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Genotype of congenital adrenal hyperplasia patients with testicular adrenal rest tumor

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ABSTRACT

Testicular adrenal rest tumor (TART) is one of the important complications that can cause infertility in male patients with congenital adrenal hyperplasia (CAH) and should therefore be diagnosed and treated at an early age. The factors that result in TART in CAH have not been completely understood. The aim of this study is to evaluate the genotype-phenotype correlation in CAH patients with TART.

Method: Among 230 malepatients with CAH who were followed upwith regular scrotal ultrasonography in 11 different centers in Turkey, 40 patients who developed TARTand whose CAH diagnosis was confirmed by genetic testing were included in this study. Different approaches and methods were used for genotype analysis in this multicenter study. A few centers first screened the patients for the ten most common mutations in *CYP21A2* and performed Sanger sequencing for the remaining regions only if these prior results were inconclusive while the majority of the departments adopted Sanger sequencing for the whole coding regions and exon-intron boundaries as the primary molecular diagnostic approach for patients with either CYP21A2 orCYP11B1 deficiency. The age of CAH diagnosis and TART diagnosis, type of CAH, and identified mutations were recorded.

Results: TART was detected in 17.4% of the cohort [24 patients with salt-wasting (SW) type, four simple virilizing type, and one with nonclassical type with 21-hydroxylase (CYP21A2) deficiency and 11 patients with 11-beta hydroxylase (CYP11B1) deficiency]. The youngest patients with TART presenting with CYP11B1 and CYP21A2 deficiency were of 2 and 4 years, respectively. Eight different pathogenic variants in *CYP21A2*were identified. The most common genotypes were c.293-13C>G/c.293-13C>G (31%) followed by c.955C>T/c.955C>T(27.6%) and c.1069C>T/c.1069C>T (17.2%). Seven different pathogenic variants were identified in *CYP11B1*. The most common mutation *in CYP11B1* in our study was c.896T>C (p.Leu299Pro).

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Conclusion: We found that 83% TART patients were affected with SW typeCYP21A2 deficiency,and the frequent mutations detected were c.955C>T (p.Gln319Ter), c.293-13C>G in *CYP21A2* and c.896T>C (p.Leu299Pro) inCYP11B1. Patients with CYP11B1 deficiency may develop TART at an earlier age. This study that examined the genotype–phenotype correlation in TART may benefit further investigations in larger series.

1. Introduction and objective

Congenital adrenal hyperplasia (CAH) is an autosomal recessive disease that occurs as a result of deficiency/insufficiency of enzymes involved in glucocorticoid and/or mineralocorticoid synthesis. The most common cause of CAH is 21-hydroxylase deficiency with an incidence between 1:10.000 and 1:20.000, and >200 variants have been identified in *CYP21A2 (*encoding21-hydroxylase enzyme) to date (El-Maouche et al., 2017; Baumgartner-Parzer et al., 2020). The severity of CAH is variable in patients with CYP21A2 deficiency depending on residual enzyme activity (Krone and Arlt, 2009). Residual enzyme activity mainly depends on the genotype and can be divided into the following four subcategories: groups 0, A, B, and C (Krone and Arlt, 2009). Phenotypically, patients are classified into three categories. Patients presenting with the symptoms of cortisol&aldosterone deficiency because of 0% enzyme activity are classified into the classical salt-wasting (SW) form, which is the most severe form. Patients with residual enzyme activity of 1–2% are classified into the classic simple virilizing (SV) form with sufficient aldosterone, low cortisol, and very high androgen levels. The nonclassical form (NC) is the mildest form of CAH with 30–50% residual enzyme activity that is characterized by only slightly elevated androgen and generally adequate cortisol&aldosterone levels. The second most common cause of CAH is CYP11B1 deficiency, which accounts for 2–8% of the reported cases (Bulsari and Falhammer, 2017). Patients with CYP11B1 deficiencycan also present with both the classical and NC types of CAH according to the clinical findings and enzymatic activity. The NC form is caused by partial impairment of enzymatic function with a phenotype resembling NCCYP21A2 deficiency (Polat et al., 2014). The treatment of patients with classic CAH comprises lifetime glucocorticoid replacement, which lowers adrenocorticotropic hormone (ACTH) and adrenal androgen levels. (El-Maouche et al., 2017; Baumgartner-Parzer et al., 2020).

