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Molecular Section Original Article

ASSOCIATION OF CORTICOTROPHIN RELEASING HORMONE RECEPTOR 1 GENE POLYMORPHISMS (RS242941 AND RS242939) WITH PERSISTENT ASTHMA AND ITS PHENOTYPE IN NORTHERN INDIAN ASTHMATIC CHILDREN: A CROSS-SECTIONAL STUDY

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ABSTRACT

INTRODUCTION: *Asthma is a common, chronic, relapsing disease in children. Corticotrophin-releasing hormone receptor 1 (CRHR1) gene is identified as a potential marker for steroid responsiveness. Genetic variations in CRHR1 are expected to diminish the capacity to secrete endogenous cortisol.* ***OBJECTIVE:*** *This study aimed to find out association of CRHR1 gene polymorphisms namely, rs242941 (G > T) and rs242939 (A > G) with persistent asthma and its phenotype in Northern Indian asthmatic children.* ***MATERIALS AND METHODS:*** *This was a hospital-based cross-sectional study. Genotyping was done for 250 asthmatic children, aged 1-12 years, using polymerase chain reaction restriction fragment length polymorphisms method.* ***RESULTS:*** *Mutant homozygous genotype (TT) and mutant allele (T) of single nucleotide polymorphism (SNP) rs242941 were found to be associated with increased risk for persistent asthma (odds ratio [OR] =4.2; 95% confidence interval [CI] =1.6-10.9; P = 0.00 and OR = 1.8; 95% CI = 1.2-2.8; P = 0.00, respectively). On analyzing genotypic and phenotypic associations, factors such as urban residence (OR = 2.01; 95% CI = 1.11-3.63; P = 0.01), family history of asthma (OR = 1.80; 95% CI = 1.00-3.24; P = 0.05), previous hospitalization (OR = 2.12; 95% CI = 1.14-3.96; P = 0.01), previous use of bronchodilators (OR = 3.64; 95% CI = 1.68-7.94; P = 0.00), previous use of inhaled corticosteroids (OR = 2.37; 95% CI = 1.15-4.93; P = 0.01), visit to doctor in last 1 year (OR = 1.82; 95% CI = 1.01-3.28; P = 0.04), seasonal variation in exacerbation (OR = 2.66; 95% CI = 1.16-6.12; P = 0.01), and lower pulmonary functions (P = 0.00) were found to be associated with SNP rs242941. Genotypes of SNP rs242939 showed no association with persistent asthma and its phenotype. Minor allele frequency for SNP rs242941 and SNP rs242939 was 43.20% and 11.00%, respectively, in Northern Indian asthmatic children.* ***CONCLUSION:*** *In conclusion, we observed an association of SNP rs242941 with persistent asthma and its phenotype in Northern Indian asthmatic children.*

Key words: *Asthmatic children, corticotrophin-releasing hormone receptor 1, Northern Indian, persistent asthma, phenotype, single nucleotide polymorphism*

INTRODUCTION

Asthma is a common chronic disease in children. It affects more than 300 million subjects worldwide.[1] In India, the prevalence of asthma in adults is 2.3% and in children, it varies from 6 to 31%.[2,3] Prevalence of asthma was 2.3% and 3.3% in the children of age group 6/7 and 13/14 years, respectively, in Lucknow, where the current study has been done.[4] Susceptibility for development of asthma is likely the result of multiple environmental risk factors as well as multiple genes.[5-9] In addition to defining the pathogenesis of asthma, genetics also define the response to treatment with different classes of drugs. Approximately, two-third of patients with asthma may not attain full control of their asthma.[10,11] Up to one-third of patients treated with inhaled corticosteroids (ICS) may not achieve objective improvements in airway function or indices of airway reactivity.[12] Drazen *et al*. found that 60-80% of the variance in drug response may be due to individual genetic differences.[13] Corticotrophin-releasing hormone receptor 1 (CRHR1) gene has been identified as a potential marker for steroid responsiveness.[14] Tantisira *et al*. observed that single nucleotide polymorphisms (SNPs) in CRHR1 were associated with improved lung function response to ICS after 6-8 weeks of treatment in three clinical trials.[14] However, Dijkstra *et al*. found no significant difference between CRHR1 genotypes with respect to both the immediate effect on forced expiratory volume in 1 s (FEV1) level and the long-term effect of ICS on the decrease in FEV1.[15]

