Primary Adrenal Insufficiency in childhood: data from a large nationwide cohort.

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Disclosure Statement: The authors have nothing to disclose.

Abstract

Context: Primary adrenal insufficiency (PAI) is a rare and potentially life-threatening condition, poorly characterized in children.

Objective: to describe causes, presentation, auxological outcome, frequency of adrenal crisis and mortality of a large cohort of children with PAI.

Patients and methods: data from 803 patients from 8 centers of Pediatric Endocrinology were retrospectively collected.

Results: the following etiologies were reported: 85% (n=682) Congenital Adrenal Hyperplasia (CAH) due to 21-hydroxylase deficiency (21-OHD); 3.1% (n=25) X linked Adrenoleukodystrophy; 3.1% (n=25) Autoimmune Polyglandular syndrome type 1; 2.5% (n=20) autoimmune AI; 2% (n=16) Adrenal Hypoplasia Congenital; 1.2% (n=10) CAH non 21-OHD; 1% (n=8) rare syndromes; 0.6% (n=5) Familial Glucocorticoid Deficiency; 0.4% (n=3) acquired AI; 9 patients (1%) did not receive diagnosis. Since 21-OHD CAH has been extensively characterized, it was not further reviewed. In 121 patients with a diagnosis different from 21-OHD CAH, the most frequent symptoms at diagnosis were fatigue (67%), hyperpigmentation (50.4%), dehydration (33%) and hypotension (31%). Elevated ACTH (96.4%) was the most common laboratory finding followed by hyponatremia (55%), hyperkalemia (32.7%) and hypoglycemia (33.7%). The median age at presentation was 6.5±5.1 years (0.1-17.8 years) and the mean length of symptoms before diagnosis was 5.6±11.6 months (0-56 months) depending on etiology. Rate of adrenal crisis was 2.7 per 100 patients years. Three patients died for the underlying disease. Adult height, evaluated in 70 patients, was -0.70±1.20 SDS.

Conclusions: we characterized one of the largest cohorts of children with PAI aiming to improve the knowledge on diagnosis of this rare condition.

Keywords: Primaryadrenal insufficiency, Addison's disease, adrenal crisis, adult height

Introduction

Primary adrenal insufficiency (PAI) is a rare, potentially life-threatening, condition due to abnormalities of steroid biosynthesis or of adrenal gland development and responsiveness (1-3). PAI is characterized by impaired secretion of glucocorticoids and can be accompanied by mineralocorticoid and adrenal androgens deficiency or excess, depending on the underlying cause (4-6). In adulthood the prevalence is about 100 to 140 cases per million (1) and the most common etiology is represented by autoimmunity (up to 90% of cases), followed by glandular infiltration by tuberculosis, adrenalectomy, neoplasia, various genetic causes and iatrogenic factors (1-2, 7-8). Conversely in childhood, PAI is more frequently associated with inherited monogenic disorders, especially enzyme defects occurring as isolated forms or as a part of a syndromic condition (3, 5, 9).

The most common form of PAI in children is indeed represented by Congenital Adrenal Hyperplasia (CAH) caused by 21-hydroxylase deficiency (21-OHD) (5, 9). Less common forms of CAH responsible of PAI include *CYP11A1*, *CYP11B1*, *CYP17A1*, *HSD3B2* and *POR* deficiencies (5, 10, 11,12). Severe disruption of *CYP11A1* causes a block in all aspects of adrenal and gonadal steroid synthesis (10); *CYP11B1* deficiency comprises glucocorticoid insufficiency along with androgen excess and hypertension (11); *CYP17A1* defect impairs both cortisol and sex steroid synthesis and causes mineralocorticoid hypertension (13) while defects of *HSD3B2* cause a severe form of CAH with glucocorticoid, mineralocorticoid and sex steroids deficiency (14). Finally, *POR* deficiency manifests with glucocorticoid deficiency, disorder of sex development and may present skeletal malformations (12).

Mutations in *STAR* usually cause lipoid CAH, a disorder characterized by gonadal and adrenal steroid deficiency (15). However, *STAR* and *CYP11A1* defects can give rise to non-classical forms of lipoid CAH and cytochrome p450 side chain cleavage deficiency, respectively, which can masquerade as familial glucocorticoid deficiency (FGD) but are not considered by most to be FGD (16-18).

Other inherited causes are represented by genetic defects causing adrenal hypoplasia (i.e. *NR0B1*, *NR5A1/SF1)*(19-20), adrenal destruction (e.g. *AIRE*, *AAAS*, *ABCD1*, *PEX1*, *LIPA*) (21-23) and FGD and related conditions (e.g. *MC2R,MRAP,MCM4*, *NNT*, *TXNRD2*) (9, 5, 24-30).

Defects in *NROB1,* located on Xp21.3 and encoding a nuclear receptor named DAX1, cause X-linked adrenal hypoplasia congenital (AHC) which comprises PAI, hypogonadotropic hypogonadism and infertility (19, 31) whereas *NR5A1* mutations cause testicular dysgenesis only rarely in combination with PAI (20). Within the group of adrenal destruction, mutations in the autoimmune regulator gene *AIRE* are responsible for Autoimmune Polyendocrinopathy Syndrome type 1 (APS-1) which typically combines PAI with chronic mucocutaneous candidiasis and hypoparathyroidism (21); X-linked Adrenoleukodystrophy (X-ALD), a peroxisomal defect due to mutations of *ABCD1* gene located on Xq28, usually presents with adrenal insufficiency and myelopathy (23) while Triple A or Allgrove syndrome, due to mutations of *AAAS* gene which encodes a nucleoporin named ALADIN, is characterized by the classical triad of alacrimia, achalasia and adrenal insufficiency, and additional neurological problems including motor and sensory peripheral neuropathy, optic atrophy and ataxia (22, 32). FGD is a rare condition characterized by lack of response to ACTH and the presence of isolated glucocorticoid deficiency (26, 33).

