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Rare forms of pediatric adrenal disorders: beyond congenital adrenal hyperplasia due to 21-hydroxylase deficiency

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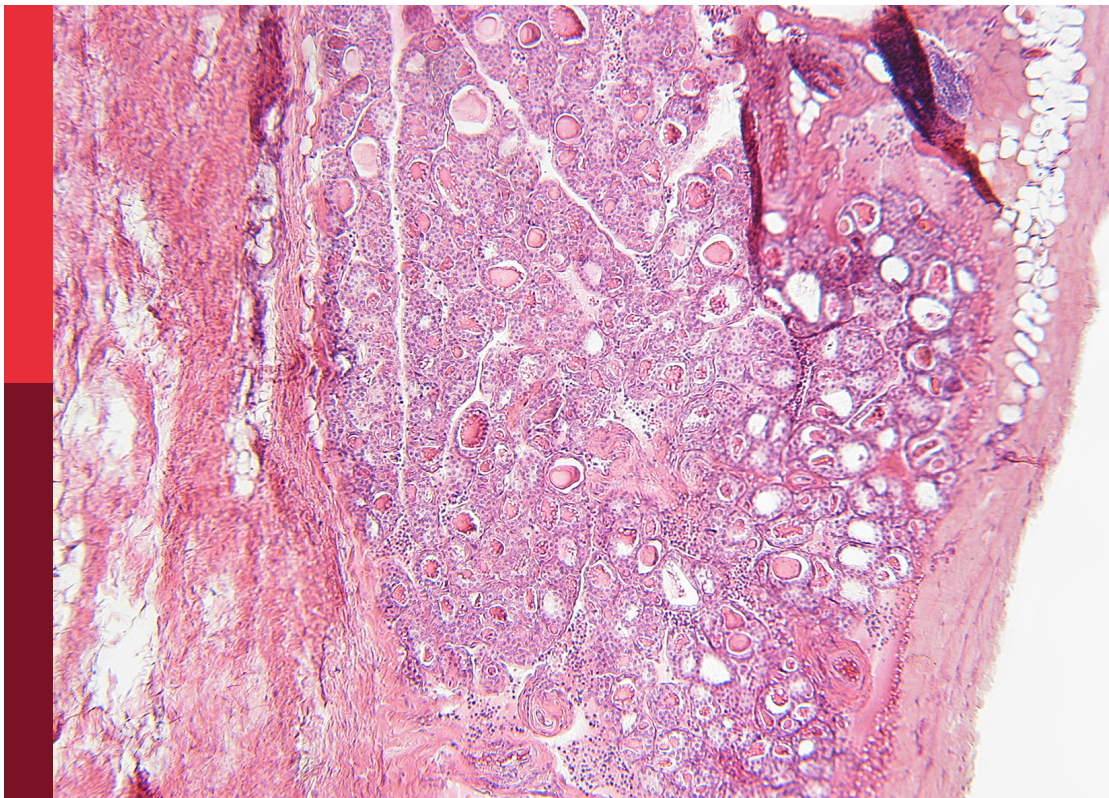

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Rare forms of pediatric adrenal disorders: beyond congenital adrenal hyperplasia due to 21-hydroxylase deficiency

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Editorial: Rare forms of pediatric adrenal disorders: beyond congenital adrenal hyperplasia due to 21-hydroxylase deficiency

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KEYWORDS

adrenal diseases, childhood, adrenal insufficiency, Cushing's syndrome, pheochromocytoma, adrenocortical carcinoma

Editorial on the Research Topic
Rare forms of pediatric adrenal disorders: beyond congenital adrenal hyperplasia due to 21-hydroxylase deficiency

Defects of the adrenal gland in childhood comprise a broad spectrum of etiologies, ranging from genetic variants resulting in adrenal insufficiency (1), autoimmune dysfunction (1), impaired stimulation by the hypothalamic-pituitary axis (1), and adrenal tumors (2–4) that may result in autonomous neoplastic hypersecretion of both adrenal cortical (3) and medullary steroids (4). Adrenocortical hypofunction can be a life-threatening condition requiring urgent diagnosis and replacement with glucocorticoids (1). In contrast, hypersecretion of cortisol as in Cushing's syndrome leads to specific clinical features such as growth failure, obesity, hirsutism and osteoporosis, which if undiagnosed can cause major morbidity and decrease in quality of life (3). Adrenal tumors, including adrenocortical carcinomas and pheochromocytomas, may be isolated or a component of familial endocrine neoplastic syndromes, requiring urgent diagnosis, genetic characterization, and surgical removal (2–4).

In this collection of articles, we describe the rare genetic causes of adrenal insufficiency and discuss the diagnosis, molecular characterization, and treatment of adrenal defects that comprise the diverse landscape of familial glucocorticoid deficiency syndromes (Liu et al., Maharaj). Autoimmune Addison's disease is also discussed which, although rare in childhood, may occur in isolation or as a component of polyendocrine autoimmune dysfunction in conjunction with hypoparathyroidism and gonadal insufficiency (Capalbo et al.). The molecular features are also described (Capalbo et al.). The genetic origins of adrenoleukodystrophy are discussed together with the pediatric features, therapeutic approaches and long-term prognostic predictions (Cappa et al.).

Finally, neoplastic disorders such as adrenocortical carcinoma, Cushing's syndrome, and pheochromocytoma are discussed in detail (Unsal et al., Zagojska et al., Guarnotta et al., Savage et al., Bima et al.). Adrenocortical carcinoma is extremely rare in childhood but is associated with a poor prognosis (Zagojska et al.). Cushing's syndrome may be ACTH-dependent, as in Cushing's disease, which is the secretion of excess ACTH by a pituitary adenoma or ectopic ACTH syndrome, or ACTH-independent, as in adrenocortical tumor or adrenocortical hyperplasia, which may be isolated or part of the genetic complex of McCune-Albright syndrome or Carney Complex (Unsal et al., Guarnotta et al., Savage et al.). Guidelines for the management of pediatric pheochromocytoma are described together with therapeutic recommendations for the management of hypertension (Bima et al.).

Our aim in bringing together this collection of articles is to provide clinical and scientific updates to inform and educate both pediatric and adult endocrinologists and their nursing support staff about the wide range of adrenal disorders that, although rare in childhood, can be life-threatening and, by definition, serious. Collaboration between pediatric and adult endocrinology staff is emphasized, particularly in disorders such as familial endocrine neoplasia and Cushing's syndrome, where few pediatricians have extensive experience in the management of these disorders.

Author contributions

RF: Writing – review & editing. MS: Writing – review & editing. MOS: Writing – original draft.

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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A rare homozygous variant of *MC2R* gene identified in a Chinese family with familial glucocorticoid deficiency type 1: A case report

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Background: Melanocortin-2 receptor (*MC2R*), a member of the G protein-coupled receptor family, is selectively activated by adrenocorticotropic hormone (ACTH). variants in *MC2R* are associated with family glucocorticoid deficiency 1 (FGD1).

Case presentation: We first reported a Chinese family with two affected siblings with a homozygotic variant of c.712C>T/p.H238Y in *MC2R*, presenting with skin hyperpigmentation, hyperbilirubinemia, and tall stature. These individuals showed novel clinical features, including congenital heart defects, not been found in other FGD1 patients.

Conclusions: We reported a Chinese family with affected siblings having a homozygotic variant of c.712C>T/p.H238Y in *MC2R*.Our report may expand the genetic and clinical spectrum of FGD1.

KEYWORDS

FGD1, *MC2R*, homozygous mutation, Chinese siblings, ACTH resistance

Background

Melanocortin-2 receptor (*MC2R*), also called ACTHR, which is a member of the G protein-coupled receptor family, is selectively activated by adrenocorticotropic hormone (ACTH). Homozygous or compound heterozygous variant in the *MC2R* is the cause of family glucocorticoid deficiency 1 (FGD1) (OMIM # 202200), and the variants cause ACTH resistance in the adrenal cortex. The clinical features include hypoglycemia, seizures, coma, skin hyperpigmentation, hyperbilirubinemia, cholestasis, tall stature, and developmental delays (1–5). Some dysmorphic features, such as a prominent forehead,

hypertelorism, a broad nasal bridge, and small, tapering fingers have also been observed (6, 7).

We here reported a Chinese family with two siblings suffering from FGD1. Our findings may broaden the genetic and clinical spectrum of FGD1.

Case presentation

Case 1

The 10-year-old boy was referred to our hospital for fever and arrhythmia. The boy was Gestation 1 Parturition 1 (G1P1) and had been delivered by caesarean section after 40 weeks of gestation. His birth weight and height were normal (3900 g, 50 cm), and there was no history of asphyxia or birth trauma. He was born with skin hyperpigmentation. The child was susceptible to infection. An atrial septal defect was found by ultrasonography when he was one year old, and atrioseptopexy was performed. He had intellectual disability and was unable to communicate. The blood glucose levels before admission were unavailable, and whether he had suffered prolonged neonatal hypoglycemia was undetermined. His parents were non-consanguineous without recognizable abnormalities.

Medical examination: He had a tall stature, height 162 cm (+3.5 SD), weight 48 kg. Skin pigmentation was evident throughout the body, especially the penis and the joints of the fingers and toes (Figures 1A, B). His heart rate was 140 beats per minute with arrhythmia. Testicular volume was about 10 ml, and the patient's penis was 12cm in length and 9 cm in circumference. No armpit hair or public hair was observed. Laboratory examination revealed low plasma cortisol levels, and the plasma ACTH was elevated (Supplementary Table 1). Thyroid hormone and androgen levels were low, while The glucose, electrolytes and renin levels remained normal (Supplementary Table 1). Based on these laboratory test results, congenital adrenal insufficiency was suspected. After obtaining the consent of the family members, multiplex ligation-dependent probe amplification (MLPA) for congenital adrenocortical hyperplasia (including *CYP21A2*) gene was performed. However, no pathogenic or likely pathogenic mutation was identified. We then performed whole-exome sequencing, and the results showed the proband to be homozygotic for c.712C>T/p.H238Y in *MC2R*. variants come from both parents Sanger sequencing corroborated this.

CNV-seq was performed to identify any possible copy number variations, and no phenotype relevant to CNV was found. Thus, the diagnosis of FGD1 was made according to the clinical phenotypes and genetic testing. Oral hydrocortisone was administered at a dose of 12 mg/m2 body surface area, delivered in three doses. Oral euthyrox was started at a dose of 50 μg/d. After the infection and arrhythmia were treated, he was discharged from the hospital. His serum cortisol levels returned to normal, while ACTH levels remained above normal levels as of one week after discharge.

Case 2

Fifteen days later, his sister was admitted to our hospital for jaundice. The patient was 27 days old, G4P2, and delivered by caesarean section after 40 weeks and 4 days of gestation. Her Apgar score at 5 min and 10min after birth was 9 and 10. her birth weight was normal (4000 g). She was also born with skin hyperpigmentation (Figures 1C, D) but never had hypoglycemia.

Jaundice appeared four days after birth. It was initially relieved by phototherapy, but gradually worsened after phototherapy was stopped. Laboratory examination revealed that the cortisol level was low, and extremely high morning ACTH was observed (Supplementary Table 2).

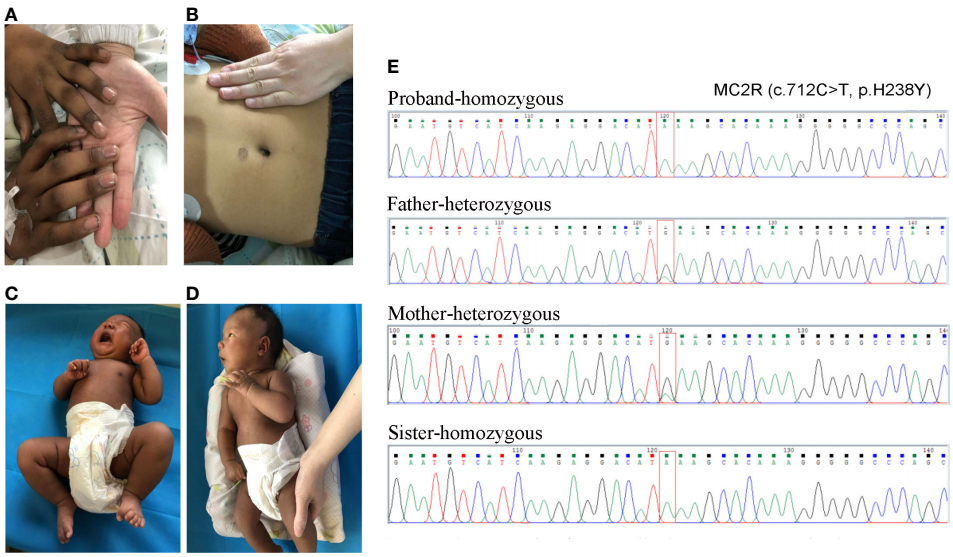

FIGURE 1
The skin pigmentation and the results of Sanger sequencing. **(A, B)** The skin pigmentation of the brother; **(C, D)** The skin pigmentation of the sister. **(E)** Sanger sequencing results of the variant.

She had normal glucose, electrolytes and renin levels (Supplementary Table 1). Thyroid hormone levels were normal, but elevated total bilirubin (Tbil), direct bilirubin (DBIL), indirect bilirubin (IBIL), and total bile acid (TBA) were observed, cardiac ultrasound revealed that she suffered from mild mitral and tricuspid regurgitation, while the electrocardiogram was normal (Supplementary Table 2). Sanger sequencing confirmed that the sister had the same homozygotic variants in c.712C>T/p.H238Y in *MC2R* (Figure 1E). The pedigree of the family is shown in Figure 2. The sister received oral hydrocortisone at a dose of 10 mg/m2 body surface area. To date, no readily visible side effects have been observed. Informed consent was obtained from the parents for the publication of this case.

This variant was located in the transmembrane domain (PM1) and not reported in the dbSNP152, gnomAD, 1000 Genome Database, or Exome Variant Server (PM2). In-silico tools predicted this variant would be pathogenic (SIFT: Damaging, Mutation taster: Disease causing, Polyphen-2: Probably damaging, CADD: Pathogenic and Revel: Damaging), and it was located at a highly conserved site (PP3), the protein model was constructed and polar contacts of wild-type and mutated amino acid residues were compared (Supplement Figure 1). A previous work showed it to be a causal variant for FGD1[8], and it was observed in the trans position against other *MC2R* pathogenic variants R145C (PM3). The variant was observed in both affected siblings (PP1). Thus, the variant produced PM1+PM2+PM3+PP1+PP3, which met pathogenic criteria.

Discussion

ACTH has been shown to activate the *MC2R* to stimulate androgen production in fetal/neonatal mice (8), and deficiency of sex hormones such as 17-alphahydroxyprogesterone, androstenediol, dehydroisoandrosterone, testosterone, progesterone, and dehydroepiandrosterone sulfate (DHAS) had been reported in several FGD1 patients (5, 7, 9–11). Although such patients sometimes had delayed development of pubic hair, other sexual characteristics have not been reported to be affected (10, 12–14). In our case, decreased levels of 17-alpha hydroxyprogesterone and dehydroepiandrosterone were found in the older brother, and slightly lower testosterone level was observed, but he had greater than usual genital size, which may not be associated with deficiency of sex hormones, and we speculated that the excess ACTH leading to the activation of other melanocortin receptors may account for it, while the affected sister showed no such abnormality for receiving timely treatment.

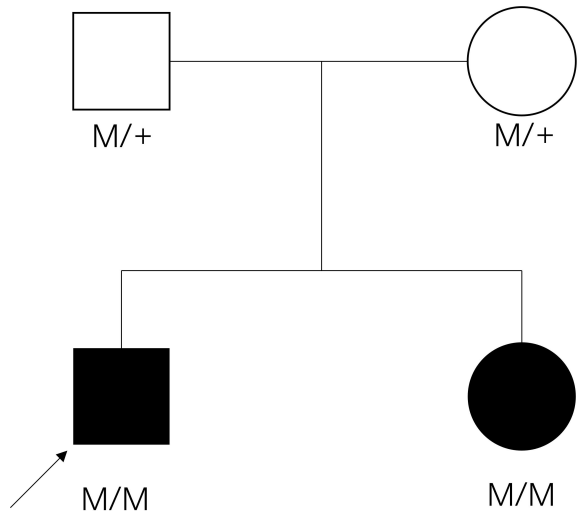

FIGURE 2
The pedigree of the family. Filled symbol: patient, unfilled symbol: unaffected parents, black arrow: the proband, plus: normal allele, M: mutant allele.

Abnormal thyroid hormone level had been observed in an FGD1 patient as associated features in few cases. Artur Mazur et al. reported hypothyroidism in a Polish patient with familial glucocorticoid deficiency and compound heterozygous p.Leu46fs/p.Val49Met mutation (3). M al Kandari reported that three out of five patients developed hypothyroidism with homozygous frameshift mutation p.Ile154fsX248 (15), and a recent study reported hypothyroidism (TSH 10.61 mIU/L) in a neonatal FGD1 patient with compound heterozygous p.R145C/p.H238Y variant, but the repeated TSH levels without hormone replacement therapy at the age of 1.4 months were normal (16). There is evidence that glucocorticoid inhibits thyrotropin (17), but it could not explain the abnormal thyroid hormone level. The mechanism underlying FGD1-assosiated abnormal thyroid hormone level remains unknown. In our cases, the older brother was found to have abnormal thyroid hormone level, with a low FT3 level and a high TSH level. His FT3 and TSH levels returned to normal after glucocorticoid replacement therapy, but the sister showed no signs of abnormal thyroid hormone level.

A tall stature with normal growth hormone levels is one of the reported characteristics of FGD1 (18). Various melanocortin receptors are expressed in bone cells, and activation of melanocortin receptors can cause increased proliferation and expression of a variety of genes in osteoblastic cells (19). In *MC2R* -/- mice, bone mineral density as well as the thickness of the cortical bone of femur increased (20). Because the FGD1 patients had excess ACTH even after cortisol treatment, activation of other melanocortin receptors in bone cells may account for their tall stature. ACTH had also been found to enhance chondrogenesis in multipotential progenitor cells and matrix production in chondrocytes (21).

Developmental delay and intellectual disability had been described previously in patients with homozygous p.Ile154fsX248 (15). Severe prolonged neonatal hypoglycemia could lead to sequelae such as brain injury, seizure, intellectual disability, cerebral palsy, and visual disturbances (21). In these two cases, the older brother had intellectual disability, but we did not have any data on his blood glucose levels in his neonatal period.

The older brother had congenital atrial septal defect, tricuspid and mitral regurgitation, atrial tachycardia, premature ventricular beats, and QT internal prolongation, while the sister only suffered from tricuspid and mitral regurgitation. To the best of our knowledge, there has been no previous report of congenital heart defects in patients with FGD1. In *MC2R* -/- mice, no

morphological abnormalities of the heart were observed, except that the heart rate was attenuated (22). Glucocorticoid receptor (GR) knockout hearts showed aberrant alignment in the compact myocardium with short and disorganized myofibrils (23), indicating a vital role of glucocorticoid signaling in heart physiology and pathophysiology. Whole-exome sequencing and CNV-seq were performed, and no other variant associated with heart disease was found. We could not rule out the possibility of other factors that could account for these defects. Further research is needed to determine the pathogenesis.

The mechanism underlying FGD1 involves unresponsiveness to ACTH due to defective trafficking of the receptor to the cell surface or impaired ligand binding (24). H238Y (located in the TMD6) in our patient was previously reported to be compound heterozygous with R145C, and the patient manifested with hypoglycemia, seizure, skin hyperpigmentation, hyperbilirubinemia, cholestasis, and tall stature (16). A round of *in vitro* site-directed mutagenesis at the same amino acid residue (H238A) resulted in lower expression of *MC2R* on the cell surface, and the binding affinity for ACTH was significantly lower than that for the *hMC2R* WT (25). It has been proposed that H238 may form a second hydrophobic binding pocket with F235 in TM6 of *hMC2R* (25). We speculated that H238Y could attenuate cortisol production by reducing the membrane localization and lowering ACTH binding affinity. Further functional assays were required to reveal the details of the underlying mechanism.

Conclusions

In this study, we reported two siblings with FGD1 from Hunan Province in China, both harboring homozygous *MC2R* variant, which broaden our understanding of the genetic and clinical spectrum of FGD1.

Data availability statement

The datasets for this article are not publicly available due to concerns regarding participant/patient anonymity. Requests to access the datasets should be directed to the corresponding author.

Ethics statement

Written informed consent was obtained from the individual(s), and minor(s)' legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

Author contributions

SPL and TZ drafted the initial manuscript, carried out the initial analyses, and reviewed and revised the manuscript. CL, DXP, QLiu, QW, QLu, and FRH designed the data collection instruments, collected data, and critically reviewed the manuscript. XX conceptualized and designed the study, coordinate and supervised data collection, reviewed, and revised the manuscript. All authors contributed to the article and approved the submitted version.

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Author was QLu employed by GeneMind Biosciences Company Limited.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1113234/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Protein modulation SWISS-model () was utilized to construct protein model by using the most similar structure (8gy7.1.F, Adrenocorticotropic hormone receptor), and Pymol software () was used to compare the polar contacts of wild-type and mutated amino acid residues.

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Case Report: Severe McCune–Albright syndrome presenting with neonatal Cushing syndrome: navigating through clinical obstacles

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Background: Café-au-lait skin macules, Cushing syndrome (CS), hyperthyroidism, and liver and cardiac dysfunction are presenting features of neonatal McCune–Albright syndrome (MAS), CS being the rarest endocrine feature. Although spontaneous resolution of hypercortisolism has been reported, outcome is usually unfavorable. While a unified approach to diagnosis, treatment, and follow-up is lacking, herein successful treatment and long-term follow-up of a rare case is presented.

Clinical case: An 11-day-old girl born small for gestational age presented with deterioration of well-being and weight loss. Large hyperpigmented macules on the trunk, hypertension, hyponatremia, hyperglycemia, and elevated liver enzymes were noted. ACTH-independent CS due to MAS was diagnosed. Although metyrapone (300 mg/m^2/day) was started on the 25th day, complete remission could not be achieved despite increasing the dose up to 1,850 mg/m^2/day. At 9 months, right total and left three-quarters adrenalectomy was performed. Cortisol decreased substantially, ACTH remained suppressed, rapid tapering of hydrocortisone to physiological dose was not tolerated, and supraphysiological doses were required for 2 months. *GNAS* analysis from the adrenal tissue showed a pathogenic heterozygous mutation. During 34 months of follow-up, in addition to CS due to MAS, fibrous dysplasia, hypophosphatemic rickets, and peripheral precocious puberty were detected. She is still regularly screened for other endocrinopathies.

Conclusion: Neonatal CS due to MAS is extremely rare. Although there is no specific guideline for diagnosis, treatment, or follow-up, addressing side effects and identifying treatment outcomes will improve quality of life and survival.

KEYWORDS

McCune Albright syndrome, neonatal Cushing syndrome, metyrapone, adrenalectomy, follow-up

Introduction

McCune–Albright syndrome (MAS) is a rare mosaic disorder of remarkable complexity with an estimated prevalence of 1/100,000 and 1/1,000,000 (1). Timing of postzygotic missense gain of function mutation of *GNAS* encoding stimulatory $G\alpha_s$ determines the extent of tissue involvement, imposing a unique clinical phenotype. Although a combination of two or more classical features, such as fibrous dysplasia of bone (FD), café-au-lait skin macules, and hyperfunctioning endocrinopathies (gonadotropin-independent gonadal function, nonautoimmune hyperthyroidism, growth hormone excess, and neonatal hypercortisolism), are diagnostic, renal, hepatobiliary, and cardiac involvement have also been reported (2–4).

Adrenocorticotropic hormone (ACTH)-independent adrenal $G\alpha_s$ activation results in the rarest endocrine feature of MAS, which almost invariably presents in the neonatal period: Cushing syndrome (CS). Due to greater burden of $G\alpha_s$-mutation-bearing cells, the presence of CS is correlated with increased number of accompanying features of MAS and a poorer outcome. Although there is spontaneous resolution in 33% of cases with neonatal CS, mortality occurs with a high rate of 20% (4).

A dilemma for the clinician is that most publications to date have been case reports, and there is as yet no guideline for diagnosis, treatment, or follow-up. Here, a rare case of severe CS due to MAS, underlining the unique clinical phenotype specific to the neonatal period, is presented. Our goal is to offer a practical approach based on 3 years of clinical experience of this rare disorder that will help navigate challenges during follow-up.

Case presentation

A baby girl, born small for gestational age with a birthweight of 2,340 g (−2.1 SDS) and a head circumference of 32.6 cm (−1.61 SDS) was admitted to the neonatal intensive care unit in the first day of life for respiratory distress. She was the second child of a healthy non-consanguineous Caucasian couple, born 38 weeks of gestation via cesarean section following an uneventful pregnancy. Alanine aminotransferase [ALT, 2,376 U/L (normal, 0–40)] and aspartate aminotransferase [AST, 875 U/L (normal, 0–40)] were elevated; gamma-glutamyl transferase and bilirubin were normal. Antibiotics were administered intravenously after a diagnosis of possible neonatal sepsis. Respiratory distress resolved, and liver enzymes decreased (ALT, 687 U/L; AST, 108 U/L). As soon as the antimicrobial treatment was completed, she was discharged in the seventh day of life.

She was referred to our center, 4 days later, for failure to thrive (2,315 g), difficulty in feeding, and deterioration of general health. On physical examination, round facies, elongated philtrum and retro-micrognatia, hyperpigmented macules both at the front and back of the trunk and on labia majora, which do not cross midline, and hypertrichosis on the forehead and extremities were noted (Supplementary Figure S1). Newborn reflexes were hypoactive, blood pressure was 100/70 mmHg, and second-degree cardiac murmur was also detected. Systems were normal otherwise. Laboratory findings revealed hyponatremia, impaired renal and liver function tests, tubulopathy, and proteinuria, while blood count was normal (hemoglobin, 10.4 g/dl; leukocyte, 25.0×10^3/µl; platelet count, 449×10^3/µl) (Table 1). Hyponatremia resolved with fluid treatment, while liver enzymes, blood urea nitrogen, and creatinine remained elevated. Further endocrine evaluation revealed an elevated serum basal cortisol [225.68 g/dl (N, 6.7–22.6 µg/dL)] and 24-h urinary free cortisol [1,129 µg/day (N, 1.4–20 µg/day)]. Serum cortisol was not suppressed during overnight high-dose dexamethasone suppression test (Table 2) (5). Thyroid hormones were consistent with non-thyroidal illness.

ACTH-independent CS and café-au-lait spots suggested MAS. Hypercortisolism-related complications emerged. On the 11th day, hyperglycemia (blood glucose, 250 mg/dl) was seen, and it persisted after cessation of intravenous fluids in the exclusively breastfed neonate; thus, 0.5 U subcutaneous neutral protamine Hagedorn insulin (NPH) (three times a day) was initiated on the 16th day of life when blood glucose was 340 mg/dl, and serum insulin was 18.10 µIU/ml. Hypertension (110/90 mmHg) and hypokalemia were triggered by mineralocorticoid action of excessive cortisol on 20th day. Spironolactone (2 mg/kg/day) was started, and nifedipine (0.5 mg/kg/day) was added in order to control blood pressure (Supplementary Figure S2). Since immunosuppressive effects of excess cortisol may increase the risk for opportunistic infections, *Pneumocystis jirovecii* prophylaxis was started and live vaccines were postponed.

TABLE 1 Laboratory investigations on admission, prior to medical treatment (19 days), after medical treatment (6 months), and post-adrenalectomy.

	11-days	19-days	6-months	9 months*	24 months*	34 months*	Normal range
Renal function tests							
BUN (mg/dl)	53.3	23.6	8.9	10.2	11.2	12.3	5-18
Creatinine (mg/dl)	0.6	0.5	0.2	0.2	0.2	0.27	0.16-0.39
Sodium (mEq/L)	110.0	134.0	136.0	138.0	138.0	140	136.0-146.0
Potassium (mg/dl)	4.5	2.3	4.6	3.4	4.8	4.1	4.1-5.3
Renin (pg/ml)	–	181.2	460.0	10.2	198.0	54.8	1-8.2
Aldosterone (pg/ml)	–	160.0	136.2	–	–		35-300

(Continued)

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TABLE 1 Continued

	11-days	19-days	6-months	9 months*	24 months*	34 months*	Normal range
Protein (spot urine) (mg/dl)	52.7	–	170.3	100.2	–	–	0-14
Creatine (spot urine) (mg/dl)	5.8	–	31.1	16.6	19.6	29.3	30.5-141.4
Protein/Cre ratio	9.0		5.8	6.0	–	–	
Beta-2 microglobulin (spot urine) (ng/ml)	19428	–	–	–	–	1085	18.8-24.7
Liver function tests							
ALP (U/L)	150	156	581	795	1143	1331	100-450
ALT (U/L)	539	539	1667	123	56	38	0-40
AST (U/L)	134	134	357	75	69	39	0-40
GGT (U/L)	420	423	907	746	126	102	0-40
Bone metabolism							
Calcium (mg/dl)	10.2	10.1	9.8	8.7	10.3	10.3	8.8-10.9
Phosphate (mg/dl)	4.8	4.7	1.8	2.2	4.4	4.9	3.9-7.7
PTH (pg/ml)	NA	–	NA	42.2	62	136.7	0-60
Tp reabsorption (%)	NA	99	70.0	72.1	–	–	>80.0

BUN, blood urea nitrogen; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; PTH, parathyroid hormone; Tp, tubular phosphate.
* represents post adrenalectomy.
NA, not applicable.

TABLE 2 Endocrine evaluation prior to medical treatment (19 days), after medical treatment (6 months), and post-adrenalectomy.

	19-days	6-months	9 months*	24 months*	34 months*	Normal range
8 AM ACTH (pg/ml)	10.5	<0.05	<0.05	7.7	5.2	0-46
8 AM cortisol (µg/dl)	225.7	35.0	4.4	1.2	0.8	6.7-22.6
8 AM cortisol (HDDST) (µg/dl)	102.3	–	–	–	–	<1.8
Urinary free cortisol, 24-hours (µg/day)	1129.6	221.2	–	–	–	1.4-20
DHEA-S (mcg/dL)	1275.4	2706.9	102.2	3.8	–	43-214
DHEA (ng/mL)	21.8	10.1	37.85	–	–	
Androstenedion (ng/dl)	2465	4817	88.9	–	–	
17-hydroxyprogesteron (ng/dl)	2.4	–	–	–	–	
TSH (uIU/ml)	0.72	1.0	0.7	4.04	3.2	0.4-5.3
Free T4 (pmol/L)	9.49	15.6	15.7	11.7	16.6	9.7-19.2
Free T3 (pmol/L)	3.4	5.4	4,8	NA	9.7	4.3-6.9
LH (IU/ml)	1.9	0.2	0.1	0.1	0.1	
FSH (IU/ml)	6.5	1	0.8	0.6	6.5	
Estradiol (pg/ml)	12.7	12.1	37.5	36.7	<11.8	
IGF-1 (ng/ml)	27 (0.6SDS)	–	–	133 (1.7SDS)	54 (-2.2SDS)	
IGFBP-3 (ng/ml)	530 (0.7SDS)	–	–	3153 (1.2SDS)	2224 (-0.1SDS)	
Growth hormone (ng/ml)	1.2	–	–	2.6	–	1-13.6

ACTH, adrenocorticotropic hormone; HDDST, high-dose dexamethasone suppression test; DHEA-S, dehydroepiandrosterone sulfate; TSH, thyroid-stimulating hormone; LH, luteinizing hormone; FSH, follicle stimulating hormone.
* represents post-adrenalectomy.
NA, not applicable.

Features of MAS and accompanying hyperfunctioning endocrinopathies were screened (Table 2). On ultrasonography, adrenal glands were hypertrophic; kidneys showed increased parenchymal echogenicity, loss of separation between the cortex and medulla, and enhanced medullary echogenicity; and size and echogenicity of the liver were normal. Magnetic resonance imaging of the abdomen confirmed that adrenal glands were hypertrophic (right and left adrenal gland were 24×22×18 mm and 18×19×20 mm in size, respectively) and lobulated. Echocardiogram revealed left ventricular hypertrophy. Bone survey verified generalized decrease in bone mass and revealed areas of irregular ossification and radiolucency in radius, ulna, and distal tibia, which were interpreted as osteoporosis due to hypercortisolism (Supplementary Figure S1).

Medical treatment

Metyrapone (300 mg/m^2/day, per oral, in four doses) was started on the 25th day (Supplementary Figure S2) (6). Since liver function tests were impaired, metyrapone was preferred over ketoconazole. Soon after metyrapone was started, hyperglycemia and hypertension improved, enabling the discontinuation of insulin and nifedipine. Spironolactone was also gradually tapered and discontinued after 13 days of metyrapone treatment, and she was discharged.