CRHR1 gene (NM_004382) is located at 17q12-q22.[16] CRHR1, encoded by *CRHR1* gene is the primary receptor mediating the release of adrenocorticotropic hormone (ACTH), which regulates endogenous cortisol levels[17,18] and the catecholaminergic response to corticotrophin-releasing hormone (CRH).[19] Genetic variation leading to decreased expression or function of CRHR1 is expected to diminish the capacity to secrete cortisol in response to inflammation, owing to decreased ACTH release. Therefore, asthmatic patients

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with alterations in this gene are expected to have lesser endogenous corticosteroid resulting in more severe asthma.

There is no study from India reporting the work on *CRHR1* gene in relation to asthma. Therefore, this study was conducted to find out the association of CRHR1 genes, SNPs rs242941 (G > T) and rs242939 (A > G) with persistent asthma and its phenotype in Northern Indian asthmatic children.

MATERIALS AND METHODS

Study design and setting

This was a hospital-based cross-sectional study, conducted in the outdoor patients (OP) facility and indoor patients (IP) facility of Department of Pediatrics at Chhatrapati Shahuji Maharaj Medical University (CSMMU), Lucknow, Uttar Pradesh, a tertiary care center in Northern India from August 2008 to May 2011.

Subjects

Totally, 250 children of Northern India, aged 1-12 years, presenting with the symptoms of bronchial asthma were included in the study. This study was approved by the CSMMU ethics committee and written informed consent for participation was obtained from parents/guardians of all recruited asthmatic children.

Inclusion and exclusion criteria for cases

Cases were those children, aged 1-12 years, presenting with symptoms of bronchial asthma. Bronchial asthma for this study was defined as the presence of one or more of the following symptoms:

(1) 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 months,
(2) relief with bronchodilators with or without short course oral steroid or on any regular medication for asthma, or
(3) currently hospitalized and diagnosed as case of bronchial asthma.

Those children who had any other respiratory disease or with alternative causes of recurrent wheezing were excluded. To assess disease severity, children were classified into four groups (mild intermittent, mild, moderate, and severe persistent asthma) based on frequency of day and night time symptoms along with spirometric parameters, namely % predicted FEV1, FEV1/forced vital capacity (FVC) in children > 6 years of age for the present episode.[1] Since the sample size was small, all the sub-groups of persistent asthma were merged together to have two groups of intermittent and persistent asthma.

Data source and measurement

We developed a standardized data collection form. Demographic, environmental, clinical, and physical examination findings were recorded for all cases included in this study.

Spirometry

A pulmonary function test was done using a Spirometer (Spiro lab II, MIT II, Longfian Scitech Company, Boading, China). This test was performed on children above 6 years of age. It included measurements of FEV1, FVC, and FEV1/FVC ratio. Measurements of FEV1 and FVC were expressed as a percentage of predicted values. Values of percent predicted FEV1 and FVC higher than 80% and FEV1/FVC ratio higher than 0.7 were defined as normal.

DNA extraction and genotyping

DNA was extracted by standardized salting out method.[20] Genotyping was done by polymerase chain reaction restriction fragment length polymorphisms (PCR-RFLP) [Figure 1].

