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IL-13 and STAT 6 Gene Variants and Atopic Disorders-A Case Control study from the Northern State of India

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Abstract

Inflammatory markers are considered as a common biochemical manifestation among subjects with atopic disorders. The current study was aimed to study the possible association of IL-13 and STAT 6 genes among atopic subjects.

A total of 528 patients diagnosed with atopy and 610 normal subjects were taken for the polymorphic analysis of IL-13 and STAT 6 genes. On stratification, we observed a significant difference between cases and controls with various demographic, life style and dietary factors. Subjects harboring homozygous variant genotypes of IL-13–C1055 T and IL-13–A1512 C showed a statistically significant increase in atopy (OR=1.8 (1.2–2.6), P=0.001) and (OR=6.7 (4.9-9.2), P<0.0001) respectively. Similarly, the variant genotype of STAT 6 G2964 A showed a substantial increase towards atopy when harbor by the subjects (OR=3.7 (2.7–5.1), P<0.0001). Additionally, the above genetic variants of all the genes significantly modified the risk of allergy when stratification was done on the basis of various lifestyle, epidemiological and clinico-pathological characteristics.

Keywords: Atopy • IL-13 • STAT 6 • Gene variants • Kashmir

Introduction

Allergic diseases are of great health concern with very wide geographical difference in its incidence. Though, the disease is being considered as an epidemic in developed countries and has doubled in past 2 to 3 decades but, currently the incidence is increasing alarmingly both in developing and developed world [1]. India is experiencing an increasing trend, which otherwise was considered as low endemic zones for such disorders. The disease is multi etiological without a single factor being established as a causal agent. Therefore, etiology remains unclear rather than conclusive. Epidemiological research over the last years has helped to identify possible environmental factors associated with allergic diseases [2]. Among the various risk factors like smoke in different forms, pollution, microbial infections, occupational exposures, diet, pesticide use etc have been associated with allergies. However, the worldwide variation in its incidence suggests a complex interplay of multiple environmental, socioeconomic, and cultural factors. Further research regarding the role of various risk factors in modulating differences in allergic disease prevalence between migrant and native populations will enhance our understanding of the complex gene-environment interactions involved in the pathogenesis of allergic disorders [3,4].

The condition that result due to exposure of body to environmental substances is characterized by raised IgE levels a feature of allergic diseases. It results due to immune reaction to a substance called an allergen, which is normally harmless. Allergen-specific IgE-antibodies cause most allergic reactions. A number of atopy susceptibility interleukin genes have been identified which regulate the production of IgE. Genome Wide Association Studies (GWAS) have identified strong linkage of different genes with atopy in different populations. IL-13 and STAT 6 genes have been found to be involved in the regulation of IgE synthesis and manifestation of allergic disorders [5,6].

A number of studies regarding the different polymorphic variants of inflammatory markers and different allergic phenotypes are available. However, such studies are lacking in this part of the world. With a suitable environment for boarding a huge variety of allergens, Kashmir valley has a significant number of atopic patients. On the basis of our outpatient department experience, there has been an increasing flow of atopic patients seeking medical attention for complaints ranging from seemingly mild symptoms such as watery and itchy eyes, cough and cold to serious asthmatic attacks. There is a general perception that visible particles in air such as fluffy seeds from poplars and blooms cause a rise in allergies in Kashmir but there is no concrete scientific data to support such observations [7].

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Since, in allergic disorders an increased level of IgE and mutations in different inflammatory genes like IL4, IL-13 etc. are a common biochemical manifestation as reported elsewhere with none of the studies available in study population, it has created the opportunity to develop novel clinical strategies for the management and treatment of these patients in study population. Therefore, we intend to identify the patients having atopy and elucidate the role of IL-13 and STAT 6 gene variants alone as well as their combinational effect on atopy in presence of different life style and environmental factors.

Materials and Methods

Patients and controls

A total of 528 confirmed cases diagnosed with "atopy" attending allergy clinics, Sher-i- Kashmir Institute of Medical Sciences (SKIMS), department of dermatology, SKIMS Medical College, Srinagar and department of dermatology, SMHS Hospital, Srinagar were included in this study. Blood samples were collected from all 528 cases. Besides blood, samples were obtained from 610 no-atopic subjects from the out patients departments of above departments. A written pre-informed consent was obtained from all cases and controls. The study was approved by the Institutional Ethical Clearance Committee of SKIMS, Srinagar vide order No. SIMS 1 131/IEC-SKIMS/2016-133 [8,9].

Data collection

Detailed information on age, sex, place of residence, ethnicity, religion, education, dietary data including and other potential confounding factors of interest was collected using a questionnaire specifically designed for the study population. Detailed information on life-long history of use of several tobacco products was obtained. Information on family history of any allergic disorder was obtained from all the participants. To assess the Socio-Economic Status (SES) of the subjects, information on potential parameters of SES was obtained including education level (highest level attained), monthly income (INR), house type and ownership of several household appliances. Similarly, the information regarding, second hand smoking was also acquired from all the subjects. For certain parameters, due to logistic constraints (or not required in some parameters) we could not analyze them in control group. The parameters which we were missed in control group include PFT, SPT, time of peak allergies, history of allergies in families, comorbidities, collateral diseases and diagnosis. For PFT parameters, we followed the guidelines as per reports published elsewhere. Briefly, In we calculated the basic parameters like Vital Capacity (VC) the maximal volume of air that can be exhaled after a maximal inhalation or the maximal volume of air that can be inhaled after a maximal exhalation; Forced Expiratory Volume (FEV), Forced Vital Capacity (FVC) and FEV/VC% (calculated as FEV/VC × 100). In most cases these variables suffice to provide all the information needed to interpret a spirogram. The normal limits of FEV and FVC is considered to be 80 to 120%, while as the normal reference range for FEV/VC% should fall within 5% of the predicted ratio [10].

Genetic analysis of IL-13 and STAT 6

Five milliliters of venous blood was collected from each subject in sterilized plastic vials containing EDTA (0.5 M; pH=8.0) and stored at -80°C before DNA extraction. Genomic DNA was extracted from collected blood samples using the phenol chloroform method and kit based method (DNASure Blood Mini Kit from Nucleo-pore) [11].

The details of PCR conditions, primers, restriction enzyme, and length of expected fragments on digestion, minor alleles and change in nucleotide position of the above genes are given in Table 1.

It is pertinent to mention that two different SNPs were simultaneously studied in case of IL-13 gene based on their reported association with different inflammatory diseases which is common manifestation among patients with allergic diseases [12].

Gene polymorphism analysis

RFLP for IL-13–C1055 T missense mutation (rs20541): PCR was setup to amplify the 372 bp product followed by restriction enzyme digestion by Hpy 199 I restriction enzyme (New England Biolabs, USA). The details of the PCR product and digestion details are given in Figure 1.

