Genotype and Phenotype Characteristics of Patients with Nonclassical Congenital Adrenal Hyperplasia due to 21-hydroxylase Deficiency

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BACKGROUND: Nonclassical congenital adrenal hyperplasia (NCCAH), which is generally presented with symptoms of androgen excess, is inherited autosomal recessive due to different kind of mutations in the *CYP21A2*. Genotype-phenotype correlation studies in large groups of patients are associated with predominantly classic congenital adrenal hyperplasia. Prediction of phenotype from genotype tends to be more difficult among patients who are compound heterozygotes for two different mutations or those carrying mutations of intermediate severity. Recently, predisposition of *CYP21A2* gene duplications for germ line de novo mutations in the next generation has been reported

AIM: To evaluate clinical and molecular characteristics of the patients with NCCAH.

METHOD: Twenty-two patients from unrelated 21 families (20F, 2M), diagnosed as NCCAH according to their clinical, hormonal and molecular (biallelic or monoallelic mutations) findings, were included and data were collected retrospectively. History and physical findings were extracted from existing medical records. Pubertal stage of all patients and Ferriman Gallwey score (FGS) of females were assessed by physical examination. Hirsutism was defined as FGS≥ 8. Menstrual irregularity or oligomenorrhea was defined as menstrual cycles ≥ 35 days or fewer than 8 cycles per year. Hormonal testing was done in the follicular phase of the cycle whenever possible. Basal serum 17-hydroxyprogesterone (17-OHP), androstenedione, cortisol, LH, FSH, estradiol, testosteron levels were measured. Measurement of serum 17-hydroxyprogesterone following stimulation with a 0.25 mg adrenocorticotrophic hormone were evaluated. Basal, 30 and 60 min samplings for serum 17-OHP and cortisol were measured. Genetic analysis was performed in Department of Medical Genetics of Istanbul Faculty of Medicine. Sequencing and multiplex ligation-dependent probe amplification (MLPA) were used for moleculer analysis.

RESULTS: Findings are summarized in Table 1 and 2. Eleven different mutations, including one novel (p.P214L) were detected in patients. Clinical and hormonal findings were consistent with NCCAH in 8 patient who had mutation in only one allel. The presenting symptoms were clinical hyperandrogenemia (hirsutism, acne, hair loss) (n=9), premature adrenarche (n=8), precoccious puberty (n=5), menstrual irregularity (n=2) and cliteromegaly (n=1). Cortisol responses to ACTH stimulation were normal whereas 17 –OHP levels were high. *CYP21A1P* (pseudogene) or *CYP21A2* (active gene) gene duplications were detected in 4 patients. Altough three of these patients had moleculer analysis results as carrier; the hormonal and clinical findings were consistent with disease. Two unrelated patients had *CYP21A2* duplication and point mutations (p.Q319X) similar to their mothers (Family 1 and 2). Interestingly, their mothers were normal clinically and had no hormonal findings. One patient carried two different (p.V282L and p.P454S) mutations in a single allel. The p. P454S was de novo in cis position according to parental analysis and her father had active gene duplication with p.Q319X mutations which wasn't inherited (Family 3). Other patient with homozygous p. V282L also had novel de novo heterozygous p.P214L mutation and *CYP21A1P* duplication (Family 4). One patient had conversion of exon 4 from mother's allel carrying heterozygous p. I173N mutation and heterozygous p.V282L mutation from father's allel (Family 5). Sequence analysis of *CYP21A2* is complicated with various micro conversion events from pseudogene. Therefore care must be taken to include overlapping PCR producers sequenced from multiple sites which are colloborated with MLPA analysis, including parenteral investigations.