Genotyping of rs242939

For performing PCR, forward and reverse primer sequences for SNP rs242939 were: Forward: TGA GGG CTG AAA ATG TTT ATC TGG AGC A; Reverse: GTT CCT GTC ATG TCC ACT TCC AGA GTG A. PCR condition consisted of an initial denaturation at 94°C for 2 min, followed by 35 cycles, each consisting of a denaturation step at 94°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 1 min. Five microliters of the PCR product were digested with 5U HinfI restriction enzyme (*Medox*™ Biotech

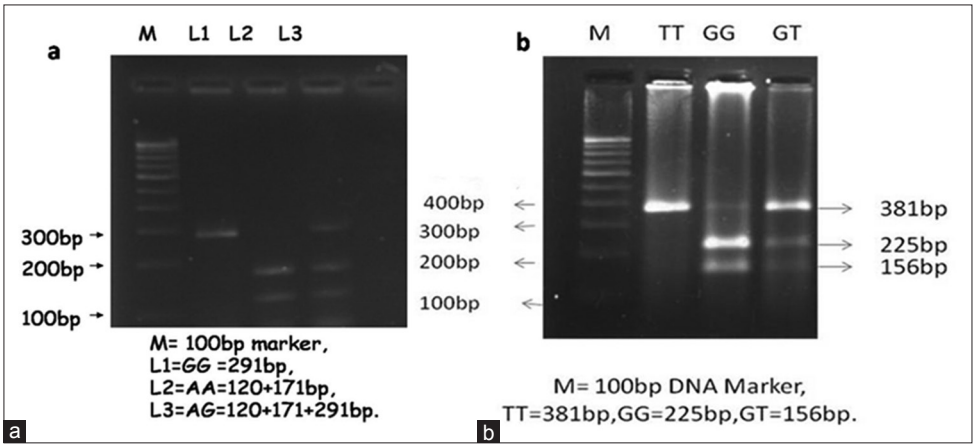

Figure 1: Gel pictures of single nucleotide polymorphisms (SNPs); (a) rs242939 and (b) SNPs rs242941 Images are loaded as separate file

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 was 120 bp + 171 bp (AA genotype), 120 bp + 171 bp + 291 bp (AG genotype), and 291 bp (GG genotype).

Genotyping of rs242941

Sequences for the forward and reverse primers for SNP rs242941 were: Forward: GAC ACT TCA GGA GGG GAC GGT GGA TAT G; Reverse: CTG AGT CCA GCA GAG AAA GGG AGC CAA T. All PCR cycle conditions were same as for SNP rs242939, except annealing temperature was 64°C. PCR product was digested by 5U AciI (*Medox*™ Biotech India PVT. LTD, India) restriction enzyme 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 was 225 bp + 156 bp (GG genotype), 225 bp + 156 bp + 381 bp (GT genotype), and 381 bp (TT genotype).

Quality control

Duplicate genotyping performed on 20% of samples by other laboratory personnel demonstrated no discordance.

Statistical analysis

EpiI6 (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) were used for statistical analysis. Univariate analysis was performed to study the frequency distribution of the variables. Chi-square and '*t*' test were used to test the association between categorical and continuous variables. The Chi-square test was also used to determine differences in genotype/allele frequencies and deviation from Hardy–Weinberg equilibrium. To assess the association between dependent and independent variables, odds ratios (OR) were calculated with 95% confidence interval (CI). On calculating OR and 95% CI between intermittent and persistent asthma, homozygous genotype for the normal allele of each SNP was used as reference. *P* values were corrected (pcorr) for multiple corrections (Bonferroni's correction) in case of further sub-grouping or stratification. The 0.05/N threshold was set up according to the Bonferroni's correction to account for multiple testing issues. N is the number of tested markers (genotypes) for each gene polymorphism. Therefore, for applying Bonferroni's correction, we have multiplied the *P* value by the number of comparisons (e.g. in case of a polymorphism having three genotypes, we multiplied the *P* value by 3 and for carrier analysis the *P* value was multiplied by 2).[21] The significance level for all statistical tests was set at *P* value < 0.05. Statistical analyses for the haplotype estimation and linkage disequilibrium (LD) were carried out using the expectation–maximization algorithm. The SNP analyzer (version 1.0; ISTECH; Istech, Kyungkido, Republic of Korea) was used to calculate D' value, the measure index of LD.