Testicular adrenal rest tumor (TART) is one of the complications observed in male patients with CAH. As TART may cause infertility in adults, early diagnosis and treatment in childhood is crucial. A study reported that the incidence of TART increases up to 94% in adults (Stikkelbroeck et al., 2001). A few studies evaluated the incidence of TART in childhood&adolescence, and some of them included pediatric and adult age groups (Avila et al., 1996; Vanzulli et al., 1992; Claahsen-van der Grinten et al., 2008; Claahsen-van der Grinten et al., 2007a; Cakir et al., 2012; Claahsen-van der Grinten et al., 2007b). It is reported that the prevalence of TART in children is above 24% (Claahsen-van der Grinten et al., 2007b). The prevalence of TART in patients with CYP21A2 deficiency aged 2–18 years was 18.3% and the youngest patient was of 6 years in our previous study conducted in 2013 (Aycan et al., 2013). TARTs that do not reach 2 cm in size are difficult to detect by physical examination. Tumor diameter may increase during patient follow-up,leading to decreased gonadal functions and infertility. Thus, the early diagnosis of TART in the childhood and adolescence in addition to their long-term follow-up are important. There are few studies on the long-term follow-up in childhood&and adolescence. In our previous study, the long-term follow-up and fertility in those with TART were investigated (Aycan et al., 2013).

The etiopathogenesis in the development of TART has not been completely elucidated. Long-term poor hormonal control was reported in the majority of the CAH patients with TART. However, not all studies showed a clear association between hormonal control and the development of TART. Therefore, other unknown factors besides poor hormonal control might play a role in the pathogenesis (Engels et al., 2019). The relationship between CAH genotype and development of TART is unknown. There is limited research on this subject. For years, we have been monitoring our male patients diagnosed with TART and investigating the relationship of identified CAH mutations in the development of TART. In this study, we aimed to evaluate the genotype-phenotype correlation in the development of TART in patients with CAH due to CYP21A2 and CYP11B1 deficiencies.

2. Method

2.1. Clinical and laboratory enrollment criteria

The Departments of Pediatric Endocrinology in Turkey were invited to participate in this study, and the data of male patients with CAH due to CYP21A2 or CYP11B1 deficiencies, who were regularly followed up with annual scrotal USG, were collected using a data collection form. A total of11 endocrinology departments participated in the study. Cases without regular follow-upor regular scrotal USG, or who did not have a proper diagnosis of TART or did not undergo genetic testing were not included in the study. This study was supported by the National Pediatric Endocrinology and Diabetes Society. The study was approved by the Local Ethics Committee of Ankara Child Health&Diseases Hematology&Oncology Health Practice&Research Center, and informed consents were obtained from all participants and/or their parents&legal guardians. All study procedures were performed in accordance with the Declaration of Helsinki of 1975, as revised in 2008.

In all patients, the phenotype of CAH was assigned by the referring endocrinologist and was based on clinical&laboratorial criteria. The classic SW form was characterized by adrenal crisis, atypical genitalia in females, and elevated basal 17-OH progesterone (17OHP). The classic SV form was characterized by ambiguous external genitalia in females or early virilization in males and elevated 17-OHP, with no evidence of SW. The NC form was defined as normal genitalia in newborns&infants and clinical signs of hyperandrogenism that appear later in childhood&adolescence, associated with elevated 17-OHP. The differentiation between the SV and NC forms was based on atypical genitalia versus normal genitalia in females and the age of onset of clinical symptoms in males (those presenting <3 years of age were classified as SV) (Santos-Silva et al., 2019).

Individualized glucocorticoid and/or mineralocorticoid treatments were administered to all patients. Follow-ups were performed every three months in infancy&childhood and then every 3–6 months. Anthropometric assessments (height-SDS, BMI-SDS, annual growth rate),were performed at each visit. In addition, serum ACTH, testosterone, 1–4 delta-androstenedione (1,4AS) and 11-deoxycortisol levels in CYP11B1deficiency and plasma renin activity and serum Na, K levels in SW CYP21A2 deficiency were determined in the peripheral blood collected in the morning before administering the drug. Patients with normal growth rate; without advanced bone age; and low serum ACTH, 17OHP, 11-deoxycortisol and 1,4AS levels were considered under good metabolic control. Tanner stages were used to evaluate the pubertal state. Greulich and Pyle atlas were used for the determination of bone age.

Scrotal USG was performed by experienced pediatric radiologists. At least three transverse and axial images and Doppler images of each testis were obtained. TART within the mediastinum of the testis can be detected on USG when they are at least 2 mm in size (Stikkelbroeck et al., 2003).

A total of 230 male children and adolescents diagnosed with CAH

and who met the inclusion criteria and filled the data collection form were included in the study. The findings of 40 patients with TART were separately evaluated. The clinical diagnoses of CAH were confirmed by molecular genetic testing in these 40 patients. Those without genetic studiesor without any pathogenic variant identified were excluded from the study.

2.2. Genetic studies

Genomic DNA was isolated from peripheral blood lymphocytes of the patients and parents (if available) either by salting out method or commercially available DNA isolation kits.

