is known that glucocorticoid treatment can potentially affect bone health. If NCCAH patients use glucocorticoids, dosages are usually comparable to the dosages used in classic patients (55), so the long-term side effects of glucocorticoid treatment are most likely similar. However, in NCCAH, glucocorticoid treatment is most often initiated at a later age, and before that time NCCAH patients are exposed to androgens which is favorable for BMD (see below). This is also illustrated in several studies where higher bone quality or BMD is reported in NCCAH patients compared to classic CAH patients (85, 89, 105, 107, 108). Finkielstain et al. (94) reported comparable BMD between classic and NCCAH patients. In this same study, older age was associated with lower femoral neck BMD in classic CAH patients but not in NCCAH patients. This might indicate that prolonged glucocorticoid treatment has a negative effect on femoral BMD in classic patients but less in NCCAH patients (94).

The inconsistency between studies may be due to differences in the patients' age of BMD determination. Besides, several factors play a role in the bone health of NCCAH patients:

Firstly, androgens stimulate osteoblast proliferation and differentiation and inhibit osteoclast formation (109), thereby, increasing BMD. This was illustrated by one study reporting higher lumbar spine BMD SDS in classic CAH prepubertal patients who did only receive glucocorticoid treatment for a relatively short period (106).

Secondly, glucocorticoids have direct effects on bone metabolism. They increase bone resorption by upregulation of osteoclasts (110) and increase their life span (111). In addition, glucocorticoids directly induce osteoblast and osteocyte apoptosis which subsequently leads to decreased bone formation (112). Furthermore, osteoblasts produce fewer vascular growth factors during glucocorticoid treatment which inhibits bone vascularization leading to bone necrosis (113). These glucocorticoid effects have a negative impact on bone formation, especially on trabecular bone (114) which is mainly present in the vertebral bodies and epiphyses of long bones (115).

Thirdly, glucocorticoids have an indirect effect on BMD by inhibiting calcium absorption in the gastrointestinal tract and calcium reabsorption in the renal tubules (116).

Lastly, glucocorticoids decrease the concentration of androgens (117), thereby attenuating the positive effect of the increased androgen concentration on BMD in NCCAH patients.

It is generally known that glucocorticoids have potential negative effects on BMD, also observed in patients who use glucocorticoids for various reasons (114). Long-acting glucocorticoids like dexamethasone and prednisolone have a less favorable effect on BMD compared to short-acting glucocorticoids like hydrocortisone (84, 118).

In general, the use of glucocorticoid treatment increases the risk of fractures (119). A recent study from Falhammar et al. (120) reported an increased frequency of fractures in classic CAH patients but not in NCCAH patients. This is consistent with the findings of another study that reported fewer non-traumatic fractures in NCCAH patients compared to CAH patients (108). However, when only major osteoporotic fractures are considered, both forms of CAH had an increased frequency (± 5% in NCCAH vs ±10% in classic CAH patients) compared to the general population (120). This indicates that glucocorticoid treatment has clinically relevant negative effects on bone health. When glucocorticoid use is discontinued, the fracture risk decreases (114, 119), indicating that the glucocorticoid effects are reversible. If patients are untreated, no routine dual-energy X-ray absorptiometry is necessary to evaluate BMD. The Endocrine Society suggests screening for BMD only in patients receiving high dosages of glucocorticoids or who suffered from a non-traumatic fracture (11).

Besides the use of glucocorticoids, other risk factors like smoking, low intake of calcium and vitamin D, and low physical activity increase the risk of osteoporotic fractures (121). It is essential to inform NCCAH patients about these risk factors to preserve their bone mineral density and reduce the risk of osteoporotic fractures later in life.

In conclusion, treatment with glucocorticoids during childhood can already negatively influence BMD later in life. Therefore, both endocrinologists and pediatric endocrinologists should include these (long-term) negative effects in the decision whether glucocorticoid treatment is beneficial for their particular patient and to properly counsel their patients. When glucocorticoids are necessary, hydrocortisone should be used, especially in children, as this short-acting glucocorticoid has fewer negative effects on BMD compared to long-acting glucocorticoids. Patients should be counseled for the potential decrease in BMD, and other risk factors for osteoporotic fractures like smoking, low calcium and vitamin D levels, and low physical activity should be brought to a minimum.

Cardiovascular and metabolic complications

NCCAH is associated with increased cardiovascular and metabolic morbidity in adulthood (122). Both glucocorticoid treatment and androgen excess are important factors in this respect (Figure 2). Supraphysiological glucocorticoid dosages are associated with a higher prevalence of obesity, insulin resistance, dyslipidemia, and hypertension, which are known risk factors for cardiovascular disease (123, 124). On the other hand, untreated or undertreated 21OHD patients suffer from androgen excess which can also be unfavorable for the cardiovascular and metabolic risk profile (125). In this paragraph, different cardiovascular and metabolic complications and risk factors in NCCAH patients will be discussed.

Several studies (92, 94, 122) reported a higher prevalence of obesity in glucocorticoid-treated NCCAH patients compared to healthy controls. Falhammar et al. (126) reported a higher BMI and waist-to-hip ratio in treated NCCAH women (compared to controls) who were ≥30 years of age, but not in patients <30 years. This indicates that the effect on body composition and fat distribution occurs later in life. Several factors may cause weight gain in CAH patients (127). Völkl et al. (128) reported a minor positive correlation (r=0.22, p=0.04) between hydrocortisone dose and BMI, suggesting that supraphysiological dosages of glucocorticoids contribute to weight gain. Ariyawatkul et al. (129) did, however, not confirm this finding, possibly because a smaller sample size was used. Zhang et al. (130) reported a higher BMI in 30 untreated female patients with classic simple virilizing 21OHD, indicating that glucocorticoids are not the only factor influencing the weight gain in classic 21OHD and that the elevated androgens are possibly a contributing factor as well. These authors suggested that the elevated androgens have a negative effect on the body fat distribution and lipid metabolism. Nonetheless, Saygili et al. (125) reported no significant difference in BMI between untreated NCCAH patients and healthy controls even though free testosterone levels were four times higher in the patient group compared to the controls. The

difference between the two studies might be explained by the fact that the former study included more severe cases of 21OHD compared to the latter (simple virilizing vs. NCCAH) and the included patients had higher concentrations of androgens. This suggests that slightly increased androgen levels in NCCAH do not affect BMI.

Another factor influencing cardiovascular and metabolic risk is insulin sensitivity. Several studies reported increased insulin resistance in both treated and untreated NCCAH patients compared to controls (90, 92, 125, 131–133). Williams et al. (132) confirmed this finding in treated NCCAH patients but did not report a higher prevalence of insulin resistance in classic CAH patients. In this study, classic CAH patients were diagnosed by newborn screening and subsequently treated. The NCCAH patients were diagnosed at a later age and, therefore, exposed to elevated androgen concentrations for a longer postnatal period. This suggests that hyperandrogenism contributes to insulin resistance in these NCCAH patients. This is confirmed by other studies (14, 134, 135) who reported higher rates on insulin resistance in women with polycystic ovarian syndrome, who also suffer from hyperandrogenism. There is a vicious circle in which hyperandrogenism leads to insulin resistance, resulting in hyperinsulinemia, which in turn leads to an aggravation of hyperandrogenism (135). On the contrary, Bayraktar et al. (136) did not report increased insulin resistance in NCCAH patients compared to controls. As genetic testing was not performed here, differences in disease severity of these patients versus patients included in other studies could not be evaluated. Besides hyperandrogenism, also glucocorticoids lead to insulin resistance by opposing the actions of insulin (137). Delai et al. (133) confirmed this in NCCAH patients and found that insulin resistance in patients was related to prolonged use of long-acting glucocorticoids.

Whether the increased insulin resistance also leads to a higher incidence of diabetes mellitus type II was unknown for a long time, because only a few patients were older than 50 years old in the described studies and diabetes usually presents later in life. Falhammar et al. (138) did not report higher incidences of diabetes in one study but sample sizes were small and the oldest included patient was 67 years old. In a later study, Falhammar et al. (122) found a higher prevalence of diabetes in a cohort of 75 treated NCCAH patients (with the oldest patient being 92 years old) compared to age- and sex-matched controls.

Another important risk factor for cardiovascular diseases is hypercholesterolemia. Hypercholesterolemia has been observed in 59% of the NCCAH females (92). This incidence was even higher compared to the classic CAH males and females (36% and 48% respectively) (92). Krysiak et al. (139) reported that atorvastatin, a statin that reduced the levels of cholesterol and androgens, decreased the cardiometabolic risk in untreated NCCAH women. If this decrease in risk also leads to fewer cardiovascular incidents in these patients, needs to be further elucidated.

In conclusion, glucocorticoid treatment has potential negative effects on the cardiovascular and metabolic system (123, 124). These complications are dose-dependent (123, 140) and, therefore, glucocorticoids should always be prescribed in the lowest effective dose (124). However, hyperandrogenism also contributes to an unfavorable cardiometabolic risk profile, and therefore adequate monitoring and balancing over- and undertreatment is necessary. Other treatment options like statins need further attention, to elucidate whether these can be used in untreated NCCAH patients with hyperandrogenism to reduce the risk for cardiometabolic complications.

Dermatological symptoms caused by hyperandrogenism

Hyperandrogenism can lead to well-known clinical symptoms such as hirsutism, acne vulgaris, and androgenetic alopecia (Figure 2). In NCCAH, these symptoms of androgens excess can present already during adolescence but are mostly observed later in life. Hirsutism is the most common symptom in adult women with NCCAH and does not correlate well with genotype (54). In childhood, hirsutism is only observed in 4% of the patients, while in adulthood the incidence rate increases to 69-78% (6, 72). Alopecia incidence rates also increase with age with peak incidences of 19% between 40-49 years of age (72). Acne is most often observed in 20- to 29-year-olds with an incidence of 37% (72). Signs of adrenal hyperandrogenism are less frequently observed in men, as testicular androgen production greatly outreaches adrenal androgen production (13).

Glucocorticoids can be effective in lowering adrenal androgen levels and thereby also decreasing the dermatological signs of hyperandrogenism. However, due to long-term negative effects on bone health and cardiovascular risk as discussed above, other drugs like oral contraceptives with antiandrogenic effects are generally used as first step treatment option in females with NCCAH suffering from hirsutism. Different mechanisms contribute to the antiandrogenic effect of oral contraceptives. First, estrogens have negative feedback on the pituitary gland, thereby lowering the levels of luteinizing hormone resulting in less ovarian androgen production in females and testicular androgen production in males (141). In addition, oral contraceptives lower adrenal androgen production by inhibiting the enzymatic activities of 17-hydroxylase and 17,20-lyase which are necessary for androgen production (142). Furthermore, estrogens in oral contraceptives increase sex hormone binding globulin (SHBG) production and thereby reduce free testosterone levels (143, 144). Lastly, progestogens in oral contraceptives inhibit the function of 5α-reductase resulting in less conversion of testosterone into the more potent androgen dihydrotestosterone (145). Although oral contraceptives can contain different progestogens with either androgenic or antiandrogenic features, the net effect of combined oral

contraceptives is always antiandrogenic (146). Antiandrogenic progestogens can also be used as monotherapy. For instance, cyproterone acetate monotherapy has been described to be superior compared to hydrocortisone in treating hirsutism in female NCCAH patients (147).

Another option interfering with the androgen pathway to reduce signs of hyperandrogenism is the administration of spironolactone. This mineralocorticoid receptor antagonist has antiandrogenic effects as it blocks the binding of (dihydro) testosterone to the androgen receptor and diminishes 5α-reductase activity in the skin (148, 149). Physicians used to be hesitant to prescribe spironolactone for hirsutism or acne treatment because carcinogenic features of spironolactone are described (3). However, this was only seen in animal models using very high dosages of spironolactone (150). Higher incidence rates for cancer were not found in humans using spironolactone (151–153) and nowadays, the use of spironolactone in the treatment of acne is increasing (154).

Other off-label treatment options for hyperandrogenism are competitive antagonists of the androgen receptor, like flutamide, and 5α-reductase inhibitors, like finasteride and dutasteride (146). However, these should not be used in childhood and in women who try to conceive or are currently pregnant. These antiandrogens can have side effects like decreased libido, headache, dizziness, and nausea (155–157). In addition, flutamide is potentially hepatotoxic and liver functions should be monitored during treatment (158).

Besides antiandrogens, also cosmetic and/or topical treatment options have to be considered to treat hirsutism in female NCCAH patients. This includes, among others, shaving, plucking, waxing, laser therapy, or topical eflornithine cream (159, 160). For acne, topical gels with benzoyl peroxide, antibiotics, retinoids, or azelaic acid can be effective (161). A combination of different treatment approaches (both topical and systemic hormonal) might be most effective (160, 162).

In conclusion, NCCAH women may suffer from signs of hyperandrogenism such as hirsutism, acne vulgaris, or androgenetic alopecia. Glucocorticoid treatment is not recommended to reduce signs of hyperandrogenism in these patients. An individualized approach with topical and/or hormonal treatment is necessary for treating signs of hyperandrogenism, and consultation with a dermatologist can be of added value for these patients.

Fertility and pregnancy in women

Oligomenorrhoea in NCCAH women may lead to sub- or infertility. However, reported pregnancy rates are normal in these women (163–166). Bidet et al. (167) reported that 57% of the pregnancies were spontaneous without any treatment and 83% of the women who conceived were pregnant within one year. Untreated mothers with 21OHD have elevated androgen levels but these androgens cannot reach the fetus because placental aromatase will convert them into estrogens protecting the female fetus from virilization (168).

If pregnancy is not achieved, temporary glucocorticoid treatment might be indicated to normalize progesterone levels (11, 167, 169). Here, hydrocortisone, prednisolone, or prednisone should be used as they can be inactivated by placental 11β-hydroxysteroid dehydrogenase type 2 and do not reach the fetus (170). Hydrocortisone or prednisone before and/or during pregnancy did not significantly change the duration of pregnancy or the child's birth weight (165, 167). Placental 11β-hydroxysteroid dehydrogenase type 2 is not able to metabolize dexamethasone and dexamethasone will reach the fetus (171) which can lead to negative side effects (172). Therefore, we recommend against the use of dexamethasone in women who try to conceive or are already pregnant. If glucocorticoid treatment is necessary, an endocrinologist specialized in CAH should be involved.