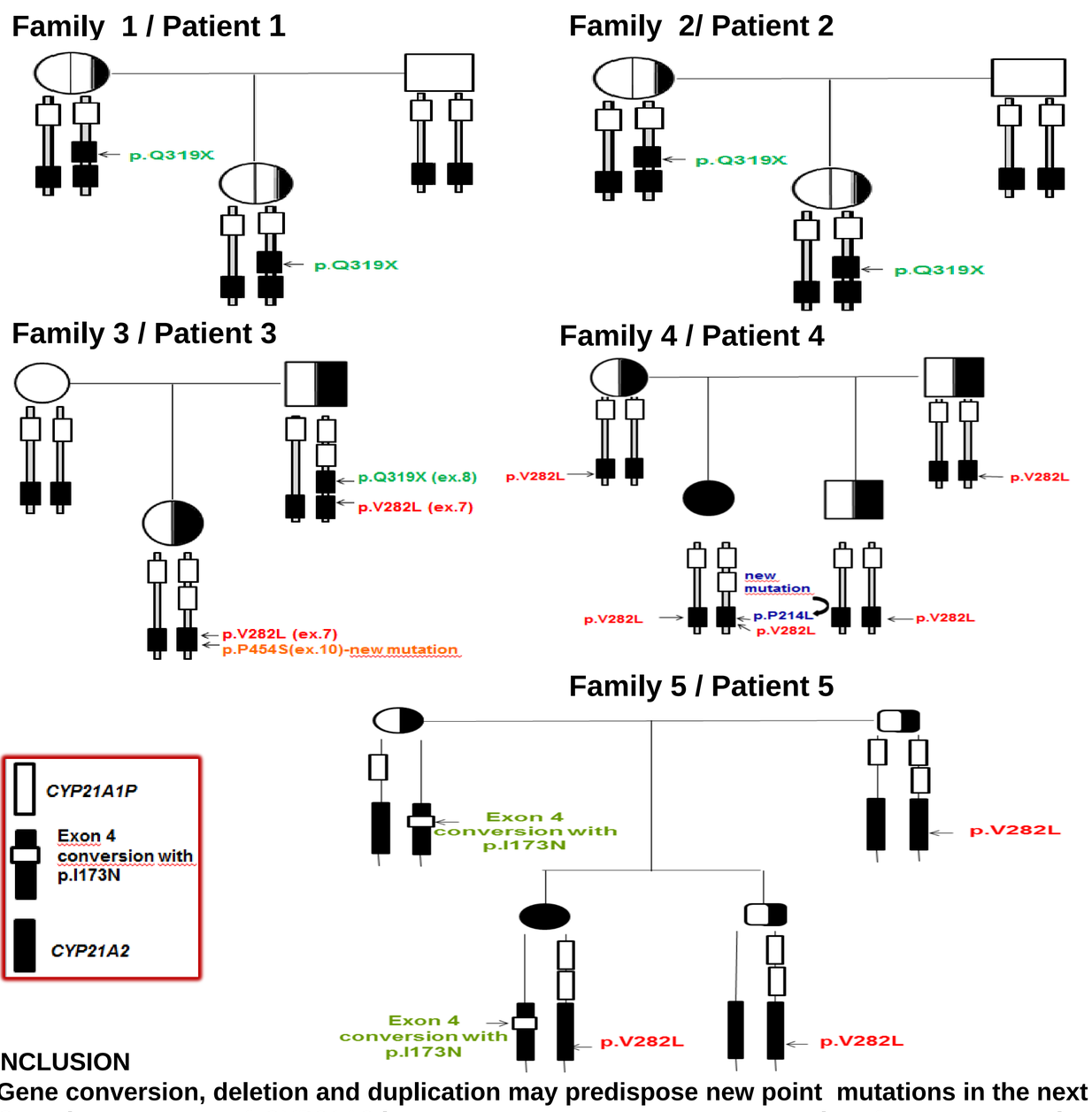
Table 1. Clinical findings of patients with NCCAH