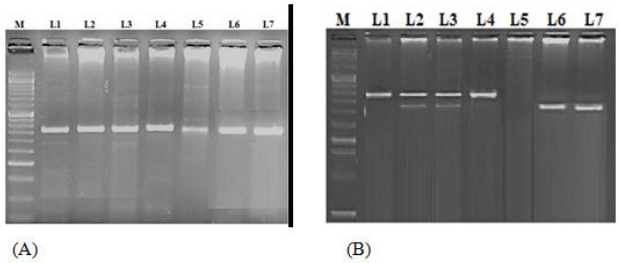

Figure 1(A). Representative gel picture of IL13 promoter region amplicon, M: 50 bp ladder, Lanes 1-7: Amplicons (product size 372 bp); (B): Restriction fragment length polymorphism pattern of IL13 – C1055T using Hpy199I restriction enzyme. Lane M: 50bp ladder, Lane 6 and 7 represent the Homozygous wild (C/C) genotype (size 339 and 33bp), Lane 1 and 4 represent homozygous variant (T/T) genotype (size 372bp uncut product), Lane 2 and 3 represent C/T heterozygous genotype (size 372,339 and 33bp), Lane 5 represents negative control.

RFLP for IL-13–C1512A SNP (rs1881457): PCR was setup to amplify the 214 bp product, which was subjected to restriction digestion with Bsh 1236I restriction enzyme (Thermo Fisher Scientific, USA). Details provided in Figure 2 and Table 1.

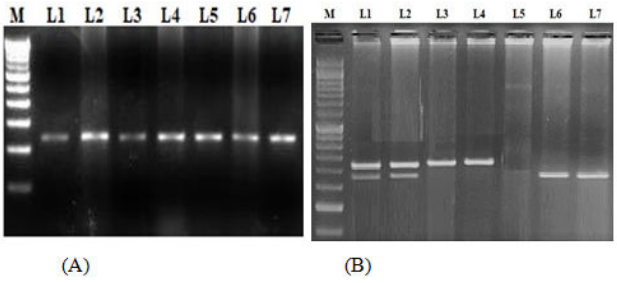

Figure 2 (A). Representative gel picture of IL13 promoter region amplicon, M: 100 bp ladder, Lanes 1-7: Amplicons (product size 214 bp); (B): Restriction fragment length

polymorphism pattern of IL13-C1512A using Bsh1236I restriction enzyme. Lane M: 50 bp ladder, Lane 6 and 7 represent the Homozygous wild (C/C) genotype (size 192 and 22 bp), Lane 3 and 4 represent homozygous variant (A/A) genotype (size 214 bp uncut product), Lane 1 and 2 represent C/A heterozygous genotype (size 214, 192 and 22 bp), Lane 5 represents negative control.

Gene	Nucleotide change	Primer sequence	Product size (bp)	DP (bp)
IL-13	Promoter (– C1055T)	FP: 5'- ACTTCTGGGAGTCAGAGCCA -3' RP: 5'- TACAGCCATGTCGCCTTTTC CTGCTCTTCCGTC- 3'	372	AP=372 HW=339 and 33 HTZ=372, 339 and 33
	Promoter (– C1512A)	FP: 5'- CAACCGCCGCGCCAGCGCC TTCTC-3' RP: 5'- CCGCTACTTGGCCGTGTGAC CGC-3'	214	AP=214 HW=192 and 22 HTZ=214, 192 and 22
STAT6	3' UTR (G2964A)	FP: 5'- GAAGTTCAGGCTCTGAGAGA C-3' RP: 5'- CCATCACCCTCAGAGAGC-3'	93	AP=93 HW=74 and 19 HTZ=93, 74 and19

FP: Forward Primer; RP: Reverse Primer; DP: Digestion Product; AP: Amplified Product; HW: Homozygous Wild; HTZ: Heterozygous; HM: Homozygous Mutant

Table 1. Primer and RFLP details of IL-13 and STAT 6 genes.

RFLP for STAT 6 G2964A (3'UTR) SNP (rs 324015): PCR was setup to amplify the 93 bp product. Enzyme digestion was carried out using BsaHI or Hin1I restriction enzymes (New England Biolabs, USA). The details are given in Figure 3.

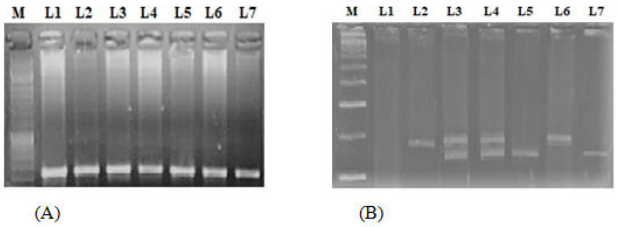

Figure 3 (A). Representative gel picture of STAT 6 3' UTR amplicon, M: 50 bp ladder, Lanes 1-7: Amplicons (product size 93 bp); (B): Restriction fragment length polymorphism pattern of STAT6 G2964A using BsaHI or Hin1I restriction enzyme. Lane M: 50 bp ladder, Lane 5 and 7 represent the Homozygous wild (G/G) genotype (size 74 and 19 bp), Lane 2 and 6 represent homozygous variant (A/A) genotype (size 93 bp uncut product), Lane 3 and 4 represent G/A heterozygous genotype (size 93,74 and 19 bp), Lane 1 represents negative control.

The European Molecular Genetics Quality Network (EMQN) good practice guidelines have been followed. A few PCR vials with all the PCR contents except the DNA, were also included per PCR batch as "negative controls". No contamination was observed and there were no "false positives". To minimize the risk of contamination, sterilized and autoclaved solutions and equipment were used during DNA isolation. The ingredients for PCR were well stored at -20°C and were thawed just before use. Retyping of samples was done at random to check for the homology of results.

Statistical analysis

Categorical variables were set for presenting and calculating numbers and percentages for different genotypes of IL-13 and STAT 6. Test for Hardy-Weinberg Equilibrium (HWE) were conducted by comparing observed and expected genotype distributions by the χ^2 goodness of fit. Statistical significance for departure of a genotype frequency from its expected frequency under the HWE model was set at $P \leq 0.05$. Conditional logistic regression models were used to calculate Odds Ratios (ORs) and corresponding 95% Confidence Intervals (95% CIs) to assess the association of the genotypes with allergies and to assess the analysis of effect modification of gene-gene and Gene-Environment Interaction (GEI). All statistical analysis was done using STATA software, version 14 (STATA Corp., College Station, TX, USA). Two sided P values <0.05 were considered as statistically significant.

Results

Characteristics of study subjects

A total of five hundred twenty-eight (n=528) consecutive patients diagnosed with atopy and six hundred ten (n=610) no atopic subjects (at least free from any sort of Allergy) were included for the polymorphic analysis of IL-13 and STAT 6 genes. Almost all the cases had attended the hospital with a clinical presentation of atopy.

