THE MEDICAL BULLETIN OF
SISLI ETFAL HOSPITAL

DOI: 10.14744/SEMB.2021.28044
Med Bull Sisli Etfal Hosp 2022;56(2):291–298

Original Research

Clinical and Genetic Characteristics of Patients with Common and Rare Types of Congenital Adrenal Hyperplasia: Novel Variants in *STAR* and *CYP17A1*

Ozge Koprulu,[1] Behzat Ozkan,[1] Sezer Acar,[1] Ozlem Nalbantoglu,[1] Beyhan Ozkaya Donmez,[1] Gulcin Arslan,[1] Filiz Hazan,[2] Semra Gursoy[3]

[1]Division of Pediatric Endocrinology, Dr. Behçet Uz Children's Training and Research Hospital, Izmir, Turkey
[2]Department of Medical Genetics, Dr. Behçet Uz Children's Training and Research Hospital, Izmir, Turkey
[3]Department of Pediatric Genetics, Dr. Behçet Uz Children's Training and Research Hospital, Izmir, Turkey

Abstract

Objectives: Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive diseases characterized by salt wasting or virilization. 21 hydroxylase deficiency (21-OHD) accounts for 90–95% of all cases of CAH and caused by the genetic defects of CYP21A2. Other forms include 3-β-hydroxysteroid dehydrogenase deficiency, 11-β-hydroxylase deficiency (11β-OHD) (%5-8), 17-α-hydroxylase deficiency (17α-OHD), and steroidogenic acute regulatory protein (*STAR*) defects (congenital lipoid adrenal hyperplasia) with mutations in *HSD3B2*, *CYP11B1*, *CYP17A1*, and *STAR*, respectively. Objectives: Herein, we aimed to present the clinical and genetic features of 64 patients with various types of CAH.

Methods: Sixty-four patients with CAH, monitored in the Izmir Dr. Behcet Uz Children Hospital Division of Pediatric Endocrinology, were retrospectively analyzed for the clinical, laboratory, and genetic data.

Results: Fifty-six patients (87.5%) had 21-OHD and four patients (6.3%) had 17α-OHD, three patients (4.7%) had 11β-OHD, and one patient (1.5%) had *STAR* defect. The most common presenting features in 21-OHD were ambiguous genitalia. Patients with 21-OHD were diagnosed earlier than the rare groups. Disease-causing variants of *CYP21A2* were identified in 46 patients. The most common mutations were IVS2, Q318X, I172N, and large deletions. Three patients with 11β-OHD were presented with enlargement of penis and early pubic hair at the median presenting age of 26 months. 17α-OHD deficiency was detected in 4 cases. Genetic analysis revealed two different homozygous *CYP17A1* variants. The patient with *STAR* defect was presented with dehydration and cholestasis in 44 days of the life. Genetic analysis of patient with *STAR* deficiency revealed a novel homozygous variant.

Conclusion: The current study reported a genotype-phenotype correlation consistent with literature data in CAH cases with 21-OHD. This study also reported novel homozygous variants in *STAR* and *CYP17A1* genes that lead to rare types of CAH.

Keywords: Congenital adrenal hyperplasia, *CYP11B1*, *CYP17A1*, CYP21A2, *STAR*

Please cite this article as "Koprulu O, Ozkan B, Acar S, Nalbantoglu O, Ozkaya B, Arslan G, et al. Clinical and Genetic Characteristics of Patients with Common and Rare Types of Congenital Adrenal Hyperplasia: Novel Variants in *STAR* and *CYP17A1*. Med Bull Sisli Etfal Hosp 2022;56(2):291–298".

Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive diseases which is the most common cause of adrenal deficiency in children. It is mainly characterized by salt wasting (SW) caused by impairment of adrenal cortisol and/or aldosterone biosynthesis or virilization due to excessive androgen biosynthesis.[1] Phenotypically,

Address for correspondence: Ozge Koprulu, MD. Dr. Behcet Uz Cocuk Egitim ve Arastirma Hastanesi, Pediatrik Endokrinoloji Bilim Dali, Izmir, Turkey
Phone: +90 506 599 46 12 **E-mail:** ozgeguclu@hotmail.com

Submitted Date: June 17, 2021 **Accepted Date:** August 31, 2021 **Available Online Date:** June 28, 2022

CAH can be divided into two forms; classical and non-classical (NC). The classic form can vary in SW and simple-virilizing (SV) phenotypes. The impairment of the enzyme activity determines the form of the disease. The incidence of classical CAH is about 1:14,000–1:18,000 live births.[2] 21 hydroxylase deficiency (21-OHD) accounts for 90–95% of all cases with CAH and caused by the genetic defects of CYP21A2. Other forms include 3-β-hydroxysteroid dehydrogenase deficiency (3β-HSD), 11-β-hydroxylase deficiency (11β-OHD) (5–8%), 17-α-hydroxylase deficiency (17α-OHD), and steroidogenic acute regulatory protein (STAR) defects (congenital lipoid adrenal hyperplasia) with mutations in *HSD3B2*, *CYP11B1*, *CYP17A1*, and *STAR*, respectively.[3]

