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Research

Pediatric Primary Adrenal Insufficiency: A 21-year Single Center Experience

Çamtosun E et al. Pediatric Primary Adrenal Insufficiency

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What is already known on this topic?
Primary adrenal insufficiency (PAI) is characterized by deficient production of glucocorticoids and/or mineralocorticoids from **the** adrenal glands **due to** dysfunction**al** or **unresponsive** adrenal tissue. Congenital adrenal hyperplasia (CAH) is the most common and well known etiology in childhood.
Non-CAH etiologies are rare, and have varying rates of distribution across different populations. There is limited epidemiological and clinical information regarding non-CAH PAI.

What this study adds?
To the best of our knowledge, this is the first cohort study about PAI in children from Turkey. We were able to determine the etiology in 95.8% of PAI patients. Non-CAH etiologies were presented in detail along with the literature review. The most common non-CAH etiology was adrenoleukodystrophy.
A potential novel p.Q301* hemizygous frameshift mutation of the *DAX-1* gene was also identified in one patient.

ABSTRACT
Background: Primary adrenal insufficiency(PAI) is a rare but potentially life threatening condition. It is usually caused by monogenic diseases in childhood. Although congenital adrenal hyperplasia (CAH) is the most common cause of childhood PAI, numerous non-CAH genetic causes have **also** been identified.
Methods: We retrospectively evaluated patients aged 0-18 years who were diagnosed with PAI between 1998 and 2019 in **a** tertiary care hospital. After the etiologic distribution was determined, non-CAH PAI patients were evaluated in detail.
Results: Seventy-three PAI patients were identified. The most common etiology was CAH (69.9%, n=51). Non-CAH etiologies accounted for 30.1% (n=22) **and included** adrenoleukodystrophy (ALD; n=8), familial glucocorticoid deficiency (FGD; n=3), Triple A syndrome (TAS; n=5), autoimmune adrenalitis (n=1), adrenal hypoplasia congenital (AHC; n=1), IMAGe syndrome (n=1), and other unknown etiologies(n=3). The median age at the time of AI diagnosis for non-CAH etiologies was 3.52 (0.03-15.17) years. The most frequent symptoms/clinical findings at onset were hyperpigmentation of skin (81.8%), hypoglycemia symptoms (40.9%), and weakness/fatigue (31.8%). Hypoglycemia (50.0%), hyponatremia (36.4%) and hyperkalemia (22.7%) were prominent biochemical findings. Diagnosis of specific etiologies were proven genetically in 13 of 22 patients. Novel p.Q301* hemizygous frameshift mutation of the *DAX1* gene was identified in one patient.
Conclusion: We determined etiologies in 86.3% of children with non-CAH PAI through specific clinical and laboratory findings with/without molecular analysis of candidate genes. ALD was the most common etiology. Nowadays, advanced molecular analyse can be utilized to establish a specific genetic diagnosis of PAI for patients who have no specific diagnostic features.
Keywords: Primary adrenal insufficiency, pediatric

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INTRODUCTION

Adrenal insufficiency (AI) is a life-threatening condition characterized by deficient production of glucocorticoids(GC) and/or mineralocorticoids(MC) from **the** adrenal glands or reduced response to these steroids. If AI **is** caused by **dysfunctional or unresponsive adrenocortical tissue,** it is **classified** as primary AI (PAI); if it is caused by **disordered function of the pituitary gland and/or hypothalamus,** it is called secondary AI (SAI) (1). A PAI diagnosis **depends** on low serum cortisol and high plasma adrenocorticotropic hormone (ACTH) **as well as** clinical findings such as hyperpigmentation of the skin, hypoglycemia, salt wasting, hypotension**,** and other non-specific symptoms such as fatigue, weight loss, failure to thrive, depression, **and convulsions (**2,3**).**

Primary AI has a prevalence of 93-140 per million and an incidence of 4.7-6.2 per million in the white adult populations (4). It is thought to be less common in pediatric population. In childhood**,** PAI is usually caused by hereditary or sporadic monogenic diseases. Congenital adrenal hyperplasia (70-85**%**) is the most common cause**, with an estimated prevalence of 1/10,000-18,000** (1,2,**5,6**). **In several studies non-CAH etiologies generally accounted for 10-30% of childhood PAI** and autoimmune AI **was** usually the most common one (1,**5,6**). Other **genetic etiologies of non-CAH** are adrenal gland developmental disorders **[**X-linked adrenal hypoplasia congenital (AHC), **s**teroidogenic factor-1 related and other syndromic causes**]**, ACTH resistance **[**familial glucocorticoid deficiency (FGD) and related conditions, Triple A syndrome (TAS), etc..**]**, metabolic causes **[**cholesterol synthesis/metabolism defects, adrenoleukodystrophy (ALD), other **defects of the peroxisome, lysosom, endoplasmic reticulum and mitochondria], glucocorticoid** resistance and aldosterone synthesis/action defects (**7,8**). Infections, infiltrative diseases, adrenal hemorrhage, bilateral adrenalectomy and some drugs are the nongenetic causes of PAI.

Non-CAH PAI cases are less common, so the exact frequencies of non-CAH etiologies are still unknown (except ALD which has an estimated prevalence of 1/17000 at birth); the literature contains limited clinical data regarding these rare subgroups (2,8,9). Sharing clinical information about these patients will raise awareness about the disease. Early diagnosis and appropriate treatment is essential for avoiding lethal outcomes in PAI patients.

The aim of this study was to review the etiologies, clinical presentations, laboratory **findings**, genetic analysis, treatments and follow up features of non-CAH PAI cases **that were** followed in a **pediatric endocrinology department** of a tertiary care hospital within 21 years.