RESULTS

This study was carried out from August 2008 to May 2011. Totally, 250 children, aged 1-12 years, diagnosed as bronchial asthma were in the study. Out of 250 asthmatic children, 88 (35.2%) had mild intermittent asthma, 93 (37.2%) had mild persistent, 50 (20.0%) had moderate persistent, and 19 (7.6%) had severe persistent asthma. All the sub-groups were merged together into two groups making one group of 88 (35.2%) children as intermittent asthma and another group of 162 (64.8%) children as persistent asthma.

Demographic and clinical profiles of children with intermittent and persistent asthma are represented in Table 1. Children with persistent asthma were older in age at recruitment (*P* = 0.04), had residence within 1.5 km from heavy traffic (OR = 4.18; 95% CI = 2.17-8.09; *P* < 0.000), and were more exposed to second-hand smoke indoor (OR = 3.59; CI = 1.19-10.97; *P* = 0.02) with ≥5 cigarettes or *"bidi"*/day smoked indoor (OR = 4.74; 95% CI = 1.60-14.30; *P* = 0.00). As compared to children with intermittent asthma, children with persistent asthma had statistically significant more previous hospitalization (OR = 2.87; 95% CI = 1.58-5.24; *P* = 0.00), previous use of bronchodilator (OR = 2.20; 95% CI = 1.03-4.72; *P* = 0.04), previous use of ICS (OR = 3.11; 95% CI = 1.55-6.32; *P* = 0.00), and visit to doctor annually (OR = 4.06; 95% CI = 2.25-7.34; *P* < 0.000). Persistent asthma group had statistically significant higher proportion of children with exacerbation (OR = 3.58; 95% CI = 1.05-12.75; *P* = 0.03). Persistent asthma group also missed more school/play days (OR = 5.52; 95% CI = 2.51-12.37; *P* < 0.000) for the present episode of exacerbation. The percent predicted FEV1 and FVC/FEV1 ratios were statistically significantly lower among children with persistent asthma as compared to children with intermittent asthma (*P* < 0.00).

Allele and genotype frequencies of CRHR1 gene polymorphisms (SNP rs242941 and rs242939) in Northern Indian asthmatic children

Table 2 represents the genotype and allele frequencies of SNP rs242941 and SNP rs242939.

Minor allele frequency of SNP rs242941 (G > T) in the study population was 43.20%, whereas minor allele frequency of SNP rs242939 (A > G) in the study population was 11.00%. Both SNPs genotyped were in Hardy–Weinberg equilibrium (*P* > 0.05).

Association of CRHR1 gene polymorphisms (SNP rs242941 and rs242939) with persistent asthma

Table 3 represents the association of *CRHR1* gene polymorphisms with persistent asthma.

Table 1: Demographic and clinical profiles of children with intermittent and persistent asthma