Adrenal insufficiency has also been reported as part of recently described multisystemic growth restriction syndromes. IMAGE syndrome, usually caused by mutations in cyclin-dependent kinase inhibitor 1 C (CDKN1C), is characterized by adrenal hypoplasia, intrauterine growth restriction, metaphyseal dysplasia and genitourinary anomalies (34); gain-of-function in Steril Alpha Motif Domain Containing 9 (*SAMD9*), comprises myelodysplasia, infections, restricted growth, adrenal hypoplasia, gonadal anomalies and enteropathy and is termed MIRAGE syndrome (35); deficiency in polymerase epsilon-1 (*POLE1*) comprises adrenal hypoplasia together with growth restriction, variable immune dysfunction and distinctive facial features (36). Deficiency in sphingosine-1-phosphate lyase-1 (*SGPL1*) has also been documented to cause association of PAI with steroid-resistant nephrotic syndrome (37).

Beyond inherited diseases, PAI in childhood may be caused by several other conditionsas autoimmunity, isolated or within the context of polyglandular autoimmune syndromes, bilateral adrenal hemorrhage, infections or drugs (5, 38).

Clinical presentation of PAI in children is highly variable, ranging from subtle and aspecific symptomatology (i.e. muscle weakness, fatigue, anorexia orweight loss) to acute onset with life-threatening adrenal crisis, which has been reported in up to 40% of affected children (5,39). Due to its insidious onset, the diagnosis of PAI is challenging and can be missed or delayed, thus increasing morbidity and mortality (40).

Once PAI is confirmed, adequate treatment should be promptly initiated in order to revert physical and biochemical alterations, avoid life-threatening adrenal crisisand allow normal physical and pubertal development (5). However, even when the disease is appropriately treated, several stressors may precipitate an adrenal crisis (41-42).

Etiology, presentation and long-term outcome of children affected with PAI have been poorly characterized, with the exception of forms associated to 21-OHD CAH (43). Only a few case series so far described causes and clinical findings of PAI not due to 21-OHD CAH in childhood (18, 39, 44-46). All these studies were based on retrospective analysis of small patients' series from single centers and were limited by several methodological biases. Recently, few studies assessed frequency of genetic defects in larger cohorts of children with PAI (24, 47-48) but information on clinical presentation or auxological outcome were detailed only for some specific rare conditions (24,47).

Finally, data on morbidity and mortality and on long-term outcome of children with PAI are scanty.

Aim of the present study was to describe causes, clinical presentation, long-term auxological outcome, frequency of adrenal crisis and mortality of a large, nationwide, multicenter cohort of children with PAI.

Patients and methods

The study design is illustrated in Figure 1. A total of 803 patients with a diagnosis of PAI followed in 8 Italian centers of Pediatric Endocrinology in the last 20 years were included in the study. The patients from this study are from Italian population which is currently estimated 60 million people (with 440.780 live births/year).

A diagnosis of 21-OHD CAH was made in 682/803 (85%) patients and 121/803 subjects (15%) had a different diagnosis; since patients with 21-OHD have been extensively characterized, they were not further reviewed. Data of the 121 patients with PAI not due to 21-OHD CAH were collected and analyzed. In these patients PAI was diagnosed on the basis of cortisol response after intravenous cosyntropin stimulation lower than 500 nmol/L (18mcg/dL) or a morning plasma cortisol level lower than 140 nmol/L (5 mcg/dL) associated with an increased ACTH level (1) in the presence of signs and symptoms of adrenal insufficiency.

Different assays and units of measurement were used across the multiple centers over the entire period of the study. Serum cortisol was assessed by chemiluminescent immunoassays (CLIA) (Immulite 2000, range 50-200 ng/mL; Immulite 2000XP, range 0.6-19.8 mcg/dL; ADVIA Centaur, range 4.3-22.4 mcg/dL) or by electrochemiluminescence immunoassay (ECLIA) (COBAS C8000, range 48-195 ng/ml; Elecsys Cortisol II, range 70-260 nmol/L). All cortisol values were converted in nmol/L to define insufficient cortisol secretion or response.

ACTH concentrations were determined by CLIA (Immulite 2000, range 10-130 pg/mL; COBAS C8000, range 7.2-52 pg/mL) or by immunoradiometric assay (Nichols ACTH IRMA, range 9-52 pg/mL). Plasma renin concentration was measured by direct radioimmunoassay method (RIA) (Sanofi-Pasteur, range 5-90 pg/mL) or by CLIA (Pantec, range 3.1-59.5 pg/ml; LIAISON XL, range 4.4-46.1 mcU/ml).

Autoantibodies against 21-hydroxilase (21-OHAb) and adrenal cortex (ACA) were performed by immunofluorescence technique or ELISA, as appropriate.

Very long chain fatty acids (VLCFA) were evaluated through Gas-chromatography-mass spectrometry using stable isotope dilution for quantification (C22:0, C24:0, C26:0).

Genetic analysis was performed through Sanger sequencing of candidate genes.

The following data were retrieved retrospectively from the patients' files: demographic data, family history, etiology, age at onset of the disease and at diagnosis, clinical signs/symptoms and the following laboratory findings at presentation: electrolytes, blood glucose, ACTH, baseline and corticotropin-stimulated cortisol, renin levels and acid-base balance. Additional information on other diagnostic tests as autoantibodies, VLCFA, genetic testing, imaging studies, were collected when available. Details on therapy regimens at the diagnosis and at last visit were obtained.

The number of episodes of adrenal crisis under steroid replacement was evaluated; adrenal crisis was defined as an acute deterioration in health status associated with an acute hemodynamic disturbance or electrolyte abnormality requiring hospitalization and resolving after parenteral glucocorticoid administration (41).

In order to define the long-term auxological outcome Target Height (TH) and Adult Height (AH), defined as a height velocity of <1 cm/year (49) or bone age ≥15 years in females and ≥17 years in males, were recorded when available. Height was normalized by age and sex in accordance with Italian standard and expressed as standard deviation score (SDS) (50).

Finally, data on mortality were also collected.

This study was approved by the Ethical Committee of University of Naples Federico II. Written informed consent was obtained from patients or their families as appropriate.