11β-OHD, is the second prevalent type of CAH following 21OHD and associated with low cortisol and variable mineralocorticoid production.[4] 17α-OHD, is one of two hypertensive forms of CAH. Frequent symptoms include mild hypocortisolism, ambiguous genitalia in 46,XY individuals or ovarian failure at puberty in 46,XX individuals, and hypokalemic hypertension due to increased DOC and corticosterone.[5] Defects in *STAR* block the influx of cholesterol from the outer mitochondrial membrane to the inner and disrupt all steroidogenesis. *STAR* mutations cause congenital lipoid adrenal hyperplasia (lipoid CAH).[6]

To date, more than 200 mutations of *CYP21A2* have been described worldwide. The most frequent mutations in CYPA21A2 are IVS-2, large conversion/deletions, I172N and R356W in Turkey.[7] Q318X mutations and large gene deletions constitute the most severe disease with low enzyme activity.[8] CAH shows a continuous phenotypic spectrum in 21-OHD. Genotyping is important in confirming the diagnosis, maintains prognostic information about severity and essential for genetic counseling.[9] Herein, we aimed to present the clinical and genetic features of 64 patients with various types of CAH.

Methods

A total of 64 patients with CAH, monitored and treated at Division of Pediatric Endocrinology in the Izmir Dr. Behcet Uz Children Hospital between 1998 and 2020 years, were retrospectively analyzed. A questionnaire was used to evaluate all clinical, biochemical data related to the diagnosis and treatment. The diagnosis for patients was based on the clinical features and serum hormone assays. Studies were performed with the approval of the Ethics Committee of the Behçet Uz Children's Hospital (2020/386). Patients and parents provided written informed consent.

The study was designed retrospectively and the clinical, laboratory and genetic data of all patients were obtained from file records. Genetic results of patients with 21-OHD were obtained by Multiplex Ligation-Dependent Probe Amplification (MLPA) and/or strip assay (Revers Dot Blot) analysis. In addition, genetic analysis of patients with 17α-OHD 11β-OHD, and *STAR* deficiency were performed by Next Generation Sequencing (NGS) or Sanger sequencing methods.

DNA Extraction

Genomic DNA of the patients and some of the parents was extracted from the peripheral blood as described in manufacturer's protocol on a DNA Isolation system (Magpurix Blood Dna Extraction Kit, Zinexts Life Science Corp., Taiwan). Primary quality control of the isolated DNA samples was performed using Qubit (Thermofisher, ABD).

MLPA Analysis

CYP21A2/CYP21A1P deletions, duplications, and large gene conversions were analyzed by MLPA with the SALSA P050 CAH MLPA kit (MRC-Holland BV, Amsterdam, Holland). It includes 27 MLPA probes with amplification products between 130 and 382 nucleotides, eight probes for *CYP21A2* gene (large rearrangements, SNP at 113 bp before start codon, IVS-12A/C-G, 706_713del8, I172N, V237E, M239K, and F306+T) and four probes for CYP21A1P pseudogene. Furthermore, the probemix contains six probes for the TNXB gene and one for the ATF6B gene to further define *CYP21A2* gene deletions. To analyse MLPA data we used ABI 3500 capillary electrophoresis system and Coffalyser software (MRC Holland, Amsterdam, The Netherlands), an Excel-based program. The area under the peak for each amplified fragment was measured and normalized to the peak areas of normal control individuals.

Strip Assay Analysis

Some *CYP21A2* variants (P30L, I2 splice, Del 8bp E3, I172N, Cluster E6, V281L, L30 frame shift, Q318X, R356W, P453S, and R483P) were analyzed by Revers Dot Blot.

NGS Analysis

46,XY DSD and 46,XX DSD Custom Target Capture NGS Panels (Celemix, Inc., Seoul, Korea) were performed to analyze 34 genes. Libraries were arranged according to manufacturer's instructions. Target capture NGS was performed on an Illumina MiniSeq NGS System (Illumina, Inc., San Diego, CA, USA). The data were analyzed using "SEQ" variant analysis software (Genomize, Istanbul, Turkey) according to the reference genome of GRCh37(h19). ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) and literature information were considered for collecting the information about known variants. We also used the search engine

Varsome (https://varsome.com/), which has information from 30 external databases, to investigate the pathogenicity of the novel variant. The pathogenicity of the identified sequence variants was reported using an automatic variant classifier that evaluated the submitted variant according to the American College of Medical Genetics (ACMG) 2015 guidelines, classifying it as one of "pathogenic," "likely pathogenic," "likely benign," "benign" or "uncertain significance."[2]

Sanger Sequencing

STAR gene was amplified by polymerase chain reaction. All of the coding exons and exon-intron boundaries of the gene were analyzed by Sanger sequencing. The sequences were analyzed with SeqScape Software V3 sequencing program (Applied Biosystems, ThermoFischer). The Ensembl database (GRCh37.p13) with ENST00000276449 transcript ID of *STAR* was used to compare the individual's and the reference sequence. For segregation analysis, primers were designed for all needed regions.

