Clinical phenotype and genotype association in patients with 21-hydroxylase deficiency.

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Introduction: Congenital adrenal hyperplasia (CAH) is an autosomal recessively transmitted disease and 95% of CAH cases are due to 21-hydroxylase deficiency (21-OHD). There are more than 100 mutations that cause CAH due to 21-OHD and the clinical expression of the disease is reported to correlate with the mutated alleles. Predicting the form that could be observed for a certain genotype may be useful in the prenatal diagnosis of CAH. Genotype may thus be important in genetic counseling before normal pregnancy or in vitro fertilization in parents who are heterozygous for CYP21A2 mutation.

The aim: The aim of this study was to investigate responsible mutations in CAH patients with 21-OHD and then to evaluate the genotype-phenotype relationship.

Subjects: Genotype was investigated in 40 cases with 21-OHD (33 classical, 7 nonclassical). Clinical and biochemical data of patients was shown in Table 2.All were brought up appropriately with their karyotype.

Methods: Mutations were firstly investigated by sequence analysis by Sanger method. When needed Multiplex Ligation-dependent Probe Amplification (MLPA) technique was applied. In 11 cases only Sanger method, in 26 cases Sanger and MLPA methods were used. In 4 compound heterozygotes genotypes were determined after the genotyping of parents. ***Mutations were grouped as of group 0, A, B or C and compared with the expected clinical phenotype i.e. Group 0: Salt wasting (SW), Group A: SW, Group B: Simple virilizing (SV), Group C: Nonclassical (NC) (Table 1).*** Positive predictive value (PPV) was determined for the different groups. PPV was calculated by dividing the total number of patients in each group to the expected phenotype and multiplying by 100.

Table1. The classification of common CYP21A2 mutations based on in vitro data (1).

Group	Null	A	B	C
Phenotype	SW	SW	SV	NC
In vitro activity of CYP21A2	0%	<1%	1–2%	20–60%
Mutations	Large deletion Large conversion 8-bp deletion exon 6 cluster p.Q318X p.R356W	IVS2-13A/C>G	p.I172N	p.V281L p.P453S p.P30L

Results: Responsible mutations were determined in 37 of the cases (n:15 SW, n:15 SV, n:7 NC) as shown in Table 2.

Table 2. Clinical and biochemical data and molecular defects of patients with 21-OH deficiency

Patient no	Karyotype	Genotype	Consanguinty	Age at diagnosis	Baseline 17-OHP (ng/ml)	Phenotype
2[1]	XX	p.Q318X/p.Q318x	-	0,04	110(u.t)	SW
3	XX	IVS-2 / IVS-2	+	0,04	>20	SW
4	XX	del/ND	-	0,01	138	SW
5[2]	XY	IVS-2 / IVS-2	+	0,02	47,6	SW
6[1]	XY	p.Q318X/p.Q318x	-	0,02	166(u.t.)	SW
7	XX	IVS2-2/p.Q318X	+	0,08	591	SW
8	XY	del/IVS2-2	-	0,01	16,50(u.t)	SW
9	XY	del /IVS2-2	-	0,29	>20	SW
10	XY	IVS-2 / IVS-2	+	0,08	42,25	SW
11	XX	del/del	+	0,05	>20	SW
12	XX	conv/conv	+	0,05	259	SW
13	XY	conv/conv	-	0,15	100	SW
15	XX	del/del	+	0.01	>20	SW
16	XX	8-bp del/del	-	0.140	>20	SW
17	XY	conv/conv	+	0,06	>20	SW
18[3]	XX	p.I172N/p.I172N	+	5,05	-	SV
19	XY	p.I172N/p.Q318X	-	0,08	591	SV
20	XX	conv/del	-	0,11	43,94	SV
21	XX	del/IVS-2	-	0,01	>20	SV
22[3]	XX	p.I172N /p.I172N	+	4,671	0,91	SV
23	XX	p.I172N /p.I172N	+	7,5	69,6	SV
24*	XX	IVS-2 / IVS-2	-	2,54	12,8	SV
25*	XX	IVS-2 / IVS-2	-	0,36	>20	SV
27	XY	p.I172N /p.I172N	-	9,29	>20	SV
28	XX	IVS-2 / IVS-2	-	4,42	>20	SV
29[2]	XY	IVS-2 / IVS-2	+	2 529	>20	SV
30	XX	p.I172N /p.I172N	-	1,84	220	SV
31	XX	IVS-2 / IVS-2	+	0,15	80	SV
32	XX	conv/p.Q318x	+	0,66	28,5(u.t)	SV
33	XX	conv/del	-	0.485	>20	SV
34	XX	p.V281L/p.V281L	+	8,4	5,9	NC
35	XX	p.V281L /p.V281L	-	6,62	32	NC
36	XX	p.V281L /p.V281L	+	17,38	8,37	NC
37	XX	p.I172N /p.I172N	-	11,06	4,4	NC
38	XX	p.I172N/p.V281L	-	17,03	28,7	NC
39	XX	p.V281L/p.Y59N	-	8,25	31,5	NC
40	XX	p.30L/8-bp del	-	7,12	4,7	NC