Statistical analysis

Statistical analysis was performed using the software SPSS (SPSS Statistics for Windows, version 18.0, IBM Corp). Continuous quantitative variables are expressed as Mean±SDand categorical variables are expressed as frequency distribution. The amount of missing data ranged between 0 and 10% depending on variable and the frequency of variables has been calculated taking into account missing data. Comparison of variables was performed using the One-way Analysis of Variance (ANOVA) or the Tukey post hoc test for multiple comparisons. Categorical variables were compared by the chi-square test. To identify predictors of AH, a stepwise logistic regression model was applied. The level of significance was set at 0.05 (two-tailed).

Results

Causes of PAI and their frequency are shown in Figure 2. As stated above, 21-OHD CAH represented the most common cause accounting for 85% (n=682) of the total PAI cases. A different etiology of PAI was found in 121 patients (15%, M/F=83/38), and also in this group the most frequent causes were inherited monogenic conditions. X-ALD was diagnosed in 25 male patients (3.1%), APS-1 was diagnosed in 25 subjects (3.1%, M/F=9/16), AHC caused by mutations in *NR0B1* were detected in 16 males (2%); among them, 4 patients had contiguous *NR0B1* gene deletions associated with Duchenne muscular dystrophy, glycerol kinase deficiency and mental retardation. Ten patients (1.2%) (M/F=8/2) had CAH other than 21-OHD: 3 males had 11-hydroxylase deficiency (11-OHD), 5 (M/F=4/1) had 3β-hydroxysteroid dehydrogenase deficiency (3β-HSD), 1 male had 17α-hydroxylase deficiency (17-OHD) and 1 female had POR deficiency (PORD). A diagnosis of FGD was made in 5 children (0.6%) (M/F=3/2): 4 of them had a mutation of *MC2R* gene and one male patient had a mutation of *MRAP* gene. Finally, in 8 patients (1%) PAI represented a component of a rare syndrome: 7 patients (M/F=5/2) were affected by Allgrove syndrome and 1 patient (a female) was affected by Pearson Syndrome, a complex syndrome caused by a mitochondrial defect (5).

With respect to not monogenic inherited causes of PAI, 20 patients (2.5%)(M/F=11/9) had a diagnosis of autoimmune AI: among them, 12 subjects (M/F=6/6) had the isolated form whereas 8 (M/F=5/3) had autoimmune AI in the context of APS type 2 (APS-2). Other acquired forms of PAI were found in 3 patients (0.4%; M/F=1/2): 1 adrenalectomy, 1 adrenal hemorrhage in neonatal period and 1 bilateral adrenal hematoma associated with Cytomegalovirus infection. Finally, in 9 patients (1%; M/F=7/2) the etiology was not yet identified.

According with the study design, only patients with non 21-OHD PAI were further characterized as regard to age and signs/symptoms at presentation, long-term auxological outcome, frequency of adrenal crisis and mortality. Clinical details of these subjects are shown in Table 1, moreover details on genetic alterations were available in 71/89 patients with monogenic inherited diseases (Table 2).

The mutation R257X was frequent among APS-1 subjects (6/20) as already described in Italian patients (51); however two of these subjects harboring R257X variant were siblings and other

patients were spread across Italy. Also among patients with X-ALD, the only recurrent mutation, R152C, was detected in 2 couples of siblings and in 2 other members of the same family.

Parents were consanguineous in 9 families of subjects affected with 11-OHD CAH, 3β-HSD CAH, APS-1, FGD and Allgrove syndrome. Eight families had multiple siblings affected.

Age at presentation and age at diagnosis

The median age at presentation in subjects with PAI not due to 21-OHD CAH was 6.5±5.1 years (range 0.1-17.8 years) and the median age at diagnosis was 6.9±5.2 years (range 0.1-17.8 years). The mean length of symptoms before diagnosis was 5.6±11.6 months, ranging between 0 and 56 months, depending on etiology (Table 1).

As expected, the onset of AI occurred earlier in children with a diagnosis of FGD (1.0±1.3 years), CAH other than 21-OHD (2.8±4.6 years), AHC (1.6±3.5 years) or rare syndromes (3.7±2.7 years) in comparison to X-ALD (9.3±2.9), APS-1 (8.6±4.4 years) or autoimmune AI (10.7±3.5years)($p < 0.001$); however, variability was observed within each form (Table1).

Symptoms, signs and laboratoryfindingsat the onset of PAI

The frequency of signs and symptoms at presentation in subjects with non 21-OHD CAH childhood onset PAI are reported in Figure 3a. The most frequent symptom at diagnosis was fatigue (67%), followed by hyperpigmentation (50.4%), dehydration (33%) and compensated hypotension (31%). Gastrointestinal symptoms, such as vomiting, abdominal pain and diarrhea were reported in 30.7%, 19.5% and 9.8% of subjects, respectively. Failure to thrive was described in 24% of patients. Behavioral disorders, as depression or anorexia, were reported in 7/121 subjects (5.7%) at diagnosis; several other neurological findings described in up to 25% of subjects were indeed features related to the specific underlying disease rather than to the onset of PAI. In particular, patients with X-ALD presented cognitive defects, behavioral problems or loss of hearing; peripheral neuropathy and Posterior Reversible Encephalopathy Syndrome (PRES) were reported in subjects with APS-1; mental retardation or not well specified other neurologic signs were also described in subjects with Allgrove

syndromes and glycerol kinase deficiency. In 13 subjects (10.7%) the disease presented at onset with an adrenal crisis leading to shock.

With regard to laboratory testing, the most frequent finding at diagnosis was elevated ACTH levels (96.4%) followed by low levels of basal cortisol (74%). Increased levels of Renin were found in almost 50% of cases; hyponatremia was frequent, occurring in 55% of patients while hyperkalemia and hypoglycemia were found in 32.7% and 33.7 % of subjects, respectively(Fig.3b).

Forty-two patients (34.7%) had isolated glucocorticoid deficiency whereas 79 (65.3%) had combined glucocorticoid and mineralocorticoid deficiency. Mean starting doses of Hydrocortisone and Fludrocortisone were 16.9±8.2 mg/m^2/day and 0.04±0.05 mg/day, respectively.

