

CRHR1 Gene SNPs and Response to Systemic Corticosteroids in Indian Asthmatic Children During Acute Exacerbation

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Abstract

Objective To determine association of corticotrophin releasing hormone receptor 1 (*CRHR1*) gene single nucleotide polymorphisms (SNPs), rs242939 (A>G) and rs242941 (G>T) with response to systemic corticosteroids in North Indian asthmatic children during acute exacerbation.

Methods This was a hospital based cross-sectional study. Sixty-eight children aged 1 to 12y with acute exacerbation of asthma were included in the study. The study was approved by the institutional ethics committee and written informed consent was obtained from parents/guardians of recruited children. GINA guidelines 2008, were used for classification and treatment of acute exacerbation of asthma. As per the GINA guidelines 2008, children who had good response to injectable corticosteroid were classified as “Corticosteroid Responders” (CR). Rest of the children with incomplete or poor response to injectable corticosteroid were classified as “Corticosteroid Non Responders” (CNR).

Results Among 68 hospitalized children, 45 (66.17 %) children were CR whereas 23 (33.83 %) children were CNR. On analyzing as dominant model, children with one or two copies of mutant allele of SNP rs242941 had statistically significant better response to systemic corticosteroid (OR=5.00; 95 %CI=1.32–19.64; *p* 0.013) as compared to children with no mutant allele.

Conclusions Thus, *CRHR1* gene SNP rs242941 polymorphism is associated with better response to systemic corticosteroid during acute exacerbation of asthma.

Keywords Asthma exacerbation • Corticotrophin releasing hormone receptor 1 gene • rs242941 • Corticosteroids

Introduction

Asthma is a common chronic disease among children affecting 300 million people worldwide [1]. Prevalence of asthma is 2.3 and 3.3 % in the children of age group 6/7 and 13/14 y, respectively, in Lucknow, North India where this study has been done [2]. Asthma is also one of the most common reasons for children to present for acute evaluation. Acute exacerbation of asthma is characterized by progressive increase in shortness of breath, cough, wheezing or chest tightness, or some combination of these symptoms. Systemic corticosteroids are given to patients who have moderate or severe exacerbations and patients who do not respond completely to initial doses of rapid acting inhaled β_2 -agonist therapy [3]. However asthma is identified as a phenotypically heterogeneous disorder with variable therapeutic response [4]. Pharmacogenetic studies of polymorphism of the β_2 -adrenergic receptor, 5-lipoxygenase (5-LO) promoter region and corticosteroid receptor have shown the influence of genetics on disease severity and response to therapy [5–10]. Clinical studies have shown that approximately 5 to 10 % of all patients with asthma and up to 35 % of those with severe disease have reduced responses to corticosteroid therapy [11]. A guided approach to corticosteroid treatment should be recommended as it favors better control of the disease and presumably a lower rate of adverse events. Corticotrophin releasing hormone receptor 1 (*CRHR1*) gene located at 17q12-q22 [12] has been identified as a potential marker for corticosteroid responsiveness. *CRHR1*, encoded by *CRHR1* gene regulates endogenous corticosteroid level [13, 14] through the release of adrenocorticotrophic hormone (ACTH). Genetic variation of *CRHR1* gene is expected to diminish the capacity to secrete corticosteroid owing to decreased ACTH release. It is hypothesized that asthmatic patients with genetic variations in *CRHR1* gene are expected to have lesser stress induced endogenous corticosteroid [15] secretion and

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therefore a better therapeutic response to exogenous corticosteroid.

Therefore this study was conducted to find out association of *CRHR1* gene SNPs (single nucleotide polymorphism), rs (restriction site) 242939 (A>G) and rs242941 (G>T) with response to systemic corticosteroids in Northern Indian asthmatic children during acute exacerbation of asthma.