The dose of metyrapone was adjusted frequently, according to clinical findings and serum cortisol levels during regular visits. However, even after gradually increasing metyrapone dose to 1,850 mg/m^2/day over the course of 6 months, total biochemical suppression of serum cortisol could not be achieved (Supplementary Figure S3A), and the patient had progressive loss of bone mineral density, persistent left ventricular hypertrophy, and a lack of catch-up growth. In addition to that, café-au-lait macules became darker, dehydroepiandrosterone sulfate (DHEA-S) gradually increased (Table 2), and previously non-existent marked clitoromegaly was noted as a side effect of high-dose metyrapone. She was also prescribed ursodeoxycholic acid (15 mg/kg/day); however, liver enzymes remained high (Table 1).

Right total and left three-quarters adrenalectomy

Right total and left three-quarters adrenalectomy was carried out at 9 months of age in light of the patient's continued clinical findings of hypercortisolism, the existence of unfavorable prognostic markers (high cortisol levels upon admission and heart and liver problems), and the adverse effects of high-dose metyrapone. The patient was administered 100 mg/m^2/day glucocorticoids (GC) perioperatively; however, she developed symptoms of adrenal insufficiency. The required GC dose to attain euglycemia, restore general well-being, and resolve adrenal insufficiency was 300 mg/m^2/day. Fludrocortisone (0.05 mg/day) was also started. Following surgery, supraphysiological doses of GC were required, as she suffered frequent symptoms of adrenal insufficiency (hypoglycemia, malaise, and loss of appetite). GC dose could be tapered very slowly, and a daily dose of 15 mg/m^2/day could be attained in 2 months.

As liver function tests, serum cortisol levels and left ventricular hypertrophy all improved following adrenalectomy (Table 1). Bilateral nodular adrenal hyperplasia was observed in the pathological evaluation of surgical specimen, while the findings of liver wedge biopsy were non-specific (Supplementary Figure S4). Sequence analysis of *GNAS* from the surgical sample of adrenal gland revealed a heterozygous, previously described missense mutation in exon 8 (c.2530C>A, p.Arg844Ser), while the sequence analysis of the *GNAS* gene from peripheral blood sample was normal. Lymphocyte activation was normal 3 months post-adrenalectomy, and immunization schedule for live vaccines was established.

Other findings of MAS

She had breast development and vaginal bleeding that lasted 2 days when she was 7 months old, which repeated five more times after the adrenalectomy till 26 months of age. Breast development was Tanner stage 3, and bone age was markedly advanced (4 years and 2 months), despite severe hypercortisolism. On pelvic ultrasonography, uterus was enlarged to 34×22×24 mm; thus, letrozole (0.625 mg, per oral) was started at 26 months of age.

She also developed marked hypophosphatemia at the age of 6 months (Table 1). Radiological investigations since birth demonstrated severe osteopenia and lytic lesions, which were attributed to severe hypercortisolism; however, overt lesions of FD were not confirmed. When she was 9 months old, FGF-23 was elevated [122 pg/ml (normal <52)], which suggested hypophosphatemic rickets associated with FD. Oral phosphate (8 mg/kg) and calcitriol (18 ng/kg) were started. At the age of 23 months, bone survey revealed sclerosis of the base of the skull and maxilla and FD in the lower extremities. She has been on oral phosphate (58.7 mg/kg/day), while calcitriol was ceased.

She is now 34 months old with severe short stature [height, 81 cm (−3.5 SDS); weight, 9,580 g (−3.7SDS)] (Supplementary Figure S3B). She had been under regular clinic visits and has been on 15 mg/m^2/day hydrocortisone and fludrocortisone 0.025 mg/day, letrozole (1×6.25 mg/day), phosphate (58 mg/kg), and ursodeoxycholic acid (100 mg/day) (Supplementary Figure S2). She has six words, cannot form two-word sentences, shows body parts, cannot stand up from supine position without support, and takes a few steps with support. Despite regular physiotherapy and ergotherapy, developmental delay is evident (Bayley Scales of Infant and Toddler Development III language scale, 13/79; motor scale, 2/46).

Discussion

ACTH-independent CS and café-au-lait macules suggested MAS in this case. Interestingly, this patient was admitted for hyponatremia and hyperglycemia requiring insulin treatment. Neonatal MAS and CS are rare conditions, and presentation of this case is quite unique (4).

The earlier the timing of somatic mutation, the greater the burden of $G_s\alpha$-mutation-bearing cells leading to widespread tissue involvement in MAS. In the current case, adrenal, hepatic, cardiac, renal, and bone tissue involvement were evident in first weeks of life, while precocious puberty and hypophosphatemic rickets were observed later. A lifetime risk of additional tissue involvement is being acknowledged. CS is the rarest endocrine manifestation of MAS, which appears in <5%–7.1%. It presents exclusively within the first year of life (median age, 3.1 months) where features may develop as early as *in utero* (2–4, 7). The fact that our case was SGA and had moon facies and hirsutism with impaired linear growth, weight gain, hyperglycemia, hypertension, and nephrocalcinosis detected in the neonatal period, suggested severe, *in utero* onset CS. Upon suspicion, both comorbidities (hyperthyroidism, excess growth hormone, FD, and cardiac and hepatobiliary function) of MAS and complications of GC excess (hypertension, hyperglycemia, hyperlipidemia, nephrocalcinosis, decreased bone mineral density, and muscle atrophy) were assessed (1, 3).

Since the initial description of MAS, only 20 neonates with CS have been described with various initial basal serum cortisol ranging from 9.6 to 80.1 μg/dl, and data regarding long-term follow-up and outcome are still developing (1, 2, 8–11). Disease course is heterogenous, and spontaneous resolution of hypercortisolism has been reported (30%) since G_s-bearing cells are mostly located in the fetal adrenal zone, which normally undergoes apoptosis after birth. However, the outcome is mostly unfavorable in cases with extensive endocrine and extra-endocrine manifestations (1, 2, 8–15). Brown et al. reported poorer prognosis and a lower likelihood of spontaneous remission of adrenal disease in patients with cardiac (cardiomyopathy) and liver involvement (hepatocellular adenomas, inflammatory adenomas, choledochal cysts, neonatal cholestasis, and hepatoblastoma). It was hypothesized that these patients have a greater burden of $G_s\alpha$ mutation (3, 4).

Treatment of neonatal CS is a long and challenging path where both cortisol excess and its complications should be targeted. Marked hypercortisolism that precipitate neonatal diabetes requiring insulin treatment like our patient is rare and was previously reported only in six patients with CS (4). Until hypercortisolism is managed, hyperglycemia should be treated with insulin. Hypertension is due to mineralocorticoid effect of excess cortisol; thus, blood pressure lowering agents of choice should be aldosterone antagonists (spironolactone) or potassium-sparing diuretics.

The treatment strategy of hypercortisolism is determined by disease severity. In a mildly affected case, medical treatment with an expectation of spontaneous resolution (due to previously stated apoptosis of fetal adrenal zone) may be of choice (3, 4, 16–19). Metyrapone, ketoconazole, and mitotane are medical options for lowering cortisol (20–23). Since our patient had impaired liver function, metyrapone, a potent, rapid acting relatively selective inhibitor of 11-hydroxylase was preferred over ketoconazole for its low risk of hepatotoxicity. Reports reviewing adult data suggest an initial dose of 500–750 mg/day and achievement of biochemical control with 1,500 mg/day (23). However, the initial and maximum dose of metyrapone in neonates is unclear; some authors recommend 300 mg/m^2/day in four equal doses (6). In our case, adequate biochemical and clinical suppression of cortisol with metyrapone was not achieved despite an increase in dose from 300 to 1,850 mg/m^2/day.

There are important issues to be considered while using a steroidogenesis inhibitor like metyrapone. Monitoring biochemical response is essential, not only for dose titration and management of cortisol excess but also for adrenal insufficiency due to possible overtreatment. Clinical signs of adrenal insufficiency should always be questioned and assessed. The 24-h urinary free cortisol is the commonly used method; however, it may be impractical due to difficulties in the collection of urine in infants. Alternative methods may be the measurement of early morning serum cortisol and ACTH (23). Low ACTH level may indicate hypercortisolism or may be a sign of suppression due to long-term exposure to hypercortisolism. However, there are deadlocks to be considered in the evaluation of these measurements. A high cortisol level measured by immunoassays does not always indicate an actual elevation. It should be kept in mind that cortisol immunoassays exhibit significant cross-reactivity with cortisol precursors that may be elevated in patients treated with a steroidogenesis inhibitor (especially with metyrapone, which is known to increase 11-deoxycortisol). Such cross-reactivity can be a cause for overestimation of cortisol and may lead to risk of overtreatment (24, 25). It has been suggested that the patients on metyrapone should be biochemically monitored via specific methods, such as mass spectrometry (24–26).

Metyrapone is a relatively selective inhibitor of 11-hydroxylase and 18-hydroxylase. Recent *in vitro* studies indicate greater inhibitory action of metyrapone on aldosterone synthase, resulting in significant reversible reduction in both cortisol and aldosterone. The loss of negative feedback leads to an increase in ACTH, which causes an accumulation of cortisol and aldosterone precursors resulting in an increase in adrenal androgens (23). Although we could not serologically prove an increase in ACTH, hyperpigmentation and the increase in adrenal androgens confirm this mechanism. As far as we know, an increase in DHEA-S causing virilization was an unreported side effect of metyrapone. Clinical (clitoromegaly and hirsutism) and laboratory (DHEA-S) signs of hyperandrogenism should be monitored when higher doses of metyrapone are required.

In the severely affected case with CS, where medical treatment is inadequate and the chance of spontaneous resolution is subsiding, adrenalectomy is indicated when medically feasible. Brown et al. suggested that the presence of comorbid cardiac and liver disease like in our case should prompt consideration for early adrenalectomy (4). Although a previous correlation with initial serum cortisol level and prognosis was not established, it may be speculated that excessively high serum cortisol level is associated with increased number of $G_s\alpha$-mutation-bearing adrenal cells. Thus, we suggest that in neonatal CS due to MAS, initial very high serum cortisol levels, like our case, may be a negative prognostic factor both for spontaneous resolution and clinical response to medical treatment. In infants with severe CS, bilateral adrenalectomy is generally performed. Alternatives like unilateral adrenalectomy and one-side total, other-side three-quarters adrenalectomy may be considered to avoid the requirement for

lifelong GC and mineralocorticoid replacement. Unilateral adrenalectomy was reported to successfully improve clinical symptoms and endocrinological status in adult studies; nevertheless, recurrence during follow-up was 23.1%, while 17.5% required contralateral adrenalectomy (27–29). Since the causes of CS in adult series are variable and different from pediatric CS due to MAS, it should be borne in mind that reproducibility of adult data is poor. In CS due to MAS, $G_s\alpha$-mutation-bearing adrenal gland cells are heterogeneously distributed, and partial adrenalectomy may carry the risk of inadequate management and recurrence. Only a few pediatric case reports addressed this issue. Unilateral adrenalectomy of the larger gland was performed in two neonates with CS due to MAS; remission was achieved for 2 years (30, 31). Itonaga et al. reported a 6-month-old neonate with MAS-associated CS treated with right-sided total adrenalectomy and left-sided half adrenalectomy with remission for 2 years (32). Although these cases were less severe [basal serum cortisol: 16.9, 18.5, and 23.4 μg/dl, respectively (N: 6.2–18.0 μg/dL)], we preferred to perform partial adrenalectomy (right total and left three-quarters adrenalectomy) and succeeded. Our patient has been in remission for more than 2 years.

In the largest case–control analysis of CS in patients with MAS, overall mortality was 20% (six cases) where four of them were deceased following bilateral adrenalectomy (66.7% of all deaths) (4). Anaphylaxis (or adrenal insufficiency), sudden cardiac arrest, sepsis, and sudden death were listed as causes of mortality in those four cases where GC dose and process of GC tapering were not clearly described. The fact that our patient required high-dose GC during peri- and postoperative period to restore well-being, tapering to maintenance dose was very slow, and she is still on maintenance dose GC, suggests that rapid tapering of GCs should be avoided and, although being speculative, may explain sudden death following adrenalectomy.

Gross motor developmental delay may be caused by prenatal exposure to excess GCs. Prenatal GC treatment for possible congenital adrenal hyperplasia or risk of premature birth have been shown to result in cognitive deficits after birth. Furthermore, children who develop CS later in life may experience a decline in cognitive and school performance where the younger the age of onset, the greater the deterioration in IQ scores (3, 4, 33, 34). Since transgenic mice with $G_s\alpha$ mutation was shown to have short- and long-term memory deficits and impaired associative and spatial learning, it may also be speculated that $G_s\alpha$ mutation may also be present in the central nervous system (35, 36).

The establishment of diagnosis of FD follows a characteristic and predictable time course. Although *GNAS* mutations are acquired early in embryogenesis, skeletal development appears to be relatively normal *in utero*, without frank clinical signs of FD at birth. Boyce et al. affirmed that FD lesions become apparent over the first several years of life and expand during childhood and adolescence, like our case. Previous case reports have also stated severe osteoporosis, rickets, polyostotic irregular lucencies, pathological fractures, and biopsy-proven FD during infancy (1, 2, 8–15). The exact pathophysiological mechanism is unclear, and $G_s\alpha$ activation in abnormally differentiated osteocytes is accused. FGF-23 overproduction is an inherent feature of FD, and most patients have elevated circulating levels of FGF-23, but frank hypophosphatemia is rare. The increase in FGF-23 is linked to substantial skeletal involvement. Although FGF-23 levels may wax and wane over time, an increase in FGF-23 usually occurs during periods of rapid growth like infancy and adolescence. Concurrent hyperfunctioning endocrinopathies like hyperthyroidism or CS may also adversely affect bone health.

Peripheral precocious puberty (PP) is the most frequent presenting feature in female patients with MAS (85%) (6). To date, a safe, effective, and long-term treatment for PP in girls with MAS has not been established. The benefits of current interventions on the ultimate outcome of interest, adult height, have not been well-established due to the rarity of the condition and heterogeneous nature of the disease. Despite the small sample size, studies have concluded that letrozole resulted in a statistically significant decrease in the bone age/chronological age ratio, growth velocity, hence increasing predicted adult height (37). Growth outcome in MAS is not only dependent on timing of pubertal onset but on several other disease components (skeletal involvement and endocrinopathies) as well. Hyperthyroidism and growth hormone excess may accelerate growth, while CS may decelerate it (37, 38).

Lack of consensus on both medical and surgical treatment strategies were major obstacles while navigating this case of severe neonatal MAS. The eminence of this report is that it presents current literature with clinical experience on this rare case of neonatal CS due to MAS. High index of suspicion for MAS in a neonate with extensive café-au-lait macules and symptoms of hypercortisolism is the key for early recognition and intervention. Initial excessive cortisol in neonatal CS may be a negative prognostic factor for spontaneous resolution and response to medical treatment, indicating early right total and left three-quarters adrenalectomy. Post-adrenalectomy survival may be related to close supervision during GC tapering.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Ethics statement

Written informed consent was obtained from the individual(s), and minor(s)' legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

Author contributions

YU collected and analyzed data, drafted the initial manuscript, and reviewed and revised the manuscript. OG collected data. İU, HH, BG, SE, and TK collected data and reviewed and revised the manuscript. ZO and EG analyzed data, conceptualized the work,

and revised and critically reviewed the manuscript for important intellectual and medical content. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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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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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1209189/full#supplementary-material

SUPPLEMENTARY FIGURE 1
(A) The findings of physical and radiologic examination. Notice cushingoid facies, hyperpigmented macules that does not cross the midline at the front of the trunk. **(B)** Anteroposterior radiographs reveal irregularities in radius, ulna and femur. Although generalized osteopenia improves at 34 months, FD lesions become prominent over months.

SUPPLEMENTARY FIGURE 2
Timeline of the course of symptoms in neonatal McCune Albright Syndrome noting adjustments made in treatment. Grey box denotes age in days for the first month of life then in months. NPH: Neutral Protamine Hagedorn insulin, CS: Cushing syndrome, PP: precocious puberty.

SUPPLEMENTARY FIGURE 3
(A) Change in serum cortisol with increased metyrapone (methyrapone was initiated on day 25). **(B)** Growth chart, the arrow represents right total and left three quarters adrenalectomy.

SUPPLEMENTARY FIGURE 4
Representative histological features of nodular adrenal hyperplasia. **(A, B)** show low-power while **(C)** Show high-power views.

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X-linked adrenoleukodystrophy and primary adrenal insufficiency

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X-linked adrenoleukodystrophy (X-ALD; OMIM:300100) is a progressive neurodegenerative disorder caused by a congenital defect in the ATP-binding cassette transporters sub-family D member 1 gene (ABCD1) producing adrenoleukodystrophy protein (ALDP). According to population studies, X-ALD has an estimated birth prevalence of 1 in 17.000 subjects (considering both hemizygous males and heterozygous females), and there is no evidence that this prevalence varies among regions or ethnic groups. ALDP deficiency results in a defective peroxisomal β-oxidation of very long chain fatty acids (VLCFA). As a consequence of this metabolic abnormality, VLCFAs accumulate in nervous system (brain white matter and spinal cord), testis and adrenal cortex. All X-ALD affected patients carry a mutation on the ABCD1 gene. Nevertheless, patients with a defect on the ABCD1 gene can have a dramatic difference in the clinical presentation of the disease. In fact, X-ALD can vary from the most severe cerebral paediatric form (CerALD), to adult adrenomyeloneuropathy (AMN), Addison-only and asymptomatic forms. Primary adrenal insufficiency (PAI) is one of the main features of X-ALD, with a prevalence of 70% in ALD/AMN patients and 5% in female carriers. The pathogenesis of X-ALD related PAI is still unclear, even if a few published data suggests a defective adrenal response to ACTH, related to VLCFA accumulation with progressive disruption of adrenal cell membrane function and ACTH receptor activity. The reason why PAI develops only in a proportion of ALD/AMN patients remains incompletely understood. A growing consensus supports VLCFA assessment in all male children presenting with PAI, as early diagnosis and start of therapy may be essential for X-ALD patients. Children and adults with PAI require individualized glucocorticoid replacement therapy, while mineralocorticoid therapy is needed only in a few cases after consideration of hormonal and electrolytes status. Novel approaches, such as prolonged release glucocorticoids, offer potential benefit in optimizing hormonal replacement for X-ALD-related PAI. Although the association between PAI and X-ALD has been observed in clinical practice, the underlying mechanisms remain poorly understood. This paper aims to explore the multifaceted relationship between PAI and X-ALD, shedding light on shared pathophysiology, clinical manifestations, and potential therapeutic interventions.

KEYWORDS

X-linked adrenoleukodystrophy, primary adrenal insufficiency, very long chain fatty acids, adrenal function, cortisol replacement

1 Introduction

1.1 X-linked adrenoleukodystrophy

X-linked adrenoleukodystrophy (X-ALD; OMIM:300100) is a progressive neurodegenerative disorder caused by a congenital defect in the ATP-binding cassette transporters sub-family D member 1 gene (ABCD1) producing adrenoleukodystrophy protein (ALDP). According to population studies X-ALD has an estimated birth prevalence of 1 in 17.000 subjects (considering both hemizygous males and heterozygous females), and there is no evidence that this prevalence varies among regions or ethnic groups (1). ALDP deficiency results in a defective peroxisomal β-oxidation of very long chain fatty acids (VLCFA). As a consequence of this metabolic abnormality, VLCFAs accumulate in different critical parts of the body, such as CNS (brain white matter and spinal cord), testis and adrenal cortex. All X-ALD affected patients carry a mutation on the ABCD1 gene. Nevertheless, patients with a defect on the ABCD1 gene can have a dramatic difference in the clinical presentation of the disease. In fact, X-ALD can vary from the most severe cerebral pediatric form (CerALD), to adult adrenomyeloneuropathy (AMN), Addison-only and asymptomatic forms.

1.2 Primary adrenal insufficiency

Primary adrenal insufficiency (PAI), also known as Addison's disease, is a rare and chronic endocrine condition characterized by the inadequate adrenal production of steroid hormones, due to damage or dysfunction of the adrenal gland. The prevalence of PAI varies among different countries, with the greatest prevalence in countries of northern Europe, where a prevalence of 15-20 affected individuals per 100.000 has been described (2). The most common cause of acquired PAI is autoimmune destruction of the adrenal gland, mediated by antibodies targeting the 21-hydroxylase enzyme (3). This condition can occur at any age, with most individuals affected between the age of 20 and 50 (4). Along with the acquired causes, PAI can be the result of inherited disorders as well. The most common form of inherited PAI is congenital adrenal hyperplasia, which refers to a heterogenous group of genetic conditions characterized by defects in different enzymes involved in adrenal and gonadal steroidogenesis. X-ALD represents a relevant cause of PAI in male children and adults (5–8). The proportion of cases in which PAI is related to ALD is age-dependent. ALD is the most common cause of adrenal insufficiency in boys before 15 years of age [18]. Adrenal function is abnormal in 90% of neurologically symptomatic ALD boys and in 70% of men with AMN. It is usually normal in female carriers (9, 10).

1.3 Objective of the paper

PAI and X-ALD are two distinct yet interconnected medical conditions. Although the association between these two disorders has been observed in clinical practice, the underlying mechanisms and the extent of their relationship remain poorly understood. This paper aims to explore the multifaceted relationship between PAI and X-ALD, shedding light on their shared pathophysiology, clinical manifestations, and potential therapeutic interventions. By addressing several key objectives, this review aims to contribute to the knowledge and understanding of these complex disorders, ultimately improving patient care and outcomes.

2 Pathophysiology of X-linked adrenoleukodystrophy

2.1 Genetic basis and inheritance pattern of X-ALD

X-ALD is primarily caused by mutations in the ABCD1 gene located on the X chromosome (Xq28), which spans for 19.9 kb and 10 exons (11). The mutations in the ABCD1 gene exhibit a wide spectrum, including missense, nonsense, frameshift, and splice-site variants. Type and location of the variant affect the severity and phenotypic variability of X-ALD (12). Nonetheless, identical variants can result in highly diverse clinical phenotypes, suggesting the presence of additional factors that influence the expression of the disease (13). The majority of affected individuals have mutations resulting in loss of ALDP protein function, while a smaller proportion may have milder mutations allowing some residual protein activity. X-ALD follows an X-linked inheritance pattern due to the location of the ABCD1 gene on the X chromosome. Nevertheless, some patients are affected by a *de novo* mutation, indicating that the mutation can occur in the germ line. Since X-ALD is an X-linked inherited disorder, males are more severely affected than females. Some heterozygous X-ALD females can exhibit symptoms due to skewed X-chromosome inactivation or other genetic factors.

2.2 Impact of VLCFA accumulation on adrenal function

ALDP is a constitutive part of the peroxisomal membrane. It transports VLCFAs from the membrane to the matrix where they eventually undergo β-oxidation. If this mechanism is hampered, the breakdown of VLCFAs is impaired, resulting in a rise of cytosolic VLCFAs concentrations. The precise role of the accumulation of VLCFAs in the pathogenesis of ALD remains unclear. Both a direct and indirect effect on cells survival and functioning has been postulated. A direct cytotoxic effect on oligodendrocyte and astrocytes has been observed in rats, following exposure to C26:0 lipids (14). Similarly, supraphysiological accumulation of C26:0 in astrocytes of ABCD1-deficient mice is followed by production of high reactive oxygen species (15, 16). These findings suggest a pivotal role of VLCFAs accumulation in the pathogenesis of ALD. It is still unknown why only few specific sites of the body are affected by the disease, while ABCD1 gene is widely expressed and VLCFAs are elevated in several other tissues (17).

The exact mechanism whereby increased levels of VLCFAs lead to toxicity in the adrenal glands is not well understood. However, it has been observed that cholesterol, along with saturated VLCFAs, accumulates in the zona fasciculata and reticularis of the adrenal glands. This accumulation starts during fetal development (18). VLCFAs preferentially accumulate in the postnatal zona fasciculata and zona reticularis, which are responsible for the production of glucocorticoids (cortisol) and androgens, respectively. Over time, this chronic accumulation is believed to trigger apoptosis and eventual shrinkage of the adrenal cortex, leading to a decrease in cortisol production (19). This observation provides further evidence supporting the idea that the buildup of VLCFAs plays a significant role in the development of adrenal insufficiency. Furthermore, function of adrenal cells has been witnessed to be disrupted by altered microviscosity of the cell-membrane when exposed to C26:0, leading to an impaired response to adrenocorticotropin (ACTH) stimulation (20). Another proposed mechanism for glucocorticoid and androgen deficiencies is a relative lack of cholesterol necessary for their production, as cholesterol is a degradation product of cholesterol esters containing VLCFA (21).

3 Clinical manifestations and diagnosis of X-ALD

3.1 Neurological manifestations of X-ALD

X-ALD primarily affects the white matter of the CNS, leading to neuroinflammation, demyelination and subsequent neurological symptoms. In approximately 85% of the individuals with clinical symptoms, brain magnetic resonance imaging (MRI) shows a characteristic pattern of symmetric confluent hyperintense lesions in T2 and FLAIR images, usually first appearing in the parieto-occipital region of both hemispheres. Contrast enhancement.is evident at the edges of the lesions, indicating inflammation and disruption of the blood-brain barrier. The progression of these lesions is unpredictable; it can be severe and rapid in the childhood cerebral form; milder and slower in forms with adult onset (11, 22).

The manifestations of X-ALD can be broadly categorized into different clinical phenotypes:

3.1.1 Cerebral ALD (CerALD)

This is the most severe form of X-ALD, typically affecting young boys (typically between 4 and 8 years of age). It is characterized by progressive inflammatory demyelination in the cerebral white matter. Symptoms initially include behavioural changes, learning difficulties, and attention deficits. As the disease progresses, individuals may develop motor abnormalities, impaired vision, hearing loss, seizures, and cognitive decline. In advanced stages, CerALD can lead to severe disability and loss of motor function (11, 22).

3.1.2 Adolescent and Adult Onset X-ALD

Adolescent and adult onset X-ALD occurs later in life and has a milder and slower progression compared to CerALD. Neurological symptoms include behavioural changes, impaired coordination, gait abnormalities, and cognitive decline. Individuals with this form of X-ALD may also experience sensory abnormalities, such as loss of vibration and positional sense. Although these forms have been classified as separated forms, nowadays many authors tend to consider them as variants of CerALD (adolescent onset X-ALD) and of AMN (adult onset X-ALD). (11, 22)

3.1.3 Adrenomyeloneuropathy (AMN)

AMN is the most common form of adult-onset X-ALD. It primarily affects hemizygous males, although heterozygous females can also be affected to a lesser extent, with a similar but later progression of symptoms (usually after the 4^{th}-or 5^{th} decade of life). It is characterized by spinal cord involvement, leading to progressive motor dysfunction and sensory deficits. Symptoms typically include weakness, muscle stiffness, difficulty walking, bladder dysfunction, and sexual dysfunction (11, 22). Patients with AMN may also present primary hypogonadism, characterized by progressive impairment of Leydig cell function, decreased testosterone levels and increased LH and FSH levels. Fertility has been described as normal in the pre-symptomatic period and gradually impaired with the progression of the disease. In men with AMN and late onset of the disease, the possibility of procreation and the resulting inheritance by their daughters should be considered. All families should have genetic counseling regarding the inheritance of X-ALD (23). Cognitive impairment is generally absent at presentation, but a secondary cerebral involvement may appear during the progression of the disease (24).

3.2 Adrenal involvement in X-ALD

The endocrine complications of X-ALD extend beyond its neurological impact, encompassing adrenal dysfunction and hypogonadism. These complications significantly contribute to the disease burden and require diligent monitoring and multidisciplinary management. Early identification, appropriate hormone replacement therapy, and continuous medical oversight are crucial to mitigate the impact of these complications and enhance the quality of life for X-ALD patients. In X-ALD, PAI is a dominant clinical aspect. During an assessment of adrenal function in a group of 49 affected boys who had not yet exhibited neurological symptoms (ranging in age from 5 months to 13 years), it was observed that 80% of them already displayed biochemical evidence of PAI, which otherwise did not manifest as clinical symptoms, at the point of their ALD diagnosis (25). Due to the absence of comprehensive prospective investigations into the natural progression of the disease, the extent to which PAI develops in individuals with X-ALD remains uncertain, with different research studies indicating a penetrance of 50-100% (26). In particular, it has been observed that the possibility of X-ALD patients to develop PAI fluctuates across different stages of life, with a higher risk occurring during the initial 10 years. According to an international retrospective review of medical records of affected

boys and male adults, the cumulative probability of experiencing PAI reached its peak by the age of 10 years (46.8%), remained notably elevated up to 40 years of age (an additional 28.6%), and subsequently declined significantly beyond that point (an additional 5.6%) (26). While plasma levels and ratios of VLCFAs are indicative of X-ALD, they appear not to correlate with the risk of developing AI, spinal cord disease, or cerebral disease based on age, as demonstrated by different research groups (27, 28). PAI was identified in X-ALD patients as young as 7 months and 5 months old. These individuals showed biochemical irregularities associated with PAI but yet did not exhibit any clinical symptoms (25, 26). Although studies on female X-ALD patients are few in number, PAI remains an exceedingly uncommon condition in heterozygous females (29–31). In our cohort of 49 female carriers we observed only 2 patients with PAI. While PAI is commonly associated with deficits in both glucocorticoid and mineralocorticoid functions, it's worth noting that in cases of X-ALD related PAI, the mineralocorticoid function can remain unaffected (9, 10). As VLCFAs mainly accumulate in the zona fasciculate and reticularis, the relative preservation of the zona glomerulosa aligns with the observation that mineralocorticoid function remains functional in approximately 50% of the patients (26). It is noteworthy that the category formerly referred to as "Addison-only" is now regarded as rare. By definition, these patients are devoid of detectable neurological involvement. Nevertheless, due to the progressive nature of the disease, a significant number of individuals within this category eventually experience neurological manifestations (32).

3.3 Diagnostic approaches for adrenal insufficiency and X-ALD/AMN

The concurrent presence of PAI, neurological symptoms and characteristic signs at brain MRI serves as a diagnostic indicator that typically leads to X-ALD diagnosis. However, the complex variability in the clinical presentation means that diagnosis across different age groups may rely on varying clinical features. In children and adults, cognitive and neurological symptoms that could potentially indicate CerALD include the sudden emergence of attention and learning problems, the onset of behavioural issues, deteriorating speech and vision and progressive difficulties with walking and coordination. Of note, PAI as well can manifest with neurological overlapping symptoms, such as malaise, fatigue and impairment in cognitive function up to confusion. A distinguishable clinical characteristic of PAI is the increased pigmentation of the skin and mucous membranes, particularly in regions exposed to sunlight and friction due to the elevated levels of circulating ACTH, that can be the first clinical manifestation of X-ALD. When suspected, the diagnosis of PAI follows the same diagnostic guidelines used for other causes of PAI. As aforementioned, dosing plasma renin and aldosterone remains important to evaluate functioning of the zona glomerulosa. In the presence of indicative signs or symptoms of X-ALD, the diagnosis can be confirmed through biochemical and genetic assessment. Elevated levels of VLCFAs in boys and men lead to unequivocal diagnosis. VLCFA levels and ratios are in the normal range in 10-15% of female carriers (33).

ABCD1 genetic testing is recommended to confirm the diagnosis. A detection of a known pathogenic ABCD1 variant validates the diagnosis. Nevertheless, *de novo* mutations and variants of uncertain significance (VUS) are common. In these cases, the diagnosis can be made when the putative causative mutation is associated with typical symptoms. Thus, diagnosis can be difficult in asymptomatic females with either VUS or *de novo* mutations. In these patients, *in vitro* fibroblasts studies can help studying the pathogenicity of the putative variant.

In cases where PAI is diagnosed in the absence of neurological symptoms, the question of who should be tested for VLFCA levels still remains a subject of debate. Among children with PAI of unknown origin, 2 out of 47 boys were found to have ABCD1 mutations consistent with X-ALD with no indicative neurological symptoms (34). This discovery suggests the importance of incorporating VLCFA assessment for male children diagnosed with PAI. Nevertheless, indications about testing children with PAI vary according to different societies and research groups. The Endocrine society guidelines for PAI (35) suggest dosing VLCFA in males with confirmed PAI and negative 21-OH antibodies (21OHab) when older than 6 months. According to the same source, VLCFAs measurement should be present in the first evaluation in the case of preadolescent boys. A recent influential Seminar (36) suggests dosing VLCFAs in male patients with PAI negative for 21OHab and with normal computed tomography scan negative for adrenal enlargement. A recent international consensus (37) recommends testing all male children and adults with PAI and negative 21-hydroxylase autoantibodies or other organ specific autoantibodies. The same paper suggests not to routinely test female patients with PAI, as PAI is considered rare in heterozygous females.