Although pregnancy rates in NCCAH women are similar to those in the general population (163–165), these women have higher rates of miscarriages compared to healthy females (165–167, 173). This might be due to dysfunction of the corpus luteum in NCCAH women (174). The progesterone production of the corpus luteum is most important for the continuation of pregnancy in the first trimester (175) and this is also the period in which most miscarriages occur (165, 167).

Moran et al. (173) showed that miscarriage rates were lower in NCCAH women who were diagnosed before conception compared to NCCAH women who were diagnosed thereafter. The exact reason for this is unclear, but it is noteworthy that almost 65% of the women in the diagnosed group were treated with glucocorticoids (either alone or in combination with clomiphene or menotropins), compared to 5% of the women in the undiagnosed group. However, this study found no significant difference in miscarriage rate between untreated women compared to women treated with glucocorticoids alone (i.e., without clomiphene and/or menotropins). Whether these treated women received glucocorticoid treatment *before* or *during* pregnancy did not influence the miscarriage rate. Moreover, also Eyal et al. (165) did not observe a difference in miscarriage rate in treated versus untreated women. On the contrary, studies by Feldman et al. (166) and Bidet et al. (167) reported normalized miscarriage rates in NCCAH women with glucocorticoid treatment. This difference might be explained by the genotype of the patients; Bidet et al. (167) described patients with a more severe genotype (but both NCCAH) compared to Eyal et al. (165), which possibly results in a greater effect of the glucocorticoids. The other two studies (166, 173) did not report mutation analyses of the NCCAH women. If glucocorticoid treatment does not lead to conception, ovulation induction with clomiphene citrate might be successful (166, 167).

As CAH is a recessive disorder, NCCAH patients have two affected alleles. Therefore, the child has at least one mutated

allele from the affected parent. If the partner is heterozygous for 21OHD or a *de novo* mutation occurs, the child can suffer from CAH with also the risk of a child with a classic CAH. Higher incidence rates of both classic and NCCAH were found in children of NCCAH mothers (165, 167, 173). Therefore, genetic counseling is recommended to inform parents about this risk and to genetically test the partners of patients with NCCAH (167).

In conclusion, reported pregnancy rates are normal in NCCAH women. Therefore, glucocorticoid treatment is generally not advised. There is no conclusive evidence whether glucocorticoid treatment during pregnancy in female NCCAH patients leads to a decreased miscarriage rate. It is recommended that an endocrinologist specialized in CAH is involved before and during pregnancy of NCCAH women. If glucocorticoid treatment is indicated, we advise prescribing hydrocortisone to prevent glucocorticoids to cross the placental barrier.

TART and fertility in men

In classic CAH, benign testicular adrenal rest tumors (TART) can lead to decreased fertility in men (176–179). Four different articles (94, 98, 180, 181) reported the incidence of TART in NCCAH men and described a total of 124 NCCAH patients of whom only two had evidence of TART. So, TART are not a common finding in NCCAH males, and fertility in men with NCCAH is generally normal. Therefore, routine ultrasound of the scrotum is not recommended in NCCAH men (3).

11OHD

The second most common form of CAH is 11OHD with a diminished conversion of 11-deoxycortisol into cortisol (Figure 1) (182). The estimated prevalence of 11OHD in the general population is 1 in 100,000 (183). As the clinical picture of NC 11OHD is variable and could resemble that of women with polycystic ovarian syndrome, its prevalence is most likely underestimated. Interestingly, a prospective study identified no NC 11OHD patients amongst 270 women with hyperandrogenism, whilst six suffered from NC 21OHD (184), indicating that the prevalence of 11OHD is probably lower than the prevalence of NC 21OHD.

In non-classic 11OHD, residual enzymatic activities ranging from 15 to 73% are reported (185, 186). Steroid hormones prior to the enzymatic block, such as 11-deoxycortisol, progesterone, 11-deoxycorticosterone, and 17-OHP increase, of which the latter is converted into adrenal androgens by the unaffected adrenal androgen pathway (Figure 1). Therefore, the non-classic form of 11OHD might also present with signs of hyperandrogenism like precocious pseudo puberty in children or irregular menstruation in females, similar to NC 21OHD patients (187, 188). Treatment management of 11OHD patients also resembles the treatment in 21OHD patients.

Classic 11OHD patients have elevated 11-deoxycorticosterone levels which can bind the mineralocorticoid receptor resulting in high blood pressure, low renin levels, and hypokalemia (189). As 11-deoxycorticosterone prevents salt wasting from happening, no salt-wasting form of 11OHD is known. Salt wasting was only observed after glucocorticoid treatment, as this gives negative feedback on the pituitary gland and, thereby, decreases the ACTH concentration, eventually leading to reduction of the 11-deoxycorticosterone concentration (190). Most NC 11OHD patients do not present with hypertension during childhood (187, 191). However, the occurrence of hypertension in some patients cannot be ruled out because residual enzymatic activity is variable and the clinical picture is a continuum as seen in 21OHD (187). In fact, hypertension is observed in some patients with a mild form of 11OHD (188, 191, 192) and Zachmann et al. (191) found a correlation between the age of diagnosis and systolic blood pressure. This indicates that hypertension can occur in the NC form as well, but reported incidence rates are lower compared to patients with classic 11OHD (191).

Glucocorticoid treatment will restore the negative feedback on the pituitary gland similar to treatment in patients with 21OHD. This might be useful in patients with classic 21OHD but potentially harmful in classic 11OHD patients. Mineralocorticoid activity in 11OHD patients relies on 11-deoxycorticosterone which is in these patients ACTH dependent (193). By administration of glucocorticoids, ACTH will decrease and less 11-deoxycorticosterone is produced, increasing the chance of salt wasting crisis in classic 11OHD patients (190). However, it is unclear to what extent this is relevant for NC 11OHD patients as residual activity of 11β-hydroxylase will probably secure sufficient aldosterone production. If hypertension is present, treatment with mineralocorticoid antagonists like spironolactone might be successful (188, 192). Similar to NC 21OHD patients, 11OHD patients who receive glucocorticoid treatment have an increased risk for an iatrogenic Addisonian crisis if adherence to glucocorticoid treatment is poor and the patient becomes sick (22).

As the clinical picture of 11OHD patients is variable and glucocorticoid treatment has both short- and long-term side effects, it should be considered per patient whether administration of glucocorticoids is beneficial.

Discussion

In this review, we focus on different clinical aspects of NCCAH patients in childhood and adulthood. Most NCCAH patients have cortisol levels within the normal reference ranges and normal ACTH levels, indicating that there is sufficient glucocorticoid activity during basal conditions (i.e., in periods without physical stress). Hence, the main problem in NCCAH

patients during basal conditions is not the decreased cortisol production, but the increased production of adrenal androgens.

Hyperandrogenism can lead among others to rapid postnatal growth, advanced bone age, and premature pubarche in childhood, as well as acne, hirsutism, menstrual irregularities (in females), and decreased insulin resistance in adulthood.

Although glucocorticoid treatment could suppress the production of ACTH in the pituitary gland and lower adrenal androgen production, most NCCAH patients do not suffer from clinically relevant glucocorticoid deficiency. Therefore, glucocorticoid treatment is generally not recommended in NCCAH. Glucocorticoid treatment can have a negative impact on the cardiovascular system, metabolic outcome, and bone health later in life. Besides, externally administered glucocorticoids can lead to iatrogenic adrenal insufficiency during periods of sickness with a risk of developing a life-threatening Addisonian crisis, whilst most untreated NCCAH patients do not develop this severe complication. Therefore, patients should be carefully evaluated and the decision for starting glucocorticoid treatment should be made for each patient individually taking into account whether treatment solely aimed to lower androgen levels outweighs the negative effects of chronic glucocorticoid treatment. Adequate counseling should be offered to patients (and their parents).

In Table 1 we present general recommendations for NCCAH patients based on the literature discussed in this review. It should

TABLE 1 Recommendations for monitoring and treatment of NCCAH patients.

Clinical parameter	Patient group	Recommendations
Daily glucocorticoid treatment	All ages	1. Daily glucocorticoid treatment is not recommended to compensate for a cortisol deficiency.
Stress dosing of glucocorticoids	All ages	1. Stress dosing is only recommended in patients with suboptimal ACTH test in periods of severe physiological stress such as major surgery, child delivery or trauma. 2. In NCCAH patients receiving basal glucocorticoid treatment stress dosing is recommended.
Growth	Childhood	1. Yearly follow up of growth and bone age until final height is reached. 2. Start glucocorticoid treatment in patients with severe accelerated bone age to improve final height only after careful counseling of patients and parents. 3. Use short-acting glucocorticoid like hydrocortisone, especially during childhood. 4. Discontinue glucocorticoid therapy when final height is reached.
Puberty	Childhood	1. Isolated premature pubarche is no indication for glucocorticoid treatment. 2. Glucocorticoid treatment is not advised to prevent central precocious puberty.
Menstruation (in girls en females)	Adolescence and adulthood	1. Daily glucocorticoid treatment is not recommended to regulate the menstrual cycle. 2. Antiandrogenic contraceptives are recommended as the first step in the treatment of menstrual irregularity.
Bone health	Adulthood	1. Screening for BMD is only recommended in NCCAH patients receiving supraphysiological dosages of glucocorticoids for a prolonged period 2. Patients using glucocorticoids should be informed about other risk factors for decreased bone mineral density such as smoking, low intake of calcium and vitamin D, and low physical activity.
Cardiovascular and metabolic system	Adulthood	1. Negative effects of glucocorticoids on the cardiovascular and metabolic system are dose-dependent and therefore glucocorticoids should always be prescribed in the lowest effective dose. 2. Both glucocorticoid treatment and elevated androgens (due to undertreatment) negatively influence the cardiovascular and metabolic system, but evidence in NCCAH is low
Dermatological signs of hyperandrogenism	Adolescence and adulthood	1. Daily glucocorticoid treatment is not recommended as a first step in the treatment of hyperandrogenism such as hirsutism, acne, or androgenic alopecia. 2. Treatment options to reduce hirsutism in adult female NCCAH patients: a. Topical treatment with eflornithine cream. b. Cosmetic treatment such as shaving, plucking, waxing, or laser therapy. c. Systemic treatment with spironolactone, competitive androgen receptor antagonists like flutamide or 5α-reductase inhibitors such as finasteride or dutasteride. d. A combination of these treatment options. 3. For acne, topical treatment with benzoyl peroxide, antibiotics, retinoids, or azelaic acid can be effective. If acne is severe, systemic treatment with antibiotics or retinoids can be used.
TART and fertility	Men in adolescence and adulthood	1. Routine ultrasound for TART screening is not recommended.
Fertility	Women in adulthood who try to conceive	1. Glucocorticoid treatment is only advised in females who do not get pregnant after a substantial period. 2. An endocrinologist specialized in CAH should be involved. 3. If glucocorticoid treatment is indicated, we advise to prescribe hydrocortisone because this glucocorticoid does not cross the placental barrier.
Pregnancy	Pregnant women	1. The use of dexamethasone during pregnancy is not recommended. 2. Genetic counseling is recommended to inform patients about the increased risk for classic and non-classic CAH in their offspring.

be noted that it is difficult to predict which NCCAH patients will develop symptoms of hyperandrogenism because the residual enzymatic activity is a gradual scale that leads to a variable degree of androgen excess in different patients. Furthermore, several genes can be variably affected by different mutations, leading to different residual activities of the affected enzyme. In addition, not all forms of NCCAH show a good genotype-phenotype correlation, and different factors contribute to the clinical presentation. Therefore, an individualized follow-up and treatment plan is of utmost importance and clinicians should always weigh which treatment options are best for their patient.

Further research is necessary to establish more evidence-based treatment recommendations specifically for NCCAH patients. Most studies described only classic 21OHD patients or cohorts of classic and NCCAH patients together. Findings in these patients cannot always be extrapolated to the situation of NCCAH patients. So, bias in the design of these studies may have resulted in misleading conclusions for NCCAH patients. Cohort studies in untreated NCCAH patients can be useful to gain more insight into the natural course of the disease. In addition, such studies will give more information about the risk of Addisonian crisis in untreated patients. Furthermore, more information is needed on differences in glucocorticoid sensitivity, glucocorticoid activity of adrenal precursor steroids, and differences in relative free cortisol levels in NCCAH patients, to better predict which patients have glucocorticoid deficiency and need glucocorticoid treatment. Until these research topics are further elucidated, clinicians should consider whether glucocorticoid treatment is beneficial for their patient based on the whole clinical picture.

Author contributions

BA carried out the literature search, collected the included articles, carried out the initial analyses of the literature, and wrote the manuscript. MS, PS, FS, and AH reviewed and revised the manuscript. HC-G conceptualized and designed the idea of this review, contributed to the initial analyses of the literature, and reviewed and revised the manuscript. All authors contributed to the article and approved the submitted version.

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Figure 2 was created with BioRender.com.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Ovarian gonadoblastoma with dysgerminoma in a girl with 46, XX karyotype 17a-hydroxylase/ 17, 20-lyase deficiency: A case report and literature review

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17α–hydroxylase/17,20–lyase deficiency (17-OHD), caused by mutations in the gene of the cytochrome P450 family 17 subfamily A member 1 (CYP17A1), is a rare type of congenital adrenal hyperplasia (CAH), usually characterized by cortisol and sex steroid deficiency combined with excessive mineralocorticoid. Gonadoblastoma is a relatively rare ovarian tumor that is frequently seen among patients with 46,XY gonadal dysgenesis. Rarely have they been reported in female patients with normal 46,XX karyotype. Here, we report an interesting case of an 11-year-old Chinese girl who presented acute abdominal pain that was later attributed to tumor rupture of right ovarian gonadoblastoma with dysgerminoma. Further evaluations revealed hypertension and hypokalemia. Hormonal findings showed increased progesterone, hypergonadotropic hypogonadism, and low cortisol levels. Her chromosome karyotype was 46,XX without Y chromosome material detected. Genetic analysis revealed that the patient had a homozygous pathogenic variant c.985_987delTACinsAA (p.Y329Kfs*90) in exon 6 of the *CYP17A1* gene and that her parents were all heterozygous carriers of this pathogenic variant. Due to the variable clinical manifestations of 17-OHD, meticulous assessment including genetic analysis is necessary. Further study is warranted to unravel the mechanism of gonadoblastoma in a patient with normal karyotypes.