SUBJECTS AND METHODS

We retrospectively evaluated patients aged 0-18 years who **had their diagnosis and follow up for** PAI between August 1998 and October 2019 **at** İnönü University, Faculty of Medicine, Turgut Özal Medical Center, Department of Pediatric Endocrinology. After the etiologic distribution was determined, non-CAH PAI patients were evaluated in detail. We retrospectively reviewed and recorded the patients' date of birth, age at diagnosis, sex, clinical characteristics, comorbidities, laboratory results (serum glucose, electrolytes, plasma ACTH, serum cortisol, plasma renin, aldosterone, plasma very long chain fatty acids (VLCFA), autoantibodies), imaging results (adrenal, central nervous system and other), mutational analysis results, AI etiologies and treatment information. All the information was obtained from clinical records and the hospital's electronic database, and reviewed by an endocrinologist. Written informed consent forms were filled out by the patients and/or their families so that medical data (including genetic analysis results) may be collected and reported for educational and/or scientific purposes.

Primary AI was diagnosed based on coexistence of at least first two of the following criteria: 1) clinical symptoms/signs were suggestive of PAI (recurrent hypoglycemia, hyperpigmentation of skin, hyponatremia with hyperkalemia etc..); 2) plasma ACTH levels at 8 AM being two times higher than the reference range and a cortisol level of <138 nmol/L. If a patient had clinical symptoms and signs suggestive of PAI, but had a serum cortisol level >138 nmol/L at 8 AM with high ACTH, a standard dose Synacthen stimulation test was performed; (serum cortisol was recorded at 0, 30 and 60 minutes after 250 μg/m^2 intravenous ACTH); if the peak plasma cortisol level was under 500 nmol/L the patient was also diagnosed with PAI (10); 3) a positive genetic analysis report indicated one of the etiologies of PAI. After PAI diagnose, CAH subtypes were evaluated by clinical and biochemical analysis initially, and a target gene sequence analysis was done in patients diagnosed with CAH. Only classical CAH patients were included in the study, non-classical ones were excluded. Non-CAH patients were then evaluated for autoimmune adrenalitis and ALD. Presence of 21-hydroxylase-antibody in serum was evaluated by enzyme immunoassay (EIA). Plasma very long chain fatty acids (pVLCFA) were analyzed with Gas Chromatography-Mass Spectrometry (GCMS). Alacrima was confirmed by using Schirmer's test. Achalasia was diagnosed based on clinical symptoms and timed barium esophagogram. Brain MRI was conducted on patients who had neurological symptoms or high pVLCFA levels. If certain genetic tests were available, with the permission of the parents, specific genetic analysis was done for target genes in PAI patients. Patients with PAI, who had high pVLCFA levels with/without neurological symptoms/leukodystrophy on brain MRI or a family history of ALD, were diagnosed as ALD clinically and DNA sequencing analysis of the *ABCD1* gene was done. In patients with PAI, who were also diagnosed with alacrima and/or achalasia, a DNA sequencing analysis of the *AAAS* gene was done to investigate TAS. Patients who had evident growth retardation and other dysmorphic features were evaluated for syndromic PAI etiologies. Patients with early onset PAI who had no specific clinical features were evaluated for *MC2R* and/or *DAX1* gene mutations using DNA sequencing. The genetic analyses had been conducted in many different commercial genetic laboratories in Turkey (Detagen, İntergen and Düzen Genetic Laboratories).

This study was approved by the ethical committee of İnönü University (Turkey) (Approval number:2019/407), and was conducted in accordance with the World Medical Association Declaration of Helsinki.

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Statistical Analysis

Data were analyzed with descriptive statistical methods. Qualitative variables were expressed as number (%). Continuous quantitative variables were expressed as mean and standard deviation if they are suitable for normal distribution; as median and range if they are not.

RESULTS

Seventy-three patients were diagnosed **with** PAI in either **an** inpatient or outpatient setting over a 21 year period.

I-Congenital Adrenal Hyperplasia

Congenital adrenal hyperplasia was the most common etiology and 51 (69.9%) patients had CAH. **Twenty-four** CAH patient had 21-hydroxylase deficiency (21OHD) **(47%** of CAH), **19** patients had 11-beta-hydroxylase deficiency(11OHD) **(37.2%** of CAH), six patient**s** had 17-alpha-hydroxylase deficiency (17OHD), one patient had 21-22 desmolase deficiency, one patient had **s**teroidogenic acute regulatory protein (StAR) deficiency. **The** CAH patients were not reviewed further.

II-Non-CAH Etiologies

Non-CAH etiologies accounted for 30.1% (n=22) of PAI (ALD in 8 patients (11%), FGD in 3 (4.1%) patients, TAS in 5 (6.8%) patients, autoimmune adrenalitis in one patient, AHC in one patient and IMAGe syndrome in one patient. Etiology of PAI could not be clarified in three patients in whom CAH was excluded (Table 1). Median age at the diagnosis of adrenal insufficiency was 3.52 (0.03-15.17) years and male/female ratio was 6.33 (19/3) for non-CAH patients (Table 2). Mean height SDS and weight SDS were normal on admission. **The most** frequent symptoms and clinical findings were hyperpigmentation of skin (81.8%), hypoglycemia symptoms (40.9%) and weakness/fatigue (31.8%). The other symptoms and clinical findings were alacrima, adrenal crisis, achalasia, neonatal prolonged jaundice, learning disability, vomiting, headache, low school performance, mental retardation, walking disability, epilepsy, and polyneuropathy. Parental consanguinity was very frequent, and was present in 77.8% (15/19) of cases. Hypoglycemia (50.0%), hyponatremia (36.4%) and hyperkalemia (22.7%) were prominent biochemical findings. Mean plasma ACTH level was very high, while mean serum cortisol level was very low (Table 2). All non-CAH PAI patients were treated with oral Hydrocortisone (HC); however, 27.3% of them also received oral Fludrocortisone (FC) treatment. The mean follow up duration was 65.04 ± 36.23 months.