According to the same consensus, in the case of known X-ALD without detected PAI, periodic screening should be started by the age of 6 months in males, with combined evaluation of basal glucocorticoid and mineralocorticoid function every 3 to 6 months until the age of 10 years. From pubertal age to adulthood, patients should be tested yearly. Of note, no routine screening is recommended in female patients. According to some authors of the aforementioned consensus, routine screening should be performed until the age of 40 years and solely if supported by symptoms after age 41 (26). Notably, Capalbo et al. reported that 32.1% of the patients were diagnosed with PAI by stimulation testing (8).

4 Management and treatment of X-linked adrenoleukodystrophy-associated adrenal insufficiency

4.1 Glucocorticoid replacement

When PAI is confirmed, hormone replacement therapy is recommended in order to prevent serious life-threatening events. As hormone replacement therapy in X-ALD does not vary

significantly from PAI caused by other conditions, it is mandatory to start hormone treatment even without an established diagnosis of X-ALD. As for glucocorticoid deficiency, treatment varies in different age groups, according to the most recent guidelines. In children, glucocorticoid replacement therapy (GCCrt) should be started with hydrocortisone (HC) at a total daily dose of 10 mg/m^2, with dose titration according to individual needs (35). The normalization of ACTH levels should not be pursued, as for the relative resistance of the adrenal gland to ACTH, the normalization would be achieved only at the cost of a significant HC overdose. Synthetic or long-acting glucocorticoids should not be used for GCCrt in children, because of their negative impact on linear growth and puberty. It is worth noting that during puberty daily requirement of HC can vary as a consequence of endogenous sexual steroids production, so that dose titration may be necessary (38). Treatment effectiveness in children with PAI must be carefully monitored by clinical evaluation of growth velocity, weight, blood pressure and energy status every 3 to 6 months (39). Notably, in children with CerALD growth and general wellbeing can be hampered by the condition itself, hence a cautious and reasonable approach may be routinely check electrolytes and carbohydrate balance, in order to detect signs of undertreatment in children with ambiguous symptoms or signs. In adults with PAI and X-ALD, GCCrt doses vary according to the treatment of choice. Standard regimens consist of oral HC (15–25 mg/day) or cortisone acetate (20–35 mg/day) in two or three divided doses. Treatment adequacy should be monitored yearly in adults with PAI. Since adult X-ALD patients can suffer for hypogonadism, which can be associated with fatigue and low energy levels, routine follow up should involve gonadal function, electrolyte balance and glucose metabolism, in order to discriminate symptoms in complex patients.

4.2 Mineralocorticoid replacement

As the adrenal zona glomerulosa can be spared from disruption, plasma renin and aldosterone levels and serum electrolyte should be always checked before considering the initiation of mineralocorticoid replacement therapy (37). When started, therapy relies on fludrocortisone (FC) oral therapy, at a starting dose of 100 mcg in children and 50-100 mcg in adults, administered once daily in the morning (40). As PAI in children with X-ALD can occur early in life, oral supplement of sodium chloride 1gr/day should be added in infants up to 1 year of age.

Given the disruption of the zona reticularis, a putative role for the supplementation of adrenal sex steroids may be hypothesized. As far as we are aware of, there is neither clear evidence nor strong recommendation for the use of dehydroepiandrosterone (DHEA) in PAI.

4.3 Emerging therapies and potential future treatments

HC therapy has been the mainstay of treatment of PAI in X-ALD. However, novel approaches have been explored to fine-tune hormonal replacement and mimic the body's natural rhythm more closely and to ameliorate patient's compliance and wellbeing. This includes the development of glucocorticoid formulations providing a more physiological hormone release, thus minimizing side effects and optimizing therapeutic benefits. The tablets commercially available in the market come with a minimum dosage of 5 mg in the USA and Europe. This dosage disparity presents a challenge when it comes to achieving precise dosing for infants and young children, as it limits the ability to finely adjust the dosage according to their specific requirements. A granulated HC formulation has recently gained approval in Europe. This formulation offers small dosages of 0.5, 1, 2, and 5 mg, which can prove useful in children taking small quantities of HC (41).

Adults with PAI for X-ALD can benefit from the dual-release HC formulation (DRHC), consisting in a system based on an outer layer for immediate release and an inner retard formulation. DRHC has proved in comparative studies to have a more natural cortisol profile. Hitherto, this formulation is considered off-label in children with PAI (42).

Lorenzo's oil, that is a mixture of monounsaturated erucic acid (C22:1) in the form of triglycerides (glyceroltrierucate), in conjunction with a mixture of monounsaturated oleic acid (C18:1) also in triglyceride structure (glyceroltrioleate), at a 4:1 ratio, was shown to normalize plasma C26:0 levels within one month when accompanied by a low-VLCFA diet (43). However, the effectiveness of this therapeutic approach has been met with skepticism, as Lorenzo's oil fails to halt the progression of pre-existing neurological symptoms, so that hitherto it is not routinely recommended by X-ALD guidelines (37). The potential benefit of Lorenzo's oil in regard to PAI has not been deeply investigated, and definitive evidence is still lacking. Nevertheless, in a perspective study on a small group of adult males with AMN, Lorenzo's oil supplementation was able to lower ACTH levels in patients with subclinical PAI, potentially linking VLCFA levels to the degree of adrenal dysfunction (44). X-ALD related PAI is probably due to a defective adrenal response to ACTH, related to VLCFA accumulation with progressive disruption of the adrenal cell membrane functions. Lorenzo's oil therapy may be able to improve VLCFA clearance and restore ACTH receptor activity in an early phase. Recently, a new nutritional approach has been proposed with a new oil mixture allowing erucic acid to cross the blood brain barrier and reduce VLCFA levels in spinal fluid (45). No data on the effect of this nutritional approach on adrenal function has been published.

Allogenic hematopoietic cell transplantation (HCT) has the potential to arrest the progression of the disease in individuals with inflammatory cerebral involvement. The post-HCT consequences are intrinsically linked to the neurological condition during the transplantation phase. Patients displaying mild neurological impairment along with discernible MRI involvement appear to achieve the most favorable outcomes (46, 47). Gene therapy is a cutting-edge approach that has shown potential in various genetic disorders, including X-ALD. A lentiviral vector has been used to introduce a wild-type copy of the ABCD1 gene into the patients' hematopoietic stem cells *ex vivo*. Subsequently, the genetically modified cells have been reintroduced into the patients (48).

We still do not fully understand how patients with cerebral ALD may achieve disease stabilization after HCT or gene therapy. Improvement in disease burden is usually not observed, and stop or minimization of progression represent the primary therapeutic goals (49). There is little data to support that myelin producing oligodendrocytes of the CNS are successfully replaced through allogeneic HCT. Patients that have undergone transplantation and fail to engraft with donor-derived cells experience progression of neuroinflammation and white matter damage, suggesting that the immune suppression associated with allogeneic HCT may decrease neuroinflammation. On this basis, the presence of a cell product expressing the wild-type ABCD1 appears critical in achieving stabilization of the disease. The ABCD1 gene product (ALD protein, ALDP) is incorporated in the peroxisomal membrane and is not released into the environment with the ability to provide 'cross correction' of adjacent cells. It remains to be elucidated whether cells expressing wild-type ABCD1 are able to localize to the brain and specifically reduce VLCFAs in the CNS, or they simply play a role in stabilizing inflammation, enhancing cellular respiration, and reducing oxidative stress (49).

In the context of PAI in X-ALD, HCT and gene therapy may potentially restore the impaired adrenal function by delivering functional genes to the adrenal glands. There are no long-term observational studies after HCT and there is no evidence so far that HCT can change the course of PAI in ALD patients. All HCT-treated patients with pre-existing PAI had to continue GCCrt after transplantation. No cases of reversed PAI have been reported in X-ALD patients who underwent gene therapy.

As already said, the mechanisms whereby elevated VLCFAs damage the steroid producing cells of the adrenal glands and testicles are not fully understood. It has been demonstrated that the accumulation of cholesterol and saturated VLCFAs in the zona fasciculata and reticularis of the adrenal cortex starts during foetal life (18), probably in conjunction with the activation of foetal adrenal steroidogenesis. This chronic accumulation triggers an early apoptosis of the adrenal cortical cells leading to irreversible shrinkage of the adrenal cortex (19). A similar (even if later) process may be at the basis of the chronic Leydig cell damage leading to adult-onset hypogonadism in patients with AMN.

While this field is still in its infancy and faces several challenges, ongoing research and advancements offer a glimpse into a future where gene therapy could dramatically change PAI outcome.

4.4 Newborn screening

HCT is potentially life-saving in patients with ALD, if initiated as soon as cerebral disease is discovered. However, as neurological impairment does not improve after HCT, an early diagnosis would allow for surveillance of cerebral disease and reduce potential residue (46). Neonatal screening for ALD was initially proposed by Moser and colleagues in 2004-2005 (50). However, at that time there was no valid test for ALD using the newborn blood spot. In the following years, a screening test for ALD by measurement of C26:0-lysophosphatidylcholine, C26:0-LPC in newborn dried blood spot was established (51). After refining the liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay of C26:0-LPC and several pilot studies, in December 2013, neonatal screening for ALD was started in the state of New York. From that time onward, some countries have implemented their neonatal screening program with ALD screening (52).

The early detection of the characteristic biochemical abnormalities associated with ALD and AMN has proven to be reliable to detect affected subjects but also poses new clinical ethical issues (52). Considering the variable and unpredictable clinical expression and prognosis of the disease, the justification of neonatal screening could be questionable and it is still a matter of discussion.

5 Conclusion

X-ALD is a complex progressive genetic disorder with a wide spectrum of clinical phenotypes.

While the neurological deterioration is a dominant clinical aspect of X-ALD, PAI and endocrine complications contribute significantly to the disease burden. The development of PAI in X-ALD can occur early, even in the absence of clinical symptoms, highlighting the importance of early screening and monitoring. Diagnosis of X-ALD relies on the assessment of VLCFA levels, genetic testing, and clinical presentation. Hormone replacement therapy remains the cornerstone of treatment for PAI in X-ALD. The dosing and management of glucocorticoid and mineralocorticoid replacement therapies differ based on age groups and individual needs. Emerging therapeutic approaches, such as modified-release formulations and gene therapy, hold promise for improving treatment outcomes and addressing the complexities of PAI in X-ALD.

While advancements have been made in understanding and managing X-ALD, further research is essential to unravel the intricate mechanisms underlying VLCFA toxicity and adrenal dysfunction. The interplay of genetics, hormone pathways, and disease progression requires continued exploration to develop more targeted and effective therapeutic strategies. As we navigate the intricate landscape of X-ALD, a multidisciplinary approach, encompassing medical, genetic, and endocrinological expertise, will be pivotal in improving the lives of individuals affected by this complex disorder.

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Case Report: Adrenocortical carcinoma in children—symptoms, diagnosis, and treatment

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Adrenocortical carcinomas are extremely rare in the paediatric population. Most of them are hormone-secretive lesions; therefore, they should be taken into consideration in a child with signs of precocious puberty and/or Cushing's syndrome symptoms. Nonetheless, differentiation from benign adrenal tumours is necessary. We report a rare case of adrenocortical carcinoma in a girl and a literature review using the PubMed database. A four-year-old girl presented with rapidly progressing precocious puberty and signs of Cushing's syndrome. Imaging of the abdomen revealed a large heterogeneous solid mass. Histopathologic evaluation confirmed adrenocortical carcinoma with high mitotic activity, atypical mitoses, pleomorphism, necrosis, and vascular invasion. After tumourectomy, a decrease of previously elevated hormonal blood parameters was observed. Genetic tests confirmed Li Fraumeni syndrome. Adrenocortical carcinoma should be suspected in children with premature pubarche and signs of Cushing's syndrome. Diagnosis must be based on clinical presentation, hormonal tests, imaging, and histopathological evaluation. Complete surgical resection of the tumour is the gold standard. Oncological treatment in children is not yet well-studied and should be individually considered, especially in advanced, inoperable carcinomas with metastases. Genetic investigations are useful for determining the prognosis in patients and their siblings.

KEYWORDS

adrenocortical carcinoma, adrenocortical tumour, precocious puberty, Cushing syndrome - therapy, Cushing syndrome

Introduction

Adrenocortical tumours (ACT) including adrenocortical carcinomas (ACC) and adrenocortical adenomas (ACA) are rare in the paediatric population, with an incidence of 0.3–0.38:1,000,000, accounting for approximately 0.2% of all paediatric neoplasms (1–4).

ACT are more common in women, with a proportion of 1.4:1. This rate varies according to age, but remains higher in women (2, 4, 5), especially in malignant adreno-cortical carcinomas; however, there are certain subtypes of ACC, such as oncocytic, with no preference for any sex (6).

A much higher percentage of ACT is observed in Brazil, with an incidence of 6.2:1,000,000 in children <10 years (7), and for ACC, the incidence is 2.2:1,000,000 in the general population and 4:1,000,000 in children <10 years (8). This is due to the greater prevalence of the TP53 mutation, especially in industrial and urban areas (7).

The majority of ACT and ACC are sporadic; however, there is an increased risk of developing the tumour in patients with genetic syndromes such as Li Fraumeni syndrome, Beckwith–Wiedemann syndrome, multiple endocrine neoplasia type 1 (MEN-1), familial adenomatous polyposis, and other hereditary cancers (6, 9, 10).

Most ACT in the paediatric population are hormone-secreting lesions (1, 2). The hypersecretion of adrenocortical hormones leads to clinical manifestation, most commonly peripheral precocious puberty with virilisation and Cushing's syndrome, and rarely, hyperaldosteronism (1, 2, 4, 5).

Non-functional ACT and ACC are much less common. The manifestation of adrenal tumours is non-specific, including abdominal or back pain, fatigue (1, 5), or acute abdomen (11, 12).

The differentiation between benign adrenocortical adenomas and ACC presents a challenge at every stage of the diagnostic process, including clinical symptom analysis, laboratory tests, imaging scans, and histopathologic evaluation (1, 2, 13, 14).

The only undisputed sign of malignancy is metastases. The most frequent sites of ACC metastases are the lung, liver, and retroperitoneal space (15). Metastatic disease is one of the most adverse, significative prognostic factors at the time of diagnosis—this raises the need for early diagnosis (1, 2, 13, 16).

The aim of this study was the presentation of a rare cancer patient and a review of the up-to-date knowledge of adrenocortical carcinoma and its diagnostics-therapeutics algorithm.

Case presentation

A four-year-old female presented with rapidly progressive precocious puberty. Pubic hair was first observed approximately two months prior to presentation. Additionally, the child's mother noticed intense perspiration and acne. The patient gained approximately 3 kg in one month and became apathetic.

The patient's medical history was insignificant: second pregnancy, second delivery (natural delivery at the 39th week of gestation), Apgar score of 10, birth weight of 3,900 g, and birth length of 55 cm. Psychomotor development was normal. She had no chronic diseases, no allergies, or no operations in the past. Her diet was normal and physical activity was regular; however, she became less active in the weeks prior to presentation.

On physical examination, the patient's general condition was good, her vital signs were stable, her height was 110 cm (90th percentile), her body weight was 23.9 kg (>97th percentile), and she appeared overweight with an excess of visceral adipose tissue, a rounded face, and prominent, reddened cheeks. She also had acne (Figure 1).

The abdomen was asymmetrically distended and a firm, non-tender, and immovable mass was palpated on the left side. Physical examination also revealed pubarche, Th1 P3 A1 on the Tanner scale.

Abdominal ultrasonography (USG) revealed a large heterogeneous solid mass between the spleen and left kidney, measuring 121 x 100 x 141 mm, with several blood flow signals. The mass shifted and modelled surrounding organs. Additionally, a small round lesion of unidentified character was observed in the right lobe of the liver.

Computed tomography of the abdomen showed a large left suprarenal mass measuring left-right (LR) 10 cm; anterior-posterior (AP) 13.5 cm; and cranio-caudal (CC) 12 cm, with calcifications (Figures 2, 3). The tumour compressed and shifted the tail of the pancreas, stomach, spleen, and left kidney. On post-contrast-enhanced CT there was a heterogenous enhancement in the lateral parts, with irregular low-density areas of tumour necrosis and lysis in the centre. A lesion measuring 11 x 13 mm, characteristic for angiomas, was found in the VI segment of the liver with contrast enhancement. Chest CT did not show any abnormalities.

The patient's bone age was six years based on the Greulich-Pyle method.

Laboratory investigations are shown in Table 1.

The girl was qualified for surgical intervention. Before surgery, she was prepared with hydrocortisone in high substitutive doses. The patient was referred to the Department of Surgery, The Children's Memorial Health Institute, where she underwent radical tumourectomy, with resection of the left kidney and the lesion in the liver. After the tumourectomy, the high doses of hydrocortisone were continued. The perioperative period was complicated by lymphorrhoea, pancreatitis, pneumonia, left side pleural effusion, and gastrointestinal obstruction that required a second laparotomy. In the subsequent days, the patient improved. The substitution of hydrocortisone was decreased, and fludrocortisone was introduced orally.

Histopathologic evaluation of the tumour confirmed ACC with high mitotic activity, atypical mitoses, pleomorphism, necrosis, and vascular invasion. The structure of the left kidney was normal. No metastases were found in the local lymph nodes. The lesion in the sixth liver segment was confirmed to be a cavernous angioma.

In the post-operative follow-up, the patient remained under observation of the Department of Oncology in The Children's Memorial Health Institute, Warsaw. In a control CT of the abdomen, chest, and pelvis, no lesions were observed. The control steroid profile showed normal daily excretion of androgens and 17-OH-progesterone metabolites.

At the request of her parents, the patient was transferred to the Department of Haematology, Oncology and Transplantology of the Children's Hospital of Lublin to continue treatment. At this stage,

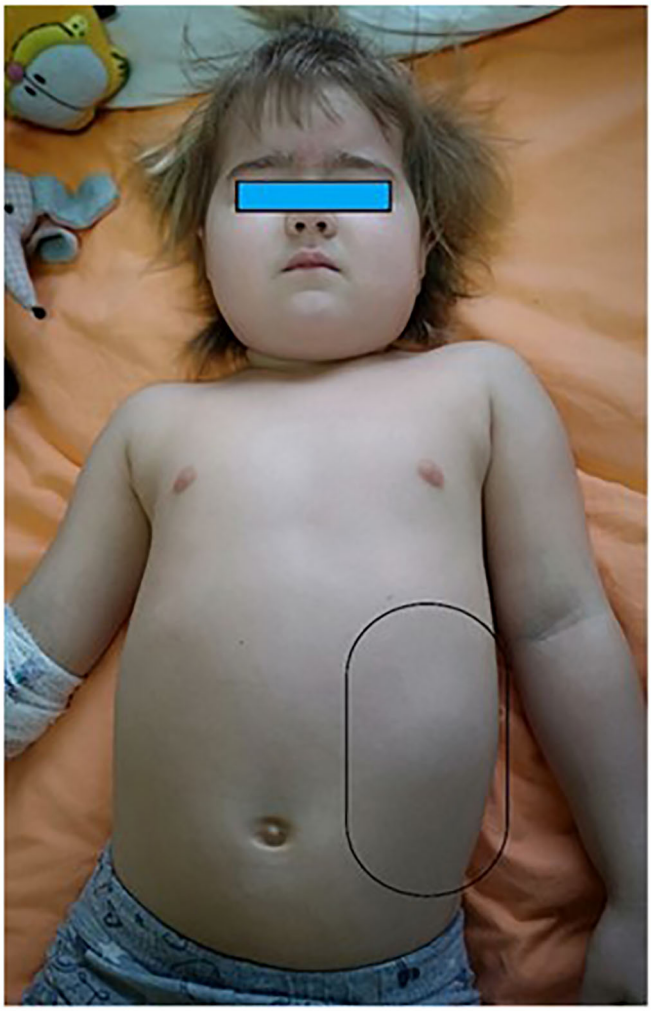

FIGURE 1
The patient's appearance. A large mass is visible in the left abdomen.

no new symptoms presented. The development of the girl is normal: her Tanner scale is Th1P2A1. The second control steroid profile was normal.

Genetic investigations confirmed Li Fraumeni syndrome in the girl. There is a need to examine the patient's siblings. The parents did not consent to oncological treatment of their daughter.

Review

Epidemiology and genetics

ACT, encompassing both benign and malignant lesions, are rare in the paediatric population, accounting for 0.2% of paediatric neoplasms. The literature reports the peak incidence of ACT in children at either <five years, or at a biphasic age distribution, with the first peak <five years and the second >10 years (1, 2, 4).

At the molecular level, two common aberrations are described —excessive expression of IGF-2 and constitutive activation of the signal route Wnt/β-catenin (4, 14, 17, 18). Identification of these aberrations correlates with a poor prognosis.

It was recently observed that germline EGFR variants (19) and vitamin D receptor hypermethylation and underexpression (20) may predispose infants to childhood ACC. High expression of Stathmin1 was also observed in paediatric adrenal tumours (21). Thus, genetic analysis in children with ACT is important, especially those with second primary cancer or a positive family history of malignancies (6, 9).

Clinical manifestation

Clinical manifestation of ACT is different in children and adults. Adults present with non-specific symptoms more often

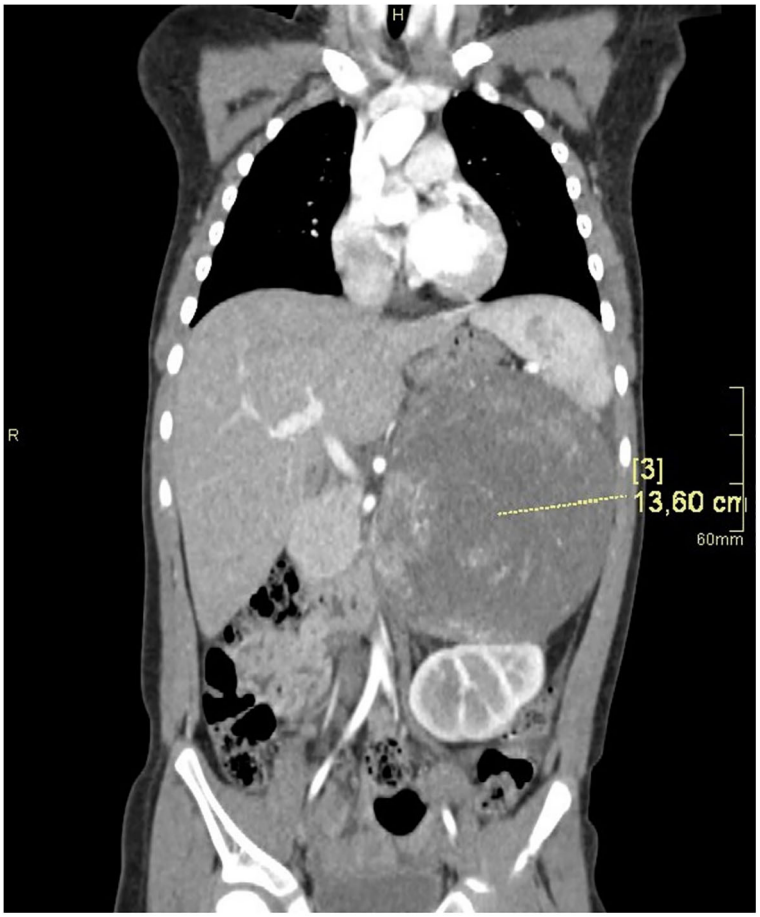

FIGURE 2
CT of the abdomen in the AP plane.

than hormonal hypersecretion due to the tumour-mass effect (4), while clinical manifestation in children is usually a consequence of elevated levels of steroids and their precursors. Based on clinical observations, it is noted that ACT, especially ACC, secrete more than one hormone, resulting in mixed symptomatology. Non-secreting tumours are rare (1, 2, 4, 5).

The most frequent symptom of ACC in children is virilisation, which is characterised by precocious development of pubic or axillary hair, penis enlargement or erection, clitoris enlargement, accelerated growth velocity with high bone-age, acne, hirsutism, and voice changes. The second most common manifestation is Cushing's syndrome, with symptoms such as central obesity, moon face, and hypertension (6, 9, 15, 19, 22, 23). There is a case described in the literature in which chronically elevated sex steroids and, therefore, activated GnRG pulse generator, lead to central precocious puberty (24). Hyperaldosteronism is uncommon (1, 5).

The appearance of precocious puberty should always prompt consideration of pathology within the adrenal cortex in the diagnostic process (16, 24–26). A sudden increase in body weight, especially in conjunction with precocious puberty, is also a red flag. It would seem that the combination of the above symptoms indicates suspicion of ACT, especially ACC (2, 24). There are several features that may suggest a malignant character of precocious adrenarche: age <four years, rapid progression, clitoral enlargement, significant acceleration of bone age paired with increased blood pressure, and features of Cushing's syndrome (13, 25–27).

Laboratory testing

Laboratory diagnostics of ACC are based on hormonal activity observed in over 90% cases. Clinical manifestation results from excessive secretion of steroids and their precursors by the tumour. The most common abnormalities are increased levels of DHEAS, androstenedione, testosterone, 17-OH progesterone, and 11-deoxycorticosterone (6, 24). Among the biochemical parameters, attention should be given to LDH, which might be useful in distinguishing between ACA and ACC. Although LDH is not a specific marker for ACC, an elevated level marks a risk of malignancy (2, 27). Other parameters such as cortisol level, DHEAS, testosterone, oestradiol, and aldosterone are not helpful in differentiating between ACC and ACA (2), although it was observed that testosterone, DHEAS, and 17A-hydroxyprogesterone are higher in childhood adrenal carcinoma than adenoma (27). Nevertheless, a laboratory workup is essential in every paediatric patient with ACT and should comprise circadian rhythm of cortisol secretion, 24 h urine cortisol

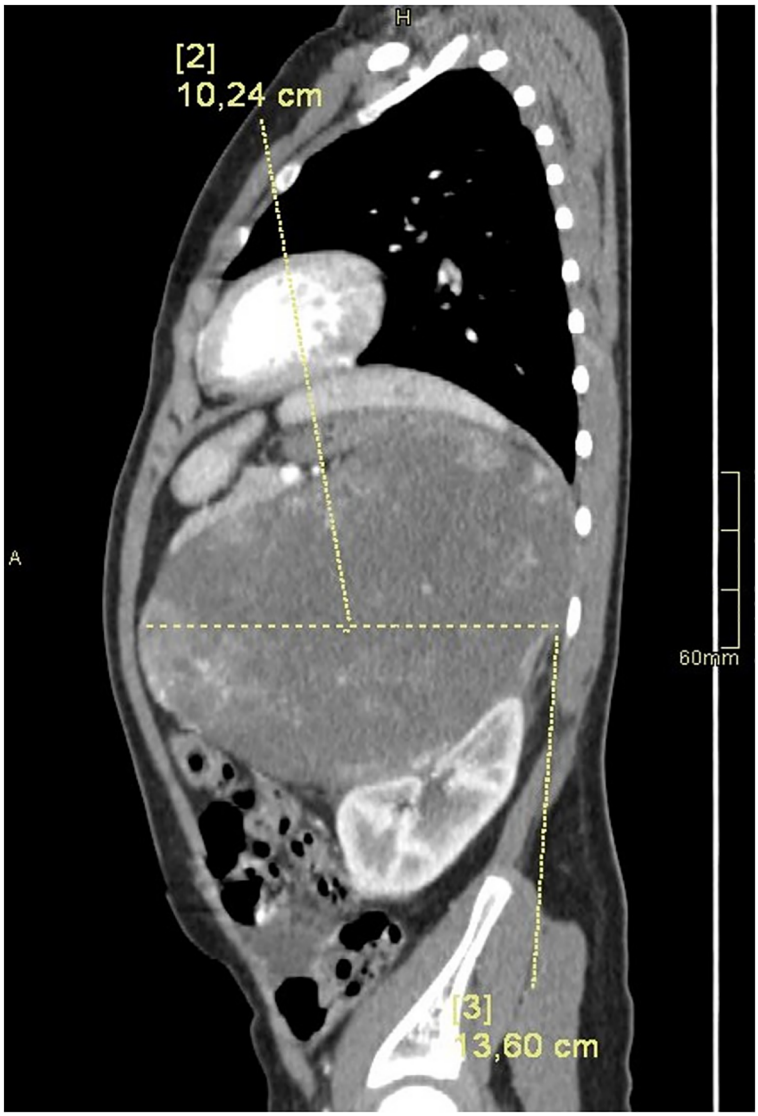

FIGURE 3
CT of the abdomen in the LR plane.

excretion, testosterone, oestradiol, DHEAS, androstenedione, 17-OH-progesterone, aldosterone, PRA, and the steroid profile in urine. The recommended laboratory technique for steroids assessment is mass spectrometry (1, 5, 13). Bearing in mind the role of the TP 53 gene mutation and other genetic factors in ACT in children, it is advisable to also perform genetic tests (5).

Imaging testing

Ultrasonography (USG) of the abdomen is the first stage of imaging in a patient with clinical signs of adrenocortical pathology. Computed tomography (CT) is the next step in the diagnostic process (1, 2) and it is the most commonly used imaging procedure to identify adrenal tumours, with the best cost-benefit ratio (28). It enables a more precise evaluation of the tumour than USG in terms of size, capsule, demarcation, areas of calcifications, necrosis, or bleeding. CT allows early diagnosis of ACT and preoperative staging to plan the operation, although it has limitations and cannot determine the benign or malignant character of the tumour.

According to Kuhlen et al. (29), however, abdominal magnetic resonance imaging (MRI) rather than CT should be preferred in addition to USG. It is suggested to perform USG, abdominal MRI, and chest CT (for the evaluation of lung metastases) in all patients, PET when there is a suspicion of high-risk ACC and bone scan or brain MRI when metastases are suspected.

Malignant tumours are usually bigger than benign ones; however, diagnosis cannot be based only on dimensions. Nonetheless, tumours ≤5 cm seem to be benign, while tumours ≥10 cm are more likely to be malignant, especially with a concomitant elevated LDH level (2).

On imaging, ACA have homogenous contrast enhancement and are well circumscribed, while ACC are usually characterised by a heterogenous structure with necrosis, haemorrhage, and

TABLE 1 Laboratory investigations before and after tumour surgery.

Parameter	Result before treatment	Results after surgery	Normal ranges	Units
Cortisol 8:00 AM	22.52	12.86	6.2–19.4	ug/dl
Cortisol 11:00 PM	21.36	5.22	2.3–11.9	ug/dl
ACTH	3.82	32.8	7.2–63.6	pg/ml
24 hr urine Cortisol	184.80	104.1	4.3–176	ug/24 h
Steroid profile GC-MS	Typical for ACC secreting cortisol and androgens, showing intensive enzymatic abnormalities—deficiency of 3BHSD (massive excretion of DHA and its metabolites), deficiency of 11-B OH, 21-OH, and increased excretion of 6B-OHF	All steroids in normal range		
Testosterone	1,366.00	6.2	6–82	ng/dl
DHEAS	1,000.00	5.92	0.47–19.4	ug/dl
Androstenedione	>30	1.25	0.3–3.3	ng/ml
Oestradiol	<5	<5	6–27	pg/ml
Progesterone	2.30	0.99	0–0.99	ng/ml
17-OHP	25.11	0.5	0.2–0.9	ng/ml
LDH	3 184	423	0–615	U/l
NSE	77.03	12.8	0–16.3	ng/ml

ACTH, adrenocorticotropic hormone; GC-MS, gas chromatography-mass spectrometry, 3BHSD; 3B-hydroxysteroid dehydrogenase; 11B-OH, 11B hydroxysteroid dehydrogenase; 21-OH, steroid 21-hydroxylase; 6B-OHF, 6B-hydroxycortisol; DHA, dehydroandrostendion; DHEAS, dehydroepiandrosterone sulphate; 17-OHP, 17-hydroxyprogesterone; LDH, lactate dehydrogenase, NSE, neuron-specific enolase.

calcifications. ACC can also invade local structures and vessels (30). The presence of metastases in imaging tests indicates ACC (1, 2, 13). Distant metastases most often invade the liver, lungs, kidneys, and bones; therefore, these areas need to be radiologically examined (31) by conducting pelvis and chest CT (1, 2, 29). FDG-PET is also used to identify metastases (29–31).

Tumour biopsy is not recommended because resection is a basis of treatment, and tumour rupture and spillage during biopsy worsen the prognosis (31).

Histopathological examination

There is no single histopathologic trait that can reveal the malignant character of adrenal tumour, apart from invasion of nearby tissues. Nonetheless, architectural disarray, reticulin framework disruption pleomorphic, and large, clear, and granular or eosinophilic neoplasm with intranuclear inclusion and nuclear atypia may help differentiate between benign and malignant adrenal tumours and are indications of ACC rather than ACA (32).