Characteristics	*Intermittent asthma N=88*	*Persistent asthma N=162*	*P value*
Age (months) at recruitment (mean±SD)	68.67±38.68	79.08±40.02	0.04
Age (months) at onset (mean±SD)	32.47±32.80	31.28±33.93	0.78
Weight (kg) (mean±SD)	17.38±7.65	18.19±7.08	0.39
Height (cm) (mean±SD)	107.32±20.62	112.19±20.44	0.07
BMI (kg/m^2) (mean±SD)	14.25±2.14	13.93±2.16	0.27
Male (n, %)	59 (67.0)	101 (62.3)	0.46
Religion (*n*, %)			
Hindu	70 (79.5)	129 (79.6)	0.98
Muslim	18 (20.5)	33 (20.4)	
Total socioeconomic status (*n*, %)			
Upper	46 (52.3)	75 (46.3)	
Lower	42 (47.7)	87 (53.7)	0.36
Urban residence (*n*, %)	52 (59.1)	93 (57.4)	0.79
Exclusive use of LPG (*n*, %)	47 (53.4)	89 (54.9)	0.81
Distance of place of residence from heavy traffic ≤1.5 km (*n*, %)	52 (59.1)	139 (85.8)	<0.00
Industrial area with emission of smoke within 2 km of residence (*n*, %)	9 (10.2)	26 (16.0)	0.20
Exposure to second hand smoke indoor (*n*, %)	30; 19 (63.3)	72; 62 (86.1)	0.02
≥5 cigarettes or "*bidi*"/day smoked indoor	30; 17 (56.7)	72; 62 (86.1)	0.00
Family history of asthma (*n*, %)	47 (53.4)	90 (55.6)	0.07
Previous hospitalization (*n*, %)	24 (27.3)	84 (51.9)	0.00
Previous use of bronchodilator (*n*, %)	69 (78.4)	144 (88.9)	0.04
Previous use of inhaled corticosteroids (*n*, %)	14 (15.9)	60 (37.0)	<0.00
>5 visit to doctors in last 1 year (*n*, %)	28 (31.8)	106 (65.4)	0.00
Children with exacerbation (*n*, %)	79 (89.8)	157 (96.9)	0.03
>5 school/play days missed for present episode of exacerbation (*n*, %)	55; 12 (21.8)	122; 74 (60.7)	<0.00
FEV1% (*n*; mean±SD)	37; 78.01±1.47	84; 63.77±5.80	<0.00
FEV1/FVC (*n*; mean±SD)	37; 0.69±0.04	84; 0.60±0.05	<0.00

BMI=Body mass index, LPG=Liquid petroleum gas, FEV1=Forced expiratory volume in 1, FVC=Forced vital capacity

Table 2: Frequencies of genotypes and alleles of single nucleotide polymorphism rs242941 and single nucleotide polymorphism rs242939

Frequencies	**SNP rs242941 (%)**	**SNP rs242939 (%)**
Genotypes		
Wild homozygous	GG-72 (28.8)	AA-200 (80.0)
Heterozygous	GT-140 (56.0)	AG-45 (18.0)
Mutant homozygous	TT-38 (15.2)	GG-5 (2.0)
Alleles		
Normal	G-284 (56.8)	A-445 (89.0)
Mutant	T-216 (43.2)	G-55 (11.0)

SNP=Single nucleotide polymorphism

After comparing genotype and allele frequencies between intermittent and persistent asthma, increased risk for persistent asthma was observed with heterozygous (GT) (OR = 1.8; 95% CI = 1.0-3.4; *P* = 0.04) and homozygous mutant (TT) (OR = 4.2; 95% CI = 1.6-10.9; *P* = 0.00) genotypes of SNP rs242941. However, after Bonferroni's correction, only mutant homozygous genotype of SNP rs242941 (TT) was associated with increased risk for persistent asthma (Bonferroni's corrected *P* value = 0.00). Similarly, mutant allele (T) of SNP rs242941 was found to be associated with increased risk for persistent asthma (OR = 1.8; 95% CI = 1.2-2.8; *P* = 0.00).

Demographic and clinical profiles of 250 asthmatic children screened for CRHR1 gene polymorphisms (SNP rs242941 and rs242939)

Table 4 represents the demographic and clinical profiles of study population screened for SNP rs242941. The frequency of mutant homozygous genotype of SNPs rs242941 and rs242939 was 15.2% and 2.0%, respectively; therefore, for statistical advantage genotypes, mutant homozygous and heterozygous were merged together and named as "Group II." Children with wild homozygous genotype were named as "Group I." Analysis was performed to find out an association between made groups (I and II).

