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Association of Glucocorticoid Receptor Gene *NR3C1* (*Tth111*I, *Bcl*i) Polymorphisms with Asthma Children in Iraq

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Abstract:
Glucocorticoids in-general are considered the most powerful and efficient treatment for asthma. The following investigation was aimed to study the effect of the glucocorticoid receptors (NR3C1) rs10052957 (*Tth111*I), rs41423247 *(BclI*) the single nucleotide polymorphisms (SNPs), on the susceptibility to asthma in children as well as assessing its effect on the response to corticosteroids (ICS). A case-control study was conducted by 200 children were diagnosed with asthma; their ages ranged from 5 to 17 years old. They subdivided into two groups: 100 patients and 100 apparently healthy as a control. The polymorphisms (*Tth111*I (rs10052957), *Bcl*I (rs41423247) were genotyped using QT PCR method. The obtained results were analyzed using SPSS, and Linkage disequilibrium analysis was performed using Haploview. A significant variance was observed between the asthmatic group and the control group regarding the *Bcl*I (rs41423247) genotype. Among the patient's group, The CC genotype was 24.0 %, and the control group was 6.0 % with P-value (0.00). The GC genotype was statistically associated with an increase in the risk of asthma. No statically significant difference was found on *Tth111*I (rs10052957) for the studied groups. The results exhibited the association of *NR3C1* gene polymorphism rs41423247 to provides risk allele for individual susceptibility to developing asthma with CC genotype while the (rs10052957) showed no association risk for exposure to developing asthma in children.
Key words*:* *asthma disease, glucocorticoid receptor, NR3C1.*

Introduction:

Asthma is a chronic disease due to a variety of factors. Many different types of cells and substances are involved in this pathogenesis. It well known that complex gene – gene and environmental interactions were induced by clinical presentation [1-4]. The gene NR3C1 is sited on chromosome 5q31- q32 with nine exons (NCBI) and has reference sequence: NM- 000176]. [4, 5] There are seven basic transcriptional variants in exon 1. The NR3C1 gene core is composed of exons 2 to 8 [6]. GCR isoforms formed by another linking (GCR alpha, GCR, GCR, GCR, and GCR-P). Moreover, only the alpha isoform is exposed active and produced by the NR3C1 gene expression product, mRNA. [7,8]. For chronic asthma, corticosteroid (ICS) is the recommended first prior therapy [9]. However, the response to ICS varies widely [10–12], reaches up to 35–40% of patients receiving ICS for 2-3 months showing no

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substantial improvement in lung function [10, 11]. Furthermore, despite frequent use of this drug, around 8-10% of patients with a chronic asthma with maintenance ICS care can stay symptomatic or at high threat with asthma attacks [13]. In addition to inadequate drug adherence, continued exposure to toxic environment, misdiagnosis, genuinely refractor steroid disease, and genetic differences can also play a role in treatment response in asthma patients[12, 14–16]. Genetic factors are thought to responsible for about 70% of the variation in ICS response. [12, 17]. For patients with respiratory diseases, identifying genes that may affect disease progression and development is important. It's useful not just for understanding progression's pathophysiology, but also for recognizing patients who may benefit from new clinical techniques and care designed to their genetic profile. This research is aimed to investigate the effect of Glucocorticoid receptors (NR3C1) *Tth111*I (rs10052957), *Bcl*I (rs41423247) single nucleotide polymorphisms (SNPs) on susceptibility to asthma in children and to assess its effect on the response to corticosteroids (ICS).

Materials and methods

The following research was conducted on asthmatic patients who had been treated in Al-Zahraa Consulting Center for Allergy and Asthma and control subjects who were recruited from Alfajer lab/ Abd- Al-Majeed Hussein private hospital and selected on a random basis. In total, 200 subjects were enrolled, including 100 asthma patients the inclusion criteria were: clinical asthma diagnosis was confirmed by a physician, history of diagnosed asthma. Treated with inhaled salbutamol according to usual care and consultation for acute asthma exacerbation. The following criteria were used to exclude patients from the study: if they were suffering from any other respiratory illness such as pulmonary disease, cystic fibrosis, bronchiectasis. In addition, cardiac insufficiency, heart disorder, cancer and genetic diseases. The asthma group comprised 52% (52) female and 48% (48) males. With a range of 5-17 years. The control healthy group involved 100 healthy subjects; 53% (53) females and 47% (47) males, with a range of 5-17 years.