2.2.1. Molecular analyses of CYP21A2

Molecular genetic analysis of *CYP21A2* was performed either by targeted analysis through screening of the most common CYP21A2 pathogenic variants or sequencing the entire coding region and exon–intron boundaries of *CYP21A2*. In this multicenter study, various molecular methodologies including allele specific polymerase chain reaction (PCR), reverse dot-blot method, PCR-RFLP, and reverse-hybridization strip-based assay were used to screen common pathogenic variants as previously described (Németh et al., 2012; Kolahdouz et al., 2015; Özyılmaz et al., 2018). Most of the inactivating mutations or deletions are generated by gene conversion or unequal crossing over between the functional (CYP21A2) gene and a highly homologous nonfunctional pseudogene *CYP21A1P* (Kolahdouz et al., 2015). Pseudogene derived common pathogenic variants (c.92C>T; p. P31L, c.293-13C>G (formerly known as: In2G, IVS2–13C>G), c.332_339delGAGACTAC; p. Gly111Valfs*21, c.518T>A; p. Ile173Asn, Exon 6 cluster (c. 710T>A; p. Ile237Asn, c.713T> A; p. Val238Glu, c.719T>A; p. Met240Lys), c.844G> T; p. Val282Leu, c.923dupT; p. Leu308PhefsTer6, c.955C >T; p. Gln319Ter, c.1069C>T; p. Arg357Trp and c.1360C>T; p. Pro454Ser) account for approximately 90–95% point mutations (Krone and Arlt, 2009; Narasimhan and Khattab, 2019). All the coding exons and exons–introns boundaries of *CYP21A2* were sequenced by Sanger sequencing using specific primer pairs to avoid pseudogene amplification as previously described (Menassa et al., 2008). *CYP21A2* deletion/duplication or large-scale conversion analysis by Southern blot or multiplex ligand probe amplification could not be performed for all patients.

2.2.2. Molecular analyses of CYP11B1

Molecular genetic analysis of *CYP11B1* was performed by Sanger sequencing using specific primer pairs to discriminate between *CYP11B1* and highly homologous *CYP11B2* as previously defined (White et al., 1991). Three pairs of primers were used to amplify only exons 1–2, 3–5, and 6–9 of *CYP11B1* and nested PCR was performed to obtain smaller amplicons spanning these individual exons before the sequencing.

2.3. Statistical analyses

Genotype–phenotype correlation was evaluated for patients with CYP21A2 deficiency. Patients were stratified according to their genotypes into groups. The genotypes for CYP21A2 deficiency were categorized according to the predicted severity of mutations: group 0 (null) (complete enzyme impairment), group A (activity <2%), group B (activity ~2%), and group C (partial impairment) (Santos-Silva et al., 2019). Genotypes categorized in group 0 were predicted to result in SW CAH, while patients with group A genotypes were anticipated to present with either SW or SV form of CAH. Those in group B were expected to manifest as a SV phenotype, and those in group C as NC CAH. Categorical data are presented as percentages. The accuracy of phenotype prediction by the genotype was evaluated by estimating the positive predictive value in each group. The positive predictive value (PPV) for each genotype group was calculated as the number of patients with the expected phenotype divided by the total number of patients (expected+observed phenotype) in the given group, and expressed as a percentage (Table 1) and these percentages were calculated using descriptive statistical methods. For example, PPV for genotype c.1069C>T/c.1069C>Tin groupA was calculated as follows: PPV equals the number of SW patients (Krone and Arlt, 2009) in this genotype divided by the total number of patients (Polat et al., 2014) with the same genotype multiplied by 100 (3/5 × 100).

The genotypes for CYP11B1deficiency were categorized into classic and NC and patients harboring variants with in vitro enzymatic activity of less than 5% were considered to have the classic form of the disease as described in literature (Parajes et al., 2010).

3. Results

TART was observed in 40 of 230 male children and adolescent patients (CYP21A2 deficiency-29patients, CYP11B1deficiency-11 patients). The incidence of TART was 17.4% in children who underwent regular scrotal USG starting from the younger age group. Further, 24 of 29 patients with TART with CYP21A2 deficiency were SW type, four were SV type, and one was NC type. TART was detected in 11 patients with 11-beta hydroxylase deficiency. The youngest patients with CYP21A2 and CYP11B1 deficiency who developed TART were of 4 and 2 years, respectively.

