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Impact of glucocorticoid receptor gene polymorphism on the metabolic profile of child patients with classical form of 21-hydroxylase deficiency

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Keywords

- Congenital adrenal hyperplasia
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- Polymorphism

Abstract

Background; Congenital adrenal hyperplasia due to 21-hydroxylase deficiency (21OHD) is a common autosomal recessive disorder caused by mutations in the CYP21A2 gene, which encodes 21-hydroxylase (an enzyme involved in aldosterone and cortisol biosynthesis), **Aim and objectives:** The aim of study was to evaluate the influence of NR3C1polymorphisms on the metabolic profile in a series of pediatric with congenital adrenal hyperplasia due to 21-hydroxylase deficiency (21OHD). **Subjects and methods**: A case control study curried out at Mansoura university children hospital in endocrinology outpatient clinic during 2019-2020. The study held on 50 children with congenital adrenal hyperplasia due to 21-hydroxylase deficiency (21OHD). **Result:** No significant differences were found between obese CAH patients versus non obese CAH patients regarding genotypes. No significant differences were found between CAH patients with MS versus CAH patients without MS regarding genotypes, **Conclusion:** Our results suggest that NR3C1 polymorphism could be involved with a susceptibility to adverse metabolic profile in pediatric CAH patients. GG genotype and G allele of rs6198 genotype have significant risk to CAH. However, rs41423247 genotype was non significantly correlated with CAH. The rs41423247 and rs6198 genotype variants and alleles were comparable between obese and non-obese CAH patients, between obese CAH patients with or without metabolic syndrome and between poor and adequate hormonal control. Our novel findings may contribute to further studies on the clinical relevance and prognostic value of assessing NR3C1 gene haplotypes towards individualized treatment for CAH patients.

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INTRODUCTION

Congenital adrenal hyperplasia due to 21-hydroxylase deficiency (21OHD) is a common autosomal recessive disorder caused by mutations in the CYP21A2 gene, which encodes 21-hydroxylase (an enzyme involved in aldosterone and cortisol biosynthesis). In individuals with the disease, ACTH levels rise due to impaired cortisol secretion, thereby stimulating the adrenal cortex, accumulating androgen precursors, and resulting in varying degrees of hyperandrogenism **(1).**

The spectrum of clinical manifestations depends on the degree of enzymatic impairment. Such impairment ranges from prenatal external genitalia virilization in females and postnatal virilization in both sexes, which may occur with or without salt loss (classical forms), to a milder form with late onset hyperandrogenic signs (nonclassical) **(2)**.

The classical forms have a prevalence of approximately one in 10,000 to one in 16,000 live births, while the nonclassical form affects approximately one in 2,500 live births **(3).**

Current CAH therapy aims to provide adequate glucocorticoid replacement and, when necessary, mineralocorticoid replacement, to avoid adrenal crisis, to suppress the increased androgen secretion (to allow the achievement of normal final height), and to avoid signs of hypercortisolism. The introduction of glucocorticoid (GC) replacement leads to significant improvement in the prognosis of classical forms **(4)**

In the general population, besides lifestyle and environmental factors, genetics variants also predispose to an adverse metabolic profile. Glucocorticoid receptor (NR3C1) gene polymorphisms are associated with increased cardiovascular risk, characterized by increased body mass index (BMI), blood pressure, and lipid levels, such as the BclI polymorphism, which is associated with increased GC sensitivity and the A3669G polymorphism linked to increased inflammatory parameters **(5)**

In addition to the variability in the prevalence of an adverse metabolic profile among CAH patients, there are few data in the literature regarding pediatric patients **(6)**

The aim of study was to evaluate the influence of NR3C1polymorphisms on the metabolic profile in a series of pediatric with congenital adrenal hyperplasia due to 21-hydroxylase deficiency (21OHD)

PATIENTS AND METHODS

A case control study carried out at Mansoura university children hospital in endocrinology outpatient clinic during 2019-2020. The study held on 50 children with congenital adrenal hyperplasia due to 21-hydroxylase deficiency (21OHD)

Inclusion criteria: Pediatric CAH patients with classical forms, Under stable glucocorticoid and mineralocorticoid therapy in the last two years, did not use enzyme inductor drugs, demonstrated good compliance, received

exclusively short-acting glucocorticoids during growth periods and normal androgen and plasma renin activity (PRA) levels in at least 3 out of 4 annual measurements.