Allele and genotyping the *Tth111*I (rs10052957), *Bcl*I (rs41423247) polymorphisms in NR3C1

Genome of DNA was isolated from whole blood utilizing the extraction kit of genomic DNA (ReliaPrep Blood gDNA Miniprep system, promega/USA). The two single nucleotide polymoprhisms were genotyped by using TaqMan real time PCR quantification (Smart cycler real time- PCR , Cephied,USA). The primer used was design for the current study to amplify the SNPS in NR3C1 rs4143247 G>C were F′ (5′-TTGCACCATGTTGACACCAA-3′) and R′ (5 ′ -GCAGAAGTACTAAAGAGCCC -3 ′) The labeled probes used for genotyping detetion 6- FAM (6-carboxyfluorescein) (5 AGAGATTGATCAGCAGACATAAC-3) and VIC (4,7,29-trichloro-79- phenyl-6-carboxyfluorescein) (5 ′ -AGATTCATCAGCAGACATAACTTG-3′).The primers used for rs rs10052957 G>A , the forward and reverse primers were F′ (5 ′-GCAGAGGTGGAAATGAAGGTG-3 ′) and R′(5 ′-TGTTGGGTGCCTGCTATGTA-3 ′), the probes used for detection the

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SNP : (FAM-probe 5 ′ -ACTCAGTCAAGGCAAGGACC-3′)and (VIC-probe5′ CAATCAAGGAAGGACCTGATC-3 ′).

Statistical Analysis

SPSS for Windows, version 22 was used to analyze the data (SPSS Inc. , Illinois, USA). The Chi-square test (χ^2) was used to compare proportions. For continuous variables, mean ± standard deviation (SD) were used. The haploview tool was used for the linkage disequilibrium between the two SNP sites was studied, and a haploblock between rs41423247 and rs10052957 was discovered. The haplotype distribution of the haploblock was then determined using Pearson's chi-square test.

Results and discussion

NR3C1 Gene SNP at 1184+646 position (rs41423247) was presented with three genotypes (GG, GC and CC) that were corresponding to two alleles (*G* and *C*). Hardy-Weinberg Equilibrium (HWE) analysis demonstrated a significant difference between obtained and anticipated frequencies of genotype in the patient groups with P-valuev0.05, but no significant difference was founded between observed and expected genotype frequencies in the control group (P-value = 0.01). Among these genotypes and alleles, as listed in table 1, it observed that the homozygous of the mutant allele (CC), has a significantly increased frequency percentage of patients in comparison with control group (24% *vs*. 6%) (P-value 0.00) and associated OR was 0.20 (95% CI: 0.07-0.52). Also it was observed that *C* allele possessed a significant increase the frequency in patients (41.5% *vs.* 16.5%; OR= 0.27; 95% C.I: 0.17-0.44) (p=0.00).

In the state of homozygosity of the wild type allele (GG), there was a decrease with significant difference observed between patients and control groups 41%*vs* 73%) (p=0.00) and the association was OR =3.89 (95% CI: 2.14 -7.05), the *G* allele showed a significant difference between patient and control groups respectively (58.5% *vs.* 83.5%; OR=3.59; 95% C.I: 2.24-5.72) p= (0.000). The heterozygous (GC) had a significant difference between two groups (35% *vs.* 21%; OR=0.49 (95% CI: 0.26-0.93) P= 0.03).

Table1: Genotype and allele frequencies of rs41423247 on NR3C1 gene for control and patients groups.

Genotype / Allele	Control group (n=100)	Patients group (n=100)	OR	95% CI	*P* value
	no. (%)	no. (%)			
GG	73 (73.00)	41 (41.00)	3.89	(2.14-7.05)	0.00
GC	21 (21.00)	35 (35.00)	0.49	(0.26-0.93)	0.03
CC	6 (6.00)	24 (24.00)	0.20	(0.07-0.52)	0.00
G	167(83.50)	117 (58.50)	3.59	(2.24-5.72)	0.00

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C	33 (16.50)	83 (41.50)	0.27	(0.17-0.44)	0.00

OR, odd ratio; CI, confidence interval.