Children with ACT have better prognosis than adults (33); however, histopathologic criteria predicting tumour behaviour in adults are unreliable in children (34). The most prevalent systems in differentiating between benign and malignant ACT are the Weiss scale, the modified Weiss scale, and the Wieneke index (1). Nevertheless, histopathological criteria that would permit unequivocal distinguishment between ACC and ACA remain difficult to establish (2). The rarity of ACT results in limited diagnostic experience. The Weiss scale and its modified version evaluate microscopic features and are also used in adult patients. The Wieneke index, preferred in the paediatric population, includes both macro- and microscopic features. Such divergence was less often noted when the Wieneke index was applied, suggesting the higher validity of this tool (1, 35, 36). Wieneke's criteria for malignancy (36) are as follows:

Tumour weight >400g
Tumour size >10.5cm
Extension into periadrenal soft tissues and/or adjacent organs
Invasion into vena cava
Venous invasion
Capsular invasion
Presence of tumour necrosis
>15 mitoses per 20 high power field (400X)
Presence of atypical mitotic figures

According to Wieneke's criteria, it is possible to determine the prognosis outcome of the patient. Two criteria indicate a benign long-term clinical outcome, three criteria indicate an intermediate/atypical/uncertain malignant potential while four or more criteria indicate a poor clinical outcome. (4, 36).

It was observed that the cellular proliferation index Ki-67 was significantly different between ACC and ACA, with a mean level of

30.2% (range 7–80%) and 9.9% (range 2–20%), respectively (27). In another study (37), it was observed that the Ki-67 labelling index of paediatric adenomas and carcinomas was much higher than in adult adrenal tumours and that a KI-67 index ≥15% could be used to presume poor outcome in the paediatric population. Currently, research is in progress on the usefulness of molecular biology techniques such as DNA methylation analysis for differentiation between benign and malignant ACT (14).

Fang et al. suppose that ACC development may depend on intracellular communications mediated by miRNA and mRNA (38).

Prognosis

According to the European Network for the Study of Adrenal Tumours (ENSAT), there are four stages of ACC. Stage I and stage II are strictly localised tumours with a size of ≤5 or >5 cm, respectively. Stage III is characterised by infiltration of surrounding tissue, positive regional lymph nodes, or a tumour thrombus in the vena cava and/or renal vein. Stage IV is defined by the presence of distant metastasis (1, 4). Stage I and II, age <4 years, smaller tumour size, and virilisation as the only symptom of childhood ACC are correlated with better prognosis (1, 23, 39, 40). It seems that the risk of recurrence is directly proportional to the size of ACT (1, 4, 5). The difference in the survival rate between a Weiss score >6 and ≤6 is also significant—the higher score, the worse the prognosis (15, 37). A similar observation was made for the Wieneke score (37). According to the National Cancer Institute (NCI) PDQ cancer information summary on childhood ACC, unfavourable prognostic factors include large tumour size, older age, incomplete resection, microscopic tumour necrosis, and metastatic disease (41).

Treatment

Complete surgical resection is the mainstay in ACT treatment as it enables full control of the disease and satisfactory clinical effects, especially in cases that are not advanced (1, 13, 42–44). In some cases, preoperative management with ketoconazole is reported, especially in hypercortisolism (42–44).

In malignant adrenal tumours, open surgery rather than laparoscopic should be prioritized (31). Increased hormones levels can decrease even after 24 h post-surgery (6). Corticosteroids should be supplemented both in the perioperative (31) and the post-operative period (24, 31) to avoid adrenal insufficiency due to a rapid decrease in hormone production. A very important procedure is substitutive treatment with hydrocortisone before and after surgery in patients with very low levels of ACTH as a result of overproduction of tumour steroids and blockade of ACTH secretion from pituitary glands. After surgery, the substitutive doses of hydrocortisone are reduced, and the addition of fludrocortisone is necessary for good electrolyte balance (42–44).

The effect of adjuvant chemotherapy in treating ACC is not satisfactory; adrenal cancer cells are resistant to common drugs. Mitotane, an inhibitor of the adrenal cortex, is commonly used in adult patients with ACT (3, 18, 42, 44). Due to its cytotoxic effect on adrenocortical cells, it inhibits adrenal steroidogenesis and decreases the risk of recurrence in adults (35, 42, 44). Experience with using mitotane in children is limited and its effect on the paediatric population is not yet well established; therefore, an individualised approach to each patient should be taken into account. Mitotane is mostly administered in inoperable tumours, tumours with positive margins, or advanced tumours with metastases (39), although there are studies describing mitotane usage in earlier stages of ACC (1, 15). There are cases of patients treated with mitotane who ended up with stable disease or complete remission (11, 15), as well as those who had poor prognosis or died as a result of the disease, despite chemotherapy (15, 22, 45–47). In cases of stable disease course, monotherapy with mitotane only may be used, while in aggressive ACC with metastases, the treatment combines both mitotane and cytostatics (42), mostly etoposide, doxorubicin, and cisplatin (EDP).

Toxicities related to combined therapy with mitotane and chemotherapy may lead to discontinuation of treatment (1, 4, 5, 48, 49).

During mitotane treatment, substitutive doses of hydrocortisone should be 2–3 times higher than those used for congenital, adrenal hyperplasia (CAH), and in some patients, fludrocortisone treatment for control of electrolytes is necessary. Serum mitotane evaluation should also be reported as well as the dose, usually every 15 days (42, 44, 47).

ACC seems to be sensitive to radiotherapy (RT). Adult patients who received RT as an adjuvant treatment had higher overall survival (p=0.004) than patients treated with surgery alone (50). Wiegering at al (51). analysed cases of children with ACC, in whom RT was administered. A systematic review shows that the majority of patients receiving RT were stage 2, although the treatment was also performed in stage 1, 3, and 4 children.

Currently, the role of immunotherapy in ACC treatment in adult patients is being investigated. The potential use of immune checkpoint inhibitors, such as pembrolizumab, which has a favourable safety profile and good tolerance in initial evaluation in adult patients (43, 52, 53), is noteworthy. The study of Iodine-131 Iodometomidate (131I MTO) targeted radionuclide therapy (54) and Yttrium-90/177Lu-DOTATOC in somatostatin expressing tumours (55) is promising. According to Akinkuotu et al. children with ACC had better survival than adults. Factors independently associated with worse survival included older age, metastatic disease, and receipt of lymph node surgery (56).

Conclusions

ACC should be suspected in children with premature pubarche and signs of Cushing's syndrome. For diagnosis, hormonal tests and imaging (especially USG and CT) are necessary. The surgical treatment of ACC is the gold standard, and oncological treatment should be individually considered as the second adrenal gland may

be destroyed. Genetic investigations are useful for determining the prognosis in patients and siblings.

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 humans were approved by Ethical Committee of Medical University in Lublin. The studies were conducted in accordance with the local legislation and institutional requirements. The human samples used in this study were acquired from a by-product of routine care or industry. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements. Written informed consent was obtained from the minor(s)' legal guardian/next of kin for the publication of any potentially identifiable images or data included in this article. Written informed consent was obtained from the parents of the child for the publication of this case report.

Author contributions

EZ, MM, AG, and IB-S made substantial contributions to the conception, design, and acquisition of data, drafting of the article, giving final approval of the version to be published, and agreeing to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. IB-S critically revised the paper for important intellectual content. 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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Adrenal Cushing's syndrome in children

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Adrenal Cushing's syndrome is a rare cause of endogenous hypercortisolism in neonatal and early childhood stages. The most common causes of adrenal CS are hyperfunctioning adrenal tumours, adenoma or carcinoma. Rarer causes are primary bilateral macronodular adrenal hyperplasia (PBAMH), primary pigmented adrenocortical disease (PPNAD) and McCune Albright syndrome. The diagnosis represents a challenge for clinicians. In cases of clinical suspicion, confirmatory tests of hypercortisolism should be performed, similarly to those performed in adults. Radiological imaging should be always combined with biochemical confirmatory tests, for the differential diagnosis of adrenal CS causes. Treatment strategies for adrenal CS include surgery and in specific cases medical drugs. An adequate treatment is associated to an improvement of growth, bone health, reproduction and body composition from childhood into and during adult life. After cure, lifelong glucocorticoid replacement therapy and endocrine follow-up are required, notably in patients with Carney's complex disease.

KEYWORDS

hypercortisolism, pediatric, childhood, adrenal tumors, adrenal hyperplasia

Introduction

Cushing's syndrome (CS) is characterized by an excess of glucocorticoids and represents a diagnostic and therapeutic challenge for clinicians. The total incidence of CS is approximately 0.7-2.4 new cases per million people per year. Of these, only about 10% occur in paediatric age (1). Paediatric and adolescent CS is quite rare, especially when compared to other disorders of this age including growth, puberty, thyroid and metabolic diseases.

The aim of this review is to discuss the causes of adrenal CS and their pathogenesis, focusing on the clinical presentation, diagnostic approach and therapeutic management, showing advantages and disadvantages in a critical way.

Epidemiology and classification

As in adult, paediatric and adolescent CS can result from an exogenous or endogenous cause. Exogenous CS, probably underdiagnosed, generally occurs in children who need to take steroid therapy. Although the majority of cases result from administration of oral or parenteral glucocorticoids, in childhood the topical and inhaled administration of supraphysiological doses requires particular attention. Since they have a thinner dermis layer of epidermis than adults, children are more vulnerable to systemic effects of topically glucocorticoids. In iatrogenic CS, it is essential for clinicians to be aware of sudden withdrawal of steroids and to inform patients and/or caregivers to prevent adrenal crisis.

Endogenous CS can be divided into ACTH dependent forms such as ectopic CS and pituitary ACTH-secreting tumours or ACTH independent forms including adrenocortical tumours (adenoma or carcinoma) and primary adrenocortical hyperplasia such as primary pigmented nodular adrenocortical disease (PNAD), primary bilateral adrenal macronodular hyperplasia (PBAMH) and McCune-Albright syndrome.

A bimodal age distribution has been reported, with higher incidence of adrenal forms before 7 years and ACTH-dependent forms over 7 years (2–4). Ectopic CS is very rare in children and adolescents with a prevalence <1% although a recent systematic review reported a median prevalence of 7% (X). Cushing's disease (CD) accounts for 75%, while adrenal causes of CS account for 15% of all cases of hypercortisolism in children.

Pathogenesis of adrenal causes of CS

The most common causes of adrenal CS are hyperfunctioning adrenal adenoma or carcinoma. Rarer causes are BAMH, PPNAD and McCune Albright syndrome.

Adrenocortical tumours

Adrenocortical tumours (ACTs) are very rare in childhood although they are an important cause of CS in this age. The reported incidence is about 0.2-0.3% of all paediatric tumours and a very low percentage of them are adrenocortical carcinomas. The incidence is different depending on the geographic area, with a higher incidence in Brazil compared to the United States (5, 6).

Paediatric adrenocortical tumours notably affect children aged from 0 to 4 years old and adolescents. In addition, a gender difference has been reported, with a female predominance before 3 years and after 13 years (5, 6). Two large studies have collected paediatric adrenocortical cancer cases, the International Pediatric Adrenocortical Tumor Registry (IPACTR) (7), which includes 254 patients, and a Brazilian monocentric study including 73 children (8).

The higher incidence in Brazil is explained by a high prevalence of p53 tumour suppression (TP53) mutation, which is involved in ACT pathogenesis. In patients without TP53 mutation, 11p15 chromosome defects have been reported (Beckwith-Wideman syndrome) (6). ACTs are generally functional and are only associated with virilizing signs and symptoms including pubic hair, faster growth and skeletal maturation and external genitalia enlargement in about 50-60% of children (9–12). In some cases virilization is combined with hypercortisolism (about 30% of cases) (13). Less frequently (10-15% of all cases) ACTs tend to present with CS, even though CS secondary to adrenocortical cancer is more frequent in adolescents and is associated with a poor survival rate (3, 14). Less than 5% of patients show feminization or hyperaldosteronism (13).

Primary adrenocortical hyperplasia

Primary pigmented adrenocortical disease

PPNAD can be isolated or associated with Carney's complex. It frequently occurs in adolescents, even though several cases have been described in childhood (15). PPNAD is characterized by black adrenocortical micronodules located in the adrenal gland that appears atrophic in the areas not involved by nodules. Further, non-pigmented adrenocortical nodular disease has also been described, characterized by a unilateral adrenal tumour and absence of pigmentation, caused by a myosin heavy chain 8 mutation (16, 17).

PPNAD is generally caused by a mutation of the protein kinase 1 regulatory subunit 1A (PRKAR1A) gene, leading to activation of the cAMP/PKA pathway associated with a glucocorticoid hypersecretion (18). However, second-line molecular events are involved in cortisol hypersecretion as shown in patients with armadillo-repeat containing 5 (ARMC5) gene mutations (19).

PPNAD is generally characterized by overt CS; it is rarely associated with a subclinical or cyclical CS (20–22). The clinical presentation can rarely be classical (23) and is more frequently characterized by hyperandrogenism and osteoporosis beyond typical symptoms of Carney complex, which can be combined, such as skin pigmentation, cardiac myxomas and endocrine and non-endocrine tumours (15).

Primary bilateral macronodular adrenal hyperplasia (former name ACTH-independent macronodular adrenal hyperplasia AIMAH)

Primary bilateral macronodular adrenal hyperplasia (PBMAH), is a bilateral adrenal hyperplasia characterized by large yellow-to brown cortisol secreting nodules, not associated with other disorders. It is very rare in childhood and adolescence, while it is more frequent in older patients. In pediatric/adolescent cases, PBMAH can be associated with dominantly inherited genetic condition. It can be sporadic or associated with genetic mutations, such as hyperexpression of the G-protein aberrant receptors and pathogenic variants of MC2R, GNAS, PRKAR1A, and PDE11A. However, the ARMC5 gene is believed to be a major genetic cause of PBMAH, accounting for more than 80% of the

familial forms (24). In patients with an aberrant expression of the gastric inhibitory polypeptide receptor (GIPR) in the adrenal glands, cortisol hypersecretion can be food-dependent (25). Clinical presentation can be overt or subclinical CS (26).

McCune-Albright syndrome

McCune-Albright syndrome, a sporadic heterogeneous disorder caused by somatic or post-zygotic activating mutations in the α subunit of G protein (Gsα gene) is the main cause of CS in the neonatal period. McCune-Albright syndrome is generally characterized by peripheral precocious puberty, *café-au-lait* skin pigmentation and polyostotic fibrous dysplasia, which can be combined with other endocrine disorders including CS, hyperthyroidism and growth hormone excess (27). In addition, it generally presents with severe CS (28–30), typically in the neonatal period, and though severe, it could be also transient. However, milder cases have also been reported (31).

Clinical presentation

The clinical presentation of paediatric CS is characterized by many different symptoms. Contrary to what is observed in pediatric CD where there is a male prevalence, non-gender differences are observed in pediatric adrenal CS.

The two most common are weight gain and growth retardation. Other signs include faciotruncular fat distribution, skin fragility, arterial hypertension, violaceus skin striae, acanthosis nigricans, hypertensive encephalopathy (32), skin infection, recurrent infection (33), nephrocalcinosis and kidney stone (34), delayed bone mineralization and puberty (35), depression, fatigability, behaviour disorders (36), ion disorders, such as hypokalaemia, and hypercalcemia, glucose intolerance or diabetes, as shown in Figure 1.

In a recent retrospective study on a Chinese paediatric population with Cushing's disease, growth retardation, weight gain, hirsutism and acne were the main clinical signs (37).

Chronic exposure to glucocorticoids results in an overall inhibition of the somatotropic axis with reduced production of GH and IGF-1. This factor, in association with some direct effects of glucocorticoids such as inhibition of collagen synthesis, cartilage sulphation and chondrocyte mitosis, contributes to growth arrest (38). Moreover, prolonged exposure to excess glucocorticoids can lead to pathological reduction of bone mass and in some cases osteoporosis, even in childhood. Another very significant aspect is pubertal development: a pseudo-precocious puberty and virilization were observed with gonadotrophin levels subnormal by a suppressive effect of chronic hypercortisolism.

In a large Brazilian study in 254 patients younger than 20 years of age with newly diagnosed or previously treated ACTs, Michalkiewicz et al. (7) reported a significant frequency of virilization (around 84% in the examined population) as a clinical onset sign in paediatric adrenal CS, either isolated or combined with other symptoms. Isolated Cushing's syndrome was rare.

The impact of CS on the psychological sphere of children can be much more devastating. Indeed, in adults an improvement in quality of life and cognitive functions are generally described, while in children a worsening of cognitive functions and the

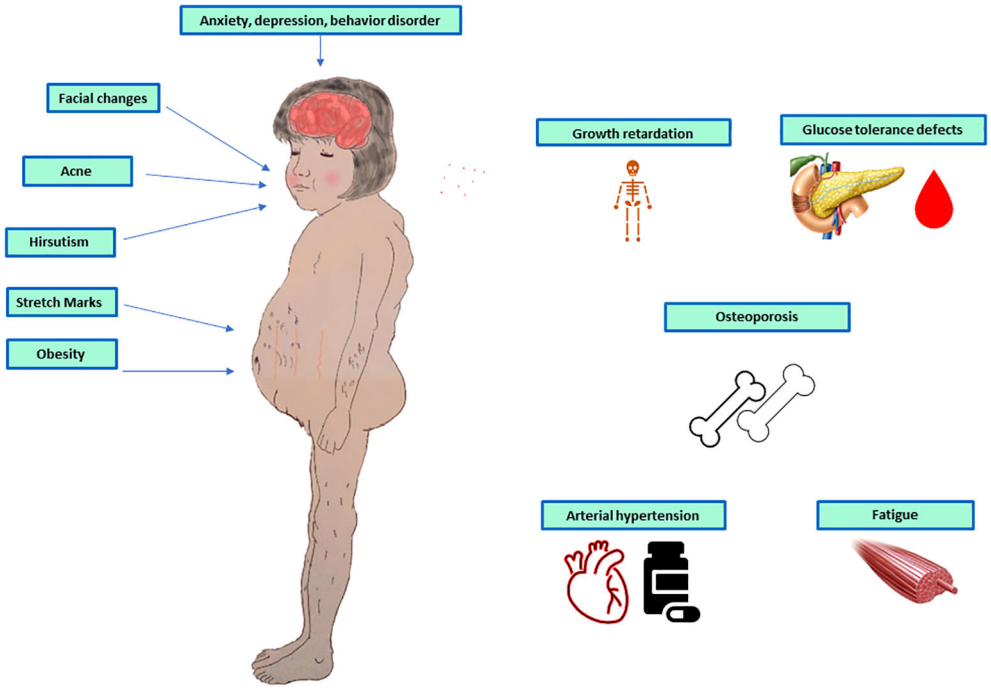

FIGURE 1
Symptoms and signs of paediatric adrenal Cushing's syndrome.

appearance of psychopathological manifestations are observed even after the remission of hypercortisolism (39, 40).

Anyway, the clinical presentation of CS is different according to the age onset. In neonatal age it is generally associated with the classical skin marks; bone dysplasia and early puberty can also occur (41). In childhood the common causes of hypercortisolism are ACTs and the most frequent symptoms are hyperandrogenism, hirsutism and acne (early puberty). Another cause is PPNAD, which is not systematically associated with delayed growth (42, 43). In the peri-puberty period the typical symptoms are slow growth and weight gain, combined with delayed puberty, and behaviour disorder (aggressiveness, fatigability, poorer tolerance of physical exercises) (36).

Diagnosis

Biochemical exams

After exogenous causes are excluded, a suspect pediatric patient should be referred to a third-level centre. Since there are no pediatric guidelines, the diagnosis and confirmation of hypercortisolism should be performed according to current guidelines for adult CS although there may be some difference (44, 45). It is important to note that none of these tests have not been used extensively in children population.

Three consecutive 24-hour urine collection, to evaluate urinary free cortisol (UFC), corrected for body surface area, are the first-level exams in older children (44). In younger children an adequate 24h urine collection could be difficult, leading to false negative results. False positive results could be obtained in cases of excessive water intake (more than 5L/day), severe obesity, anorexia, malnutrition, and physical and emotional stress, which could be indicators of pseudo-Cushing states (46).

The loss of circadian rhythm represents an hallmark of CS. Late-night salivary cortisol can be easily performed in children, notably in younger children and babies, even though it is characterized by greater inter-laboratory variability and normal levels in children are uncertain (47–49). Recently, in 320 healthy children (174 girls) between 4 and 16 years of age, bedtime and morning salivary cortisol and cortisone were measured by LC-MS/MS (49). The cutoff level for bedtime salivary cortisone was 12.0 nmol/L and salivary cortisol was 2.4 nmol/L, slightly lower than that of adults using the same method (2.8 nmol/L) supporting that age and gender-specific cutoff levels are not required for bedtime salivary cortisol or cortisone. Salivary cortisone and cortisol can be used interchangeably. In addition, exogenous hydrocortisone-contaminated samples can easily be identified by LC-MS/MS because cortisone/cortisol ration is very low.

An overnight suppression test can be performed by administering 25 μg/kg of dexamethasone at 11 p.m./midnight (maximum dose 1 mg) and serum cortisol measurement on the following day at 8 AM (50). Another biochemical test is the 2mg-2days dexamethasone suppression test (DST), which consists in the administration of 20-30 μg/kg/day of dexamethasone (maximum dose 2 mg/day) divided into 0.5 mg doses every 6 h, given at 09.00, 15.00, 21.00 and 03.00 h for 48 h (51, 52). The cortisol cut-off level should be <1.8 ug/dl (50 nmol/L).

Once the diagnosis is confirmed we should distinguish between ACTH dependent and ACTH independent forms, by assaying ACTH levels. If ACTH value is less than 5 pg/mL (1.1 pmol/L), it is strongly indicative of ACTH-independent CS. ACTH levels between 5 and 29 pg/mL (1.1-6.4 pmol/L) can be considered a grey zone, while ACTH values greater than 29 pg/mL (6.4 pmol/L) are strongly suggestive for ACTH-dependent conditions. See diagnostic work-up in Figure 2 (53).

Additional exams are the high dose dexamethasone suppression test (HDDST) which can be performed for the PPNAD diagnosis. Indeed, in patients with PPNAD, HDDST can provide a paradoxical answer, with an increase of UFC/cortisol instead of a reduction. However, the sensitivity of the HDDST is quite low (39%) (12, 54, 55). Recently some studies identified a cut-off of UFC post HDDST/UFC pre HDDST of 1.08 reporting a sensitivity of 84% and a specificity of 75.6% (56). In patients with a high suspicion for PPNAD, the genetic analysis to identify mutations of the PKAR1A could confirm the diagnosis of PPNAD, notably in patients with combined Carney's complex.

Modest elevations in plasma 17-hydroxyprogesterone or urinary 17-OH corticosteroids levels are frequently found in PBAMH (57, 58). In these patients, the 250 mcg ACTH-stimulation test leads to increased plasma cortisol and 17-hydroxyprogesterone levels reflecting the large hyperplastic adrenal glands with relative 21-hydoxylase and other steroidogenic enzyme deficiencies (57, 59). However, the suppressed ACTH levels allow one to distinguish PBAMH from congenital adrenal hyperplasia.

Radiological imaging

Adrenal imaging is essential for the definition of the adrenal CS cause. For the differential diagnosis, an upper abdomen ultrasound, which can be considered the first-line radiological exam, can be performed, followed by adrenal CT or MRI, which can show more clearly a single adrenal tumour or bilateral hyperplasia.

Adrenal CT scan and MRI show higher sensitivity and accuracy than ultrasound and ensure a better definition of fat richness, providing information on size, calcifications and heterogeneity of the lesion, and the anatomical relationship with adjacent organs. A CT scan using 3 mm or less in thickness can reveal micronodules inside the adrenal gland (60). MRI shows several advantages compared to CT scan, including absence of ionizing radiation, capability of imaging multiple planes, and improved tissue contrast differentiation. For this reason, an MR scan should be preferred in patients who need long-term follow-up.

High-resolution 18FDG PET could be useful to distinguish benign from malignant tumours (61, 62). However, an increased uptake of radiotracer has been reported in PBAMH, with an uptake similar to that of a malignant lesion (maximum standardized uptake value > 3.1), reflecting the high glycolytic activity of nodular lesions (63). Single-photon emission computed

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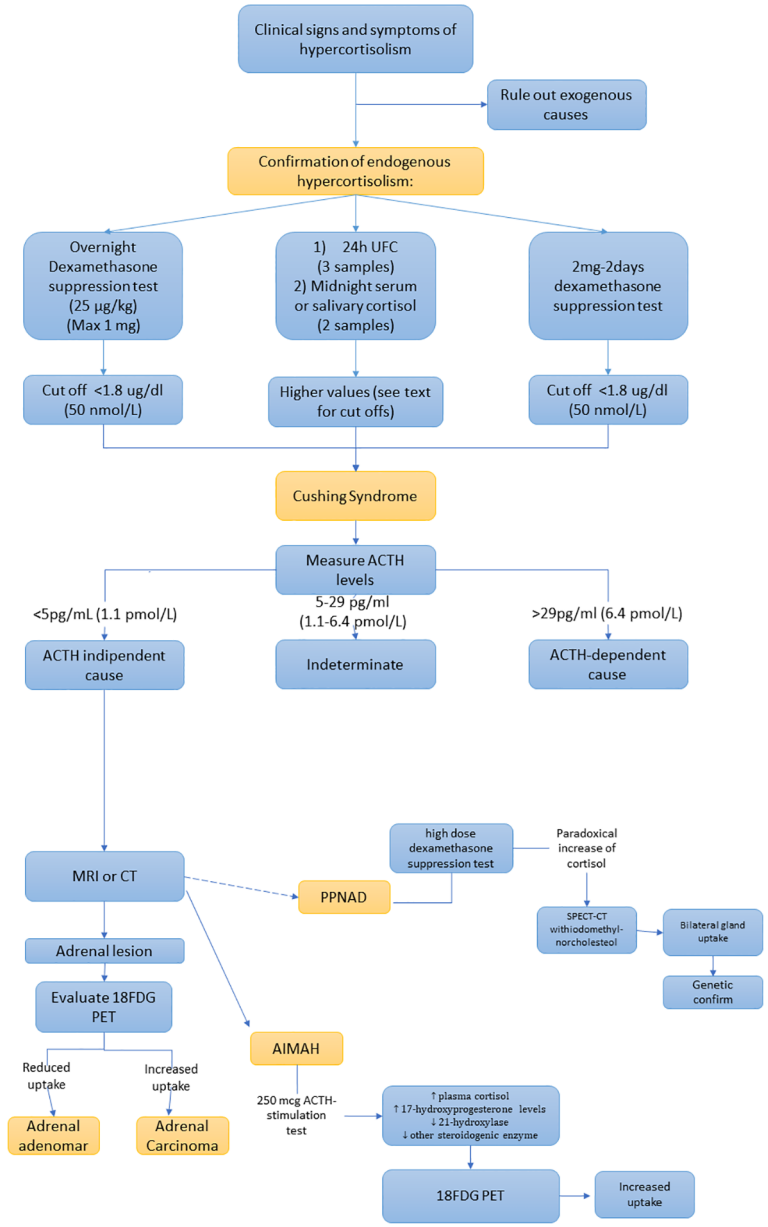

FIGURE 2
Diagnostic work-up of adrenal Cushing's syndrome.

tomography (SPECT-CT) with the iodomethyl-norcholesterol (I-131) radiotracer can be useful in patients with PPNAD, showing bilateral gland uptake (64). However, considering the high radioactive exposure, the use of this method should be reserved for exceptional cases.

Treatment

The therapeutic management of adrenal causes of CS depends on the primary cause. Adrenalectomy is the first-choice treatment. However, when surgery is contraindicated or refused, medical therapy should be recommended.

Unilateral adrenalectomy is indicated for unilateral benign cortisol-secreting adenomas.

Surgery is also the first-line therapeutic approach for adrenocortical cortisol-secreting cancers (10, 65). Contrasting findings are currently available on the usefulness of lymph node dissection (66). Mitotane is often used in adults as adjuvant therapy, but due to its neurotoxicity, in children it should be used with caution (67, 68). A nonrandomized single-arm study showed better survival in those children who achieved a mitotane level greater

than 14 mg/L (69). In patients with unresectable adrenocortical cancers adjuvant chemotherapy could be used. Chemotherapy in children generally includes cisplatin and etoposide with or without doxorubicin combined with mitotane. Despite this multimodality approach, prognosis of paediatric adrenocortical cancer with metastatic disease remains poor, with an estimated 5-year survival below 20% (70, 71).

In cases of adrenocortical cancers with distant metastases, checkpoint inhibitors could be recommended. Notably, some encouraging findings have been reported with pembrolizumab (10, 72).

Bilateral adrenalectomy is strongly recommended in patients with PPNAD (73). Surgery is associated with biochemical remission, growth catch-up, weight loss and improvement of CS phenotype (74). Unilateral adrenalectomy can be associated with remission of symptoms without risk of adrenal insufficiency. However, in some patients recurrence of CS could occur (75, 76).

In patients with PBAMH the suggested treatment consists in surgical removal of one or both adrenal glands, and rarely in specific drugs inhibiting excessive cortisol secretion (77, 78). The best surgical approach is still on debate. Indeed, unilateral adrenalectomy has a remission rate of hypercortisolism reaching 96%, but with a recurrence rate of 23% (75, 79, 80). However, a post operative follow-up is strongly required in order to prevent adrenal insufficiency and CS recurrence (81, 82).

In patients with contraindications or refuse of surgery, drugs inhibiting adrenal cortisol synthesis (ketoconazole, metyrapone and recently osilodrostat) have been used in rare cases (83). At the moment, there are no medications approved by EMA or FDA for use in pediatric CS.

Recently, a child with mild, cyclical CS due to PBAMH, who carries a novel germline pathogenic variant in KCNJ5, was treated at 4 years 9 months with very low doses of ketoconazole (300 mg/day), increased to 400 mg/day at 8 years. She showed improved linear growth and normalization of BMI, along with resolution of behavior changes and normalization of blood pressure. However, the discontinuation of the drug for 6 weeks led to recurrence of CS (84).

Osilodrostat has not yet been approved for pediatric patients, but is currently being evaluated in a small Phase II trial (NCT03708900) in the pediatric population (<18 years) and the results are expected at the end of 2023. Recently, in a 14-year-old male with ectopic CS due to metastatic pancreatic tumor, osilodrostat (18 mg twice daily) was well tolerated and obtained a rapid improvement and normalization of UFC (85).

Glucocorticoid replacement therapy has a significant role in the management of patients with adrenal CS who have undergone adrenalectomy. Generally glucocorticoid replacement therapy should be recommended in the pre- and perioperative periods and after surgery for at least 6 months from the adrenalectomy (1). In patients who have undergone bilateral adrenalectomy, a chronic steroid replacement therapy is required, except for patients who have undergone adrenal sparing surgery with preservation of healthy cortical tissue.

Conclusions

Adrenal CS is a quite rare condition in childhood that occurs more frequently in children aged less than 7 years. Adrenal CS can result from adrenocortical tumours including adenoma or carcinoma or from primary adrenal hyperplasia including PPNAD, PBAMH or McCune Albright syndrome. The clinical presentation can vary according to the age of onset. However, weight gain, clinical hyperandrogenism and growth disorders are the most frequent signs and symptoms. Early diagnosis is essential to improve clinical symptoms and to better manage the primary cause. Indeed, in cases of adrenocortical cancer an early diagnosis is associated with a better survival. Unilateral or bilateral adrenalectomy is the first-line therapeutic approach. In patients who have undergone bilateral adrenalectomy lifelong glucocorticoid replacement therapy is required. The prognosis is generally good, except for patients with adrenocortical cancer, for which it depends greatly on the initial stage.

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Familial Glucocorticoid Deficiency: the changing landscape of an eponymous syndrome

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Familial Glucocorticoid Deficiency encompasses a broad spectrum of monogenic recessive disorders that theoretically solely abrogate cortisol biosynthesis. In reality, delineating clear genotype-phenotype correlations in this disorder is made complicated by marked phenotypic heterogeneity even within kindreds harbouring identical variants. Phenotypes range from isolated glucocorticoid insufficiency to cortisol deficiency plus a variety of superimposed features including salt-wasting and hypoaldosteronism, primary hypothyroidism, hypogonadism and growth defects. Furthermore, mutation type, domain topology and perceived enzyme activity do not always predict disease severity. Given the high burden of disease and implications of a positive diagnosis, genetic testing is crucial in the management of patients warranting detailed delineation of genomic variants including viable functional studies.

