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CYP11B1 GENE MUTATIONS IN PATIENTS WITH CONGENITAL ADRENAL HYPERPLASIA IN TURKEY

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Background

Congenital adrenal hyperplasia (CAH) due to 11β-hydroxylase deficiency (11OHD) is the second most common form of CAH, representing 5-8% of the total cases, with the incidence of 1:100.000 of live births (OMIM #202010). It is found to be more common in Israeli population of Moroccan Jewish origin affecting 1:5000 to 1:7000 of live births. In two different clinical studies, the distribution of 11OHD in all CAH patients was found high and varied between 13 and 16.5% in Turkish population. Recessive loss of function mutations in the 11OHD encoding gene (*CYP11B1*) results glucocorticoid deficiency, hyperandrogenism and hypertension. Females born with this deficiency have masculinized external genitalia, while male patients undergo isosexual precocious puberty.

Objective and hypotheses

To investigate the specific causative mutations of CAH in *CYP11B1* gene and to examine for genotype-phenotype correlations.

Methods

21 patients (n=9, 46,XX; n=12, 46,XY) with the classical 11OHD from 20 unrelated Turkish families were included in this study. Most of the patients initially presented with premature adrenarche, rapid somatic development and hypertension. Diagnosis of 11OHD was based on both clinical and hormonal criteria.

Screening of all the coding and exon-intron boundaries of *CYP11B1* (NM_000497) gene was performed by Sanger sequencing.

Known mutations were confirmed by database and literature search. Novel mutations were analyzed by *in siico* prediction tools (PolyPhen-2, SIFT and MutationTaster).

Results

Clinical, hormonal and genetic findings of the patients are summarized in Table 1. The age of diagnosis at onset ranged from 6 days to 12.5 years. The rate of consanguinity was very high (75%). Four out of nine 46,XX patients received a late diagnosis (age 2-8.7 years) and were raised as males due to severe masculinization (Prader genital stages IV and V). These patients were reassigned to the male sex after reconstructive surgery and bilateral salpingo-oophorectomy and hysterectomy. Histopathological examination of both ovaries of the Patient 4 revealed a steroid tumor.

Mutation analyses in 20 index patients revealed twelve different mutations in *CYP11B1* gene. These mutations were homozygous c.896T>C (p.L299P) (30%, 6/20), homozygous c.421C>T (p.R141*) (10%, 2/20), homozygous c.954G>A (p.T318=) (silence, cryptic splicing; 10%, 2/20), homozygous c.1398+2T>C (IVS8+2T>C) (novel splice-donor mutation, 5%, 1/20), compound heterozygous c.[896T>C (p.L299P)]; [c.1398+2T>C (IVS8+2T>C)]; 5%,1/20], homozygous c.348G>T (p.W116C) (5%, 1/20), homozygous c.1151G>A (p.R384Q) (5%,1/20), homozygous c.1342C>T (p.R448C) (5%, 1/20), homozygous c.1449_1451delGGT (5%, 1/20), compound heterozygous c.[G393+3A>G (IVS2+3A>G)]; [896T>C (p.L299P)] (5%, 1/20), homozygous c.1179_1180dupGA (novel frame shift; 5%, 1/20) and homozygous c.328G>C (p.R143P) (novel missense; 5%, 1/20). One patient had mutation in only one allele c.953C>T (p.T318M). There was no definitive correlation between genotype and phenotype.

Table 1. Some clinical and laboratory findings of patients with 11OHD

Patient	At diagnosis				Karyotype	Reared gender	External genitalia (Prader)	Mutations	Follow-up Surgery (age-yr)
	Age	Findings	Bone age (yr)	11-DOC (ng/ml) (<8)					
1	2.5 yrs	AG,PA, RSD, HT	7	ND	XX	F	3	p.W116C/ p.W116C	C & V (3.5)
2	6.5 yrs	PA, RSD, HT	14	16	XY	M		p.R141*/ p.R141*	
3	2 yrs	PA, RSD, HT	12	15	XY	M		p.R141*/ p.R141*	
4	8.7 yrs	AG,PA, RSD, HT	17	ND	XX	M	4	p.L299P/ p.L299P	BSOH and male reconstruction (9.1) **Streoid cell tumor**
5	4.5 mos	AG, HT	ND	ND	XX	M	4	p.L299P/ p.L299P	BSOH (11) & male reconstruction (11.9)
6	2 yrs	PA, RSD, HT	5.5	22	XY	M		p.L299P/ p.L299P	
7	1.3 yrs	PA, RSD, HT	12.5	16	XY	M		p.L299P/ p.L299P	
8	3.6 yrs	PA, RSD, HT	11	16	XY	M		p.L299P/ p.L299P	
9	2 yrs	PA, RSD, HT	12.5	16.3	XY	M		p.L299P/ IVS8+2T>C	
10*	2 yrs	AG, PA, RSD, HT	13.5	32	XX	M	5	IVS8+2T>C/ IVS8+2T>C	BSOH (4.5)
11*	12.5 yrs	PA, RSD, HT	16	38	XY	M		IVS8+2T>C/ IVS8+2T>C	
12	4.7 yrs	PA, RSD, HT	14	85	XY	M		IVS2+3A>G/p.L299P	
13	1.8 yrs	AG, PA, RSD, HT	7	ND	XX	M	5	p.R384Q/p.R384Q	BSOH (10), testicular prosthesis (17.3)
14	1.3 yrs	PA, HT	2.7	ND	XY	M		p.T318M/ND	
15	7 days	AG		596	XX	F	3	c.1449_1451delGGT /c.1449_1451delGGT	C & V (0.6)
16	6 days	AG		ND	XX	F	4	p.R448C/p.R448C	C & V (1.3)
17	3 yrs	PA, RSD, HT	6	22	XY	M		p.L299P/ p.L299P	
18	7.5 mos	AG, PA, HT	ND		XX	F	4	c.1179_1180dupGA/ c.1179_1180dupGA	C & V (3.3)
19	4 mos	AG, PA, HT	ND	120	XX	F	5	c.954G>A/ c.954G>A	C & V (0.8)
20	2 yrs	PA, RSD, HT	8	10	XY	M		c.954G>A/ c.954G>A	
21	5.3 yrs	PA, RSD, HT	12	135	XY	M		p.R143P/p.R143P	

*: cousin; AG: Ambiguous Genitalia; PA: Premature Adrenache; HT: Hypertension; RSD: Rapid Somatic Development; DOC:Desoxycorticosterone; F: Female; M:Male; C: Cliteroplasty; V: Vaginoplasty; BSOH: Bilateral salphingo-oophorectomy and hysterectomy; ND: Not determined
Novel mutations.are showed red colour.

Conclusions

- In this study, three different novel mutations were detected and the p.L299P was found to be the most common mutation.
- The results of the study might contribute to the establishment of molecular screening strategies. Identification of the disease causing mutations provides reliable information for genetic counseling for the families.

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