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Clinical Research Article

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Catch-up Growth and Discontinuation of Fludrocortisone Treatment in Aldosterone Synthase Deficiency

Busra Gurpinar Tosun,[1] Yasemin Kendir Demirkol,[2] Tuba Seven Menevse,[1] Sare Betul Kaygusuz,[1] Mehmet Nuri Ozbek,[3] Selda Ayca Altincik,[4] Jamala Mammadova,[5] Atilla Cayir,[6] Esra Doger,[7] Elvan Bayramoglu,[8] Ozlem Nalbantoglu,[9] Gul Yesiltepe Mutlu,[10] AghaRza Aghayev,[11] Serap Turan,[1] Abdullah Bereket,[1] and Tulay Guran[1]

[1]Department of Pediatric Endocrinology and Diabetes, Marmara University, School of Medicine, Istanbul, Turkey; [2]Department of Pediatric Genetics, Umraniye Research and Training Hospital, University of Health Sciences, Istanbul, Turkey; [3]Department of Pediatric Endocrinology and Diabetes, Gazi Yasargil Education and Research Hospital, Diyarbakir, Turkey; [4]Department of Pediatric Endocrinology and Diabetes, Pamukkale University, Denizli, Turkey; [5]Department of Pediatric Endocrinology and Diabetes, Ondokuz Mayis University, Samsun, Turkey; [6]Department of Pediatric Endocrinology and Diabetes, Erzurum Education and Research Hospital, Erzurum, Turkey; [7]Department of Pediatric Endocrinology, Gazi University, Faculty of Medicine, Ankara, Turkey; [8]Department of Pediatric Endocrinology, Haseki Training and Research Hospital, Istanbul, Turkey; [9]Department of Pediatric Endocrinology, Dr. Behcet Uz Children's Hospital, Izmir, Turkey; [10]Department of Pediatric Endocrinology and Diabetes, Koç University Hospital, Istanbul, Turkey; and [11]Medical Genetics Department, National Hematology and Transfusiology Center, Baku, Azerbaijan

ORCiD numbers: 0000-0002-7588-9807 (B. Gurpinar Tosun); 0000-0001-8457-8042 (S. B. Kaygusuz); 0000-0002-5172-5402 (S. Turan); 0000-0002-6584-9043 (A. Bereket); 0000-0003-2658-6866 (T. Guran).

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Abstract

Background: Aldosterone synthase deficiency (ASD) caused by mutations in the *CYP11B2* gene is characterized by isolated mineralocorticoid deficiency. Data are scarce regarding clinical and biochemical outcomes of the disease in the follow-up.

Objective: Assessment of the growth and steroid profiles of patients with ASD at the time of diagnosis and after discontinuation of treatment.

Design and method: Children with clinical diagnosis of ASD were included in a multicenter study. Growth and treatment characteristics were recorded. Plasma adrenal steroids were measured using liquid chromatography-mass spectrometry. Genetic diagnosis was confirmed by *CYP11B2* gene sequencing and in silico analyses.

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Results: Sixteen patients from 12 families were included (8 females; median age at presentation: 3.1 months, range: 0.4 to 8.1). The most common symptom was poor weight gain (56.3%). Median age of onset of fludrocortisone treatment was 3.6 months (range: 0.9 to 8.3). Catch-up growth was achieved at median 2 months (range: 0.5 to 14.5) after treatment. Fludrocortisone could be stopped in 5 patients at a median age of 6.0 years (range: 2.2 to 7.6). Plasma steroid profiles revealed reduced aldosterone synthase activity both at diagnosis and after discontinuation of treatment compared to age-matched controls. We identified 6 novel (p.Y195H, c.1200 + 1G > A, p.F130L, p.E198del, c.1122-18G > A, p.I339_E343del) and 4 previously described *CYP11B2* variants. The most common variant (40%) was p.T185I.

Conclusions: Fludrocortisone treatment is associated with a rapid catch-up growth and control of electrolyte imbalances in ASD. Decreased mineralocorticoid requirement over time can be explained by the development of physiological adaptation mechanisms rather than improved aldosterone synthase activity. As complete biochemical remission cannot be achieved, a long-term surveillance of these patients is required.

Key Words: aldosterone synthase deficiency, hypoaldosteronism, CYP11B2, catch-up growth, steroid hormone profile, follow-up, children

Aldosterone synthase deficiency (ASD) caused by inactivating biallelic variants in *CYP11B2* gene is a rare disorder typically characterized by isolated mineralocorticoid deficiency (1,2). The *CYP11B2* gene encodes the cytochrome P450 enzyme aldosterone synthase (CYP11B2), a mitochondrial enzyme that catalyzes the final 3 steps in aldosterone biosynthesis: namely, the 11β-hydroxylation of 11-deoxycorticosterone (DOC) to form corticosterone, the 18-hydroxylation of corticosterone to form 18-hydroxycorticosterone, and finally, the 18-oxidation of 18-hydroxycorticosterone to form aldosterone (2,3).

Biochemical presentation of ASD is hyponatremia, hyperkalemia, elevated renin, and plasma renin activity (PRA) and low aldosterone concentrations. PRA is remarkably elevated in affected children, but it may be normal in adults. Measurement of adrenal steroids, such as DOC, corticosterone, and 18-OH corticosterone, is of importance in diagnosis. Concentrations of DOC and corticosterone are increased in plasma and their metabolites are elevated urine (1,4,5).

Clinical symptoms of ASD include frequent vomiting, dehydration, hypovolemia, and failure-to-thrive in the first months of life. The infants and children are successfully treated with oral sodium supplementation and fludrocortisone (FC), although severely symptomatic infants may require intravenous fluids. In treated infants with ASD, electrolyte abnormalities are resolved, but PRA may not return to normal for several months. Children older than 3 to 4 years of age usually have normal serum electrolytes even in an untreated state. Adults are usually asymptomatic, but they compensate for salt-wasting less effectively than unaffected individuals (1,4-7).