Although there was overlap in clinical and biochemical features, presentation varied on the basis of underlying disease.

X-ALD

PAI was the first disease manifestation preceding neurological signs in 10/25 (40%) subjects with X-ALD and was mainly characterized by isolated glucocorticoid deficiency. The most common signs were hyperpigmentation (72%) and fatigue (68%); all patients had increased ACTH concentrations at diagnosis. In 8% of patients an acute onset with shock was reported.

AHC

All subjects with AHC were diagnosed in early infancy; dehydration and salt loss occurred at presentation in 90% of cases. Hypogonadotropic hypogonadism was diagnosed in 8/9 patients who had reached puberty at the time of the study.

FGD

As expected, all patients with FGD had isolated cortisol deficiency and none developed salt loss. However, transient hyponatremia was reported in 3/5; recurrent and severe hypoglycemia and very high concentrations of ACTH were present in all patients. Hyperpigmentation was reported in 4/5 patients.

CAH

Nine patients had a diagnosis of CAH other than 21-OHD (11-OHD, 3β-HSD, 17-OHD, PORD) and were initially investigated for atypical genitalia or pubertal delay depending on underlying defect.

Autoimmune AI

Forty-five patients had a diagnosis of autoimmune disease with positive adrenal autoantibodies; 25 had APS-1 and 20 had APS-2 or isolated autoimmune PAI. Other autoantibodies were present in subjects with APS; thyroid autoimmunity was observed in 51% of patients, antibodies against islets cells (ICA), glutamic acid decarboxylase (GAD) and/or islet antigen type 2 (IA2) in 37.8%, parietal cell antibodies were present in 24.4% of patients. In subjects with isolated form or APS-2, fatigue was the most common reported symptom at diagnosis (90%) followed by hyperpigmentation (50%), hypotension (50%) and gastrointestinal symptoms (40%). In children with APS-1 fatigue was reported at diagnosis in 88% of cases whereas other symptoms were less frequent since adrenal failure occurs generally later than other component of PAI and the diagnosis was often made in a presymptomatic phase.

Allgrove syndrome

Seven patients were affected by Allgrove Syndrome; two of them were siblings born from consanguineous parentsand the youngest one was diagnosed soon after birth in a presymptomatic phase. Among other patients, fatigue (85%) and hyperpigmentation (71%) were frequent at presentation; 4 of them also presented frequent episodes of severe hypoglycemia.

Long- term outcome

At last follow-up mean age of subjects was 15.9±8.3 years and mean duration of follow-up was 8.7±8.2 years (range 0-28 years). Patients were receiving a mean dose of Hydrocortisone of 15.4±4.8 mg/m^2/day and a mean dose of Fludrocortisone of 0.06±0.08 mg/day.

During the follow-up, 23% of subjects experienced 1 adrenal crisis, 4.9% had 2 and 4.1% had 3 events, accounting for 2.7 adrenal crisis cases per 100 patient years. Acute events were more

frequently observed in patients with autoimmune conditions (60% of subjects experiencing adrenal crisis). Multiple regression analysis did not reveal any significant correlation between treatment at onset or at last visit and occurrence of adrenal crisis.

Three patients (2.5%), 2 of which affected with X-ALD and 1 affected with APS-1, died during follow-up for complications related to the underlying disease.

Data on AH were available for 70/121 patients (M/F = 50/20) and are shown in Table 1. Mean AH was -0.70±1.20 SDS. In males, AH (-0.73±1.20 SDS) was significantly lower than TH (-0.29±0.84, p=0.03); also in females AH (-0.56±1.30 SDS) was lower than TH (-0.09±0.85) but this difference did not reach statistical significance (Fig. 4). As expected, AH depended on underlying diagnosis; a significant lower AH was observed in subjects with AHC and CAH other than 21-OHD in comparison to patients with X-ALD ($p<0.05$ and $p<0.03$ respectively), APS-1 ($p<0.02$), and autoimmune AI ($p<0.04$ and $p<0.03$, respectively) and also in comparison to rare syndromes, although these differences were not statistically significant (Table 1). However, in the majority of cases the differences in AH between groups reflected the difference in TH. In each group AH was slightly but not significantly lower than TH except for subjects with X-ALD ($p<0.008$) and autoimmune AI ($p<0.04$) (Table1). Regression analysis did not reveal significant effects of diagnostic delay nor treatment regimens at the diagnosis or end of follow-up on AH.

Discussion

Causes of PAI in childhood are heterogeneous and different from adults. While in adulthood the most common cause is autoimmunity, in children inherited disorders of steroidogenesis constitute the largest group of PAI (9,25). Among them, 21-OHD CAH is certainly the most frequent and the better characterized. However, in the last decades several other genetic alterations responsible of PAI have been unraveled (9), although poorly characterized.

Indeed, to date only a few studies examined childhood-onset PAI due to different conditions. (18,24,39,45-46). Most of these studies reported only etiology or clinical and biochemical presentation from small, single-center cohorts of patients.

Perry et al (44) reported incidence and etiology, but not clinical data, in a cohort of 103 children documenting that 21-OHD CAH accounts for the majority of PAI patients (71.8%), while autoimmune adrenal failure is the second leading cause, followed by other inherited conditions.

Few recent studies provided a genetic characterization on larger cohorts of childhood onset PAI (24,47-48). In a study on 77 Turkish patients, Guran et al (24) characterized the molecular genetics of childhood-onset non-CAH PAI reporting that 95% of defects occurred in six genes: *MC2R*, *NR0B1*, *STAR*, *CYP11A*, *MRAP* and *NNT*. The most frequent conditions detected were FGD due to mutation in *MC2R* gene (26%) and AHC due to *NR0B1* mutations (13%). Amano et al (47) identified genetic defects in 86% of a cohort of Japanese patients with biochemically uncharacterized PAI; the relative proportion of genetic defects differed from Turkish cohort being characterized by an higher proportion of *STAR* defects and a lower proportion of *MC2R* defects. Finally in a study by Chan et al. (48), whole exome sequencing (WES) provided a genetic diagnosis in 17/43 patients referred for FGD; only in 3 of the 17 subjects FGD was confirmed while other patients had a different inherited cause of PAI, emphasizing the role of WES in successfully identifying genetic variants causing PAI.