Adrenoleukodystrophy

Eight male patients from four different families had been diagnosed with ALD (Table 3). Patient (P)1 and P2 were brothers and their family history revealed that four of their brothers had died from AI. P3 was a nephew, who had died of encephalitis when he was 7 years old, and P4 was a distant relative of them. P5 and P6 were also brothers from a different family. The other two patients (P7, P8) were from different unrelated families.

Median age at AI diagnosis was 7.17 (2.89-15.17) years for ALD patients. All but one of them presented with hyperpigmentation of the skin. Other symptoms/findings were adrenal crisis, headache, vomiting and hypoglycemia symptoms. In laboratory analysis three patients had hyponatremia and two of them also had hyperkalemia. All patients had low serum cortisol (mean level 105.71± 66.79 nmol/L) and very high plasma ACTH levels. Four patients presented with neurological problems (Table 3). Plasma VLCFA levels were evaluated in four patients, and revealed high plasma C26 levels in three and high C26/C22 levels in all of them. Four patients showed signs of white matter involvement on brain MRI, and one demonstrated a thin corpus callosum and hydrocephalus. Four patients were diagnosed were based on clinical and laboratory findings as well as molecular analysis; they all had the same p. P543L (c.C1628T) mutation in the *ABCD1* gene. The other four patients' diagnoses were based on clinical and laboratory findings with /without family history. All patients were given HC at a median dose of 17,5 mg/m2/d. Three patients had MC deficiency (P1 and P3 had mild hyponatremia and high plasma renin activity, P2 presented with severe hyponatremia and hypercalemia), and needed fludrocortisone at a dose of 0,05 mg/d orally. One patient (P4) also had transient hyponatremia and high renin level but he did not need MC replacement. Three patients were prescribed Lorenzo's oil (LO) and Lovastatin.

Triple A (Allgrove) Syndrome

Five patients (P9-13) (three males, two females) from three different families were diagnosed **with** TAS. P9 and P10 were siblings and P11 was their cousin, the other two patients were from different families without a consanguineous marriage. All patients were living in the same city in east Turkey. Three patients had **a** classical triad of achalasia, alacrima, AI; **the** other two had two **symptoms from the triad** (alacrima and AI). Four of **the** patients presented with hypoglycemia symptoms and convulsions, all patients had hyperpigmentation of skin, two patients had mild facial dysmorphism, (wide/depressed nasal root, down slanting palpebral fissures, short philtrum), one had mild mental retardation, nasal speech, thenar atrophy and short stature, the other one had normocytic anemia and corpus callosum hypoplasia. The earliest symptom was alacrima for all patients, which was noticed in early infancy. Hyperpigmentation of skin was noticed at 3-5 years old. **The median** age **at diagnosis** of AI was 6 (3.19-7.83) years old. Transient hyponatremia was seen in one patient; hyperkalemia was not seen. Median serum cortisol level was 30.36 (13.8-218.04) nmol/L, plasma ACTH level was 275 (186.34-440) pmol/L. Molecular analysis of patients revealed the same homozygous mutation p.L356Vfs*8 (c.1066_1067delCT) in the *AAAS* gene. According to the Clinvar database, this mutation (variation ID:264992) was a previously reported pathogenic mutation (**11**). All patients were given HC at a median dose of 15 mg/m2/d orally, but none of them needed FC. Surgery for achalasia was undertaken for P9 and P10. All patients were prescribed eye drops for preventing dry eye.

Familial glucocorticoid deficiency

Three patients (P14-16) (two males, one female) from three different families were diagnosed FGD with genetic confirmation. **The age** at onset of AI **was** 1.5, 21 and 2.5 months old**,** respectively. Two of them presented with hyperpigmentation of skin and hypoglycemia. One patient (P14) also had pes equinovarus and posterior embriyotoxone of **the** eyes. All of them had

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history of prolonged jaundice. **Laboratory analysis revealed very low cortisol levels, very high plasma ACTH levels and normal plasma VLCFA levels for all patients.** One patient had transient **hyperthyrotropinemia.** MRI of **the** adrenal glands was normal **in all patients**. Genetic analysis showed; P14 and P15 had **a** homozygous p.G116V (c.347 G>T) mutation in **the** *MC2R* gene; and P16 had **a** homozygous p.L225R (c.674T>G) mutation in **the** *MC2R* gene. All patients were given **oral** HC at a median dose of 12.5 mg/m2/d, but none of them needed FC.

Congenital Adrenal Hypoplasia

A twenty-one months old boy **(P17),** diagnosed with PAI in another medical center at 16 days old **was** admitted to our hospital. He had salt wasting during AI onset. Medical history revealed that his brother **had** died **due to** AI at 10 months old. Biochemical results had shown **that he had** hyponatremia and hyperkalemia in **the** neonatal period. **At 16 days old his serum cortisol level was low, plasma ACTH level was high,** and serum gonadotropin levels were normal. Congenital adrenal hyperplasia was excluded. He had normal external genitalia. **MRI of the adrenal glands was normal**. Brain MRI (T2A) showed bilateral hyperintense changes in occipital white matter. Genetic analysis **through DNA sequencing** revealed **a** p.Q301* (c.901C>T) hemizygous **non-sense** mutation in **the** *DAX1* gene (Figure 1). **This variation was not described in the literature before; however, it had led to a stop codon and was most likely pathogenic according to both Mutation Taster and ClinVar data. (12,13).** Genetic analysis of the mother couldn't be performed. He was treated with HC 15 mg/m2/d and FC 0.05 mg/d orally.

