Original article

The effect of BclI polymorphism of NR3C1 gene on asthma phenotypes in Egyptian children

Background: *BclI is the promoter polymorphism observed within human glucocorticoid receptor gene (hGR/NR3C1) which plays an important role in the development of bronchial asthma (BA) and resistance to Glucocorticosteroids (GCs) in the severe BA.* ***Objective:*** *To assess the influence of BclI gene (rs41423247) polymorphisms on phenotypic expression of bronchial asthma in a group of Egyptian asthmatic children.* ***Methods:*** *This case control study included 135 asthmatic children with varying degrees of asthma severity. They were recruited from Allergy and Pulmonology Outpatient Clinic, Cairo University. Ninety healthy age and sex matched children served as the control group. Determination of BclI single nucleotide polymorphism (SNP) was done by polymerase chain reaction restriction fragment length polymorphism (PCR- RFLP).* ***Results:*** *Our results revealed that the variants of BclI polymorphism: CC/CG/GG was found with frequency 73.3%, 26.7%, 0% in control group. While in asthmatic children, their frequency was 42.2%, 51.1%, 6.7%, respectively. This revealed a significant difference in distribution between cases and control, similarly there was a significant difference in frequency of allele G between both groups (P-value <0.001). The frequency of allele G/C showed statistically significance association with increased severity of bronchial asthma (P-value<0.001), with uncontrolled asthma and hospitalization (P value <0.001).* ***Conclusion:*** *The Bcl I polymorphism of hGR/NR3C1 gene is significantly associated with bronchial asthma. The GG phenotype is significantly associated with increased susceptibility to the development of severe asthma and uncontrolled asthma symptoms, with increased risk of hospitalization.*

Keywords: Bronchial asthma (BA), Glucocorticoids (GCs), Glucocorticoid receptor (GR).

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INTRODUCTION

Bronchial asthma (BA) is a chronic inflammatory disease of the airways characterized by variable and recurring symptoms, reversible airflow obstruction and bronchospasm. It has increasing incidence and prevalence worldwide.[1]

Bronchial asthma is determined by many environmental and genetic factors, the clinical picture occurs as a result of complex interactions among genes, and the mutual influence of a genotype and environment upon each other.[2,3]

Glucocorticoids (GCs) constitute the basic group of medication used to control inflammatory condition in patient with bronchial asthma; they regulate expression of specific genes within the cell nuclei via the Glucocorticoids \ Glucocorticoid receptor complex (GC\GR).[4]

It has been estimated that approximately 100 genes are involved in the pathogenesis of asthma, NR3C1 gene (nuclear receptor subfamily 3, group C, member 1) is one of them, it encodes glucocorticoid receptor (GR) h-GR/NR3C1 which is localized on chromosome 5q31-q32 and consists of nine exons.[5] The molecular mechanism of action of GCs involves binding of the specific ligand/glucocorticoid receptor to the sequences of regulator genes encoding the synthesis of anti-inflammatory proteins determining the clinical effects of GCs.[6] About 2571 polymorphisms of this gene are known, but the most common is the Bcl1 gene polymorphism of GR.[7]

BclI (rs41423247) is formed as a result of changes in a single base. A C/G single nucleotide polymorphism (SNP) within the h-GR/NR3C1 gene promoter has been localized in the intron 647 bp away from the exon/intron binding site.[8,9] BclI polymorphism (C/G) within h-GR/NR3C1 gene promoter demonstrates correlations with sensitivity to steroids, and may play an important role in the development of BA and resistance to GCs in severe bronchial asthma.[10,11]

Therefore, in the present study we aimed at assessing the influence of BclI gene polymorphisms on phenotypic expression of bronchial asthma in a group of Egyptian asthmatic children, using polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP).

METHODS

Study design

This controlled cross-sectional study was carried out on 135 asthmatic children, all were recruited from the outpatient Allergy Clinic of the Allergy and Pulmonology Unit of the Specialized Children Hospital, Cairo University, along with 90 ages and sex matched healthy children as control with no history of atopic diseases, no chronic upper or lower airway diseases or history of 1st degree relative of BA.