Among cases 60.2% (n=318) were females and 39.8% (n=210) were males with a male: female ratio of 0.7:1. In controls 58.2% (n=355) were females and 41.8% (n=255) were females. On the basis of age, the patients were grouped into two categories, less than 40 years (<40) and greater or equal to 40 years of age (≥ 40). The number of cases in the age group of <40 years (n=414; 78.4%) exceeded than ≥ 40 years (n=114; 21.6%) with a mean age of 29.7 ± 13.9 years in cases while as in control subjects in the age group of <40 (n=459; 75.0%) exceeded than ≥ 40 years (n=151; 25.0%). The mean age of the control subjects was 29.45 ± 14.2 years. Based on the smoking status, 63 (12%) patients were smokers as compared to 38 (6.2%) smokers among control group. Among cases we found 525 (99.4%) patient with elevated IgE levels while as only 123 (20.2%) controls presented with higher IgE levels. Other study variables of the study subjects did not showed any statistically significant association with atopy (P>0.05) Table 2.

Characteristics	Cases (n=528)	Controls (n=610)	P value
Age			
<40	414 (78.4)	459 (75.0)	>0.05
≥ 40	114 (21.6)	151 (25.0)	
Gender			
Male	210 (39.8)	255 (41.8)	>0.05
Female	318 (60.2)	355 (58.2)	
Dwelling			
Urban	240 (45.5)	281 (46.1)	>0.05
Rural	288 (54.5)	329 (44.9)	
Smoking status			
Smokers	63 (12.0)	38 (6.2)	>0.05
Non-smokers	165 (31.2)	345 (56.5)	
Passive smokers	300 (56.8)	227 (37.3)	
IgE levels			
Normal	03 (0.6)	487 (79.8)	<0.05
Elevated	525 (99.4)	123 (20.2)	
Eosinophil count			
Normal	297 (56.3)	519 (85.1)	>0.05
Elevated	231 (43.7)	91 (14.9)	
Vitamin D levels (ng/ml)		503 (82.4)	>0.05
Deficient (<20)	345 (65.3)	49 (8.0)	
Insufficient (20-30)	111 (21.0)	58 (9.6)	
Sufficient (31-100)	72 (13.7)	00 (0.0)	
Upper safety limit (>100)	00 (0.0)		
PFT			
Normal	324 (61.4)	NA	
Abnormal	204 (38.6)		
SPT			
+HDM	72 (13.6)	NA	
+Pollens	39 (7.3)		
+HDM/Pollens	114 (21.6)		
+HDM/Pollens/Fungi/AE	66 (12.5)		
+HDM/Pollens/Few foods/AE	225 (42.6)		
Negative	12 (2.2)		
Seasonal/year round			
Year round	387 (73.3)	NA	
Seasonal	141 (26.7)		
Peak time			
Morning	255 (48.2)	NA	
Evening	249 (47.1)		
Both	24 (4.5)		
Family history			
Yes	393 (74.5)	NA	
No	135 (25.5)		
Triggers			
Dust	174 (32.9)	NA	
Dust/Irritant	210 (39.7)		
Temperature	12 (2.3)		
Irritants	132 (25.0)		
Co-Morbidity			
Diabetes	24 (4.5)	NA	
Hypertension	27 (5.1)		

Diabetes/Hypertension/Obesity	16 (2.7)	
Hypothyroidism	51 (9.6)	
PCOD	21 (3.9)	
Nil	389 (73.6)	
Collateral diseases		
Drug allergy	60 (11.5)	NA
Migraine	120 (22.7)	
Food allergy	90 (17.0)	
Migraine/Food allergy	09 (1.7)	
Migraine/Drug allergy	06 (1.1)	
Nil	243 (46.0)	
Diagnosis		
AR/BA	210 (39.7)	NA
AR/AD	57 (10.8)	
AR/CU	36 (6.8)	
AR/BA/AD	120 (22.7)	
AR/BA/CU	75 (14.2)	
AR/BA/Conjunctivitis	12 (2.3)	
BA/CU	18 (3.4)	

IgE=Immunoglobulin E; ESR=Eosinophil Sedimentation Rate; LSCS=Lower Segment Caesarian section; TSH=Thyroid Stimulating Hormone; PFT=Pulmonary Function Test; HDM=House Dust Mite; AE=Animal Epithelia; AR=Atopic Rhinitis; BA=Bronchial Asthma; AD=Topic Dermatitis; CU=Chronic Urticaria

Table 2. General characteristics of study subjects.

Polymorphic analysis of IL-13

Analysis of I0L-13-C1055T polymorphism (rs20541): The representative gel pictures showing amplified products of IL-13 5' UTR and PCR-RFLP of the IL-13–C1055T polymorphism are shown in Figure 1. The frequency of variant allele (CT+TT) was found to be higher in cases (n=321; 60.8%) than controls (n=284; 46.4%), a difference which is statistically significant (OR= 1.8 (1.4-2.2), P<0.0001) suggesting that the presence of variant allele predisposes a person to allergic disorders (Table 3).

Genotypes	Cases n=528 (%)	Controls n=610 (%)	OR (95%CI)	P Value
IL-13- C1055 T				
CC	207 (39.2)	327 (53.6)	1.8 (1.2-2.6)	0.001
TT	81 (15.4)	70 (11.4)	1.8 (1.3-2.3)	<0.0001
CT	240 (45.4)	213 (35.0)		
Allele frequencies				
C (wild allele)	654 (62.0)	867 (71.0)	1.5 (1.2-1.7)	<0.0001
T (minor allele)	402 (38.0)	353 (29.0)		
IL-13- A1512 C				
AA	99 (18.8)	345 (56.5)	6.7 (4.9-9.2)	<0.0001
CC	231 (43.8)	119 (19.5)	4.7 (3.4-6.4)	<0.0001
AC	198 (37.4)	146 (24.0)		
Allele frequencies				
A (wild allele))	396 (37.5)	836 (68.5)	3.6 (3.0-4.3)	<0.0001
C (minor allele)	660 (62.5)	384 (31.5)		
STAT6 G2964 A				
GG	156 (29.6)	365 (60.0)	3.7 (2.7-5.1)	<0.0001
AA	174 (33.0)	108 (17.7)	3.4 (2.5-4.5)	<0.0001
GA	198 (37.4)	137 (22.3)		
Allele frequencies				
G (wild allele)	510 (48.2)	867 (71.0)	2.6 (2.2-3.1)	<0.0001
A (minor allele)	546 (51.8)	353 (29.0)		

HW=Homozygous Wild; HM=Homozygous Mutant; HTZ=Heterozygous

Table 3. Genotypic and allelic frequency distribution of study genes among cases and controls.

On further analyzing IL-13–C1055T genotypes and clinico-pathological variables for possible association with, it was found that patients with <40 years of age and were having higher frequency of variant allele (CT+TT) were significantly associated with the disease (OR=2.0, P<0.0001). Female showed increased risk of atopy when they harbor variant allele (CT+TT) (OR=2.1, P<0.0001). Rather unusually we observed increased risk of atopy among passive and non-smokers when they also contain variant form of IL-13–C1055T polymorphism (P<0.0001). Majority of the patients with elevated IgE levels were also harboring variant genotype (CT+TT), hence elevated IgE levels were strongly associated with the IL-13–C1055T polymorphism (P<0.0001). We observed a higher frequency of variant alleles of IL-13 gene in patients with normal eosinophil count (P<0.0001). Fascinatingly, the subjects with no family history and harbor CT+TT alleles had higher risk of developing atopy than patients with family history of the disease (P=0.02).