The polymorphisms in the NR3C1 gene can inhibit the creation of GR/GCs complexes, diminish transcription, and affect the trans repression of the synthesized proteins genes encoding in the form of cellular responses to GCs. It can also lower GR expression, resulting in a reduced response to GCs and GCR impairment [18]. As documented by Kostik *et al*., glucocorticoid resistance is the major obstacle in many inflammatory diseases, causing clinical management difficult [19]. Our findings are consistent with those of Pietras *et al*., who examined a group of Polish patients with severe asthma and found significant differences between the genotypes investigated, with a significant increase in the CC genotype among severe asthmatic patients in comparison to controls. [20].

In addition, the findings of this study are consistent with those of Panek *et al*., who found a lower frequency of the 646 C>G homozygote (GG) and a higher frequency of the homozygote (CC) in asthmatic patients group in comparison with controls [21]. The NR3C1 Gene SNP at -13-6284 position (rs10052957) was presented with three genotypes (GG, GA and AA) that were corresponding to two alleles (*G* and *A*) in two investigated groups. There was no significant alteration between detected and expected genotype frequencies in controls (P-value= 0.27) and patients (P-value=0.84) groups. There was significant difference in distribution of homozygous wild type (GG) between patients and controls (18.0 % *vs* 33.0 %; OR=2.24 ; 95% C.I (1.16 -4.33) P = 0.02). In addition, no significant difference was detected in the mutant allele (AA) in comparison with two groups (32.0% vs 23.0%; p=0.15; OR=0.63; 95% C.I: 0.33-1.18). Comparing patients to control revealed that *G* and *A* allele frequency were significant differences, the *G* allele decrease in patients compared to control (43.0% *vs.* 55.0%; P=0.02; OR=0.1.62; 95% C.I (1.09-2.40), P=0.02) and the *A* allele increase in patients compared to control group (57.0% *vs.* 45.0%; P=0.02; OR= 0.61; 95% C.I: 0.41-0.91). The heterozygous genotype has non significant difference between two groups (50.0% *vs.* 33.0%; P= 0.39; OR= 0.78; C.I (0.45-1.37), P=0.02) as mentioned in table 2.

Table 2 : Genotype and allele frequencies of rs10052957 on NR3C1 gene for control patients groups.

Genotype / Allele	Control group (n=100)	Patients group (n=100)	OR	95% CI	*P* value
	no. (%)	no. (%)			
GG	33 (33.00)	18 (18.00)	2.24	(1.16-4.33)	0.02
GA	44 (44.00)	50 (50.00)	0.78	(0.45-1.37)	0.39
AA	23 (23.00)	32 (32.00)	0.63	(0.33-1.18)	0.15
G	110 (55.00)	86 (43.00)	1.62	(1.09-2.40)	0.02

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A	90 (45.00)	114 (57.00)	0.61	(0.41-0.91)	0.02

OR, odd ratio; CI, confidence interval.

The function of NR3C1 gene polymorphisms in the pathogenesis of obstructive syndromes is a multifactorial interpretation of the role of SNPs in the aetiology of pulmonary diseases. according to this approach on asthma, the analyzed Single nucleotide polymorphism *Tth111*I (rs10052957) were found to have non-significant differences. The Tth*111*I polymorphism is detected in an intron nearby the initiation site, 3807 pb above the first site in exon 2 where transcription starts. It induces a G>A substitution in the promoter region and is associated to the ER22/23EK SNP in the NR3C1 gene's located on exon 2 [22]. Single nucleotide polymorphisms in the NR3C1 gene domain can influence structural changes in the A/B region of the GCR and functional changes within the AF1 functional domain, which influences GCR activity and allows interaction with a variety of transcriptional factors when combined with ER22/23EK. [23].