Ethical considerations

The study objectives were explained to the patient's parents before data collection and informed consent was obtained. Study protocol was approved from the Ethical Committee at the Faculty of Medicine, Cairo University and is in accordance with the current version of the Helsinki Declaration.

Methods

Asthma diagnosis was established according to GINA, 2018 based on clinical asthma symptoms and lung function test. The degree of asthma severity, the level of control and the severity of asthma exacerbations was determined on the basis of GINA guidelines.[1] The study included children above 6 years with varying degree of asthma severity; persistent (mild, moderate, severe), and were adherent to controller inhaled corticosteroids (ICS) (as verified by routine follow up in the outpatient Allergy clinic). Exclusion criteria were asthmatic children less than 6 years (since lung function measurements are considered to be unreliable in children < 6 years), patient using drugs which might induce resistance to glucocorticoids as rifampicin, ephedrine, phenobarbital and phenytoin or suffering from other chronic lung disease.

Sample collection and processing

For analyzing BclI promoter polymorphism using PCR-RFLP technique, two ml venous blood samples were collected from all study participants. DNA extracted using spin column (G-spin™ Total DNA Extraction Kit, Boca Scientific, Florida, USA).

BclI polymorphism by PCR-RFLP

Amplification of DNA segments for Bcl I SNP of h-GR/ NR3C1 was conducted by PCR using a forward primer 5`- GAG AAA TTC ACC CCT ACC AAC-3` and a reverse primer 5`- AGA GCC CTA TTC AAA CTG-3` .

PCR reactions were carried out in a final volume of 25 ul using12.5 µL MyTaq™ Red Mix (Bioline, London UK) and 1µL Primers (20µM each) and finally water is added .

The PCR reaction tubes were placed in the thermal cycler (GeneAmp PCR system 9700, Germany). Primers Sequence and PCR cycling conditions are demonstrated in table in appendix. Amplification yielded a 418 bp amplicon which was further digested using 1ul of the restriction enzyme Fast Digest BclI (Thermo Scientific™ catalog number: ER0721) according to the manufacturing recommendations. Digested products were visualized using 2% agarose gel electrophoresis and allele assignments were homozygous G/G allele, for the undigested 418 bp DNA fragment as for the homozygous C/C allele it contains a recognition site for the BclI restriction enzyme, so the digestion yields two DNA fragments 263 and 151 bp while heterozygous C/G allele, yields three DNA fragments 418, 263 and 151 bp. As shown in figure 1.

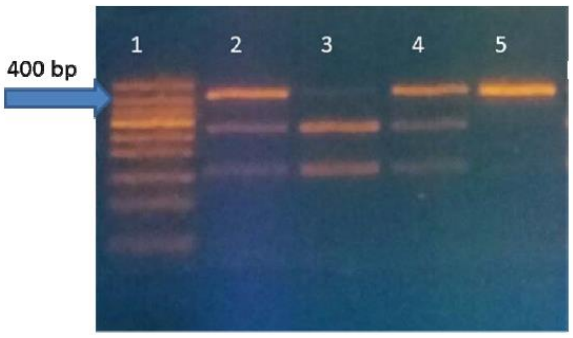

Figure 1. Agarose gel electrophoresis of BclI digestion products of NR3C1 at position 646
Lane 1: DNA marker (50 bp)
Lane 2 and 4 represent heterozygous (CG) genotype, 3 bands=418,263,155 bp
Lane 3 shows homozygous (CC) genotype, 2 bands= 263, 155 bp
Lane 5 shows homozygous (GG) genotype, 1 band=418 bp

Statistical Methods

Data was coded and entered using the statistical package SPSS version 23. Data was summarized using mean and standard deviation for quantitative variables and frequencies (number of cases) and relative frequencies (percentages) for categorical variables. Comparisons between groups were done

using unpaired t test. For comparing categorical data, Chi square (χ^2) test was performed. Exact test was used instead when the expected frequency is less than 5. Genotype and allele frequencies were compared between the disease and the control groups using logistic regression. Odds ratio (OR) with 95% confidence intervals was calculated. Univariate Logistic regressions was done to detect if BclI carrier act as independent predictor of asthma and asthma severity. P-values less than 0.05 were considered as statistically significant. All genome types were examined, and they were in Hardy-Weinberg equilibrium.