KEYWORDS

adrenocorticotropin, steroidogenesis, hypocortisolaemia, zona fasciculata, multi-systemic

1 Introduction

1.1 Historical origins of Familial Glucocorticoid Deficiency

Unsubstantiated reports of Addisonian-like familial disease associated with hyperpigmentation were recorded from as early as 1900 but the disorder first came to prominence in 1959 when Shepard and colleagues described a unique form of hypocortisolaemia in two siblings with preserved mineralocorticoid function (1). Over subsequent decades, the phenotypic spectrum evolved to include a distinct constellation of features including hyperpigmentation, recurrent hypoglycaemia, seizures, high plasma deoxycorticosterone and tall stature (2–9). Initially designated as hereditary

adrenocortical unresponsiveness to adrenocorticotropin (ACTH) (4), this syndrome was eventually termed Familial Glucocorticoid Deficiency (FGD).

1.2 Physiological regulation of cortisol production

Higher brain centres regulate the synthesis of endogenous cortisol through an intricate negative feedback pathway governed by the tropic Corticotrophin releasing factor (CRF) secreted by the hypothalamo-adenohypophyseal portal system (Figure 1). CRF preferentially binds to the CRF type 1 G-protein coupled receptor (CRF-1R) and acts as a secretagogue to potentiate ACTH release from the anterior pituitary. ACTH then acts as an agonist for the melanocortin 2 receptor (MC2R), expressed in adrenocortical cells of the adrenal zona fasciculata, thereby producing cortisol via modulation of adenylate cyclase/protein kinase A signalling. Cortisol mediates the negative feedback loop via the centrally expressed glucocorticoid receptor. Glucocorticoid mediated feedback may be divided into: (i) non-genomic rapid inhibition of glutamate release at the hypothalamic paraventricular nucleus via endocannabinoid synthesis, (ii) genomic prefrontal limbic regulation and, (iii) destabilization of hypothalamic pituitary adrenocortical axis-activating neuropeptide mRNA (10). Loss of feedback regulation forms the basis of ACTH resistance syndromes of which defects in *MC2R* were the first to be elucidated.

2 Familial glucocorticoid deficiency (GCCD1/FGD Type 1) – melanocortin-2 receptor (*MC2R*) defects (OMIM #202200)

The ACTH receptor is a seven transmembrane domain receptor encoded by the *MC2R* gene, which maps onto the short arm of chromosome 18 and consists of 2 exons, the first of which is non-coding/untranslated whilst the latter encodes the full sequence of the receptor. Following isolation and sub-cloning of the human ACTH receptor in 1992 (11), the first reported kindreds with primary adrenal insufficiency secondary to defects in *MC2R* were characterised by Clark et al. and Tsigos et al. in 1993 (12, 13). Since these initial reports, around 48 mutations have been described in association with FGD (Human Gene Mutation Database http://www.hgmd.cf.ac.uk) (14), the majority of which are missense/nonsense variants (Figure 2) in addition to small genomic deletions

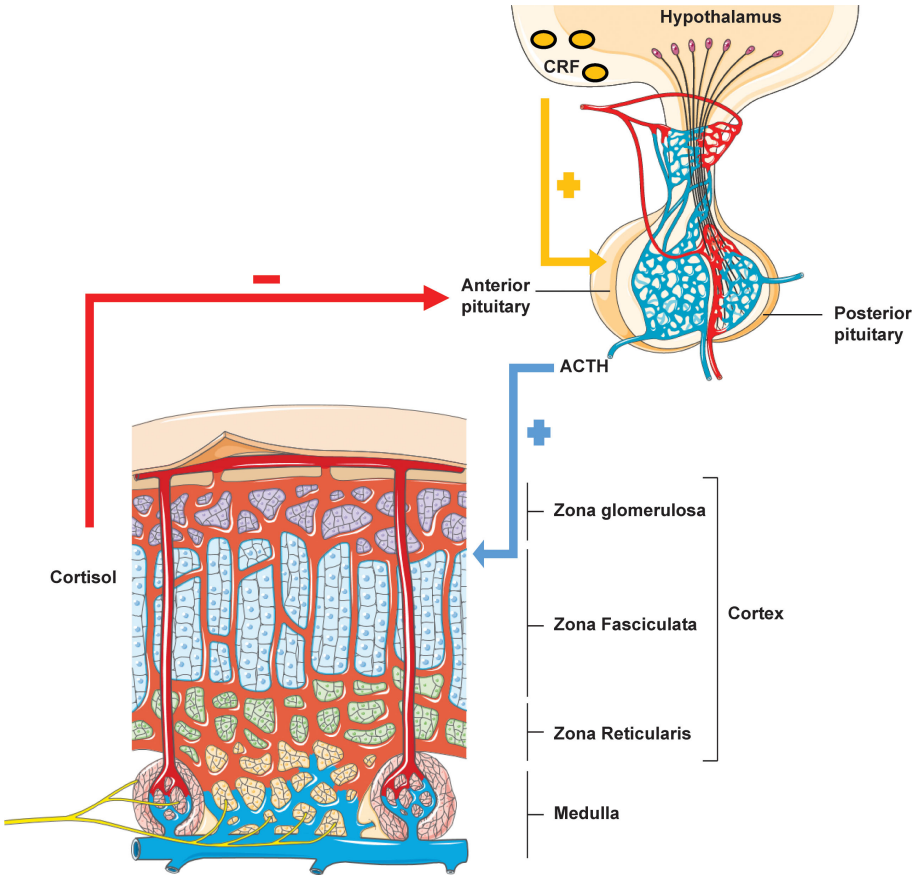

FIGURE 1
Hypothalamic-pituitary-adrenal axis. Rhythmicity of the HPA axis is driven by hypothalamic tropic factor CRH which stimulates anterior pituitary ACTH production. ACTH acts on the adrenal zona fasciculata to mediate glucocorticoid biosynthesis. Cortisol itself then exerts feedback inhibition of both ACTH and CRH via a negative feedback loop.

and insertions. Variants in *MC2R* account for about 25% of FGD cases, the majority of which are non-conservative, single amino acid substitutions that likely abrogate cyclic adenosine monophosphate (cAMP) generation and impair receptor trafficking (17–19).

Classically in FGD Type 1, patients are mineralocorticoid replete however, in clinical practice, the picture may be less unambiguous. Several reports have alluded to mild disruptions of the renin-angiotensin-aldosterone axis in patients with *MC2R* defects, in whom early glucocorticoid replacement may mask aldosterone insufficiency (20, 21). Furthermore, several patients with homozygous frameshift truncating variants have presented in the neonatal period with transient salt wasting (20). Interestingly *Mc2r*$^{-/-}$ mice demonstrate reduced serum aldosterone levels (22). This disparity may reflect the fact that the majority of human subjects harbour missense mutations that retain some degree of enzyme activity. Patients also invariably present with early and significant hyperpigmentation due to the action of markedly increased ACTH on the Melanocortin 1 receptor (*MC1R*). This association was clearly demonstrable in a patient with FGD harbouring homozygous missense variants in both *MC2R* and *MC1R* and lacking the 'classic' hyperpigmented phenotype (23).

An often overlooked but increasingly recognised feature of FGD Type 1 is hypothyroidism (18, 24–29). Patient phenotypes range from transient neonatal hypothyroidism that normalise with short term thyroxine replacement to subclinical hypothyroidism and persistent thyroid hypo-function (28, 29). Despite assertions that elevated ACTH levels may inhibit thyroid stimulating hormone release (30), the exact mechanism underlying the incidence of hypothyroidism in patients with FGD remain to be elucidated. Interestingly, another feature recognised in patients with ACTH receptor defects is reduced adrenal androgen production or lack of adrenarche. Weber et al. demonstrated consistently sub-optimal serum DHEAS levels in 6 patients with *MC2R* variants (31). This model of 'functional' ACTH deficiency suggests that ACTH at least partially regulates adrenarche given that patients with central ACTH deficiency (hypopituitarism) also exhibit low levels of adrenal androgens (31–34).

Tall stature is an inconsistent but historically common feature of FGD type 1 despite an unaffected Growth hormone-IGF-1 axis (35). Although the exact mechanism underlying this phenotype is unclear, it is likely theorised to be due to the sustained effect of markedly elevated Adrenocorticotropin levels on the growth plate (35, 36). This also correlates to a later median age at diagnosis (2.0 years) when compared to FGD Type 2 (37). ACTH has been shown *in vitro* to increase rat chondrocyte progenitor cell proliferation and matrix production (38). Interestingly, growth trajectories return to normal once glucocorticoid treatment is instituted. *Mc2r* knockout mice do not recapitulate this trait and demonstrate similar body lengths to wild-type littermates (22). There is however little genotype phenotype correlation and disease severity and onset is highly variable (39).

3 Familial glucocorticoid deficiency (GCCD2/FGD Type 2) – melanocortin-2 receptor accessory protein (*MRAP*) (OMIM #607398)

After initial characterisation of loss of function defects in *MC2R* as a primary cause of FGD, it became apparent that other genetic

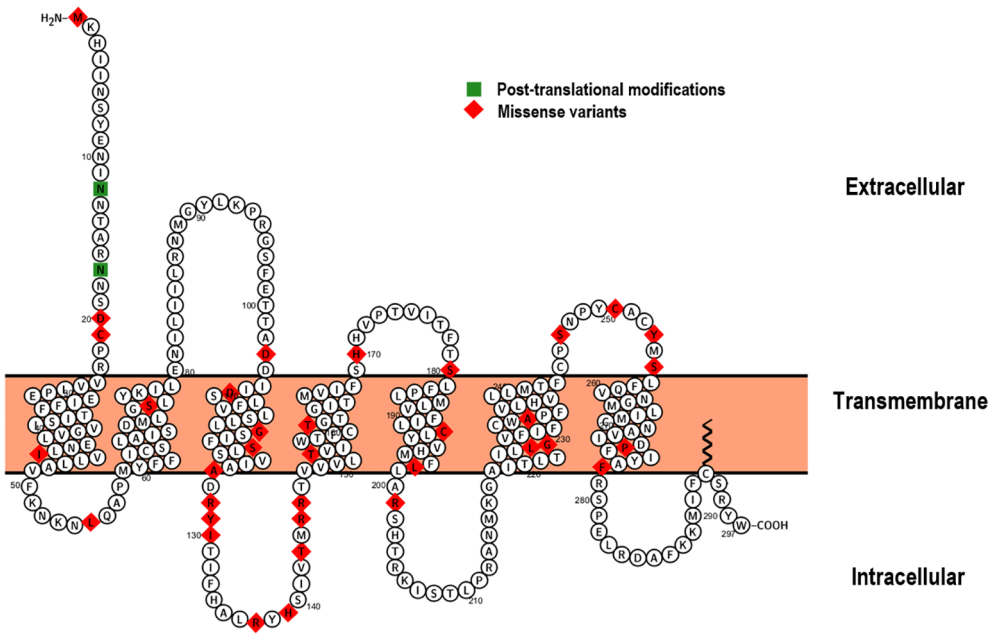

FIGURE 2
Proteoform structure of the ACTH receptor (15). The topological (extracellular, transmembrane and cytoplasmic) domains are outlined and sites of missense variation highlighted (red). The majority of *MC2R* variants causing FGD Type 1 are associated with defective receptor trafficking and mainly occur within the transmembrane domains (16). Sites of post-translational modifications are indicated in green.

factors were involved in pathogenesis of FGD given the existence of several families with an isolated adrenal insufficiency phenotype and negative *MC2R* genomic screening. *In vitro* expression studies by Noon et al. revealed that a fluorescent tagged *MC2R* cDNA clone was able to traffic to the cell membranes of adrenocorticotropin resistant murine adrenocortical tumour cells but remained restricted to the endoplasmic reticulum in non-adrenal cells. This suggested that an adrenal derived co-factor was necessary for MC2R expression (40, 41) (Figure 3).

In 2005, Metherell et al. conducted autozygosity mapping in a single family in which runs of homozygosity identified a 2.2 Mbp region. Within this region, the adrenally expressed chromosome 21 open reading frame 61 (*C21orf61*) gene previously encoding a fat-associated low-molecular-weight protein, was identified. Variants in this gene, retitled the melanocortin 2 receptor accessory protein (*MRAP*), were subsequently identified in several families with FGD (42). Variants in *MRAP* (FGD Type 2) account for 20-25% of FGD cases, with around 15 mutations being described since initial characterisation of this gene (37, 42–45). The majority of variants are splice site/nonsense that ultimately lead to a truncated and non-functional receptor. Patients generally present at an earlier age (median age of 0.08 years at diagnosis) with normal stature when compared to subjects with *MC2R* variants (37, 45, 46). This suggests reduced exposure to the unfettered actions of ACTH on the growth plate seen in FGD Type 1. *Mrap*$^{-/-}$ mice phenocopy the isolated glucocorticoid deficiency and normal mineralocorticoid function of human subjects. Interestingly, the adrenals from knockout mice are small with indistinct cortical zonation and dysregulated accumulation of WNT4/β-catenin (47).

4 Partial loss of function mutations in steroidogenic acute regulatory protein (*STAR*) and cytochrome P450 side chain cleavage enzyme (*CYP11A1*)

4.1 *STAR* (GCCD3, OMIM #609197)

Defects in *STAR* disrupt steroidogenesis globally resulting in classic congenital lipoid adrenal hyperplasia (CLAH). Patients invariably present with hyper-reninaemic hypoaldosteronism in the setting of hypocortisolaemia, enlarged adrenals due to progressive lipid deposition and gonadal insufficiency. A two hit model has been proposed to account for pathogenesis of CLAH (Figure 4); the first 'hit' being lack of STAR and the second being

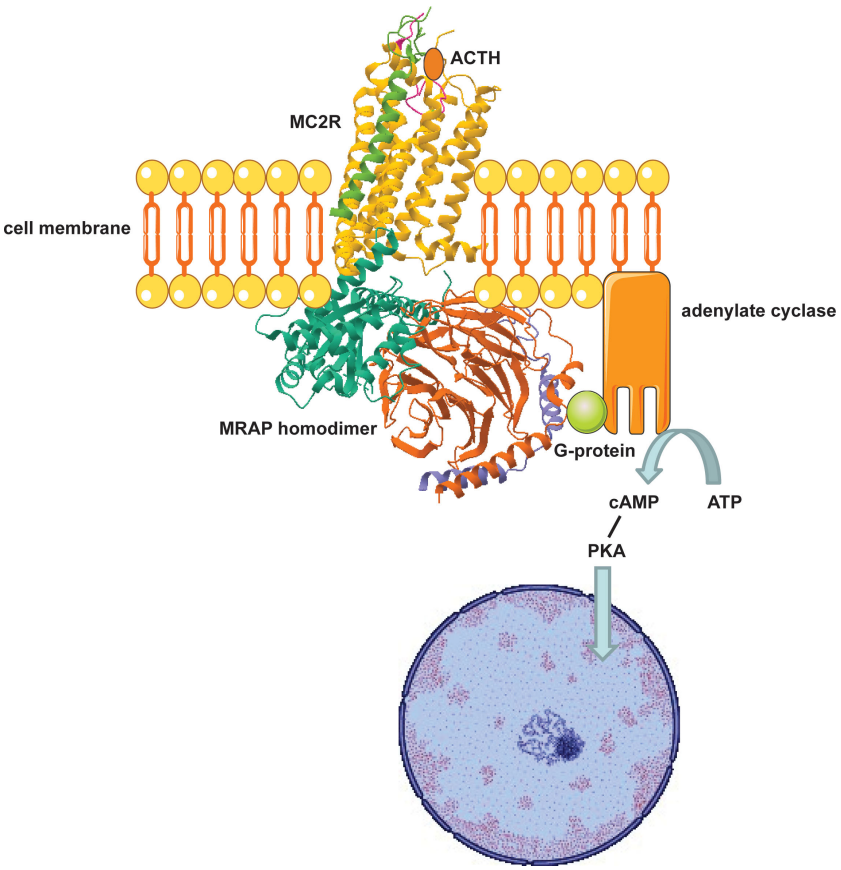

FIGURE 3
Adrenocortical cell surface MC2R-MRAP complex mediates glucocorticoid biosynthesis. MRAP traffics MC2R to the cell surface where ligand binding (ACTH) induces a G-protein coupled increase in cAMP which phosphorylates Protein Kinase A (PKA). PKA then induces transcription of specific intra-nuclear factors that mobilise STAR activity and expression of enzymes involved in steroidogenesis.

cholesterol mediated oxidative damage. In STAR deficient adrenocortical and testicular Leydig cells, steroidogenesis is markedly diminished with the exception of minimal residual STAR-independent steroid biosynthesis. The primary biosynthetic defect leads to compensatory increases in ACTH and LH which promote LDL receptor mediated cholesterol uptake and *de novo* synthesis. Progressive lipid accumulation leads to mitochondrial oxidative damage and cellular stress ultimately abolishing residual STAR-independent steroidogenic capacity (48, 49). Early fetal destruction of Leydig cell integrity in 46,XY subjects leads to lack of testosterone and feminized external genitalia (50). The fetal ovary, on the contrary, is relatively preserved until puberty when gonadotrophin stimulation leads to cholesterol accumulation.

In some patients, mutations in *STAR* are associated with retention of up to 20% of wild type activity and a mild phenotype. These partial loss of function variants result in non-classic CLAH (NCLAH), often indistinguishable from FGD due to an isolated adrenal phenotype (51–55). Age at presentation is highly variable, ranging from 18 months to adulthood (51, 56). NCLAH cortisol deficient patients exhibit normal external genitalia and consonant pubertal development however, primary gonadal failure may occur progressively over time (51, 53).

Expression of a mutant Star protein (N47-StAR) in knockout mice ($Star^{-/-}$) gave rise to a partial loss of function phenotype. When compared to wild type transgenic mice, $Star^{-/-N47Tg}$ mice had lower basal and stimulated corticosterone levels. $Star^{-/-N47Tg}$ mice had normal external genitalia but exhibited progressive gonadal insufficiency with aging (57).

4.2 Cholesterol side-chain cleavage enzyme, P450scc (*CYP11A1*) (OMIM #613743)

CYP11A1, a mitochondrial monooxygenase regulates placental production of progesterone required for maintenance of gestational viability (48, 58). Absolute deficiency of this enzyme was previously thought to be incompatible with term gestation i.e. embryonically lethal, however, several patients with side chain cleavage enzyme deficiency have been reported. It is postulated that in these instances, persistence of the corpus luteum throughout pregnancy compensates for the placentation defect (59). Severe loss of function mutations in *CYP11A1* produce a phenotype not dissimilar to that of CLAH due to *STAR* deficiency. Primary adrenal failure in combination with 46, XY sex reversal characterise these patients although unlike *STAR* deficient subjects, adrenal size is unaffected.

However, like *STAR*, partial loss of function mutations can produce a phenotype consistent with non-classic CLAH (60). Furthermore, partial inactivating mutations in *CYP11A1* have been implicated in development of an isolated glucocorticoid deficiency. Parajes et al. (2011) described a homozygous mutation (p.R451W) in two 46, XY male siblings who presented with primary

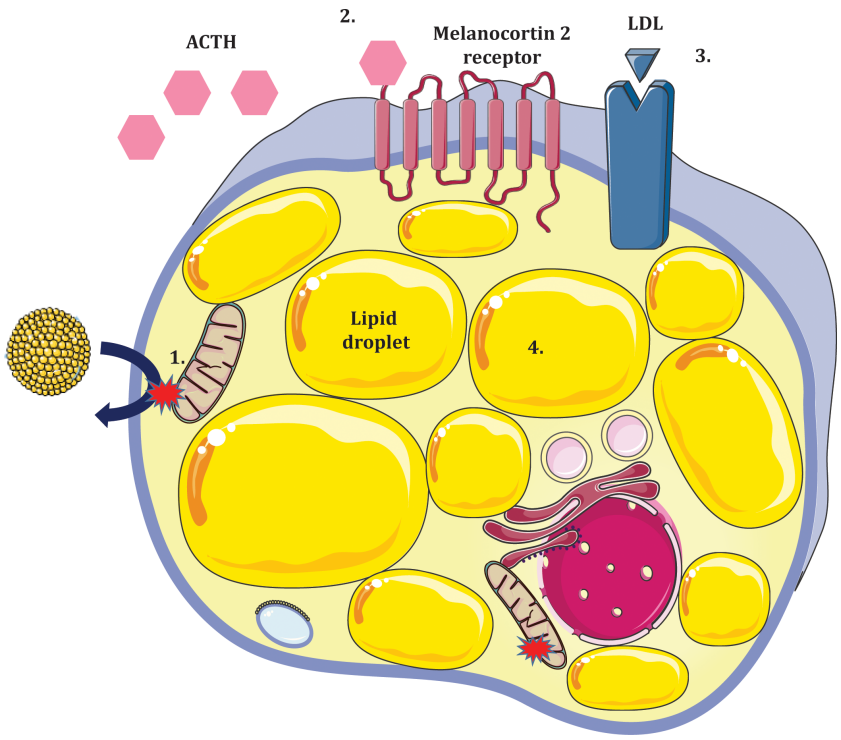

FIGURE 4
Two 'hit' model of lipoid congenital adrenal hyperplasia due to deficiency of STAR in adrenocortical cells. 1. Absence of STAR prevents cholesterol import to the inner mitochondrial membrane (first hit). Some residual STAR-independent steroidogenesis occurs but relative hypocortisolaemia ensues. 2. Loss of negative feedback to the anterior pituitary results in increased ACTH production. 3. Steroidogenic stimuli promote expressivity of LDL receptors which increase adrenocortical cholesterol uptake. 4. Accumulation of intracellular lipids causes oxidative damage and cellular stress (second hit) further impeding steroidogenic potential leading to hyperplastic adrenals.

adrenal insufficiency and normal external genitalia without hypogonadism (61). Unlike severe enzyme deficiency which presents within the first 10 days of life, partial loss of function variants in *CYP11A1* are associated with later ages of onset ranging from 6 months to 15 years (62–65).

Maharaj et al. (64) described a heterozygous missense variant (rs6161, c.940G>A, p.Glu314Lys) in *CYP11A1*, previously designated benign, in a cohort of 19 probands (13 families) with isolated glucocorticoid deficiency. The variant occurred *in compound heterozygosity* with a second gene disrupting allelic hit in *CYP11A1* in 17 probands and in combination with two synonymous single nucleotide variants (p.Thr330= and p.Ser391=) in two probands. *In vitro* splicing assays demonstrated exon skipping due to aberrant splicing for these three variants. The impact of the rs6161 variant on splicing was further corroborated by several subsequent groups (66–69).

Cyp11a1$^{-/-}$ mice do not survive weaning and demonstrate marked corticosterone and aldosterone deficiency when compared to wild-type litter mates. Interestingly, corticosteroid administration prolonged survival until adulthood. *Cyp11a1*$^{-/-}$ XY males exhibit sex reversal with feminization of external genitalia and disorganization of internal genitalia. Similar to human counterparts, knockout adrenals are small however they demonstrate progressive lipid droplet accumulation (70).

5 FGD-like syndrome due to altered cellular redox status (*NNT*, *TXNRD2*)

5.1 Nicotinamide nucleotide transhydrogenase (*NNT*) (GCCD4, OMIM #614736)

Single nucleotide polymorphism genotyping and targeted exome sequencing of consanguineous kindreds with adrenal insufficiency identified variants in the gene encoding the inner mitochondrial membrane enzyme, nicotinamide nucleotide transhydrogenase (*NNT*) (71). The initial 15 probands characterised by Meimaridou et al. presented with a classical FGD phenotype indistinguishable from mutations in *MC2R* and *MRAP*. Patients were diagnosed before age 40 months with biochemical evidence of marked hypocortisolaemia, elevated ACTH and normal renin and aldosterone levels. A significant number of patients (8 out of 15) presented with sequelae of hypoglycaemia. A further study noted that some patients were mineralocorticoid deficient highlighting a degree of phenotypic variability (46, 72).

Lentiviral knockdown of *NNT* in an H295R (adrenocortical cancer) cell line demonstrated increased mitochondrial reactive oxygen species generation in the knockdown condition with a lowered reduced glutathione (GSH) to oxidized glutathione (GSSG) ratio. This was indicative of an altered cellular redox balance with subsequent perturbations in adrenal steroidogenesis (71, 73). A reduced GSH : GSSG ratio has also been seen in other *in vitro* models of adrenal disease where oxidative stress is implicated in the pathogenic mechanism, including *TXNRD2* and *AAAS*-KD H295R adrenocortical cell lines (74, 75). Similarly, metabolic profiling of glucocorticoid deficient *fdx1b*$^{-/-}$ zebrafish revealed significant alterations to glutathione metabolism and biochemically lowered GSH to GSSG ratios in ferredoxin null zebrafish larvae (76).

Heterozygous loss-of-function *NNT* mutations have been linked to left ventricular non-compaction (LVNC) in two probands and their families. *Nnt* deficient zebrafish cardiomyocytes demonstrated reduced proliferation and contractility leading to cardiac oedema. *In vivo*, co-injection of wild-type human *NNT* mRNA was able to rescue the cardiac oedema phenotype whilst mutagenized human *NNT* ORF constructs failed to rescue morpholinos-induced cardiac dysfunction (77). Roucher-Boulez et al. subsequently identified a novel homozygous *NNT* mutation (p.R379*) in a single patient who exhibited features of glucocorticoid insufficiency with progressive left ventricular hypertrophy (78).

C57BL/6J mice possess an intrinsic inactivating mutation in *Nnt* resulting in an untranslated protein. Interestingly, C57BL/6J *Nnt*-null mice do not develop cardiomyopathy (79) but they do have a lower adrenal reserve with attenuated corticosterone levels, both basally and following ACTH provocation. Adrenal histology revealed a markedly disorganized zona fasciculata with increased apoptosis (71). Mitochondria isolated from *Nnt* null mice exhibited an increased oxidized/reduced glutathione ratio and impaired ability to metabolize organic peroxide suggesting that loss of *Nnt* leads to redox imbalance (80). At protein level, *Nnt* null mice exhibited a 65% reduction in expression of *Cyp11a1*, which catalyses the rate limiting step of steroidogenesis (73).

5.2 Thioredoxin reductase 2 (*TXNRD2*) (GCCD5, OMIM #617825)

Amongst the genetic causes of FGD, *TXNRD2* is perhaps the most enigmatic. Until recently, only one consanguineous kindred was known to harbour a deleterious homozygous variant in *TXNRD2* in association with a phenotype of glucocorticoid insufficiency. In 2014, Prasad et al. characterised 7 individuals from a Kashmiri kindred who were found to have a stop gain variant, p.Y447*, associated with loss of TXNRD2 (74, 81). The age of presentation was highly variable ranging from 0.1 to 10.8 years (74). Of the glucocorticoid deficient family members genotyped, only one had co-morbid heart defects precipitating cardiac failure; cardiomyopathy being a recognised feature of *TXNDR2* haploinsufficiency (82).

Sibbing et al. identified two heterozygous mutations in *TXNRD2* (p.G375R and p.A59T) in 3 individuals with dilated cardiomyopathy (DCM). When cell survival was used as a marker of *Txnrd2* function, neither mutant construct was able to rescue *Txnrd2* function in *Txnrd2*$^{-/-}$ GSH depleted mouse embryonic fibroblasts in contrast to a wild type construct (83).

In 2022, a new study highlighted a novel homozygous missense variant in *TXNRD2* (c.1081G>A, p.V361M) in a proband with glucocorticoid and gonadal insufficiency but normal cardiac

function (84). This adds credence to the impact of TXNRD2 as a player in the pathogenesis of isolated glucocorticoid deficiency and further broadens the phenotypic continuum of this disorder.

6 FGD-like syndrome due to defective DNA replication (Minichromosome maintenance 4, *MCM4*) (OMIM #609981)

In 2012, dual reports of partial *MCM4* deficiency in 14 probands from the Irish Traveller population highlighted a unique syndrome that encompassed adrenal insufficiency, intrauterine and postnatal growth restriction, microcephaly and natural killer cell (NK) deficiency (85, 86). The variant highlighted (and to date, the only variant associated with this disorder) was a homozygous splice site substitution c.71-2A>G leading to a single base cDNA insertion c.70_71insG and frameshift truncation, p.P24Rfs*4. Unlike other forms of FGD, the adrenal phenotype was relatively mild with onset of adrenal insufficiency often in late childhood. In healthy control derived peripheral lymphocytes, two MCM4 isoforms are detectable at 96KDa and 85KDa. In patient cells, only the minor isoform is present. This smaller protein is touted to lack the N-terminal MCM4 domain and may partially rescue patient phenotype (85, 86).

Complete loss of *Mcm4* in mice is lethal. Hypomorphic $Mcm4^{Chaos3/-Mcm3+/-}$ mice are viable and demonstrate abnormal adrenal morphology. The adrenal capsule is thinned with significant numbers of non-steroidogenic *Cyp11a1*/*Cyp11b1* negative cells that are on the contrary, Gata-4 and Gli1-positive. This remodelled adrenal cortex demonstrates a lack of steroidogenic output in keeping with human disease (85, 87).

7 Disorder of sphingolipid metabolism due to SGPL1 deficiency (OMIM #617575) leading to primary adrenal insufficiency and steroid resistant nephrotic syndrome

Until recently, inborn errors of sphingolipid metabolism due to single enzyme defects within the sphingolipid pathway have been characterised by their predominantly neurological phenotype. Sphingosine-1 phosphate lyase insufficiency syndrome (SPLIS) due to defects in *SGPL1*, which coordinates the final degradative step in the sphingolipid pathway, is a newly described multi-systemic disorder, in which adrenal failure features prominently (88–90).

Sphingolipid synthesis involves a series of tightly regulated, enzyme-catalysed steps that initiate in the endoplasmic reticulum from non-sphingolipid precursors to biosynthesis of higher order complex glycosphingolipids within the Golgi apparatus. Despite the diversity within the biosynthetic pathway, sphingolipid metabolism begins with a common entry point and exit via a single degradative pathway. This common initial step involves the coupling of cytosolic serine and palmitoyl CoA to 3-ketodihydrosphingosine through the action of serine palmitoyltransferase (*SPT*) whilst *SGPL1* executes the penultimate step of the metabolic pathway, catalytic cleavage of sphingosine-1 phosphate (S1P), into 2*E*-hexadecanal and phosphoethanolamine (91, 92). *SGPL1* is the major modulator of S1P signalling (93). Under normal physiological conditions, S1P is largely pro-proliferative, suppressing the pro-apoptotic actions of ceramide however loss of function mutations in *SGPL1* result in a pathological accumulation of S1P which studies have shown to be associated with induction of apoptosis.

In 2017, Prasad et al. (88) described loss of function human mutations in *SGPL1* and a novel syndrome characterised by primary adrenal insufficiency. Extra-adrenal phenotypic features included steroid-resistant nephrotic syndrome, hypothyroidism, ichthyosis, neurodevelopmental delay, hypogonadism and lymphopenia. Mass spectrometric analysis of plasma sphingolipids in one patient and heterozygous parents revealed elevated ceramides and S1P levels when compared to age and sex matched controls suggesting that the underlying multi-system pathology in these patients may be due to organ-specific cytosolic accumulation of sphingolipid intermediates. Correspondingly, Lovric et al. described a cohort of 7 families who were found on next generation sequencing to harbour 9 unique, recessive mutations in *SGPL1* (89). Several studies have subsequently corroborated these initial findings and expanded the phenotypic spectrum to include microcephaly, sensorineural deafness, and progressive neurological deterioration (94). A further neurological phenotype has been described in siblings bearing compound heterozygous loss of function mutations in *SGPL1*, involving axonal mononeuropathy giving rise to Charcot Marie Tooth-like disease (95).

$Sgpl1^{-/-}$ mice exhibit early postnatal mortality but those that survive demonstrate disrupted adrenal morphology. Adrenocortical zonation is disordered and cells of the zona fasciculata have reduced expression of steroidogenic enzymes and contain fewer lipid droplets when compared to wild type mice (88). Electron microscopy of $Sgpl1^{-/-}$ kidneys demonstrated foot process effacement and absent slit diaphragms (89). Tamoxifen-inducible *Sgpl1*-ablated ($SPL^{Flox/Flox}$ Cre+) mice with partial lyase deficiency, interestingly, demonstrated glomerulopathy with progressive proteinuria and markedly increased intra-renal S1P levels (96). These mice additionally showed dermal irritation and hyperkeratosis whilst other phenotypic features of *Sgpl1* silencing were less evident suggesting that some degree of wild type activity may be protective against developing multi-systemic disease.

8 Conclusion

From initial descriptions of defects in the ACTH receptor to disorders of sphingolipid metabolism, the genomic landscape of FGD has dramatically transformed over the last three decades.

Pending the discovery of novel genes implicated in pathogenesis of glucocorticoid deficient disorders, the likelihood of oligogenic inheritance is augmented in the diagnosis of unsolved FGD cases. One report suggests that digenic, tri-allelic inheritance of variants in both *STAR* and *CYP11A1* account for an isolated case of adrenal failure (97). Oligogenic heterozygosity is increasingly pertinent particularly in cases of haplotype ambiguity. Given the increasing accessibility of next generation sequencing techniques, the heritability of adrenal insufficiency-related phenotypes may be more easily uncovered allowing earlier patient intervention with significantly disease modulating impacts.