In these studies only sparse information on clinical presentation or auxological outcome mainly limited to some rare genetic conditions were provided. To our knowledge, we have described one of the largest cohorts of subjects with childhood-onset PAI providing data on causes, presentation and long-term outcome.

According with previous studies (44,46), we confirm that 21-OHD CAH is the most common cause of PAI in childhood, accounting for 85% of the cases. Also in the remaining 15% of cases (n=121), leading causes were rare inherited conditions documented in 73.5% of subjects, followed by autoimmune PAI in 16.5% of patients. Among genetic diseases, the most commonly represented were X-ALD due to mutation in *ABCD1* geneand APS-1 due to mutation in *AIRE* gene followed by AHC due to mutations in *NR0B1* gene, non 21-OHD CAH, genetic syndromes (Allgrove or Pearson) and FGD. Similarly, a recent single-center study (46) reporting the distribution of etiology in 434 Chinese patients with childhood-onset PAI documented that among non-CAH etiologies (11.3% of the entire cohort) the most common cause was X-ALD, followed by AHC. The prevalence of inherited

disorders or single genetic mutation or variant is different in our cohort in comparison to other studies (24,46-47) probably reflecting different demographic characteristics different founder effects as well as collection bias. Differently from the study by Guran et al (24) we did not find regional recurrent genetic mutations and in our study the recurrent variants were familial or spread across Italy. Probably these results are affected by the small number of patients in each single group disease.

In addition to describe the etiology of PAI in children, another aim of the study was to characterize children with non 21-OHD PAI. In this group of subjects the actual frequencies of signs and symptoms at onset of PAI in childhood have been poorly characterized. Hsieh and White (45) reported that hypotension and hyponatremia were usually present at diagnosis while hyperpigmentation and hyperkalemia occurred inconsistently in 42 children with non-CAH PAI (45). Conversely in the study by Guran et al (24) and Wijaya et al (46) hyperpigmentation was the most common presenting sign in non-CAH children with PAI (94% and 85.7% respectively). Wijaia et al (46) found that neurologic alterations were the second most common finding at presentation (28.6%). Discrepancies in frequency of signs at presentation between studies can be explained by different characteristics of the population included and the preponderance of specific etiology in each study. Moreover, no data on age at presentation or on diagnostic delay were provided in these studies.

The clinical picture at presentation is indeed highly dependent on the underlying etiology. Our cohort included several inherited and acquired conditions representative of the wide spectrum of etiology underlying childhood-onset PAI. In our study the most frequent symptom at onset of AI was fatigue (67%), followed by hyperpigmentation (51%) and then by compensated hypotension and dehydration, whereas neurologic alterations were described in a minority of patients and were mainly characterized by behavioral changes. Also in subjects with X-ALD, symptoms of AI preceded in many cases neurological involvement. The frequency of fatigue and hyperpigmentation in our patients is similar to that described in adults with PAI (2, 7, 52); differently from data in adults, our patients did not complain muscle or joint pain at diagnosis. As regard to biochemical findings at onset, the most common abnormality reported at diagnosis in the entire cohort was increased ACTH (96.4%) followed by low levels of basal cortisol (74%). According to other studies in adults and children (2, 7,

46,52-53) we confirmed that hyponatremia occurs more frequently than hyperkalemia; therefore, unexplained hyponatremia not associated to hyperkalemia should rise the suspicion of adrenal insufficiency.

An acute onset was more frequently reported in children with AHC or FGD, whereas nonspecific signs may dominate the clinical picture in children with autoimmune PAI or X-ALD. Moreover, symptoms tended to occur earlier in subjects with AHC, FGD or CAH than in those with autoimmune forms or X-ALD where AI became clinically evident generally around 9-10 years of age. The mean length of symptoms before diagnosis was also shorter in the former group thus pointing attention on the importance of increasing awareness in older children in which nonspecific symptoms may dominate the clinical picture for long-time. Indeed, in autoimmune PAI and X-ALD hormone deficiencies result from progressive destruction of adrenal gland and become clinically evident when most of adrenal cells are impaired.

Each year, approximately 6-8% of adult patients with AI have an incident adrenal crisis which contributes to mortality of patients with hypoadrenalism and the adrenal crisis-associated rate of death in adults may reach 6% of crisis (41,53-54). Only a few studies addressed this issue in children; a frequency of adrenal crisis between 6.5-10.9 per 100 patients years has been reported in pediatric patients with CAH (55-57). A recent study, including also children with both PAI and central AI, documented a frequency of adrenal crisis of 3.4 per 100 patients years (42). In our study the rate of events was slightly lower and we registered 28 adrenal crises during follow-up, accounting for 2.7 adrenal crisis cases per 100 patient/years. These events occurred more frequently in children with autoimmune AI according to data in adults (53), while no association of hydrocortisone dose with incidence of adrenal crisis was found. Noteworthy, the evaluation of the frequency of adrenal crisis is affected by heterogeneous criteria defining adrenal crisis in retrospective studies and a prospective assessment is needed to better estimate incidence of adrenal crisis in childhood. However, these findings underline that adrenal crises continue to occur despite preventive intervention and highlight importance of improving key strategies to decrease their incidence in children with PAI.

Finally, our study is the first reporting data on the auxological outcome in subjects with non 21-OHD CAH childhood-onset PAI. Mean AH in 70 subjects was normal and, as expected, varied between etiologies and depending on TH. However, not all subjects approached their parental height, suggesting a potential role of the disease on linear growth. Regression analysis did not reveal significant effects of treatment regimens at the diagnosis or end of follow-up on AH; however we acknowledge that further studies evaluating treatment regimens during the entire follow-up are needed to unravel the relation between treatment dosages and long-term outcome.