Statistical Analysis

The values statistically analyzed by SPSS (Statistical Package for the Social Sciences) v25 (IBM, Armonk, NY, USA). Results were given as median (range). To calculate the median value of a group, Mann–Whitney U test was used.

Results

Characteristics of CAH Patients

This retrospective study included 64 subjects (39 females and 25 males) with CAH from 61 unrelated families. Parents of patients were consanguineous in 40 (65%) of the 61 unrelated families, whereas 21 families did not declare consanguinity. Frequency of consanguinity was 65% in all patients: 59% in the 21-OHD patients, and 66% in the patients with 11β-OHD. All the parents of the patients with 17α-OHD were consanguineous.

Fifty-six of the patients (87.5%) had 21-OHD and four patients (6.3%) had 17α-OHD, three patients (4.7%) had 11β-OHD, and one patient (1.5%) had *STAR* defect. The most common presenting features in 21-OHD were ambiguous genitalia (46%), vomiting/fatigue (32%), premature pubarch (11%), jaundice (4%), and diarrhea (4%). Ambiguous genitalia was the presenting symptom in 22 of the 32 female patients with 21-OHD whereas vomiting was the most common presenting symptom in males.

Patients with 21-OHD

Patients with 21-OHD were classified according to their phenotype: SW (n: 39, 70%), SV (n: 13, 23%), and NC forms (n: 4, 7%). The median presenting age of the groups was 19 days (0–74 days), 3.23 years (0–8.1 years), 14 years of age (8.69–17 years) in SW, SV, and NC forms, respectively. Patients with 21-OHD were diagnosed earlier than the rare groups; median ages at diagnosis were 20 days (0–14.6 years) versus 2.1 years (1.7–2.5 years) and 11.08 years (3.1–14 years) in 11β-OHD, 17α-OHD, respectively. Among 21-OHD patients; female patients were diagnosed earlier than males; median ages at diagnosis were 9 versus 31 days, respectively. Considering the psychiatric evaluations and family request, a 21-OHD patient with SV whose genital appearance was compatible with Prader stage 5 reared as male despite of 46,XX karyotype.

A total of 47 patients with 21-OHD were analyzed for the *CYP21A2* gene variants. Disease-causing mutations were identified in 46 patients. Thirty-seven patients (80.4%) were homozygous and eight patients (17.3%) were compound heterozygous. The most common mutation was IVS2 (n: 13, 28.2%), followed by Q318X (n: 6, 13%), I172N (n: 5, 10.8%), and large deletions (n: 4, 8.7%). The genotype and phenotype of patients with 21-OHD are shown in Table 1.

Table 1. Variants of CYP21A2 and clinical classifications

Genotype	Number of cases	Clinical type		
		SW	SV	NC
Large deletions	3	3	0	0
IVS2 (a)	9	8	1	0
I172N (a)	4	1	3	0
R356W (a)	3	3	0	0
P30L (a)	3	0	2	1
Q318X (a)	3	3	0	0
del8bp (a)	1	0	1	0
IVS2, Q318X (a)	1	1	0	0
I172N, E6cluster (a)	1	1	0	0
P30L, IVS2, del8bp (a)	3	3	0	0
V281L, Q318X (a)	1	1	0	0
Q318X, L307fs (a)	1	1	0	0
Q318X (a), IVS2 (b), del8bp (b)	1	1	0	0
I172N/V237E+M239K (CH)	1	1	0	0
I172N/V281L (CH)	2	0	1	1
IVS2/R356W (CH)	2	1	1	0
V281L/Q318X (CH)	2	1	0	1
IVS2/P453S (CH)	1	0	1	0
Large del/I172N, V237E, M239K	1	1	0	0
P454S (a), V281L (b), R340H (b)	1	0	0	1
V281L/ND	1	0	1	0
IVS2/ND	1	1	0	0

SW: Salt wasting; SV: Simple virilizing; NC: Nonclasiccal; a: Homozygous pattern; b: Heterozygous pattern; CH: Compound heterozygous; ND: Non-determined.