Exclusion criteria: all patients with the following excluded: Patients without adequate hormonal control and patients refused to participate in the study.

Sample size:40 cases of CAH , and 20 normal child, age range from 2y to 18 years

Methods

Metabolic syndrome was defined according to the National Cholesterol Education Program, Adult Treatment Panel III criteria (NCEP ATPIII), adapted to the pediatric group **(7).**

For prepubertal patients, testosterone and androstenedione levels were maintained ≤14 ng/dL and 2ng/mL, respectively, and for all older patient's androstenedione ≤3.5 ng/dL and testosterone levels ≤50 ng/dL for older females.

Regarding mineralocorticoid replacement, the PRA levels of these patients were maintained in the upper normal limit. In this period, no patient presented suppressed 17-OHP or PRA levels **(8)**.

Mean daily glucocorticoid doses were calculated using body surface area (mg/m^2) and were also evaluated retrospectively in the last 2 years. The glucocorticoid doses were converted to hydrocortisone equivalents (30 mg hydrocortisone = 37.5 cortisone acetate = 0.75 dexamethasone) and presented as mg/m^2 **(9)**.

Tools:

Patients' data including the following parameters: Demographic data as weight, age, height and gender, BMI. Children were classified as obese if their BMI was ≥95th percentile, overweight if their BMI was between the 85th and 95th percentiles, and healthy weight if their BMI was between the 5th and 85th percentiles according to age-sex tables (Centers for Disease Control and Prevention), waist circumference. Abnormal waist circumference was defined as circumference >90th percentile for age and sex **(10)**.

Blood pressure, Increased systolic or diastolic blood pressure was defined as pressures >90th percentile for age and sex **(7),** Duration and onset of symptoms, systemic examination and number and type of system affection and family history including paternal consanguinity and similar conditions.

Blood tests: Lipid profile, TC, HDL-c, LDL-c and TG at a fasting state, before the subjects took their hormonal replacement therapy, increased triglyceride levels were characterized as values ≥110 mg/dL, since all patients were less than 18 years old. Abnormal HDL-c levels were characterized as values ≤40 mg/dL

Blood glucose level increased impaired glucose levels were characterized as values ≥100 mg/dL. Insulin resistance was assessed by the homeostasis model assessment for insulin resistance (HOMA-IR). Genetic analysis, PCR amplification of the glucocorticoid receptor gene regions was carried out using primer sequences and amplification conditions as previously described. The A3669G polymorphism was genotyped by sequencing. PCR products were

sequenced using the Big Dye Terminator Sequencing KitTM (Applied Biosystem, Inc., Foster City, CA, USA) and capillary electrophoresis on an ABI PRISM 3100 sequencer (Applied Biosystem, Inc.). The BclI polymorphism was screened by an allele-specific PCR as previously described. The results of the allele-specific PCR were confirmed by direct sequencing **(11)**.

Ethical consideration: Study protocol submitted for IRP approval (Institutional research Board). Informed consent will be obtained from the legal guardians of all children enrolled in the study.

Confidentiality and personal privacy will be respected in all levels of the study. Data will not be used for any other purposes.

Statistical analysis: The (Statistical Package for the Social Sciences, version 20.0, SPSS Inc, Chicago, III, USA) (SPSS 20) was used used for data analysis. Quantitative data will be presented as mean ± standard deviation. Quantitative variables will be compared by the student t test. Qualitative data will be presented as frequency and percentage and comparison between qualitative data will be done by chi-square test. Differences will be considered significant if P values are less than 0.05 and highly significant if ≤0.001. Other appropriate statistical tests will be used when needed.