Approaches to date have recommended discontinuation of oral sodium supplements when PRA/renin has decreased to normal, but mineralocorticoid replacement therapy is frequently maintained through childhood (1). There are no biochemical guidelines regarding when to terminate FC replacement. Furthermore, data about the effect of treatment on catch-up growth and the changes in the steroid hormone profiles of the patients whose need for FC treatment disappeared are missing.

In this study, we aimed to investigate the growth characteristics of ASD patients and the change in steroid profiles of ASD patients whose need for FC treatment disappeared in search of a biochemical parameter that might be useful in the decision regarding discontinuation of FC treatment.

Patients and Methods

We included 16 patients with clinical diagnosis of ASD (8 females) from 12 unrelated families followed up in 9 pediatric endocrinology centers in Turkey. All patients presented in early infancy with hyponatremia. All had normal cortisol levels and had no signs or symptoms of adrenal insufficiency. Treatment with FC and salt effectively restored clinical and biochemical abnormalities.

Data on the clinical characteristics were obtained from the medical records. Changes in weight and height SD scores (SDS)s during follow-up were calculated, and the presence or absence of catch-up was subsequently classified. Increase in weight SDS above 0.67 SDS compared to that at diagnosis was accepted as the catch-up growth. Time to catch-up growth was calculated accordingly (8).

Steroid hormone profiles were investigated by the liquid chromatography-mass spectrometry (LC-MS/MS) method both at off-treatment state and reanalyzed at least 3 months after the discontinuation of FC treatment. LC-MS/MS–based panel of plasma adrenal steroids was evaluated using Eureka kit (Eureka Lab Division, Ancona, Italy) as previously described (9). The Poroshell 120 EC-C18 (50 × 2.1 mm, 2.7 μm; Agilent Technologies, Santa Clara, CA, USA) column was used. Analyses were performed on a Shimadzu LCMS 8050 tandem mass spectrometer equipped with a Nexera XR LC-20AD HPLC system (Shimadzu Corporation, Japan) that was operated using electrospray ionization source in positive and multiple reactions monitoring mode. Method validation was performed according to the Clinical and Laboratory Standards Institute C62-A guideline.

Adrenal steroids measured in the patient group were compared to those of sex- and age-matched controls. Control plasma samples were obtained from children examined for other conditions including well-controlled type 1 diabetes, euthyroid hypothyroidism on treatment, and simple growth retardation but without adrenal or pubertal problems.

Genetic Analysis

Genomic DNA was extracted from EDTA-anticoagulated peripheral blood by using a semiautomated robot as recommended by the manufacturer (Qiagen). The library preparation for next-generation sequencing was performed using a capture-based Clinical Exome Solution Kit by Sophia Genetics, including a gene panel for primary adrenal insufficiency (*CYP11A1*, *CYP11B1*, *CYP11B2*, *CYP21A2*, *HSD3B2*, *CYP17A1*, *POR*, *STAR*, *AAAS*, *ABCD1*, *MC2R*, *MRAP1*, *NR0B1*, *NR5A1*, *NNT*, *TXNRD2*, *MCM4*, *AIRE*, *SGPL1*, *SAMD9*, *CDKN1C*, *POLE1*).

NextSeq 500 (Illumina, USA) was used as the sequencing platform. Data quality control, alignment, variant calling, and variant annotations were performed by using the Sophia DDM analysis tool (version 5.2). NCBI Build37 (hg19) version of the human genome was used as a reference. As a primary variant filtering strategy, variants located within ±10 base pairs boundary of targeted exons with minimum read depth 50 × were selected. Any variants that fall outside these regions, variants in homopolymer regions and exonic variants with a variant fraction of less than 20% were considered as false positives and not analyzed. All variants were manually inspected by using the Integrative Genomics Viewer visualization tool.

The interpretations of the variants were performed according to the 2015 American College of Medical Genetics and Genomics standards and guidelines (10). 1000 genome project, dbSNP ExAC, and GnomAD population frequency databases were used as the control population. The possible effects of the variants on protein function were determined by using pathogenicity prediction tools such as SIFT (11), Polyphen (12), MutationTaster (13), and VarSome (14).

Sanger sequencing was performed to patients and parents to confirm the *CYP11B2* variation identified by the next-generation sequencing. All coding regions of *CYP11B2* and the exon-intron splicing junction boundaries were amplified by polymerase chain reaction using the primers and conditions as previously described (15), and then the amplimers were sequenced using the Big Dye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems; Life Technologies, Darmstadt, Germany) on an Applied Biosystems 3130 Genetic Analyzer. Analysis of the *CYP11B2* gene was based on the Ensembl transcript ENST00000323110 and variants nomenclature was according to the Human Genome Variation Society Sequence Variant Nomenclature guidelines (16).

Full amino acid sequence of wild and 2 coding mutants (pLeu130 and pHis195) of human CYP11B2 protein (UniProtKB/Swiss-Prot P19099) introduced into I-Tasser server and 3-dimensional (3D) structures protein models were created based on multiple-threading alignments by Local Meta-Threading-Server (LOMET) using in silico tool, I-TASSER (http://zhanglab.ccmb.med.umich.edu/I-TASSER). The structural representations were generated with PyMOL, a Python-based viewer for visualization of macromolecular structures.

Studies were performed with approval of the Ethics Committee of the Marmara University Faculty of Medicine, Istanbul, Turkey (09.2020.883). Participants provided written informed consent, and all studies were conducted in accordance with the principles of the Declaration of Helsinki.