Patients with 17α-OHD

17α-OHD was detected in four cases from two unrelated families. The first patient from the family one (patient 1) was presented with puberty tarda and the diagnosis of CAH in the index patient prompted investigations in other siblings (patient 2,3). Patient four was referred for puberty tarda at the age of 14 years. LH and FSH levels were significantly elevated, consistent with gonadal failure. A laparoscopic examination and gonadectomy was planned for patient four who had a 46,XY karyotype, which was not accepted by the parents. This patient was followed up with laboratory markers and imaging for the development of malignancy. Genetic analysis revealed two different homozygous *CYP17A1* variants (c.1319 G>A, c.991 G>A). c.1319 G>A (p.Arg440His) variant was previously reported in a patient with absent pubertal development, mild hypertension and hypergonadotropic hypogonadism.[10]

c.991 G>A (p.Glu331Lys) was interpreted as "Uncertain Significance" according to ACMG criteria. Moreover, this variant has not been identified in HGMD (http://www.hgmd.cf.ac.uk) and the Exome Aggregation Consortium. In addition, this variant was strongly predicted to be a disease-causing variant with in silico analyses with MutationTaster (mutationtaster.org), SIFT (sift.jcvi.org), and PolyPhen-2 (genetics.bwh.harvard.edu/pph2). This 46,XY individual was referred us because of puberty tarda at age of 14 years with female appearance. Parents and unaffected sibling were carriers.

Detailed clinical and laboratory findings of the rare forms CAH are provided in Table 2.

Patients with 11β-OHD

All three patients with 11β-OHD were presented with enlargement of penis and early pubic hair. The median presenting age of these three patients was 26 months. Of the patients with 11β-OHD, two patients had 46,XY karyotype, and one patient had 46,XX karyotype. All patients with 11β-OHD reared as males. The weight SDS and height SDS of the 11β-OHD cases were +2.9, +3.9, +3.8 SDS and +3.1, +2.9, +3.6 SDS, respectively. Bone age of all patients with 11β-OHD was advanced and median bone age was 7 years. Two different variants that were previously reported were detected in two patients who underwent *CYP11B1* genetic analysis among three patients with 11β-OHD (c.896 T> C, c.1342 C> T).[11,12]

Patient with *STAR* Deficiency

The patient with *STAR* defect was presented with dehydration, vomiting, inability to weight gain (her birth weight was 4000 g and her current weight was 3800 g), and prolonged jaundice in 44 days of life. As a result of investigations, elevated total and direct bilirubin (17.5 and 10.5 mg/dL), moderate AST elevation (119 IU/L), mild hyponatremia (131 mmol/L), elevated serum level of ACTH, and low level of cortisol and hepatomegaly (3 cm palpable) were detected (Table 2). Her external genital appeared normal female without palpable gonads and she had both vaginal and urethral openings. Pelvic ultrasonography and MRI revealed no uterus or fallopian tubes; however, bilateral gonad structures were detected in the inguinal canal. Chromosome analysis from peripheral blood cells revealed 46,XY. Hydrocortisone treatment was given for adrenal insufficiency. In the follow-up, signs of cholestasis, elevated AST, inability to weight gain, and hyponatremia were completely improved. After *STAR* deficiency was diagnosed clinically and genetically, the patient underwent gonadectomy and was reared as a girl. Gonad pathology was compatible with atrophic testis and malignant degeneration was not detected. Genetic analysis of patient with *STAR* deficiency revealed a novel homozygous variant in the 3rd exon of *STAR* gene (c.219_223delGGCCT and p.Ala74SerfsTer16), which has not been identified in the Human Gene Mutation Database (HGMD) or Genome Aggregation Database (gnomAD). This variant that caused a frame shift mutation generating downstream stop codon was interpreted as "Pathogenic" according to ACMG criteria and was strongly predicted to be a disease-causing variant with MutationTaster. Parents were obligatory carriers.

Discussion

CAH is a group of autosomal recessive diseases causing enzyme deficiencies in the adrenal steroidogenesis. The most prevalent cause of CAH is 21-OHD. In our population, the incidence of classical 21-OHD and 11β-OHD is approximately 1:15,000 and 1:60,000, respectively.[13] In spite of CAH is an autosomal recessive disorder, the high female/male ratio of CAH suggests that female CAH cases are usually diagnosed at birth due to ambiguous genitale, while males are usually missed, misdiagnosed or died early due to SW crisis.[14,15]

In studies from the screened populations it was reported that the diagnosis rates of males increased and death rates due to SW crisis decreased with screening. As a result, the female/male ratio was equal in these populations.[16] Moreover, parental consanguinity rate among the patients was higher than the general population in Turkey (78% vs. 24%).[17] In the current study included 39 females and 25 males (female/male ratio was 1.56) and the frequency of consanguinity was found to be 65% in all cases and 59% in 21-OHD cases, and these rates were high in line with the literature data. Likewise, recent studies, in our study, female patients were diagnosed earlier than males; median ages at diagnosis were 14 versus 38 days, respectively. This was similar among 21-OHD patients too (9 days vs. 31 days).

