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Original Article

Protective association of IKAP T3214A polymorphism with asthma in a North Indian population: a case-control study

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ABSTRACT: IKAP participates in activation of NF-κB and activated NF-κB is associated with over production of various inflammatory cytokines, chemokines and eotaxin genes in asthma. Genotyping of IκB kinase complex associated protein (IKAP) G2295A, T3214A and C3473T polymorphisms were carried out in a total of 964 individuals (483 healthy controls and 481 asthma patients) from a North Indian population using the PCR-RFLP method. Statistical analysis revealed a significant protection from asthma in TA genotype (OR=0.75, p=0.046) and combination of TA+TT genotype (OR=0.76, p=0.043) of T3214A polymorphism. Some asthma phenotypes were also found to be protective with this polymorphism. However, no polymorphism was observed in G2295A and non-significant association was found for C3473T polymorphism. Haplotype analysis of T3214A and C3473T polymorphisms found TA and TT to be significantly associated with asthma (p=0.016 and 0.012). The present study concludes that T3214A polymorphism confers protection from asthma among the studied North Indian population.

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KEYWORDS: Asthma; case- control study; IKAP polymorphisms; PCR-RFLP; total IgE

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INTRODUCTION

Asthma is a heterozygous disease that involves complex interactions of both genetic as well as environmental factors. It is characterized by chronic inflammation of the bronchial airways owing to an over expression of the inflammatory mediators that require various transcription factors[1]. To localize some candidate genes of asthma, a number of genome-wide linkage studies have been conducted that identified almost 20 linkage regions possessing 100 potential candidate genes[2,3]. However, specific genes that directly influence this complex disease have not yet been found.

IKK complex associated protein (IKAP) is a well-conserved 150 kDa protein isolated from cells secreting IL-1. Originally, IKAP was described as a scaffold protein for the IκB kinase (IKK) complex[4] that participates in the activation of NF-κB. The IKK are serine/threonine kinases having three catalytic domains namely IKKα (85 kDa), IKKβ (87 kDa) and IKKγ or NEMO (48-kDa) (Figure 1). NF-κB is a ubiquitous transcription factor that plays a pivotal role in various inflammatory and immune responses[1]. It exists in the cytoplasm in an inactive form associated with inhibitory proteins (IκB). The serine present in the N-terminal regulatory domain of IKKα and β are rapidly phosphorylated when cells encounter a pro-inflammatory stimulus from TNF-α, IL-1β, bacterial LPS, radiation, stress signals and pathogenic assaults[5]. This results in complete destruction of IκBs inhibitory protein and spares the NF-κB complex for translocation to the nucleus that transcribes various cellular genes. The translocation of NF-κB complex also helps in the regulation of pro-inflammatory cytokines (TNF-α, IL-1β, IL-5, IL-6 and IL-8)[6,7], increased expression of nitric oxide synthase in the airway epithelial cells[8] and macrophages in patients with asthma[9]. NEMO is only activated in canonical pathway in which α and β catalytic subunits becomes more redundant[10].

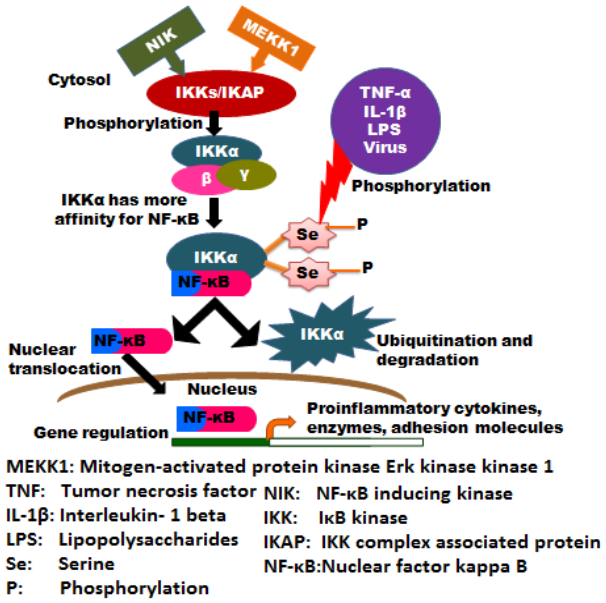

Figure 1. Systematic representation of role of IKAP in asthma.