Isolated Autoimmune AI

A thirteen-year old male patient (P18) presented with hyperpigmentation of skin, weakness and fatigue. He had no neurological signs. Laboratory analysis showed severe hyponatremia (Na:117 meq/L) and mild hyperkalemia (K:5.5 meq/L). Serum glucose was normal. Serum **cortisol level was low, and plasma ACTH level was high**. He was **examined** for autoimmune diseases; thyroid autoantibodies, and celiac antibodies were not detected. Plasma VLCFA test **revealed a normal C26 level and high C26/C22 ratio**. Mutational analysis of **the** *ABCD1* gene was normal. In his follow up serum 21-hydroxylase antibody was detected positive**, and** he was diagnosed isolated autoimmune AI. Left renal agenesis was also **discovered** during **the** evaluations. He was treated with HC 10-15 mg/m2/d. In the follow up period (47 months)**,** he became obese and **demonstrated** insulin resistance, type 2 DM, hypertension, hepatosteatosis and dyslipidemia; but he did **not have** any other autoimmune disease.

Syndromic PAI

One male patient (P19) was admitted to another center with adrenal crisis when he was 10 days old, he was diagnosed **with** PAI, treated with HC **and** FC**,** and **subsequently referred** to our hospital after one month of hospitalization in **an** intensive care unit. He was born at 41 weeks of gestation with **a weight of** 2400g (small for gestational age). He had facial dysmorphic features (frontal bossing, deeply located eyes, hypertelorism, depressed nasal bridge), bilateral undescended testicles, penile hypospadias. His karyotype was 46, XY, SRY locus (+). **His testes exhibited normal hormonal functions**. Diagnosis of CAH was excluded. Echocardiogram showed thin patent ductus arteriosus. Abdominal US revealed ectopic and fusioned kidneys. Brain and lomber **MRI revealed** multiple hemorrhagic lesions in **the** brain and **a** 35x4 mm syringohydromyelia at **the** lomber region. It was considered that he **could have** syndromic PAI, IMAGe (Intrauterine growth retardation-metaphyseal dysplasia-adrenal hypoplasia congenita-genital anomalies) syndrome, but genetic analysis couldn't be performed yet.

Unknown Etiology

A total of three male patients were diagnosed non-CAH PAI with unknown etiology.

One male patient (P20) presented with hyperpigmentation and an incidental mass in **the** right adrenal gland at 13.7 years old. **He also had** hereditary spherocytosis. He had no neurologic**al**/psychiatric complaints or findings. **Other than hypoglycemia biochemical analysis was normal**; hormonal assays revealed only partial PAI. **Basal level of serum cortisol was normal, but plasma ACTH levels were very high. A standard dose Synacthen test resulted in a peak serum cortisol level of 331.08 nmol/L (low)**. He had no **MC** deficiency. Plasma VLCFA levels were normal. Adrenal antibodies could **not** be analyzed**,** but thyroid autoantibodies and tissue transglutaminase antibodies were negative. Adrenal MRI showed a 4.3x3.1 cm mass in right adrenal gland. **Following right adrenalectomy, the lesion pathology was revealed to be adrenal hemorrhage**. He was given HC replacement.

A two-year-old boy (P21) presented with weakness, hyperpigmentation of skin, hypoglycemic symptoms (even convulsions). He had no other neurologic**al** symptoms or signs. **Laboratory analysis showed apparent adrenal insufficiency, normal plasma VLCFA levels. Adrenal antibodies were negative.** Molecular analysis showed no mutation in **the** *DAX1* gene; he **was** treated with HC. The patient was thought to have FGD; however, molecular analysis could not be done.

A seven-**month** old boy (P22) presented **with** hypoglycemia symptoms and hyperpigmentation of skin. Medical history revealed that he was term with normal birthweight, he had adrenal crisis and hypoglycemic episodes in **the** newborn period, laboratory analysis showed hyponatremia, hyperkalemia, hypoglycemia, low serum DHEAS, very high plasma ACTH. He was treated with HC **and** FC in another medical center. **Physical examination in our hospital revealed that** he had microcephaly, diplopia and motor mental retardation. Plasma VLCFA analysis showed **a** high C26 level, but C26/C22 was normal. His Brain MRI revealed cerebral atrophy, calcification in **the** basal ganglia**,** and corpus callosum atrophy. Further genetic analysis could not be performed. He **was** treated with HC and FC. (for two years).

DISCUSSION

Primary AI in childhood is a relatively rare but potentially life-threatening condition. **Although PAI is mostly caused by monogenic diseases in children, it is often acquired in adults** (2).

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We reviewed 73 children with PAI **over a period of** 21 years **in** a single tertiary center from Turkey. **Non-CAH PAI patients were especially reviewed** in detail and compared with **the** literature. **To the best** of our knowledge this is the **first cohort** of PAI in children from Turkey. **In a previously conducted nationwide cohort study,** Güran et al. reported clinical and molecular genetic characteristics of children with PAI of unknown etiology (patients with CAH, ALD, autoimmune AI or obvious syndromic PAI like TAS were excluded) from Turkey (**14**). We were able to determine **the** etiology in 95.8% (70/73) of **all** PAI patients and in 86.3% (19/22) of non-CAH PAI patients in our cohort **through** clinical and laboratory findings, and **some being proven genetically.**

Although CAH is still the most common cause of **childhood PAI** today, numerous non-CAH genetic causes have been identified in the last 25 years, **but their prevalance** in children with PAI **is not yet clear** (1,2,**5,6**). Among CAH etiologies**,** 21OHD is the most common one (90-95%) (**5,6,15**). In our cohort**,** CAH was **also** the most frequent etiology (69.9%) and 21ODH **was** the most common CAH **(47%).** But unlike the literature, **11OHD (19 cases from 15 families) (37,2%) was also very common in our study. Diagnosis of 11OHD was confirmed with genetic analysis in 18 of the cases.** Racial characteristics and frequent consanguineous marriage in our region might have caused this difference. **In a review of 273 Turkish patients with CAH, Kandemir et al. reported that** 11OHD **was** the second most common **cause of** CAH **and accounted for** 13.5% of cases (high **compared to** other populations **in** whom it is 5-8%) (**16**). **Similar to the literature, non-CAH causes accounted for 30.1% of childhood PAI in our cohort (Table 1)**. In contrast, Hsieh et al reported **a higher rate (54.5%) of** non-CAH etiologies within 77 pediatric PAI patients (3). Many studies from western countries reported that autoimmune etiologies were the most common cause and accounted for %30-55 of non-CAH PAI in children (1,3,**5,17,18**). **In their Chinese cohort, Wijaya et al reported that** ALD (44.9%) and AHC (40.8%) were the most common etiologies in **the** non-CAH group, autoimmune etiologies only accounted for 6.1% (**6**). In our cohort, ALD was the most common etiology (36,3%, n=8), and autoimmune AI was rare (only one patient) **similar to** Wijaya's study. This suggests that racial features affect the etiological distribution of adrenal insufficiency.