Table 2. Clinical characteristics of patients with rare forms of CAH

Patients	Findings at onset	Genital examination	Age at onset	Reared gender	Consanguinity	Karyotype	Pub. Stage	Hypertension	Na/K mmol/l	FSH/LH mIU/ml	ACTH pg/mL	Cortisol mcg/dL	Testosterone ng/dL	17-OHP ng/mL	1,4-AS ng/mL	DHEA-S mcg/dL	11 DOC ng/mL	Variant (c.DNA, p.DNA)
17 α-hydroxylase deficiency																		
Family 1																		
Patient 1	Puberty tarda	Normal female Nonpalpable gonad	14 y	F	Yes	46,XY	1	No	141/ 4.2	22/5	351	<1.0	0.44	0.13	<0.3	29	NA	CYP17A1 (c.1319 G>A/ p.Arg440His)
Patient 2	Family screen	Normal female Nonpalpable gonad	11 y	F	Yes	46,XX	1	No	142/ 3.5	30/11.5	80	<1.0	1.6	NA	<0.3	8.2	NA	
Patient 3	Family screen	Normal female Nonpalpable gonad	3.1 y	F	Yes	46,XX	1	No	140/ 4.3	22.2/0.2	68	<1.0	2	NA	<0.3	NA	NA	
Family 2																		
Patient 4	Puberty tarda	Normal female Nonpalpable gonad	14 y	F	Yes	46,XY	1	No	141/ 3.5	12.8/25	96.9	4.2	7.7	NA	<0.3	21	NA	CYP17A1 (c.991G>A/ p.Glu331Lys) (novel)
11β-hydroxylase deficiency																		
Patient 5	Premature pubarch, macropenis	Normal male Bilateral palpable testes	21 m	M	Yes	46,XY	1	No	132/ 4.6	NA	875	4.8	59	4.1	NA	32	275	CYP11B1 (c.896T>C/ p.Leu299Pro)
Patient 6	Premature pubarch, macropenis	Normal male Bilateral palpable testes	30 m	M	Yes	46,XY	2	No	142/ 4	NA	926	2.1	32	5.7	5.7	28	NA	CYP11B1 (c.1342C>T/ p.Arg448Cys)
Patient 7	Premature pubarch, macropenis, hypospadias	Ambiguous genitale Nonpalpable gonad (Prader stage 4)	24 m	M	Yes	46,XX	2	Yes	146/ 3.8	NA	392	6	291	22	NA	NA	31	Nondetermined
StAR defect																		
Patient 8	Prolonged jaundice, vomiting, inability to weight gain	Normal female Nonpalpable gonad	44 d	F	Yes	46,XY	1	No	131/ 6.2	NA	>1200	0.9	0.1	NA	NA	4.79	NA	STAR (c.219_223delGGCCT/ p.Ala74SerfsTer16) (novel)

y: Year, m: Month, d: Day, M: Male, F: Female, Na: Sodium, K: Potassium, FSH: Follicle stimulating hormone, LH: Luteinizing hormone, ACTH: Adrenocorticotropic hormone, 17-OHP: 17-OH-Progesterone, 1,4-AS: 1,4-delta androstenedione, DHEA-S: Dehydroepiandrosterone sulphate, 11-DOC: 11-deoxycorticosterone, NA: Not available. Normal references of the parameters as follow: Na: 135-145 mEq/L, K: 3.5-5.0 mEq/L, FSH: 0.2-11.1 mIU/ml , LH<0.2 mIU/ml, ACTH: 0-46 pg/mL, Cortisol: 3.7-19.4 mcg/dL, Testosterone: <20 ng/dL, 1,4 Androstenedione: 0.3-3.3 ng/mL, DHEA-S: 65.3-214.1 mcg/dL, 11-Deoxycortisol: <8 ng/mL.

In most populations; 21-OHD accounts for about 90–95% of CAH.[9] A cohort from Turkey reported this percent as 85%,[18] Click or tap here to enter text.similar to the rate of 21-OHD (88%) in our study. The distribution of the 21-OHD patients was 70%, 23%, and 7% in the SW, SV, and NC form, respectively, which also coincided with the previous published data.[13,15,19] The distribution of the forms was similar whether underwent neonatal CAH screening or not. The unexpected low rates of NC form may be due to inability of NC cases to be diagnosed in neonatal screening too. In the current study, all four of NC cases were female. This shows that NC male cases are undiagnosed because they are asymptomatic.