RESULTS

This sample of individuals was selected randomly from population in Dakahlia Governorate in Delta, Lower Egypt. Applying Hardy Weinberg equation revealed that rs6198 genotypes and rs41423247 genotypes in two groups (CAH children and normal children)are in HW equilibrium (HWE). Frequency of two genes among studied groups reported in the next figure. Table (2)

No significant differences were found between obese CAH patients versus non obese CAH patients.regarding genotypes. Table (3)

No significant differences were found between CAH patients with MS versus CAH patients without MS regarding genotypes. Table (4)

No significant differences were found between in poor control CAH patients versus adequate control CAH patients regarding genotypes. Table (5)

No significant differences were found between in poor control CAH patients versus adequate control CAH patients regarding genotypes and alleles. Table (6)

There was significant elevation of BMI z score and LDL in AG+GG genotype group compared to AA genotype group. Otherwise no other significant could be detected. Table (7)

BMI z score, appendicular fat mass and FM/FFM, were significantly elevated in CG+GG genotype group compared to CC genotype group. Otherwise no other significant could be detected. Table (8)

Table (1).Genetic features of studied SNPs according to National Center for Biotechnology Information (NCBI).

SNP ID	**rs6198**
Alleles	A/G
Ancestral Allele:	A
Cytogenetic location	5q31.3
Gene	NR3C1 gene encode the human glucocorticoid receptor (hGR)
Nucleotide change	**A to G substitution at nucleotide 3669 located in the 3' end of the exon**

Mann-whitney tests, Chi-Square test*, independent sample T test**

NR3C1 (Nuclear receptor subfamily 3, group C, member 1)

Table (2): Assessment of Hardy Weinberg equilibrium for studied genes

		Control (n=20)		**CAH patients (n=40)**	
Frequency		**Observed**	**Expected**	**Observed**	**Expected**
rs6198	**AA**	7	7.8	5	7.2
	AG	11	9.4	24	19.6
	GG	2	2.8	11	13.2
	P (HW)	0.438		0.149	

HW, Hardy Weinberg.**Mann-whitney tests, Chi-Square test*, independent sample T test****

This sample of individuals was selected randomly from population in Dakahlia Governorate in Delta, Lower Egypt. Applying Hardy Weinberg equation revealed that rs6198 genotypes and rs41423247 genotypes in two groups are in HW equilibrium (HWE). Frequency of two genes among studied groups reported in the next figure.

odds ratio

**significant (P value < 0.05)

Table (3): Distribution of rs41423247 genotype variants and alleles in CAH patients with MS versus CAH patients without MS

		CAH without MS patients (n=19)	**CAH with MS patients (n=21)**	**Relative risk of MS in CAH**			
				OR	**95%CI**		**P**
CC	**Count**	7	10	**1**	**-**	**-**	**R**
	%	36.8%	47.6%				
CG	**Count**	11	11	0.700	0.195	2.150	0.584
	%	57.9%	52.4%				
GG	**Count**	1	0	0.238	0.008	6.685	0.399
	%	5.3%	0.0%				
CG+GG	**Count**	12	11	0.641	0.181	2.275	0.
	%	63.2%	52.4%				
C	**Count**	25	31	0.682	0.261	1.782	0.435
	%	65.8%	73.8%				
G	**Count**	13	11				
	%	34.2%	26.2%				

odds ratio

Mann-whitney tests, Chi-Square test*, independent sample T test**

*significant (P value < 0.05)

Table (4): Distribution of rs6198 genotype variants and alleles in poor control CAH patients versus adequate control CAH patients.

		Adequate control CAH patients (n=21)	Poor control CAH patients (n=19)	Relative risk of poor control in CAH			
				OR	95%CI		P
AA	**Count**	3	2	**1**	**-**	**-**	**R**
	%	14.3%	10.5%				
AG	**Count**	14	10	1.071	0.150	7.642	0.945
	%	66.7%	52.6%				
GG	**Count**	4	7	2.625	0.299	22.99	0.383
	%	19.0%	36.8%				
AG+GG	**Count**	18	17	1.416	0.210	9.548	0.720
	%	85.7%	89.5%				
A	**Count**	20	14	1.55	0.636	3.814	0.331
	%	47.6%	36.8%				
G	**Count**	22	24				
	%	52.4%	63.2%				

odds ratio **Mann-whitney tests, Chi-Square test*, independent sample T test****

**significant (P value < 0.05)

Table (): Distribution of rs6198 genotype variants and alleles in CAH patients versus control. GG genotype and G allele have significant risk to CAH. Otherwise no other significant differences were found between CAH patients versus control regarding genotypes.