The human IKAP gene is located on chromosome 9q31.3. Cloning and genomic characterization of mouse Ikbkap gene suggests its homology with human IKBKAP (IKAP)[11]. Ikbkap encodes a protein of 1332 amino acids with a molecular weight of approximately 150 kDa and 80% of the amino acids show identity with human IKAP. It was also observed that mutations in the IKAP gene results in the autosomal recessive disease, familial dysautonomia (FD)[12,13]. In addition, while studying childhood asthma in a Japanese population Takeoka *et al.* [14] found six SNPs in the coding region of the IKAP gene. They are C819T (Leu273Leu), G2295A (Gly765Gly), A2446C (Ile816Leu), A2490G (Ile830Met), T3214A (Cys1072Ser) and C3473T (Pro1158Leu).

Till date, literature refers to only two studies, conducted on Japanese and the Caucasian populations where association of IKAP gene polymorphisms with asthma was investigated. Their results were found to be inconsistent[14,15]. The present case-control study was conducted for the first time on a North Indian population to evaluate the role of IKAP gene polymorphism in asthma etiology.

MATERIALS AND METHODS

Ethical clearance

This study was conducted after receiving ethical clearance from the Ethics Committee, PGIMER, Chandigarh, India, vide approval memo no. PG-1Trg-10 on 21.9.2010 and strictly followed the ethical guidelines for human samples handling proposed by the ‘‘Central Ethics Committee on Human Research (CECHR) ICMR-2000’’ as well as the ‘‘Declaration of Helsinki’’. After doctor’s diagnosis, each patient was informed about the study and a due written consent was taken from each patient prior to inducting him/her in the study. However, only patients fulfilling the criteria of GINA (Global Initiative for Asthma) guidelines were recruited for the study.

Inclusion/ Exclusion criteria

Recruitment of patients for this study were from different states of North India including Chandigarh, Punjab, Haryana, Himachal Pradesh, Uttaranchal, Jammu and Kashmir, Rajasthan, Uttar Pradesh, and New Delhi. A total of 481 patients were enrolled as cases visiting OPD (Out Patient Department), Pulmonary Medicine at Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh. The other recruits were 483 age-matched, healthy controls. Those subjects that did not possess any symptoms of atopic, pulmonary disease, any other co-morbid disease or smoking habits were recruited as controls.

Asthma patients with any other history of respiratory illness like COPD, tuberculosis, pneumonia, bronchitis were excluded from this study. Pregnant females and individuals with any other co-morbid illnesses such as diabetes mellitus, hypertension were also excluded.

Pulmonary function test (Spirometry)

Spirometer measures several aspects of lung function that are important in determining both the severity and the prevention of asthma. Spirometry tests were performed strictly in accordance with Association of Respiratory Technician and Physiologists (ARTP) guidelines[16] for generating pneumotachographs, helpful in assessing conditions such as asthma, COPD *etc.* using device Spiro 233 (PK Morgan, Rainham, Kent, UK).

Out of 481 asthma patients, spirometry was done on 377 asthmatics which were further categorized according to their severity. The frequency of mild obstruction was found to be higher in the studied population (Table 1).

Allergy screening tests

Allergic diseases including asthma are characterized by an increase of serum Immunoglobulin E (IgE) levels. IgE initiates and propagates an inflammatory cascade which leads to allergic responses in asthma. Total serum IgE was measured using ImmunoCAPs with the device, Phadia 100 IDM version 5.43 (Thermo Fisher Scientific Inc., USA) in serum samples of both control (n=125) and asthma patients (n=213) in order to screen for allergy.

Skin Prick Test (SPT) and serum specific IgE against *Aspergillus fumigatus* were also done in some patients to distinguish asthma and ABPA (Allergic bronchopulmonary aspergillosis). Only SPT negative patients with specific IgE < 0.35 KUA/L were recruited in the study (Table 1). Usually the accepted upper limit of serum IgE is between 150 to 300 IU/mL but they can range from 150 to 1,000 IU/mL[17] as observed in the present study. These variations in the range are due to changes in the diet, genetic background, geographical location *etc.*[18]

Body Mass Index (BMI)

Increased BMI exaggerates the risk of acquiring asthma. BMI is a measure of human body shape based on an individual's weight and height which indicate

underweight (<18.5 kg/m^2), normal (18.5–24.9 kg/m^2), overweight (25–29.9 kg/m^2) or obese (>30 kg/m^2).