Material and Methods

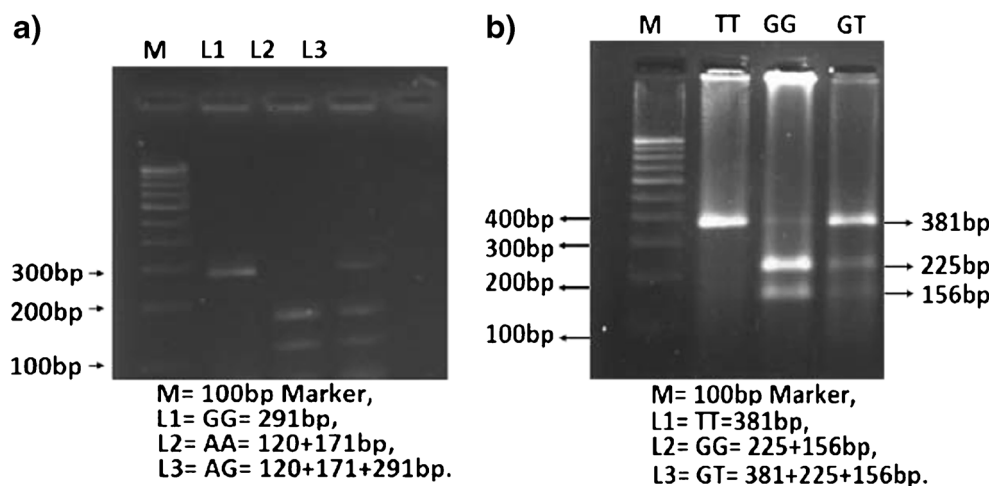
This was a hospital-based cross-sectional study, conducted in the indoor patients (IP) facility of Department of Pediatrics at King George's Medical University (KGMU), Lucknow, Uttar Pradesh, a tertiary care centre in Northern India from August'2008 through August'2011. The total number of patients who were screened for the study included 236 children with acute exacerbation of asthma, out of which 168 children were treated on OPD basis as they had mild exacerbation of asthma and 68 children with severe exacerbation were admitted to IP facility. Therefore the present study included 68 children of Northern India, aged 1–12 y, admitted with the severe exacerbation of bronchial asthma. The present study was approved by the KGMU ethics committee and written informed consent for participation was obtained from parents/guardians of all recruited asthmatic children. Cases were children, aged 1 to 12 y, presenting with symptoms of bronchial asthma. Bronchial asthma for the present study was defined as the presence of one or more of the following symptoms: (i) Current presence of wheeze in any child with a history of more than four episodes of documented wheeze or use of bronchodilators in the preceding 12 mo, or (ii) Relief with bronchodilators with or without short course oral steroid or on any regular medication for asthma, or (iii) Currently hospitalized and diagnosed as case of bronchial asthma [16]. Those children who had any other respiratory disease or with alternative causes of recurrent wheezing were excluded [17]. The children who had an exacerbation of asthma or had been treated with systemic steroids 8 wk prior to the study were also excluded. A standardized data collection form was developed. Demographic, environmental, clinical, and physical examination findings including oxygen saturation measured by Novamatrix Oxypleth Pulse Oxymeter, use of intravenous (i.v.) fluid, use of oxygen, use of specific medications for asthma [inhaled salbutamol, injection hydrocortisone, injection aminophylline, injection terbutaline, injection Magnesium sulphate (MgSO_4) and antibiotics], outcome [discharge, leave against medical advice (LAMA) and death] and duration of hospital stay were recorded for all cases included in the present study. Absolute eosinophil count was calculated as the percentage of eosinophils multiplied by the white blood cell count.

Acute exacerbation severity was assessed as per GINA guidelines, 2008. In children >5 y of age, exacerbation was

categorized into four categories namely mild, moderate, severe and imminent respiratory arrest on the basis of parameters named as breathlessness, ability to speak, alertness, respiratory rate (RR), accessory muscle use, wheeze, heart rate (HR) and SpO_2 . In children <5 y of age exacerbation was graded into two categories: mild and severe on the basis of parameters like state of consciousness, ability to speak, respiratory rate (RR), wheeze, HR and SpO_2 . In children >5 y of age, all the subgroups were merged together to have two categories of mild and severe exacerbation. Response to treatment was assessed as good response, incomplete response and poor response according to GINA guidelines, 2008. Response was "good" when response was sustained for 60 min after last treatment, physical examination was normal, SpO_2 > 90 % and there was no respiratory distress. Response was "incomplete" when mild-moderate symptoms/signs were present and SpO_2 % was not improving. Response was "poor" when symptoms/signs suggestive of impending respiratory failure were present. All the children were treated as per the GINA guidelines 2008 for acute exacerbation. Salbutamol (0.15 mg/kg/dose) through nebulizer with Oxygen (6 L/min) was given every 20 min, up to one hour. Since all the admitted children had severe exacerbation [17] they were given injection hydrocortisone sodium succinate (20 mg/kg/d). Children who had incomplete or poor response [17] within 4 h as assessed clinically by treating physician were subsequently administered injection aminophylline and/or injection terbutaline and/or injection MgSO_4 and were labeled as "Corticosteroid Non Responder" (CNR). Rest of the children who had good response [17] to injection hydrocortisone within 4 h as assessed clinically by treating physician were labeled as "Corticosteroid Responder" (CR).