Autoimmune PAI can be isolated or a component of APS. Detecting anti-adrenal antibodies in serum of patients with PAI leads to the diagnosis of autoimmune PAI. Mutations in the autoimmune regulator gen (*AIRE*), are responsible for APS-1 in which PAI **is usually** combined with hypoparathyroidism and mucocutaneus candidiasis. APS-2 typically combines PAI with autoimmune thyroid disorders and Type 1 **d**iabetes mellitus, and shows **a** complex inheritance pattern (HLA-DR3/DR4, CTLA-4) **similar** isolated autoimmune **PAI (2,7). Our male** adolescent patient had positive serum anti 21-OH antibodies, he had no **other** accompanying autoimmune disease (or family history) **over** nearly four years of follow up, and so **was ultimately** diagnosed **with** isolated autoimmune PAI.

ALD is an X- linked hereditary metabolic disorder **caused by** mutations of the *ABCD1* gene, which encodes a peroxisomal transport protein necessary for **VLCFA degradation** (≥C22). **Toxic accumulation of VLCFA in plasma and multiple tissues (white matter of the brain, spinal cord and adrenal cortex) is associated with a proinflammatory state and eventual cell death (19). In male patients**; **A**ddison only, cerebral ALD (childhood, adolescent, adult onset), **and** adrenomyeloneuropathy (AMN) phenotypes can be seen **(9)**. **Perry et al reported four ALD patients in their study (two Addison only, one childhood and one adolescent CALD)**. In our cohort, three **patients** had Addison only phenotypes, three had childhood CALD, and two had adolescent CALD (**5**). **Hyperpigmentation of skin was the most common symptom.** All patients had either neurologic**al** symptoms/white matter involvement, elevated plasma VLCFA**,** or family history of ALD**. ALD causes GC deficiency, and MC deficiency may also be detected**. Three of eight ALD patients in our cohort needed MC treatment. **Perry reported MC deficiency in one of four ALD patients, while Wijaya reported zero cases of MC deficiency in 22 ALD patients (5,6). If ALD is suspected in a male with neurological symptoms (with or without typical brain MRI abnormalities) or Addison's disease, diagnosis is made based on elevated VLCFA levels in plasma; genetic confirmation is useful for genetic counselling (9). For molecular confirmation of ALD, a sequence analysis of the *ABCD1* gene is first performed, and if no pathogenic variant is found, it is followed by a gene-targeted deletion/duplication analysis. This is because, the sequence analysis method has a reported diagnostic value of 97% when identifying mutations of the *ABCD1* gene, and the remaining 3% can be detected using deletion/duplication analysis by way of the multiplex ligation-dependent probe amplification (MLPA) method (20). In addition, mutations in regions (such as the promoter region) that play a role in regulating gene expression may not fall within the sequenced region in sequencing analysis.** Four of our patients had **a** p.P543L mutation in exon 6 of **the** *ABCD1* gene, which was reported before (**21**); **in two cases *ABCD1* sequencing analysis revealed no mutation, and subsequent deletion / duplication analysis was not performed.** Genotype–phenotype correlation or the trigger for cerebral disease has not been described. The only currently available standard therapy is hematopoietic stem cell transplantation (HSCT) **which is done in the** early stage of demyelination in boys with **CALD.**

Triple A (Allgrove) syndrome (OMIM 231550), characterized **by** achalasia, alacrima, and AI as well as neurologic**al** (central, peripheral and autonomic nervous system) and dermatologic**al** problems, is caused by homozygous or compound heterozygous mutations in the gene encoding aladin (*AAAS*) on chromosome **12q13. Palmoplantar hyperkeratosis (PH),** hypothenar atrophy, short stature, facial dysmorphism, deafness, mental retardation, nasal speech can also be seen (**22,23**). **Even with the same *AAAS* gene mutation, patients have phenotypical heterogenity (23,24).** Alacrima was the earliest and the most consistent finding in our five TAS patients; hyperpigmentation of skin and hypoglycemia symptoms were **also** common in accordance with **the** study of Polat et al who reported a large Triple A cohort (23 patients from 14 families) from Turkey (**23). Therefore** alacrima and achalasia symptoms must be **questioned in** all non-CAH PAI patients for **the investigation** of TAS. **Although Polat et al reported short stature and PH in more than half of the cases**, our cohort **contained** only one patient **with** short stature and **PH was not present at all** (**23**). Since our study **has a retrospective design**, **PH may have been overlooked** or not noted. **On the other hand PH was only present in patients with the p.R478* mutation in the aforementioned study. This finding may be specific to the mutation**. Grant et al reported fissured palms **in** half of 20 **patients in whom molecular analysis was not**

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conducted (25). All of our patients with TAS had the homozygous p.L356Vfs*8 mutation in the *AAAS* gene, which was previously reported in a nine year old Turkish boy who had presented with achalasia, alacrima, stimulated cortisol deficiency, pitosis, pallor of optic disc, anisocoria and dry skin (26).