Statistical Analysis

Statistical evaluation was performed using GraphPad Prism® V5.0 software (GraphPad Software Inc., San Diego, California, USA). Pairwise comparisons were performed using Student *t* test. The nonparametric Mann-Whitney U test was used when the assumptions of Student *t* test could not be respected. Data were expressed as median and range or mean ± SD. Statistical significance was set at $P < 0.05$.

Results

Mean age at diagnosis of the ASD patients was 3.2 ± 2.2 months (median: 3.1, range: 0.4 to 8.1). Gestational ages ranged between 36 and 40 gestational weeks. Birth weights ranged between 1780 and 3900 g,

and birth weight SD scores were –0.4 ± 1.1 (median: –0.9, range: –2.4 to 1.1).

The most common presenting complaint was poor weight gain (n = 9; 56.3%). All patients had hyponatremia (125.7 ± 4.3 mEq/L, median: 127, range: 115 to 131), and 14 patients had hyperkalemia (6.4 ± 0.8, median: 6.5, range: 5.1 to 7.7) at the diagnosis. In 15 patients, the renin or PRA measurements were available at the presentation. Concentration of renin or PRA was high in 10 patients (10/15; 66.6%). PRA was within the normal range in 2 patients and low in 1 patient. Serum aldosterone concentration was low in 6 patients at the presentation (6/14; 42.8%). Clinical, biochemical, and molecular characteristics of the patients at the time of diagnosis are illustrated in Table 1.

Initial treatment was FC and salt supplementation for all patients. FC was initiated at age 3.6 ± 2.3 months (median: 3.6, range: 0.9 to 8.3). F11P1 had been initially misdiagnosed as adrenal insufficiency before biochemical and steroid profiles were documented and hydrocortisone (20 mg/m^2/day) had been started. The detailed treatments on follow-up of the patients are shown in Table 2.

The median follow-up duration of the patients was 2.6 years (range: 0.8 to 14.3). Catch-up weight gain was observed in 15 patients. Average catch-up time was 3.3 ± 3.7 months (median: 2, range: 0.5 to 14.5) after the initiation of the therapy. The changes in weight SDS of the patients after therapy are illustrated in Figure 1A. The mean weight and height of the patients were –0.2 ± 0.9 (median: –0.4, range: –2.0 to –1.2) and –0.7 ± 1.0 SD (median: –0.6, range: –2.5 to 1.0) at the last visit, respectively, compared to –2.5 ± 1.0 (median: –2.6, range: –4.4 to –0.6), and –1.7 ± 1.1 (median: –1.8, range: –3.0 to 0.7) at diagnosis (Fig. 1A and 1B). There was a statistically significant difference in both weight SDS values ($P < 0.0001$) and height SDS values ($P = 0.027$) between those at diagnosis and the last visit.

Salt supplementation was no longer needed in the patients after an average duration of 3 months. FC treatment could also be discontinued in 6 patients (37.5%) (Table 2). FC had to be restarted in 1 patient (F4P1) due to hyponatremia (Na = 130 mEq/L), which was detected in an emergency admission with diarrhea and vomiting the third month after the cessation of treatment. Mean age of discontinuation of FC in the remaining 5 patients who no longer needed FC was 5.5 ± 2.0 years (median: 6.0, range: 2.2 to 7.6).

Steroid hormone profiles were analyzed at diagnosis and after a minimum of 4 months after discontinuation of treatment in cases whose treatment could be stopped. Comparison of steroid hormone profiles of the patients at diagnosis and after their treatment could be discontinued demonstrated the followings: corticosterone ($P = 0.014$) and DOC ($P = 0.027$) concentrations were significantly lower in the latter, but no statistically significant difference was found between aldosterone levels ($P = 0.974$) and corticosterone/aldosterone ratios ($P = 0.876$). Nevertheless, when compared to control individual corticosterone, DOC, and corticosterone/aldosterone ratios were higher and aldosterone concentrations were lower in patients both at diagnosis and after their treatment could be discontinued (Fig. 2.A, B). Three patients (F2P1, F3P1, F4P2) who have LC-MS/MS–based steroid measurements at multiple time points after discontinuation of FC therapy also showed higher corticosterone and DOC and low/normal aldosterone concentrations compared to controls [Supplemental Figure 1 (17)]. The age of control group before treatment measurements (n = 340, median: 0.005, range: 0.05 to 0.74 years) and after discontinuation of treatment measurements (n = 216, median: 9.9, range: 4 to 14 years) were similar to the patient groups ($P = 0.12$ and 0.48, respectively).

In patients whom FC treatment was no longer required, plasma renin or renin activity remained high despite normonatremia, normokalemia, and normovolemia (Table 3).

Molecular Characteristics of *CYP11B2* Mutations

Sixteen patients from 12 families were identified with biallelic mutations in *CYP11B2*. Thirteen patients (81%) were homozygous, and 3 (19%) were compound heterozygous for *CYP11B2* mutations. A total of 10 pathogenic variants were identified including 5 missense, 1 nonsense, 3 splicing mutations, and 2 deletions. Of the 10 sequence variants identified, 6 have not been reported before (p.Y195H, c.1200 + 1G > A, p.F130L, p.E198del, c.1122-18G > A, p.I339_E343del), and 4 sequence variants (p.T185I, c.395 + 1G > T, p.E255X, p.I263N) have been described in previous reports (18-25) (Table 1, Fig. 3A). The most frequently detected variant was c.554C > T (p.T185I) (n = 13; 40%).

In in silico assessment, among the 5 strongest 3D models created by the server with highest confidence score were selected and used for analysis. The spatially superimposition of the native and mutant 3D models were shown in Figure 3B, in which 2 substitutions of amino acid residues 130 from Phe to Leu and 195 from Tyr to His were amplified.