Table (5): Distribution of rs41423247 genotype variants and alleles in poor control CAH patients versus adequate control CAH patients.

		Adequate control CAH patients (n=21)	Poor control CAH patients (n=19)	Relative risk of poor control in CAH			
				OR	95%CI		P
CC	**Count**	11	6	**1**	**-**	**-**	**R**
	%	52.4%	31.6%				
CG	**Count**	9	13	2.648	0.715	9.798	0.144
	%	42.9%	68.4%				
GG	**Count**	1	0	0.589	0.020	16.67	0.756
	%	4.8%	0.0%				
CG+GG	**Count**	10	13	2.383	0.654	8.67	0.187
	%	47.6%	68.4%				
C	**Count**	31	25	1.465	0.560	3.828	0.435
	%	73.8%	65.8%				
G	**Count**	11	13				
	%	26.2%	34.2%				

odds ratio

significant (P value < 0.05) Mann-whitney tests, Chi-Square test*, independent sample T test****

Table (6): Comparison of clinical and biochemical data among data among studied CAH cases as regard rs6198 genotype variants.

Parameter		AA genotype (n=5)	AG+GG genotypes (n=35)	P value
Gender*	Male	1 (20.0%)	14 (40.0%)	0.633
	Female	4 (80.0%)	21 (60.0%)	
Age (years)**	Mean ± SD	7.1 ± 4.3	9.0 ±3.6	0.394
Age of diagnosis (month)	Median (IQR)	4.0 (0.8-36.0)	1.0 (0.03-6.0)	0.279
HTN (positive)*		0 (0.0%)	3 (8.6%)	1.00
SBP	Median (IQR)	90.0 (80-127.5)	90.0 (90-120)	0.605
DBP	Median (IQR)	60.0 (45.0-5.0)	60.0 (60.0-80.0)	0.578
Hydrocortisone dose	Median (IQR)	18.9 (9.1-27.3)	23.4 (15.0-30.0)	0.337
Hydrocortisone *	Physiological dose	1 (20.0%)	9 (25.7%)	1.00
	Supraphysiological dose	4 (80.0%)	26 (74.3%)	
Fludrocortisone (received in)*		3 (60.0%)	20 (57.1)	1.00
17 alpha OH progesterone	Median (IQR)	9.0 (1.9-10.5)	9.7 (6.0-10.0)	0.691
Level of disease control	Adequate control	3 (60.0%)	18 (51.4%)	1.00
	Poor control	2 (40.0%)	17 (48.6%)	
Height Z	Median (IQR)	-2.6 (-3.1/1.9)	-0.58 (-2.0/1.5)	0.498
BMI Z	Median (IQR)	-1.8 (-4.5/-0.05)	1.8 (0.4/2.3)	**0.001**
Waist C (cm)	Median (IQR)	55.0 (37.5-68.7)	60.0 (55.0-80.0)	0.103
Fat %	Median (IQR)	19.0 (10.5-34.6)	22.7 (15.8-39.0)	0.605
FM (Kg)	Median (IQR)	5.9 (2.3-12.4)	11.4 (3.0-15.5)	0.524
FFM (Kg)**	Mean ± SD	18.5 ± 8.5	25.1 ±10.6	0.171
Trunk FM (Kg)	Median (IQR)	1.3 (0.2-4.9)	1.8 (1.0-6.4)	0.244
Appendicular FM (Kg)	Median (IQR)	1.6 (0.5-6.9)	2.5 (1.8-8.4)	0.157
Trunk FM/appendicular FM	Median (IQR)	0.66 (0.41-0.78)	0.72 (0.50-0.85)	0.551
FM/FFM	Median (IQR)	0.011 (0.005-0.04)	0.014 (0.009-0.02)	0.578
Cholesterol**	Mean ± SD	112.8 ± 25.0	136.1 ± 25.1	0.106
TG**	Mean ± SD	66.2 ± 30.4	90.7 ± 42.8	0.233
HDL**	Mean ± SD	23.6 ± 6.2	28.3 ± 10.7	0.349
LDL**	Mean ± SD	66.3 ± 31.2	105.4 ± 24.8	**0.049**
Insulin	Median (IQR)	14.3 (4.1-16.3)	15.9 (9.4-21.1)	0.157
HOMA-IR	Median (IQR)	3.1 (0.83-4.2)	4.3 (2.0-5.9)	0.183
Insulin resistance (positive cases)	Count (%)	3 (60.0%)	23 (65.7%)	1.00
Metabolic syndrome (positive cases)	Count (%)	2 (40.0%)	19 (54.3%)	0.654