Familial glucocorticoid deficiency (FGD), is characterized by isolated **GC** deficiency in early infancy or in childhood, and presents with hyperpigmentation and hypoglycemia symptoms. **All three of our patients were younger than two years and had prolonged jaundice or history of it, two of them presented with hyperpigmentation of skin and hypoglycemia.** Akın et al also reported the case of a 17-day-old newborn diagnosed with FGD type 1 who presented with hyperbilirubinemia and hyperpigmentation **(27)**. Therefore, hyperpigmentation, persistent hypoglycemia and prolonged jaundice should suggest the possibility of adrenal insufficiency in infancy. **Although MC need and transient hyponatremia has been occasionally reported (14,28),** none of our FGD patients had MC deficiency. Mutations in the *MC2R* gene (encoding the ACTH receptor protein) and *MRAP* gene (encoding MC2R accesory protein) are well described causes (almost 50%) of FGD (**7**). **Although other rare genetic defects are also reported as causes in FGD,** the underlying cause is unknown in about 40% of cases (2**,7,29).** These genetic defects manifest **as** phenotypically indistinguishable. Molecular genetic analysis of our patients revealed two different mutations in **the** *MC2R* gene which were previously reported **(14,27,30).**

Mutations in the *NR0B1 (DAX1)* gene located on Xp21.3-p21.2 and deletions in Xp21 (contiguous gene deletion) lead to problems with the development of the adrenal glands, hypothalamus, pituitary gland and gonads and cause AHC. Adrenal insufficiency typically begins in early infancy or in childhood, **and** rarely begins in adulthood **(31)**. Patients can also have hypogonadotropic hypogonadism (HH)**,** which is characterized by undescendent testes, micropenis, delayed puberty or infertility related with low levels of **gonadotropins. It was reported** that AHC due to *DAX1* mutation is a relatively frequent cause of non-CAH PAI in Chinese children **(6,32)**. Wijaya et al reported 20 male AHC **cases** (19 had *NR0B1* **gene mutation) among 49 children with PAI; five patients presented with a typical adrenal crisis, 10 with salt craving, three with generalized hyperpigmentation at onset; and six patients with HH during follow-up (6). In this study the age at onset of AHC was < 3 months in 13 of 20 patients, and ≤ 2 years in 17 of 20 patients**. **Lin et al reported *DAX1* mutations in** 58% (37 of 64) of 46, XY phenotypic boys with **AI** (not caused by **CAH, ALD**, or autoimmune disease) and in all boys (eight of eight) with **HH** and a family history suggestive of **AI** in males **(33)**. Adrenal insufficiency had begun in early infancy in 81% of patients in their study. Only one male patient **was** diagnosed **with** AHC in our cohort, who **had** presented with salt wasting in **the** neonatal **period. He** had normal external genitalia and gonadotropin levels. **Molecular analysis revealed a p.Q301* hemizygous non-sense mutation (novel) in the *DAX1* gene.**

In recent years, many syndromic diseases that can cause PAI have been identified (Table 1). IMAGe syndrome was **primarily defined by a** spectrum of intrauterine growth restriction, metaphyseal dysplasia, congenital adrenal hypoplasia and genital anomalies. Patients can also have dysmorphic craniofacial features, hypocalcemia, scoliosis **(34)**. **This disease is caused by gain** of function mutations in **the** cyclin dependent kinase inhibitor (CDKN1C) gene, which regulates prenatal and postnatal growth. MIRAGE syndrome **which is another known cause of syndromic PAI emerges** due to heterozygous SAMD9 gain **of function** mutations**; it is characterized by** myelodysplasia, infection, restriction of growth, adrenal hypoplasia, genital anomalies and enteropathy. Neurologic**al** findings such as microcephaly, hydrocephalus, white matter abnormalities, and perivascular calcifications were also described **(35)**. **Our two patients with neonatal onset PAI had dysmorphic features**. One of them (P19) had severe pre and postnatal growth retardation, dysmorphic facial features and urogenital anomalies accompanying PAI **and was clinically diagnosed as IMAGe syndrome**. **The other one (P22) unlike these two syndromes had no growth retardation, so it was considered** that **the** neurologic**al** findings of the patient could **have beeen** due to severe hypoglycemic episodes and electrolyte imbalances **during the** newborn period, or **caused by a** peroxisomal or undefined syndromic disorder.

According to **both** the literature **and our** study; in at least 80% of children with non-CAH PAI, the etiology can be determined by specific clinical and laboratory findings with or without molecular analysis of **a** candidate gene. For patients who **do not exhibit** specific **clinical findings, predicting the** exact etiology **can be** very difficult. **Nevertheless**, establishing a specific genetic diagnosis **in** PAI is very valuable for 1) providing clear information about disease spectrum, potential comorbidities and prognosis 2) modifying treatments**, ie. the need for MC**. 3) genetic counseling of affected individuals and their families, identifying presymptomatic children before onset of potentially life-threatening symptoms 4) increasing knowledge about the normal biology and pathomechanisms of PAI (2). Comprehensive diagnostic algorithms for PAI in children **are** available in the literature **(5,7)** For unsolved patients, gene panel based next generation sequencing(NGS), whole exome sequencing(WES), whole genome sequencing(WGS) or array **comparative genomic hybridization** (CGH) can be applied today.

Study limitations

One of the limitations of our study was its retrospective design. Another limitation was that diagnostic molecular analysis could not be performed for all patients due to the limit in resources especially in previous years.

CONCLUSION

Because PAI is a life threatening condition, early recognition and proper treatment are crucial. Signs such as hyperpigmentation of skin, recurrent hypoglycemic episodes with or without prolonged jaundice, chronic fatigue and hyponatremia with **hyperkalemia** could most likely suggest adrenal insufficiency in children. During diagnostic studies, CAH must initially be excluded. Afterwards, specific clinical and laboratory features must be evaluated and proven with appropriate candidate gene analysis. Because of its many advantages, advanced molecular analysis should be considered for patients who have no specific diagnostic features.