KEYWORDS

congenital adrenal hyperplasia, *CYP17A1* gene, ovarian gonadoblastoma, dysgerminoma, 17α-hydroxylase/17,20-lyase deficiency

Introduction

Congenital adrenal hyperplasia (CAH) refers to a group of syndromes caused by inherited deficiencies in one of five enzymes involved in the biosynthesis of cortisol from cholesterol (1). The majority of CAH cases (90–95%) are caused by 21α-hydroxylase deficiency, whereas 17α-hydroxylase/17,20-lyase deficiency (17-OHD) is the least frequent form of the condition and only accounts for 1% of all CAH cases (2). The cytochrome P450c17 enzyme catalyzes both 17α-hydroxylase activity and 17,20-lyase activity, which play a pivotal role in the production of cortisol and sex hormones. This enzyme is encoded by the *CYP17A1* gene, located on chromosome 10q24.3. Mutations in the *CYP17A1* gene lead to a deficiency in 17α-hydroxylase/17,20-lyase. Due to blockage of sex hormone synthesis, 17-OHD causes differences/disorder of sex development (DSD) in 46,XY, while sexual infantilism and primary amenorrhea in 46,XX. Moreover, insufficient glucocorticoids in turn increase adrenocorticotropic hormone (ACTH) secretion, leading to adrenal hyperplasia and excessive mineralocorticoid, causing hypertension and hypokalemia (3).

Gonadoblastomas are relatively rare ovarian tumors consisting of sex cord and primitive germ cell components (4). Although the great majority of gonadoblastomas occur in individuals with 46,XY gonadal dysgenesis, a substantial number arise in individuals with normal 46,XX karyotype (5). In this study, we reported a case of an 11-year-old girl with 46,XX karyotype who initially presented with ovarian gonadoblastoma with dysgerminoma and was finally diagnosed with 17-OHD caused by p.Y329Kfs*90 homozygous pathogenic variant.

Case report

An 11-year-old girl was admitted to the local hospital for abdominal pain in April 2022. CT revealed a large pelvic mass measuring 10 × 8 cm in diameter, with suspicion of adnexal torsion or tumor rupture. Serum tumor markers, including carbohydrate antigen (CA) 125, CA199, carcinogenic embryonic antigen (CEA), alpha-fetoprotein (AFP) and β-human chorionic gonadotropin (β-HCG) were in the normal range. An emergent laparoscopy showed a ruptured ovarian tumor measuring 10 × 8 × 6 cm on the right side, along with approximately 50 ml of intra-abdominal bleeding. The root of the right adnexa was twisted 720 degrees. The uterus and the left ovary were small in size, and no enlarged retroperitoneal lymph nodes were noted. Right salpingo-oophorectomy was performed. The sample was placed in an endo bag and exteriorized through the incision. Microscopically, a mixture of germ cells, sex cord elements, and hyaline bodies were arranged in large nests and lobules. The sex cord cells were arranged at the periphery of these nests (Figure 1A). The dysgerminoma component showed positive immunohistochemical staining (IHC) for OCT4 (Figure 1B), SALL4 and CD117 (Figure 1C). An α-inhibin IHC staining showed positivity in the component of the sex cord of the nests (Figure 1D). Other IHC markers, including estrogen receptor (ER), progesterone receptor (PR), AFP, CEA, β-HCG, testis-specific protein Y-encoded (TSPY), and epithelial membrane antigen (EMA), were negative. Based on these findings, the diagnosis of "gonadoblastoma with dysgerminoma" was made.

The patient was then referred to our hospital's outpatient in May 2022 for further evaluation and treatment. Menstruation has not yet begun. Physical examination showed that her blood pressure was 148/106 mmHg, her body weight was 40 kg, and her height was 160 cm. Her breasts were in Tanner stage I, without pubic or axillary hair. There was no clitoromegaly and the external genitalia were phenotypically female and infantile. Blood biochemistry tests showed hypokalemia and normal plasma sodium and chlorine level. The following hormonal tests revealed low estradiol (E2) and testosterone (T), along with increased levels of progesterone (P), follicle stimulating hormone (FSH) and luteinizing hormone (LH). Furthermore, decreased cortisol and elevated ACTH levels were observed (Table 1). Serum tumor markers were still within the normal range.

An abdominal CT scan revealed no apparent hyperplasia in the bilateral adrenal gland (Figure 2A). Based on the X-ray, her bone age was 3 years younger than her actual age (Figure 2B). Karyotype analysis from the peripheral blood sample revealed a normal female 46,XX pattern and ruled out the possibility of mosaicism. To further exclude the possibility of a low level of Y chromosome material, fluorescence *in situ* hybridization (FISH) analysis using a probe specific for the *sex-determining region of Y-chromosome (SRY)* gene and *zinc-finger Y (ZFY)* gene locus at the Y chromosome was performed and no signal was detected for the *SRY* and *ZFY* locus. To exclude the possibility of somatic mosaicism, additional molecular analyzes were conducted in both the gonadal stroma and tumor tissue. Quantitative fluorescence-polymerase chain reaction (QF-PCR) results showed no amplification of the *SRY* in both the gonadal stroma and tumor tissue. Further whole-exome sequencing of the patient indicated that she was homozygous for c.985_987delTACinsAA (p.Y329Kfs*90) in exon 6 of the *CYP17A1* gene (Figure 3A). No other genetic variant in gonadal development was found. She has no siblings and peripheral blood samples from her parents were also sent to analyze the genetic sequence of CYP17A1. Although her parents were apparently not consanguineous, they were all heterozygous carriers of this pathogenic variant (Figures 3B, C).

As no Y-chromosome material was identified in peripheral blood, gonadal stroma, and tumor, the risk of malignancy of the left ovary was estimated to be low. Therefore, the contralateral ovary was preserved. According to the International Federation of Gynecology and Obstetrics (FIGO) staging classification for cancer of the ovary, the pathological staging was stage I gonadoblastoma with dysgerminoma, and no adjuvant

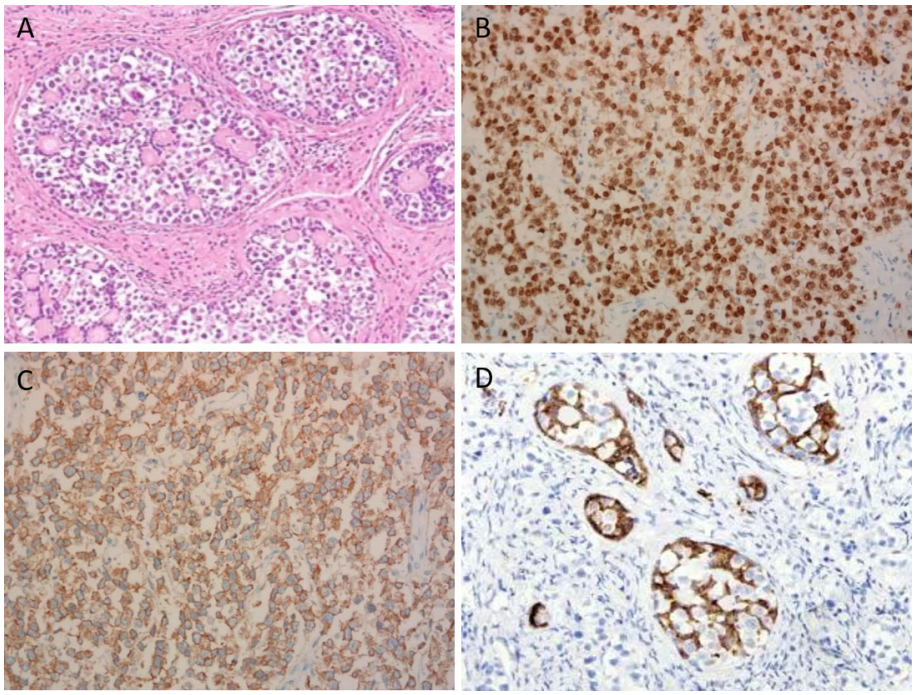

FIGURE 1
Microscopic view of the ovarian gonadoblastoma with coexisting dysgerminoma (x200). **(A)** A mixture of germ cells, elements of the sex cord, and hyaline bodies were placed in large nests and lobes. **(B)** Dysgerminoma component demonstrating positive nuclear staining for OCT4. **(C)** Dysgerminoma component demonstrating positive membranous staining for CD117. **(D)** Sex cord component demonstrating positive cytoplasmic staining for α-inhibin.

chemotherapy was needed based on the National Comprehensive Cancer Network (NCCN) guidelines. Glucocorticoid supplementary treatment was initiated. The patient was prescribed prednisone starting at 5 mg/day. Three months later, her blood pressure varied between 117-130/72-85 mmHg, and plasma levels of potassium, cortisol, and ACTH returned to the normal range. After communicating with the patient and her parents about her condition, supplementary treatment with sex hormones will be used later to promote the development of secondary sexual characteristics and induce an artificial menstrual cycle. During the follow-up period, physical examination, blood parameters, including sex hormone, cortisol, ACTH, aldosterone, blood electrolyte levels, and pelvic ultrasound will be regularly monitored. Currently, the follow-up is still ongoing.

Discussion

17-OHD is a rare kind of congenital adrenal hyperplasia (CAH) characterized by the failure to synthesize cortisol, adrenal androgens, and gonadal steroids (6). Defects in 17α-hydroxylase/17,20-lyase activities result in low cortisol levels and a reduction in dehydroepiandrosterone (DHEA) and androstenedione, which in turn results in low estradiol and testosterone levels (7). Negative feedback is diminished by lower levels of cortisol, and consequentially induces ACTH overproduction, adrenal hyperplasia, and excessive synthesis of mineralocorticoid precursors, such as 11-deoxycorticosterone and corticosterone, resulting in low-renin hypertension and hypokalaemia. A deficiency of sex hormones causes primary amenorrhea in females, and 46,XY DSD in males, which manifests with small testicles, small penis, and mammary gland development.

Biglieri et al. described the first 17-OHD case in 1966, which involved a 35-year-old woman with hypertension and delayed menstruation as the primary symptoms (8). To date, more than 100 pathogenic variants have been reported in the *CYP17A1* gene, and most are associated with a classic phenotype of combined 17α-hydroxylase/17,20-lyase deficiency. A smaller number of *CYP17A1* missense variants have been reported to exhibit partial impairment of 17α-hydroxylase/17,20-lyase activity. Based on the degree of enzyme deficiency, 17-OHD is classified chiefly into complete and partial type deficiency. Specific *CYP17A1* gene variants have been reported to cause a partial loss of 17a-hydroxylase/17,20-lyase activities or dissociation of the 17a-hydroxylase and 17,20-lyase functions (9). Hypergonadotropic hypogonadism and high serum levels of

TABLE 1 Hormone levels and biochemical parameters of the patient.

Items	Results	Reference range
E2 (ng/mL)	<0.02 ↓	Follicular phase, 0.039-0.375
T (ng/mL)	<0.02 ↓	<0.41
P (ng/mL)	5.83 ↑	Follicular phase, <0.31
FSH (IU/L)	105.92 ↑	Follicular phase, <10.00
LH (IU/L)	34.25 ↑	Follicular phase, 2.12-10.89
PRL (ng/mL)	13.20	<30.0
Pregnenolone (ng/mL)	3.51 ↑	<2.30
11-Deoxycorticosterone (ng/mL)	1.322 ↑	<0.300
Corticosterone (ng/mL)	116.60 ↑	0.18-19.70
ACTH (pg/mL)	68.20 ↑	7.20-63.30
Cortisol-8am (ng/mL)	<0.40 ↓	48.2-195
Aldosterone (ng/mL)	<0.02	<0.22
Potassium (mmol/L)	3.12 ↓	3.5-5.5
Sodium (mmol/L)	142	135-145
Chlorine (mmol/L)	107	96-111

E2, estradiol; T, testosterone; P, progesterone; FSH, follicle-stimulating hormone; LH, luteinizing hormone; PRL, prolactin; ACTH, adrenocorticotropic hormone.
The symbols ↑ means above normal, the symbols ↓ means below normal.

ACTH and mineralocorticoids are found in complete and partial 17-OHD patients. Patients with partial 17-OHD have different clinical characteristics such as the development of breasts and pubic hair, and oligomenorrhea or secondary amenorrhea, due to the various degrees of estrogenic and androgenic impacts (10).

Until now, numerous pathogenic variants in the *CYP17A1* gene have been identified in 17-OHD patients, and differ among racial and geographic areas. Tyrosine at amino acid position 329 is particularly critical for the appropriate functioning of the P450c17 enzyme (11). Tyrosine (Y)-329-Lysine (K) is the first impacted amino acid due to a frameshift in exon 6 of the *CYP17A1* gene, which occurs when the nucleotide 985 to 987 (TAC) was changed by AA. This frameshift pathogenic variant finally leads to the premature stop codon of 418TGA and generates a truncated protein containing only 417 amino acids without the heme-binding region of codons 435 and 455, which plays a key role in the catalytic function of the enzyme. Therefore, the loss of the pivotal functional domain results in the complete loss of 17-hydroxylase/17,20-lyase activity.

In 2003, a Korean compound heterozygote patient with primary amenorrhea, hypokalemia, and hypertension, was first diagnosed with this pathogenic variant (12). Subsequently, this pathogenic variant was found in many Chinese 17-OHD patients. Tian et al. reported cases with the pathogenic variant p.Y329Kfs*90 and assumed that this was common in Asian people (13). Another study reported that 10 out of 15 (66.7%) Chinese 17α-OHD patients carried the pathogenic variant p.Y329Kfs*90 (14). To clarify the prevalence of 17-OHD pathogenic variants in China, Wang et al. (15) searched PubMed for all published English papers about 17-OHD and summarized all reported *CYP17A1* gene pathogenic variants. In total, genetic variants were reported in 181 Chinese cases; of them, 70 (38.6%) had a pathogenic variant p.Y329Kfs*90.