Blood sample collection

Approximately 5mL blood from each patient as well as control subjects was taken in EDTA coated vials for extraction of genomic DNA and stored at -80^{o}C. Genomic DNA was isolated from frozen whole blood samples using SSC Buffer method[19] and checked on 0.8% agarose gel by electrophoresis before storage at -20^{o}C for further use.

Genotyping

Detection of the IKAP polymorphisms was done by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method using the following specific primers[15]:

G2295A (Gly765Gly): 5'-CTGTTTAATGAAGGTTTCCAGATT-3'
5'-CCCTAAGGTAACTTTCTAAGCTG-3'
T3214A (Cys1072Ser): 5'-AGGAATTGAGTTTACCTGGGGAC-3'
5'-AGTCAACTGCTGCTTATTGTCTC-3'
C3473T (Pro1158Leu): 5'-GTAGTTCGAGAGCTCAAGGA-3'
5'-TGCCACTCACGACACTGCT-3'

The PCR was carried out in a thermal cycler, in a total volume of 25µl containing: 10X PCR Buffer, 3 mM $MgCl_2$, 1 mg/mL nuclease free BSA , 50 pmol of each primer, 10 mM of each dNTP, 0.125 U *Taq* polymerase and 2µl genomic DNA. Amplification conditions were 95°C for 5 min, followed by 35 cycles of 94°C for 30 s, 60°C for 30 s and 72°C for 30 s with a final extension for 5 min at 72°C. PCR products of both the IKAP G2295A and T3214A polymorphisms were digested with the *Hin*fI (New England BioLabs, UK) at 37°C for 4h and C3473T was digested with *Kpn*I (New England BioLabs, UK) at 37°C for 4h.

Identification of IKAP G2295A and T3214A polymorphisms

For IKAP G2295A and T3214A polymorphisms, 10µL of the PCR product was digested at 37°C for 4h with 5U of restriction endonuclease, *Hin*fI. In G2295A polymorphism, no digested bands were observed in any sample, only PCR amplified product were obtained (Figure 2). For T3214A polymorphism, an uncut band of 105 bp indicated the wild type TT genotype. The heterozygote TA genotype was indicated by the digested bands of 105 bp, 84 bp and 21 bp sizes while the mutant AA genotype was indicated by bands of 84 bp and 21 bp (Figure 3).

Identification of IKAP C3473T polymorphism

For IKAP C3473T polymorphism, 10µL of the PCR product was digested at 37°C for 4h with 5U of restriction endonuclease, *Kpn*I. After digestion, bands of 545 bp and 58 bp indicated wild type CC genotype. The heterozygote CT genotype was indicated by bands of 603 bp, 545 bp and 58 bp sizes while the mutant TT genotype was indicated by an uncut band of 603 bp (Figure 4).

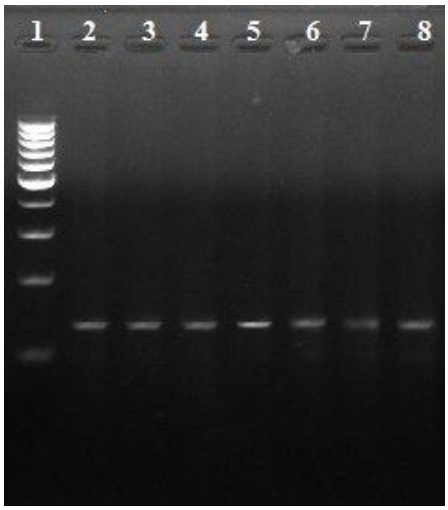

Figure 2. Restriction digestion (*Hin*fI) products of IKAP G2295A polymorphism on 2% agarose gel.

Lane 1: 100 bp ladder
Lanes 2-8: homozygous mutant AA (133bp)

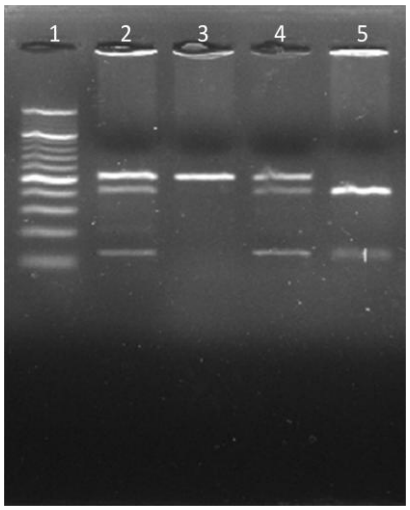

Figure 3. Restriction digestion (*Hin*fI) products of IKAP T3214A polymorphism on 2% agarose gel.