Mann-whitney tests, Chi-Square test*, independent sample T test P,** between 2 groups

**significant (P value < 0.05)

**significant (P value < 0.05)

Table () reported that, There was significant elevation of BMI z score and LDL in AG+GG genotype group compared to AA genotype group. Otherwise no other significant could be detected.

Table (7): Comparison of clinical and biochemical data among data among studied CAH cases as regard rs41423247 genotype variants.

Parameter		CC genotype (n=17)	CG+GG genotypes (n=23)	P value
Gender*	Male	7 (41.2%)	8 (34.8%)	0.680
	Female	10 (58.8%)	15 (65.2%)	
Age (years)**	Mean ± SD	7.5 ±3.0	9.7 ±3.9	0.055
Age of diagnosis (month)	Median (IQR)	1.3 (0.03-13.5)	1.6 (0.3-18.0)	0.085
HTN (positive)*		0 (0.0%)	3 (13.0%)	0.248
SBP	Median (IQR)	90.0 (90-120)	100.0 (90-120)	0.498
DBP	Median (IQR)	60.0 (60-80)	70.0 (60-80)	0.277
Hydrocortisone dose	Median (IQR)	23.4 (15.0-30.0)	26.4 (15.7-30.0)	0.978
Hydrocortisone *	Physiological dose	4 (23.5%)	6 (26.1%)	1.00
	Supraphysiological dose	13 (76.5%)	17 (73.9%)	
Fludrocortisone (received in)*		12 (70.6%)	11 (47.8%)	0.150
17 alpha OH progesterone	Median (IQR)	9.0 (2.9-10.0)	10.0 (6.0-11.0)	0.386
Level of disease control	Adequate control	11 (64.7%)	10 (43.5%)	0.216
	Poor control	6 (35.3%)	13 (56.5%)	
Height Z	Median (IQR)	-0.34 (-1.6/0.84)	-1.7 (-2.6/1.96)	0.588
BMI Z	Median (IQR)	0.73 (-1.14/1.98)	1.85 (0.95/2.38)	**0.037**
Waist C (cm)	Median (IQR)	57.5 (52.5-67.7)	67.5 (57.5-80.0)	0.051
Fat %	Median (IQR)	18.3 (15.4-31.1)	24.2 (15.8-39.8)	0.277
FM (Kg)	Median (IQR)	5.3 (2.5-14.1)	11.6 (3.1-25.5)	0.221
FFM (Kg)**	Mean ± SD	23.0 ± 9.7	25.2 ± 11.1	0.499
Trunk FM (Kg)	Median (IQR)	1.3 (0.9-4.0)	3.7 (1.3-10.0)	0.075
Appendicular FM (Kg)	Median (IQR)	1.8 (1.3-5.3)	5.8 (1.8-13.0)	**0.032**
Trunk FM/appendicular FM	Median (IQR)	0.66 (0.55-0.81)	0.72 (0.44-0.85)	0.935
FM/FFM	Median (IQR)	0.01 (0.009-0.01)	0.02 (0.01-0.03)	**0.045**
Cholesterol**	Mean ± SD	132.6 ± 30.4	133.6 ± 22.9	0.910
TG**	Mean ± SD	79.0 ± 33.1	94.0 ± 52.2	0.272
HDL**	Mean ± SD	30.1 ± 10.4	25.9 ± 10.1	0.217
LDL**	Mean ± SD	101.1 ± 27.9	100.2 ± 29.6	0.922
Insulin	Median (IQR)	15.3 (8.6-20.2)	16.9 (11.0-21.1)	0.448
HOMA-IR	Median (IQR)	3.7 (1.6-5.9)	4.5 (2.2-5.4)	0.570
Insulin resistance (positive cases)	Count (%)	12 (70.6%)	14 (60.9%)	0.524
Metabolic syndrome (positive cases)	Count (%)	10 (58.8%)	11 (47.8%)	0.491