Gonadoblastoma, an uncommon ovarian tumor, occurs almost always in patients with dysgenetic gonads associated with DSD and an aberrant karyotype (4). Although gonadoblastomas are benign themselves, they are frequently associated with invasive germ-cell malignant tumors. The most common malignancy is pure dysgerminoma, whereas other variants include immature teratoma, embryonal carcinoma, yolk sac tumor, and choriocarcinoma (16). In 1970, Scully reviewed 74 cases of ovarian gonadoblastoma and more than

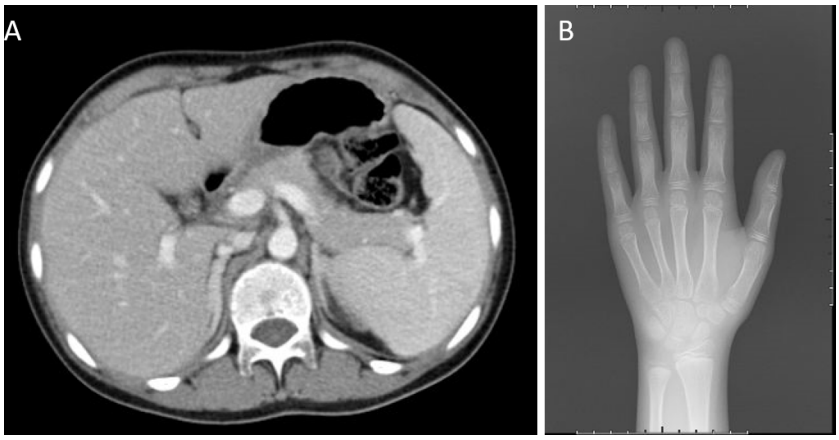

FIGURE 2
Imaging examinations. **(A)** An abdominal CT scan revealed no apparent hyperplasia in the bilateral adrenal gland. **(B)** X-ray showed her bone age was 8 years old.

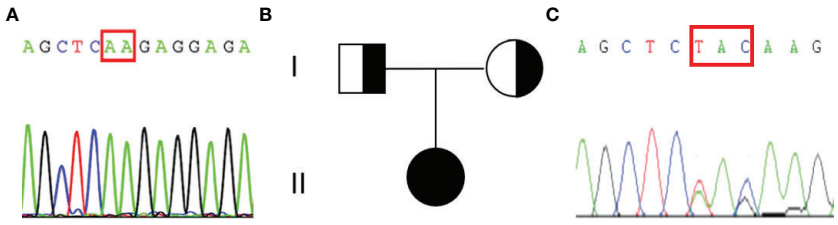

FIGURE 3
Results of the sequencing of CYP17A1 gene and the pedigree of the patient's family. **(A)**The patient was homozygous for the pathogenic variant c.985_987delTACinsAA (p.Y329Kfs*90). The red rectangular represents the altered nuclear acid. **(B)** Pedigree of the patient's family. **(C)** Her parents were all heterozygous for c.985_987delTACinsAA (p.Y329Kfs*90) pathogenic variant.

90% of the reviewed cases had a Y chromosome. As a result, the Y chromosome is believed to be the locus of an oncogene necessary for the development of a gonadoblastoma (17). Subsequently, it was postulated that GBY (gonadoblastoma locus on the Y chromosome) exerts oncogenic effects in dysgenetic gonads and the *TSPY* gene was established as the putative gene for GBY (18). Y chromosome increases the risk of gonadal malignancy by 15% to 50% in patients who have partial or total gonadal dysgenesis, therefore, preventive bilateral gonadectomy is usually recommended (19). Due to the young age at diagnosis, it is crucial to rule out the presence of Y chromosomal material. Typically, peripheral blood lymphocyte karyotyping is performed to check for Y chromosomes in the germline; however, a small chromosomal fragment containing Y chromosome material may be missed (20). The unique approach of incorporating molecular techniques, such as FISH and QF-PCR, were used to confirm the absence of Y chromosome material in peripheral blood and tumor tissue to exclude gonadal mosaicism or the presence of a fragment of the Y chromosome too small to be detected using routine cytogenetic analysis, therefore accurately determining risks to the contralateral gonad (21).

Although most cases of gonadoblastoma occur in sexually abnormal patients with a Y chromosome, a substantial number of cases arise in females with a normal 46,XX karyotype. We reviewed the literature since 1990 and identified 13 female cases of gonadoblastoma with a normal 46,XX karyotype (22–34). Table 2 summarized the clinical characteristics of the 13 cases that we found in the literature. The age at diagnosis varied between 9 to 34 years old. Of the 14 cases, 8 (57%) occurred in women older than 18 years, 4 of whom were fertile. Most (86%) gonadoblastomas were unilateral. Preoperatively, most of them reported abnormally elevated serum tumor markers, such as β-HCG, AFP, and lactic dehydrogenase (LDH). Only one patient reported elevated levels of testosterone, while others did not report hormone levels. Kanaga et al. (29) reported a 14-year-old girl who presented with abdominal distension, excessive hair growth over the body, and hoarseness of voice. An elevation of LDH, β-HCG, and testosterone levels suggested the probable diagnosis of germ cell tumor, mostly dysgerminoma. Laparotomy revealed a large left ovarian gonadoblastoma with dysgerminoma. After surgery and combination chemotherapy, the levels of β-HCG, LDH, and testosterone were reduced to normal. Patients with gonadoblastoma and dysgerminoma generally have an excellent prognosis. When gonadoblastoma is accompanied by more aggressive germ cell tumors, such as yolk sac tumor, embryonal carcinoma, immature teratoma, and choriocarcinoma, the prognosis is different. Even though no hypothesis for the occurrence of these neoplasms has yet been put out in the literature, several pathological studies suggested that they likely developed *via* a completely distinct molecular pathway from those that arose in people who have a DSD (4, 5).

At present, there is limited evidence on the gonadal-malignancy risk of patients with 17-OHD, and there is no clear guidance regarding prophylactic gonadectomies. According to our experience, of the 20 female 17-OHD patients with 46,XY, two (10%) patients had gonadal tumors, one with a Leydig cell tumor and the other with a Sertoli cell tumor (35). The gonads of these two patients were located in the abdomen. Genetic analysis of the *CYP17A1* gene showed that one patient had a homozygous pathogenic variant p.T390R in exon 7 of *CYP17A1*, and another had a homozygous pathogenic variant p.Y329Kfs*90, whose variant type was the same as the case we reported here. Given the fact that the malignancy rate for these patients is quite high, 17-OHD patients with Y chromosome material are recommended to undergo prophylactic gonadectomy to prevent gonadal malignancy. In our case, no Y chromosome material was identified in peripheral blood, gonadal stroma, and tumor, and the risk of malignancy of the contralateral ovary was estimated to be low. So, the second surgery for contralateral ovary resection was avoided.

In the management of 17-OHD, glucocorticoid administration is the key therapy to suppress adrenal hyperplasia and normalize blood pressure as well as plasma potassium level. Treatment should be individualized and the dose should be adjusted according to the patient's blood pressure, plasma potassium, and hormone levels

TABLE 2 Summary of published female cases of gonadoblastoma with normal 46, XX karyotype.

No.	Author	Year	Age at diagnosis	Clinical presentation	Fertile status	Hormonal abnormalities	Laterality	Surgical procedure	Pathological findings in addition to gonadoblastoma	Adjuvant therapy
1	Erhan Y et al (22)	1992	26	Abdominal mass during pregnancy	Fertile	NR	Right	TAH + BSO	Dysgerminoma	Chemotherapy
2	Obata NH et al (23)	1995	10	Abdominal discomfort	No	NR	Bilateral	USO + right ovarian cystectomy	Left with dysgerminoma, right with dysgerminoma and yolk sac tumor	Chemotherapy
3	Zhao S et al (24)	2000	27	Abdominal pain	Fertile	NR	Right	USO + chemotherapy + later TAH + USO + LND + omentectomy	Choriocarcinoma, embryonal carcinoma, yolk sac tumor, immature teratoma and dysgerminoma	Chemotherapy
4	Erdemoglu E et al (25)	2007	19	Abdominal mass and pain	No	NR	Unilateral	USO	Endodermal sinus tumor	None
5	Yilmaz B et al (26)	2010	20	Abdominal mass	No	NR	Bilateral	BSO	Bilateral with dysgerminoma	Radiation and chemotherapy
6	Koo YJ et al (27)	2011	34	Vaginal bleeding	Fertile	NR	Left	USO + para-aortic LND	Dysgerminoma	Chemotherapy
7	Esin S et al (28)	2011	15	Vaginal bleeding	No	NR	Right	USO	Dysgerminoma	None
8	Kanagal DV et al (29)	2013	14	Abdominal distension	No	Elevated testosterone level	Left	USO + pelvic and para-aortic LND + infra-colic omentectomy	Dysgerminoma	Chemotherapy
9	Kulkarni MM et al (30)	2016	20	Abdominal pain	Fertile	NR	Left	USO + omental biopsy	Dysgerminoma	None
10	McCuaig JM et al (31)	2017	20	Oligomenorrhea and menorrhagia	No	NR	Left	USO	Dysgerminoma	None
11	Roth LM et al (32)	2019	9	Abdominal pain and a right adnexal mass	No	NR	Right	USO	Mixed germ cell tumor.	Chemotherapy
12	Raafey MA et al (33)	2020	10	Abdominal pain, abdominal distention and fever	No	NR	Left	USO	Dysgerminoma	Chemotherapy
13	Chandrapattan P et al (34)	2022	9	Signs of virilization and contrasexual pubertal development	No	NR	Right	USO + pelvic LND + infra-colic omentectomy	Dysgerminoma	Chemotherapy

BSO, bilateral salpingo-oophorectomy; USO, unilateral salpingo-oophorectomy; LND, lymph node dissection; TAH, total abdominal hysterectomy; NR, not reported.

(36). In cases where glucocorticoids alone cannot control blood pressure and potassium levels well, mineralocorticoid receptor antagonists could be added. Supplementation therapy of sex steroid hormones should be initiated in adolescent female patients to promote the development of secondary sex characteristics. Additionally, cyclical estrogen and progestin therapy are required in patients with an intact uterus to induce menstruation (37).

Conclusion

In summary, 17-OHD is uncommon and challenging in clinical practice. We describe the extremely rare case of a 17-OHD female patient with normal 46,XX karyotype accompanied by ovarian gonadoblastoma with dysgerminoma. Due to the variable clinical characteristics of 17-OHD patients, a meticulous assessment is necessary, including genetic analysis. Further study

is warranted to unravel the mechanism of gonadoblastoma in patient with normal karyotypes.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by Peking Union Medical College Hospital. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

MY: Writing and literature search. JY and QT: Concept, design, and medical practice. XZ: Analysis and interpretation. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Publisher's note

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Clinical characteristics and molecular etiology of partial 17α-hydroxylase deficiency diagnosed in 46,XX patients

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Introduction: Complete 17α-hydroxylase deficiency (17OHD) is relatively common, with typical juvenile female genitalia, severe hypertension, hypokalemia, and the absence of sexual development, but partial (or non-classical) 17OHD (p17OHD) is extremely rare. The p17OHD patients can present with a broad spectrum of symptoms in 46,XX karyotype including various degree of spontaneous breast development after puberty, recurrent ovarian cysts, oligomenorrhea and infertility depending on specific gene mutations and other influencing factors.

Methods: This paper is a retrospective analysis of p17OHD cases from 1997 to 2021 in a Chinese tertiary hospital. Eight patients were recruited from unrelated families according to clinical data. Genotypes of patients were determined by sequencing the *CYP17A1* genes. Clinical characteristics were summarized based on manifestations, hormone profiles, and responses to treatments.

Results: All seven post-pubertal patients had abnormal menses. All patients had enlarged multilocular ovaries, and six (6/8) had a history of ovarian cystectomy prior to a definite diagnosis of p17OHD. All eight patients' sex hormone levels were in accord to hypogonadism with mildly elevated follicle-stimulating hormone levels, and oral contraceptives effectively suppressed the ovarian cysts. Of the four patients who underwent plasma renin activity tests, all showed results below the reference range. Fourteen alleles with a *CYP17A1* mutation were found. Exon 6 was the most frequent mutation site (5/14), and four out of these five mutations were c.985_987delTACinsAA, being the most common one. In Case 2, c.1220dupA was a newly reported mutation of *CYP17A1*.

Conclusions: 46,XX p17OHD patients were born with highly fragile ovarian reserve due to diverse mutations of *CYP17A1*. However, their multi-ovarian cysts can be managed conservatively for fertility preservation. This study focuses on p17OHD in 46,XX by locating the complex genetic causes in novel mutations, summarizing the puzzling spectrum of clinical manifestations, and illustrating the significance of fertility preservation in these scarce cases.

KEYWORDS

disorders of sex development, *CYP17A1*, partial 17α-hydroxylase deficiency (p17OHD), recurrent ovarian cyst, fertility preservation

Introduction

17α-hydroxylase/17,20-lyase deficiency (17OHD) is a rare condition that accounts for only 1% of congenital adrenal hyperplasia (CAH), occurring in 1 out of 1,000,000 births worldwide (1). Cytochrome P450 17α-hydroxylase (P450c17) is encoded by *CYP17A1*, which is located on chromosome 10q24–25 (2) and contains eight exons and seven introns (3). Similar to other CAH, 17OHD follows an autosomal recessive inheritance pattern and equally affects individuals harboring the 46,XY or 46,XX karyotypes (4). Depending on the retained degree of 17α-hydroxylase/17,20-lyase activities of P450c17, 17OHD can be either complete or partial.

Complete or classical 17OHD is relatively common and occurs when 17α-hydroxylase and 17,20-lyase activities are completely lost. As a result, complete 17OHD presents with an overproduction of mineralocorticoids, particularly 11-deoxycorticosterone (DOC), accompanied by an impairment in the production of glucocorticoids, androgens, and estrogens. Phenotypically, the disease is characterized by typical juvenile female genitalia, severe hypertension, hypokalemia, and the absence of secondary sex development (5).

Nonetheless, partial or non-classical 17OHD (p17OHD) is an extremely infrequent condition, characterized by isolated 17,20-lyase deficiency (ILD) and the partial loss of 17α-hydroxylase and 17,20-lyase function. The key difference between partial and complete17OHD is whether P450c17 exhibits, even a little, function. With some remaining P450c17 enzymic activity, clinical presentations of 46,XX patients with p17OHD include spontaneous menarche, irregular menses, oligomenorrhea or primary amenorrhea, spontaneous breast development, sparse or absent axillary and pubic hairs, juvenile genitalia, and the presence/absence of mild hypertension or hypokalemia. Laboratory results for p17OHD show increased plasma adrenocorticotropic hormone (ACTH) levels with (in ILD) or without (in p17OHD combined with 17,20-lyase deficiency) increased 17α-hydroxyprogesterone (17OHP) levels. Levels of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) remain in the range of normal to mildly elevated, in contrast to the significantly elevated FSH (>40IU/L) in the complete type. Progesterone (P) levels were abnormally higher, while estradiol (E2) and testosterone (T) levels were lower when comparing to normal 46,XX female.