At present, more than 200 mutations of the *CYP21A2* gene have been reported. Over the last two decades, has seen significant progress in our understanding of the genotype-phenotype correlation of 21-OHD.[20] There are numerous studies about this issue worldwide and in our country. [7,8,19,20] The first study on the molecular basis of CAH patients in Turkish population, included 56 patients. In this study; the most frequent mutations were IVS-2, large conversion, I172N, R356W, and large deletions.[7] Larger cohort study by Turan et al. confirmed that IVS-2 was the most prevalent mutation in Turkey and mutation frequencies of patients were closely similar. Our study shows similar frequencies of the most common mutations with recent studies published from Turkey.[19] The studies display the most frequent mutation is IVS-2 worldwide, the only exception was the V281L in the studies from America. This is likely due to the large number of NC cases or the large number of Ashkenazi Jews in study population.[21-23] In our study, the most common variants were IVS2, I172N and P30L in the SW, SV, NC forms, respectively. The previous studies display V281L is the most frequent in NC cases. Compared with other studies, the difference may be due to small number of NC cases in our study.[7,19]]

The most challenging cases were those showing multiple mutations which had more than one homozygous or more than two heterozygous mutations and two heterozygous genotype cases. Multiple mutations thought to be result from parental consanguinity. The two cases with heterozygous mutations should be evaluated further.

11β-OHD, the second common form of CAH representing 5–8% of the total cases. In our study, the ratio was similar with previous studies. In another study among Turkish population, the distribution of 11β-OHD was higher.[18] The distinction may be due to regional differences. The most common clinical features of 11β-OHD are ambiguous genitalia in 46,XX fetuses and hyporeninemic hypokalemic hypertension due to high concentrations of deoxycorticosterone (DOC). In classic 11β-OHD, extreme androgen production causes virilization of external genitalia and isosexual precocious puberty in females and males, respectively. Similarly, all three of the patients with 11β-OHD were presented with enlargement of the penis and early pubic hair at a median age of 24 months (21–30 months). Hypertension, occurs in approximately two-third of the cases due to the potent mineralocorticoid DOC.[4] In recent study, hypertension is observed one of the 3 cases at the time of diagnosis (33%).[24] The study reported by Kandemir et al. showed similar hypertension frequency in 11β-OHD.[25] In affected females, gender identity may be either male or female. This is thought to be due to pre and postnatal androgen exposure or the extent of genital virilization.[26] In our study group; one of the patients with the 11β-OHD was reared as male, despite of 46,XX karyotype. In two patients who had performed genetic analysis, two different variants was detected in *CYP11B1*, one of which is the most prevalent variant (c.896 T>C, p.Leu299Pro) in Turkey according to Baş et al., suggesting founder effect and additionally, c.1342 C>T (p.Arg448Cys) variant was also previously reported in some Turkish families.[27]

17α-OHD is a rare form of CAH. The most common presentation is puberty tarda in the literature.[28,29] In our study, the index case (Patient 1) was presented likewise. In this index case and two siblings; c.1319G>A variant, previously reported by Fardella et al. was detected in *CYP17A1*.[10] Reports suggest that 15% of the 17α-OHD patients may be normotensive.[30] Interestingly, in our study none of the patients were hypertensive. Single blood pressure measurements to evaluate hypertension may be misleading in childhood due to technical difficulties, blood pressure monitoring methods (e.g., holter devices) may be more helpful in these patients. Variable degree of hypertension in the 17OHD patients suggests, duration of this decompensated situation should be an important factor to the clinical severity of hypertension.[31] A novel variant was found in one of the four cases (from 2 unrelated families) with 17OHD. Considering the proper segregation of the parents, in silico analysis results and clinical phenotype, this variant was considered pathogenic.

STAR defects prevent the transport of cholesterol into mitochondria and lead to lipoid CAH. Congenital lipoid adrenal hyperplasia is characterized by severe SW and 46,XY DSD. Due to two-hit hypothesis age at onset may be later than the other SW CAH forms. Furthermore, the genotype may affect the severity of the disease.[32,33] Most *STAR* mutations are located between exon 5 and 7, and represent no measurable enzyme activity and thus cause severe disease. Some mutations, retaining 10–20% *STAR* protein activity, are related to milder form of lipoid adrenal hyperplasia. In

milder form typically adrenal insufficiency develops after infancy, mineralocorticoid secretion is minimally affected and masculinization can be affected to varying degrees. Most patients with lipoid adrenal hyperplasia have female external genitalia regardless of karyotype.[34] Our case was admitted for jaundice at 44 days of the life and had direct hyperbilirubinemia, mild hyponatremia, hyperkalemia, and external female genitalia with hyperpigmentation. Our case showed intermediate phenotype with severe insufficient masculinization and without neonatal adrenal crisis. Hormone analysis revealed low plasma cortisol, 17OH-progesterone and DHEA-S levels, while elevated ACTH level, suggesting primary adrenal insufficiency, and adrenals were in normal size. Following molecular genetic studies of this patient, we identified a novel homozygous variant in the *STAR*.

Conclusion

The current study reported a genotype-phenotype correlation consistent with literature data in CAH cases with 21 OHD. Determining the mutation type using molecular genetic methods in 21-hydroxylase deficiency, which is the most common cause of CAH, may be suggestive in terms of predicting the clinical course. This study also reported two different novel homozygous variants in *STAR* and *CYP17A1* genes that lead to rare types of CAH (*STAR* deficiency, 17α-OHD).