DNA was extracted by standardized salting out method [18]. Genotyping was done by polymerase chain reaction restriction fragment length polymorphisms (PCR-RFLP) (Fig. 1). PCR products of SNP rs242939 was digested with 5U *HinfI* restriction enzyme (*Medox*TM Biotech India PVT.LTD, Chennai, Tamilnadu, India) for overnight at 37 °C whereas PCR product of SNP rs242941 was digested by 5U *Acil* restriction enzyme (*Medox*TM Biotech, India PVT.LTD, Chennai, Tamilnadu, India) for overnight at 37 °C. The digested products were then separated on ethidium bromide-stained 2 % agarose gels. The expected size of the digested products for SNP rs242939 were 120bp+171bp (AA genotype), 120bp+171bp+291bp (AG genotype), and 291bp (GG genotype). The expected size of the digested products for SNP rs242941 were 225bp+156bp (GG genotype), 225bp+156bp+381bp (GT genotype), and 381bp (TT genotype) [15]. Duplicate genotyping performed on 20 % of samples by other laboratory personnel demonstrated no discordance. Epi6 (available from the Centers for Disease control and Prevention, Atlanta, GA, USA; <http://www.cdc.gov/epo/epi/epiinfo.htm>) and SPSS 11.5 (Chicago, IL, USA)

Fig. 1 Gel pictures of single nucleotide polymorphisms **a** SNP rs242939 (A>G) and **b** SNP rs242941 (G>T)



were used for statistical analysis. Chi-square and Students *t*-test were used to test the association between categorical and continuous variables respectively. To assess the association between dependent and independent variables, odd's ratios were calculated with 95 % confidence interval. Variables that were entered in the adjusted logistic regression model to assess the factors associated with response to systemic corticosteroid were those which on univariate analysis were found to be associated with response to systemic corticosteroid with *p* value <0.1. Using a 2-tailed distribution, *p* value of <0.05 was considered statistically significant.

Results

The present study was carried out from August'2008 through August'2011. Sixty-eight children, aged 1 to 12 y, diagnosed as bronchial asthma admitted to IP facility with severe exacerbation were included in the study. Among 68 hospitalized children, 45 (66.17 %) children were CR whereas 23 (33.83 %) children were CNR (Fig. 2). Demographic and clinical profiles of CR and CNR children are represented in Table 1. The CR children had statistically significant higher baseline serum absolute eosinophil count ($P<0.015$).

Table 2 represents association of *CRHR1* gene SNPs, rs242939 and rs242941 with response to systemic corticosteroid during acute exacerbation of asthma. Since the sample

size was small therefore, for statistical advantage, analysis was done as *dominant model* (heterozygous + mutant homozygous). On comparing genetic variant of SNP rs242941, children with one or two copies of mutant allele (heterozygous and mutant homozygous) had statistically significant better response to systemic corticosteroid as compared to children with no mutant allele (wild homozygous) (OR=5.00; 95%CI=1.32–19.64; p 0.013). There was no statistically significant difference among the genetic variant of SNP rs242939.

Adjusted logistic regression analysis was performed to assess the factors associated with response to systemic corticosteroid during acute exacerbation of asthma (Table 3). Variables entered in the adjusted logistic regression model were genotypes of SNP rs242941, persistent asthma and absolute eosinophil count; adjusting for age, sex, socioeconomic status, place of residence and previous use of inhaled corticosteroid (ICS). Response to systemic corticosteroid during acute exacerbation of asthma demonstrated statistically significant better association with genotypes of SNP rs242941 (OR=6.23; 95 %CI=1.47–26.32; p 0.013).

Discussion

The present study was carried out to find out association of *CRHR1* gene SNPS, rs242939 (A>G) and rs242941 (G>T)

Fig. 2 Schematic representation of drugs administered to hospitalized children

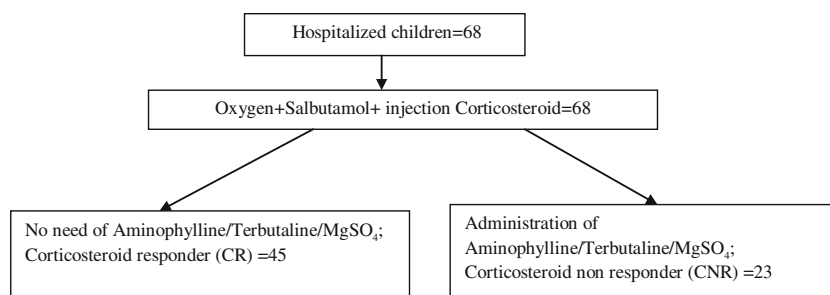


Table 1 Demographic and clinical profiles of Corticosteroid responder and Corticosteroid non responder asthmatic children