Mann-whitney tests, Chi-Square test*, independent sample T test P,** between 2 groups
**significant (P value < 0.05)

DISCUSSION

This cross-sectional observational study was conducted in Mansoura university children hospital in endocrinology outpatient clinic from 2019 to 2020. This study was conducted on 50 children with congenital adrenal hyperplasia due to 21-hydroxylase deficiency (21OHD).

In the current study the sample was of individuals randomly selected from population in Dakahlia Governorate in Delta, Lower Egypt.

Applying Hardy Weinberg equation revealed that rs6198 genotypes and rs41423247 genotypes in two groups are in HW equilibrium (HWE).

In agreement with the current study in a Brazilian cohort **Villela et al., (12)** reported that the distribution of genotypes for each NR3C1 SNP showed no deviation from Hardy-Weinberg equilibrium.

Also, the study by **Moreira et al., (13)** reported that Allelic frequencies of BclI and A3669G NR3C1 polymorphisms were in Hardy-Weinberg equilibrium.

In addition, **Moreira et al., (14)** found that Allelic frequencies of the BclI and A3669G polymorphisms were in HardyWeinberg equilibrium.

Distribution of rs6198 genotype variants and alleles in CAH patients versus control. GG genotype and G allele have significant risk to CAH. Otherwise, no other significant differences were found between CAH patients versus control regarding genotypes. Also, Distribution of rs41423247 (BclI) genotype variants and alleles in CAH patients versus control. No significant differences were found between CAH patients versus control regarding genotypes and alleles

However, the study by **Villela et al., (12)** found no significant differences in allele frequencies at any SNP when the 21-OHD patients and healthy subjects (controls) were compared ($P > 0.05$). Conversely, heterozygous subjects for the BclI SNP (CG) were more frequent in controls ($p = 0.049$). they also found a reduced frequency of BclI variant and BclI haplotype in 21-OHD patients as compared to controls.

Regarding the distribution of rs6198 genotype variants and alleles in obese CAH patients versus non obese CAH patients, we found that no significant differences were found between obese CAH patients versus non obese CAH patients. Similarly, we found no significant differences between obese CAH patients versus non obese CAH patients regarding rs41423247 genotypes and alleles.

In agreement with our results **Moreira et al., (13)** reported that there was no significant difference in the frequency of the A3669G (rs6198) polymorphism between the obese and non-obese CAH patients, 12.5% vs. 19.2%, respectively. However, they reported that the heterozygous BclI carriers showed higher body mass index, waist circumference and higher systolic BP as compared to wild-type subjects.

Also, in harmony with our results **Moreira et al., (14)** reported that There was no significant difference in the frequency of the A3669G polymorphism between the obese and nonobese CAH patients, 42.8% versus 29.4%, respectively. There was no significant difference in the frequency of the BclI polymorphism between the obese and nonobese CAH patients, 33.3% versus 30.4%, respectively.

Literature showed that BclI (rs41423247) is the most frequent and commonly studied NR3C1 variant, it is a specific fragment of DNA located between exon 2 and 3 that is removed by an

endonuclease-restriction enzyme, it has been associated with increased body fat in adults, and higher cardiovascular risk in individuals with autoimmune adrenal insufficiency **(15).**

Also, **Maneschijn et al., (16)** reported that the BclI polymorphism has been linked to increased GC sensitivity and consequently to higher BMI, waist circumference and lipid levels, compared to wild type carriers.

Regarding the distribution of rs6198 genotype variants and alleles in CAH patients with MS versus CAH patients without MS we found that there was no significant differences were found between CAH patients with MS versus CAH patients without MS regarding rs6198 genotypes. Similarly, that there was no significant differences were found between CAH patients with MS versus CAH patients without MS regarding rs41423247 genotypes

In agreement with our results **Moreira et al., (13)** reported that there was no significant difference in the frequency of the A3669G (rs6198) polymorphism between the patients with and without metabolic syndrome, 20% vs. 17.4%, respectively.