Association between IL-13–C1055T genotypes and demographic/ clinico-pathological characteristics is shown in Table 4. The majority of clinic-pathological characteristic showed a statistically significant difference when compared cases and controls (P<0.05). However, majority of these factors did not showed any significance with IL-13–C1055T polymorphism when compared among cases only (P>0.05) except peak time and diagnosis of atopy (P<0.05).

Characteristics /overall genotype	Cases			Controls	OR (95%CI)	P Value		
	Number (n)	CC n (%)	CT+TT n (%)	Number (n)			CC n (%)	CT+TT n (%)
	n=528	207 (39.2)	321 (60.8)	n=610	327 (53.6)	283 (46.4)	1.8 (1.4-2.2)	<0.0001
Age								
<40	414 (78.4)	159 (76.8)	255 (79.4)	459 (75.0)	255 (78.0)	204 (72.1)	1.0 (1.5-2.6)	<0.0001
≥ 40	114 (21.6)	48(23.2)	66 (20.6)	151 (25.0)	72 (22.0)	79 (27.9)	1.2 (0.7-2.0)	0.3
Gender								
Male	210 (39.8)	96 (46.4)	114 (35.5)	255 (41.8)	135 (41.3)	120 (42.4)	1.3 (0.9-1.9)	0.1
Female	318 (60.2)	111 (53.6)	207 (64.5)	355 (58.2)	192 (58.7)	163 (57.6)	2.1 (1.6-2.9)	<0.0001
Dwelling								
Urban	240 (45.5)	75 (36.2)	165 (51.4)	281 (46.1)	145 (44.3)	136 (45.1)	2.3 (1.6-3.3)	<0.0001
Rural	288 (54.5)	132 (63.8)	156 (48.6)	329 (44.9)	182 (55.7)	147 (51.9)	1.4 (1.06-2.0)	0.01
Smoking status								
Smokers	63 (12.0)	30 (14.5)	33 (10.3)	38 (6.2)	23 (7.0)	15 (5.3)	1.6 (0.7-3.8)	0.2
Non-smokers	165 (31.2)	60 (29.0)	105 (32.7)	345 (56.5)	126 (38.5)	101 (35.7)	2.1 (1.4-3.2)	<0.0001
Passive smokers	300 (56.8)	117 (56.5)	183 (57.0)	227 (37.3)	178 (54.4)	167 (59.0)	1.6 (1.2-2.2)	0.001
IgE levels								
Normal	03 (0.6)	00 (0.0)	03 (0.9)	487 (79.8)	266 (81.3)	221 (78.1)	4.8 (0.5-43.3)	0.1
Elevated	525 (99.4)	207(100))	318 (99.1)	123 (20.2)	61 (18.6)	62 (21.9)	1.5 (1.01- 2.24)	0.04
Eosinophil count								
Normal	297 (56.3)	111 (53.6)	186 (57.9)	519 (85.1)	284 (86.8)	235 (83.0)	2.0 (1.5-2.7)	<0.0001
Elevated	231 (43.7)	96 (46.4)	135 (42.1)	91 (14.9)	43 (13.2)	48 (17.0)	1.25 (0.7-2.0)	0.3
Vitamin D levels (ng/ml)								
Deficient (<20)	345 (65.3)	147 (71.1)	198 (61.7)	503 (82.4)	268 (82.0)	235 (83.0)	1.5 (1.1-2.0)	0.002
Insufficient (20-30)	111 (21.0)	39 (18.8)	72 (22.4)	49 (8.0)	25 (7.6)	24 (8.5)	1.9 (0.9-3.8)	0.06
Sufficient (31-100)	72 (13.7)	21 (10.1)	51 (15.9)	58 (9.6)	34 (10.4)	24 (8.5)	3.4 (1.6-7.1)	0.0007
PFT								
Normal	324 (61.4)	126 (60.9)	198 (61.7)				1.03 (0.7-1.5)	0.8
Abnormal	204 (38.6)	81 (39.1)	123 (38.3)					
SPT								
HDM	72 (13.6)	30 (14.5)	42 (13.1)				-	0.3
Pollens	39 (7.3)	12 (5.8)	27 (8.4)					
HDM/Pollens	114 (21.6)	45 (21.7)	69 (21.5)					
HDM/Pollens/ Fungi /AE	66 (12.5)	21 (10.1)	45 (14.0)					

HDM/ Pollens/Few foods/AE	225 (42.6)	93 (45.0)	132 (41.1)		
Negative	12 (2.2)	06 (2.9)	06 (1.9)		
Seasonal/Year round					
Year round	387 (73.3)	159 (76.8)	228 (71.0)	0.7 (0.5-1.1)	0.1
Seasonal	141 (26.7)	48 (23.2)	93 (29.0)		
Peak time					
Morning	255 (48.2)	120 (58.0)	135 (42.0)	0.6 (0.4-0.8)	0.005
Evening	249 (47.1)	87(42.0)	162 (50.5)	0.04 (0.006-0.3)	<0.0001
Both	24 (4.5)	00 (0.0)	24 (7.5)		
Family history					
Yes	393 (74.5)	165 (79.7)	228 (71.0)93	0.6 (0.4-0.9)	0.02
No	135 (25.5)	42 (20.3)	-29		
Triggers					
Dust	174 (32.9)	69 (33.3)	105 (32.7)	-	0.07
Dust/Irritant	210 (39.7)	93 (44.9)	117 (36.4)		
Temperature	12 (2.3)	03 (1.4)	09 (2.8)		
Irritants	132 (25.0)	42 (20.3)	90 (28.0)		
Co-Morbidity					
Diabetes	24 (4.5)	09 (4.3)	15 (4.6)	-	0.5
Hypertension	27 (5.1)	12 (5.7)	15 (4.6)		
Diabetes/ Hypertension/ Obesity	16 (2.7)	00 (0.0)	16 (5.0)		
Hypothyroidism	51 (9.6)	21 (10.1)	30 (9.3)		
PCOD	21 (3.9)	09 (4.3)	12 (3.7)		
Nil	389 (73.6)	156 (75.4)	233 (72.6)		
Collateral diseases					
Drug allergy	60 (11.5)	27 (13.0)	33 (10.3)	-	0.5
Migraine	120 (22.7)	45 (21.7)	75 (23.4)		
Food allergy	90 (17.0)	33 (15.9)	57 (17.8)		
Migraine/Food allergy	09 (1.7)	09 (4.3)	00 (0.0)		
Migraine/Drug allergy	06 (1.1)	00 (0.0)	06 (1.9)		
Nil	243 (46.0)	93 (44.9)	150 (46.7)		
Diagnosis					
AR/BA	210 (39.7)	87 (42.0)	123 (38.3)	-	0.01
AR/AD	57 (10.8)	27 (13.0)	30 (9.3)		
AR/CU	36 (6.8)	18 (8.7)	18 (5.6)75		
AR/BA/AD	120 (22.7)	45 (21.7)	-23.4		
AR/BA/CU	75 (14.2)	18 (8.7)	57 (17.7)		
AR/BA/ Conjunctivitis	12 (2.3)	06 (2.8)	06 (1.9)		
BA/CU	18 (3.4)	06 (2.8)	12 (37.4)		

IgE=Immunoglobulin E; GAD=General Anxiety Disorder; MDD=Major Depressive Disorder; ESR=Eosinophil Sedimentation rate; LSCS=Lower Segment Caesarian Section; TSH=Thyroid Stimulating Hormone; PFT=Pulmonary Function Test; HDM =House Dust Mite; AE=Animal Epithelia; AR=Atopic Rhinitis; BA=Bronchial Asthma; AD=Topic Dermatitis; CU=Chronic Urticaria.