We are aware that the major limitation of this study is represented by its retrospective design, so there is the potential for heterogeneity in the data recording and detailed extensive medical records are not available in some patients; moreover, different hormone assays have been used in the participating centers. However, the major strength of our study consists in the description of a large, multicenter cohort of subjects, providing a complete characterization of etiology, clinical presentation, and long term outcome.

In conclusion, we provided data on the largest cohort of subjects with rare causes of childhood onset PAI. Although in recent years a substantial amount of progress has been made in optimizing recognition of rare diseases associated to development of PAI, this condition in children is not well characterized and still underdiagnosed. Increasing knowledge on this rare disease will help improving diagnosis.

Data availability statement: The datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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Legends to tables and figures

Table 1. Characteristics of 121 patients with non 21-OHD childhood-onset Primary Adrenal Insufficiency

Table 2.Mutations detected in subjects with non 21-OHD CAH inherited causes of childhood-onset Primary Adrenal Insufficiency

Figure 1. Overview of recruitment of subjects with childhood-onset Primary Adrenal Insufficiency

Figure 2. Causes of childhood-onset Primary Adrenal Insufficiency in a cohort of 803 subjects

Figure 3. Signs and symptoms (a) and laboratory findings (b) at diagnosis in subjects with non 21-OHD CAH childhood onset Primary Adrenal Insufficiency

Figure 4. Adult height compared to Target Height in males (n=50) and females (n=20) with childhood onset non 21-OHD CAH Primary Adrenal Insufficiency

Acknowledgments

The centers of Bologna, Milan, Naples and Padua that participate to this publication are members of the European Reference Network for Rare Endocrine Condition (Endo-ERN – Project ID number 739527).

Table 1. Characteristics of 121 patients with non 21-OHD childhood-onset Primary Adrenal Insufficiency

Etiology	Number	Age at presentation (years)	Age at diagnosis (years)	Diagnostic delay (months)	TH SDS (n=70)	AH SDS (n=70)
X-ALD	25	9.3±2.9	10.2±3.1	10.6±16.1	0.0±0.6	-0.6±0.9[d]
APS-1	25	8.6±4.4	9.4±4.3	8.0±13.7	-0.3±1.0	-0.2±1.3
AHC	16	1.6±3.5[a]	1.9±3.5	3.1±12.0	-0.7±0.8	-1.2±1.0[b]
Non 21-OHD CAH		2.8±4.6[a]	3.0±4.9	1.4±3.4	-1.2±0.3	-1.5±1.3[c]
11-OHD	3					
3β-HSD	5					
17-OHD	1					
PORD	1					
FGD	5	1.0±1.3[a]	1.3±1.5	3.4±5.1	NA	NA
Syndromes		3.7±2.7	4.2±3.0	6.4±12.8	-0.4±1.1	-0.9±1.4
Allgrove	7					
Pearson	1					
Autoimmune AI		10.7±3.5	11.0±3.5	3.8±5.0	0.3±0.9	-0.4±1.1[e]
Isolated	12					
APS-2	8					
Other acquired conditions		2.9±5.0	3.0±5.0	NA	NA	NA
Adrenalectomy	1					
Adrenal hemorrhage	1					
CMV infection	1					
Undiagnosed	9	3.5±4.7	3.5±4.7	0.6±1.3	NA	NA

[a] $p<0.001$ vs X-ALD, APS-1 and Autoimmune AI.

[b] p<0.05 vs X-ALD, p< 0.02 vs APS-1 and p<0.04 vs Autoimmune AI ; [c] p<0.03 vs X-ALD, p<0.02 vs APS-1, p<0.04 vs Autoimmune AI.
[d] p<0.008 vs TH, [e] p<0.04 vs TH.

Abbreviations: 21-OHD, 21-Hydroxylase Deficiency; PAI, Primary Adrenal Insufficiency; SDS, standard deviation score; AH, Adult Height; TH, Target Height; X-ALD, X-linked Adrenoleukodistrophy; APS-1, Autoimmune Polyglandular Syndrome type 1; AHC, Adrenal Hypoplasia Congenital; non 21-OHD CAH, Congenital Adrenal Hyperplasia not due to 21-Hydroxylase Deficiency; FGD, Familial Glucocorticoid Deficiency; AI, Adrenal Insufficiency; APS-2, Autoimmune Polyglandular Syndrome type 2; CMV, Cytomegalovirus. NA= not applicable

Table 2. Mutations detected in subjects with non 21-OHD CAH inherited causes of childhood-onset Primary Adrenal Insufficiency

Disease	***Gene*** **(Chromosome)**	**N**	**Mutations**	**n**
X-ALD[a]	*ABCD1* (Xq28)	22	p.G116R	1
			p.R128Afs*67	1
			p.R152C	6
			p.L173Rfs*23	1
			p.Y181C	1
			p.L229P	1
			p.E291G	1
			p.G343V	1
			p.R401Q	1
			p.R418W	1
			p.S514R	1
			p.P560L	1
			p.Y617C	1
			p.D629N	1
			p.L670Sfs*19	3
APS-1	*AIRE* (21q22.3)	20	p.T16M/p.T16M	1
			p.T16M/p.W78R	1
			p.A21V/p.A21V	2
			p.A21V/p.W78R	1
			p.A21V/p.R257*	2
			p.A21V/p.C322fs*372	1
			p.W78R/p.W78R	2
			p.R139*/p.R257*	2
			p.R139*/p.C302fs*10	1
			p.R203*/p.R257*	2
			p.R203*/ c.994+5G>T	1
			p.R257*/p.R257*	1
			p.R257*/p.C322fs*372	3
AHC[a]	*NR0B1 (DAX-1)* (Xp21.2)	9	p.G168fs*17	1
			p.K249fs*49	1
			p.L318P	1
			p.H341D	1
			p.F364C	2
			p.N430del	1
			p.F449Sfs*13	2
	DMD-GK (Xp21)	4	Xp21del	4
CAH (other than 21-OHD)	*HSD3B2* (1p12)	3	p.P130L/p.P130L	1
			p.V319Afs*49	2
	CYP11A1 (15q24.1)	2	p.E314K/R465W	2
	POR (7q11.23)	1	p.A287P/-	1
FGD	*MC2R* (18p11.21)	4	p.R146H/R146H	1
			p.T159K/-	1