Phe130Leu is a conserved residue located on a loop between B'- and C-helix. F130 is conserved in all CYP11B isozymes. This residue is thought to lie within a region that may form part of the access route for the substrate; point mutations in this region significantly alter substrate

Table 1. Clinical, biochemical, and molecular characteristics of the aldosterone synthase deficiency patients at presentation

F	P/Sex	CYP11B2 variants (transcript NM_000498, ENST00000323110)	Initial features				Laboratory at diagnosis				
			Age at onset (day)	Height SDS	Weight SDS	Signs/symptoms	Na (mEq/L)	K (mEq/L)	Renin (pg/mL)	PRA (ng/mL/h)	Aldosterone (pg/mL)
F1	P1/female	c.554C > T p.T185I Homozygous	25	NK	−3.46	Dehydration, hypotonia, microcephaly	125	7.7	>150	NA	192
	P2/male	c.554C > T p.T185I Homozygous	243	−2.96	−1.34	Poor feeding, poor weight gain, dehydration	128	6	NA	NA	NA
F2	P1/female	c.395 + 1G > T Homozygous	114	−1.45	−3.46	Poor weight gain, vomiting, hypotonia	128	5.7	NA	5.6	39.3
F3	P1/female	c.554C > T p.T185I Homozygous	109	−1.31	−3.01	Poor weight gain, weakness	126	6.5	1100	NA	199
F4	P1/male	c.554C > T p.T185I Homozygous	119	−2.01	−2.63	Vomiting, weakness, restless	121	6	>150	NA	144
	P2/female	c.554C > T p.T185I Homozygous	143	0.12	−2.69	Poor weight gain	128	5.4	NA	64	290
F5	P1/female	c.554C > T p.T185I Homozygous	76	0.65	−2.1	Poor weight gain, dehydration	126	6.4	NA	1.15	191.5
F6	P1/female	c.763G > T/c.554C > T p.E255X/p.T185I Compound heterozygous	208	−2.64	−4.05	Poor weight gain, vomiting, dehydration	129	5.1	42.3	NA	NA
F7	P1/male	**c.583T > C p.Y195H** Homozygous	70	NK	−2.85	Polydipsia, dehydration	127	6.5	>150	NA	<10
	P2/male	**c.583T > C p.Y195H** Homozygous	41	−1.8	−1.66	Weakness, diarrhea, dehydration	118	6.5	NA	10.4	29.7
F8	P1/male	c.788T > A p.I263N Homozygous	138	−1.06	−2.26	Vomiting, weakness, fever, dehydration	127	5.82	NA	8.64	62.2
	P2/male	c.788T > A p.I263N Homozygous	23	NK	−1.07	Poor weight gain	125	7.48	NA	0.48	114
F9	P1/female	**c.1200 + 1G > A** Homozygous	62	−2.51	−3.06	Vomiting, poor feeding	131	7.31	NA	168.2	81.61
F10	P1/female	**c.390C > G/c.593_595del p.F130L/p.E198del** Compound heterozygous	112	−3.02	−4.4	Poor weight gain, growth retardation	129	5.9	>150	NA	<3.7
F11	P1/male	c.788T > A/**c.1122-18G > A** p.I263N/**c.1122-18G > A** Compound heterozygous	12	−0.29	−2.58	Poor weight gain	128	7.2	>150	NA	<3.7
F12	P1/male	**c.1016_1030del15bp p.I339_E343del** Homozygous	36	NK	−0.59	Poor feeding, poor weight gain, dehydration	115	6.9	371.6	NA	4.08

Novel variants are marked in bold. PRA normal range: 1 to 6.5 ng/mL/h; renin normal range: 0.8 to 16.5 pg/mL; aldosterone normal range: 35 to 300 pg/mL; Na normal range: 135 to 145 mEq/L; and K normal range: 3.5 to 5.5 mEq/L.

Abbreviations: F, family; P, patient; NA, not available, NK, not known; PRA, plasma renin activity, SDS, SD score.

Table 2. Treatment characteristics of the patients with aldosterone synthase deficiency

Patient	Initial treatment			Last visit treatment			Maximum FC dose (µg/day)	Age at the discontinuation of FC (year)
	Age (day)	FC dose (µg/day)	Salt (mEq/kg/day)	Age (year)	FC dose (µg/day)	Salt (mEq/kg/day)		
F1P1	25	100	3.5	6.88	–	–	200	5.04
F1P2	243	200	6.92	1.86	200	–	200	
F2P1	114	50	–	7.79	–	–	100	7.61
F3P1	109	200	5.10	6.11	–	–	200	6.03
F4P1	119	50	4	5.08	100	–	200	
F4P2	143	100	6	9.43	–	–	100	6.55
F5P1	76	100	4	14.52	–	–	100	2.24
F6P1	208	100	6.51	3.34	100	–	100	
F7P1	70	50	3	10.18	100	–	100	
F7P2	41	200	14	1.12	200	–	200	
F8P1	138	100	6.5	2.88	100	–	100	
F8P2	23	100	4.8	1.01	150	3.5	150	
F9P1	62	200	3.00	1.41	100	–	200	
F10P1	112	50	10.83	1.06	100	–	100	
F11P1	12	100	7	1.36	200	1.5	300	
F12P1	36	100	14.5	2.62	150	–	300	

All of the patients were exclusively breastfeeding for the first 5 to 6 months, and none of them received formula.
Abbreviations: F, family; FC, fludrocortisone; P, patient.