Table 4. Association between IL13IL-13–C1055T genotypes and clinicopathologic characteristics of cases and controls.

Analysis of IL-13–C1512A polymorphism (rs1881457): The representative gel pictures showing amplified products of IL-13 5' UTR and PCR-RFLP of the IL-13–C1512A polymorphism are shown in Figure 2. The genotype distributions of IL-13–C1512A polymorphism in the cases and controls are shown in Table 3. The frequency of variant allele (CC+AC) was found to be higher for cases (n=429; 81.2%) than controls (n=265; 43.5%), a difference which is statistically significant (P<0.0001) suggesting that the presence of variant allele predisposes a person to atopy.

On stratifying IL-13–C1512 A genotypes with clinico-pathological variables of study subjects, it was found that the frequency of wild (AA) and variant genotypes (CA+AC) between cases and controls

with respect to all the subgroups of age, gender, dwelling, smoking status, eosinophil count and vitamin D levels did not showed any significant difference statistically. However, the presence of variant genotype (CA+AC) was significantly associated with elevated IgE levels (OR=3.5, P<0.0001), similarly, the frequency of CA+AC genotypes was greater in patients having morning allergic attacks as compared to patients with no usual peak time for atopy (OR=4.6, P=0.0006). Similarly, IL-13–C1512 A genotypes were significantly associated with all the differential diagnosis of atopy cases (P<0.0001) Table 5.

Characteristics /Overall Genotype	Cases			Controls	OR (95%CI)	P Value		
	Number (n)	AA n (%)	AC+CC n (%)	Number (n)			CC n (%)	CA+AA n (%)
	n=528	99 (18.7)	429 (71.3)	n=610	345 (56.5)	265 (43.5)	5.6 (4.3-7.3)	<0.0001
Age								
<40	414 (78.4)	84 (84.8)	330 (76.9)	459 (75.0)	251 (72.6)	208 (78.5)	4.7 (3.5-6.4)	<0.0001
≥ 40	114 (21.6)	15 (15.2)	99 (23.1)	151 (25.0)	94 (27.2)	57 (21.5)	10.8 (5.7- 20.5)	<0.0001
Gender								
Male	210 (39.8)	42 (42.4)	168 (39.2)	255 (41.8)	153 (44.3)	102 (38.5)	6.0 (3.9-9.1)	<0.0001
Female	318 (60.2)	57 (57.6)	261 (60.8)	355 (58.2)	192 (55.6)	163 (61.5)	5.4 (3.7-7.6)	<0.0001
Dwelling								
Urban	240 (45.5)	36 (36.4)	204 (47.6)	281 (46.1)	156 (45.2)	125 (47.2)	7.0 (4.6-10.8)	<0.0001
Rural	288 (54.5)	63 (63.6)	225 (52.4)	329 (44.9)	189 (54.8)	140 (52.8)	4.8 (3.4-6.8)	<0.0001
Smoking status								
Smokers	63 (12.0)	12 (12.1)	51 (11.9)	38 (6.2)	24 (6.9)	14 (5.3)	7.3 (2.9-18.1)	<0.0001
Non-smokers	165 (31.2)	27 (27.3)	138 (32.2)	345 (56.5)	127 (3.8)	100 (37.7)	6.5 (3.9-10.5)	<0.0001
Passive smokers	300 (56.8)	60 (60.6)	240 (55.9)	227 (37.3)	194 (56.2)	151 (57.0)	5.1 (3.6-7.3)	<0.0001
IgE levels								
Normal	03 (0.6)	00 (0.0)	03 (0.7)	487 (79.8)	289 (83.7)	198 (74.7)	5.8 (0.6-12.1)	0.09
Elevated	525 (99.4)	99 (100)	426 (99.3)	123 (20.2)	56 (16.2)	67 (28.3)	3.6 (2.3-5.4)	<0.0001
Eosinophil count								
Normal	297 (56.3)	57 (57.6)	240 (55.9)	519 (85.1)	295 (85.5)	224 (84.5)	5.5 (3.9-7.7)	<0.0001
Elevated	231 (43.7)	42 (42.4)	189 (44.1)	91 (14.9)	50 (14.5)	41 (15.5)	5.4 (3.2-9.3)	<0.0001
Vitamin D levels (ng/ml)								
Deficient (<20)	345 (65.3)	69 (69.7)	276 (64.3)	503 (82.4)	278 (80.6)	225 (84.9)18 (6.8)	4.9 (3.5-6.7)	<0.0001
Insufficient (20-30)	111 (21.0)	27 (27.3)	84 (19.6)	49 (8.0)	31 (9.0)	22 (8.3)	5.3 (2.6-11.0)	<0.0001
Sufficient (31-100)	72 (13.7)	03 (03.0)	69 (16.1)	58 (9.6)	36 (10.4)		37.6 (10.5- 94.2)	<0.0001
PFT								
Normal	324 (61.4)	54 (54.5)	270 (62.9)				1.4 (0.9-2.2)	0.1
Abnormal	204 (38.6)	45 (45.5)	159 (37.1)					
SPT								
HDM	72 (13.6)	12 (12.1)	60 (13.9)				-	0.3
Pollens	39 (7.3)	06 (06.1)	33 (7.7)					
HDM/Pollens	114 (21.6)	15 (15.1)	99 (23.1)					
HDM/Pollens/ Fungi /AE	66 (12.5)	09 (9.1)	57 (13.3)					
HDM/ Pollens/Few foods/AE	225 (42.6)	54 (54.5)	171 (39.9)					
Negative	12 (2.2)	03 (3.0)	09 (2.1)					
Seasonal/Year round								
Year round	387 (73.3)	69 (69.7)	318 (74.1)				1.2 (0.7-2.0)	0.3
Seasonal	141 (26.7)	30 (30.3)	111 (25.9)					
Peak time								
Morning	255 (48.2)	45 (45.5)	210 (48.9)				0.9 (0.6-1.5)	0.8
Evening	249 (47.1)	42 (42.4)	207 (48.2)				4.6 (1.9-11.0)	0.0006
Both	24 (4.5)	12 (12.1)	12 (2.8)					
Family history								