Lane 1: 20 bp ladder
Lane 3: homozygous wild TT (105 bp)
Lanes 2, 4: heterozygous TA (105 bp, 84 bp and 21 bp)
Lane 5: homozygous mutant AA (84 bp and 21 bp)

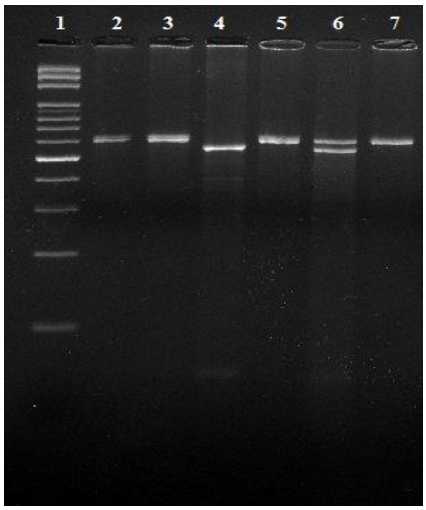

Figure 4. Restriction digestion (*Kpn*I) products of IKAP C3473T polymorphism on 2% agarose gel.

Lane 1: 100 bp ladder
Lanes 2, 3, 5, 7: homozygous mutant TT (603 bp)
Lane 4: homozygous wild CC (545 bp and 58 bp)
Lane 6: heterozygous CT (603 bp, 545 bp and 58 bp)

STATISTICAL ANALYSIS

All statistical analyses were performed using the SPSS software for Windows version 20.0 (SPSS, Inc., Chicago, IL, USA) and Epi Info version 3.4.7 (Centers for Disease Control and Prevention, Atlanta, GA, USA). Chi-square analysis was used to check the deviation from Hardy-Weinberg equilibrium (HWE) and to compare the genotype and allele frequency between asthma and control groups. Odds ratio (OR) and 95% confidence

interval (CI) were used for the assessment of risk factors and p value < 0.05 was considered as statistically significant. PLINK v1.07[20] was used for calculation of haplotypes and Haploview v4.2[21] for calculating linkage disequilibrium.

RESULTS

Studied population characteristics

In the present study, three IKAP polymorphisms (G2295A, T3214A and C3473T) were genotyped in a total of 964 subjects, including 483 healthy controls and 481 asthma patients. Many variables including total IgE, smoke exposure, family history, spirometry diagnosis, cough, occurrence, severity, BMI, *etc*., were examined, which have a significant role in asthma severity. The mean age of asthma patients were 37 years and age matched healthy controls with mean age of 34 years were found to be significantly associated (p=0.001). Mean disease duration was more than 9 years in patients and 28% asthmatic had a family history of asthma. Total serum IgE concentration (IU/mL) was assessed for 213 asthma patients and 125 control subjects and the average of total IgE was higher in the asthmatics (2651.7 IU/mL) than the controls (776.1 IU/mL) having p=0.012. Spirometry data was only available for asthma patients so we were unable to apply the statistics (Table 1).

Table 1. Characteristic of studied population.

Phenotypic traits	Asthma patients n (%)	Controls n (%)	p
Gender	481	483	
Males	191 (39.7)	189 (39.1)	0.854
Females	290 (60.3)	294 (60.9)	$\chi2$=0.034
Age (Mean ±SD; years)	37.22±14.1 (range 18-57 years)	34.29±12.2 (range 18-60 years)	0.001* t=3.43
Disease duration (years)	9.23	0	
Rhinitis			0.000*
Allergic rhinitis	397 (82.5)	0	$\chi2$=674.37
No rhinitis	84 (17.5)	483	
Allergy			0.000*
Allergic to at least 2 provoking factors	400 (83.5)	0	$\chi2$=683.11
Non- allergic	81 (16.8)	483	
Smoking status			0.000*
Ever-smoker	56 (11.6)	0	$\chi2$=57.59
Non-smoker	425 (88.5)	483	
Spirometry data[a]	(n=377)	nd	
FVC observed	2.94±1.1		
FVC predicted	2.23±1.6		
FEV1 observed	2.74±1.1		
FEV1 predicted	2.61±0.66		
FEV1/FVC observed (%)	68.92%		
FEV1/FVC predicted (%)	87.81%		
Family history	135 (28%)	0	0.000* $\chi2$=155.32
BSA (m^2)	1.61±0.20	1.59±0.28	0.585 t=0.547
BMI (kg/m^2)	23.7±2.1	23.96±12.3	0.606
Underweight ≤18.5	16.6	17.4	t=0.516
Normal weight = 18.5–24.9	21.15	21.3	
Overweight = 25–29.9	26.85	26.7	
Obesity ≥ 30	33.7	30.8	
IgE (IU/mL)[b]	2651.7	776.1	0.012*
Asthma severity[a]	n=377	nd	
Normal	126		
Mild obstruction (FEV1%pred > 60%)	153		
Moderate obstruction (40% < FEV1%pred > 60%)	65		
Severe obstruction (FEV1%pred < 40%)	33		