Patient	Gender	Presentation					
		Age(yrs)	Findings	Weight SDS	Height SDS	BMI SDS	Bone age (yrs)
1 (ABU)	Female	16.5	Menstrual irregularity	-0.4	-0.3	-0.3	18
2 (CŞ)	Female	17.1	Menstrual irregularity, Hirsutism	-0.7	-1.0	0.04	
3 (ES)	Female	7.3	Precocious puberty	0.6	0.4	0.6	7.8
4 (MÖ)	Female	14.7	Hirsutism	1.9	0.4	1.7	18
5(TS)	Female	6.9	Precocious puberty	0.8	0.2	0,9	8.3
6 (DB)	Female	17	Hirsutism	0,4	-1,4	1,4	
7 (ED)	Female	8.3	Precocious puberty	-0.08	-0.4	0.2	7.8
8 (HA)	Female	13.4	Hirsutism, gaining weigth	2.4	-1.4	2.9	15
9 (SD)	Female	8.1	Premature adrenarche, gaing weight	2.5	1.7	2.0	7,8
10 (TBY)	Male	10.2	Precocious puberty	1.1	0.3	1.2	12.5
11(LB)*	Female	16.6	Hirsutism	-1.3	-2.0	0.01	17
12 (RB)*	Female	13	Hirsutism	2.1	-0.3	2.3	
13 (SG)	Female	6.6	Premature adrenarche	0.1	-0.7	0.7	7.8
14 (FK)	Female	12.6	Hirsutism	-0.3	0.08	-0.3	
15 (MÇ)	Female	8.4	Premature adrenarche Cliteromegaly	0.5	2.0	-0.6	13
16 (KA)	Female	6	Premature adrenarche, hirsutism	-1.5	-0.6	-1.9	7.8
17 (NY)	Male	5.8	Premature adrenarche	-.2.4	-0.9	-3.2	5
18 (NA)	Female	7.5	Precocious puberty	0.5	1.8	-0.5	10
19 (OC)	Female	9.8	Premature adrenarche	-0.1	-0.3	0	11
20 (ES)	Female	3.1	Premature adrenarche	5.2	3.2	4.2	5
21 (DD)	Female	7.5	Premature adrenarche	0,8	-0.4	1.3	8.5
22 (MG)	Female	11.1	Hirsutism, hair loss	0.1	-0.4	0.4	10.5
Mean±SD (Ranges)		10.3±4.2 3.1/17.1		0.6±1.6 -2.4/5.1	0.01 ±1.2 -2/3.2	0.6 ±1.6 -3.2/4.2	10.6±4.1 5/18

*Siblings

Table 2. Hormonal and molecular findings of patients with NCCAH

Patient	17-OH progesterone (ng/ml)		Cortisol (µg/dl)		Mutation	
	Basal	After ACTH	Basal	After ACTH	Allel 1	Allel 2
1	29.3	54.6	13.9	34.7	p.Q319X *CYP21A2* duplication	Wild
2	3.0	12.7	10.9	32.6	p.Q319X *CYP21A2* duplication	Wild
3	2.3	27.0	8.8	29.9	p. V282L p. P454S *CYP21A1P* duplication	Wild
4	13.6	16.4	22.7	26.0	p. V282L p. P214L (novel) *CYP21A1P* duplication	p. V282L
5	4.0	57.9	10.4	32.6	p.I173N Exon 4 conversion	p.V282L *CYP21A1P* duplication
6	5.4	34.0	13.4	22.1	p.P31L	p.V282L
7	6,0	21.2	19.1	38.7	c.293-13C/A>G	Wild
8	2.1	13.0	13.4	42.2	p.V282L	Wild
9	17.8	20.0	16.3	20.4	p.V282L	8bp del
10	11.0	60.8	12.5	17.8	p. V282L	p. V282L
11	35.1	-	22.1		p.P31Q c.293-13C/A>G	Wild
12	84.0	-	17.9		p.P31Q c.293-13C/A>G	Wild
13	1.7	9.5	10.0	33.2	8bp del	8bp del
14	9.5	16.0	13.5	25.3	p.V282L	8bp del
15	22.7	22.3	15.0	16.3	p. V282L c.293-13C/A>G	p. V282L
16	6,0	8.5	19.0	41.3	p. Q319X	p. Q319X
17	10,0	25.0	18.8	20.1	p.I173N	p. V282L
18	43.8	50.2	15.1	16.7	p. R340H p. P454S	p. R340H p. P454S
19	42.0	54.0	24.9	24.7	p.V282L	p.V282L
20	32.8	69.0	20.6	23.7	p. R340H p. P454S	p. R340H p. P454S
21	5.4	34	8.3	19.3	Large gene deletion	p. R340H
22	3.0	16.9	25.5	42.2	p. V282L	Wild
Mean± SD	17.7±20	31.1±19.4	16.0±5.0	28.0±8.7		



CONCLUSION

- **Gene conversion, deletion and duplication may predispose new point mutations in the next generations.**
- **Complex structure of *CYP21A2* locus requires that sequencing results of patients should be evaluated in conjuction with deletion / duplication analysis tests, further accompained with parental investigations.**
- **Analysis of more number of patients with parental analysis will be valuable to understand the molecular patology underlining NCCAH.**

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