RESULTS

Our study enrolled 135 asthmatic children along with 90 age and sex matched controls, with mean ages 9.3±3.6 years and 9.3±3.4 years, respectively. Seventy-eight (57.8%) of the cases were males and 57 (42.2%) were females. Among controls, males were 52(57.8%) and females were 38(42.2%). According to asthma severity classification, 60 (44.4%) asthmatic children had mild asthma, 45 (33.3%) had moderate, and 30 (22.2%) had severe asthma. Included patients had different levels of asthma control. Basic demographic and clinical characteristics of the study population were shown in table 1.

Frequency of BclI polymorphism of GR gene (CC, CG and GG) together with the distribution of its allele variants (C and G alleles) showed a significant difference between cases and control with *P* value <0.001. Carriers for G (GG, CG) allele were more susceptible to asthma than control, and homozygous for the G allele had a higher risk of the disease than the heterozygous, as were shown in table 2 and figure 2.

On studying the correlation between asthma phenotype and BclI polymorphisms, the frequency of the allele G was significantly associated with increased severity of bronchial asthma in the investigated population; *P* value <0.001. Also, the frequencies of the GG genotype were associated with hospitalization with severe asthma exacerbation; *P* value <0.001. On analyzing the relation between BclI gene polymorphism and the associated allergic manifestations, there was a statistically significant difference between the frequency of allele G versus C regarding the presence of conjunctivitis and eczema; *P* value for each was <0.001, as were shown in table 3.

These results were emphasized with post Hoc pairwise comparison test that showed a statistical significance on pairing BclI polymorphism (CC, GG) against asthma severity, hospitalization with severe asthma exacerbation and allergic conjunctivitis (p <0.001), furthermore pairing BclI polymorphism(CG, GG) showed statistical significance with both asthma severity and hospitalization with severe asthma with p <0.001 and 0.003 respectively. Also pairing BclI polymorphism (CC, CG) showed statistical difference with asthma severity, allergic conjunctivitis and eczema with p= 0.006, <0.001, <0.001, respectively.

Regarding the relation between BclI gene polymorphisms and level of asthma control among the studied group, there was increased incidence of uncontrolled asthma with the presence of the allele G, where in CC, CG, GG polymorphisms uncontrolled asthma was recorded in (0.0%, 64.3%, and 100%), respectively with *P* value < 0.001, as shown in table 4.

Post Hoc pairwise comparison test was done and showed statistically significant difference between well controlled and partially controlled and between well controlled and uncontrolled regarding BclI polymorphism.

Table 1. Basic demographic and clinical data of the study population

Demographic characteristics	Asthmatic children n= 135	Control n= 90	*P value
Age (years)			
Mean ± SD	9.3±3.6	9.3±3.4	0.99
Gender n (%)			
Male	78 (57.8%)	52 (57.8%)	1
Female	57 (42.2%)	38 (42.2%)	
Asthma characteristics		NA	NA
Degree of Asthma severity n (%):			
Mild persistent	60 (44.4%)		
Moderate persistent	45 (33.3%)		
Severe persistent	30 (22.3%)		
Associated allergic manifestations n (%):			
Allergic rhinitis	93 (68.9%)		
Conjunctivitis	81(60%)		
Eczema	90(66.7%)		
Food allergy	33(24.4%)		
Level of asthma control:			
Well controlled	86(63.7%)		
Partially controlled	35(25.9%)		
Uncontrolled	14(10.4%)		

SD: standard deviation, NA: not applicable, p<0.05 is statistically significant

Table 2. Genotype and allele frequencies of the Bcl I polymorphisms in asthmatic children versus control group