Herein, we retrospectively summarized eight clinically diagnosed 46,XX p17OHD patients in our department over the past 24 years and analyzed their clinical presentations, hormonal changes, genetic mutations, treatments, and prognosis. Because of 46,XX p17OHD patients' atypical manifestations, they are often misdiagnosed and may receive inappropriate medical or operational treatments. To our knowledge, this is the first ever study to focus specifically on 46,XX p17OHD. We hope to clarify its genetic basis, differentiate its diagnosis from similar diseases, and guide the management of p17OHD in 46,XX, especially in treatments relevant to fertility.

Materials and methods

Patients and clinical data

Patients with p17OHD were included in this study if their clinical and biochemical manifestations were in line with p17OHD, if their peripheral blood karyotype was 46,XX, and if medical histories were completely documented. This study was approved by the Ethics Committee of Peking Union Medical College Hospital (IRB number: JS-2510). Written informed consent was obtained from the individuals ≥18-year-old and minors' legal from guardian for patient < 18 year-old for the publication of any potentially identifiable images or data included in this article.

Medical data from 1997 to 2021, including age, gender, sexual intercourse history, chief complaints, diagnosis and treatment process, menstrual history, and family history, were retrospectively collected from medical records. Although genetic tests for *CYP17A1* mutations are crucial for the final diagnosis,

initial diagnosis upon visit primarily depends on clinical manifestations and laboratory findings.

Physical examination and developmental status were recorded at the first visit. Breast and pubic hair development were assessed using the Tanner rating scale. Laboratory investigations included (1) gonadal function (FSH, LH, E2, P and T) and transvaginal/transabdominal sonography for the female reproductive system; and (2) ACTH, 17OHP, cortisol (F), urine free cortisol (UFC), dehydroepiandrosterone sulfate (DS), aldosterone (ALDO), plasma renin activity, serum potassium (K^+), and CT for adrenal glands. The fasting blood sampling is taken at around 8 a.m. Since all postpubescent patients suffer from amenorrhea or oligomenorrhea, sexual hormones are measured on first visit at clinics without specific timing of period. Hormone tests were performed using an automated Elecsys immunoanalyzer (Beckman Coulter UniCel DXI800, Beckman Coulter; Brea, CA, USA). The follow-up time ranged from two months to more than 20 years.

Mutation analysis of *CYP17A1* genes by Sanger sequencing

Blood samples were collected for sequencing of the *CYP17A1* gene (NM_000102.3). Genomic DNA from peripheral blood was extracted using the Lab-Aid 820 Automatic DNA Extraction Kit (Xiamen Zhishan Biotechnology Co., Ltd., China), according to the manufacturer's instructions. Exons and intron-exon boundaries of *CYP17A1* were tested using Sanger sequencing.

Bioinformatic analyses were performed using an in-house pipeline. Briefly, sequencing reads that passed quality filtering were aligned with the human reference genome, hg19, using the Burrows–Wheeler Aligner program. Variants were identified using a combination of the FreeBayes, Genome Analysis Toolkit, CNVnator, and in-house software programs. All mutations were compared with databases such as dbSNP, ExAC, or 1000 Genomes Project, and the frequency of occurrence was calculated for different population groups. Predicted pathogenicity data were obtained using SNPEff, Ensembl Variant Effect Predictor, Sorting Intolerant from Tolerant, Polymorphism Phenotyping version 2, and dbscSNV databases. Gene-phenotype association was characterized from structured resources, including the online Mendelian Inheritance in Man database, and unstructured resources, including the literature, using natural language processing techniques. The average sequencing depth was >100×. Candidate variants identified in the exome data were validated using Sanger sequencing.

Results

General clinical characteristics

Eight patients were eligible for analysis (Table 1). All patients were presented as female with the 46,XX karyotype. Their first visits were between the ages of 11 and 36 years, and their initial presentation was primarily due to abnormal menses (7/8, 87.5%) or recurrent ovarian cysts before menarche (1/8, 12.5%). Except for one pediatric patient (Case 4), patients after puberty (>18 years) all exhibited spontaneous breast development. However, their breasts

TABLE 1 General characteristics of eight patients with partial 17α-hydroxylase deficiency.

Case	Age at first visit (years)	Chief complain	Height (cm)	Weight (kg)	Breast (Tanner)	Pubic /axillary hair (Tanner)	Juvenile female genitalia	Menstrual history	Blood pressure (mmHg)
1	19	Irregular menses	160	48	III	I	+	1~8 d/15~180d	140~160/110~120
2	18	Primary amenorrhea	169	54	II	I	+	N/A	120/80
3	27	Secondary amenorrhea for 13 years	158	61	IV	I	+	Secondary amenorrhea since 14yrs	110/60
4	11	Recurrent ovarian cysts	149	39	I	I	+	N/A	125/50
5	25	Primary amenorrhea	160	45	II	I	+	N/A	121/70
6	36	Oligomenorrhea for 6 years	160	51	V	I	+	4~7d/60~210d	130/80
7	20	Primary amenorrhea	164	49	II	I	+	N/A	90/60
8	21	Secondary amenorrhea for 3 years	179	65	III	I	+	Secondary amenorrhea since 18yrs	110/60

d, days; N/A, not available.

tended to be less well developed in relation to other peers of the same age (Tanner stages II-IV). None of the patients had well-developed axillary or pubic hairs. The external genitalia were underdeveloped, considering their ages, and showed juvenile patterns (hypoplastic labia majora and minora). Only Cases 3, 5, and 6 attempted to have sexual intercourses, but had unsatisfactory results due to the dryness of vagina.

Gonadal axis function

The function of the hypothalamus-pituitary-ovary axis in our patients indicated hypogonadism (low serum E2 and T levels) with mildly to moderately elevated FSH and LH levels, despite that their serum P levels were abnormally high (16.7 ± 5.4 ng/mL) (Table 2). Each of the eight patients had ipsilateral or bilateral enlarged ovaries with a multilocular morphology (Figure 1A). Except for Cases 5 and 6, all other patients underwent ovarian cystectomy at least once, and their pathologies fit the diagnosis of luteinized cysts. Cases 2, 3 and 8 received combined oral contraceptives (COC) following surgery, and no relapse of ovarian cysts occurred (Figure 1B). Case 1 underwent oophorectomy after a persistent recurrence of multilocular cysts in both ovaries, even after COC treatment, and the cysts turned out to be serous cystadenoma. All patients' ovarian tumor markers were within the normal reference range.

Adrenal axis function

All patients had abnormally high ACTH. Cases 1, 2, 4, 5, and 8 had reduced serum F levels (Table 3). Apart from Cases 4, 5, and 8, the other five patients had increased serum 17OHP levels. Four patients (Cases 1, 2, 3, and 7) underwent the plasma renin activity test, and the results were remarkably below the lower reference. Low serum K^+ levels were identified in Cases 1, 5, and 8. In addition, hypertension was observed only in Case 1 (Table 1). Computed tomography revealed enlarged adrenal glands in Cases 1, 2, and 4. No case received ACTH stimulation test.

Detection of genetic mutations

The detected gene mutations are summarized in Table 4 and Figure 2. Among the eight patients clinically diagnosed with

TABLE 2 Function of gonadal axis of eight patients with partial 17α-hydroxylase deficiency.

Case	FSH (IU/L)	LH (IU/L)	E_2 (pg/mL)	P (ng/L)	T (ng/mL)	PRL (ng/mL)	Bone age	Tumor markers	Ovaries under ultrasonography
1	14.6	15.8	34.1	9.8	0.1	6.7	2-3 yrs delayed	CA_{125} 9.8U/ml	Left ovary: 5.6 cm×4.8cm, multilocular cysts Right ovary: 6.2 cm×4.2cm, multilocular cysts
2	18.4	26.5	18.5	23.6	<0.1	15.6	5-6 yrs delayed	CA_{125} 25~65U/ml	Left ovary: 5.9 cm×2.4cm, multilocular cysts Right ovary: 6.6 cm×5.4cm, multilocular cysts
3	9.4	18.2	15.8	36.8	<0.1	36.8	N/A	N/A	Both polycystic ovarian morphology with a 3.6cm cyst in the right ovary
4	11.2	22.7	17.6	17.9	<0.1	5.1	N/A	AFP 1.1 ng/ml,CEA 0.7 ng/ml, CA_{199} 6.2 U/ml,CA_{125} 14.5U/ml	Both polycystic ovarian morphology with a 5.0cm cyst in the right ovary
5	9.5	15.0	29.2	28.9	0.4	12.8	N/A	N/A	Left ovary: 7.2 cm×5.2cm, multilocular cysts Right ovary: 5.4 cm×3.0cm, multilocular cysts
6	4.3	12.5	19.9	8.5	1.7	7.9	N/A	AFP 2.19ng/ml,CA_{125}10.74U/ml	Left ovary: 6.0 cm×3.5cm, multilocular cysts Right ovary: 5.5 cm×3.2cm, multilocular cysts
7	18.6	26.8	10.5	21.7	<0.1	5.7	3-5 yrs delayed	CA_{125} 27.15U/ml,AFP 1.18 ng/ml	Left ovary: 6.6 cm×6.0cm, multilocular cysts Right ovary: 4.8 cm×2.6cm, multilocular cysts
8	10.3	19.3	32.0	17.8	<0.1	14.0	N/A	CA_{125} 35U/ml,CA_{199} 4.9 U/ml,CEA 0.4 ng/ml,AFP 1.0ng/ml	Left ovary: nothing abnormal detected Right ovary: 10.3 cm×6.3cm, multilocular cysts, Doppler: resistance index 0.48

E2, estradiol; FSH, follicle stimulating hormone; LH, luteinizing hormone; PRL, prolactin; T, testosterone; yrs, years old.
N/A, not available.

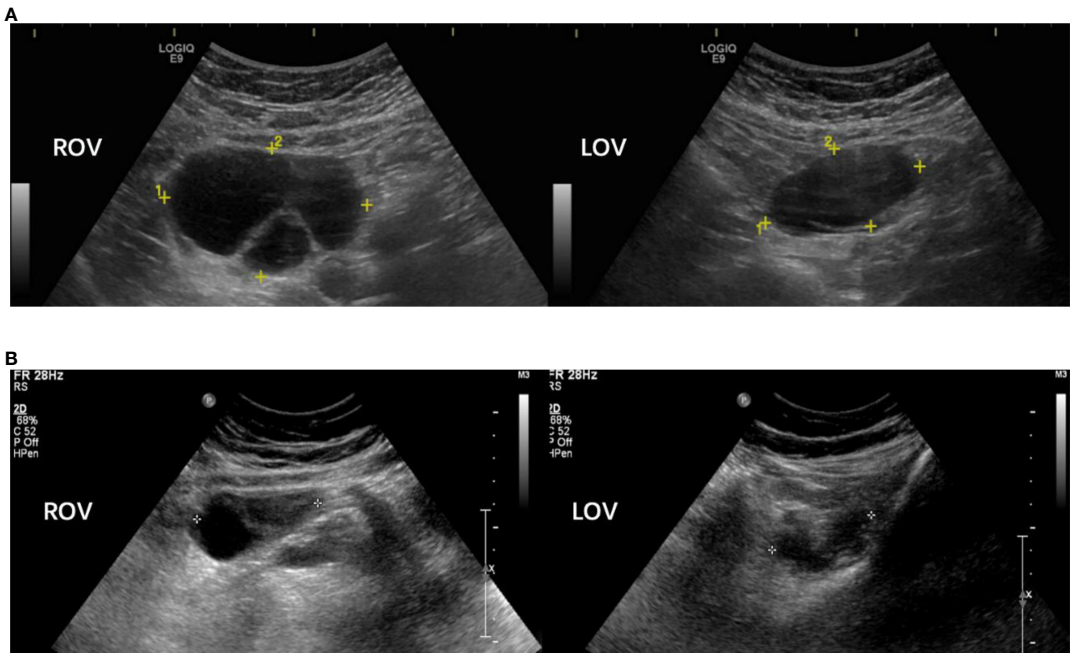

FIGURE 1
Sonographic pictures of a 18 year-old p17OHD patient's multi-locular ovaries before **(A)** and after **(B)** combined oral contraceptive treatment (Case 2). Note: Rov, right ovary; Lov, left ovary.

TABLE 3 Function of adrenal axis of eight patients with partial 17α-hydroxylase deficiency.

Case	ACTH (pg/mL)	17OHP (ng/mL)	F (ug/dL)	DS (μg/dl)	UFC (μg/24h)	ALDO (ng/dL)	PRA (μg/L/h)	K^+ (mmol/L)
REF	0~46.0	0.3~1.7	6.3~19.3	12~133	12.3~103.5	6.5~22	0.93~6.56	3.5-5.5
1	446.3	5.1	3.1	N/A	9.5	32.8	0	3.4
2	133.1	2.9	1.8	<50	<0.2	14.7	0.8	4.7
3	129.4	9.3	9.6	3.6	3.2	9.3	0	4.6
4	63.0	0.5	5.0	3.0	N/A	N/A	N/A	4.2
5	316.2	1.2	3.7	N/A	N/A	N/A	N/A	3.1
6	53.8	1.9	12.6	N/A	N/A	N/A	N/A	4.0
7	47.7	7.4	8.3	10.1	N/A	12.0	0.3	4.0
8	163.6	0.7	5.0	5.0	N/A	N/A	N/A	2.9

17OHP, 17α-hydroxy progesterone; 24h UFC, urine free cortisol; ACTH, adrenocorticotropic hormone; ALDO, aldosterone; DS, dehydroepiandrosterone sulfate; F, cortisol; PRA, plasma renin activity; REF, reference range.
N/A, not available.

p17OHD, *CYP17A1* gene mutations were detected in all patients but only 14 alleles mutations (6*2+2*1) were identified. Six patients had two heterozygous allele mutations, and two patients had only one single allele mutation (Cases 6 and 8). All mutations were heterozygous among those with two known mutant alleles. Exon 6 (5/14) was the most frequent mutation site, and four out of these five mutations were c.985_987delTACinsAA (p.Tyr329LysfsTer90), which was the most common mutation pattern. The c.1220dupA of *CYP17A1* from Case 2 was a newly reported mutation. According to the 2015 American College of Medical Genetics and Genomics (ACMG) guideline, this new variant was defined as a variant of pathogenic (PVS1+PS2).