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Conflict of interest

The author declares 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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Paediatric Cushing's disease: long-term outcome and predictors of recurrence

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Paediatric Cushing's disease (CD) is characterized by excess ACTH secretion from a pituitary adenoma, leading to hypercortisolism. It has approximately 5% of the incidence of adult CD and is a rare disorder in the paediatric age range. The four most specific presenting features of hypercortisolism are: change in facial appearance, weight gain, decreased linear growth and virilisation shown by advanced pubic hair for the stage of breast development or testicular volume. The main diagnostic priority is the demonstration of hypercortisolism followed by distinction between its ACTH-dependent and ACTH-independent origin, thus leading to identification of aetiology. All treatment options aim to resolve or control hypercortisolism. Consensus favours transsphenoidal (TSS) pituitary surgery with selective removal of the corticotroph adenoma. TSS in children with CD is now well established and induces remission in 70-100% of cases. External pituitary radiotherapy and bilateral adrenalectomy are second-line therapeutic approaches in subjects not responding to TSS. Long-term medical treatment is less frequently adopted. Recurrence in paediatric CD cases is low with factors predicting relapse being higher post-TSS cortisol and ACTH levels and rapid recovery of the hypothalamic-pituitary-adrenal axis after TSS. In summary, complete excision of the microadenoma with histological and biochemical evidence for this, predicts a low rate of recurrence of CD. Due to the need for rapid diagnosis and management to avoid the burden of prolonged exposure to hypercortisolism, tertiary university centres comprising both paediatric and adult endocrinology specialists together with experienced pituitary surgery and, eventually, radiotherapy units are recommended for referral of these patients.

KEYWORDS

Cushing's, pituitary adenoma, transsphenoidal surgery, pituitary radiotherapy, recurrence

Introduction

Cushing's disease (CD), characterised by hypercortisolism due to excess ACTH secretion by a pituitary adenoma, is essentially an adult disorder which occasionally presents in children (1, 2). Its incidence in the paediatric age range is considered to be approximately 5% of that seen in adults (3). Consequently, a paediatric endocrinologist is

likely to see only a few cases during their career, which strengthens the case for specialist university centres, staffed by both paediatric and adult endocrinologists, neurosurgeons specialising in transsphenoidal pituitary surgery, and specialised pituitary radiotherapists, to be the optimal institutions for the care of these patients. There is no doubt that collaboration and cooperation between paediatric and adult endocrinologists in the components of clinical management is beneficial to patient care (4).

Because the morbidity of hypercortisolism, also named Cushing's syndrome (CS), in children is serious, early and rapid investigation is indicated in suspected cases. Two main goals dominate the investigational process. First, it is essential to confirm or exclude the presence of endogenous CS. Secondly, the etiologic cause of CS needs to be defined. Cushing's syndrome can be divided into two main aetiological groups, namely ACTH-dependent and ACTH-independent CS: the first group comprises CD and ectopic ACTH syndrome, whereas the second one comprises adrenal cortical neoplasms, both benign and malignant, and adrenocortical hyperplasia. Exogenous glucocorticoid administration should be ruled out in the early phases of the diagnostic work-up to avoid needless investigations.

Clinical presentation of paediatric CD

There are four key presenting features which are currently recognised to have a relevant diagnostic value (Table 1), whereas other features tend to be non-specific and are therefore less reliable in suspecting CD in children. These four key diagnostic features are; change in facial appearance with rounding of the face; weight gain, which is almost universal; retardation of linear growth, which may or may not lead to clinical short stature (i.e. height <-2 SDS); and increased virilisation (4). Although being present in 100% of cases in an English series of 41 patients (7), the change in facial appearance usually occurs slowly over several months or years, thus it may not be recognised as being pathological by parents or family doctors. In the same English series, weight gain was present in 98% of cases, and it usually leads to a marked increase in few months, thus being more easily recognized as a pathological sign by patients' relatives. Growth retardation was present in all cases where low height velocity was documented, but actual short stature was reported only in 42% (10) and 56% of cases (6) in two large paediatric CD series, so short stature should not be considered as a CS sign *per se* (11). Virilisation presents as a disharmony of secondary sexual characteristics with Tanner stage pubic hair growth inappropriately advanced compared to breast development or testicular volume (12), although it may not be easy to identify in patients which are already in pubertal age. Other features which are less specific, but nevertheless important, include osteopaenia, hirsutism, mood changes, headache, striae, hypertension, acne, and pubertal delay (4). Bone age is usually within the normal range and not significantly decreased despite the short stature. Increased adrenal androgen secretion may contribute to this (12). Hypertension was common at diagnosis, being present in 36 -71% of cases (Table 1). Growth hormone responses to stimulation are usually normal at presentation, however gonadotrophin deficiency is a complication of long-term hypercorticism and the combination of decreased testicular volume or decreased breast development and advanced pubic hair growth is suggestive of Cushing's syndrome (12).

Diagnostic investigations

Clinical skills remain important, and the history must exclude administration of exogenous glucocorticoids. Height and weight measurements, pubertal development using Tanner's criteria and bone age assessment should also be performed. Confirmation of hypercortisolism can be assessed using three tests (4); 24-h urinary-free cortisol, late-night sleeping salivary/serum cortisol, and dexamethasone testing. None of these tests has 100% diagnostic accuracy, so multiple tests are usually required to confirm hypercortisolism. Among them, late night serum cortisol had the highest sensitivity and specificity in children (13, 14).

Confirmation of aetiology

ACTH-dependent CS needs to be differentiated from ACTH-independent CS. First, hormonal investigations should be performed. In CD, morning plasma ACTH is typically detectable (>5 pg/ml) compared with suppressed values in primary adrenal disorders (13). The CRH stimulation test may also be useful but is not widely available. Patients with excess ACTH-secreting pituitary adenomas typically give an exaggerated response to CRH, resulting in anelevated cortisol response (15). Second, imaging techniques are required. Pituitary MRI is the optimal method for pituitary visualisation and should be performed after hormonal confirmation of ACTH-dependent CS in patients with suspect CD. However, MRI was only able to positively identify an adenoma in 16-71% of CD cases and in an English series of 41 patients appearances were consistent with a microadenoma in 55% (21/38) of paediatric patients compared with 76% (50/66) of adult patients *(P=0.045)* (7).

Although a positive MRI is beneficial for adenoma identification and supports the diagnosis of CD, the relatively low prediction rate requires a more precise localisation technique, the bilateral inferior petrosal sinus sampling (BIPSS). BIPSS with CRH stimulation (1 μg/kg, max 100 μg/kg) has been suggested in paediatric patients with a negative MRI and confirmed hypercortisolism (16). The best practical organisation is for the BIPSS procedure to be performed by a radiologist who has extensive experience of this investigation in adults with CS. The aim of this investigation is to demonstrate excess central ACTH secretion, thereby excluding ectopic ACTH syndrome, which remains very rare in children.

Treatment and therapeutic outcome

The overall opinion of expert adult endocrinologists treating Cushing's disease is that the treatment of choice is removal of the

TABLE 1 Frequency of clinical findings at diagnosis of paediatric Cushing's disease.

	Devoe 1997 (5)	Shah 2011 (6)	Storr 2011 (7)	Lonser 2013 (8)	Guemes 2016 (9)
Total number of patients	42	48	41	200	16
Mean age/median age (range)	13.4 y[a] (6.5-18)	14.85[b] ± 2.5 y (9-19)	12.3 [b] ± 3.5 y (5.7-17.8)	10.6 [b] ± 3.6 y (4-19)	10 y[a] (7-15.5)
Clinical symptoms and signs (%)					
Weight gain	92	98	98	93	94
Growth retardation	84	83	100	63[c]	63
Short stature		56			
Facial changes	46	98	100	63	
Irregular menses (females)				49[d]	
Osteopaenia	74				
Fatigue or weakness	67		61	48	38
Hirsutism	46		59	56	38
Virilization			76		
Psychiatric disorders	44[e]			31[f]	
Mood changes		46	59[g]		44[h]
Headache	26		51	38	
Striae	36	58	49	55	44
Hypertension	63	71	49	36	50
Acne	46		44	47	50
Pubertal delay or arrest	60				19
Early secondary sexual development					31
Easy bruising	28	17		25	19
Dorsal cervical or supraclavicular fat pad	28			69	
Hyperpigmentation					13
Acanthosis nigricans		75		32	
Muscle weakness		48			
Sleep disturbances					19
Glucose intolerance or diabetes		25		7	
Bone fractures				4	
Hypokalaemia					6
Infection		15			

Y, years; a median age; b mean age; c pre-pubertal patients (n = 91) showed growth retardation in 85%of cases, post-pubertal patients (n = 109) showed growth retardation in 44% of cases; d primary or secondary amenorrhea; e compulsive behaviour; f depression, anxiety, mood swings; g emotional lability/depression; h mental changes changes/poor school performance.

microadenoma by transsphenoidal pituitary resection (TSS) (17). This neurosurgical approach was developed in the 1980s and has now become the standard therapy for Cushing's disease. TSS can be very challenging in children because the microadenomas are often extremely small and difficult to locate and remove, as well as due to the specific features of skull base in paediatric patients, including anatomy of the sellar region varying with age, variable pneumatisation of the sphenoid bone according to age, reduced inter-carotid distance in younger children, and high frequency of anatomic variants, namely shorter nare-sellar and vomer-clivus distances and smaller transsphenoidal angles (18). Total excision of a corticotroph microadenoma results in immediate post-operative ACTH and cortisol deficiency (19). The histological appearance of normal corticotroph cells surrounding the adenoma are morphologically abnormal, the appearance being known as Crooke's change.

Definition of success of transsphenoidal surgery

There is no international consensus on the definition of success of TSS. The term 'cure' has generally been replaced by 'remission', which is usually defined as <5 μg/dL (<138 nmol/L) or urinary free cortisol <28-56 nmol/day (<10-20 μg/day) within 7 days of selective tumour resection (20). According to these criteria, remission rates of 70-98% have been reported (8, 14, 21, 22), but also remission rates of 100% and 69% were reported in two large paediatric CD series which used stricter definitions of remission, namely post-TSS serum cortisol of <1 μg/dL (28 nmol/L) and <1.8 μg/dL (50 nmol/L), respectively (7, 23). In summary, remission rates of ~70% or greater are now expected from specialist centres with experience of paediatric CD. The most common complication of TSS was post-operative diabetes insipidus, present in 5% of patients at discharge from neurosurgical care (8). Overall, TSS is effective and safe first-line treatment for paediatric CD. An endo-nasal approach with endoscopy may be also used for access to the pituitary and this is now gaining popularity, particularly in adult patients. A small paediatric series of patients from an English reference centre showed biochemical remission in 5 out of 6 patients, with no recurrence after a mean follow-up of 55 months (24). Second-line therapy in the form of reoperation, pituitary radiotherapy, bilateral adrenalectomy or long-term medical therapy to control cortisol synthesis may be required in approximately 30% of cases.

Recovery of the hypothalamic-pituitary-adrenal axis

Adrenal insufficiency follows successful pituitary adenoma resection and may persists for many months. A median time of recovery of the HPA axis in 106 paediatric patients successfully treated by TSS at the NIH was 12.3 months with a range of 3-35 months (25). The level of pre-operative urinary free cortisol related to recovery time with high values being associated with longer intervals of recovery of normal adrenal function.

Recurrence of Cushing's disease following remission

There are relatively few studies of recurrence of CD following treatment during the paediatric age range. Yordanova et al. studied long-term follow-up of 21 paediatric CD patients in remission following definitive TSS or pituitary radiotherapy. During an interval of 2 to 7.6 years, the recurrence rate was 14.3% (26). The most comprehensive data comes from the NIH. Interesting results were reported showing a relationship (p=0.0342) between a shorter HPA axis recovery time and the likelihood of recurrence of CD (25). All patients with recurrence of hypercortisolism had recovery of the HPA axis by 15 months post-TSS. It is helpful to look at risk of recurrence in the context of prediction of short-term remission following TSS – because the two phenomena are linked. Essentially the factors significantly predicting remission (P=<0.05) after TSS are: lack of prior surgery, younger age, identification of adenoma during surgery, the presence of a positive ACTH-producing immunohistochemical adenoma and a non-invasive and smaller adenoma (8) (Figure 1). All these factors favour complete removal of the adenoma. In a series of 78 paediatric CD patients from the NIH, 94% had sustained remission for 5.8 – 18.3 years with 6 patients showing recurrence of CD. Children who remained in remission had; lower morning ACTH and cortisol levels during the post-operative period after TSS compared to those who relapsed (P <0.001). Relapse was associated with; higher cortisol response to CRH pre-TSS, lack of histological confirmation of adenoma at surgery, normal serum cortisol and ACTH (as opposed to

In favour of recurrence	Against recurrence
• Early recovery of the hypothalamic-pituitary-adrenal axis • Higher response to CRH pre-TSS • Lack of histological confirmation of adenoma at surgery • Normal serum cortisol and ACTH (as opposed to subnormal values) post-TSS • The need for glucocorticoid replacement for less than 6 months after surgery	• Minimum morning serum cortisol of <1 μg/dL (28 nmol/L) after surgery • Early biochemical remission after TSS • Lower morning ACTH and cortisol levels after TSS compared to those who relapsed (P <0.001) • Lack of prior surgery • Younger age • Identification of adenoma at surgery • Immunohistochemical ACTH-producing adenoma • Non-invasive ACTH adenoma • Smaller adenoma

FIGURE 1
Factors in favour and against recurrence of paediatric Cushing's disease.

subnormal values) post-TSS and the need for glucocorticoid replacement for less than 6 months after surgery, ie a rapid recovery of the HPA axis (10). The confirmation of these factors, in an expanded report of 179 NIH patients (8) reinforced the message that a positive predictive value for lasting remission (96%), was associated with a minimum morning cortisol of <1 µg/dL (28 nmol/L) during the immediate post-operative period. Paediatric patients harbouring somatic *USP8* mutations were reported to have a higher likelihood of recurrence of CD following TSS compared with patients without mutations (46.2% vs 10.3%, P=0.009) (27).

Paediatric radiotherapy

External pituitary radiotherapy (RT) is an optional second-line therapy for patients not in remission after TSS. However, this treatment in children is controversial because of concern related to cognitive effects and possible post-RT hypopituitarism. Corticotroph microadenomas respond well to conventional fractionated external RT, as first documented in 1977 (28). An English series of 7 paediatric patients reported treatment by a 6-MV linear accelerator delivering a dose of 45 Gy in 25 fractions over 35 days, which induced remission in all subjects at a mean interval of 0.94 years (range 0.25-2.86) (29). These data were later confirmed in a multi-centre study focusing on the gamma knife radiotherapy technique on 24 children with recurring CD, which induced remission in 87.5% of subjects after a mean interval of 12 months (30). The effects of RT take several months during which control of hypercortisolism is required using medical therapy.

Bilateral adrenalectomy

Bilateral adrenalectomy may represent a life-saving procedure in children with very severe hypercortisolism and life-threatening clinical morbidities *per se* or which may prevent safely approaching more definitive treatments, as TSS. Virtually, bilateral adrenalectomy has a remission rate of 100%, as it removes the source of cortisol production, and only patients with subtotal or incomplete surgical removal may experience recurrence after bilateral adrenalectomy (4). However, bilateral adrenalectomy requires life-long replacement treatment with glucocorticoids and mineralocorticoids and may be associated with Nelson's syndrome. The current definition of Nelson's syndrome is 'radiological progression or new detection of a pituitary tumour on thin-section MRI' (31). Clinically this is associated with increasing levels of ACTH causing hyperpigmentation, that may even occur several years after bilateral adrenalectomy (31). Although few paediatric series have been reported, a mean interval of 8.4 years post-adrenalectomy was reported in one series (32). The mean cumulative incidence of Nelson's syndrome was considerably higher (45%, 25–67%) compared to results in adult patients (31). This emphasizes the importance of life-long follow-up of all paediatric CD patients irrespective of the therapy they have received.

Medical therapy

Whereas extensively used in adult CD patients (33), few reports are available for the medical treatment of children with CD. Ketoconazole, a multi-enzyme steroidogenesis inhibitor, is the most frequently reported drug in children with CD, mainly in patients needing fast symptomatic relief from CS comorbidities and in patients waiting for pituitary radiotherapy to be effective (4). A French study reported the effects of "low-dose" mitotane, an adrenolytic drug used in adrenocortical cancer and in patients with very severe CS. In 9 CD children, there was significant improvement in weight and growth rate after 12 months of treatment, as well as a general improvement of clinical features, although the high rate of reported adverse events suggests caution in the widespread use of this treatment in children (34). Therapeutic trials are currently in progress in children using the 11-beta hydroxylase blocking agent osilodrostat, which is now approved for treatment of adults with CD (35).

Linear growth and pituitary function following TSS and/or radiotherapy

Growth hormone deficiency (GHD) is common after TSS in children (5) and may also be a complication of pituitary RT (9, 20). In an English series, GHD occurred in 86% of paediatric patients treated with RT, but after 10 years of follow-up 3/4 boys showed recovery of GH secretion to a peak GH value of >10 ng/ml (9). Gonadotrophin deficiency was also present in 9/20 subjects causing delayed puberty and several required sex steroid replacement (26). A major goal in the management of paediatric CD is the restoration of normal linear growth during remission after successful TSS. As CD often presents shortly before or during puberty, the time for catch-up growth is frequently limited and decreased adult height in paediatric CD has been well documented (5, 11, 36) (Figure 2). We advocate testing for GH deficiency 3 months after TSS and a low threshold for hGH replacement, if necessary in combination with a GnRH analogue (4).

There are follow-up data on two other variables, namely body composition and bone mineral density. In 14 patients treated by TSS alone (n=8) or TSS followed by external pituitary radiotherapy (n=6), body mass index (BMI) SDS was elevated at +2.7 (0.8 - 5.1) at diagnosis. At a mean interval of 4.1 (1.1 -10.7) years after remission of hypercortisolaemia (postoperative serum cortisol <50 nmol/L), BMI SDS remained elevated above the mean at 1.7 SDS, being lower than at diagnosis ($P < 0.05$), but elevated compared to the normal population ($P < 0.01$) (37). It is often difficult for children to normalise their BMI after remission. Careful dieting is required to minimise the risk of continuing insulin resistance.

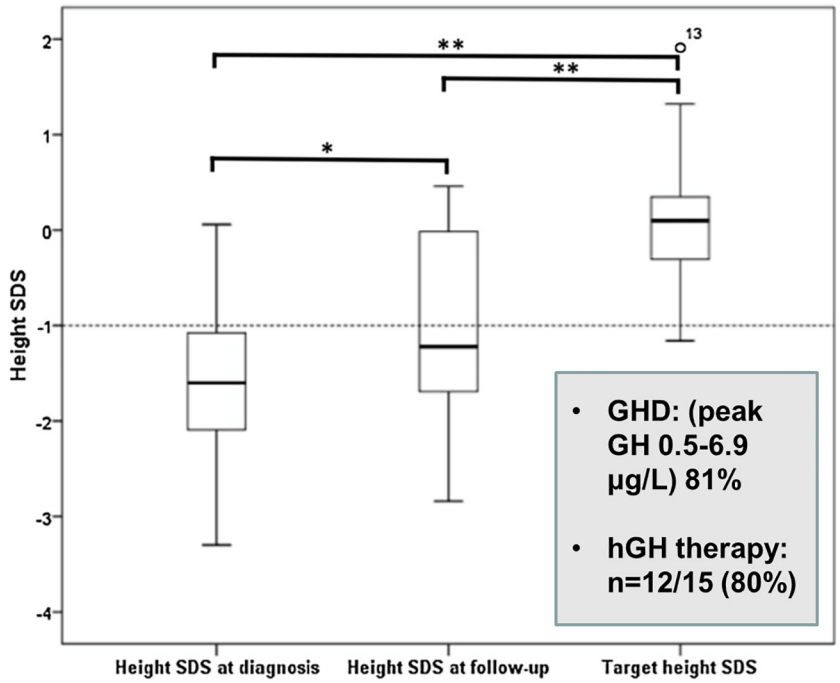

FIGURE 2
Height SDS values at diagnosis, latest assessment and target height in paediatric Cushing's disease (26). *p = 0.033, **p = 0.000.

Bone mineral density (BMD) is frequently reduced at diagnosis of paediatric CD (4). Two groups of patients were studied using DEXA, the first comprising 8 patients, mean age 12.4 yr (8.2 – 16.8 yr) had a mean L2-L4 volumetric BMD at diagnosis of -1.04 (-3.2 – 0.11) with values corelating negatively with midnight serum cortisol (P = < 0.05) (38). The second group comprised 11 subjects with hypercortisolaemia in remission following TSS or external RT, studied at a mean of 4.5 yr after remission, during which 8/11 had received hGH replacement (37). Mean L2-L4vBMD SDS was -0.38 (-1.0 – 0.13) with mean femoral neck areal BMD SDS of 0.14 (-1.62 – 2.46). These data show variable BMD SDS at diagnosis and near normal BMD SDS after induction of remission in paediatric CD (38).

Conclusions

Paediatric CD can be regarded as a niche disorder, which ideally requires joint input from paediatric and adult endocrinologists in terms of diagnosis and therapeutic strategy. Ideally a small number of tertiary centres should be reserved for the management of these patients. Following diagnosis, it is of crucial importance that a pituitary surgeon with prior experience of transsphenoidal surgery in children is identified for referral and becomes part of the management team. Complete selective excision of the corticotroph adenoma is difficult due to its very small size, but is directly related to long-term post-operative remission and quality of life. In experienced hands, the prognosis is good and the rate of recurrence is low. Post-operative challenges relate to catch-up linear growth and resumption of normal body composition. Life-long follow-up is required.

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Prevention and management of hypertensive crises in children with pheochromocytoma and paraganglioma

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Hypertensive crises in pediatric patients are rare conditions. However, determining their precise prevalence is more challenging than in adults due to the heterogeneity in the definition itself. These crises frequently occur without a prior diagnosis of hypertension and may indicate an underlying cause of secondary hypertension, including pheochromocytoma/paraganglioma (PPGL). The mechanisms of hypertensive crises in the pediatric population with PPGL are directly related to different types of catecholamine excess. Noradrenergic tumors typically present with sustained hypertension due to their predominant action on α1-adrenoceptors in the vasculature. Conversely, adrenergic tumors, through epinephrine binding to β2-adrenoceptors in addition to stimulation of α1- and α2-adrenoceptors, more frequently cause paroxysmal hypertension. Furthermore, the biochemical phenotype also reflects the tumor localization and the presence of a genetic mutation. Recent evidence suggests that more than 80% of PPGL in pediatric cases have a hereditary background. PPGL susceptibility mutations are categorized into three clusters; mutations in cluster 1 are more frequently associated with a noradrenergic phenotype, whereas those in cluster 2 are associated with an adrenergic phenotype. Consequently, the treatment of hypertensive crises in pediatric patients with PPGL, reflecting the underlying pathophysiology, requires first-line therapy with alpha-blockers, potentially in combination with beta-blockers only in the case of tachyarrhythmia after adequate alpha-blockade. The route of administration for treatment depends on the context, such as intraoperative or pre-surgical settings, and whether it presents as a hypertensive emergency (elevated blood pressure with acute target organ damage), where intravenous administration of antihypertensive drugs is mandatory. Conversely, in cases of hypertensive urgency, if children can tolerate oral therapy, intravenous administration may initially be avoided. However, managing these cases is complex and requires

careful consideration of the selection and timing of therapy administration, particularly in pediatric patients. Therefore, facing these conditions in tertiary care centers through interdisciplinary collaboration is advisable to optimize therapeutic outcomes.

KEYWORDS

juvenile hypertension, catecholamines, pheochromocytoma, paraganglioma, secondary hypertension, hypertensive crisis, children

Introduction and epidemiology

The prevalence of arterial hypertension in pediatric patients varies from 0.5-1% to 5% depending on the studies considered (1, 2). However, determining the prevalence of hypertensive crises in children is more challenging due to the heterogeneity in its definition. Generally, a hypertensive crisis in pediatric patients is suspected when blood pressure exceeds the limits of stage II hypertension (3). Specifically, the European Society of Hypertension (ESH) guidelines define severe hypertensive crisis in children as blood pressure values 20% above the stage II hypertension limit (4). Hypertensive crises can be categorized as hypertensive urgency and emergency, with the latter defined by elevated blood pressure values associated with acute target organ damage (2).

In adults, hypertensive crises are often associated with poor compliance with antihypertensive treatment. While in children, they frequently occur without a known previous diagnosis of hypertension and may reflect an underlying cause of secondary hypertension, predominantly of renal origin (70-80% of cases), but also of an endocrine one, including the diagnosis of pheochromocytoma/paraganglioma (PPGL) (3, 5). PPGLs are rare neuroendocrine tumors that can secrete catecholamines (in about the 80% of cases), arising from chromaffin cells in the adrenal medulla or sympathetic paraganglia. The incidence of PPGL is approximately 1 in 300,000 per year, with only 10-20% occurring in pediatric age, with an estimated incidence of 0.5-2 per million children, with pheochromocytomas (PCC) representing 80-85% of cases compared to paragangliomas (PGL) (6–8). A retrospective study by Pamporaki et al. (9) showed a high percentage of hereditary (up to 70-80%), extra-adrenal, metastatic, multifocal, and recurrent disease in pediatric-onset tumors (10).

Among children with arterial hypertension, the incidence of PPGL is high, around 1.7%, compared to 0.2-0.6% in hypertensive adults (8, 10). Clinical presentation varies, with signs and symptoms of catecholamine hypersecretion often overlooked in children due to their high level of physical activity compared to adults. Children with PPGL are more likely to have sustained arterial hypertension, up to 60-90% of them, while adults often exhibit paroxysmal hypertension in about 50% of cases (5, 8, 10). The clinical presentation of PPGL reflects the underlying catecholamine secretory phenotype and genotype, with epinephrine-secreting tumors more associated with an acute and explosive presentation than norepinephrine and dopamine-secreting lesions (10).

Treating arterial hypertension, particularly hypertensive crises, in children presents significant challenges. Given the rarity of PPGL in pediatric patients and the limited data available in the literature, the objective of this review is to provide guidance for the clinical management of PPGL-induced hypertensive crises in children. This includes advocating for interdisciplinary collaboration among endocrinologists, pediatric intensivists, anesthesiologists, nephrologists, emergency physicians and surgeons to ensure comprehensive care.

Pathophysiology

The mechanisms of hypertensive crisis in pediatric population with PPGL is directly related to the different types of catecholamine excess (10, 11). From a physiological point of view, norepinephrine and epinephrine are released in different pattern from chromaffin cells tumors and show variable binding affinities for adrenoreceptors, leading to different clinical manifestations as the results of their effect on hemodynamics and metabolism (11). Approximately 50% of pheochromocytomas produce norepinephrine almost exclusively, whereas the other half secretes a combination of norepinephrine and epinephrine.

Adrenoreceptors are G-protein coupled receptor mediating the actions of epinephrine and norepinephrine. The major types of human adrenoreceptors are: α1, α2 and β, each having more subtypes. α1-adrenoceptors are mainly located in the vasculature and their stimulation induces vasoconstriction and increased peripheral vascular resistances, resulting in sustained hypertension. Instead, stimulation of presynaptic α2-adrenoceptors can reduce neuronal norepinephrine release, decreasing blood pressure, through a negative feedback mechanism. β1-adrenoceptors, localized on cardiomyocytes and the cardiac conduction system, mediate increase in blood pressure, through their action on heart rate and cardiac output. In contrast, stimulation of β2-adrenoceptors, present both on blood vessels and cardiomyocytes, could induce vasodilatation and reduced cardiac inotropy, and consequently hypotension (12). Norepinephrine exerts its cardiovascular effects by working mainly on α1-adrenoceptors in the vasculature, with low

activity on cardiac β1-adrenoceptors. Instead, epinephrine is responsible for its hemodynamic and metabolic actions through its binding to β2-adrenoceptors, in addiction to stimulation of both α1- and α2-adrenoceptors (12).

Form the clinical point of view, the evaluation of catecholamine O-methylated metabolites is of primary importance because their production and release are continuous and independent from catecholamine secretion, making them reliable disease markers. The main metabolites are normetanephrine, metanephrine and 3-methoxytyramine, deriving from norepinephrine, epinephrine and dopamine, respectively (11, 13).

However, regardless of underlying etiology, hypertensive crisis is characterized by some common pathways that could contribute to the severity of hypertension itself and to end-stage organ damage (14). As shown by Harrison et al. (15), the initial stimulus to hypertension could involve several systems, determining the activation of renin-angiotensin-aldosterone system, oxidative stress and endothelial dysfunction. The consequent protein fragmentation and formation of neoantigens result in activation of T-cells and release of cytokines able to increase vasoconstriction and sodium and water retention. This theory thus recognizes a crucial role of inflammation in the pathogenesis of hypertensive crisis and possible organ damage (16).

The abovementioned pathophysiology of cathecolamine-induced hypertensive crises and related cardiovascular complications is depicted in Figure 1.

Clinical manifestations and biochemical phenotypes

In childhood functional PPGLs frequently manifest with sustained, rather than paroxysmal, hypertension present in approximately 60-90% of patients (5, 8). Furthermore, the classic "triad" including diaphoresis, headaches, and palpitations has been reported in up to 54% of children with PPGLs (8). In PPGL the cardiovascular complication of hypertensive crisis could derive, apart from marked and abrupt elevation in blood pressure values, also from catecholamine direct damage of the heart and vessels (17). However, the most common manifestations of hypertensive crisis in children are neurologic (14). Clinical signs and symptoms typically depend on type of hormonal secretion (11, 12). The specific secretion profile results in three different biochemical phenotypes: noradrenergic, adrenergic and dopaminergic, according to the predominant increase in normetanephrine, metanephrine and 3-methoxytyramine, respectively (12, 13). Moreover, there is a rare PPGL subtype that does not produce and release catecholamines, named biochemically silent (12). Noradrenergic tumors typically occur with sustained hypertension and possible consequences of excessive α1-adrenoceptors stimulation on cardiovascular system, such as flash pulmonary edema, coronary artery vasospasm and myocardial infarction, or aortic dissection. Instead, adrenergic tumors, in addition to the sequalae of α1-adrenoceptors stimulation, could also

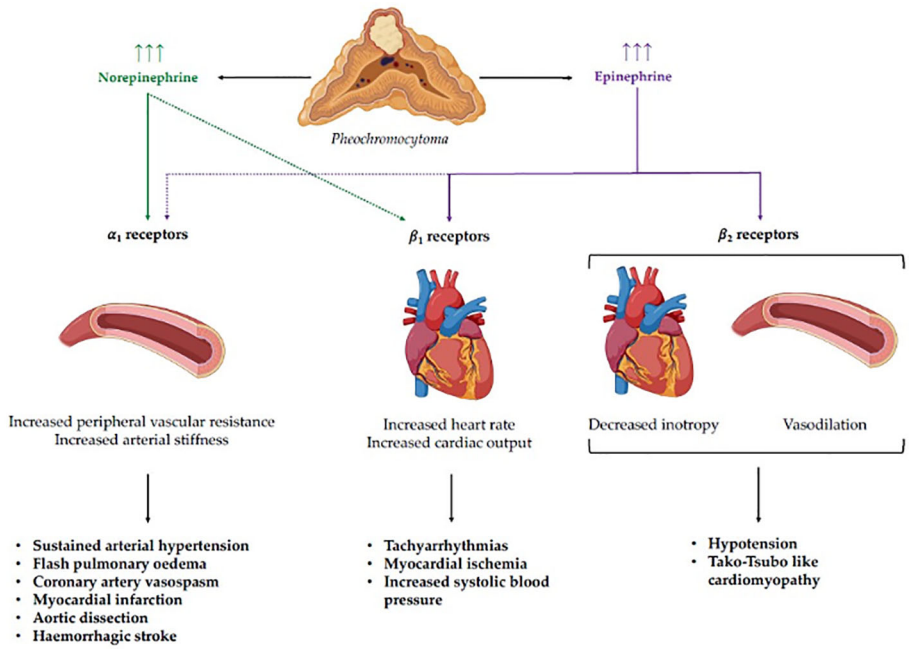

FIGURE 1
Pathophysiology of catecholamine-induced hypertensive crises and related cardiovascular complications. Norepinephrine and epinephrine display different binding affinities for adreno-receptors, leading to distinct clinical phenotypes according to the specific secreting pattern of the pheochromocytoma. Stimulation of $\alpha 1$ receptors, which show a higher affinity for norepinephrine, mediates an increase in peripheral vascular resistance, leading to sustained arterial hypertension and complications such as flash pulmonary edema, coronary artery vasospasm, myocardial infarction, aortic dissection, and hemorrhagic stroke. On the other hand, stimulation of cardiac $\beta 1$ receptors, which have a greater affinity for epinephrine, leads to a positive chronotropic and inotropic effect, with the potential development of tachyarrhythmias, myocardial ischemia, and systolic hypertension. Finally, the stimulation of cardiac and vascular $\beta 2$ receptors by epinephrine can lead to negative inotropism and vasodilation with subsequent hypotension and Tako-Tsubo like cardiomyopathy.

manifest more sequelae of excessive β-adrenoceptor stimulation, resulting in tachyarrhythmia, myocarditis, and demand myocardial ischemia or infarction (17, 18). Moreover, because of excessive β2-adrenoceptor binding, adrenergic tumors may also manifest with hypoglycemia, suppression of myocardial contractility (as in Takotsubo-like cardiomyopathy), orthostatic hypotension, and even hypotensive shock (8). Conversely, patients with dopamine-secreting tumors, could be diagnosed by mass effect symptoms, being longer normotensive and asymptomatic (5).