Characteristics	Corticosteroid responders (CR) (N=45)	Corticosteroid non-responders (CNR) (N=23)	p value
Age (mo) at recruitment (Mean±SD)	62.36±39.93	47.04±31.36	0.114
Age (mo) at onset (Mean±SD)	29.09±32.47	21.87±24.19	0.351
Weight (kg) (Mean±SD)	15.54±7.30	13.37±4.94	0.206
Height (cm) (Mean±SD)	103.66±22.39	95.69±16.99	0.139
BMI (kg/m ²) (Mean±SD)	13.99±2.57	13.52±2.07	0.450
Male; n (%)	27(60.0)	15(65.2)	0.675
Total socioeconomic status; n (%)			
Upper	22(48.9)	10(43.5)	0.672
Lower	23(51.1)	13(56.5)	
Urban residence; n (%)	24(53.3)	11(47.8)	0.799
Exposure to SHS indoor; n (%)	26; 22(84.6)	9; 5(55.6)	0.162
Persistent asthma; n (%)	35(77.8)	13(56.5)	0.069
FH of asthma; n (%)	27(60.0)	10(43.5)	0.211
PH of allergic rhinitis; n (%)	20(44.4)	8(34.8)	0.603
PH of allergic dermatitis; n (%)	2(4.4)	1(4.3)	1.000
PH of allergy; n (%)	27(60.0)	13(56.5)	0.800
Any previous hospitalization; n (%)	23(51.1)	16(69.6)	0.197
Previous use of ICS; n (%)	29(64.4)	13(56.5)	0.602
Use of BD for present episode; n (%)	24(53.3)	12(52.2)	1.000
Absolute eosinophil count (Mean±SD)	1033.51±967.096	514.52±286.748	0.015
HR (beats/min) at the time of admission in IP facility (Mean±SD)	123.73±17.55	121.91±10.77	0.651
HR (beats/min) 2 h after administration of corticosteroid (Mean±SD)	114.71±14.59	116.43±11.99	0.627
RR (breaths/min) at the time of admission in IP facility (Mean±SD)	48.80±16.52	50.78±14.31	0.627
RR (breaths/min) 2 h after administration of corticosteroid (Mean±SD)	39.33±14.90	45.13±14.05	0.127
SpO ₂ at the time of admission in IP facility (Mean±SD)	90.96±2.41	90.00±3.13	0.165
Use of i.v. fluid; n (%)	36(80.0)	22(95.7)	0.146
Duration of i.v. fluid in days (Mean±SD)	2.64±2.12	2.61±2.98	0.955
Duration of stay in days (Mean±SD)	4.84±5.31	4.30±3.68	0.664
Outcome; n (%)			
Discharge	43(95.6)	21(91.3)	0.370
LAMA	2(4.4)	1(4.3)	
Death	0(0.0)	1(4.3)	

BMI Body mass index; SHS Second hand smoke; FH Family history; PH Personal history; ICS Inhaled corticosteroid; BD Bronchodilator; HR Heart rate; RR Respiratory rate; IV Intravenous; LAMA Leave against medical advice

with response to systemic corticosteroids in Northern Indian asthmatic children during acute exacerbation of asthma. The authors observed a statistically significant better association of mutant allele of SNP rs242941 with response to systemic corticosteroid during acute exacerbation of asthma.

In the study population, mutant allele of SNP rs242941 was found to be statistically significantly related with better response to systemic corticosteroid. Corticotrophin releasing hormone (CRH) has been related to the pathogenesis of asthma [19] as it influences the immune system through activation of the hypothalamic-pituitary-adrenal axis, sympathetic

system and through local modulatory actions of peripheral CRH. Therefore it is postulated that presence of variation in the *CRHR1* gene is expected to alter the hypothalamic-pituitary-adrenal pathway resulting in alteration of diverse physiological processes mediated by *CRHR1* gene [20–22]. Genetic variation of *CRHR1* is expected to diminish the capacity to secrete corticosteroid in response to inflammation, as a result of decreased ACTH release. Therefore, asthmatic patients with alterations in this gene are expected to be having lesser endogenous corticosteroid [15, 23] and thus a better response to exogenous corticosteroids. The index data support this hypothesis that children with lesser endogenous

Table 2 Association of *CRHR1* SNPs rs242939 (A>G) and rs242941 (G>T) with response to systemic corticosteroid