On comparing Group I and Group II for SNP rs242941, Group II was showing statistically significant association with urban residence (OR = 2.01; 95% CI = 1.11-3.63; *P* = 0.01) and family history of asthma (OR = 1.80; 95% CI = 1.00-3.24; *P* = 0.05). Children in this group also had higher frequency of previous hospitalization (OR = 2.12; 95% CI = 1.14-3.96; *P* = 0.01), previous use of bronchodilator (OR = 3.64; 95% CI = 1.68-7.94; *P* = 0.00), previous use of ICS (OR = 2.37; 95% CI = 1.15-4.93; *P* = 0.01), seasonal variation in exacerbation (OR = 2.66; 95% CI = 1.16-6.12; *P* = 0.01), and visit to doctor in last 1 year (OR = 1.82; 95% CI = 1.01-3.28; *P* = 0.04). The percent predicted FEV1 and FEV1/FVC ratios were also statistically significantly lower in Group II as compared to Group I (*P* = 0.00; insignificant data on demographic and clinical profiles are not shown).

There were no significant differences in the distribution of any demographic and clinical profiles of study population among genetic variants of SNP rs242939 (data not shown).

Association of CRHR1 gene SNPs rs242941 and rs242939 diplotypes with risk of persistent asthma

Distributions of diplotypes between intermittent and persistent are shown in Table 5. After the estimation, total four set of diplotypes were found. Comparison was made between intermittent and persistent asthma. The TA diplotype having mutant allele and wild allele of SNP rs242941 and rs242939, respectively, was found to be associated with risk of persistent asthma (OR = 1.51; 95% CI = 1.01-2.26; *P* = 0.04), whereas the GA diplotype having wild allele of both SNPs showed protective association with respect to persistent asthma (OR = 0.65; 95% CI = 0.44-0.95; *P* = 0.02). Conversely, TG diplotype having mutant allele of both SNPs depicted a tendency of association with persistent

Table 3: Association of corticotrophin releasing hormone receptor 1 gene polymorphisms with persistent asthma

SNP rs242941			
Genotypes	Intermittent asthma *N*=88 (%)	Persistent asthma *N*=162 (%)	OR; 95% CI, *P* value
Wild homozygous (GG) *N*=72	34 (38.6)	38 (23.5)	[1]
Heterozygous (GT) *N*=140	47 (53.4)	93 (57.7)	1.8 (1.0-3.4) 0.044*1
Mutant homozygous (TT) *N*=38	7 (8.0)	31 (19.1)	4.2 (1.6-10.9) 0.003*2
Alleles	Intermittent asthma *N*=176 (%)	Persistent asthma *N*=324 (%)	OR; 95% CI, *P* value
Wild type (G) *N*=284	115 (65.3)	169 (52.2)	[1]
Mutant type (T) *N*=216	61 (34.7)	155 (47.8)	1.8 (1.2-2.8) 0.00
SNP rs242939			
Genotypes	Intermittent asthma *N*=88 (%)	Persistent asthma *N*=162 (%)	OR; 95% CI, *P* value
Wild homozygous (AA) *N*=200	158 (89.8)	287 (88.6)	[1]
Heterozygous (AG) *N*=45	18 (10.2)	37 (11.4)	0.7 (0.3-1.4) 0.39
Mutant homozygous (GG) *N*=5	0 (0.0)	5 (3.1)	NC
Alleles	Intermittent asthma *N*=176 (%)	Persistent asthma *N*=324 (%)	OR; 95% CI, *P* value
Wild type (A) *N*=445	158 (89.8)	287 (88.6)	[1]
Mutant type (G) *N*=55	18 (10.2)	37 (11.4)	0.7 (0.3-1.4) 0.39

CI=Confidence interval, NC=Not calculated, Bonferroni's corrected *P* value *1=0.132; *2=0.009, OR=Odds ratio, SNPs=Single nucleotide polymorphism

Table 4: Demographic and clinical profile of 250 asthmatic children screened for single nucleotide polymorphism rs242941