The biochemical phenotype also influences tumor localization. In fact, PPGL with noradrenergic phenotype is typically extra-adrenal instead adrenergic PPGLs are characterized by location within the adrenal glands (12).

Genetic background

Overall, PPGL are characterized by the highest rate of hereditary background compared to other human neoplasms. In fact, approximately 40–50% of cases are related to germline mutations in one of the known susceptibility genes and in 40-60% of sporadic cases a somatic mutation is found (19–21). Moreover, the hereditary rate rises to 70-80% in the pediatric setting (7, 9, 22). Baush et al. (7) reported an even higher 80% frequency of germline mutations among pediatric cases. Early onset of disease, bilateral multifocal, extra-adrenal, and malignant tumors are the clinical hallmarks of hereditary disease. Moreover, children usually presented symptomatic and potentially life-threatening disease (6).

Specific germline mutations have been attributed to at least 25 tumor-susceptibility genes (19, 20) that could be divided into three cluster groups based on transcriptomic profiles revealed by gene expression microarray analyses (23–26). This proportion of implicated genes will progressively increase as mutations impacting new genes are found (21).

Cluster 1 tumors comprehend those related to the following mutations: von Hippel-Lindau (*VHL*) suppressor, the four subunits of the succinate dehydrogenase complex (*SDHA, SDHB, SDHC*, and *SDHD*), and less commonly, the enzyme responsible for flavination of the SDHA subunit (*SDHAF2*), fumarate hydratase (*FH*), malate dehydrogenase 2 (*MDH2*), prolyl hydroxylase (*PHD*), and somatic gain-of-function pathogenic variants in the hypoxia-inducible factor 2 alpha gene (*HIF2A* or *EPAS1*) and some newly discovered genes that will be detailed in Table 1. Cluster 1 mutations result in stabilization of hypoxia-inducible factors and activation of the hypoxia signaling pathways (25, 26).

Instead, cluster 2 tumors include neoplasms due to mutations of the neurofibromatosis type 1 (*NF1*) tumor suppressor gene, the rearranged during transfection (*RET*) proto-oncogene, genes encoding transmembrane protein 127 (*TMEM127*), MYC-associated factor X (*MAX*) and *HRAS*. Mutations of cluster 2 genes regard activation of kinase receptor signaling pathways, translation initiation, protein synthesis, and pathways involved in maintenance of neural/neuroendocrine identity (26).

Cluster 3 comprehends somatic *CSDE1* (Cold shock domain–containing E1 gene) mutations and *MAML3* (a member of the Mastermind-like family of transcriptional co-activators) fusion variants implicated in Wnt-pathway signal alterations (25).

The specific genetic background influences the biochemical and clinical phenotypes because the underlying mutation determines a variable expression of biosynthetic enzymes involved in the synthesis of catecholamines by the tumors (28). In more detail, cluster 1 neoplasms are typically characterized by noradrenergic phenotype, on the contrary cluster 2 tumors by adrenergic phenotype (29). Literature data showed that noradrenergic PPGLs typically manifest in younger age that adrenergic ones (30). Based on these assumptions, a study performed by Pamporaki et al. (9) on large cohort of pediatric and adult patients with PPGLs demonstrated, not only a childhood predominance of extra-adrenal, multifocal, metastatic, recurrent, and hereditary PPGLs, but also the link between these phenotypic features to a higher prevalence of noradrenergic and related cluster 1 hereditary tumors in pediatric than adult patients.

As mentioned above, the biochemical properties of PPGL are related to the underlying genetic mutations able to influence differentiation of tumor progenitor cells and consequently the secretory pathways and the epigenetic remodeling profiles (21).

The complex relationship between genotype and phenotype in PPGLs and the characteristics of the main tumor-susceptibility genes and their related hereditary syndromes are summarized in Table 1.

Diagnostic work-up

Biochemical testing is recommended as the initial step in evaluation of suspected PPGL and should include plasma or 24-hours urinary free metanephrines measurement, performed using liquid chromatography assay (5, 8). Taking into account that 24-hour urine collection may not be feasible in young children, a plasma sample is often the initial biochemical test obtained in childhood. Indeed, two studies documented a high diagnostic accuracy of plasma free metanephrines for pediatric patients (31, 32). Pre-analytical considerations are specific challenges that impact upon the interpretation of biochemical tests in pediatric patients. In fact, sympathoadrenal activation triggered by upright posture, distress associated with venepuncture, emotional stress, as well as many medications (e.g. acetaminophen, tricyclic antidepressants, amphetamines,.) should be carefully evaluated. In general, normetanephrine or metanephrine values three-/four-fold or higher above the upper limit of the reference ranges require anatomical imaging for tumor localization and staging. Initial imaging studies include either contrasted enhanced computed tomography (CT) or magnetic resonance imaging (MRI), given their similar diagnostic performance (33). However, MRI is the preferred imaging modality for patients with head and neck PPGL and in those with metastatic disease and it is necessary if the initial imaging of the abdomen and pelvis does not identify the neoplasia. Moreover, functional imaging is a complementary technique useful for disease staging, detection of metastases or recurrent/multiple tumors. However, in childhood the indication for functional

TABLE 1 Characteristics of the main PPGL-susceptibility genes (5, 8, 12, 19–21, 27).

Gene	Gene type	Most common PPGL location	Biochemical phenotype	Related syndromes and other manifestations	Malignancy risk
Cluster 1 (pseudohypoxic signaling)					
SDHA	Germline	sPGL, HNPGL, PCC (very low penetrance)	NE; NE+DA; NS	PGL6 RCC; GIST; pituitary adenoma	0-14%
SDHB	Germline	sPGL, HNPGL (intermediate penetrance) PCC (low penetrance)	NE; NE+DA; NS	PGL4 RCC; GIST; pituitary adenoma; pulmonary condroma	34-70%
SDHC	Germline	HNPGL, sPGL (intermediate penetrance) PCC (low penetrance)	NS; NE +DA	PGL3 RCC; GIST; pituitary adenoma	0-28%
SDHD	Germline	Multifocal HNPGL (high penetrance) sPGL (low penetrance) PCC (low penetrance	NE; NE +DA; NS	PGL1 RCC; GIST; pituitary adenoma; pulmonary condroma	<5%
SDHAF2	Germline	Multifocal HNPGL (high penetrance)	NS	PGL2	/
FH	Germline	PCC + sPGL	NE	Hereditary Leiomyomatosis and Renal Cell Cancer (HLRCC)	/
MDH2	Germline	sPGL	NE	Early-Onset Severe Encephalopathy (homozygous germline mutations)	/
VHL	Germline/ somatic	PCC (high penetrance) usually bilateral (50%) sPGL (low penetrance) also multifocal HNPGL (very low penetrance)	NE	VHL 2A: retinal and CNS hemangioblastomas, endolymphatic sac tumors, epididymal cystadenomas 2B: renal and pancreatic cell cysts and carcinomas, retinal and CNS hemangioblastomas, endolymphatic sac tumors, epididymal cystadenomas	< 10%
EPAS-1 (HIF2A)	Postzygotic/ somatic	Multifocal sPGL (high penetrance) PCC (high penetrance)	NE	Pacak–Zhuang syndrome; Somatostatinoma; polycythemia; ocular lesions	29%
IRP1	Somatic	/	NE	/	/
DLST	Germline	sPGL (multiple)	NE	/	/
SLC25A11	Germline	/	NE	/	Increased risk of metastatic disease
SUCLG2	Germline	/	NE	/	/
Cluster 2 (kinase signaling)					
RET	Germline/ somatic	PCC (high penetrance) usually bilateral (50-80%) sPGL (very low penentrance) HNPGL (very low penetrance)	E	MEN2 2A: medullary thyroid carcinoma, hyperparatiroidism, cutaneous lichen amyloidosis 2B: medullary thyroid carcinoma, multiple mucocutaneous neuromas, marafanoid habitus, intestinal ganglioneuromas	< 5%
NF1	Germline/ somatic	PCC (low penetrance) sPGL, HNPGL (very low penetrance)	E and NE	Von Recklinghausen's disease Café-au-lait spots, neurofibromas, Lisch nodules, Optic pathway/CNS gliomas, GIST	< 10%

(Continued)

TABLE 1 Continued

Gene	Gene type	Most common PPGL location	Biochemical phenotype	Related syndromes and other manifestations	Malignancy risk
Cluster 2 (kinase signaling)					
TMEM127	Germline	PCC (intermediate penetrance) bilateral (33-39%) sPGL (low penetrance) HNPGL (very low penetrance)	E	PGL5 RCC	<5%
MAX	Germline/ somatic	PCC (common bilateral) sPGL (very low penetrance)	E and NE	PGL7 renal oncocytoma; pituitary adenoma	7-25%
HRAS	Somatic	PCC PGL	/	/	/
MET	Germline/ somatic	/	/	/	/
MERTK	Germline	/	/	/	/
DNMT3A	Germline	Multifocal HNPGL	/	/	/
Cluster 3 (Wnt signaling)					
CSDE1	Somatic	PCC	E	/	Increased risk of recurrent and metastatic disease
UBTF-MAML3	Fusion	PCC	E	/	Increased risk of recurrent and metastatic disease

PPGL, pheochromocytoma-paraganglioma; SDHA, succinate dehydrogenase complex flavoprotein subunit A; sPGL, sympathetic paraganglioma; HNPGL, head and neck paraganglioma; PCC, pheochromocytoma; NE, norepinephrine; DA, dopamine; NS, non-secreting; RCC, renal cell carcinoma; GIST, gastrointestinal tumor; SDHB, succinate dehydrogenase complex flavoprotein subunit B; SDHC, succinate dehydrogenase complex flavoprotein subunit C; SDHD, succinate dehydrogenase complex flavoprotein subunit D; SDHAF2, Succinate dehydrogenase complex assembly factor 2; FH, fumarate hydratase; MDH2, malate dehydrogenase 2; VHL, von Hippel-Lindau; EPAS-1, Endothelial PAS domain protein 1; HIF2A, hypoxia-inducible factor 2 alpha; IRP1, Iron Regulator Protein 1; DLST, Dihydrolipoamide S-Succinyltransferase; SLC25A11, Solute Carrier Family 25 Member 11; SUCLG2, Succinyl Co-A Ligase G2; RET, rearranged during transfection; E, epinephrine; MEN2, multiple endocrine neoplasia type 2; NF1, neurofibromatosis type 1; CNS, central nervous system; TMEM127, transmembrane protein 127; MAX, Myelocytomatosis-associated factor X; HRAS, Harvey rat sarcoma viral gene homologue; MET, Mesenchymal to Epithelial Transition; MERTK, Tyrosine Kinase Protooncogene; DNMT3A, DNA methyltransferase 3 alpha; CSDE1, Cold shock domain containing E1; UBTF-MAML3, Upstream Binding Transcription Factor Mastermind-like Transcriptional Coactivator 3.

imaging must be carefully balanced against the radiation risk. The different functional imaging modalities include 68Ga-DOTATATE, 18F-fluorodopa (FDOPA), 18F-fluorodeoxyglucose (FDG) PET/TC and 123I-metaiodobenzylguanidine (123I-MIBG). The choice of the specific functional tracers should be guided by the known or suspected genetic mutation of the patient (34).

Prevention and treatment of PPGL-induced hypertensive crisis

The most important way to approach catecholamine-induced hypertensive crisis is the prevention of their onset. Therefore, the first part of this section is dedicated to the preoperative medical management.

Preoperative prevention of hypertensive crisis

To reduce the impact of hypertensive crises, potentially occurring during intraoperative manipulation of the tumor, adequate preoperative oral anti-hypertensive treatment is required.

From a pathophysiological perspective, a sequential approach with initial α-adrenergic blockade followed by β-adrenergic blockers is recommended to avoid reflex tachycardia (Table 2). Normalization of blood pressure and heart rate in pediatric patients requires longer treatment than in adults; therefore, compared with adults, it is recommended to start the treatment at least 14 days before surgery. This is probably related to the low-dose regimen and progressive titration of drugs to avoid side effects and to the fact that children are more sensitive to sympathetic overactivity (9, 11). In addition to antihypertensive therapy, adequate salt and water supplementation is required to avoid orthostatic hypotension related to volume contraction due to catecholamine release and drug side effects. After the initiation of α-blocker therapy, a supplementation of 6 to 10 grams per day of salt, depending on patient's body surface area and an increase in fluid intake up to 1.5 times the weight-corrected levels are recommended.

The goal is to achieve a blood pressure within the 50-90th percentile for age, gender and height, aiming to obtain values closer to the 50th percentile in the very last preoperative days.

The most used drugs are long-acting non-selective α1/α2 receptor blockers such as phenoxybenzamine or long-acting selective α1 receptor blockers such as doxazosin, prazosin or terazosin. The most frequent side effect is orthostatic

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TABLE 2 Preoperative management of PPGL in children (8–11).

		Drug	Mechanism	Dose	Side effects	Comments
Pre operative (start 14 days prior to the surgery)	First-line treatment	Doxazosin	Selective α1-blocker	1-2 mg/day in 1-2 doses; Max 4-16 mg/day	Orthostatic hypotension, dizziness, fatigue, drowsiness	No reflex tachycardia, long action (>24h)
		Prazosin		0.05-0.1 mg/kg/day in 3 doses Max 0.5 mg/kg/day (20 mg/day)		Less used
		Terazosin		1 mg/day; Max 20 mg/day		Less used
	Sequential approach	Phenoxy-benzamine	Non selective α1/α2-blocker	0.2-0.25 mg/kg/day (max 10 mg/dose) Max 2-4 mg/kg/day (60 mg/day)	Orthostatic hypotension, nausea reflex tachycardia, nasal congestion, central sedation, abdominal pain	Associate β blocker 3-4 days prior to the surgery
		Atenolol	Selective β1-blocker	0.5-1 mg/kg/day in 1-2 doses; Max 100 mg/day	Dizziness, Fatigue	Never give before α-blocker
		Metoprolol		1-2 mg/kg/day in 1 dose; Max 200 mg/d		
		Propranolol	Non selective β1/β2-blocker	1-2 mg/kg/day in 2-4 doses; Max 640 mg/day	Dizziness, fatigue, bronchoconstriction	
		Labetalol	α1/β-blocker	1-3 mg/kg/day in 2 doses; Max 1200 mg/day		
	Adjunctive agents	Amlodipine	Calcium channel blocker	0.05-0.1 mg/kg/day; Max 10 mg/day	Peripheral edema, palpitations, gingival hyperplasia, headache	Use as adjunct
		Metyrosine	Tyrosine hydroxylase inhibitor	20 mg/kg/day in 4 doses; Max 2500 mg/day	Lethargy, extrapiramidal symptoms, diarrhea, crystalluria	Never give before α-blocker
	Salt	6-10 g/day				
	Fluids	1.5 times maintenance fluids per kg				

hypotension. Non-selective α1/α2 receptor blockers additional side effects also include reflex tachycardia, central sedation, nasal congestion, nausea and abdominal pain. Selective α1 receptor blockers generally have a better side effect profile, with no reflex tachycardia and more prolonged activity (>24 hours). The risk of postoperative hypotension with selective blockers is also low, therefore they are used preferentially in many centers.

Second-line or adjunctive agents include calcium channel blockers, such as amlodipine or nifedipine and tyrosine hydroxylase inhibitors, such as metyrosine. The hypotensive effect of calcium channel blockers is minor, and their use is usually limited when blood pressure control with selective and nonselective α-blockers is ineffective or in case of severe adverse effects. Metyrosine is used for short periods before surgery in combination with α-blockers to provide better pre- and intraoperative blood pressure control. Side effect profile include extrapyramidal symptoms, diarrhea, orthostatic hypotension, drowsiness, xerostomia, and neuromuscular symptoms, as well as crystalluria, therefore its use in pediatric age is limited.

When nonselective α-blockers are used, β-adrenergic blockers are often added a few days before surgery to relieve α-blockers-related reflex tachycardia. β-adrenergic blockers should never be started before α-blockers because catecholamine-related vasoconstriction would trigger a hypertensive crisis. Selective β1-adrenergic blockers, such as atenolol and metoprolol, are preferred over nonselective β1/β2-blockers (propranolol) because of the risk of bronchoconstriction. Another nonselective β-blocker agent, with combined selective α1-blockade is labetalol. The latter should not be used alone for the high risk of hypertensive crisis (8–11).

General and intraoperative management of PPGL-induced hypertensive crisis

In case of hypertension urgency where children can tolerate oral therapy, intravenous administration may be initially avoided. However, hypertensive crises in children with PPGL occur predominantly during intraoperative manipulation of the tumor, even despite adequate preoperative treatment. Therefore, continuous invasive blood pressure monitoring by intra-arterial catheterization is essential during surgery to help anesthesiologists in assessing blood pressure fluctuations (8). In case of intraoperative hypertensive crisis, intravenous administration of short-acting antihypertensive drugs is mandatory. These indications can be also applied to the general management of hypertensive crisis out of the surgical setting.

From a pathophysiological perspective, as mentioned earlier, the drugs of choice to treat PPGL hypertensive crises are α-blockers, such as urapidil, a combined peripheral selective α1 receptor antagonist and central serotoninergic 1A receptor agonist, or phentolamine, a non-selective α1/α2 receptor antagonist. Urapidil is particularly useful, as it does not induce reflex tachycardia and is not associated with alterations of the renin-angiotensin-aldosterone system, thereby minimizing side effects. However, there are limited case series on the use of this drug in the pediatric population (14, 35). One of these is a multicentric Italian retrospective survey on treatment of hypertension in children with neuroblastoma. Intraoperative hypertension management was somewhat dissimilar among the participating centers, apart from a certain consistency in the intraoperative use of urapidil (36). Side effects associated with α-blockers in PPGL patients are rare and generally not severe. The main side effects reported in literature include

TABLE 3 Management of hypertensive crises induced by catecholamine secreting tumors in children (8, 10, 11, 14).

Drug	Mechanism	Dosage	Side effects	Contraindications
FIRST LINE TREATMENT				
Urapidil	Selective α1-adrenergic receptor antagonist - central serotoninergic 1A receptor agonist	Initial 0.5–4.0 mg/kg per hour Maintenance 0.2–2.0 mg/kg per hour	Hypotension, tachycardia, dizziness, central sedation, nausea and nasal congestion	Athero-venous shunt, stenosis of the aortic isthmus
Phentolamine	Competitive non selective α1/α2 adrenergic receptor antagonist	Bolus 0.1-5 mg/Kg	Hypotension, tachycardia, dizziness, central sedation, arrythmias and nasal congestion	//
Labetalol (in case of concomitant tachyarrhythmias, after adequate α-adrenergic blockade)	Combinate α1/β-adrenergic blocker (ratio 1:7)	0.25–3 mg/kg/hour Titrate slowly Max: 3 mg/kg/hour	Orthostatic hypotension, dizziness	Asma, sinuses bradycardia, atrio-ventricular block, heart failure
Esmolol (in case of concomitant tachyarrhythmias, after adequate α-adrenergic blockade)	Selective β1-adrenergic blocker	Bolus of 500–600 μg/kg over 2 min. Maintenance 200 (50–250) μg/kg/min. Max 500 μg/Kg/min	Hypotension, bradycardia, risk of atrio-ventricular block	Asma, sinuses bradycardia, sick sinus syndrome, atrio-ventricular block, hypotension, heart failure, cardiogenic shock, pulmonary hypertension
SECOND LINE TREATMENT				
Sodium nitroprusside	Vasodilator (nitro-derivates)	Starting: 0.3–0.5 μg/kg/min. Titrate by 0.1 μg/kg/min every few minutes. Max: 10 μg/kg/min	Tachycardia, flushing, palpitations, and hypotension. Monitor for risk of cyanide and thiocyanate toxicity (so protect from light)	Renal and/or hepatic failure, hypothyroidism, deficit of vitamin B12
Nicardipine	Dihydropyridine calcium channel blocker	Starting: 0.5–1 μg/kg/min. Max: 4–5 μg/kg/min	Tachycardia, flushing, palpitations, and hypotension, edema, headache	Pathological hyperlipemia, nephrosis or acute pancreatic inflammation secondary to hyperlipemia
OTHER TREATMENTS				
Magnesium sulphate	Vasodilator, inhibits catecholamine release from adrenal medulla and sympathetic paraganglia	Loading dose: 40–60 mg/kg over 10 minutes. Maintenance: 15–30 mg/kg/hour	Neuromuscular paralysis	Use with caution in those with neuromuscular disease (risk of paralysis)
Dexmedetomidine	Central α2-agonist	Loading dose: 0.5–1 μg/kg/dose over 10 minutes. Maintenance: 0.2–0.5 μg/kg/hour	Respiratory depression, bradycardia, xerostomia	In those with reduced respiratory drive

excessive hypotension, tachycardia, dizziness, and nasal congestion (8, 10, 37).

In cases of hypertensive crises accompanied by tachyarrhythmias, when α-blockade is achieved, short-acting intravenous β-blockers may be utilized in combination, including labetalol, an α1-β blocker with a 1:7 ratio for intravenous administration, and esmolol, a selective β1-antagonist (10).

Although the majority of experience with esmolol, especially in little children younger than 6 years, is in management hypertension during and after intervention for aortic coarctation repair (38). Romero et al. (39) in a retrospective analysis of medical records of 10 children (from the age of 6 months to 18 years), that were diagnosed with a catecholamine secreting tumor from 2005–2013 and underwent surgical removal, showed how 80% of these patients experimented hypertension crises during surgery and most of them were treated with esmolol, labetalol, but also sodium nitroprusside. The disadvantage is the same as in all β-blockers, namely its negative inotropic effect and potential for bronchoconstriction. Consequently, it should be avoided in children with asthma or those suffering from decompensated or unstable congestive heart failure (14). The principal side effects of this class of drugs are dizziness, bradycardia and risk of atrio-ventricular block (10).

Second-third line treatments for hypertensive crises in children with PPGL include calcium-channel blockers, particularly second-generation dihydropyridines such as nicardipine, and nitroderivatives. Among these options, sodium nitroprusside, a preferential arterial vasodilation, is the drug of choice, because nitroglycerin, reducing cardiac preload by venous vasodilation, can potentially induce significant reflex tachycardia (10, 14). There is notable experience in using nicardipine for managing severe hypertension in children (40, 41). The main side effects observed were related to its vasodilatory effects, including tachycardia, flushing, palpitations, and hypotension. Sodium nitroprusside treatment provides additional benefits for controlling coronary vasospasm. However, it requires careful monitoring due to the potential risks of cyanide and thiocyanate toxicity. In addition, precautions must be taken to protect it from exposure to light (10, 14). Finally, in children with hypertensive crises due to PPGL, as an adjunctive therapy, treatment with dexmedetomidine, a central α2-agonist, and magnesium sulphate, a vasodilator that also inhibits catecholamine release from the adrenal medulla and sympathetic nerve endings, has been described (8, 10). In particular, magnesium sulphate is a valid and safe alternative for the pediatric population and is also suitable for use in pregnant women (42, 43). The main side effects of dexmedetomidine include respiratory depression and bradycardia. Conversely, magnesium sulphate should be used with caution in individuals with neuromuscular disorders due to the risk of paralysis (8, 10). Details about all these treatments are described in Table 3. In case of intraoperative hypotension, intravenous infusion of crystalloid or colloid fluids and administration of vasoactive agents may be necessary, with particular awareness in case of catecholamine-induced cardiomyopathy, as these patients are at risk for pulmonary edema secondary to volume overload.

Postoperative complication management

Continuous monitoring in the first 48 hours after surgery is indicated for a high risk of hemodynamic instability. Multifactorial hypotension, responsive to colloid/crystalloid infusion, may occur due to the downregulation of adrenergic receptors and acute withdrawal of catecholamines after surgical removal of the mass, as well as the prolonged action of antihypertensive agents used in the preoperative period and the short-term effect of intraoperative management. Hypoglycemia may also occur postoperatively due to rebound hyperinsulinemia, which results from the loss of the inhibitory action of catecholamines on pancreatic β-cells. This condition can be treated with the infusion of glucose-containing fluids (8, 10).

Conclusions

Although hypertensive crises in children are rare conditions, the precise determination of their prevalence is more challenging than in adults, due to the heterogeneity in its definition. Catecholamine excess represents a rare cause of hypertensive crisis, the management of which is complex and requires careful selection and timing of therapy administration, even more in pediatric patients. Therefore, it would be advisable to manage these cases in tertiary care centers through interdisciplinary collaboration involving endocrinologists, pediatric intensivists, anesthesiologists, nephrologists, emergency physicians and surgeons to optimize therapeutic success (8–10).

Author contributions

CB: Project administration, Writing – original draft, Writing – review & editing. CL: Project administration, Writing – original draft, Writing – review & editing. GT: Validation, Writing – review & editing. JM: Validation, Writing – review & editing. SA: Validation, Writing – review & editing. MP: Validation, Writing – review & editing. MB: Conceptualization, Supervision, Validation, Visualization, Writing – review & editing. MM: Project administration, Supervision, Validation, Visualization, Writing – review & editing. LS: Conceptualization, Supervision, Validation, Visualization, Writing – review & editing. MP-C: Project administration, Supervision, Validation, Visualization, Writing – review & editing.

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The multiple faces of autoimmune Addison's disease in children

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Primary adrenal insufficiency (PAI) is a rare medical condition, characterized by a deficiency in adrenal hormones. Although rare, PAI is a life-threatening disease requiring prompt recognition and treatment. However, symptoms of PAI are often non-specific and diagnosis can be challenging, causing frequent diagnostic delays. In adults, autoimmunity is the most common cause of PAI in industrialized countries, whereas in children, the most frequent etiology is represented by congenital defects of steroidogenesis and, in particular, by congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency. A few recent case series from different countries have reported that autoimmunity is the second most common etiology of PAI in the pediatric age group. However, data on autoimmune PAI in children are still scant and the exact epidemiology, clinical manifestations, and long-term outcomes of this condition have yet to be defined. The scope of this review is to summarize the current knowledge on the etiology, presentation, and treatment of autoimmune PAI in childhood and to increase physicians' awareness of the signs that should raise an early suspicion of this condition.

KEYWORDS

primary adrenal insufficiency, autoimmune Addison's disease, autoimmune polyendocrine syndromes, pediatric age, adrenal crises

Introduction

Primary adrenal insufficiency (PAI) is a rare medical condition, described for the first time by Thomas Addison in 1855, characterized by the impaired production of all hormones from the adrenal cortex. PAI is mainly characterized by a deficiency in glucocorticoids, but mineralocorticoid deficiency and androgen deficiency or excess may also occur (1). Although rare, PAI is a life-threatening disease requiring prompt recognition

and treatment. However, diagnosis and management can be challenging, particularly in children, and there is a high chance of a greatly delayed diagnosis.

Several conditions, either congenital or acquired, can be responsible for PAI (2, 3). Autoimmune Addison's disease (AAD) is the most common cause in adults in industrialized countries (4–7), whereas genetic defects are the most frequent etiology in children (2); indeed, congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency (21OH-D) represents the main cause of childhood-onset PAI. Nevertheless, among all possible causes of childhood-onset PAI, 21OH-D is the only condition that has been extensively characterized (8, 9); all other forms have not been adequately described, and the epidemiology, etiology, and long-term outcome of adrenal insufficiency in childhood still need to be well defined. Studies from the USA (10), Canada (11), and Finland (12) reported autoimmunity as the second most common cause of PAI in children following 21-OHD; accordingly, we confirm these data in a recent study describing the etiology of childhood-onset PAI in a large nationwide cohort from Italy (13). However, studies from Australia (14) and China (15) did not confirm this predominance.

It is well known that autoimmune adrenal insufficiency may develop in the context of several different conditions either in isolation or associated with other autoimmune disorders, and that clinical presentation differs depending on several factors, including the underlying disease. However, although many authors have described the epidemiology, presentation, and long-term outcomes of autoimmune forms in adults, data regarding the presentation and outcome of the autoimmune conditions responsible for AAD in children are still scant.

The aim of this review is to summarize the current knowledge on the etiology, presentation, and treatment of AAD in childhood.

Case 1

A 13-year-old girl developed a loss of appetite associated with chronic fatigue and self-injurious behavior and was managed with psychological intervention. In the next 2 years, her condition progressively worsened; she continued to lose weight and started to present recurrent episodes of nausea, vomiting, and abdominal pain. Several gastrointestinal disorders were excluded. She eventually received a diagnosis of primary eating disorder and depression and started treatment with antidepressants without significant improvement. At the age of 15 years, she was hospitalized for acute deterioration in health status. At admission, she was dehydrated, tachycardic, and hypotensive (blood pressure, 90/50 mmHg). Biochemistry revealed the presence of metabolic acidosis, hyponatremia (sodium levels, 125 mmol/l, n.v. 135–145), hyperkalemia (potassium levels, 6.5 mmol/l, n.v. 3.5–5.0), and hypoglycemia (glucose levels, 49 mg/dl). Skin hyperpigmentation localized at gums and knuckles, was noted. Owing to the presence of hyperpigmentation and typical electrolyte abnormalities, adrenal function was assessed. Undetectable serum cortisol (<0.20 µg/dl), elevated adrenocorticotropic hormone (ACTH) concentration (1,237 pg/ml, n.v. 10–130), low aldosterone levels (<37 pg/ml, n.v. 40–432), and increased renin levels (500 pg/ml, n.v. 3.1–59.5) led to a diagnosis of PAI, and she was started on iv hydrocortisone treatment with a significant improvement in her clinical condition and electrolyte abnormalities. A diagnostic workup to assess the underlying etiology of the disease revealed the presence of adrenal autoantibodies, leading to a definitive diagnosis of AAD.

Case 2

A boy with an uneventful history presented with protracted bloody diarrhea, nausea, and vomiting at the age of 9.9 years. Stool culture revealed the presence of *Campylobacter jejuni* and he received treatment with antibiotics. However, after a transient improvement, he continued to present recurrent vomiting (approximately two episodes/day) and developed severe constipation associated with long-lasting fatigue. Several investigations excluded gastrointestinal disorders. Eight months later, his conditions worsened, limiting his daily activities, and he had lost more than 5% of his body weight; therefore, he was referred to a pediatric hospital. Blood examination revealed hyponatremia (sodium, 121 mmol/l, n.v. 135–145) without other overt electrolyte abnormalities. At physical examination, the patient appeared to be slightly dehydrated and hypotensive; mild hyperpigmentation of the gums and labial mucosa was also noted, raising suspicion of AI. Adrenal function was therefore assessed and showed a significant increase in ACTH levels (877 pg/ml, n.v. 10–130), with low-normal levels of cortisol (7 µg/dl) and an increased concentration of plasma renin (2,398 pg/ml, n.v. 3.1–59.5). A standard-dose Synacthen test (SDSST) was performed because of equivocal levels of morning cortisol and definitively confirmed the diagnosis of PAI, revealing an insufficient peak of cortisol after stimulation (7.7 µg/dl). Investigations on the causes of primary adrenal failure, including steroid synthesis intermediates, very-long-chain fatty acid concentrations, and screening for infections, were negative. Adrenal cortex and 21-OH antibodies were positive, thus allowing a diagnosis of AAD.