Characteristics	Corticosteroid responders n (%) (N=45)	Corticosteroid non-responders n (%) (N=23)	OR (95%CI), <i>p</i> value
SNP rs242939 (A>G)			
Genotypes			
Wild homozygous (AA) <i>N</i> =51	36(80.0)	15(65.2)	0.47(0.13–1.66), 0.300
Heterozygous and mutant homozygous (AG+GG) <i>N</i> =17	9(20.0)	8(34.8)	
Alleles			
Wild type (A) <i>N</i> =116	76(84.5)	40(86.9)	
Mutant type (G) <i>N</i> =20	14(15.5)	6(13.1)	
SNP rs242941(G>T)			
Genotypes			
Wild homozygous (GG) <i>N</i> =16	6(13.3)	10(43.5)	5.00(1.32–19.64), 0.013
Heterozygous and mutant homozygous (GT+TT) <i>N</i> =52	39(86.7)	13(56.5)	
Alleles			
Wild type (G) <i>N</i> =71	42(46.6)	29(63.1)	
Mutant type (T) <i>N</i> =65	48(53.4)	17(36.9)	

corticosteroid respond better to exogenous corticosteroid. On the other hand, genetic variant of SNP rs242939 was not found to be associated with response to systemic corticosteroid.

There is no study assessing the association of *CRHR1* polymorphism with response to systemic corticosteroid during acute exacerbation of asthma however association of *CRHR1* polymorphism with response to ICS has been studied. Tantisira et al. investigated the genetic contribution to the variation in response to ICS therapy in asthma [23]. SNP rs242941 mutant allele was observed to be associated with significant improvement in lung function from baseline. Rogers et al. identified that the minor allele of SNP rs242941 was associated with poor lung function response [24] and Dijkstra et al. found no significant association of *CRHR1* SNPs including rs242941 and rs242939 with FEV1 decrease, in all subjects before the start of ICS use as well as the long term effect of ICS on the decrease in FEV1 [25]. In the Korean population Kim et al. identified wild allele of SNP rs242941 to be

associated with better improvement in lung function over a period of 12 wk in patients with chronic obstructive pulmonary disease (COPD) [26].

Since there is heterogeneity in response to corticosteroid, the effect of the *CRHR1* polymorphism on the response to corticosteroid in asthmatic subjects has yet to be definitively defined. This heterogeneity can be attributed to interaction of genes with each other as well as environmental factors resulting in poor replicability. Admittedly, the field of personalized medicine in the care of children with asthma is still in its infancy. There is no stratified asthma treatments based on genetic information, although this time may be fast approaching.

Despite an increased understanding of pathogenesis of asthma and its treatment, asthma continues to be a leading cause of morbidity in children. This necessitates studies for new insights into the cause and treatment of asthma. Strength of the index study lies in the fact that the authors are reporting probably for the first time association of *CRHR1*

Table 3 Logistic regression analysis to assess the factors associated with response to systemic corticosteroid

Variables (Coding)	Corticosteroid responders (N=45)	Corticosteroid non-responders (N=23)	Adjusted OR (95%CI) ^a , <i>p</i> value
SNP rs242941 (G>T); n (%) (Wild genotype=0; Heterozygous and mutant homozygous=1)	39 (86.7)	13 (56.5)	6.23 (1.47–26.32); 0.013
Persistent asthma; n (%) (No=0; Yes=1)	35 (77.8)	13 (56.5)	2.98 (0.79–11.11); 0.104
Absolute eosinophil count; n (%) (<1000=0; ≥1000=1)	11 (24.4)	1 (4.3)	3.91 (0.69–21.88); 0.121

^a Adjusted Odds Ratio for age, sex, place of residence, socio-economic status and previous use of inhaled corticosteroid

polymorphism with response to systemic corticosteroid in acute exacerbation of asthma. Care was taken to have a precise clinical definition of asthma. Weakness of the index study is that the sample size was small. Moreover response to treatment was assessed clinically by the treating physician. Therefore further studies with a larger sample size are required to document the role of the *CRHR1* gene polymorphism with response to corticosteroid in asthma.

Conclusions

In conclusion the authors found that *CRHR1* gene SNP rs242941 polymorphism is associated with better response to systemic corticosteroid during acute exacerbation of asthma.

Contributions SA: Responsible for conception, design, clinical examination, analysis and interpretation of data, drafting the article and revising it critically for important intellectual content; SG: Responsible for conception, design, acquisition of data, clinical examination, analysis, interpretation of data and drafting the article; SAg: Responsible for genetic analysis and interpretation of data; NS: Responsible for laboratory work. All of them were responsible for final approval of the version to be published. SA will act as guarantor for this paper.

Conflict of Interest None

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