n- Number of subjects sampled, FVC- Forced Vital Capacity, FEV1- Forced Expiratory Volume in 1 second, BSA- Body Surface Area, BMI- Body Mass Index, nd- not done, %- frequency, pred-predicted, t- Student's t test, $\chi2$-chi square, *- significant

[a]Spirometry test was conducted for 377 asthma patients

[b]IgE levels were confirmed for 213 asthma patients and 125 controls and given as average in IU/mL

Prevalence of allelic and genotypic frequencies in IKAP polymorphisms

Statistical analysis could not be performed for the IKAP G2295A polymorphism as only the A allele was prevalent in both the asthmatic as well as the control populations.

Statistical analysis of IKAP T3214A (rs3204145) polymorphism indicated that the overall distribution of the wild (T) allele had slightly increased frequency among the asthma patients (83.3%) as compared to the controls (79.9%) while mutant (A) allele had increased frequency among controls (20.1%) as compared to the asthmatics (16.7%) with non-significant association (OR=0.94, p=0.582) (Table 2).

Genotypic frequencies revealed the homozygous wild (TT) genotype to be more prevalent in asthma patients (71.1%) than the controls (65.0%). The heterozygous genotype (TA) was more prevalent among the controls (29.8%) as compared to asthma patients (24.3%) with protective association (OR=0.75, p=0.046). The homozygous mutant AA genotype was more prevalent in the controls (5.2%) than in the asthmatics (4.6%) with a non-significant association (OR=0.81, p=0.480). However, the TA+AA genotypic combination again had protective association with asthma (OR=0.76, p=0.043) (Table 2).

Comparison of allelic frequencies of IKAP C3473T (rs1538660) polymorphism indicated that the overall distribution of the wild (C) allele had slightly increased frequencies among the controls (65.0%) as compared to the asthma patients (64.2%), while the mutant (A) allele was slightly more prevalent among the asthmatics (35.8%) than in the controls (35.0%) having non-significant association (OR=0.97, p=0.724) (Table 2).

Genotypic frequencies revealed that the homozygous wild (CC) genotype was more prevalent in the controls (40.4%) than in the asthmatics (36.8%). The heterozygous genotype (CT) had increased trends among the asthmatics (49.3%) as compared to the controls (54.9%) having no association (OR=1.22, p=0.143). The homozygous mutant (TT) genotype was more prevalent in the controls (10.4%) than in the asthmatics (8.3%) with a non-significant association (OR=0.88, p=0.593). The CT+TT genotypic combination also had no association with asthma (OR=1.16, p=0.254) (Table 2).

Table 2. IKAP (T3214A and C3473T) genotypic and allelic frequencies.

Polymorphisms	Asthma patients 481 (%)	Controls 483 (%)	χ^2	OR(95% CI)	p
Genotypic frequencies					
IKAP(T3214A) rs3204145					
TT	342 (71.2)	314 (65.0)		Ref (1.0)	
TA	117 (24.3)	144 (29.8)	3.99	0.75 (0.55-1.01)	0.046*
AA	22 (4.6)	25 (5.2)	0.50	0.81 (0.43-1.52)	0.480
TT vs TA+AA	139 (28.9)	169 (35.0)	4.11	0.76 (0.57-1.00)	0.043*
IKAP(C3473T) rs1538660					
CC	177 (36.8)	195 (40.4)		Ref (1.0)	
CT	264 (54.9)	238 (49.3)	2.14	1.22 (0.93-1.61)	0.143
TT	40 (8.3)	50 (10.4)	0.29	0.88 (0.54-1.44)	0.593
CC vs CT+TT	304 (63.2)	288 (59.7)	1.30	1.16 (0.89-1.52)	0.254
Allelic frequencies					
IKAP(T3214A) rs3204145					
T	801 (83.3)	772 (79.9)		Ref (1.0)	
A	161(16.7)	194 (20.1)	0.30	0.94 (0.74-1.19)	0.582
IKAP(C3473T) rs1538660					
C	618 (64.2)	628 (65.0)		Ref (1.0)	
T	344 (35.8)	338 (35.0)	0.12	0.97 (0.80-1.17)	0.724