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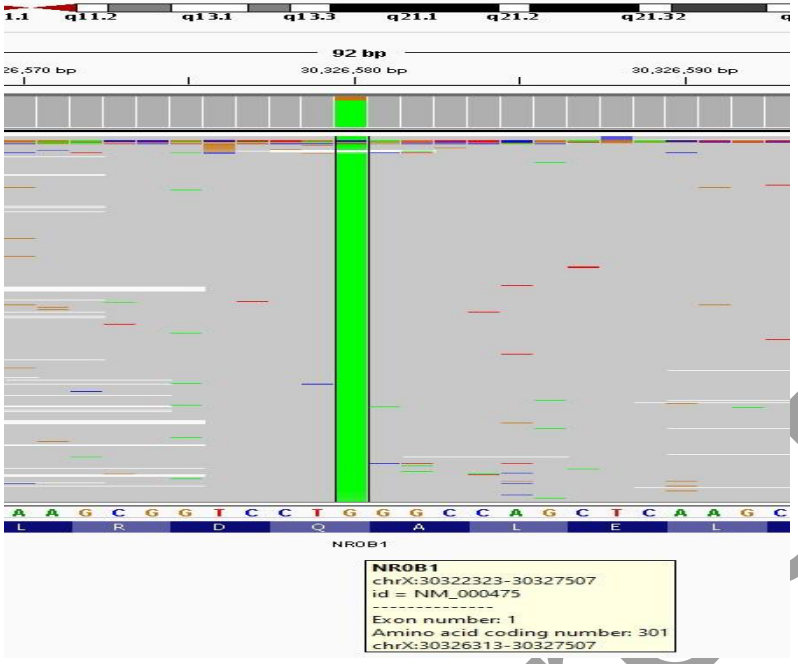

Figure 1: DNA sequence analysis of the patient with adreal hypoplasia congenital.

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Table 1: Etiologies of PAI in different cohorts

Etiologies	**Our cohort 2020**	**Simm 2004 (11)**	**Perry 2005 (4)**	**Hsieh&White 2011 (3)**	**Tsai 2016 (12)**	**Ventura 2019 (1)**	**Wijaya 2019 (8)**
Congenital adrenal hyperplasia(CAH)	51 (69.9%)	Not reviewed	74 (71.8%)	35 (45.5%)	Not	35 (85.3%)	362 (83.4%)
21-hydroxylase deficiency	24		72	Subgroups were	reviewed		351
11-hydroxylase deficiency	19		-	not reviewed			3
3β-hydroxy steroid dehidrogenase	-		2				-
17α hydroxylase deficiency	6		-				5
21-22 desmolase	1		-				-
StAR deficiency	1		-		5		2
Non-CAH etiologies	22 (30.1%)	16	29 (28.2%)	42 (54.5%)	6	6 (14.6%)	49 (11,3%)*
ALD	8	5	4	3	-	1	22
Triple A	5	-	1	-	-	-	2
FGD	3	-	-	4	1	-	-
Autoimmun adrenalitis (all)	1	5	13	23	3	3	3
Isolated	1		4	18	-		-
APS	-		9	5	3		3
Congenital adrenal hypoplasia	1	5	1	2	-	-	20
IMAGe	1?	1	-	(1)?	-	-	-
Wolman disease	-	-	3	-	-	-	-
Zelweger disease	-	-	1	-	-	-	-
Steroidogenic factor 1 deficiency	-	-	-	-	-	-	1
Pearson disease	-	-	-	-	-	1	-
Bil. adrenal hemorrhage	-	-	-	2	-	1	-
Bil. Adrenalectomy	-	-	-	5	-	-	1
Unknown etiology	3	-	6	3	2	-	23*
Total PAI	73	16	103	77	11	41	434

*Unknown etiologies classified as a separate group in this study, they were not included in non-CAH group.

Table 2: Non-CAH PAI patients' characteristics, treatment and follow up.

Number of patients	22
Male/Female	19/3
Age at diagnosis. median (min-max) years	3.52 (0.03-15.17)
Height SDS at diagnosis, mean ±SD	-0.63±1.56
Weight SDS at diagnosis mean ± SD	-0.95±1.61

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Symptoms and clinical findings	
Hyperpigmentation of skin	81.8% (18/22)
Hypoglycemia symptoms Convulsions	40.9% (9/22) 27.3% (6/22)
Weakness/Fatigue	31.8% (7/22)
Alacrima	22.7% (5/22)
Adrenal crisis	22,7 % (5/22)
Achalasia	13.6% (3/22)
Neonatal prolonged jaundice	13.6% (3/22)
Learning disability	13.6% (3/22)
Vomiting	9.1% (2/22)
Headache	9.1% (2/22)
Low school performance	1/22
Mental retardation	1/22
Walking disability	1/22
Epilepsy	1/22
Polyneuropathy	1/22
Parental consanguinity	77.8 % (14/18)
Laboratory findings	
Hypoglycemia	50.0% (11/22)
Hyponatremia	36.4% (8/22)
Hyperkalemia	22.7% (5/22)
ACTH (reference range < 10.56 pmol/L)	284.68 ± 69.95
Cortisol (reference range 138-580 nmol/L)	85.56 ± 87.49
Medical treatment given to patients	
Hydrocortisone **(mg/m2/d)**	100% (22/22) (14.5± 2.65)
Fludrocortisone **(mg/d)**	27.3% (6/22) (0.05)
Follow up period (months)	65.04 ± 36.23

To convert ACTH pmol/L to pg/ml divide by 0.22. To convert cortisol nmol/L to µg/dL divide by 27.6

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Table 3: Etiologies and characteristics of non-CAH PAI patients