Discussion

In p17OHD, P450c17, with some remaining function, maintains certain activity in estrogen synthesis that result in various degrees of female secondary sexual characteristics development and mild symptoms of mineralocorticoid hyperactivity [hypertension, 12.5%

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TABLE 4 Summary of gene mutations in eight patients with partial 17α-hydroxylase deficiency.

Case	Gene	Base change	Amino acid change	Location	Type of mutation	dbSNP	Scores of SIFT prediction	Prediction (cutoff=0.05)	ACMG guideline
1	*CYP17A1*	c.985_987delTACinsAA	p.Tyr329LysfsTer90	Exon 6	Frameshift	N/A	–	LOF	pathogenic
	CYP17A1	c.1193C>T	p.Ala398Val	Exon 7	Missense	rs1315561755	0.00	Deleterious	pathogenic
2	*CYP17A1*	c.1220dupA*	p.His407GlnfsTer8	Exon 7	Frameshift	N/A	–	LOF	pathogenic
	CYP17A1	c.1497G>A	p.Trp499Ter	Exon 8	Nonsense	rs776625979	–	LOF	pathogenic
3	*CYP17A1*	c.985_987delTACinsAA	p.Tyr329LysfsTer90	Exon 6	Frameshift	N/A	–	LOF	pathogenic
	CYP17A1	c.1345C>T	p.Arg449Cys	Exon 8	Missense	rs371825363	0.00	Deleterious	pathogenic
4	*CYP17A1*	c.985_987delTACinsAA	p.Tyr329LysfsTer90	Exon 6	Frameshift	N/A	–	LOF	pathogenic
	CYP17A1	c.1039C>T	p.Arg347Cys	Exon 6	Missense	rs104894149	0.00	Deleterious	pathogenic
5	*CYP17A1*	c.1226C>G	p.Pro409Arg	Exon 7	Missense	rs367833709	0.00	Deleterious	pathogenic
	CYP17A1	c.1459_1467del	p.Asp487_Phe489del	Exon 8	Deletion	rs756135168	0.00	Deleterious	pathogenic
6	*CYP17A1*	c.626T>C	p.Leu 209 Pro	Exon 3	Missense	N/A	0.00	Deleterious	pathogenic
	N/D	N/D	–	–	–	–	–	–	–
7	*CYP17A1*	c.287G>A	p.Arg96Gln	Exon 1	Missense	rs104894153	0.00	Deleterious	pathogenic
	CYP17A1	c.1345C>T	p.Arg449Cys	Exon 8	Missense	rs371825363	0.00	Deleterious	pathogenic
8	*CYP17A1*	c.985_987delTACinsAA	p.Tyr329LysfsTer90	Exon 6	Frameshift	N/A	–	LOF	pathogenic
	N/D	N/D	–	–	–	–	–	–	–

ACMG, American College of Medical Genetics and Genomics, dbSNP, single nucleotide polymorphism database; LOF, loss of function; N/A, not available; N/D, not detected; rs, reference SNP; SFIT, sorts intolerant from tolerant.
*a novel mutation.

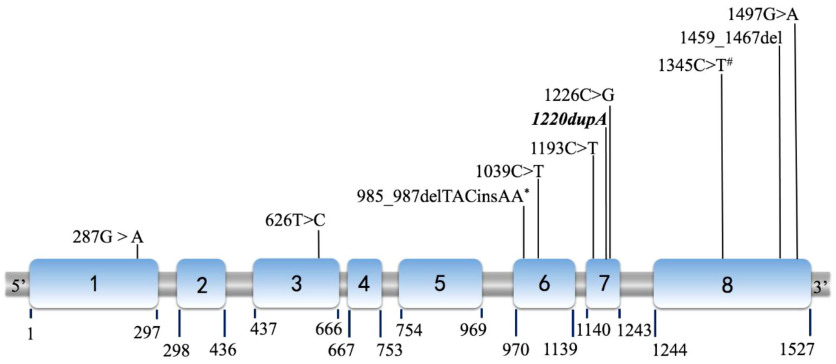

FIGURE 2
Diagram of the P450c17 cDNA (NM_000102) with location of mutations. The locations shown as Italic and Bold indicate novel mutations (1220dupA in case 2). Among all 14 mutations detected, mutations marked by asterisk (*) were discovered in 4 cases (Case 1, 3, 4 and 8); and mutations marked by hashtag (#) were found in both case 3 and 5. Other 8 mutations were identified as unique for each case respectively.

(1/8); hypokalemia, 37.5% (3/8)] due to the limited overproduction of DOC. Therefore, all post-pubertal cases exhibited various extents of spontaneous breast development, although their breasts tended to be less developed in relation to other peers of the same age, due to the impaired and limited activity of P450c17. In 46,XX patients, p17OHD is atypical in endocrinology but the recurrent multiple ovarian cysts distinguish p17OHD from the more common complete 17OHD (6). Therefore, summarizing the characteristics of p17OHD is of great clinical significance.

Mutations in patients with p17OHD

Human *CYP17A1* is located on chromosome 10q24.3, and spans 6.6 kb and eight exons (7). According to previous studies, the c.985_987delTACinsAA frameshift mutation is the most prevalent *CYP17A1* mutation among Chinese patients, and is present in approximately 50% of patients with complete 17OHD (8). Due to the founder's effect in Asia, this mutation has been reported mainly in China and Korea (9). Similarly, the c.1459_1467del mutation found

in Case 5 is a deletion of GACTCTTTC commonly seen in China and Thailand (10). Mutations in exons 6 and 8 are the most prevalent mutations among Chinese patients with 17α-hydroxylase/17,20-lyase deficiency (11).

In our series, four patients had mutations in exon 6, four in exon 8, and three in exon 7. The carboxyl (C) terminus of P450c17 is fundamental for heme (an electron carrier) binding, so even the slightest C terminal alternation can destroy almost all enzyme activities (12). For example, according to the computed model of human P450c17, p.Arg347Cys caused by c.1039C>T (Case 4) results in a charged aspartate, weakening the hydrophobic interaction and therefore destabilizing the entire enzyme structure (12). The mutation is located in the redox-partner interaction domain that contains key residues involved in the interaction with redox proteins and contributes to the positive charges on the proximal surface of P450c17. Geller et al. reported that the absence of charged amino acids in that domain selectively impaired 17,20-lyase activity without substantially reducing 17α-hydroxylase activity (13). Mutations p.Arg347His or Cys and p.Arg358Gln map to the P450c17 surface, which impair the electrostatic interactions of P450c17 with POR and CYB5A (14), explaining the selective ILD observed. An *in vitro* study showed that the p.Arg347Cys mutant protein had 13.6% and <1% residual activities of 17α-hydroxylase and 17,20-lyase, respectively (15). Residue Ala398 comprises part of the C terminus of the K helix, a motif present in all known cytochrome P450 systems (16). The structural importance of this motif corresponds to the detrimental effect of p.Ala398Val (c.1193C>T, Case 1). In addition, the novel mutation p.His407GlnfsTer8 (c.1220dupA, Case 2) seems to have a similarly damaging effect, because of its identical location in the K helix. Both p.Pro409Arg (c.1226C>G, Case 5) and p.Arg449Cys (c.1345C>T, Cases 3 and 7) lay in a region critical for heme binding within a contiguous three-dimensional space (12).

Only one mutant allele was detected in Cases 6 and 8, which is paradoxical to the etiology of an autosomal recessive disease. Both patients had mild manifestations, particularly mineralocorticoid symptoms (Table 3). This could be due to large fragment deletions of the *CYP17A1*. Nevertheless, introns also influence *CYP17A1* expression. In this study, we sequenced the exons and flanking intronic DNA. Mutations in the un-sequenced intron area may affect the splicing process, making the spliceosome unable to properly remove introns and join both exon ends of the *CYP17A1* mRNA (17). The precise identification of the splice site is demanding, because the consensus sequences are very brief and there are many other sequences similar to the consensus motifs of the classical splice sites. Further analysis of *CYP17A1* introns may provide a deeper understanding of the regulation and expression of *CYP17A1*.

Pathophysiology of p17OHD and related functional ovarian cysts in 46,XX p17OHD

Although all eight patients had ACTH above 46 pg/mL and five had F below 6.3 μg/dL (cases 1, 2, 4, 5 and 8), none of them received glucocorticoid replacement after receiving correct diagnosis and before the consideration of fertility treatment. Glucocorticoid therapy induces adrenal axis suppression and possible failure to augment appropriate corticosterone production during acute stress (18). Since DOC accumulates upstream of the block by the failed enzyme, this mineralocorticoid causes low-renin hypertension, although it is slightly less potent than ALDO (4). The amount and activity of DOC are not sufficient to saturate the mineralocorticoid receptor under p17OHD conditions. This is consistent with our seven patients (case 2 to 8), none of whom had symptoms of glucocorticoid deficiency or an excess of mineralocorticoid. All four patients that underwent plasma renin activity tests showed results below the reference range, but only the ALDO of case 1 is dramatically elevated, which accords with her hypertension and hypokalemia.

Pathologically and persistently elevated P level is a basic indicator in most types of CAH, while the 17OHP and DHEA are substantial intermediate products in the steroid metabolism (5). The variation of them often indicates the non-classical type of a particular enzymatic deficiency in CAH. For example, in patients with p17OHD due to the combined defect of the partial 17-hydroxylase and 17,20-layse, we can observe an increased P and 17OHP, but decreased DHEA. For patients with ILD, the elevation of 17OHP and decreased DHEA can be more obvious than those with 17-hydroxylase dysfunction alone. Due to the distinct allele mutation and variant combination of both mutated alleles, the remaining of enzymic function varies dramatically. Therefore, the specific value of a particular biochemical parameter is not practical in the determination of p17OHD, but they can be helpful for clinical suspicion of a certain non-classical type in CAH.

A signature symptom in p17OHD patients with the 46,XX karyotype is recurrent ovarian cysts (19). In our study, recurrent ovarian cysts occurred in all patients, and 75% (6/8) had ovarian cystectomy at least once. Furthermore, three cases had undergone two or more invasive ovarian operations, where the youngest patient (Case 4) was only 10 years old at the time of surgery. Most patients underwent unnecessary ovarian operations without realizing the presence of p17OHD. The mechanism underlying recurrent ovarian cysts might be related to persistent stimulation of raised gonadotropins (FSH and LH) on residual ovarian follicles. But the compromised secretive function of follicles in p17OHD patients leads to low level of estrogen and weak negative pituitary feedback. Such phenomenon is rarely seen in complete 17OHD, because there are no viable follicles in the complete form due to extremely severe enzymatic defects. As a result, no follicle genesis or response to the high levels of gonadotropins exists.

The COCs provide an effective conservative management for the above type of recurrent cysts. Large cysts shrink soon after COC administration in most cases through suppressing the gonadotropin stimulation from hypophysis, but ovarian cysts may reappear after the cessation of COC treatment and it is still effective to use COC again to repress the cyst. However, if ovarian cysts persist after COC

intervention, the possibility of non-functional tumors (e.g., Case 1, serous cystic adenoma) should be excluded.

Fertility in 46,XX patients with p17OHD

Fertility is an important issue in patients with p17OHD and their families. Almost all patients of reproductive age in this study had primary infertility. Currently, it is impossible for patients with complete 17OHD to be fertile spontaneously because of the premature exhaustion of primordial follicles (20). For p17OHD, patients have reduced fertility due to the insufficiency of E2 and T synthesis, and asynchronism of endometrium by chronic P excess, and there have been no reports of spontaneous natural pregnancy in patients with the 46,XX p17OHD. Fortunately, with the development of assisted reproductive technology (ART), pregnancy can be achieved by decreasing the high P with corticosteroids and creating an artificial menstrual cycle for *in vitro* fertilization and embryo transfer (21). During a successfully controlled ovarian stimulation, E2 remains relatively low compared to the number of growing follicles, but the low E2 may not affect viable embryo development (22). Yet, the persistent abnormally high P will interfere with the simultaneous development of the fertilized egg and endometrium (23, 24). Therefore, successful artificial endometrial preparation can be achieved with glucocorticoid supplementation and conventional hormone replacement in frozen-thaw cycles. Consequently, pregnancy can be achieved with ART if 46,XX p17OHD are correctly diagnosed and ovaries are well protected by carefully avoiding unnecessarily repeated cystectomy or even oophorectomy, as cysts spontaneously disappear following the commencement of COC (25). Doing so is essential for protecting the diminished ovarian reserve and saving the chance for future ART in 46,XX p17OHD patients.

Limitations

This study has several limitations. Primarily, as p17OHD is a very rare type of CAH, the sample size is very limited; however, we will continue to collect and update data. Secondly, some laboratory works can be modified if without consideration of cost [e.g., mass spectrometry for steroids applied in all patients and assessment of adrenal function by serum F before and after ACTH stimulation, which is the accepted standard for CAH diagnosis (18)]. Last but not the least, the functional changes of the genetic mutations we found have not been examined and confirmed *in vitro* or *in vivo*, and their family pedigrees can be investigated.

Conclusion

The p17OHD patients are left with different residual activity of P450c17, and varied function in the remaining follicles in 46, XX female patients contribute to distinct degrees of female secondary sexual characteristic development. Recurrent ovarian cysts are commonly occurring in these patients while not observed in the complete form of 17OHD. These cysts can be effectively suppressed using COCs instead of repeated ovarian cystectomy. Early diagnosis, timely treatments, and ART may make it possible for 46,XX p17OHD patients to retain fertility and have their own biological children. The mutant alleles in these patients were more likely to be heterozygous and we discovered that c.1220dupA was a novel detrimental mutation leading to frameshift mutations of *CYP17A1*.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of Peking Union Medical College Hospital (IRB number: JS-2510). The patients/participants or legal guardians of the children provided their written informed consent to participate in this study. Written informed consent was obtained from the individuals ≥18-year-old AND minors' legal guardian for patients < 18-year-old for the publication of any potentially identifiable images or data included in this article.