Ref- reference, %- frequency, χ2-chi square, OR- odds ratio, CI- confidence interval, *- significant

Both the polymorphisms (T3214A and C3473T) in the studied population followed Hardy-Weinberg equilibrium (HWE). In addition, the TA and TT haplotypes in the IKAP T3214A and C3473T polymorphisms were found to be significantly associated with asthma with p=0.016 and 0.012 respectively. Linkage disequilibrium between markers was calculated by D′ and r^2 statistics from parental haplotypes and was observed to be 0.797 and 0.264 respectively for both the polymorphisms in the patients as well as the controls (Table 3).

Table 3. Haplotype frequency and LD of polymorphisms at IKAP T3214A and C3473T.

Haplotype	Frequency (Asthma)	Frequency (Control)	χ2	p	LD	
					D'	r2
TA	0.139	0.179	5.84	0.016*	0.797	0.264
CA	0.028	0.020	1.26	0.262		
TT	0.214	0.169	6.26	0.012*		
CT	0.618	0.631	0.318	0.573		

LD-Linkage disequilibrium, χ2-chi square, *- significant

Comparison of SNPs allele frequency with phenotypic characteristics of the asthma patients and the controls

From the detailed patient proforma phenotypic characteristics of the asthma patients were further categorized on the basis of the disease (Table 4). This revealed a protective association between T3214A polymorphism and asthma when the occurrence of seasonal symptoms (OR=0.69, p=0.008) and patients without rhinitis (OR=0.57, p=0.020) was compared while none of the phenotypic parameters were significantly associated with C3473T polymorphism and asthma (p>0.05) (Table 5).

Table 4. Phenotypic characteristics and IKAP T3214A polymorphism.

Phenotypic traits	n (%)	T/T n (%)	T/A n (%)	A/A n (%)	T n (%)	A n (%)	χ^2	OR (95% CI)	p
Controls	483	314(65.0)	144(29.8)	25(5.2)	772(79.9)	194(20.1)		Ref (1.0)	
Males	189(39.1)	128(40.8)	50(34.7)	11(44.0)	306(80.9)	72(19.1)			
Females	294(60.9)	186(59.2)	94(65.3)	14(56.0)	466(79.3)	122(20.7)			
Asthma patients	481								
Sex									
Males	191(39.7)	137(71.7)	49(25.7)	5(2.6)	323(84.6)	59(15.4)	1.73	0.78(0.52-1.15)	0.189
Females	290(60.3)	205(70.7)	68(23.4)	17(5.9)	478(82.4)	102(17.6)	1.88	0.82(0.60-1.10)	0.170
Occurrence									
Seasonal	295(61.3)	219(74.3)	65(20.0)	11(3.7)	503(85.3)	87(14.7)	7.05	0.69(0.52-0.92)	0.008*
Perennial	186(38.7)	123(66.1)	52(28.0)	11(5.9)	298(80.1)	74(19.9)	0.01	0.99(0.72-1.35)	0.935
Severity									
Wheeze onExertion	126(26.2)	92(73.0)	29(23.0)	5(4.0)	213(84.5)	39(15.5)	2.74	0.73(0.49-1.08)	0.098
Wheeze at Rest	355(73.8)	250(70.4)	88(24.8)	17(4.8)	588(82.8)	122(17.2)	2.25	0.83(0.64-1.07)	0.134
Family History									
Family History (Nil)	346(71.9)	243(70.3)	88(25.4)	15(4.3)	574(82.9)	118(17.1)	2.42	0.82(0.63-1.06)	0.119
Family History (+ve)	135(28.1)	99(73.3)	29(21.5)	7(5.2)	227(84.1)	43(15.9)	2.35	0.75(0.52-1.10)	0.125
Rhinitis									
Rhinitis (Nil)	84(17.5)	65(77.4)	17(20.2)	2(2.4)	147(87.5)	21(12.5)	5.36	0.57(0.34-0.94)	0.020*
Rhinitis (+ve)	397(82.5)	277(69.8)	100(25.2)	20(5.0)	654(82.4)	140(17.6)	1.70	0.85(0.66-1.09)	0.192
Allergy									
Allergy (Nil)	81(16.8)	57(70.3)	19(23.5)	5(6.2)	133(82.1)	29(17.9)	0.42	0.87(0.55-1.36)	0.519
Allergy (+ve)	400(83.2)	285(71.3)	98(24.5)	17(4.2)	668(83.5)	132(3.73)	3.73	0.79(0.61-1.01)	0.053
Smoking Status									
Non Smoker	425(88.4)	302(71.1)	104(24.5)	19(4.4)	708(83.3)	142(16.7)	3.42	0.80(0.62-1.02)	0.064
Ever Smoker	56(11.6)	40(71.4)	13(23.2)	3(5.4)	93(83.0)	19(17.0)	0.62	0.81(0.47-1.40)	0.432
Cough									
Cough (Nil)	302(62.8)	211(69.9)	77(25.5)	14(4.6)	499(82.6)	105(17.4)	1.76	0.84(0.64-1.10)	0.185
Long-lasting Cough	179(37.2)	131(73.2)	40(22.4)	8(4.4)	302(84.4)	56(15.6)	3.36	0.74(0.53-1.03)	0.066