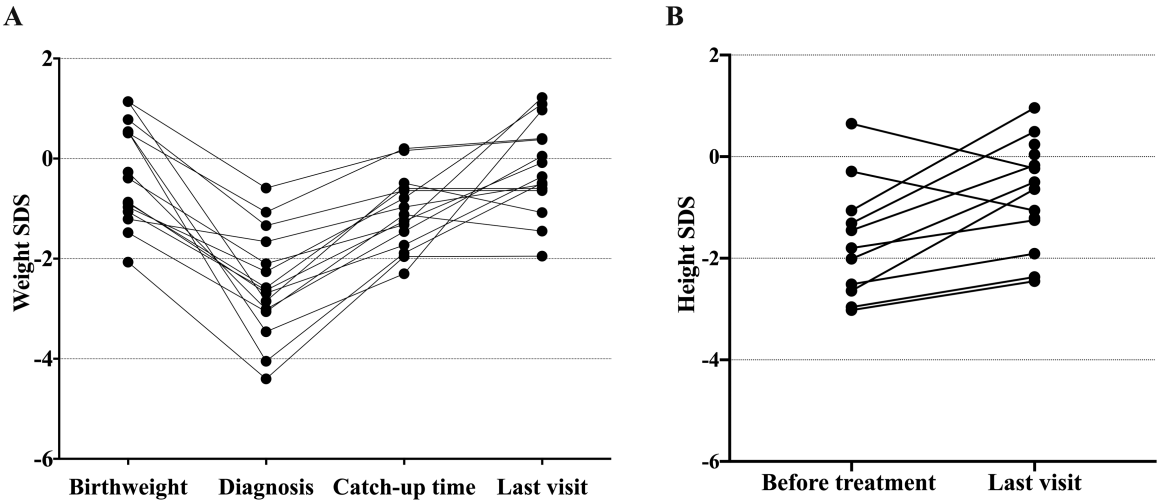

Figure 1. Plots of weight and height SD score (SDS) values of patients with aldosterone synthase deficiency (ASD) over time. (A) Change in weight SDS values in patients with ASD before and after treatment showing individual patterns of catch-up growth ($P = 0.0002$). (B) Change in height SDS values of patients between before treatment and last visit ($P = 0.027$).

specificity in different P450s (26). The side chain of Phe forms hydrogen bonds, the backbone of N133 and R110 and the side chains of H109 and R448 (27). Mutation to Leu disrupts these hydrogen bonds, resulting in the loss of the local structure of the loop (Fig. 3B).

Tyr195His is a highly conserved residue located on E-helix and close to L463-L464 in the L helix, which is involved in heme binding (28). A mutation to Gly results in the loss of these interactions and destabilization of the structure (Fig. 3B).

Discussion

Our study provides insights into 2 clinical outcomes of patients with ASD: growth and the need for FC therapy as the children age.

We have demonstrated that infants with ASD rapidly catch up in growth after initiation of FC treatment as early as 2 months and maintain it afterward. It has been reported that PRA levels varied inversely with dietary sodium content and weight gain in rats (29). In zebrafish larvae, activation of mineralocorticoid receptor increases protein deposition,