Disclosures

Ethics Committee Approval: Studies were performed with the approval of the Ethics Committee of the Behçet Uz Childrens Hospital (2020/386).

Peer-review: Externally peer-reviewed.

Conflict of Interest: None declared.

Authorship Contributions: Concept – O.K., B.O., S.A.; Design – O.K., S.A., S.G.; Supervision – B.O., S.G; Materials – O.N., B.O.D., G.A., F.H., Data collection &/or processing – O.K., O.N.; Analysis and/or interpretation – O.K., S.A., Literature search – O.K., S.A., O.N.; Writing – O.K., S.A., S.G.; Critical review – S.A., B.O.

References

1. Kirkgoz T, Guran T. Primary adrenal insufficiency in children: Diagnosis and management. Best Pract Res Clin Endocrinol Metab 2018;32:397–424. [CrossRef]
2. Speiser PW, Arlt W, Auchus RJ, Baskin LS, Conway GS, Merke DP, et al. Congenital adrenal hyperplasia due to steroid 21-hydroxylase deficiency: An Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab 2018;103:4043–88. [CrossRef]
3. Witchel SF. Congenital adrenal hyperplasia. J Pediatr Adolesc Gynecol 2017;30:520–34. [CrossRef]
4. Bulsari K, Falhammar H. Clinical perspectives in congenital adrenal hyperplasia due to 11β-hydroxylase deficiency. Endocrine 2017;55:19–36. [CrossRef]
5. Kater CE, Biglieri EG. Disorders of steroid 17 alpha-hydroxylase deficiency. Endocrinol Metab Clin North Am 1994;23:341–57.
6. Miller WL. Mechanisms in endocrinology: Rare defects in adrenal steroidogenesis. Eur J Endocrinol 2018;179:R125–41. [CrossRef]
7. Baş F, Kayserili H, Darendeliler F, Uyguner O, Günöz H, Yüksel Apak M, et al. CYP21A2 gene mutations in congenital adrenal hyperplasia: genotype-phenotype correlation in Turkish children. J Clin Res Pediatr Endocrinol 2009;1:116–28. [CrossRef]
8. Tardy V, Menassa R, Sulmont V, Lienhardt-Roussie A, Lecointre C, Brauner R, et al. Phenotype-genotype correlations of 13 rare CYP21A2 mutations detected in 46 patients affected with 21-hydroxylase deficiency and in one carrier. J Clin Endocrinol Metab 2010;95:1288–300. [CrossRef]
9. Hannah-Shmouni F, Chen W, Merke DP. Genetics of congenital adrenal hyperplasia. Endocrinol Metab Clin North Am 2017;46:435–58. [CrossRef]
10. Fardella CE, Hum DW, Homoki J, Miller WL. Point mutation of Arg440 to His in cytochrome P450c17 causes severe 17 alpha-hydroxylase deficiency. J Clin Endocrinol Metab 1994;79:160–4.
11. Riedl S, Nguyen HH, Clausmeyer S, Schulze E, Waldhauser F, Bernhardt R. A homozygous L299P mutation in the CYP11B1 gene leads to complete virilization in 46,XX individuals with 11-beta-hydroxylase deficiency. Horm Res 2008;70:145–9. [CrossRef]
12. Geley S, Kapelari K, Jöhrer K, Peter M, Glatzl J, Vierhapper H, et al. CYP11B1 mutations causing congenital adrenal hyperplasia due to 11 beta-hydroxylase deficiency. J Clin Endocrinol Metab 1996;81:2896–901. [CrossRef]
13. Güran T, Tezel B, Çakır M, Akıncı A, Orbak Z, Keskin M, et al. Neonatal screening for congenital adrenal hyperplasia in Turkey: Outcomes of extended pilot study in 241,083 infants. J Clin Res Pediatr Endocrinol 2020;12:287–94. [CrossRef]
14. Sack J, Front H, Kaiserman I, Schreiber M. 21-Hydroxylase deficiency: screening and incidence in Israel. Horm Res 1997;48:115–9. [CrossRef]
15. Elmougy F, Elsharkawy M, Hafez M, Atty SA, Baz H, Ibrahim A, et al. Genetic profiling of CAH Egyptian children: rapid guide to clinical interpretation of common mutations. J Endocrinol Invest 2021;44:83–93. [CrossRef]
16. Thil'en A, Nordenström A, Hagenfeldt L, von Döbeln U, Guthenberg C, Larsson A. Benefits of neonatal screening for congenital adrenal hyperplasia (21-hydroxylase deficiency) in Sweden. Pediatrics 1998;101:E11. [CrossRef]
17. Koç İ, Eryurt MA. The causal relationship between consanguineous marriages and infant mortality in TURKEY. J Biosoc Sci 2017;49:536–55. [CrossRef]
18. Kandemir N, Yordam N. Congenital adrenal hyperplasia in Turkey: a review of 273 patients. Acta Paediatr 1997;86:22–5. [CrossRef]
19. Turan I, Tastan M, Boga DD, Gurbuz F, Kotan LD, Tuli A, et al. 21-Hydroxylase deficiency: Mutational spectrum and Genotype-