Also, in line with our results **Moreira et al., (14)** reported that there was no significant difference in the frequency of the A3669G polymorphism between the patients with and without metabolic syndrome, 14.3% versus 14.7%, respectively, There was no significant difference in the frequency of the BclI polymorphism between the obese and nonobese CAH patients, 33.3% versus 30.4%, respectively, between the patients with and without metabolic syndrome, 22.2% versus 8.7%, respectively, or between the patients with and without hypertension, 16.7% versus 4.3%, respectively.

Regarding the distribution of rs6198 and rs41423247 genotype variants and alleles in poor control CAH patients versus adequate control CAH patients, we found that there were no significant differences were found between in poor control CAH patients versus adequate control CAH patients regarding genotypes and alleles.

To exclude the effects of increased androgens levels, **Moreira et al., (13), and Moreira et al., (14)** selected only patients with adequate hormonal control, and interestingly, **Moreira et al., (13)** reported that the mean androgen levels over the last 2 years of therapy were inversely correlated with lower HDL-c levels in our female patients. Although these patients presented normal androgen levels, glucocorticoid therapy probably does not reproduce or allow a normal adrenal androgen secretion.

Comparison of clinical and biochemical data among data among studied CAH cases as regard rs6198 genotype variants showed that There was significant elevation of BMI z score and LDL in AG+GG genotype group compared to AA genotype group. Otherwise, no other significant could be detected.

Finally, Comparison of clinical and biochemical data among data among studied CAH cases as

regard rs41423247 genotype variants showed that BMI z score, appendicular fat mass and FM/FFM, were significantly elevated in CG+GG genotype group compared to CC genotype group. Otherwise, no other significant could be detected.

In line with our results **Moreira et al., (14)** reported that A3669G carriers had higher LDL-c levels compared to wild-type carriers in the *t*-test analysis, which maintained significance after adjustment by sex, age, and clinical form. There were no significant differences observed in the HOMA value and blood pressure between carriers and noncarriers of the A3669G polymorphism. There was no significant difference in the frequency of the A3669G polymorphism between the patients with and without hypertension, 14.3% versus 8.8%, respectively. Although BclI carriers showed a tendency to adverse metabolic profile, these differences were not statistically significant. There was no significant difference in the frequency of the BclI polymorphism between the patients with and without hypertension, 16.7% versus 4.3%, respectively.

This finding was consistent with the results of previous study by **Yan et al., (17)**, in which GG genotype was identified to be more frequent in patients with MetS.

Koeijvoets et al., (18) reported that BclI polymorphism in patients with MetS are slightly similar for G allele frequency and the same study showed that men with the BclI haplotype were associated with cardiovascular disease.

Additionally, **Yan et al., (17)** showed that only GG homozygotes had higher BMI and SBP and lower plasma glucose and triglycerides. In this study high C-peptide level among homozygous GG carriers than among C allele carriers was found only in women.

CONCLUSION

Our results suggest that NR3C1 polymorphism could be involved with a susceptibility to adverse metabolic profile in pediatric CAH patients. GG genotype and G allele of rs6198 genotype have significant risk to CAH. However, rs41423247 genotype was non significantly correlated with CAH. The rs41423247 and rs6198 genotype variants and alleles were comparable between obese and non-obese CAH patients, between obese CAH patients with or without metabolic syndrome and between poor and adequate hormonal control. Our novel findings may contribute to further studies on the clinical relevance and prognostic value of assessing NR3C1 gene haplotypes towards individualized treatment for CAH patients.

Strength and weakness

Our study found that there is at, There was significant elevation of BMI z score and LDL in AG+GG genotype group compared to AA genotype group.

One weakness is small sample size

Conflict of interest: no conflicts of interest.