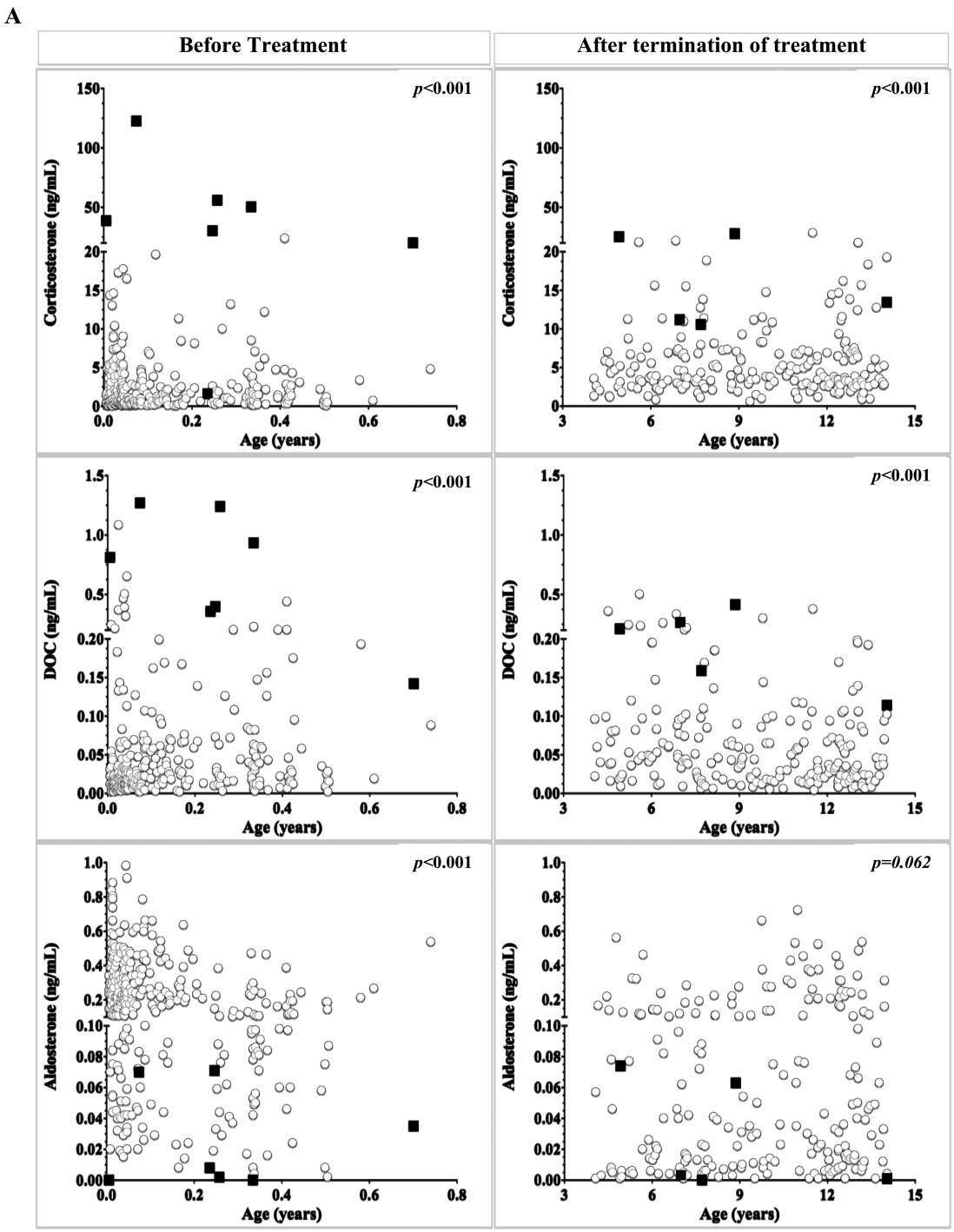

Figure 2. Adrenal steroid hormones of the patients with aldosterone synthase deficiency (ASD). (A) 11-deoxycorticosterone (DOC), corticosterone, and aldosterone concentrations of ASD patients before treatment and after discontinuation of treatment compared to control group. (B) Corticosterone/aldosterone ratios of ASD patients before treatment and after discontinuation of treatment compared to control group. Black squares and open circles indicate patients and controls, respectively. The *P*-values indicated in the upper right corner of each graph were obtained by comparing with the patients and age-matched control group in the related graph. To convert ng/mL to nmol/L, multiply by 2.77 for aldosterone, 2.89 for corticosterone, and 3.03 for DOC.

which modulates postnatal growth (30). Several publications reported that a better growth is obtained when mineralocorticoid substitution is maintained during childhood in patients with congenital adrenal hyperplasia and other disorders with aldosterone deficiency including Addison's and autoimmune polyglandular disease (31-34). These studies and our observation in ASD patients show that adequate sodium balance is essential for normal growth. As

B

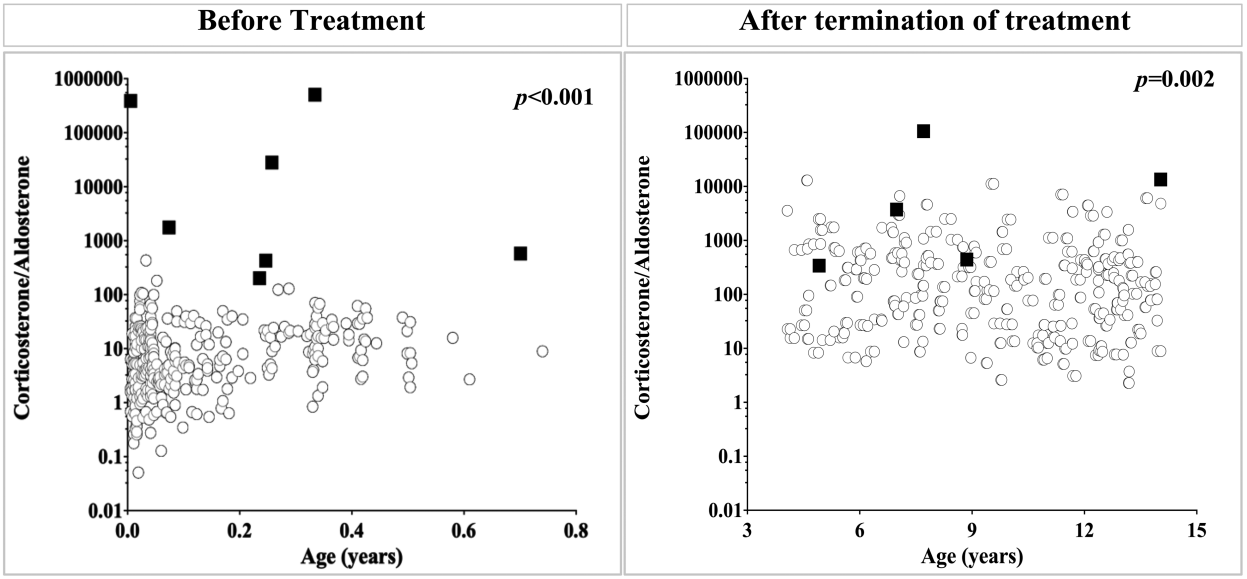

Figure 2. Continued.

the reduced activity of aldosterone synthase persists and there is a potential of salt loss in patients with ASD, we recommend careful monitoring of growth in these patients until adult height is achieved.

Hyponatremia was invariably present in all patients, but hyperkalemia was absent in 2 cases in our cohort. Similar cases have been reported with variability of serum potassium (7,21,35). Aldosterone synthase knockout mice were shown as normokalemic with a physiologic dietary K^+ load. It was concluded that renal adaptation to a physiologic, but not supraphysiologic, K^+ load can be achieved in aldosterone deficiency by aldosterone-independent activation of the renal outer medullary potassium and epithelial sodium channels (36). More recently, Yang et al concluded that aldosterone-independent mechanisms may be recruited and contribute to K^+ excretion at times before hormone levels as PRA/renin increase (37). However, in our study, initial PRA/renin levels of these 2 patients with normal potassium concentrations were already elevated >3- to 4-fold of the reference values.

Aldosterone concentrations indicated inadequate aldosterone production in patients before treatment, which were either low or inappropriately normal for the clinical signs of mineralocorticoid deficiency including hyponatremia, hypotension, failure to thrive, and elevated renin. Although aldosterone deficiency has a significant mortality in infancy, the morbidity of ASD is usually not as severe as in the salt-wasting form of congenital adrenal hyperplasia. This presumably reflects normal synthesis of deoxycorticosterone, corticosterone, and cortisol in ASD as in our patients, which ameliorate the development of hyponatremia and shock especially after infancy period.