In the group of CYP21A2 deficiency and TART, there were 9,13, and 7 patients aged 4–10, 11–15, and 16–18 years, respectively. In the group of CYP11B1 deficiency and TART, there were five, five, and one patient aged 2–10, 11–15, and 18 years, respectively. Further, 20 of the 24 patients with SW type CYP21A2 deficiency were diagnosed during the neonatal period, whereas one and three patients were diagnosed at the ages of 1 and 2 years, respectively. While 10 patients were in good metabolic control, 30 were found to be under poor control. In addition, 75% (n:30/40) of 40 patients with TART were in the pubertal period. Patients with TART were followed up for an average of 3.2 ± 1 years. 36 patients were administered hydrocortisone therapy at a high dose to suppress ACTH. TART disappeared in eight (20%) patients during follow-up, and 19 (47.5%) had a reduction in tumor size. TART dimensions remained unchanged or were enlarged in nine patients with treatment non-compliance. Testis-sparing surgery was performed in four patients with pain complaints.

In the molecular analysis, eight different pathogenic variants in *CYP21A2* were identified (Fig. 1, Table 2). The most common mutation was c.293-13C>G. The most common genotypes in the cohort were c.293-13C>G/c.293-13C>G (31.03%) followed by c.955C>T/c.955C>T(27.6%) and c.1069C>T/c.1069C>T(%17.2) (Table 2). The most common mutations in patients with SW form were c.293-13C>G and c.955C>T (p.Gln319Ter)with frequencies of 33.3% each while the most frequent mutation in the SV form wasc.1069C>T (p. Arg357Trp) with a frequency of 50%. Table 3 presents the alleles detected in *CYP21A2*, allele frequencies, and the mechanisms of loss of function.

In *CYP11B1*, we identified seven different pathogenic variants (Fig. 2). In *CYP11B1*, c.896T>C (p.Leu299Pro) was detected with a frequency of 31.8% and c.1120C>G (p.Arg374Gly) with a frequency of 27.2%, and they were the most frequently detected mutations (Table 4). All cases with CYP11B1deficiency had classical form of the disease.

4. Discussion

TART was detected in 40 of 230 male CAH patients who underwent regular scrotal USG starting fromat a young age, and the incidence of TART was found to be 17.4%. In our study, the youngest patient with TART was of 2 years. Literature review showed that the patient diagnosed with TART at the youngest age had CYP11B1 deficiency, as in our study, and was diagnosed at the age of 1.8 years (Dumic et al., 2017). In our study, TART occurred in early childhood with CYP11B1 deficiency, and approximately half of the patients had TART before the age of 10

Table 1
Genotype–phenotype correlation and positive predictive value for the identified genotypes.

Enzymatic activity	Genotype	Expected Phenotype	Observed Phenotype			Total No of Patients	Positive Predictive Value
			SW	SV	NC		
Null (0)	Comp.del/Comp.del	SW	3			3	100%
	c.955C>T/c.955C>T	SW	8			8	100%
	c.1069C>T/c.1069C>T	SW	3	2		5	60%
	Gene conv./c.1069C>T	SW	1			1	100%
Group A	c.293-13C>G/c.293-13C>G*	SW/SV	8	1		9	100%
Group B	c.518T>A/c.518T>A	SV		1		1	100%
	c.518T>A/c.332_339delGAGACTAC	SV	1			1	0%
Group C	c.293-13C>G/c.1360C>T	NC			1	1	100%
Total			24	4	1	29	82.5%

SW, classical salt-wasting type; SV, classic simple virilizing; and NC, nonclassic form.
*c.293-13C>G: SW or SV form may be observed depending on variable splicing of the transcripts.