Case 3

A boy 10.7 years of age with Hashimoto's thyroiditis was admitted at the emergency department after a few syncopal episodes occurring in the last 6 months. He complained about long-lasting fatigue and recurrent nausea. Blood tests were all within normal range except for slight hyponatremia (sodium levels ranging from 130 to 134 mmol/l, n.v. 135–145). No organic causes of nausea and hyponatremia were detected; therefore, the boy was discharged after iv hydration and an improvement in his general condition. Over the following 4 months, he continued to complain of weakness and nausea and started to present episodes of vomiting and walking difficulties. Physical examination revealed the presence of slight hypotension (blood pressure, 95/60 mmHg) and tachycardia (heart rate, 100 bpm). Moreover, slight skin hyperpigmentation localized at the gums, knuckles, and palmar creases was detected. Laboratory evaluation confirmed

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hyponatremia (128 mmol/l, n.v. 135–145) and normal levels of potassium (4.5 mmol/l, n.v. 3.5–5.0). Owing to the persistence of unexplained hyponatremia and hyperpigmentation in a patient with autoimmune thyroid disorder, adrenal function was assessed, revealing very low cortisol levels (3 μg/dl) associated with a moderate increase in ACTH (260 pg/ml, v.n.10–130) and renin concentrations (239 pg/ml, v.n. 0.9–13), leading to a diagnosis of PAI. Considering that he was affected by a comorbid known autoimmune condition, diagnostic workup started with an evaluation of adrenal autoantibodies, which was positive, confirming AAD.

Autoimmune Addison's disease

Etiology

In AAD, the impaired production of adrenal hormones is due to the autoimmune destruction of the adrenal cortex, which typically first affects the zona glomerulosa and subsequently the zona fasciculata and reticularis. As for other autoimmune diseases, environmental triggers as viral infections, therapeutic agents, and mental health disorders are thought to initiate an autoimmune attack of the adrenal cortex in susceptible individuals (3). The effector cells of this autoimmune attack are thought to be CD8 and CD4 lymphocytes directed against 21-OH (3, 5, 16). Although autoimmune destruction of the adrenal cortex is due to cell-mediated immunity, AAD is characterized by the presence of non-pathogenic autoantibodies against the adrenal cortex, which are currently considered the gold standard for the diagnosis, monitoring, and prediction of AAD (3, 5, 16).

AAD can occur as an isolated condition, although in more than two-thirds of patients it is associated with other autoimmune disorders in the context of autoimmune polyglandular syndromes (APSs) (3, 7, 16, 17). APSs include a heterogeneous group of diseases sharing fundamental characteristics (18) (Table 1). Although there is no unanimous consensus on their classification, currently, APSs are mainly classified as APS type 1 (APS-1) and type 2 (APS-2) (19), and AAD represents the bridge between these two entities.

APS-1, also named autoimmune polyendocrinopathy candidiasis ectodermal dystrophy (APECED), is a rare monogenic disease caused by mutations in the autoimmune regulator gene (*AIRE*) located on chromosome 21. Classically APS-1 was considered an autosomal recessive disease, but recently dominant negative mutations associated with milder autoimmune manifestations have been identified (19, 20). *AIRE* is expressed in medullary thymic epithelial cells and induces the expression of self-proteins expressed in other tissues, allowing their presentation to developing T cells and inducing apoptosis of self-reactive thymocytes (18–20). Moreover, AIRE promotes the generation of self-antigen-specific regulatory T cells that are involved in maintaining self-tolerance (18–20). Therefore, the absence of AIRE determines an impairment in deletion and in the peripheral control of autoreactive T cells with autoimmune attack against multiple tissues and organs (18, 19). In addition, reduced T-cell tolerance determines a dysregulation of B cells with a consequent production of autoantibodies directed against cytokines and tissue antigens that are considered specific markers of corresponding organ-specific autoimmune diseases (20, 21). Indeed, patients with APS-1 present multiple autoantibodies directed against tissue-specific antigens that often precede the onset of the corresponding clinical manifestation; among them, the most specific are autoantibodies against interferon-α and interferon-ω, which are almost invariably detected in affected subjects and have been proposed as a valid diagnostic aid (21, 22).

Despite its monogenic etiology, great variability in the spectrum and severity of APS-1 has been documented. A widely variable clinical picture has been observed even in patients carrying the same *AIRE* mutation, thus suggesting the influence of other environmental, immunological, or genetic factors on the expression of the disease (16, 19, 20, 23). An example are MHC alleles; studies on large cohorts of subjects have indeed demonstrated that patients with APS-1 carrying the DRB1*03 allele have a higher prevalence of AAD than those with different HLA haplotypes (24).

APS-1 is a rare disease and its prevalence is estimated at 1:100.000 and is higher in some particular populations, such as Iranian Jewish (1:9.000), Finland (1:25.000), Norway (1:90.000), Poland (1:129.000), and Ireland (1:130.000) (25, 26). APS-1 usually presents in childhood or in early adolescence (19, 27) and is clinically defined by the presence of at least two of the following

TABLE 1 The main features of autoimmune polyglandular syndromes (APSs).

	APS-1	APS-2
Prevalence	1:100,000 (variable)	1:20,000
Typical age at onset	Childhood	Adolescence-early adulthood
Inheritance	Monogenic	Polygenic
Genetic background	AIRE	HLA-DR3 and HLA-DR4 CTLA4 PTPN22
Main clinical features	Chronic mucocutaneous candidiasis Chronic hypoparathyroidism Autoimmune Addison's disease	Autoimmune Addison's disease Autoimmune thyroiditis Type 1 diabetes
Additional features	Hypergonadotropic hypogonadism Type 1 diabetes Autoimmune thyroiditis Autoimmune hypophysitis Alopecia Vitiligo Recurrent urticariod rash Nail dystrophy Autoimmune gastritis Autoimmune hepatitis Chronic diarrhea Constipation Malabsorption Autoimmune intestinal dysfunction	Hypergonadotropic hypogonadism Vitiligo Chronic atrophic gastritis Autoimmune hepatitis Celiac disease Myasthenia gravis Alopecia

conditions: chronic mucocutaneous candidiasis (CMC), chronic hypoparathyroidism (CH), and Addison's disease. Usually, CMC is the first manifestation appearing before the age of 5 years, followed by CH before the age of 10 years and then AAD (28), although a precise chronological order of presentation of different components is not always present (20).

The onset of Addison in APS-1 patients generally occurs within the second decade of life, and the mean age varies between 11 and 16 years, depending on the clinical series (18, 26); however, earlier onset has also been documented; according to current literature, the earliest age of presentation of AAD in patients with APS-1 is 2 years (12, 26, 29, 30).

The prevalence of AAD in patients with APS-1 is approximately 65%, with no gender difference, but a high variability in different case series has been reported. In a recent Italian series evaluating 158 subjects, the prevalence increased with age (77.2% at the end of follow-up). The clinical phenotype of APS-1 is complex and also includes several other endocrine and non-endocrine components (19, 20, 27). Hypergonadotropic hypogonadism, type 1 diabetes, autoimmune thyroiditis, and hypophysitis are the most common endocrinopathies, and ectodermal involvement is characterized by alopecia, vitiligo, recurrent urticariod rash, or nail dystrophy (20, 28). Gastrointestinal manifestations represented by autoimmune gastritis and hepatitis, chronic diarrhea, constipation, malabsorption, and autoimmune intestinal dysfunction are also common (20, 28, 31). Finally, rare features such as autoimmune pneumonitis, inflammatory demyelinating polyneuropathy, tubular interstitial nephritis, keratitis, and asplenia have been described in these patients (20, 28).

APS-2 is a more common condition characterized by at least two of the following endocrinopathies: AAD, autoimmune thyroiditis, and type 1 diabetes (19, 27). APS-2 prevalence is estimated at 1:20.000, with a male-to-female ratio of 1:3 (19, 25, 27). In contrast to APS-1, which typically presents in childhood, the peak incidence of APS-2 is in the third and fourth decade of life and the number of children reported in the literature is still limited (32). APS-2 is a multifactorial and polygenic disorder with a complex heritability. Indeed, genetic studies on APS-2 patients showed that the same genetic variants are associated with an increased risk of several autoimmune diseases (19). These variants are mainly localized in genes regulating immune system function. Specifically, the HLA system has been considered to have an important role in genetic predisposition; indeed, the HLA-DR3 and HLA-DR4 alleles have been associated with an increased risk of APS-2, although the underlying mechanism is still not completely understood (16, 33). In addition, polymorphisms in other genes involved in the regulation of the immune system, such as *CTLA4* and *PTPN22*, have been reported to be risk factors. However, to date, there is no genetic or immunological diagnostic marker of APS-2 and the diagnosis only relies on the co-occurrence of typical autoimmune diseases.

Autoimmune thyroid disease represents the most frequent autoimmune endocrinopathy in APS-2, occurring in approximately 70% of patients; type 1 diabetes occurs in approximately 40–60% of patients, and is often the first manifestation, and ADD occurs in 40–50% of affected subjects (25). Other possible manifestations in the context of APS-2 are primary hypogonadism, vitiligo, chronic atrophic gastritis, hepatitis, celiac disease, myasthenia gravis, and alopecia (27).

APS-2 is an evolutive disorder, and a large time interval between first and second manifestations can occur (25). For example, it has been estimated that up to 50% of patients with AAD may develop another autoimmune disease throughout their lives (34). Relevant autoantibodies may be detectable years before the onset of AAD and thus testing for autoantibodies may be helpful in assessing disease risk (19).

As already mentioned, so far only a few patients with a diagnosis of APS-2 during childhood and adolescence have been described in detail (32, 35–49). Age at the presentation of APS-2 varied between 8 and 17 years, and the disease was only diagnosed before the age of 10 years in a few patients. In these pediatric reports, AAD was highly frequent and represented the most common manifestation of APS-2 in association with autoimmune thyroiditis; other manifestations variably associated were type 1 diabetes, celiac disease, vitiligo, and hypergonadotropic hypogonadism. The diagnosis was made through periodic screening in subjects with a pre-existing autoimmune manifestation or following a life-threatening event, such as adrenal crisis or diabetic ketoacidosis.

In addition, other patients with childhood-onset APS-2 have been reported in several cohorts of subjects with PAI from Italy (13, 50), Canada (11), the USA (10), and Finland (12), although clinical presentation or outcome have not been provided in detail (Table 2).

TABLE 2 The prevalence of autoimmune PAI, isolated Addison's, and APSs in pediatric case series.

	Perry et al. (2005) (11)	Hiesh et al. (2011) (10)	Capalbo et al. (2021) (13)	Borchers et al. (2023) (12)
Country	Canada	USA	Italy	Finland
Study population (n)	29	42	121	42
AAD (%)	45%	55%	37%	67%
- Isolated AAD (%)	31%	73%	20%	47%
- APS-1 (%)	38%	7%	55%	28%
- APS-2 (%)	31%	20%	25%	25%

AAD, Autoimmune Addison's disease; APS, autoimmune polyglandular syndrome.

Finally, isolated AAD also presents high heritability as APSs. So far, several genetic variants associated with an increased risk of isolated AAD have been described. As for APS-2, HLA-DR3 and HLA-DR4 alleles as well as polymorphisms in *CTLA4* and *PTPN22* have been associated with an increased risk of AAD but also variants in several genes (*BACH2*, *GATA3*, *LPP*, *IKZF4*, *SH2B3*, *CIITA*, *SULT1A2*, *CLEC16*, *MIC-A*, *MIC-B*, *NLRP1*, *SIGLEC5*, *LIME1*, and *UBASH3A)* have been reported to represent genetic risk factors (3, 16, 33, 51).

Epidemiology

Only few case series on autoimmune PAI in children are available and the frequency of AAD in childhood still needs to be established. Overall, AAD prevalence can be influenced by the patient's age (18), as well as by the underlying etiology and the ethnicity. So far, AAD has been reported as the most common cause of PAI in children after 21-OHD CAH by some authors. Indeed, data from US, Canadian, Italian, and Finnish studies have identified an autoimmune etiology in up to 67% of non-CAH PAI children (10–13). The prevalence of autoimmune PAI, as well as the prevalence of APSs in comparison with isolated Addison's, varies on the basis of the population, as reported in Table 2.

Presentation

Autoimmune destruction of the adrenal cortex is a slow-progressive process; moreover, symptoms occur when up to 90% of adrenal cells are destroyed (5). As a consequence, AAD may typically remain subclinical for long periods before signs of AI become clinically evident (52). Even when adrenal insufficiency becomes manifest, clinical signs are often highly unspecific, including malaise, weakness, musculoskeletal pain, anorexia, reduced weight growth, orthostatic hypotension, salt craving, dizziness, abdominal pain, nausea, and vomiting (7), which can all be symptoms common to other different diseases. The vague nature of symptoms may often lead to misdiagnosis and cause considerable diagnostic delay. In a recent study, we reported that diagnostic delay is related to the underlying diagnosis being greater in children with AAD or X-ALD than in children with inherited monogenic conditions (13). Among all signs of PAI, hyperpigmentation is the most specific and represents the most remarkable clues for the diagnosis (3). A reduction in cortisol levels leads to the increased production of pro-opiomelanocortin (POMC), an ACTH precursor, and from its cleavage, alpha-melanocyte stimulating hormone (a-MSH) is also produced. Thus, PAI patients present increased levels of a-MSH, which acts on melanocytes favoring the production of eumelanin with a consequent increase in skin pigmentation, which is more evident in areas exposed to the sun (face, neck, back of the hand, and knuckles), normally hyperpigmented (nipple, scrotum, and labia), or subjected to friction and microtrauma (3) (Figure 1). Moreover, an increase in nevi, darkening of scars, and mucosae pigmentation are typical findings (53). Although being specific, hyperpigmentation may not be significant at the onset and therefore it is useful in identifying chronic but not acute adrenal insufficiency (2).

Typical laboratory findings of PAI include hyponatremia, hyperkalemia, and hypoglycemia. Hyponatremia is the most

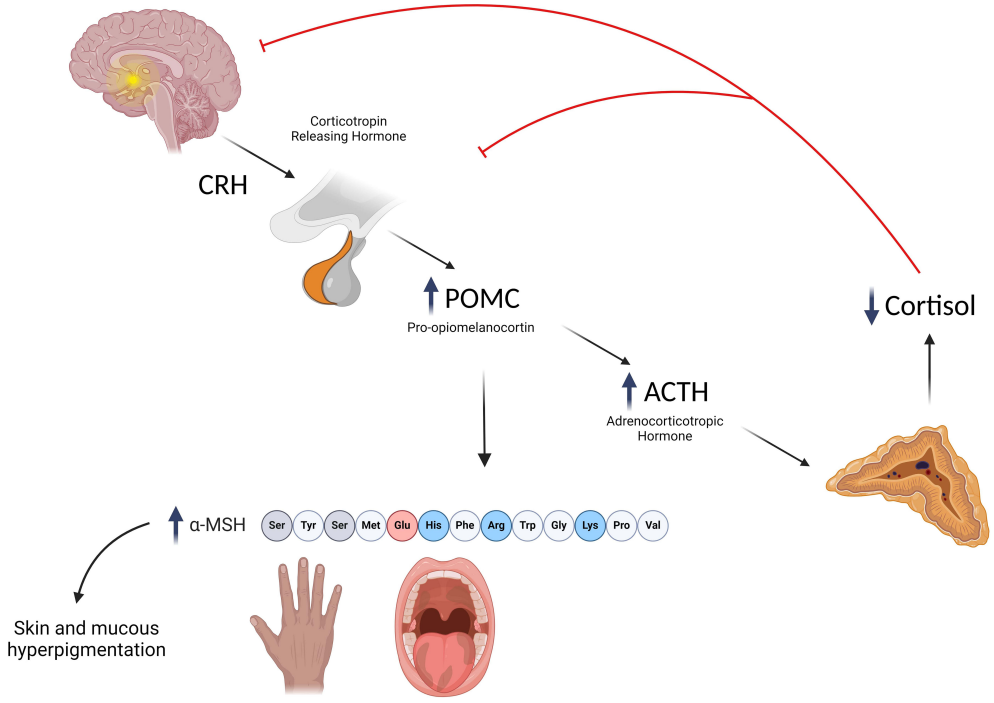

FIGURE 1
Mechanisms underlying the development of hyperpigmentation in primary adrenal insufficiency. Created with BioRender.com.

common alteration and even when isolated should raise the suspicion of AI (2, 3, 5, 13, 17, 53). Hypercalcemia, anemia, lymphocytosis, and eosinophilia can be rarely present (5, 53).

Owing to delays in the identification of symptoms and the variable length of time between presentation and definitive diagnosis, in many patients, a life-threatening adrenal crisis may be the first presentation of AAD (2, 3). Adrenal crisis is a serious condition characterized by weakness, muscular pain, vomiting, abdominal pain, dehydration, hypotension, impaired consciousness eventually associated with hyponatremia, hyperkalemia, and acidosis, which can lead to hypovolemic shock and death (54). However, clinical features at onset can be non-specific as well, and adrenal crisis can go unrecognized, especially in children (55). Common triggers for an adrenal crisis are acute stress as illness, infection, a surgical procedure, trauma, or severe psychological stress (7).

Finally, when AAD develops in the context of APSs, other autoimmune signs or diseases typical of APS type 1 or 2 (e.g., CMC, CH, and type 1 diabetes) may be present and in some cases may precede the onset of Addison's, representing an important clue favoring an early diagnosis of PAI.

Diagnostic approach

Once suspicion of adrenal insufficiency has been raised on the basis of clinical findings or history, an evaluation of cortisol and ACTH should be performed. A serum cortisol <5 µg/dl (<140 nmol/l) associated with increased ACTH (twice the upper normal limit) in the morning or during stress is diagnostic of PAI (1). In equivocal cases, a dynamic evaluation of adrenal function after SDSST can be performed to increase the sensitivity and specificity of basal hormonal measurement. Traditionally, a peak cortisol level of <18.0 µg/dl (500 nmol/l) after SDSST indicates adrenal insufficiency. However, this threshold is based on older serum assays having a high cross-reactivity with non-cortisol steroids. As the normal range of cortisol depends on the assay used, guidelines clearly indicate that checking the reference ranges of the laboratory is always recommended (1, 7). Indeed, newer more-specific immunoassays or LC-MS/MS may have lower thresholds for normal secretion and a recent study has suggested a new cortisol cutoff after SDSST of 14 (386 nmo/L) to 15 µg/dl (414 nmol/L) to define AI (depending on the specific assay) when using newer methods (56, 57), although specific ranges of normality still need to be established in children.

Recently, salivary cortisol has been proposed as valid alternative to serum cortisol (58), but standardization of the assays and accurate cutoffs are so far lacking (2).

High renin concentrations/activity and low aldosterone levels confirm mineralocorticoid deficiency (1). Of note, an increase in renin levels is an early marker of autoimmune adrenal insufficiency and precedes the alterations in ACTH and cortisol concentrations, and deserves particular attention. Indeed, autoimmune destruction typically first affects the zona glomerulosa, leading to mineralocortioid deficiency before glucocorticoid insufficiency (5).

A stepwise diagnostic approach guided by age, the clinical presentation, and associated diseases is recommended (1, 2) (Figure 2). Autoimmune etiology is mainly supported by the presence of adrenal autoantibodies (17). These antibodies are directed against steroidogenic P450 autoantigens: 21-OH, 17α-OH, and cholesterol side-chain cleavage enzyme (17, 53, 59); 21-OH is typically expressed in all areas of the adrenal cortex, and 17α-OH and cholesterol side-chain cleavage enzyme are expressed in all steroid-producing cells, thus increasing the risk of primary autoimmune hypogonadism.

Autoantibodies targeting 21-OH are highly sensitive and they are currently considered markers of AAD, although they do not

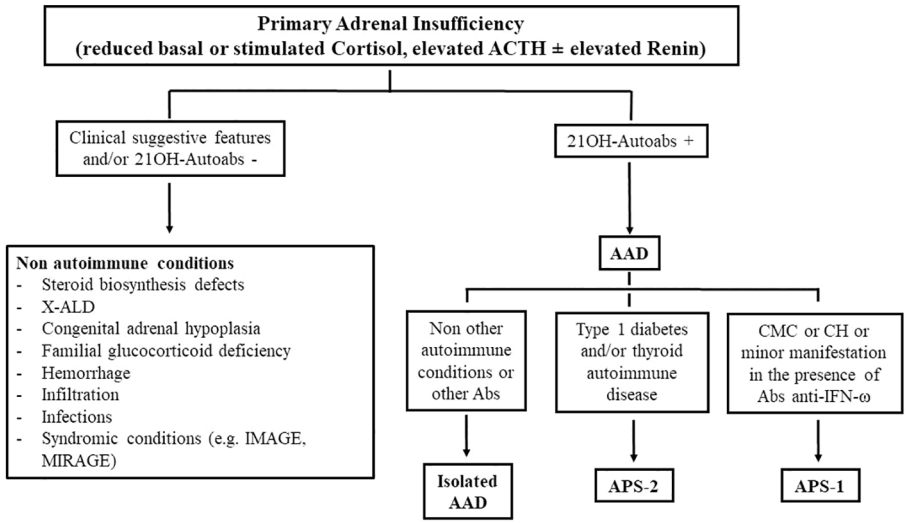

FIGURE 2
The diagnostic approach in autoimmune and non-autoimmune primary adrenal insufficiency. 21-OH, 21-hydroxylase; autoabs, autoantibodies; X-ALD, X-linked adrenoleukodystrophy; AAD, autoimmune Addison's disease; CMC, chronic mucocutaneous candidiasis; CH, chronic hypoparathyroidism; IFN, interferon.

have a defined pathogenic role in the autoimmune destruction of the adrenal gland (53, 60). Indeed, 21-OH autoantibodies have been detected in 80–90% of subjects with PAI in cross-sectional studies after the exclusion of non-autoimmune conditions (61, 62). Moreover, so far, adrenal autoantibodies have been reported in very few patients without AAD and in particular in 1.4% of patients with non-adrenal autoimmune diseases, 5% of relatives of patients with AAD, and 0.6% of healthy individuals (63), thus confirming their high specificity.

The presence of adrenal antibodies might hint at a future risk of developing PAI as they may anticipate the clinical manifestation of AAD (17).

Indeed, it has been well established that the evolution of AAD presents different biochemical phases: stage 0, an isolated presence of autoantibodies with no impairment in adrenal function; stage 1, an isolated increase in renin concentrations/activity; stage 2, a reduced cortisol response after a stimulation test; stage 3, increased basal ACTH levels; and stage 4, reduced cortisol levels (5, 53) (Table 3). Therefore, owing to their predictive role, adrenal autoantibody testing in patients with pre-existing autoimmune diseases may help in identifying subjects at risk of developing AAD in a preclinical phase (64). In these patients, a periodical evaluation of adrenal function, including renin concentrations/activity, allows early diagnosis that can prevent a life-threatening adrenal crisis (27, 53). However, not all patients with positive adrenal autoantibodies will develop overt AI; the risk of progression toward clinical AAD is higher in patients with APS-1 (74–90%) than in patients with isolated AAD or APS-2 (0–44%) (64). In a recent Italian case series, although all APS-1 patients at stage 1 developed overt AAD, approximately 50% of APS-2 patients remained at stage 1 for a long period and 12% improved to stage 0; moreover, few APS-2 patients (2.5%) became negative during follow-up (64). However a lower threshold for the suspicion of AI in patients with pre-existing autoimmune morbidites is advisable. On the other hand, periodic screening for additional autoimmune diseases in patients diagnosed with AAD should be encouraged to correctly diagnose APSs (3, 7). For example, the association of AAD with autoimmune thyroiditis or type 1 diabetes is suggestive of APS-2 (19, 27), whereas the association with chronic mucocutaneous candidiasis or hypoparathyroidism suggests a diagnosis of APS-1, which needs be confirmed by an analysis of the *AIRE* gene (19, 20, 27) (Figure 2). With regard to the diagnosis of APS-1, recently diagnostic criteria seem to be expanding as it has been documented that, in some cases, minor manifestations may precede the onset of a classic dyad; among these manifestations, urticariod rash, enamel hypoplasia, and intestinal malabsorption were the most common (20). In these patients, screening for autoantibodies against interferon-ω or *AIRE* gene analysis should be performed to confirm diagnosis (19–22).

Treatment and management

The treatment of autoimmune PAI mainly relies on hormone replacement therapy and treatment of complications. After PAI has been confirmed or in cases with highly suggestive severe symptoms, patients should begin replacement treatment with glucocorticoids and mineralocorticoids, without awaiting etiological confirmation (1). The adrenal cortex normally produces approximately 10 mg/m^2 of cortisol every day with a specific circadian rhythm characterized by high levels in the early morning and low concentrations during the night. Oral hydrocortisone is the standard replacement treatment used in children with PAI. For AAD patients, hydrocortisone at a dose of 8–10 mg/m^2 per day divided into 3–4 doses is recommended, with a half to two-thirds of the total dose in the morning (1–3). A deficit in mineralocorticoids is almost invariably present in patients with AAD and fludrocortisone (9-alphafluorohydrocortisone) at a dosage of 0.05–0.2 mg/day in the morning should be added (1–3, 53). The treatment of adrenal crisis consists of the administration of iv saline and glucose and administration of hydrocortisone intravenously at a dose of 50–100 mg/m^2 followed by 50–75 mg/m^2 per day divided into four doses or as a continuous infusion (2, 3).

A key point in the management of AAD patients is represented by the prevention of adrenal crises, which still contribute to the mortality of PAI patients and are commonly triggered by infections, surgical procedures, intense physical activity, or treatment withdrawal (7, 53). The education of parents and patients plays a key role and should be repeated periodically during clinical evaluations (65). Indeed, all caregivers of PAI patients must understand the need to increase the glucocorticoid dose in case of illness, severe psychological/physical stress, or minor surgery (54) or administer intramuscular glucocorticoids when necessary; they must be also able to manage an incipient adrenal crisis (65). Furthermore, clinicians should recommend that patients wear an item indicating their condition in case of emergency (54, 66, 67).

TABLE 3 Biochemical phases of the evolution of autoimmune Addison's disease.

	Adrenal autoantibodies	Renin levels/activity	ACTH levels	Cortisol levels	Cortisol levels after stimulation	Symptoms
Stage 0	Present	Normal	Normal	Normal	Normal	Absent
Stage 1	Present	High	Normal	Normal	Normal	Absent
Stage 2	Present	High	Normal	Normal	Low	Absent
Stage 3	Present	High	High	Normal	Low	Absent
Stage 4	Present	High	High	Low	Low	Present

In cases of APS-1 or APS-2, additional management consists of the adequate replacement of missing hormones and treatment of complications (19, 27).

It is suggested that children with AAD are seen by a pediatric endocrinologist or a healthcare provider with endocrine expertise (1). The suggested frequency of monitoring visits varies with age and associated morbidities or conditions. APS-1 patients are best followed by a multidisciplinary team led by an endocrinologist at a specialized center and with a minimum of two follow-up visits per year (19).

To date, no single biomarker for monitoring glucocorticoid treatment is available and the adequacy of treatment should be modulated on clinical evaluations, signs of overtreatment or undertreatment, general wellness, and growth (1, 3). Instead, salt craving, blood pressure, electrocyte levels, and renin concentrations/activity are used to assess mineralocorticoid treatment adequacy (2, 3, 53). As renin activity may lack accuracy, renin concentration is considered a more clinically relevant marker of the biochemical status and fludrocortisone dose (68, 69).

Physicians should always be aware that patients with AAD, in particular those affected with APSs, are at an increased risk for the development of another organ-specific autoimmunity, and periodic screening of autoantibodies or suggestive signs and symptoms of autoimmune diseases is advisable (3, 27). This screening includes an evaluation of thyroid function, diabetes mellitus, premature ovarian failure, celiac disease, and autoimmune gastritis, although the optimal frequency of screening is still not established and depends on the clinical evaluation as well as the diagnosis and complexity of the entire condition (7).

Back to the patients

In the three described cases, Addison's disease was diagnosed with a considerable delay as non-specific symptoms initially pointed to a wrong diagnosis. In all cases, hyperpigmentation helped in the recognition of PAI; however, hyperpigmentation is not significant at onset and is useful in identifying chronic but not acute adrenal insufficiency. Of note, each patient presented some specific clues that could have prompted an earlier diagnosis. In case 1, the diagnostic delay was largely related to her anchoring psychiatric diagnosis and she was correctly diagnosed only after adrenal crisis developed. This bias is common and PAI can be easily misdiagnosed with psychiatric diseases (70–73) as anorexia, weight loss, sleep disturbances, and behavior changes can be presenting symptoms of both diseases. Up until now, this patient has received a diagnosis of isolated AAD as autoimmune diagnostic work-up has not revealed any other autoantibody positivity or adjunctive manifestations during the follow-up.

In case 2, vomiting and abdominal pain dominated the clinical picture at onset thus leading to an erroneous suspicion of GI disorder. Indeed, gastrointestinal symptoms are common in the early stages of adrenal insufficiency (40, 74–77); in particular, nausea progressing to vomiting is a typical presentation of the disease. In this particular case, the index sign was the combination of vomiting, hyponatremia, and hyperpigmentation, which should have prompted a suspicion of AI. Although no other major sign was present, the early onset of the disease and the presence of specific signs as alternating constipation/diarrhea, raised a suspicion of APS-1 and the patient was tested for autoantibodies against interferon-ω, which was positive. *AIRE* gene analysis confirmed the diagnosis, revealing causative homozygous mutations (c.47C>T) of the gene. No other components of the disease have developed up to his last follow-up at the age of 11.5 years. The patient is currently on hydrocortisone and fludrocortisone replacement treatment. This case confirms the huge heterogeneity in the presentation of APECED, the need for high awareness, even in the absence of a classic triad, and the importance of an increased alert for adrenal insufficiency in case of unexplained GI symptoms (20, 28, 31).

Finally, in case 3, the presenting sign was characterized by isolated mild hyponatremia. Hyponatremia is indeed the most common laboratory finding in PAI (13), even in the absence of hyperkalemia. In this case, the pre-existing autoimmune thyroiditis hinted at a diagnosis and association of AAD, and thyroiditis led to a diagnosis of APS-2. During the follow-up, no adjunctive autoimmune diseases or signs were diagnosed. This history highlights how unexplained protracted hyponatremia should raise the suspicion of adrenal insufficiency, even in the absence of hyperkalemia or other specific signs (13), and points out the importance of an increased awareness for AI in children with other pre-existing autoimmune manifestations.

Conclusion

AAD is an important cause of PAI in children, either in isolation or in the context of APSs. Clinical and biochemical signs are evident only when most adrenal function is impaired and are often non-specific, thus pointing to a wrong diagnosis, which puts patients at risk of a life-threatening adrenal crisis. Autoimmune PAI in children is associated with a greater diagnostic delay than inherited genetic conditions. Therefore, it is important to increase physicians' awareness on signs that should raise an early suspicion of adrenal insufficiency, such as unexplained weakness, gastrointestinal or psychiatric disorders, or hyponatremia. An autoimmune condition should be suspected in all those subjects with adrenal insufficiency and without specific signs related to other genetic/syndromic disease, at any age. Of note, a low threshold for suspicion should also be kept in patients with known pre-existing autoimmune conditions. In these cases, the screening of adrenal autoantibodies is helpful in identifying at-risk subjects who deserve careful adrenal function monitoring.

The management of AAD patients should include a program of adrenal crisis prevention through the continuous education of patients and families in managing stressful events. Studies on large cohorts of children with autoimmune PAI are needed to increase the current knowledge on the presentation, natural history, and long-term outcomes of the disease in childhood and to improve the early recognition and treatment of the disease.

Author contributions

DC: Writing – original draft, Writing – review & editing. AE: Writing – original draft, Writing – review & editing. VG: Writing –

review & editing, Writing – original draft. PL: Writing – review & editing. SV: Writing – review & editing. RD: Writing – review & editing. MS: Writing – original draft, Writing – review & editing.

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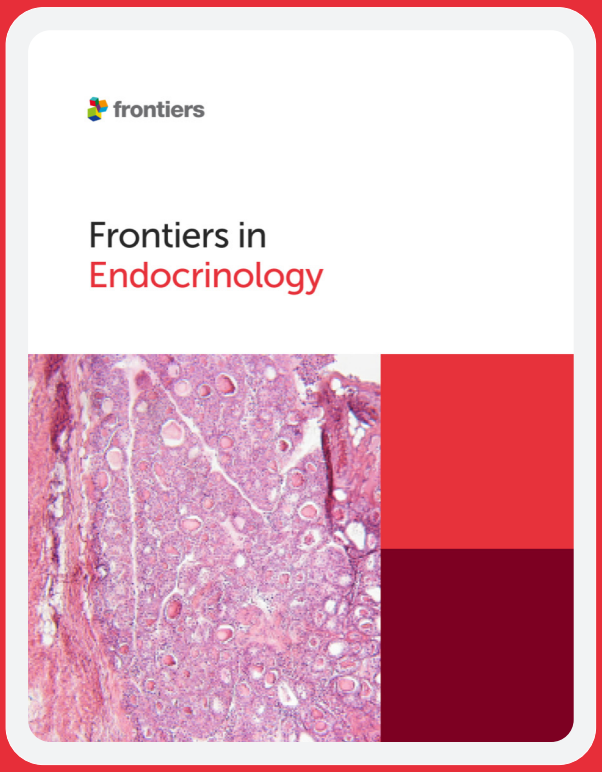

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