No	Sex	Age at onset AI (year)	Clinical presentation	Additional pathologies and imagination findings	Serum cortisol nmol/L	Serum ACTH pmol/L	Serum VLCFA	Etiologies	Gene mutation and variant	Treatment
1[α]	M	9.44	Hyperpigmentation of skin, weakness, learning disability	VSD, enuresis At 15 year: White matter involvement	121.44	>275.0	High	ALD	*ABCD1* p.P543L mutation	HC, FC, Lorenzo's oil Lovastatin
2[α]	M	5.15	Hyperpigmentation of skin, adrenal crisis	Thin corpus callosum, mild hydrocephalus	<27.6	>275.0	-	ALD	*ABCD1* p.P543L mutation	HC, FC
3[α]	M	2.89	Hypoglycemia symptoms (**sweating, confusion**), vomiting	-	245.64	>275.0	-	ALD	Not done	HC
4[α]	M	9.19	Hyperpigmentation of skin	White matter involvement	88.87	>275.0	High	ALD	*ABCD1* p.P543L mutation	HC Lorenzo's oil Lovastatin
5[β]	M	3.65	Hyperpigmentation of skin, hypoglycemia symptoms	-	146.28	>275.0	-	ALD	*DAX-1*, *ABCD1*, *MC2R* no mutation	HC, FC 5 years
6[β]	M	3.21	Hyperpigmentation of skin	Motor-mental retardation, epilepsy, stereotypic movements, cerebral atrophy, White matter involvement	67.62	>275.0	High	ALD	*ABCD1, MC2R* no mutation	HC
7	M	15.17	Hyperpigmentation of skin, weakness, learning disability, headache**. walking** difficulty	White matter involvement	121.44	>275.0	-	ALD	*ABCD1* p.P543L mutation	HC Lorenzo's oil Lovastatin
8	M	11.39	Hyperpigmentation of skin	Polyneuropathy, status epilepticus	<27.6	>275.0	High	ALD	Not done Lost the follow up	HC
9#	F	7.83	Hyperpigmentation of skin, hypoglycemia symptoms, convulsions, achalasia, alacrima, learning disability	Mental retardation, short stature,facial dysmorphism, thenar atrophy, nasal speech	30.36	>275.0	-	Tripple A sydrome	*AAAS* p.L356Vfs*8 homozygous mut	HC Surgery for achalasia
10#	M	7.39	Hyperpigmentation of skin, achalasia, alacrima,	-	41.4	>275.0	-	Tripple A sydrome	*AAAS* p.L356Vfs*8 homozygous mut	HC Surgery for achalasia
11#	F	6.07	Hyperpigmentation of skin, hypoglycemia symptoms, convulsions, achalasia, alacrima, vomiting, headache	-	218.04	186.34	-	Tripple A sydrome	*AAAS* p.L356Vfs*8 homozygous mut	HC
12	M	3.39	Hypoglycemia symptoms, convulsions, weakness, fatigue, alacrima, hyperpigmentation of skin	Mild fasial dismorphism, normocytic anemia, posterior corpus callosum hypoplasia	<13.8	>440.0	-	Tripple A sydrome	*AAAS* p.L356Vfs*8 homozygous mut	HC

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13	M	3.19	Hyperpigmentation of skin, hypoglycemia symptoms, convulsions, weakness, alacrima	-	<13.8	>440.0	N	Triple A syndrome	*AAAS* p.L356Vfs*8 homozygous mut	HC
14	F	0.13	Hyperpigmentation of skin, hypoglycemia, prolonged jaundice	Pes equinovarus, posterior embriotoxone,	<27.6	>275.0	-	FGD	***MC2R* p.G116V homozygous mut**	HC
15	M	1.78	Hyperpigmentation of skin, hypoglycemia symptoms, convulsions, prolonged jaundice	-	33.12	>275.0	-	FGD	***MC2R* p.G116V homozygous mut**	HC
16	M	0.22	Prolonged jaundice	Transient hypertirotropinemia, ASD, hypomyelinization of periventricular white matter	<27.6	>275.0	-	FGD	*MC2R* p.L225R homozygous mut	HC
17	M	0.04	Adrenal crisis in newborn	White matter involvement	79.21	>275.0	-	Congenital Adrenal Hypoplasia	*DAX-1* p.Q301* hemizygous mut (novel)	HC, FC
18	M	13.49	Hyperpigmentation of skin, weakness, fatigue, adrenal crisis	Obesity, metabolic syndrome, unilateral renal agenesis	<27.6	>275.0	C26:N C26/24 high	Autoimmune AI (isolated)	-	HC, Metformin, ACEinhibitor
19	M	0.03	Adrenal crisis in newborn	Facial dysmorphism, bilateral undescendent testes, penil hypospadias, renal ectopy, patent ductus arteriosus, growth and mental retardation	.	.	-	MIRAGe syndrome? Syndromic PAI	Genetic analyse for Wolf Hirschorn was normal.	HC, FC
20	M	13.69	Hyperpigmentation of skin	Hereditary spherocytosis, unilateral surrenal hematoma, unilateral undescendent testis	328.68	120.34	N	UD	*DAX1* no mutation	HC
21	M	2.09	Hyperpigmentation of skin, hypoglycemia symptoms, convulsions, weakness	-	<**27.6**	>275.0	N	UD	*DAX-1*, *DMD* no mutation	HC
22	M	0.03	Adrenal crisis in newborn, hypoglycemia symptoms, hyperpigmentation of skin	Microcephaly, diplopia, cerebral and corpus callosum atrophy, calcification in basal ganglia	.	**391.6**	C26 high	UD	-	HC, FC **(2 years)**

α:relatives β: siblings #:relatives. M: male F: female N: normal levels. To convert cortisol nmol/L to µg/dL divide by 27.6. To convert ACTH pmol/L to pg/ml divide by 0.22. HC: hydrocortisone, FC: fludrocortisone

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