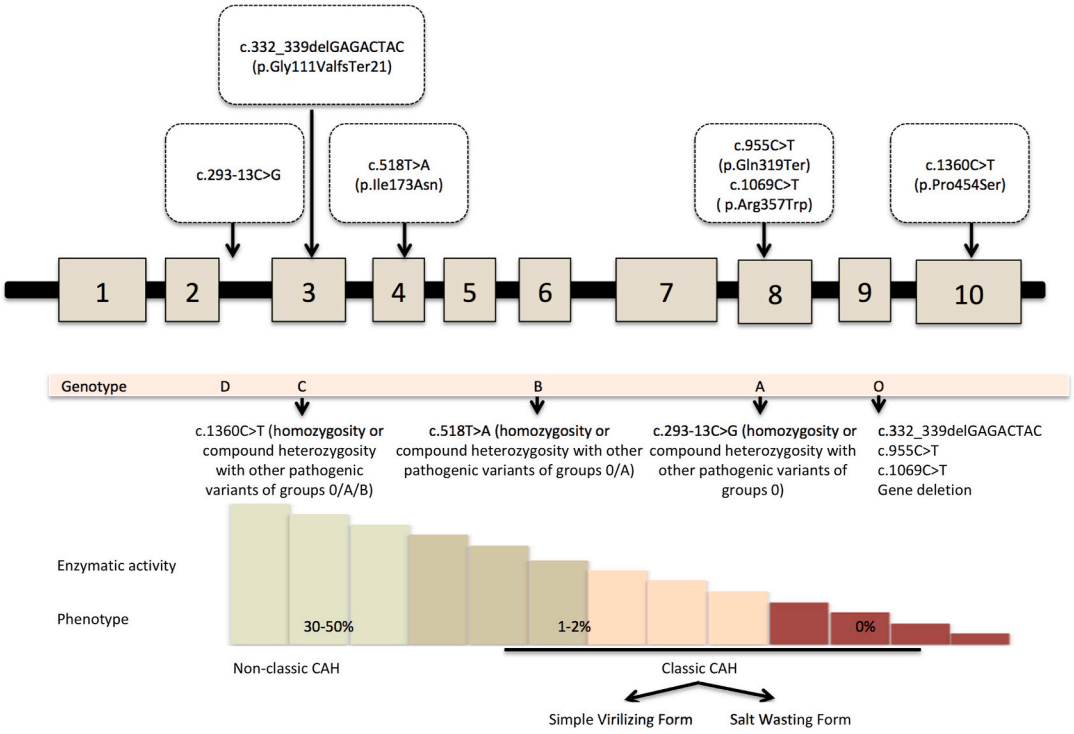

Fig. 1. Mutations in patients diagnosed with 21-hydroxylase deficiency.

years. Although larger series is needed, we recommend performing TART screening earlier in male patients with CYP11B1 deficiency. The incidence of TART reported in studies that included adult patients is higher than in those that included children&adolescents (Engels et al., 2019). Accordingly, some studies have reported an increase in the incidence of TART with age, especially during the pubertal period (Engels et al., 2019; Dumic et al., 2017; Claahsen-van der Grinten et al., 2014). In our previous study, we found the incidence of TART to be 18.3% in childhood, and we suggested that TART screening can be performed from childhood (Aycan et al., 2013).

Poor hormonal control has been reported in 58% of the studies evaluating CAH and TART (Engels et al., 2019). In our study, it was determined that 75% of patients with TART had poor metabolic control. However, notall studies were able to elucidate a definite relationship between hormonal control (increased ACTH, 17-OHP, 1,4AS levels) and TART. In a study including patients with CYP21A2 deficiency, poor metabolic control and the development of TART were not found to be related (Reisch et al., 2013). In addition, TART has been reported in those with good metabolic control (Engels et al., 2019; Claahsen-van der Grinten et al., 2014). ACTH is considered to be the most important stimulatory factor in the development of TART. However, other unknown factors might play a role in the pathogenesis of TART. One of the aims of our study was to evaluate whether genomic background of the patients which are thought to be one of these factors, predispose to the development of TART. In recent studies, TART was observed in 40% (14–89%) patients (Engels et al., 2019). The incidence of TART is related to the severity of the disease, and the highest prevalence is reached in patients most affected with CAH (groups 0, A, SW type) (Falhammar et al., 2012). In patients with a milder form of the disease (group B, SV), this rate decreases to 20% (Engels et al., 2019). In our study, 82.7% patients who were followed up with a diagnosis of CYP21A2 deficiency and TART had SW type. Majority of the mutations detected in these patients were of group 0 and A mutations. In our study, a significant number of the mutant alleles detected in SV type with TART harbored group 0 and A mutations. TART is rarely reported in patients with NC form (Falhammar et al., 2012). Despite the rare prevalence, TART was diagnosed in patient 27 with NC form, who had poor metabolic control, with a genotype of group A and C variants in a compound heterozygous state. However, in another study, a patient with NC form, who achieved tight hormonal control, also had TART (Kocova et al., 2018). Therefore,

Table 2
Genetic mutations, phenotype, and age of TART diagnosis in patients with 21-hydroxylase deficiency, CYP21A2 transcript no: NM 000500.9