Ref- reference, %- frequency, χ2-chi square, OR- odds ratio, CI- confidence interval, *- significant

Table 5. Phenotypic characteristics and IKAP C3473T polymorphism.

Phenotypic traits	n (%)	C/C n (%)	C/T n (%)	T/T n (%)	C n (%)	T n (%)	χ^2	OR (95% CI)	p
Controls	483	195(40.4)	238(49.3)	50(10.3)	628(65.0)	338(35.0)		Ref (1.0)	
Males	189(39.1)	84(44.4)	87(49.1)	18(9.5)	255(67.5)	123(32.5)			
Females	294(60.9)	111(37.8)	151(51.4)	32(10.8)	373(63.4)	215(36.6)			
Asthma patients	481								
Sex									
Males	191(39.7)	75(39.3)	97(50.8)	19(9.9)	247(64.7)	135(35.3)	0.66	1.13(0.83-1.55)	0.415
Females	290(60.3)	102(35.2)	167(57.6)	21(7.2)	371(64.0)	209(36.0)	0.04	0.98(0.76-1.25)	0.851
Occurrence									
Seasonal	295(61.3)	112(38.0)	157(53.2)	26(8.8)	381(64.6)	209(35.4)	0.03	1.02(0.82-1.27)	0.862
Perennial	186(38.7)	65(34.9)	107(57.5)	14(7.5)	237(63.7)	135(36.3)	0.20	1.06(0.82-1.37)	0.656
Severity									
Wheeze on Exertion	126(26.2)	51(40.5)	68(54.0)	7(5.5)	170(67.5)	82(32.5)	0.53	0.90(0.66-1.22)	0.466
Wheeze at Rest	355(73.8)	126(35.5)	196(55.2)	33(9.3)	448(63.1)	262(36.9)	0.65	1.09(0.88-1.34)	0.420
Family History									
Family History (Nil)	346(71.9)	117(33.8)	203(58.7)	26(7.5)	437(63.2)	255(36.8)	0.61	1.08(0.88-1.34)	0.436
Family History (+ve)	135(28.1)	60(44.4)	61(45.2)	14(10.4)	181(67.0)	89(33.0)	0.38	0.91(0.68-1.23)	0.536
Rhinitis									
Rhinitis (Nil)	84(17.5)	41(48.8)	38(45.2)	5(6.0)	120(71.4)	48(28.6)	2.63	0.74(0.51-1.08)	0.105
Rhinitis (+ve)	397(82.5)	136(34.3)	226(56.9)	35(8.8)	498(62.7)	296(37.3)	0.99	1.10(0.90-1.35)	0.319
Allergy									
Allergy (Nil)	81(16.8)	34(42.0)	37(45.7)	10(12.3)	105(64.8)	57(35.2)	0.00	1.01(0.70-1.45)	0.961
Allergy (+ve)	400(83.2)	143(35.8)	227(56.8)	30(7.5)	513(64.1)	287(42.1)	0.15	1.04(0.85-1.27)	0.699
Smoking Status									
Non Smoker	425(88.4)	152(35.8)	237(55.8)	36(8.5)	541(63.6)	309(36.4)	0.37	1.06(0.87-1.29)	0.545
Ever Smoker	56(11.6)	25(44.6)	27(48.2)	4(7.1)	77(68.8)	35(31.2)	0.62	0.84(0.54-1.31)	0.431
Cough									
Cough (Nil)	302(62.8)	110(36.4)	166(55.0)	26(8.6)	386(63.9)	218(36.1)	0.20	1.05(0.84-1.30)	0.657
Long-lasting Cough	179(37.2)	67(37.4)	98(54.7)	14(7.8)	232(64.8)	126(35.2)	0.00	1.01(0.78-1.31)	0.944