Bcl I polymorphisms	Asthmatic children n= 135	Control n= 90	OR	95% CI	*P value
CC	57(42.2%)	66(73.3%)	Ref.	Ref.	
CG	69(51.1%)	24(26.7%)	3.329	1.85-5.97	< 0.001
GG	9(6.7%)	0	22.0 [a]	1.3-386	0.999
Allele C	183(67.7%)	156(86.7%)	Ref.	Ref.	
Allele G	87(32.2%)	24(13.3%)	3.09	1.87-5.09	< 0.001

OR: odds ratio, CI: Confidence interval , *P <0.05 is considered statistically significant,a: Haldane-Anscombe correction was done to enable caclculation of OR and 95%CI.

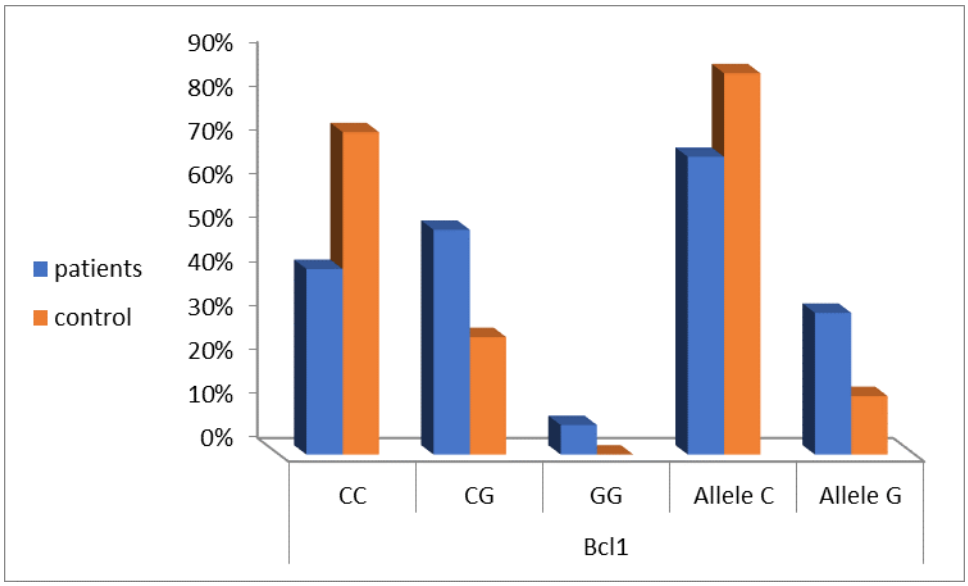

Figure 2. Frequency of Bcl1 polymorphism and distribution of its allele variants in cases and controls

Table 3. Association between asthma phenotype and Bcl I polymorphisms

Bcl I polymorphisms	CC	CG	GG	*P value
Asthma severity				
Mild persistent	36(63.2%)	24 (34.8%)	0 (0%)	
Moderate persistent	15(26.3%)	30(43.5%)	0 (0%)	< 0.001
Severe persistent	6(10.5%)	15(21.7%)	9 (100%)	
Hospitalization with severe asthma exacerbation	18 (31.6%)	33 (47.8%)	9 (100%)	< 0.001
Allergic rhinitis	36 (63.2%)	51(73.9%)	6(66.7%)	0.409
Allergic conjunctivitis	15(26.3%)	57(82.6%)	9(100%)	< 0.001
Eczema	27(47.4%)	57(82.6%)	6(66.7%)	< 0.001
Food allergy	9(15.8%)	21(30.4%)	3(33.3%)	0.119

*P < 0.05 is considered statistically significant.

Table 4. Association between Bcl I polymorphisms and level of asthma control

Group / Bcl I polymorphisms	Well controlled (n= 86)	Partially controlled (n=35)	Uncontrolled (n=14)	*P value
CC	53(61.6%)	4(11.4%)	0	
CG	33(38.4%)	27(77.2%)	9(64.3%)	
GG	0(0%)	4(11.4%)	5(35.7%)	< 0.001

*P < 0.05 is considered statistically significant.