Patient		Pathogenic Variant	Phenotype	Age at diagnosis (year)	Age at diagnosis of TART (year)
Homozygous					
Intron 2	1	c.293-13C>G	SW	Newborn	4
	2.	c.293-13C>G	SW	Newborn	4
	3.	c.293-13C>G	SW	Newborn	7
	4.	c.293-13C>G	SW	Newborn	10
	5.	c.293-13C>G	SW	1	10
	6.	c.293-13C>G	SW	2	11
	7.	c.293-13C>G	SW	Newborn	11
	8.	c.293-13C>G	SW	Newborn	12
	9.	c.293-13C>G	SV	3	17
Exon 4	10.	c.518T>A (p.Ile173Asn)	SV	15	16
Exon 8	11.	c.955C>T (p.Gln319Ter)	SW	Newborn	12
	22.	c.955C>T (p.Gln319Ter)	SW	Newborn	12
	13.	c.955C>T (p.Gln319Ter)	SW	Newborn	13
	14.	c.955C>T (p.Gln319Ter)	SW	Newborn	13
	15.	c.955C>T (p.Gln319Ter)	SW	Newborn	16
	16.	c.955C>T (p.Gln319Ter)	SW	Newborn	16
	17.	c.955C>T (p.Gln319Ter)	SW	Newborn	18
	18.	c.955C>T (p.Gln319Ter)	SW	Newborn	18
	19.	c.1069C>T (p.Arg357Trp)	SW	Newborn	6
	20.	c.1069C>T (p.Arg357Trp)	SW	Newborn	9
	21.	c.1069C>T (p.Arg357Trp)	SV	1	12
	22.	c.1069C>T (p.Arg357Trp)	SW	2	12
	23.	c.1069C>T (p.Arg357Trp)	SV	5	15
Gene Deletion	24.	Complete CYP21A2 deletion	SW	Newborn	10
	25.	Complete CYP21A2 deletion	SW	Newborn	15
	26.	Complete CYP21A2 deletion	SW	Newborn	17
Compound Heterozygous					
Intron2 /Exon 10	27.	c.293-13C>G/c.1360C>T (p.Pro454Ser)	NC	3	4
Exon 8/Conv.	28.	Gene conversion/c.1069C>T(p.Arg357Trp)	SW	2	12
Exon3/Exon 4	29.	c.518T>A (p.Ile173Asn)/c.332_339delGAGACTAC (p.Gly111ValfsTer21)	SW	Newborn	13

TART, testicular adrenal rest tumor; SW, classical salt-wasting type; SV, classic simple virilizing; and NC, nonclassic form.

Table 3
Alleles, allele frequency, and the mechanism of loss of function detected in *CYP21A2*.

Alleles	Allele frequency	Loss of function mechanism
c.293-13C>G	19/58 32.8%	Associated with aberrant splicing due to upstream activation of a splice acceptor site <5% of residual enzyme activity (Narasimhan and Khattab, 2019)
c.955C>T (p.Gln319Ter)	16/58 27.6%	Associated with disruption of H bonding and loss of enzyme activity
c.1069C>T (p.Arg357Trp)	11/58 19%	Associated with disruption of H bonding and loss of enzyme activity
Complete CYP21A2 gene deletion	6/58 10.3%	Complete loss of enzyme activity
c.518T> A (p.Ile173Asn)	3/58 5.2%	Associated with loss of the hydrophobic pocket, reduction of enzyme activity to 2% (Narasimhan and Khattab, 2019) Generally associated with SV form.
c.332_339delGAGACTAC (p. Gly111ValfsTer21)	1/58 1.7%	Results in a premature termination codon and complete loss of enzyme activity (Narasimhan and Khattab, 2019)
c.1360C>T (p.Pro454Ser)	1/58 1.7%	Residual activity + Causes nonclassical form of the disease (Krone and Arlt, 2009)

it may not be appropriate to attribute the presence of TART in NC form to only poor hormonal control.

Unlike CYP21A2 deficiency, molecular genetic studies of CYP11B1deficiency are relatively fewer. In a study by Kandemir et al. p. Thr318Thr was the most frequent pathogenic variant identified in patients with CYP11B1 deficiency followed by p. Arg141Ter with a frequency of 12.5% and p. Leu299Pro, p. Gln189Hisfs*70, and c.1398+5G>C, each with an allele frequency of 8.3% (Kandemir et al., 2017). In our study, TART was detected in 11 patients who were followed up with a clinical diagnosis of CYP11B1 deficiency. In previous studies, the incidence of TART was reported to be 40% in those with CYP11B1deficiency (Engels et al., 2019). In a recent study,Baş et al. presented the data of 25 Turkish families with *CYP11B1* mutations; they reported that the most common mutation was c.896T>C (p.Leu299Pro), as observed as the most frequent one in our CYP11B1 deficiency patients with TART (Baş et al., 2018). In the study,p.Thr318Thr and p. Asn394ArgfsTer37 variants were the second and third highest in frequency in their cohort, respectively. However, they could not find a definite correlation between specific pathogenic variants and clinical or laboratory findings. Nevertheless, TART was observed in five patients with similar mutations (p.Arg141Ter, p. Leu299Pro, c.1398 + 2T> A, and p. Thr318Thr) found in our cohort.

Previous studies showed that in vitro activities of less than 5% were considered to be severe and consistent with classic CYP11B1deficiency (Parajes et al., 2010). In our cohort, it is intriguing that all identified pathogenic variants in CYP11B1 are either associated with the classical form of the disease or are truncating (frameshift, nonsense, or splice) or missense variants presumed to cause complete loss of the enzymatic activity. Our study also adds to the spectrum of pathogenic variants responsible for CYP11B1 deficiency in Turkey as c.1398+2T>A and p. Arg374Gly variants are reported for the second time in the literature to the best of our knowledge.