Yes	393 (74.5)	75 (75.7)	318 (74.1)	0.9 (0.5-1.5)	0.7
No	135 (25.5)	24 (24.2)	111 (25.9)		
Triggers					
Dust	174 (32.9)	36 (36.3)	138 (32.2)	-	0.1
Dust/Irritant	210 (39.7)	30 (30.0)	180 (41.9)		
Temperature	12 (2.3)	06 (6.1)	06 (1.4)		
Irritants	132 (25.0)	27 (27.2)	105 (24.5)		
Co-Morbidity					
Diabetes	24 (4.5)	03 (3.0)	21 (4.9)	-	0.1
Hypertension	27 (5.1)	03 (3.0)	24 (5.6)		
Diabetes/ Hypertension/ Obesity	16 (2.7)	03 (3.0)	13 (3.0)		
Hypothyroidism	51 (9.6)	06 (6.1)	45 (10.5)		
PCOD	21 (3.9)	06 (6.1)	15 (3.5)		
Nil	389 (73.6)	78 (78.8)	311 (72.5)		
Collateral diseases					
Drug allergy	60 (11.5)	12 (12.1)	48 (11.2)	-	0.4
Migraine	120 (22.7)	18 (18.2)	102 (23.8)		
Food allergy	90 (17.0)	21 (21.2)	69 (16.1)		
Migraine/Food allergy	09 (1.7)	00 (0.0)	09 (2.1)		
Migraine/Drug allergy	06 (1.1)	03 (3.0)	03 (0.7)		
Nil	243 (46.0)	45 (45.4)	198 (46.1)		
Diagnosis					
AR/BA	210 (39.7)	27 (27.3)	183 (42.6)	-	<0.0001
AR/AD	57 (10.8)	03 (3.0)	54 (12.6)		
AR/CU	36 (6.8)	03 (3.0)	33 (7.7)		
AR/BA/AD	120 (22.7)	24 (24.2)	96 (42.4)		
AR/BA/CU	75 (14.2)	24 (24.2)	51 (11.9)		
AR/BA/ Conjunctivitis	12 (2.3)	12 (12.1)	00 (0.0)		
BA/CU	18 (3.4)	06 (6.1)	12 (2.8)		

IgE=Immunoglobulin E; GAD=General Anxiety Disorder; MDD=Major Depressive Disorder; ESR=Eosinophil Sedimentation rate; LSCS=Lower Segment Caesarian Section; TSH=Thyroid Stimulating Hormone; PFT=Pulmonary Function Test; HDM=House Dust Mite; AE=Animal Epithelia; AR=Atopic Rhinitis; BA=Bronchial Asthma; AD=Topic Dermatitis; CU=Chronic Urticaria.

Table 5. Association between IL-13-A1512 C genotypes and clinic opathologic characteristics of cases and controls.

Analysis of STAT 6 G2964A 3'UTR polymorphism (rest 324015): The representative gel pictures showing amplified products of IL-13 5' UTR and PCR-RFLP of the STAT6 G2964 A polymorphism are shown in Figure 3. The genotype distributions of STAT6 G2964 A polymorphism in the cases and controls are shown in Table 3. There was a significant difference in the genotypic variants among cases and controls (P<0.0001). The frequency of variant allele (GA+AA) was found to be significantly higher for cases (n=372; 70.5%) than controls (n=245; 40.2%) (P<0.0001) suggesting that the presence of variant allele predisposes a person to allergic disorders (Table 3).

On further classification of STAT6 G2964 A genotypes and clinico-pathological variables was analyzed, it was found that there was a statistically significant (P<0.0001) difference in the frequency of wild (GG) and variant alleles (GA+AA) between cases and controls with respect to all the subgroups of age, gender, dwelling, smoking status, eosinophil count and vitamin D levels. Majority of the Atopy patients with elevated serum IgE levels were harboring variant genotype (GA +AA), thus suggesting that an elevated IgE levels in combination with STAT6 G2964A polymorphism could act as a common feature among atopy subjects (OR=3.5, P<0.0001). STAT6 G2964A genotypes were significantly associated with all the triggers (P<0.0001) and co-morbidities (P=0.01) associated with atopy. Similarly, STAT6G2964A genotypes were significantly associated with all the differential diagnosis of atopy patients (P=0.002) (Table 6).

Characteristics /Overall genotype	Cases			Controls	OR (95%CI)	P Value		
	Number (%)	GG n (%)	GA+AA n (%)	Number (%)			GG n (%)	GA+AA n (%)

	n=528	**156 (29.5)**	**372 (70.5)**	**n=610**	**365 (59.8)**	**245 (40.2)**	**3.5 (2.7-4.5)**	**<0.0001**
Age								
<40	414 (78.4)	129 (82.7)	285 (76.6)	459 (75.0)	279 (76.4)	180 (73.5)	3.4 (2.5-4.5)	<0.0001
≥ 40	114 (21.6)	27 (17.3)	87 (23.4)	151 (25.0)	86 (23.6)	65 (26.5)	4.2 (2.4-7.3)	<0.0001
Gender								
Male	210 (39.8)	66 (42.3)90 (57.7)	144 (38.7)	255 (41.8)	155 (42.5)	100 (40.8)	3.4 (2.3-4.9)	<0.0001
Female	318 (60.2)		228 (61.3)	355 (58.2)	210 (57.5)	145 (59.2)	3.6 (2.6-5.0)	<0.0001
Dwelling								
Urban	240 (45.5)	63 (40.4)	177 (47.6)	281 (46.1)	170 (46.8)	111 (45.3)	4.3 (2.9-6.2)	<0.0001
Rural	288 (54.5)	93 (59.6)	195 (52.4)	329 (44.9)	195 (53.4)	134 (54.7)	3.0 (2.1-4.2)	<0.0001
Smoking status								
Smokers	63 (12.0)	21 (13.5)	42 (11.3)	38 (6.2)	22 (6.0)	16 (6.5)	2.7 (1.1-6.3)	0.01
Non-smokers	165 (31.2)	63 (40.4)	102 (27.4)	345 (56.5)	129 (35.4)	98 (40.0)	2.1 (1.4-3.2)	0.0002
Passive smokers	300 (56.8)	72 (46.1)	228 (61.3)	227 (37.3)	214 (58.6)	131 (53.5)	5.1 (3.6-7.2)	<0.0001
IgE levels								
Normal	03 (0.6)	03 (1.9)	00 (0.0)	487 (79.8)	292 (80.0)	195 (79.6)	0.3 (0.04-3.3)	0.3
Elevated	525 (99.4)	153 (98.1)	372 (100)	123 (20.2)	73 (20.0)	50 (20.4)	3.5 (2.3-5.3)	<0.0001
Eosinophil count								
Normal	297 (56.3)	81 (51.9)	216 (58.1)	519 (85.1)	308 (84.4)	211 (86.1)	3.9 (2.8-5.3)	<0.0001
Elevated	231 (43.7)	75 (48.1)	156 (41.9)	91 (14.9)	57 (15.6)	34 (13.9)	3.4 (2.1-5.8)	<0.0001
Vitamin D levels (ng/ml)								
Deficient (<20)	345 (65.3)	93 (59.6)	252 (67.7)	503 (82.4)	302 (82.7)	201 (82.0)	4.0 (3.0-5.4)	<0.0001
Insufficient (20-30)	111 (21.0)	42 (26.9)	69 (18.6)51 (13.7)	49 (8.0)	29 (8.0)34 (9.3)	20 (8.2)	2.3 (1.2-4.7)	0.01
Sufficient (31-100)	72 (13.7)	21 (13.5)		58 (9.6)		24 (9.8)	3.4 (1.6-7.1)	0.0007
PFT								
Normal	324 (61.4)	87 (55.8)	237 (63.7)				1.4 (0.9-2.0)	0.08
Abnormal	204 (38.6)	69 (44.2)	135 (36.3)					
SPT								
HDM	72 (13.6)	15 (9.6)	57 (15.3)				-	0.7
Pollens	39 (7.3)	18 (11.5)36 (23.1)	21 (5.6)					
HDM/Pollens	114 (21.6)	18 (11.5)	78 (21.0)					
HDM/Pollens/ Fungi /AE	66 (12.5)		48 (12.9)					
HDM/ Pollens/Few foods/AE	225 (42.6)	63 (40.4)	162 (43.5)					
Negative	12 (2.2)	06 (3.8)	06 (1.6)					
Seasonal/Year round								
Year round	387 (73.3)	111 (71.2)	276 (74.2)				1.1 (0.7-1.7)	0.5
Seasonal	141 (26.7)	45 (28.8)	96 (25.8)					
Peak time								
Morning	255 (48.2)	72 (46.2)	183 (49.2)				1.1 (0.8-1.6)	0.4
Evening	249 (47.1)	78 (50.0)	171 (46.0)				0.8 (0.3-2.2)	0.7
Both	24 (4.5)	06 (3.8)	18 (4.8)					
Family history								
Yes	393 (74.5)	120 (76.9)	273 (73.4)				0.8 (0.5-1.2)	0.4
No	135 (25.5)	36 (23.1)	99 (26.6)					
Triggers								
Dust	174 (32.9)	42 (26.9)	132 (35.5)				-	<0.0001
Dust/Irritant	210 (39.7)	57 (36.5)	153 (41.1)					
Temperature	12 (2.3)	06 (3.8)	06 (1.6)					