Treatment of ASD is achieved by oral sodium supplementation and FC treatment (100-300 µg/d) effectively. As the patients get older, the requirement for treatment decreases. In the patients published to date, no clear information has been reported about the time of treatment cessation. In our study, the mean age of discontinuation of replacement was achieved safely at 5.5 ± 2.0 years (median: 6.0, range: 2.2 to 7.6). This observation may serve as a guidance for clinicians about when they can attempt to discontinue FC treatment.

Our results demonstrate that plasma renin level or activity cannot be used as a guidance for discontinuation of FC treatment. In the follow-up after discontinuation of treatment, we observed no clinical deterioration or salt-wasting despite elevated renin in our patients. Elevated renin in these asymptomatic patients may suggest ongoing reduction in aldosterone synthase activity compensated by homeostatic mechanisms. Maturation of renal tubules and increased capacity for sodium reabsorption and sensitivity to mineralocorticoids combined with increased sodium intake and alternative pathway of mineralocorticoid biosynthesis have been offered as possible mechanisms (21). Recently, a physiological partial aldosterone resistance has been demonstrated in the newborn infants (38). In addition, newborn diets (especially breast milk) have a low sodium content, and dietary sodium is increased with the shift to table food with age (39,40). The observation of F3P1 and F5P1, whose FC discontinued but who prefer salty foods, is consistent with this hypothesis. In contrast, F4P2 is fed without salt due to hypertension since age of 6 years.

A unique aspect of the present study is the comparison of steroid hormone profiles at diagnosis and after

discontinuation of the treatment to search for any biochemical parameters, which might be useful regarding the feasibility of treatment discontinuation. In the literature, there has been no study that evaluates the steroid hormone profile after discontinuation of treatment in ASD and no hormonal guidelines exist to guide discontinuation of FC replacement. Our results demonstrate that DOC and corticosterone concentrations were significantly decreased in patients after discontinuation of treatment compared to those at diagnosis, which likely reflects reduced angiotensin II. Usually a fall in renin reflects the correction of volume depletion. However, as a limitation of our study, renin was reassessed after treatment for only 2 patients with high renin at baseline: In 1, the renin fell, and in the other, renin was >150 pg/mL at both measurements and thus might have changed but cannot be determined. Therefore, decreased DOC and corticosterone concentrations may be due to a partial restoration in aldosterone synthase activity or decrease in peripheral resistance to aldosterone with age and the increase in tubular response as an adaptation mechanism. However, aldosterone concentrations and corticosterone/aldosterone ratios reflect

Table 3. Renin and plasma renin activity concentrations of patients with aldosterone synthase deficiency before treatment and after discontinuation of treatment

Patient	Before treatment		After discontinuation of treatment	
	Renin, pg/mL	PRA, ng/mL/h	Renin, pg/mL	PRA, ng/mL/h
F1P1	>150	–	>150	–
F2P1	–	5.6	–	27.9
F3P1	1100	–	–	4.37
F4P2	–	6.4	141.6	–
F5P1	–	1.15	55.4	21

PRA normal range: 1 to 6.5 ng/mL/h; renin normal range: 0.8 to 16.5 pg/mL.
Abbreviation: F, family; P, patient.