Intronic c.1398 + 2T>A variant was identified in three hypertensive patients, of whom two were second-degree cousins (Baş et al., 2018). One of the cousins was a female patient who presented with obesity, insulin resistance, and severe virilization. Different nucleotide change at

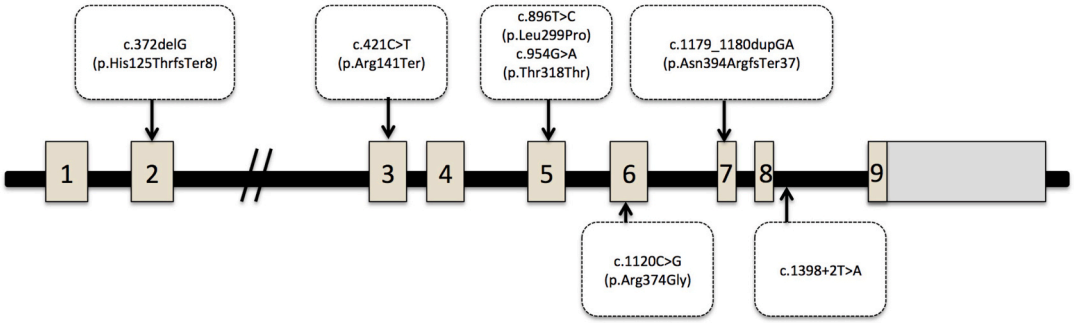

Fig. 2. Mutations detected in patients diagnosed with 11-beta hydroxylase deficiency.

Table 4
Genetic mutations, phenotype, and age of TART diagnosis in patients with 11-beta hydroxylase deficiency, CYP11B1 transcript no: NM_000497.4

Patient		Pathogenic variant	Classical form	Age at diagnosis (year)	Age at diagnosis of TART (year)
Homozygous					
Exon 2	1.	c.372delG (p.His125ThrfsTer8)	Enzymatic activity 0% Truncating variant (Polat et al., 2014)	2	14
Exon 3	2.	c.421C>T (p.Arg141Ter)	Truncating variant	2	15
Exon 5	3.	c.896T>C (p.Leu299Pro)	Enzymatic activity 1.2%	2	2
	4.	c.896T>C (p.Leu299Pro)	Enzymatic activity 1.2%	2	3
	5.	c.896T>C (p.Leu299Pro)	Enzymatic activity 1.2%	Newborn	18
	6.	c.954G>A (p.Thr318Thr)	Synonym variant affects splicing (Parajes et al., 2010)	1	14
Exon 6	7.	c.1120C>G (p.Arg374Gly)	NA	2	5
	8.	c.1120C>G (p.Arg374Gly)	NA	Newborn	11
	9.	c.1120C>G (p.Arg374Gly)	NA	Newborn	13
Exon 7	10.	c.1179_1180dupGA (p.Asn394ArgfsTer37)	Enzymatic activity 1.2%	3	5
Compound Heterozygous					
Exon 5 /Intron 8	11.	c.896T>C (p.Leu299Pro)/c.1398 + 2T> A	Enzymatic activity 1.2% /Donor site variant	Newborn	7

TART, testicular adrenal rest tumor; NA: not available.

the same donor splice site position, c.1398+2T>C variant was also observed in a Turkish patient who presented with TART at the age of 11 years. p. Arg374Gly was reported in a Turkish patient who presented with TART at the age of 19 years (Papatya Çakır et al., 2012). Different missense variants at the same position such as p. Arg374Trp and p. Arg374Gln have been reported in patients with CYP11B1deficiency; structural protein models predicted that p. Arg374Trp is associated with the severe form of the disease as the 374th residue is responsible for maintaining the heme-binding site of CYP11B1. Patients harboring p. Arg374Trp had high Prader scores (4/5), severe hypertension, and profoundly advanced bone age (Curnow et al., 1993; Khattab et al., 2017). Identification of these two variants solely in patients of Turkish origin may raise the possibility of a founder effect. Additionally, TART diagnosis in all male patients with these two variants may be due to these variants being more common in Turkish patients or these variants may be associated with a predisposition to TART development. Therefore, close follow-up regarding TART development is recommended in the future especially for patients with these two variants.