Irritants	132 (25.0)	51 (32.7)	81 (21.8)		
Co-Morbidity					
Diabetes	24 (4.5)	09 (5.8)	15 (4.0)	-	0.3
Hypertension	27 (5.1)	06 (3.8)	21 (5.5)		
Diabetes/ Hypertension/ Obesity	16 (2.7)	03 (1.9)	13 (3.5)		
Hypothyroidism	51 (9.6)	12 (7.7)	39 (10.5)		
PCOD	21 (3.9)	09 (5.8)	12 (3.2)		
Nil	389 (73.6)	117 (75.0)	272 (73.1)		
Collateral diseases					
Drug allergy	60 (11.5)	12 (7.7)	48 (12.9)	-	0.01
Migraine	120 (22.7)	27 (17.3)	93 (25.0)		
Food allergy	90 (17.0)	30 (19.2)	60 (16.1)		
Migraine/Food allergy	09 (1.7)	06 (3.8)	03 (0.8)		
Migraine/Drug allergy	06 (1.1)	03 (1.9)	03 (0.8)		
Nil	243 (46.0)	78 (50.0)	165 (44.4)		
Diagnosis					
AR/BA	210 (39.7)	60 (38.5)	150 (40.3)	-	0.002
AR/AD	57 (10.8)	09 (5.8)	48 (12.9)		
AR/CU	36 (6.8)	06 (3.8)	30 (8.1)		
AR/BA/AD	120 (22.7)	42 (26.9)	78 (21.0)		
AR/BA/CU	75 (14.2)	24 (15.4)	51 (13.7)		
AR/BA/ Conjunctivitis	12 (2.3)	06 (3.8)	06 (1.6)		
BA/CU	18 (3.4)	09 (5.8)	09 (2.4)		

IgE=Immunoglobulin E; GAD=General Anxiety Disorder; MDD=Major Depressive Disorder; ESR=Eosinophil Sedimentation rate; LSCS=Lower Segment Caesarian Section; TSH=Thyroid Stimulating Hormone; PFT=Pulmonary Function Test; HDM=House Dust Mite; AE=Animal Epithelia; AR=Atopic Rhinitis; BA=Bronchial Asthma; AD=Topic Dermatitis; CU=Chronic Urticaria

Table 6. Association between STAT6 G2964A 3'UTR genotypes and clinicopathologic characteristics of cases and controls.

Discussion

Different cytokines as modulating factors of the immune response and inflammatory reactions are involved in the pathogenesis of Atopy. It is an inflammatory disorder of upper airways and skin resulting from environmental and genetic factors individually as well as complex interactions between the two. Among various clinico-pathological characteristics, the IgE levels were substantially increased among cases. Similarly, a good number of cases were having a history of atopy among blood relatives. Among studied genotypes, variant genotypes were significantly associated with increased risk of atopy. The above risk predisposing genotypes proved to be important modifiers of environmental exposure and life style factors in determining the risk of atopic disorders. Additionally, there was an increased serum IL-13 and STAT6 levels among atopic patients as compared to controls [13-15].

IgE plays an important role in the development of allergic disorders. The generation of IgE requires the activation of the adaptive immune system. B cells in the secondary lymphoid organs are stimulated to produce mature antibodies. Current evidence shows that high-affinity IgE in blood stream of allergic individuals derives mainly from the mucous membrane. Also, mucosal synthesis of IgE can occur in the absence of systemic atopy. Higher serum IgE levels have been reported to be correlated with the clinical expression of allergy and asthma. During allergic responses, respiratory mucosa is a site of IgE production. Evidences of presence of sIgE in AR are numerous. Studies have identified presence of 90% specific IgE in nasal secretions of allergic patients. A study from early 1990 s demonstrated that house dust mite-specific IgE increased faster in the nasal secretions than in serum after allergen exposure. Similarly AR patients on exposure to pollen showed significant changes in IgE repertoires in nasal mucosa [16,17].

Our results are completely in agreement with earlier epidemiological studies which reported higher IgE levels are associated with bronchial responsiveness, a major component of the asthma phenotype, rhinitis and AD. The stimulus upon exposure of allergens in the mucosa might produce a sequential switching to IgE from IgG or IgA. In recent years, a high serum levels of IgE is used to predict the development of asthma, independent of other allergic factors. Therefore, an understanding of the mechanism regulating total serum IgE levels will valid efforts to scrutinize the components of different atopic disorders.

Although reports are available regarding heritability of serum IgE levels, however, the precise mode of genetic control has remained indefinable. There have been multiple complex segregation analyses investigating the mode of inheritance of total serum IgE within families. Several studies have found evidence for a recessive gene

regulating IgE levels, with different estimates of gene frequencies and mean IgE levels [18].