Author contributions

Conceptualization: QT and FY. Data curation: FY, YW and TT. Data collection: QT, SD, ML. Funding acquisition: QT. Investigation: FY and TT. Methodology: FY and DZ. Writing – original draft: DZ. Writing – review & editing: QT and FY. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Infertility treatment for Chinese women with P450 oxidoreductase deficiency: Prospect on clinical management from IVF to FET

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Cytochrome P450 oxidoreductase deficiency (PORD) is a rare recessive disease with multiple clinical manifestations, which is usually diagnosed in neonates and children because of ambiguous genitalia or skeletal malformations. Moreover, the paucity of studies does not allow us to establish whether adult-onset PORD is associated with infertility. Here, we report clinical and laboratory findings in two phenotypically normal women diagnosed with PORD who underwent *in vitro* fertilization (IVF) and frozen embryo transfer (FET). We modified the gonadotropin stimulation protocol during controlled ovarian hyperstimulation (COH) and suggest the use of the vaginal 17β-estradiol route for endometrium preparation in hormone replacement therapy (HRT) cycles. We presume that PORD may be associated with infertility in several aspects, including disordered steroidogenesis, endometrium impairment, attenuation of drug metabolism, and the high risk of miscarriage. Our observations will help the early diagnosis and make a tailored approach to infertility management in adult-onset PORD.

KEYWORDS

PORD, infertility, IVF, FET, HRT, pregnancy

Introduction

Cytochrome P450 oxidoreductase (POR) serves as an electron donor to all microsomal cytochrome P450 (CYP) enzymes, including both drug-metabolizing and some steroidogenic enzymes (1, 2). PORD (OMIM: 613571 and OMIM: 201750) is a rare autosomal recessively inherited disease caused by homozygous or heterozygous

mutations of POR, which mainly affect activities of P450c17 (17α-hydroxylase/17,20-lyase), P450c21 (21-hydroxylase), P450aro (aromatase), and lanosterol CYP 51 (14α-demethylase) (3, 4). Therefore, PORD is characterized by multiple clinical phenotypes depending on the severity of the causative POR mutations and the subsequent relative impairment of POR-dependent enzymes (5).

Currently, 119 patients have been reported with PORD worldwide, including 46 male and 73 female patients (6). The majority of PORD patients are diagnosed during infancy or childhood because of ambiguous genitalia or skeletal malformations (7, 8). Relatively few adults with PORD have been described (9, 10). Female patients with adult-onset PORD mainly exhibit primary amenorrhea, infertility, delayed pubertal development, or cystic ovaries. Patients are at risk of being misdiagnosed as suffering from 21-OHD or polycystic ovary syndrome (PCOS). The adrenal steroidogenesis was mainly affected in 21-OHD. PCOS is always accompanied by hyperandrogenemia, while PORD female adults mainly exhibit impaired sex hormone synthesis with combined 17α-hydroxylase and 21-hydroxylase deficiency (11). To date, only five women with PORD became pregnant after assisted reproductive techniques (ARTs) have been reported (10, 12–14). The *in vitro* fertilization (IVF) and frozen embryo transfer (FET) protocols have not been well summarized. Moreover, integrated approaches for infertility management and the potential complications of adult-onset PORD are not clear due to the limited number of patients.

Herein, we investigated two patients with a diagnosis of PORD who underwent ART therapy. We described their endocrine characteristics and specific pattern of response to controlled ovarian hyperstimulation (COH) based on clinical and laboratory findings. Moreover, the IVF and FET protocols leading to pregnancy were summarized. Furthermore, the relationship between PORD and infertility was hypothesized.

Hypothesis

This report provides new insight into the relationship between infertility and adult-onset PORD, suggesting that infertility treatment of IVF and FET may improve the pregnancy outcomes in adult-onset PORD female patients.

Clinical observations

In this study, two adult-onset PORD female patients presented with persistent high blood progesterone, low estradiol, and multiple ovarian cysts and received individual COH and FET protocols. Endocrine characteristics and pregnancy outcomes were observed.

Case 1

A 35-year-old primary-infertile woman was enrolled in IVF treatment in February 2017. Menarche occurred at 14 years of age; thereafter, she had oligomenorrhea until hormone replacement therapy (HRT). She underwent cystectomy of multiple ovarian cysts three times when she was 15, 30, and 34, respectively. Her sister also has frequent ovarian cysts and received operations twice. Physical examination showed a height of 160 cm, a BMI of 24 kg/m^2, a blood pressure of 114/65 mmHg, Tanner stage IV for breast development, Tanner stage II for axillary and pubic hair, normal genitalia, the absence of clinical hyperandrogenism, and no skeletal malformation. Adrenal cortical hyperplasia (CAH) remained undetectable on CT scans. Ultrasound showed a 6 × 5 cm cystic mass on the right ovary and a 3 × 2 cm cystic mass on the left ovary. Serum levels of basal estradiol (E2), dehydroepiandrosterone sulfate (DHEA-S), and testosterone (T) decreased while levels of follicle-stimulating hormone (FSH), progesterone (P4), and sex hormone-binding globulin (SHBG) increased (Table 1). Cortisol and adrenocorticotropic hormone (ACTH) levels as well as autoimmune antibodies were within the normal range (Table 1).

She was identified with POR heterozygous variants NM_000941.2: c.1370G>A and NM_000941.2: c.1361T>C by whole-exome sequencing (WES) (Table 2). It is difficult to perform pedigree verification because her father died and her mother suffered from paralysis. Genetic testing results used GRCh37/hg19 as the human genome reference. The variant c.1370G>A (p.Arg457His) is predicted to be disease-causing. Though the variant c.1361.T>C (p.Leu454Pro) has not been reported in the population genetic database (gnomAD) or the pathogenic mutation database (Clinvar, HGMD), it was predicted to be deleterious and the damaging score is 0.91 (VarCards). Moreover, the region where the variation is located is highly conserved among different species. The variants identified by next-generation sequencing were subsequently validated using Sanger sequencing. Based on these data, the variant was classified as "Likely Pathogenic" according to the American College of Medical Genetics and Genomics (ACMG) guidelines.

The IVF cycle was carried out on the fourth day of the menstrual cycle after oral contraceptive pretreatment for 1 month. The start dose was letrozole (LE) plus urine follicle-stimulating hormone (uFSH) 150 IU for 3 days and then uFSH 150 IU sustained for 4 days. Human menopausal gonadotropin (HMG) 300 IU was sustained for 8 days until human chorionic gonadotropin (HCG) trigger (Figure 1C). During FSH stimulation, serum E2 levels were only modestly increased and P4 levels were sustained above 5 ng/ml (Figure 1B). Luteinizing hormone (LH) levels were elevated to 17.42 mIU/ml on day 11 of the menstrual cycle, but decreased thereafter. The endometrium

TABLE 1 Synopsis of the basal hormonal results.

	Patient 1	Patient 2	Normal range
Basal estradiol (pg/ml)	20.05	22.24	12.4–233
Basal progesterone (ng/ml)	1.14	7.93	0.2–1.5
Basal FSH (mIU/ml)	17.59	10.8	3.5–12.6
Basal LH (mIU/ml)	8.67	8.51	2.4–12.6
Basal total testosterone (ng/dl)	0.06	0.03	0.06–0.82
SHBG (nmol/l)	116.5	170.4	26.1–110
DHEA-S (μg/dl)	90.77	14.24	180–3910
TSH (mIU/ml)	1.41	4.68	0.27–4.2
TgAb (IU/ml)	18.90	8.00	0–115
Anti-TPOAb	19.01	100.26	0–34
ANA	Negative	Negative	
Antiβ2GP I antibody (U/ml)	2.6	24.18 first 26.48 second	0–18
ACA (MPL/ml)	0.9	11.92	0–12

Normal range: These gonadal hormone values represent normal values for female patients in the follicular phase; TgAb, thyroglobulin antibody; Anti-TPOAb, antithyroperoxidase antibody; ANA, antinuclear antibody; ACA, anti-cardiolipin antibody; Antiβ2 GP I antibody, antiβ2 glycoprotein I antibody, 1 month for the second time of re-examination.

was less than 6 mm during COH (Figure 1A). It is noteworthy that E2 decreased for the first 3 days during COH because of letrozole (LE). A 10,000-IU dose of urine-derived HCG was administered to induce final follicular maturation, and oocytes were retrieved 36 h later. Three MII (Metaphase II) oocytes were picked up and three high-quality embryos with eight cells, even size, regular shape, and 5% fragmentation were cultivated 3 days later.

Hysteroscopy was conducted before FET. The hysteroscopic diagnosis of endometritis was based on the identification of diffuse hyperemia or hemorrhagic spots in the uterine cavity. After anti-inflammation therapy for 2 weeks and oral prednisone (5 mg/day) pretreatment, she underwent a subsequent FET cycle. A long-acting gonadotropin-releasing hormone (GnRH) agonist was used to suppress the pituitary–ovarian axis. She received oral estradiol valerate (Progynova, BAYER, Germany), 8 mg per day for 7 days, and endometrium thickness was 5 mm determined by transvaginal ultrasound (TVUS). The 2-mg daily dose of vaginal 17β-estradiol (Femoston, Abbott Biologicals, the Netherlands) was added for another 11 days. On the 18th day of HRT, when the endometrium thickness was 7.4 mm, oral dydrogesterone (Abbott Biologicals, the Netherlands) 40 mg daily was supplemented with Crinone (UK, Fleet Laboratories Ltd) *via* the vaginal route. After 3 days of progesterone administration, two embryos with eight cells were transferred on the fourth day. Pregnancy was diagnosed 14 days after embryo transfer by a β-HCG test, and a singleton pregnancy was confirmed 4 weeks later by transvaginal ultrasonography. She had high blood pressure just after 20 weeks of pregnancy and premature birth occurred after 36 weeks of pregnancy. A female baby was born by cesarean section in 2018 with no

TABLE 2 Summary of genetic molecular analyses.

	Patient 1		Patient 2	
	Mutation 1	Mutation 2	Mutation 1	Mutation 2
Transcripts	NM_000941	NM_000941	NM_000941	NM_000941
Nucleotide changes	c.1370G>A	c.1361T>C	c.1631T>C	c.1723G>A
Genotype	Het	Het	Het	Het
Inheritance	Not verified	Not verified	Father	Mother
ACMG	Pathogenic	possible	Possible	Possible
Disease	PORD	PORD	PORD	ABS

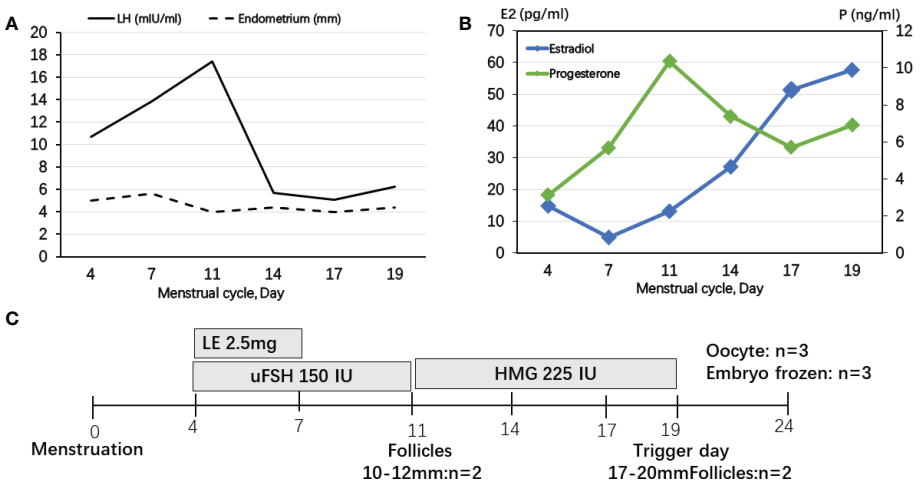

FIGURE 1
Controlled ovarian stimulation cycle in patient 1. **(A)** LH levels were slightly elevated to the maximum of 17.42 mIU/ml at day 11 of menstrual cycle, but decreased thereafter, but the endometrium was less than 6 mm during COH. **(B)** Serum E2 levels were only modestly increased and P levels were sustained above 5 ng/ml. **(C)** A protocol with letrozole (LE) and gonadotropins was used in patient 1. Ovulation was triggered by HCG on Day 19.

obvious abnormalities. The newborn length was 49 cm and the weight was 2.6 kg.

Case 2

This primary-infertile 36-year-old patient visited our reproductive medical center in August 2020 after repeated attempts of IVF at other hospitals. She underwent two FET cycles and had an early pregnancy loss in the second cycle and then she was diagnosed as having PORD. No family history was found and the result of WES was NM_000941 c.1631T>C and NM_000941 c.1723G>A. The mutants were inherited from her father and mother, respectively, and classified as "Likely Pathogenic" according to the ACMG guidelines (Table 2).

She experienced primary amenorrhea and took dexamethasone for long-term treatment. Physical examination showed the following: a height of 165 cm, a BMI of 20.6 kg/m^2, a blood pressure of 123/85 mmHg, Tanner stage V for breast development, difficulty in bending the metacarpophalangeal joints, narrow vagina and incomplete transverse vaginal septum, no clinical hyperandrogenism, and obvious skeletal malformation. Her first-visit ultrasound showed 2.6 × 2.2 cm, 2.0 × 1.9 cm, and 1.4 × 6.0 cm cystic masses on the right ovary and 2.7 × 2.4 cm, 1.7 × 1.3 cm, and 1.4 × 1.4 cm cystic masses on the left ovary. After HRT, a 1.9 × 1.2 cm cyst on the right ovary and a 1.4 × 0.9 cm cyst on the left ovary were found by the TVUS re-examination. No abnormality in the pituitary was found by magnetic resonance imaging (MRI) examination, as well as the adrenal CT scan. Cortisol levels were within the normal range. The serum level of anti-mullerian hormone (AMH) was 2.1 ng/ml. Serum levels of E2, DHEA-S, and T were reduced. Meanwhile, P4, SHBG, and TSH levels were increased. Moreover, her thyroid peroxidase auto-antibody (Anti-TPOAb), Antiβ2-GPI antibody, and anti-cardiolipin antibody (ACA) levels were increased (Table 2).

The IVF cycle was carried out on the fourth day of the menstrual cycle after oral contraceptive and dexamethasone pretreatment for 1 month. The start dose was urine-derived FSH 300 IU for 3 days and then GnRH antagonist 0.25 mg was added until the day before the trigger day (Figure 2C). During FSH stimulation, serum E2 levels were only modestly increased and P4 levels were controlled by dexamethasone (Figure 2B). LH levels were elevated on the fourth day of FSH stimulation. The endometrium was less than 5 mm during COH (Figure 2A). A 10,000-IU dose of urine-derived HCG was administered for final oocyte maturation. Oocyte aspiration was performed 35 h after the HCG trigger. The proportion of mature oocytes relative to the total number of oocytes aspirated was 90% (9/10). On day 6, two blastocysts evaluated as 3BB and 4BC using the Gardner system were frozen (15).