In a study conducted in Turkey, the most common mutations found in those with CYP21A2 deficiency were c.293-13C>G (22%), large conversions (14.3%), p. Ile173Asn (9.9%), p. Arg357Trp (8.8%), and large deletions (6.6%) (Baş et al., 2009). In the same study, p. Gln319Terwas only detected in the patients who had SW form of the disease with a frequency of 3.3%. In another study within a Turkish cohort of CYP21A2 deficiency, c.293-13C>G, large rearrangements, and p. Gln319Ter were identified as the most common pathogenic variants with allele frequencies of 33.3%, 14.4%, and 12.2%, respectively (Toraman et al., 2013). Additionally, in a recent study from Turkey conducted inpatients with CYP21A2 deficiency, large deletions were reported as the most frequent pathogenic variant with an allele frequency of 23% followed by p. Gln319Ter (16%) and c.293-13C> G and p. Ile173Asn each with an allele frequency of 14.2% (Özyılmaz et al., 2018). In our study, in those with CYP21A2 deficiency and TART, the most common mutation was c.293-13C>G with a rate of 32.8%. In our study, c.955C>T (p.Gln319Ter) was the second frequent (27.6%) mutation. Large deletions were detected in 10.3% patients. In our study, slight differences were observed between the frequency of mutations and the previously reported data of Turkey. This difference may be due to the more frequent occurrence of TART in the presence of some mutations that may predispose to development of TART. Moreover, although the data in our study were obtained from different endocrine centers, it may not homogeneously reflect the data of Turkey. In our cohort, the presence of poor metabolic control as well as the genetic background of the patients may have contributed toward the development of TART. However, our study has the largest number of patients in literature and contributes toward identifying possible predisposition mutations in the development of TART. The limitation of our study is the lack of comparison with patients harboring similar mutations who did not develop TART. Further investigations can be performed in the light of the information provided by the present study. In the study of Claahsen-van der Grinten et al. from the Netherlands, genetic data of 16 patients who had TART was reported (Claahsen-van der Grinten et al., 2014). In this study, deletion or large conversion in 13 alleles, c.518T>A (p.Ile173Asn) in six, c.1069C>T (p.Arg357Trp) in four, c.293-13C>G in three, and c.955C>T (p.Gln319Ter) in two were determined. However, similar to our study, genetic studies were performed only for patients who developed TART. Thus, the data of those without TART could not be compared. Although the incidence of mutations varies among

different studies, it should be noted that the frequent mutations in patients with TART are known common variants in CYP21A2 deficiency. In our study, six of these 10 common variants were detected in patients with TART. New studies are needed on this subject. We continue to investigate the genotype of TART in a larger patient series by increasing our number of TART patients.

There is no definitive treatment option for TART or to prevent the development of TART. There are studies mostly focusing on ensuring fertility in adult patients (Engels et al., 2019). As the first treatment option, glucocorticoid therapy at supraphysiological doses is recommended to suppress ACTH levels (Falhammar et al., 2012). However, there is no prospective study evaluating the efficacy of glucocorticoid therapy in TART patients. Surgery is especially indicated for those with severe pain. Surgery is found to have no effect on fertility (Engels et al., 2019). In our study, it was observed that the tumor disappeared in 20% (n = 8) patients with high-dose glucocorticoid treatment. In 22.5% (n = 9) patients, TART size did not decrease or increase despite high-dose glucocorticoid treatment. Surgery was performed in 10% (n = 4) patients in our study.

In conclusion, we determined that 82.7% patients with TART and CYP21A2 deficiency had SW type of the disease, and most of the mutations were in groups 0 and A. In addition, a significant number of mutant alleles of the SV-type TART cases included group 0 and A mutations. The most common mutations detected inTART cases due to CYP21A2 deficiency werec.293-13C>G and c.955C>T (p.Gln319Ter). The most common mutation was c.896T>C (p.Leu299Pro)in TART-CYP11B1deficiency.

Further studies are required to determine whether there are variants in *CYP21A2*, *CYP11B1,*or other possible regulatory genes that may be associated with an increased risk independent of poor hormonal control for the development of TART, which is associated with infertility risk and does not have a definite treatment option in patients with CAH.

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CRediT authorship contribution statement

Zehra Aycan: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition. **Melikşah Keskin:** Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition. **Naz Güleray Lafcı:** Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Şenay Savaş-Erdeve:** Resources. **Firdevs Baş:** Resources. **Şükran Poyrazoğlu:** Resources. **Pınar Öztürk:** Resources. **Mesut Parlak:** Resources. **Oya Ercan:** Resources. **Tülay Güran:** Resources. **Nihal Hatipoğlu:** Resources. **Seyit Ahmet Uçaktürk:** Resources. **Gönül Çatlı:** Resources. **Nesibe Akyürek:** Resources. **Aşan Önder:** Resources. **Suna Kılınç:** Resources. **Semra Çetinkaya:** Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing, Visualization, Supervision.

Declaration of competing interest

All authors declare that there is no financial or other potential conflict of interest. All authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Data availability

Data will be made available on request.

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