REFERENCES

1. **Turcu, AF, Auchus, RJ. (2015).** Adrenal steroidogenesis and congenital adrenal hyperplasia. Endocrinology and Metabolism Clinics 44: 275-296.
2. **El-Maouche, D., Arlt, W., & Merke, D. P. (2017).** Congenital adrenal hyperplasia. The Lancet, 390(10108), 2194-2210.
3. **de Carvalho DF, Miranda MC, Gomes LG, et al (2016).** Molecular CYP21A2 diagnosis in 480 Brazilian patients with congenital adrenal hyperplasia before newborn screening introduction. European Journal of Endocrinology 175: 107-116.
4. **Porter J, Blair J, Ross RJ. (2017).** Is physiological glucocorticoid replacement important in children? Archives of Disease in Childhood 102: 199-205.
5. **Reimondo G, Chiodini I, Puglisi S, et al (2016).** Analysis of BCLI, N363S and ER22/23EK polymorphisms of the glucocorticoid receptor gene in adrenal incidentalomas. PloS one 11: e0162437.
6. **Moreira, R. P., Gomes, L. G., Mendonca, B. B., et al (2012).** Impact of glucocorticoid receptor gene polymorphisms on the metabolic profile of adult patients with the classical form of 21-hydroxylase deficiency.
7. **Dhuper S, Cohen HW, Daniel J. (2007).** Utility of the modified ATP III defined metabolic syndrome and severe obesity as predictors of insulin resistance in overweight children and adolescents: a cross-sectional study. Cardiovascular diabetology 6: 4.
8. **Auchus RJ. (2010).** Congenital adrenal hyperplasia in adults. Current Opinion in Endocrinology, Diabetes and Obesity 17: 210-216.
9. **Liddie GW. (1961).** Clinical pharmacology of the anti-inflammatory steroids. Clinical Pharmacology & Therapeutics 2: 615-635.
10. **Fernández JR, Redden DT, Pietrobelli A. (2004).** Waist circumference percentiles in nationally representative samples of African-American, European-American, and Mexican-American children and adolescents. The Journal of pediatrics 145: 439-444.
11. **Gergics P, Patocs A, Majnik J. (2006).** Detection of the Bcl I polymorphism of the glucocorticoid receptor gene by single-tube allele-specific polymerase chain reaction. The Journal of steroid biochemistry and molecular biology 100: 161-166.
12. **Villela, T. R., Barra, C. B., Belisário, A. R., et al (2021).** Glucocorticoid receptor Gene (NR3C1) Polymorphisms and Haplotypes in patients with congenital adrenal hyperplasia. Molecular and Cellular Endocrinology, 536, 111399.
13. **Moreira RPP, Gomes LG, Mendonca BB. (2012).** Impact of Glucocorticoid Receptor Gene Polymorphisms on the Metabolic Profile of Adult Patients with the Classical Form of 21-Hydroxylase Deficiency. PloS one 7: e44893.

14. **Moreira, R. P., Gomes, L. G., Madureira, G., et al (2014).** Influence of the A3669G glucocorticoid receptor gene polymorphism on the metabolic profile of pediatric patients with congenital adrenal hyperplasia. International journal of endocrinology, 2014.
15. **Geelen, C. C., Van Greevenbroek, M. M., Van Rossum, E. F., et al (2013).** 't Hart LM, Schalkwijk CG, Ferreira I, van der Kallen CJ, Sauerwein HP. Dekker JM, Stehouwer CD, Havekes B. BclI glucocorticoid receptor polymorphism is associated with greater body fatness: the Hoorn and CODAM studies. J Clin Endocrinol Metab, 98(3), E595-E599.
16. **Maneschijn, L., van den Akker, E. L. T., Lamberts, S. W. J., et al (2009).** Clinical features associated with glucocorticoid receptor polymorphisms. Ann. NY Acad. Sci, 1179, 179-198.
17. **Yan, Y. X., Dong, J., Zhang, J., et al (2014).** Polymorphisms in NR3C1 gene associated with risk of metabolic syndrome in a Chinese population. Endocrine, 47(3), 740-748.
18. **Koeijvoets, K. C., van der Net, J. B., van Rossum, E. F., et al (2008).** Two common haplotypes of the glucocorticoid receptor gene are associated with increased susceptibility to cardiovascular disease in men with familial hypercholesterolemia. The Journal of Clinical Endocrinology & Metabolism, 93(12), 4902-4908.