Haplotyping of NR3C1 gene in asthmatic patients

The haplotypes were accomplished using two *NR3C1* SNPs (rs10052957+rs41423247) with the patients and controls. There was found four different haplotypes (AG, GG, AC, GC). The *GG* haplotype recorded a significant decrease allele frequency in asthma patients compared to controls (24.2% *vs*. 46.3%; P= 0.000). The odd ratio was strong associated OR= 2.7 (95% C.I.: 1.76-4.13) , preventive fraction (PF) value was 28.9%, therefore, protective effect against asthma development was suggested. Furthermore, *AC* haplotype observed a significant increase in frequency between the patients and control groups (23.2% *vs.* 7.3%, P=0.000), the OR corresponding haplotype frequency was (OR=0.27), the etiological factor (16.8%) . In *GC* haplotype, has shown a significant increase in asthma group compared with control (18.8% *vs.* 8.7%,P=0.006), the odd ratio was 0.42 (95% CI (0.23-0.77) the associated etiological fraction value was 11.0%. It was probably to suggest that *AC and GC* haplotypes is a predisposing for risk haplotype in asthmatic patients as indicated in Table 3. There was non-significant difference in AG haplotype among the investigated group (37.7% vs. 33.8%, OR=0.86, 95%CI (0.57-1.29), PF=5.3, P=0.53).

Table 3 : Association study with haplotype consisting alleles of *rs*10052957 and *rs*41423247 in *NR3C1 gene* in both control Asthmatic patients groups

rs10052957 + rs 41423247 Haplotype	Estimated Haplotypes				PF	*EF*	*P*	Odds Ratio	95% C.I.
	Control (100) (200 Chromosomes)		Patients (100) (200 Chromosomes)						
	No.	%	No.	%					
AG	75	37.7	68	33.8	5.3	------	0.53	0.86	0.57 -

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									1.29
GG	92	46.3	48	24.2	28.9	------	0.000	2.7	1.76 – 4.13
AC	15	7.3	46	23.2	-------	16.8	0.000	0.27	1.15 – 0.50
GC	18	8.7	38	18.8	------	11.0	0.006	0.42	0.23 – 0.77

EF: Etiological fraction; PF: Preventive fraction; *p*: Two-tailed Fisher's exact probability; C.I.: Confidence interval, Significant at 0.05 level.

The result demonstrated the associations of NR3C1 haplotypes. The D´ value for the paris of SNPs NR3C1 were not in linkage disequilibrium LD (D´=0.032; r^2 =0.001; LOD=0.02) as shown in Figure (1)

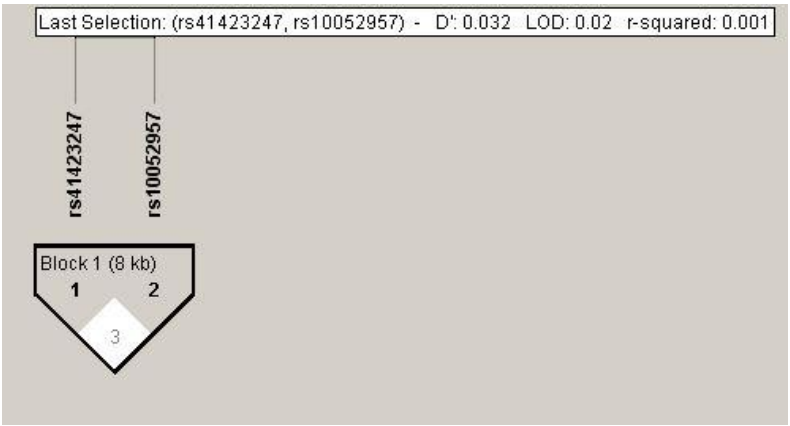

Figure (1): Linkage disequilibrium (LD) analysis between the two SNPs rs10052957 (gene promoter) and rs41423247 (Intron 2) in NR3C1 among asthmatic patients. White color block corresponding to no LD (LOD < 2, D' < 1).

Our findings showed no relation between GR polymorphisms and asthma, which consistent with the results of several other reports that have looked into the possibility of a correlation between GR SNP and corticosteroid response. In a group of pediatric asthma patients, researchers found no correlation between the GR polymorphisms *Tth111*I (10052957) and *Bcl*I (rs41423247) and the dose of ICS needed to achieve asthma control [24-26]. Furthermore, two other studies, one an association study and the other a mutational analysis, reported that NR3C1gene does not play an important role in corticosteroid resistance [27]. When a comparison was made between a control and asthmatic groups, the frequencies of the *Tth111*I, polymorphisms of the NR3C1 gene were not statistically different.

In conclusion the association of *NR3C1* gene polymorphism *Bcl*I (rs41423247) provides risk allele for individual susceptibility to developing asthma with CC genotype while the *Tth111*I (rs10052957) was no associated with risk for children susceptibility to developing asthma in children.

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