Phenotype relations analyses by next-generation sequencing and multiplex ligation-dependent probe amplification. Eur J Med Genet 2020;63:103782. [CrossRef]
20. Concolino P, Costella A. Congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency: A comprehensive focus on 233 pathogenic variants of CYP21A2 gene. Mol Diagn Ther 2018;22:261–80. [CrossRef]
21. de Carvalho DF, Miranda MC, Gomes LG, Madureira G, Marcondes JA, Billerbeck AE, et al. Molecular CYP21A2 diagnosis in 480 Brazilian patients with congenital adrenal hyperplasia before newborn screening introduction. Eur J Endocrinol 2016;175:107–16. [CrossRef]
22. Marino R, Ramirez P, Galeano J, Perez Garrido N, Rocco C, Ciaccio M, et al. Steroid 21-hydroxylase gene mutational spectrum in 454 Argentinean patients: genotype-phenotype correlation in a large cohort of patients with congenital adrenal hyperplasia. Clin Endocrinol (Oxf) 2011;75:427–35. [CrossRef]
23. New MI, Abraham M, Gonzalez B, Dumic M, Razzaghy-Azar M, Chitayat D, et al. Genotype-phenotype correlation in 1,507 families with congenital adrenal hyperplasia owing to 21-hydroxylase deficiency. Proc Natl Acad Sci U S A 2013;110:2611–6. [CrossRef]
24. Zhou Q, Wang D, Wang C, Zheng B, Liu Q, Zhu Z, et al. Clinical and molecular analysis of four patients with 11β-hydroxylase deficiency. Front Pediatr 2020;8:410. [CrossRef]
25. Kandemir N, Yilmaz DY, Gonc EN, Ozon A, Alikasifoglu A, Dursun A, et al. Novel and prevalent CYP11B1 gene mutations in Turkish patients with 11-β hydroxylase deficiency. J Steroid Biochem Mol Biol 2017;165:57–63. [CrossRef]
26. Bin-Abbas BS, Sakati NA, Al-Ashwal AA. Gender identity in congenital adrenal hyperplasia secondary to 11-hydroxylase deficiency. Ann Saudi Med 2006;26:239–41. [CrossRef]
27. Baş F, Toksoy G, Ergun-Longmire B, Uyguner ZO, Abalı ZY, Poyrazoğlu Ş, et al. Prevalence, clinical characteristics and long-term outcomes of classical 11 β-hydroxylase deficiency (11BOHD) in Turkish population and novel mutations in CYP11B1 gene. J Steroid Biochem Mol Biol 2018;181:88–97. [CrossRef]
28. Zhang M, Sun S, Liu Y, Zhang H, Jiao Y, Wang W, et al. New, recurrent, and prevalent mutations: Clinical and molecular characterization of 26 Chinese patients with 17alpha-hydroxylase/17,20-lyase deficiency. J Steroid Biochem Mol Biol 2015;150:11–6.
29. Rosa S, Steigert M, Lang-Muritano M, l'Allemand D, Schoenle EJ, Biason-Lauber A. Clinical, genetic and functional characteristics of three novel CYP17A1 mutations causing combined 17alpha-hydroxylase/17,20-lyase deficiency. Horm Res Paediatr 2010;73:198–204. [CrossRef]
30. Kardelen AD, Toksoy G, Baş F, Yavaş Abalı Z, Gençay G, Poyrazoğlu Ş, et al. A rare cause of congenital adrenal hyperplasia: clinical and genetic findings and follow-up characteristics of six patients with 17-hydroxylase deficiency including two novel mutations. J Clin Res Pediatr Endocrinol 2018;10:206–15. [CrossRef]
31. Wang YP, Zhao YJ, Zhou GY, He B. CYP17A1 gene mutations and hypertension variations found in 46, XY females with combined 17α-hydroxylase/17, 20-lyase deficiency. Gynecol Endocrinol 2014;30:456–60. [CrossRef]
32. Bose HS, Sugawara T, Strauss JF 3rd, Miller WL; International Congenital Lipoid Adrenal Hyperplasia Consortium. The pathophysiology and genetics of congenital lipoid adrenal hyperplasia. N Engl J Med 1996;335:1870–8. [CrossRef]
33. Guran T, Buonocore F, Saka N, Ozbek MN, Aycan Z, Bereket A, et al. Rare causes of primary adrenal insufficiency: genetic and clinical characterization of a large nationwide cohort. J Clin Endocrinol Metab 2016;101:284–92. [CrossRef]
34. Miller WL, Bose HS. Early steps in steroidogenesis: intracellular cholesterol trafficking. J Lipid Res 2011;52:2111–35. [CrossRef]