Characteristics	*Group 1 (wild homozygous; N=72)*	*Group 2 (mutant homozygous+heterozygous; N=178)*	*P value*
Age (months) at recruitment (mean±SD)	73.18±37.18	76.32±40.86	0.57
Age (months) at onset (mean±SD)	36.71±33.51	29.67±33.34	0.13
Weight (kg) (mean±SD)	17.52±6.66	18.06±7.53	0.59
Height (cm) (mean±SD)	109.73±19.87	110.78±20.73	0.71
BMI (kg/m^2) (mean±SD)	13.69±1.99	14.18±2.21	0.10
Male (*n*, %)	47 (65.3)	113 (63.5)	0.78
Religion (*n*, %)			
Hindu	56 (77.8)	143 (80.3)	0.64
Muslim	16 (22.2)	35 (19.7)	
Total socioeconomic status (*n*, %)			
Upper	31 (43.1)	90 (50.6)	0.28
Lower	41 (56.9)	88 (49.4)	
Urban residence (*n*, %)	33 (45.8)	112 (62.9)	0.01
Family history of asthma (*n*, %)	32 (44.4)	105 (59.0)	0.03
Children with exacerbation (*n*, %)	67 (93.1)	169 (94.9)	0.55
>5 school/play days missed for present episode (*n*, %)	19 (37.3)	59 (46.8)	0.07
Previous hospitalization (*n*, %)	22 (30.6)	86 (48.3)	0.01
Previous use of bronchodilator (*n*, %)	52 (72.2)	161 (90.4)	0.00
Previous use of inhaled corticosteroids (*n*, %)	13 (18.1)	61 (34.3)	0.01
Presence of seasonal variation (*n*, %)	57 (79.2)	162 (91.0)	0.01
>5 visit to doctors in last 1 year (*n*, %)	31 (43.1)	103 (57.9)	0.04
FEV1% (mean±SD)	71.98±7.13	66.55±8.12	0.00
FEV1/FVC (mean±SD)	0.66±0.06	0.61±0.06	0.00

FEV1=Forced expiratory volume in 1, FVC=Forced vital capacity, BMI=Body mass index

Table 5: Distribution of corticotrophin releasing hormone receptor 1 gene single nucleotide polymorphism diplotypes and association with severity of asthma

Diplotypes	*Intermittent asthma N=176*(%)*	*Persistent asthma N=324 (%)*	*OR (95% CI)*	*P value*
GA	100 (56.5)	149 (46.1)	0.65 (0.44-0.95)	0.02
TA	58 (33.2)	138 (42.5)	1.51 (1.01-2.26)	0.04
GG	15 (8.8)	20 (6.0)	0.71 (0.33-1.50)	0.42
TG	3 (1.4)	17 (5.4)	3.19 (0.87-13.90)	0.09

CI=Confidence interval, OR=Odds ratio

asthma (OR = 3.19; 95% CI = 0.87-13.90; *P* = 0.09). Studied SNPs were not in LD (Yates's corrected *P* value < 0.05 and $|D'| \neq 0$).

DISCUSSION

This study was carried out to find out the association of *CRHR1* gene polymorphisms, rs242941 (G > T) and rs242939 (A > G), with persistent asthma and its phenotype in Northern Indian asthmatic children. We observed a statistically significant association of mutant homozygous genotype and mutant allele of SNP rs242941 with persistent asthma. Children with homozygous mutant and heterozygous genotype of SNP rs242941 were analyzed as a single group (Group II), in comparison to those with wild genotype (Group I) and Group II was observed to be statistically significantly associated with urban residence and family history of asthma. Group II also had statistically

significant more previous hospitalization, previous use of bronchodilator, previous use of ICS, seasonal variation in exacerbation and visit to doctor in last 1 year as compared to Group I. The percent predicted FEV1 and FVC ratios were also statistically significantly lower among Group II children. The TA diplotype was found to be statistically significantly associated with risk of persistent asthma, whereas the GA diplotype showed protective association with respect to persistent asthma.