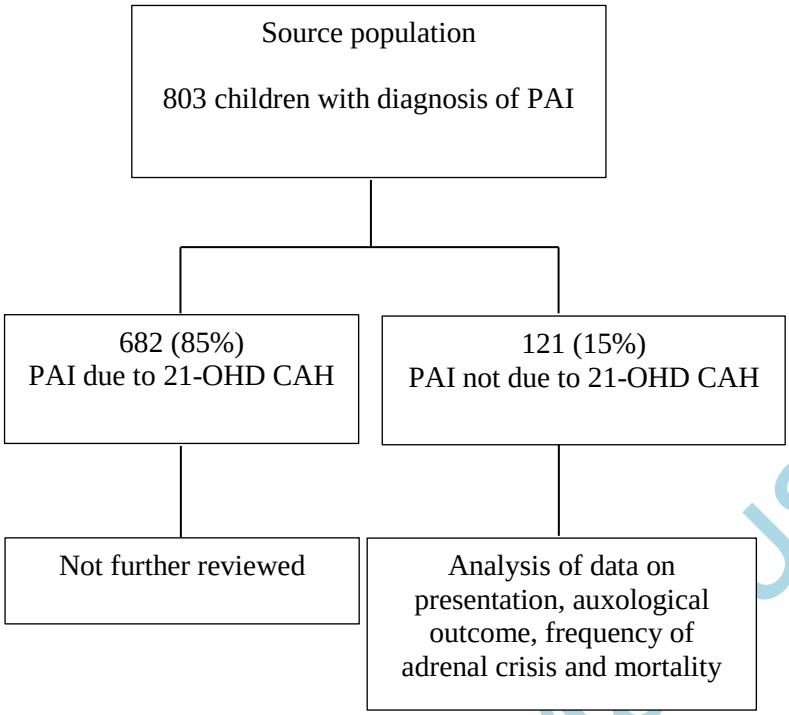
			p.T159K/A233D p.L283R/L283R	1 1
Allgrove syndrome	*AAAS* (12q13.13)	6	p.G14fs/L469P p.Q15K/Q15K p.R194*/R194* p.L381R/L381R c.1331+1G>A/ c.1331+1G>A	1 1 2 1 1
Total		71		

Details on mutations were available in 71/89 patients with monogenic inherited diseases; in the remaining patients the diagnosis was made on clinical criteria (n=7) or the gene was known but the mutation was not available (n=11).

Abbreviations: PAI, Primary Adrenal Insufficiency; X-ALD, X-linked Adrenoleukodistrophy; APS-1, Autoimmune Polyglandular Syndrome type 1; AHC, Adrenal Hypoplasia Congenital; non 21-OHD CAH: Congenital Adrenal Hyperplasia not due to 21-Hydroxylase Deficiency; FGD, Familial Glucocorticoid Deficiency.

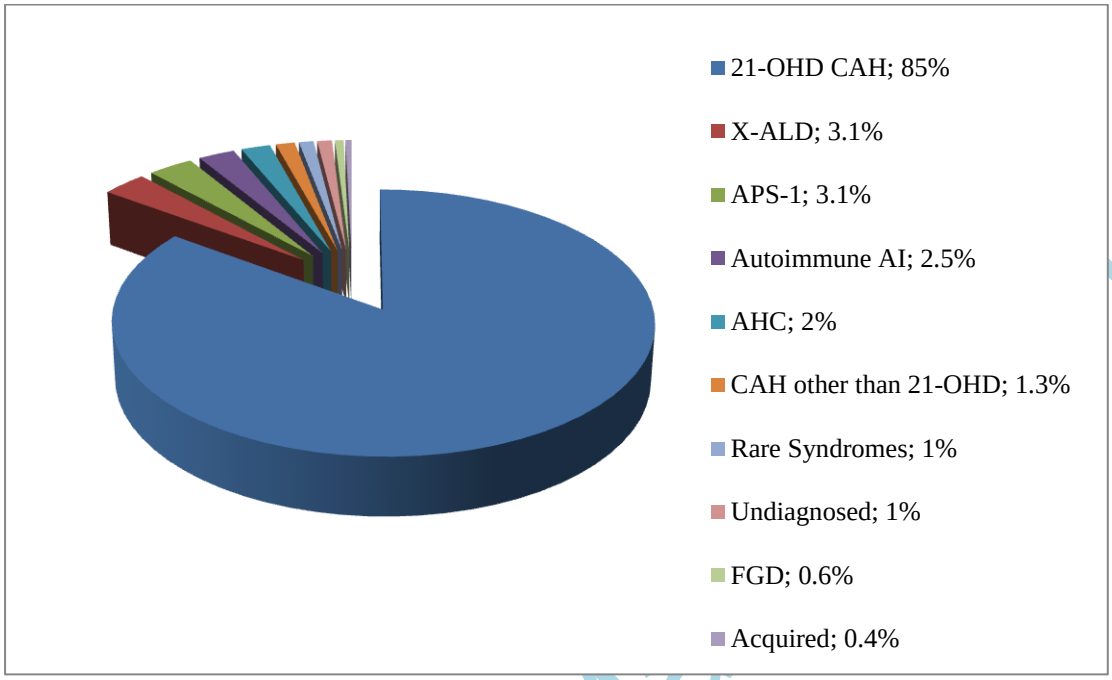
[a]hemizygous mutations in X-lined genes

Figure 1. Overview of recruitment of subjects with childhood-onset Primary Adrenal Insufficiency



Abbreviations: PAI, Primary Adrenal Insufficiency; 21-OHD CAH: Congenital Adrenal Hyperplasia due to 21-Hydroxylase Deficiency.