A functional SNP contributing to amino acid substitution in IL-13 was described to change the binding strength of IL-13 to its receptor, causing higher activity of IL-13 variants. Computer modeling of rs20541 variant has shown that this substitution affects the signal strength between interleukin 13 and its receptor. In atopic patients, Th2 phenotype predominates, leading to increased production of IL-13 and class-switching in B lymphocytes to synthesize IgE antibodies. Arima et al. have found, using animal model, that IL-13 with Gln110 variant has higher activity and is present in blood for a longer time. This was confirmed by Chen et al., who performed the experiments on mouse cell line with stable expression of human IL-13R, that had the ability to bind human IL13. This polymorphism was associated with asthma and AD in number of studies including few GWAS. A meta-analysis study which was further confirmed in cohort studies reported an association of variant genotype of IL-13 with atopic disorders. An association between AD and higher IgE serum levels was also indicated by earlier reports.

Signal Transducer and Activator of Transcription (STAT6) is a central molecule in the signal transduction pathway used by IL-4 in IgE isotype switching and Th2 cytokines production 53. STAT6 2964 G/A polymorphism is located in the 3'UTR region of the STAT6 gene at chromosome 12 q13.3-q14 and has been reported to be associated with allergic diseases. Another common polymorphism is located in intron 2 of STAT6 gene; 2892 C/T conferred risk for asthma and elevated serum IgE levels in various ethnic groups [19,20].

The current study results were reported by different studies, were variant genotypes of STAT6 gene were significantly associated with the pathogenesis of different atopic disorders. Another study reported an association between G2964A polymorphism and mild atopic asthma in Japanese population and childhood nut allergy in the United Kingdom. However, few studies could not reproduce our results and showed no association between the variant of the STAT6 gene and AR and asthma in Korean and Chinese population respectively. Similarly, no association was observed in Japanese British, and Caucasian populations. These findings suggest that there are marked differences not only between different ethnic groups but also within one ethnic group, indicating genetic heterogeneity of allergic diseases.

Furthermore, our study demonstrated significant increase in the levels of serum IgE in variant genotype (GA+AA) as compared to homozygous wild GG genotype. This result indicates that genetic variants of STAT6 gene significantly contribute to the regulation of serum IgE levels. In agreement with our result, other studies indicated that STAT6 3'-UTR polymorphisms significantly contributed to elevated serum IgE levels. However, Pykalainen, et al. reported results which were in disagreement with our results. In this study variant genotypes of STAT6 gene among Korean AR patients and Finnish asthmatic families did not showed any significant association with serum IgE levels.

The strength of the study is that previous studies have mostly reported the effect of various environmental and genetic factors on atopy individually. However, the current study reported the effect of such factors individually as well as in combination Other major strength is comparatively larger sample size and confounding of the results with the probable allergy risk factors. However, selection or recall bias could be the weak point of this study, although the same hospital setting and only a single interviewer lessen this bias.

Conclusion

In conclusion, the variant forms of IL-13 and STAT6 genes showed a strong association towards atopy which was further increased among subjects harboring these variant genotypes in presence of different clinico-epidemiological and clinico-pathological characteristics.

References

1. Asher MI, Montefort S, Bjorksten B and Christopher KW Lai. Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC Phases One and Three repeat multicountry cross-sectional surveys. *Lancet* 368 (2006): 733-743.
2. Deckers IA, McLean S, Linssen S and Mommers M, et al. Investigating international time trends in the incidence and prevalence of atopic eczema 1990-2010: a systematic review of epidemiological studies. *PloS one* 7 (2012): 39803.
3. Paramesh H. Epidemiology of asthma in India. *Indian J Pediatr* 69 (2002): 309-312.
4. Barr RG, Aviles-Santa L and Davis SM, et al. Pulmonary Disease and Age at Immigration among Hispanics. Results from the Hispanic Community Health Study/Study of Latinos. *Am J Respir Crit Care Med* 193 (2016): 386-395.
5. Cabieses B, Uphoff E, Pinart M and Anto JM, et al. A systematic review on the development of asthma and allergic diseases in relation to international immigration: the leading role of the environment confirmed. *PloS one* 9 (2014): 105347.
6. Eldeirawi K, McConnell R and Furner S. Associations of doctor-diagnosed asthma with immigration status, age at immigration, and length of residence in the United States in a sample of Mexican American School Children in Chicago. *J Asthma* 46 (2009):796-802.
7. Yao J and Sbihi H. Prevalence of non-food allergies among non-immigrants, long-time immigrants and recent immigrants in Canada. *Can J Public Health* 107 (2016): 461-466.
8. Akdis CA. Allergy and hypersensitivity: mechanisms of allergic disease. *Curr Opin Immunol* 18 (2006): 718-726.
9. Chatzi L, Alegakis A, Tzanakis N and Siafakas N, et al. Association of allergic rhinitis with pesticide use among grape farmers in Crete, Greece. *Occup Environ Med* 64 (2007): 417-421.
10. Marsh DG, Hsu SH and Roebber M. HLA-Dw2: a genetic marker for human immune response to short ragweed pollen allergen Ra5. I. Response resulting primarily from natural antigenic exposure. *J Exp Med* 155 (1982): 1439-1451.
11. Barnes PJ. Immunology of asthma and chronic obstructive pulmonary disease. *Nat Rev Immunol* 8 (2008): 183-192.
12. Hirota T, Takahashi A and Kubo M. Genome-wide association study identifies eight new susceptibility loci for atopic dermatitis in the Japanese population. *Nat Genet* 44 (2012): 1222-1226.
13. Munir S, Ber Rahman S and Rehman S. Association analysis of GWAS and candidate gene loci in a Pakistani population with psoriasis. *Mol Immunol* 64 (2015): 190-194.
14. Weidinger S, Willis-Owen SA and Kamatani Y. A genome-wide association study of atopic dermatitis identifies loci with overlapping effects on asthma and psoriasis. *Hum Mol Genet* 22 (2013): 4841-4856.
15. Weidinger S, Klopp N and Wagenpfeil S. Association of a STAT 6 haplotype with elevated serum IgE levels in a population based cohort of white adults. *J Med Genet* 41 (2004): 658-663.

16. Liu X, Beaty TH and Deindl P. Associations between total serum IgE levels and the 6 potentially functional variants within the genes IL4, IL13, and IL4RA in German children: the German Multicenter Atopy Study. *J Allergy Clin Immunol* 112 (2003): 382-388.
17. Liu X, Nickel R and Beyer K. An IL13 coding region variant is associated with a high total serum IgE level and atopic dermatitis in the German multicenter atopy study (MAS-90). The J Allergy Clin Immunol 106 (2000):167-170.
18. Basehore MJ, Howard TD and Lange LA. A comprehensive evaluation of IL4 variants in ethnically diverse populations: association of total serum IgE levels and asthma in white subjects. *J Allergy Clin Immunol* 114 (2004): 80-87.
19. Liu S, Li T and Liu J. Interleukin-4 rs2243250 polymorphism is associated with asthma among Caucasians and related to atopic asthma. *Cytokine* 59 (2012): 364-369.
20. Mitsuyasu H, Izuhara K and Mao X-Q. Ile50Val variant of IL4Rα upregulates IgE synthesis and associates with atopic asthma. *Nat Genet* 19 (1998): 119.

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