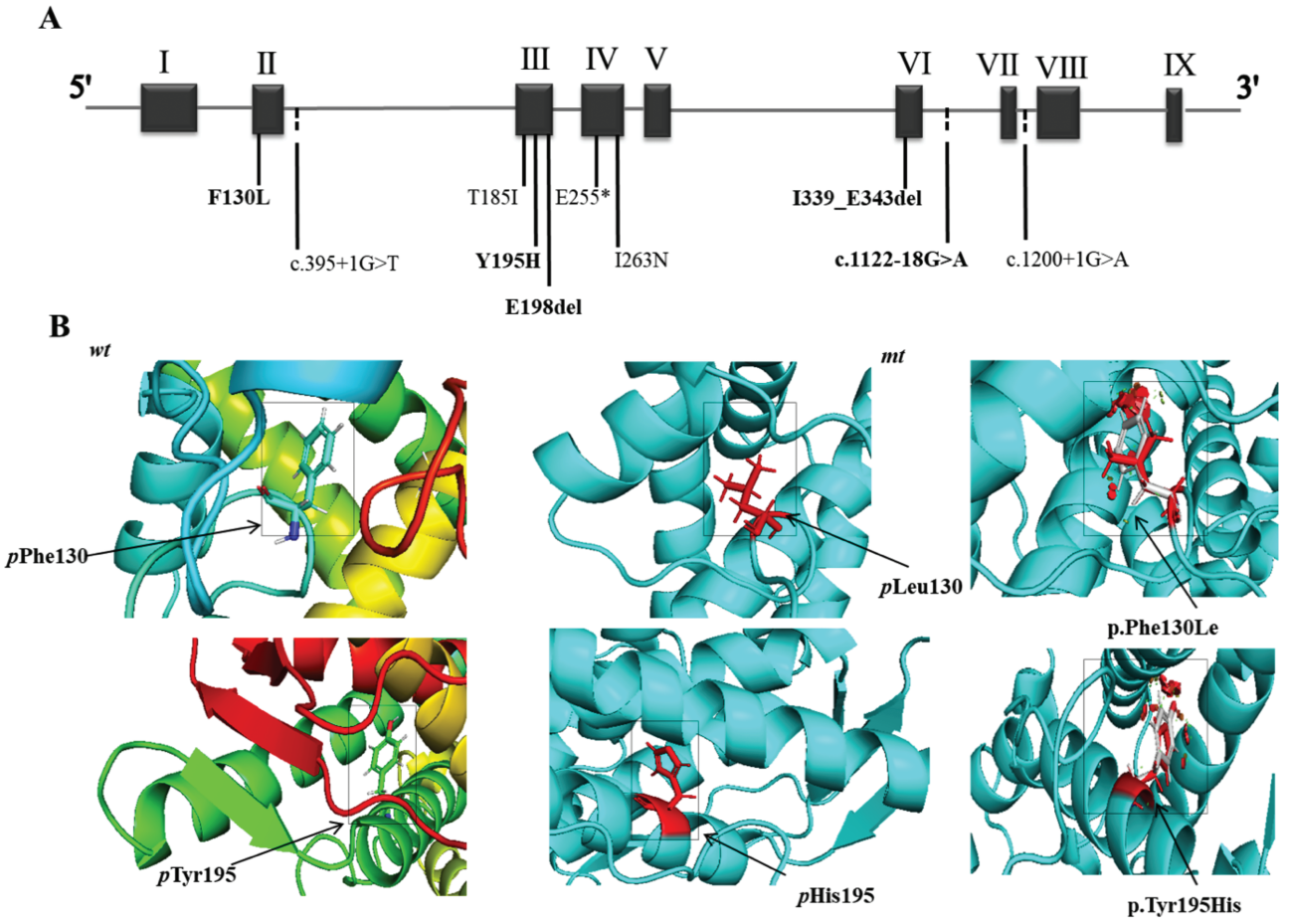

Figure 3. (A) Schematic representation of *CYP11B2* gene demonstrating the sequence variants identified in our patients with aldosterone synthase deficiency (ASD). The novel mutations are indicated in bold characters. Upper panel shows exon numbers. (B) Overview of the CYP11B2 model and 2 missense sequence variants. Locations of *p*Phe130 and *p*Tyr195 (left column) of wild-type CYP11B2 and *p*Leu130 and *p*His195 (middle column) and changes in the CYP11B2 due to amino acid variations (right column) are presented in magnified frame. Structure prediction is based on I-TASSER server. The models are visualized by PyMol, with rainbow painting starting from dark blue at N terminal and ending red at C terminal. Elements of residues are painted in stick format and the view zoomed to 20 Å below for each model.

the ultimate aldosterone synthase activity more precisely, in which there was no statistically significant difference between at diagnosis and after discontinuation of FC treatment. Furthermore, the corticosterone, DOC, and corticosterone/aldosterone ratios were higher and aldosterone concentrations were lower in patients compared to control individuals both before treatment and after discontinuation of treatment conditions during the follow-up. Taken together, our results show that, although they became able to maintain eunatremia and weaned of FC treatment over the course of the disease, complete normalization of aldosterone synthase activity is not possible. Decrease in DOC and corticosterone levels might be regarded as supportive findings besides eunatremia regarding decision of treatment discontinuation; nevertheless, a lifelong precaution and follow-up should still be advised. Indeed, 1 adult patient was diagnosed when he underwent bowel preparation for a barium enema (41).

To date, more than 60 pathogenic variants in *CYP11B2* gene (mostly missense and nonsense) have been reported (42). In the present study a total of 10 variants were identified, 6 of which were novel including 3 splicing, 5 missense, 1 nonsense mutations, and 2 deletions expanding the genotypic spectrum of ASD. The most frequently detected pathogenic variant was c.554C > T (p.T185I) (40%). This variant was first reported in a Hungarian ASD patient of Romani descent (22) and later described in patients of Slavic and Albanian origin (24,35,43). In a cohort published in recent years, composed of Greek and Albanian patients, p.T185I represents the most common pathogenic variant by far suggesting that this may be a founder mutation in the southern part of the Balkans and Turkey (44). The second most frequent variant in our cohort was c.788T > A (p.I263N) (n = 3). This variant has been previously reported as homozygous in Turkish ASD patients from 3 unrelated families, suggesting an ethnic specificity (19-21). The p.E255X variant, which has been previously reported, causes a premature stop codon (18). The predicted enzyme has lost the 5 terminal exons encoding for several α-helices and β-strands, which contain important residues for proton transfer, accessory protein binding, heme binding, and substrate binding (45).

The limitations of our study include unavailability of 18-hydroxycorticosterone measurements of the patients, and the absence of in vitro testing of the effects of newly discovered mutations by tissue and enzymatic studies. Although these limitations avoided us to differentiate type 1 or type 2 ASD in the patients, steroid profiles, clinical characteristics of the patients, and segregation of the phenotype within the families ensured the diagnosis of ASD.

In conclusion, this largest Turkish cohort of ASD patients provides novel data regarding clinical biochemical and genetic aspects of the disease. Infants with ASD rapidly catch up in weight SDS at 2 months after initiation of treatment and maintain it thereafter. FC replacement can be terminated on average 6 years after treatment owing to the compensatory mechanisms. Elevated renin concentrations persist despite patients being asymptomatic, and eunatremic, hence, is not helpful in guiding time for discontinuation of treatment. Decrease in DOC and corticosterone levels might be regarded as supportive findings besides eunatremia regarding decision of treatment discontinuation. Diminished aldosterone synthase activity, albeit subclinically, continues in the patients after discontinuation of the treatment. Therefore, long-term monitorization of these patients for growth and electrolyte disturbances is recommended.

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Additional Information

Correspondence: Tulay Guran, MD, Marmara University School of Medicine, Department of Pediatric Endocrinology and Diabetes, Fevzi Cakmak Mh. Mimar Sinan Cd. No 41. 34899, Ustkaynarca/Pendik, Istanbul, Turkey. E-mail: tulayguran@yahoo.com; tulay.guran@marmara.edu.tr.

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Data Availability: Some data sets analyzed during the current study are included in the data repositories listed in References.

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