Hysteroscopy was conducted before FET. The hysteroscopic diagnosis of endometritis was based on the identification of diffuse hyperemia or hemorrhagic spots in the uterine cavity. After anti-inflammation therapy for 2 weeks, the HRT protocol was suggested for the patient. In the FET cycle, the vaginal and oral routes of 17β-estradiol (Femoston, Abbott Biologicals, the Netherlands) 8 mg per day were used for 20 days, and the endometrium finally reached 6.3 mm. FET under oral prednisone (5 mg/day) supplementation was performed. All of

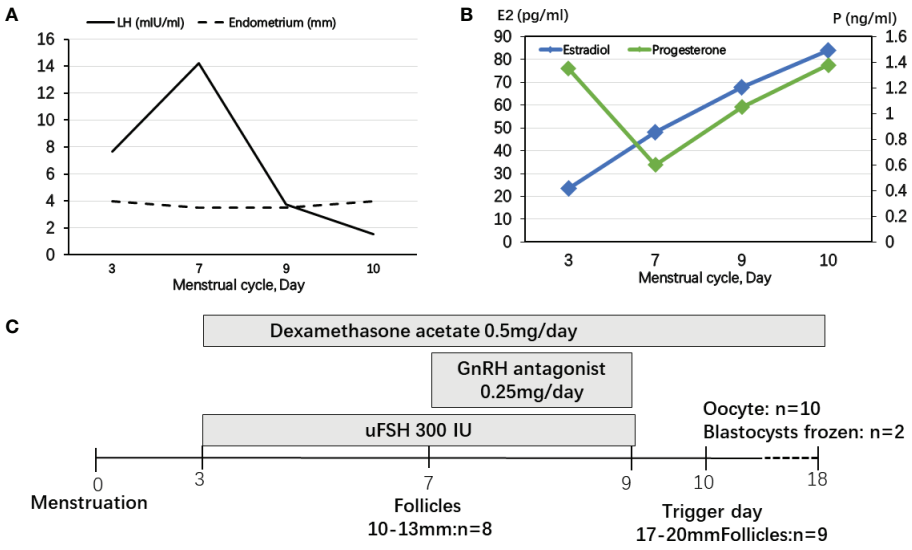

FIGURE 2
Controlled ovarian stimulation cycle in patient 2. **(A)** LH levels were elevated at the fourth day of FSH stimulating and decreased after using GnRH antagonist. The endometrium was less than 5 mm during COH. **(B)** During FSH stimulation, serum E2 levels were only modestly increased and P4 levels were controlled by dexamethasone. **(C)** The start dose was uFSH 300 IU for 4 days and then GnRH antagonist 0.25 mg was added until the day before the trigger day.

the day 6 blastocysts were transferred. Unfortunately, miscarriage occurred on the seventh week of pregnancy.

Discussion

PORD is a complex disorder with many possible mutations affecting a large number of enzymes, and the most common mutations were R457H (25%) and A287P (24%) in 180 individual POR mutations from 90 patients (16). The present study provided two patients with complicated mutations in the POR gene and finally diagnosed as having PORD. Notably, primary amenorrhea/oligomenorrhea and infertility could be the main clinical manifestations when PORD female patients reached reproductive age after spontaneous puberty. Both patients were initially misdiagnosed as having 21-OHD or 17-OHD due to some overlapping features. Consistent with the five reported PORD female patients (14), our cases presented with impaired E2 and T production and elevated P4, whereas basal cortisol and ACTH remained within the normal range. Recurrent ovarian cysts also existed in our cases. Moreover, we found that the serum level of DHEA-S were low in both patients, accompanied by an elevated blood SHBG. Currently, the diagnostic criteria for adult-onset PORD have not been definitively established. PORD is the most complex of the various forms of CAH, because it affects the activity of several steroidogenic enzymes and yields a complex and variable pattern of abnormal steroid hormones. The 17,20-lyase activity of P450c17 is more sensitive to perturbations in electron transfer than its 17-hydroxylase activity; hence, the synthesis of DHEA, androstenedione, testosterone, and estradiol is more severely affected (17). Thus, adult women with PORD will have mildly elevated 17-OHP and low 19-carbon steroids. Some asymptomatic adult women with primary amenorrhea and cystic ovaries may seek infertility treatment (18, 19).

In previous studies, the GnRH agonist protocols were used in PORD female patients (10, 14, 20). P4 secreted by the corpus luteum strongly inhibited pulsatile GnRH and LH secretion (21). Progestin-primed ovarian stimulation (PPOS) is an ovarian stimulation protocol that can block the LH surge through oral progesterone instead of the traditional down-regulating or GnRH antagonist (22). In patient 1 without glucocorticoid pretreatment, we presumed that endogenous LH secretions could be suppressed in a PORD female patient with elevated P4, which is the "natural PPOS" protocol. P4 increased from cycle day 11 and no premature endogenous LH surge occurred thereafter (Figure 1). However, in patient 2, dexamethasone was used for pretreatment and P4 was decreased below 1 ng/ml. GnRH antagonist was used from cycle day 7 to prevent a premature endogenous LH surge (Figure 2). Thus, we recommend tailored COH strategies according to the P4 and LH variations during stimulation. In the present study, a 10,000-IU dose of urine-derived HCG was administered for final oocyte maturation. In both cases, the serum levels of E2 on trigger day were below 90 pg/ml due to PORD and clinicians freeze all embryos in fresh cycles because of elevated P4 levels during the

follicular phase. In case 1, there were two follicles of 17–20 mm in diameter on the day of the HCG trigger (Figure 1). In case 2, there were nine follicles of 17–20 mm in diameter on the trigger day (Figure 2). Therefore, the probability of early- and late-onset ovarian hyperstimulation syndrome (OHSS) was decreased. Moreover, HCG trigger appears to improve the rate of oocyte recovery, and it is important for loosening the attachment of cumulus oophorus to the follicular wall so as to facilitate their recovery by follicle aspiration (23). There is uncertainty in the literature regarding both the optimal amount of time, "lag time", from trigger administration to oocyte aspiration and whether an increased lag time translates into increased mature oocyte yields and better clinical outcomes (24–26). The ESHRE Working Group on Ultrasound in ART reports that intervals from 34 to 38 h are commonly used (27). In patient 1, a transvaginal ultrasound-guided oocyte aspiration was performed 34.5 h after the HCG trigger. In patient 2, oocyte aspiration was performed 35 h after the HCG trigger. The proportion of mature oocytes relative to the total number of oocytes aspirated was 100% (3/3) and 90% (9/10), respectively. Regarding the maturation rate, our data indicate that a 34- to 35-h lag time seems to be sufficient.

Except for the recently reported five cases, no other studies focused on female infertility management in patients with PORD, especially for the HRT protocol. In the present study, hysteroscopy was performed before FET and vaginal administration of 17β-estradiol was recommended during HRT. Coincidentally, the results revealed endometritis in both cases. Recent literature suggests that endometritis is caused not only by local microbial infection but also by sex hormone imbalances (28, 29). Thus, the disordered ovarian sex steroids in PORD adults, especially E2 and P4, which are responsible for orchestrating the dynamic tissue remodeling in the uterus, were hypothesized by us to lead to the inflammatory state of endometrium (Figure 3). Additionally, PORD may account for reduced hepatic drug metabolization (30). In case 1, estradiol valerate was used orally for 7 days without obvious endometrium proliferation. Thus, we added the vaginal administration of 17β-estradiol. In case 2, we used 17β-estradiol in the whole HRT course according to previous experience. Estradiol valerate is a synthetic hormone that is extensively metabolized to estradiol in the liver. We propose that 17β-estradiol by vaginal administration would attenuate the hepatic first-pass effect and should be used for tailored infertility treatment in PORD (Figure 3). We suggest that hysteroscopy needs to be conducted before FET. The glucocorticoid treatment and sufficient luteal support may be beneficial in reducing the miscarriage rate.

In our cases, both women became pregnant after FET. However, one delivered a healthy baby, while the other

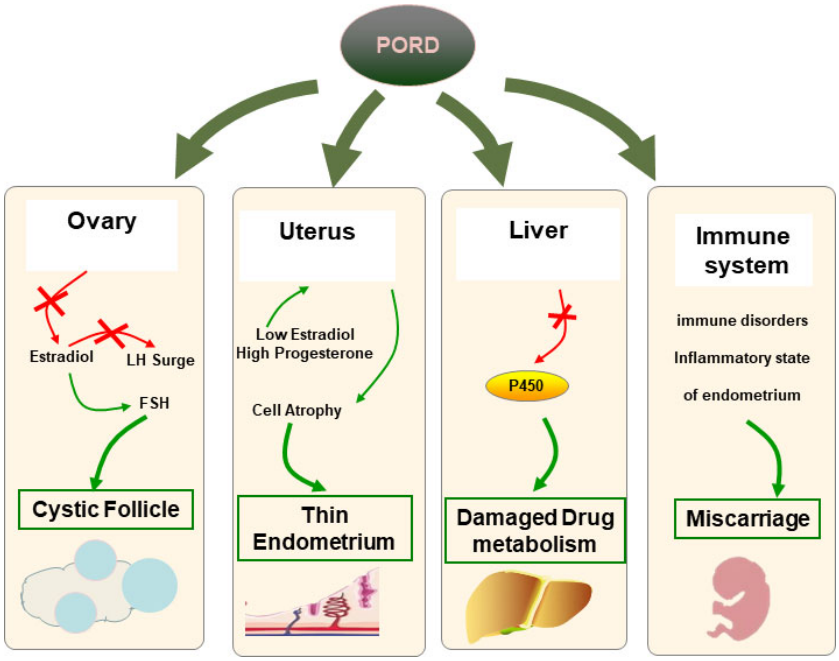

FIGURE 3
Hypothesis of associations with infertility in PORD. In ovaries, FSH fails to trigger the ovaries to produce enough E2 because of the impaired 17-α hydroxylase activities. Multiple cysts are caused by the dysfunction of the positive feedback loop to trigger the LH surge. The endometrium epithelial and stromal cells lack E2 stimulation and begin to atrophy. PORD may account for reduced hepatic drug metabolization. The risk of miscarriage may rise due to a dysfunctional endometrium and a disordered immune system.

experienced repeated early pregnancy loss with elevated autoimmune antibodies. As mentioned above, PORD is the most complex of the various forms of CAH. The miscarriage rate was significantly elevated in classic and non-classic CAH patients, but the reason remains unclear (31). PORD is associated with a decrease in E2 production and excessive ACTH stimulation. Emerging data suggest that estrogen deficiency can influence the immune system, including all levels of the innate (neutrophils, macrophages/monocytes, natural killer cells, and dendritic cells) and adaptive immune system (T and B cells) (32, 33). Also, the overactivation of the hypothalamic–pituitary–adrenal (HPA) axis may trigger the release of neurohormones, and subsequently, the activation of the HPA axis stimulates upregulation of stress hormones such as corticotropin-releasing hormone, ACTH, and glucocorticoids, which can consequently lead to altered inflammatory pathways and immune cell function affecting reproductive function (34). As reported, female patients respond more aggressively to self-antigens and are more susceptible to autoimmune diseases compared to their male counterparts (35). Thus, we propose that PORD may be associated with miscarriage in dysfunctional steroidogenesis and a disordered immune system (Figure 3).

In summary, considering the dysfunction of P450 enzymes, we made tailored COH strategies and modified the estradiol supplementation in PORD female patients. Furthermore, we suggest that hysteroscopy needs to be conducted before FET. The glucocorticoid pretreatment and sufficient luteal support may be beneficial to reducing miscarriage rate. Our findings may help the early diagnosis of late-onset PORD and expand the spectrum of appropriate infertility strategies. However, the findings of current research based on limited data need to be confirmed in more studies. The lack of patient data for side effect profiles for the long-term use of steroid supplementation is another limitation of the study. We hope that our findings will stimulate the research community to test our hypothesis further. In particular, it would be interesting to measure the immune response in women with PORD.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors. Requests to access these datasets should be directed to 1162031398@qq.com.

Ethics statement

Ethical approval was not provided for this study on human participants because Since this study used only de-identified patient data, no approval from our institutional review board was required. The patients/participants provided their written informed consent to participate in this study.

Author contributions

YL contributed to the conception and design of the study and to the acquisition and interpretation of the data, prepared the first draft, and revised the manuscript. S-DZ and C-LZ contributed to the conception and design of the study. Each author listed on the manuscript has seen and approved the submission of this version of the manuscript and takes full responsibility for the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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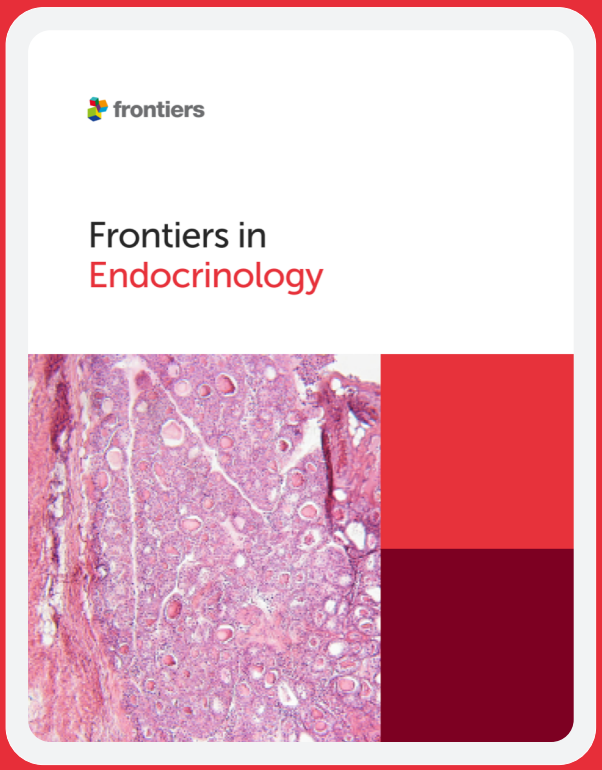

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