Association of persistent asthma with closer proximity of residence to road with heavy traffic, father smoking indoors >5 cigarettes or *"bidi"*/day, previous use of ICS, previous hospitalization, visit to doctor in last 1 year, proportion of children with exacerbation, and number of school days missed for the present episode of exacerbation in comparison to intermittent asthma has been published earlier.[22] In this study, we found persistent asthma to be statistically significantly associated with previous use of bronchodilators and lower pulmonary function in comparison to intermittent asthma.

In the study population, mutant homozygous genotype and mutant allele of SNP rs242941 were found to be statistically significantly associated with persistent asthma. CRH has been assumed to be related to the pathogenesis of asthma[23] as CRH influences the immune system through activation of the hypothalamic–pituitary–adrenal axis and sympathetic system and through local modulatory actions of peripheral CRH. The 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 it.[24-26] Therefore, alterations of any of the functions, mediated by the CRHR1 gene, have the potential to influence the pathogenesis of asthma. Genetic variation of CRHR1 is expected to diminish the capacity to secrete cortisol in response to inflammation, owing to decreased ACTH release. Therefore, asthmatic patients with alterations in this gene are expected to be having lesser endogenous corticosteroid resulting in more severe asthma. Our data support this hypothesis that risk for persistent asthma was associated with the mutant allele. On the other hand, genetic variant of SNP rs242939 was not found to be associated with persistent asthma. This may be due to compound effects of multiple alleles, multiple genes, and environmental factors. Since ast hma is a multi-factorial disorder, combination of many genes and environmental factor (such as, type of asthma and ethnicity) may affect asthma. Moreover, it was observed that TA diplotype having mutant allele of SNP rs242941 and wild allele of SNP rs242939 was associated with risk of persistent asthma; GA diplotype having wild allele of both SNPs was protective for risk of persistent asthma, whereas TG diplotype having mutant allele of both SNPs depicted a tendency for the risk of persistent asthma.

It has been affirmed earlier that increasing asthma severity is associated with more use of anti-asthma drugs,[27] more visits to doctors,[27] and reduced lung function.[28-30] These risk and impairment features of persistent asthma were also observed to be associated with SNP rs242941. This information further supports our hypothesis that mutant allele of SNP rs242941 is associated with persistent asthma.

In the study population, the minor allele frequency of CRHR1 SNPs, rs242941 and rs242939, was 43.20% and 11.00%, respectively. Childhood Asthma Management Program (CAMP) trial reported that Minor allele frequency of SNP rs242941 and SNP rs242939 was 30.0% and 3.0%, respectively, in children with mild to moderate asthma. While the Asthma Clinical Research Network (ACRN) trial conducted on subjects with persistent asthma reported that minor allele frequency of SNP rs242941 was 32.0% and that of SNP rs242939 was 7.0%.[31]

In this study, SNP rs242941 was found to be associated with family history of asthma and urban residence. This association had not been reported earlier. Since asthma is a multi-factorial disease and many gene and environmental factors together contribute to disease, further studies are needed to confirm this association.

The strength of our study was that this is the first study from Northern India reporting work on SNP rs242941 and rs242939 of *CRHR1* gene in asthmatic children. Care was taken to have a precise clinical definition of asthma. However, few limitations of study need to be acknowledged. (a) We classified children according to the severity though newer GINA guidelines had recognized level of asthma control in determining appropriate therapy, since asthma severity fluctuates over time. However, our recent original article reported statistically significant association between persistent asthma and uncontrolled asthma.[22] (b) Our objective was to assess the association of CRHR1 gene polymorphism with persistent asthma, not with the occurrence of asthma; therefore, we did not recruit healthy controls. Nonetheless, further case–control studies with a larger sample size and with long-term follow-up will undoubtedly strengthen the role of the *CRHR1* gene polymorphism in childhood asthma.

CONCLUSIONS

In conclusion, we found *CRHR1* gene SNP rs242941 to be associated with persistent asthma and its phenotype in Northern Indian asthmatic children.

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