Ref- reference, %- frequency, χ2-chi square, OR- odds ratio, CI- confidence interval

DISCUSSION

In the present study, genotyping of three out of six SNPs in the coding region of IKAP (G2295A, T3214A and C3473T) were carried out in 964 individuals (483 healthy controls and 481 asthmatics) and for the first time we report a protective role of T3241A (rs3204145) polymorphism in a North Indian population with asthma. The other three polymorphisms of IKAP gene show consistent results (non-significant association) as previously reported in the Japanese and the Caucasian populations[14, 15], so these were excluded from the study. Our results revealed a significant protective association between TA (p=0.046) as well as the combination of TA+AA genotype (p=0.043) of T3241A polymorphism (Table 2). Although it is a weak association, some of the phenotypic parameters of the disease strongly support the above findings (Table 4).

Ubiquitination of IκB inhibitor proteins activate NF-κB that over expresses various inflammatory cytokines (IL-1β, TNF-α, GM-CSF), chemokines (IL-8, MIP-1α, MCP-1, RANTES) and eotaxin genes in asthma[22,23]. A study conducted on 20 patients with COPD revealed that hypoxia is inversely related to mRNA expression of NF-κB inhibitor IκBα and to the regulatory proteins IKKγ and IKAP[24]. Another sib pair study conducted on German and Swedish population using 97 families (415 persons and 156 sib pairs) observed a locus linked to bronchial asthma susceptibility on chromosome 9q34, where the IKAP gene lies with elevated serum IgE levels (IgE; p=0.0098) and positive radio-allergosorbent test (RAST; p=0.0025)[25].

Although, these studies found association of IKAP and asthma, the exact mechanism of IKAP in asthma pathogenesis is still unclear.

Until now, only two studies on the IKAP polymorphisms and asthma have been conducted so far. The very first study was conducted on a Japanese childhood asthmatic population in which they genotyped six SNPs in the coding region of the IKAP and detected a strong allelic association of T3214A and C3473T polymorphisms with asthma (p=0.000 and 0.001). The other four polymorphisms (C819T, G2295A, A2446C and A2490G) had no association with the disease in their study[14]. This is partly consistent with our data in which we found a protective association of IKAP T3214A with asthma in TA genotype (p=0.046) and phenotypic parameters such as occurrence of seasonal symptoms (p=0.008) and patients with no rhinitis (0.020). Takeoka *et al.*[14] had found the TGAAAT haplotype to be probably associated with early-onset of asthma. Our study also revealed a significant association of haplotypes TA and TT in T3214A and C3473T polymorphisms with disease susceptibility.

The second study conducted on 682 Caucasian subjects including 373 patients and 309 healthy controls investigated the role of five out of the six known SNPs in the IKAP gene (T819C, G2295A, A2490G, T3214A and C3473T). They found no polymorphism in position G2295A, where only the A allele was detected in both the patients as well as the control groups. This result is in accordance with our findings, wherein we also found no polymorphism in position G2295A. They also observed no significant differences in the genotypic and allelic distributions for any of the SNPs in the IKAP gene in the patients or the subgroup of patients with atopic asthma as compared to the healthy controls[15]. In the present study non-significant association was observed in the genotypic and allelic distributions for IKAP C3473T polymorphism.

Although, there is little evidence till now about the role of IKAP and NF-κB activation thereby the production of inflammatory and immune responses[24], the present study found a protective association between them in the inflammatory disease development. This might be due to ethnic differences, experimental scenario and most importantly due to the complex interplay of both genetic as well as environmental factors associated with the disease.

CONCLUSIONS

The aim of the present study was to shed light on the role of IKAP polymorphism on asthma pathogenesis among North Indian population. Our findings suggest that the T3214A polymorphism contributes protection from asthma. However, the detailed signal transduction pathway of IKAP is yet to be discovered, therefore further studies are needed to reveal novel insights between IKAP and responses to allergic diseases.

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