DISCUSSION

We detected a statistically significant difference in genotype frequencies (GG, CG, and CC) of the BclI polymorphism of the human glucocorticoid receptor gene (hGR/NR3C1) in asthmatic patients compared with healthy controls. The present analysis of BclI polymorphism revealed a significant relationship between BclI gene polymorphisms and level of asthma control, where we observed an increased incidence of uncontrolled asthma in the presence of the allele G (CG, GG). Furthermore, this SNP is significantly associated with the severe asthma phenotype, what could be explained on the basis that, the observed polymorphic changes cause modifications of function or synthesis of glucocorticoid receptor which in turn affects the phenotypic characteristics of bronchial asthma.[12]

Our findings are in line with a study conducted by **Panek et al.,** 2012 which studied a group of Polish patients with severe asthma and observed a significant increase in the GG genotype among severe asthmatic patients compared with controls.[12]

On the contrary, **Panek et al.** 2013 demonstrated a lower frequency of the NR3C1 646 C>G GG homozygote and a higher frequency of the CC homozygote among asthmatics (which included mild, moderate, and severe asthmatics) in comparison to the control group with no statistically significant differences in frequencies of NR3C1 646 C>G polymorphism when severe asthma was compared with the control group.[13] Similarly, Szczepankiewicz A. et al. study showed that polymorphisms of the GR gene are not associated with asthma susceptibility nor influence response to inhaled glucocorticoids in their sample.[14]

Polymorphisms present within the h-GR/NR3C1 gene may inhibit formation of GR/GCs complexes, reduce transcription and cause transrepression of the genes encoding proteins synthesized within the framework of cellular response to GCs and decreased expression of GR that leads to a reduced response to GCs and impairment of GCR.[8,15] Accordingly, this study found that these genotypes (CG, GG) were associated with more loss of control of asthma symptoms causing hospitalization with severe exacerbations.

This study records for the first time that there is significant association between Bcl I polymorphism of h-GR/NR3C1 gene with the allergic conjunctivitis and eczema. But this was inconsistent with Mohamed et al. 2015 who reported that there is no relationship between atopy and this gene polymorphism, [16] but it is difficult to compare the results of our study with theirs due to their smaller sample size.

In addition, the current study showed a significant difference in BclI genotypes and alleles frequencies between asthmatic children with different levels of asthma control, where, we demonstrated that well controlled asthmatic children had a greater frequency of the CC genotype distribution as well as C allele carriers than the partially controlled and uncontrolled asthmatics. This finding is supported with

Mohamed et al. 2015 who conducted a study on two groups of GCs sensitive and GCs resistant asthmatics adult Egyptian asthmatics and they detected a significant difference in genotype distribution between GCs sensitive and GCs resistant asthmatics, with a greater frequency of the CC genotype distribution as well as C allele carriers among GCs sensitive asthmatics than among GCs resistant asthmatics.[16] Also, in agreement with our study, Khaled et al. 2020 noted among asthmatic children that the CC genotype was statistically associated with controlled asthma symptoms 3 months after treatment and the GG genotype was associated with poor asthma symptom control.[17]

So, identification of genetic variation influencing treatment outcome in such heterogeneous disease (BA) with different endotypes and phenotypes might identify novel targets for biomarker-guided asthma treatment and preventative strategies.[18,19]

In addition to this, the interaction with the environment that might play a major role in driving a predisposed genetic background toward severe and uncontrolled asthma phenotype, revealing the importance of the emerging Genome-wide interaction studies (GWIS) which assess the interaction between genome-wide genetic variance and environmental factors that might help in understanding this complex process.[20]

CONCLUSION

The Bcl I polymorphism of hGR/NR3C1 gene is significantly associated with bronchial asthma. The GG phenotype is significantly associated with increased susceptibility to the development of severe asthma and uncontrolled asthma symptoms, with increased risk of hospitalization due to severe asthma exacerbation.

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