Figure 2. Causes of childhood-onset Primary Adrenal Insufficiency in a cohort of 803 subjects



Abbreviations: PAI, Primary Adrenal Insufficiency; 21-OHD CAH: Congenital Adrenal Hyperplasia due to 21-Hydroxylase Deficiency; X-ALD, X-linked Adrenoleukodistrophy; APS-1, Autoimmune Polyglandular Syndrome type 1; AI, Adrenal Insufficiency; AHC, Adrenal Hypoplasia Congenital; Non 21-OHD CAH: Congenital Adrenal Hyperplasia not due to 21-Hydroxylase Deficiency; FGD, Familial Glucocorticoid Deficiency.

Figure 3. Signs and symptoms (a) and laboratory findings (b) at diagnosis in subjects with non 21-OHD CAH childhood onset Primary Adrenal Insufficiency

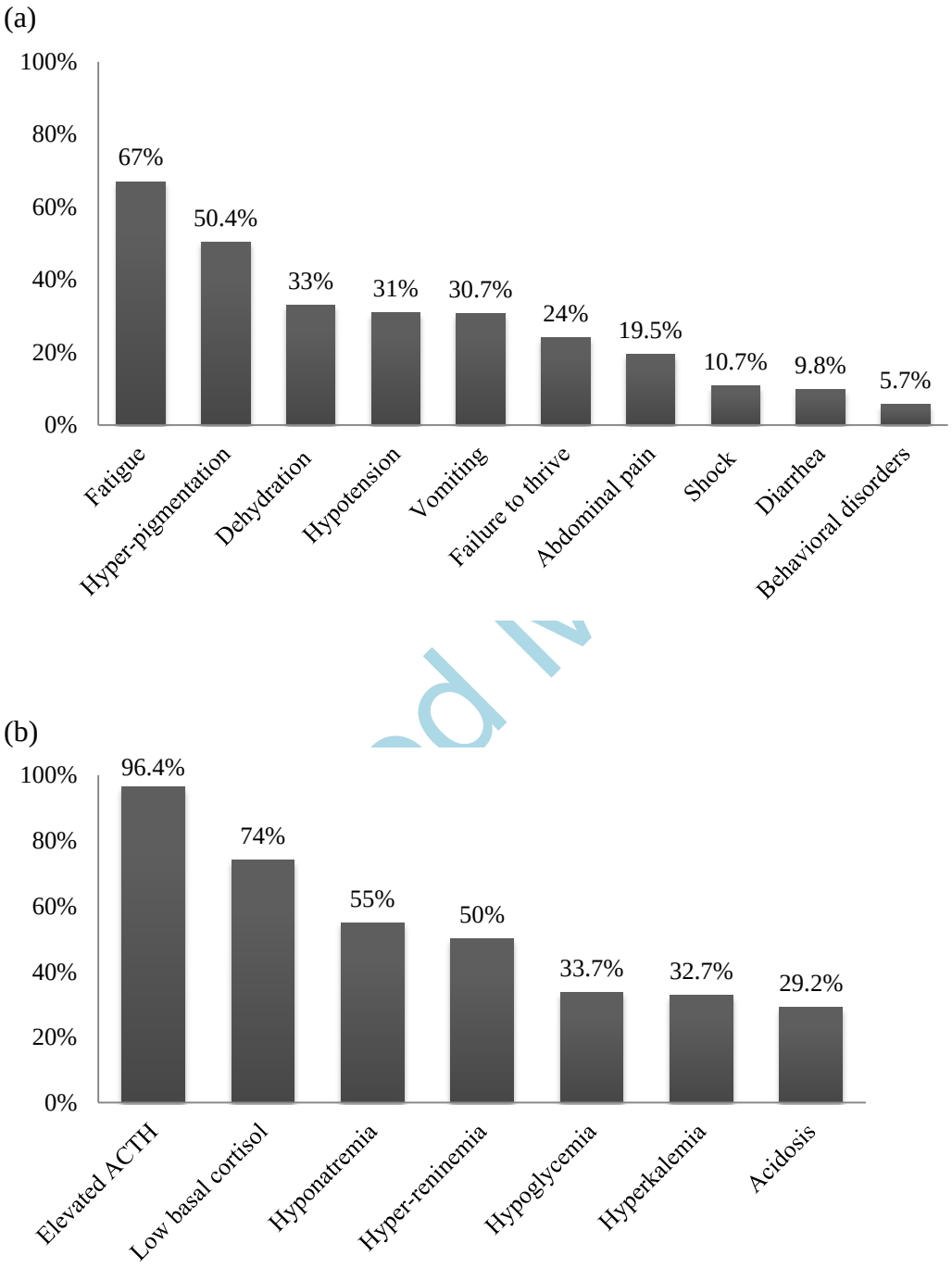
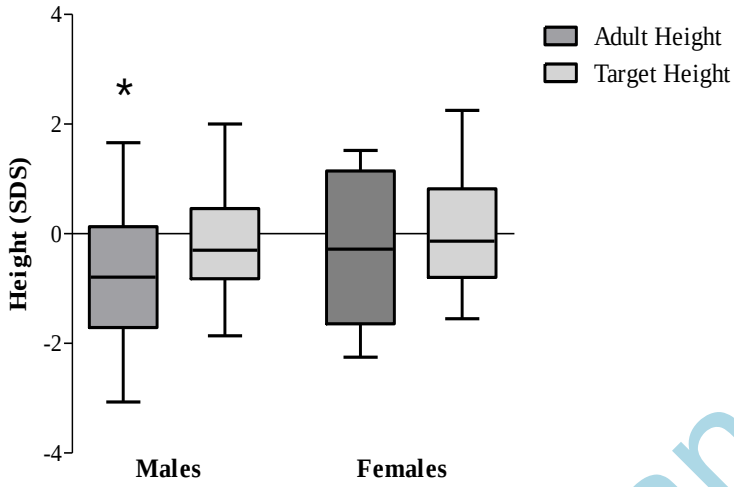


Figure 4. Adult height compared to Target height in males (n=50) and females (n=20) with childhood onset non 21-OHD CAH Primary Adrenal Insufficiency



*p=0.002 Adult height vs target height