ND: not detected; 17-OHP=17 hydroxy progesterone; SW: salt wasting; SV: simple virilizing; NC: non-classical conv: 5' end large conversion, desplice site mutation (IVS2-13C>G); *1,2,3,4,: affected siblings; u.t: under treatment. Patients 1, 14 and 26 whose genotype could not l: large deletion, 8-bp del: 8-bp deletion IVS-2: intron 2 be determined are not included in the table

Eight patients (27.5%) had a positive family history: Two pairs of sisters and two sister-brother pairs are indicated in Table 2 with top digits. A pair of siblings with homozygous genotype IVS2-13A/C>G / IVS2-13A/C>G were found to have different phenotypes (patients 5 and 29; SW and SV). In all patients, the most common mutation was IVS2-13A/C>G (28.3%), followed by p.I172N mutation (17.5%) and large gene deletions (14.7%) as shown in Table 3.

One patient with nonclassic CAH was heterozygous for p.Y59N mutation. This is the first report of a patient with p.Y59N mutation in Turkey. In salt-losing patients, the most common mutation after IVS2-13A/C>G mutation was large gene deletion (26%). In the simple virilizing form, the common mutation after IVS2-13A/C>G mutation was p.I172N. In the non-classical form, the most common mutation was p.V281L.

Table 3. Distribution of mutations obtained in patients with CAH due to 21-OHD

Mutations	SW n (%)	SV n (%)	NC n (%)	Total number	Total frequency (%)
Large deletion	9 (30)	12 (40)	0(0)	21	28.3
Large conversion	6(20)	3(10)	0(0)	9	12.1
8-bp deletion	1(3.3)	0(0)	1(7.1)	2	2.7
p.R356W	0(0)	0(0)	0(0)	0	0
p.Q318X	5(16.6)	2(6.7)	0(0)	7	9.4
IVS2-13 A, C>G	9(30)	12(40)	0(0)	21	28.3
p.I172N	0(0)	10(33.3)	3(27)	13	17.6
p.P30L	0(0)	0(0)	1 (7.1)	1	1.4
p.V281L	0(0)	0(0)	8 (57.1)	8	10.8
p.Y59N	0(0)	0(0)	1 (7.1)	1	1.4
Not detected	1(3.3)	0(0)	0(0)	1	1.4
Total	30 (100)	30 (100)	14 (100)	74	100

In group 0, PPV was 72% (8 SW, 3SV); in group A, PPV was 50% (6 SW,6 SV); in group B, PPV was 85% (6 SV,1NC); in group C, PPV was 100% (6/6 NC). Genotype-phenotype correlation was found to be less in simple virilizing CAH (6/15) than salt wasting (14/15) and non-classical types (6/7) of CAH (Table 4).

Table 4. Genotypes categorized into mutation groups according to predicted severity

Mutation group (enzyme activity)	Genotype	Predicted phenotype	SW	SV	NC	Patient number	Genotype-phenotype correlation (ppv)	
Null (0%)	Del /Del	SW	2	0	0	2	100%	72%
	Conv/Conv		3	0	0	3	100%	
	Del/Conv		0	2	0	2	0	
	p.Q318X /p.Q318X		2	0	0	2	100%	
	p.Q318X/Conv		0	1	0	1	0	
	8-bp del/del		1	0	0	1	100%	
A (1%)	IVS-2 / IVS-2	SW	3	5	0	8	37.5%	50%
	IVS-2 /p.Q318X		1	0	0	1	100%	
	IVS-2 /Del.		2	1	0	3	66.6%	
B (1-2%)	p.I172N/p.I172N	SV	0	5	1	6	83.3%	85%
	p.I172N/p.Q318X		0	1	0	1	100%	
C (20-50%)	p.V281L/ p.V281L	NC	0	0	3	3	100%	100%
	p.V281L/ p.Y59N		0	0	1	1	100%	
	p.V281L/p.I172N		0	0	1	1	100%	
	p.P30L/ 8bp del		0	0	1	1	100%	
Not classified*	Del/nondetected		1	0	0	1	0	0

* In this patient no mutation was found in one allele of the CYP21 gene.

Conclusion: In conclusion, the mutations of the CYP21A2 gene in SW, SV and NC forms of CAH were confirmed in 37/40 patients. The SW and NC forms showed a good correlation between genotype and phenotype, but the SV form showed inconsistency between genotype and phenotype. Here we also report p.Y59N mutation in CAH for the first time in Turkey.

Reference: 1.Hannah-Shmouni F, Chen W, Merke D.P. Genetics of Congenital Adrenal Hyperplasia. Best Pract Res Clin Endocrinol Metab. 2009 April; 23(2): 181–192