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ORIGINAL ARTICLE

Genetic association of key Th1/Th2 pathway candidate genes, *IRF2*, *IL6*, *IFNGR2, STAT4* and *IL4RA,* with atopic asthma in the Indian population

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Asthma is a complex, multifactorial disease resulting due to dysregulated immune responses. Genetic factors contribute significantly to asthma pathogenesis, and identification of these factors is one of the major goals in understanding the disease. Th1/Th2 helper differentiation has a critical role in modulating the phenotypes associated with atopic asthma. This study was aimed at identifying genetic modifiers of asthma in selected genes involved in T helper differentiation. A total of 354 single-nucleotide polymorphisms (SNPs) in 33 candidate genes were genotyped in a case–control cohort (cases = 147, controls = 199) and families (*n* = 247) using Illumina's Golden Gate Assay. Five SNPs, rs3733475A/C (*IRF2*), rs2069832A/G (*IL6*), rs2012075G/A (*IFNGR2*) and rs1400656G/A (*STAT4*) and rs1805011C/A (*IL4RA*) were found to be associated with asthma in family based as well as in case–control analyses ($P = 0.002$, $P = 0.001$, $P = 0.004$, $P = 0.003$ and $P = 0.001$, respectively). Interestingly, the minor alleles at these loci showed a protective effect. A five loci haplotype, TAACG, in *IRF2* gene, was significantly associated with asthma in families ($P = 1.1 \times 10^{-6}$) and in case–control cohort ($P = 0.01$). In conclusion, our studies led to identification of some key candidate genes, namely *IRF2*, *IL6*, *IFNGR2, STAT4* and *IL4RA* that modulate genetic susceptibility to asthma in the Indian population. Also, this is the first report of independent association of *IL6* gene polymorphism with atopic asthma.
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INTRODUCTION

Asthma is a multifactorial, heterogeneous disease characterized by reversible airway obstruction.[1] A strong genetic basis for asthma is indicated by high estimates of heritability,[2] and motivating genetics/population genomics studies aiming to identify the genetic susceptibility factors.[3–5] A number of asthma susceptibility genes have been identified to date, but the findings have not been consistent across different populations/studies.[3–5] This may partly relate to heterogeneity within asthma and variations across study populations.[3–5] Although the understanding of asthma pathogenesis has been enhanced in recent years, further research is required for its better understanding. Among various asthma subtypes, prevalence of extrinsic/allergic/atopic asthma seems to be the highest among all, particularly in the Indian scenario.[6,7]

Helper T (Th) cell responses, particularly, balance between Th1/Th2 responses are critical for onset and progression of the disease where Th1 responses are supposed to be protective toward asthma, whereas Th2 responses aid in the development of asthma phenotype(s).[3,4,8,9] The micro-environment in the vicinity of the naive Th-cells, antigenic dose, co-stimulators on antigen presenting cells, genetic modifiers and so on have a significant role in deciding whether naive $CD4^+$ T cells would assume Th1 or Th2 effecter phenotype.[8,10] A number of studies suggest that polymorphisms in Th1/Th2 candidate genes may modulate the respective gene products, thereby acting as genetic modifiers of the disease.[3,4,11] In our laboratory, a number of Th1/Th2 candidate genes have been found to be associated with asthma or related phenotypes in the Indian population.[11] Technological advancements in recent times have enabled genotyping of a large number of single-nucleotide polymorphisms (SNPs) simultaneously. Using high-throughput genotyping platform, we have performed candidate gene association studies where we selected genes modulating Th1/Th2 development, differentiation and function, either directly or indirectly. Our major focus was on the key candidate genes from IL4, IFNG and IL12 signaling pathways together with genes that are known to modulate these pathways. We also included genes from microarray data, which were found to be differentially expressed in allergen-induced asthma model potentially modulating apoptosis, cell cycle and so on with indirect evidences that these pathway genes may influence the differentiation or maintenance of Th1/Th2 subsets.[11]

We report the association of some important Th1/Th2 candidate genes (*IRF2*, *IL6*, *IFNGR2*, *STAT4* and *IL4RA*) with asthma. Importantly, the minor alleles at these loci seem to have protective effect with respect to asthma susceptibility. Also, to the best of our

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knowledge, this is the first report of an independent association of *IL6* gene polymorphism with atopic asthma.

MATERIALS AND METHODS

Subjects

The inclusion criteria of subjects, families, asthmatics, normal controls have been previously described.[12,13] In brief, in the present study, asthmatic patients ($n=147$, 38.5±15 years) and normal controls ($n=199$, 37.0±13.5 years) were recruited from our collaborating centers following clinician's diagnosed asthma (Table 1) and a set of inclusion/exclusion criteria (Supplementary Table A), after obtaining ethical clearance from our institute and the participating centers. Written consents were obtained from individuals for participating in the study, performing skin prick test and obtaining blood samples. A standard questionnaire was filled by the candidates giving clinical details, migration status, environmental history, family history of diseases and so on. The clinical test used for ascertaining asthma phenotypes were pulmonary function test (FEV_1, bronchial reversibility (>15%) test using β_2-agonist inhaler (albuterol/salbutamol)), skin prick test (wheal reaction >3mm diameter) to a panel of 15 local environmental allergens and total serum immunoglobulin (IgE) estimation as described earlier.[12,13] Normal controls were recruited from general population taking details of migration status, ethnicity and no self-reported history of asthma or allergic diseases. All individuals with smoking history and parasitic/helminthic infections in the past were excluded from the study. For the family based association studies, 247 families were collected as ascertained through probands (15.6±11.2 years) who were asthmatics along with both the parents affected/unaffected. Families were further extended wherever consents were obtained, and a total of 1022 individuals were recruited with average family size of 3.94 (range from 3 to 12 members). The disease status was confirmed in a manner similar to case–control study. The genetic homogeneity between patients and controls was confirmed by genotyping loci as yet unknown to be associated with asthma as described previously.[12,13]

Gene and SNP selection

We have selected 33 genes, which potentially modulate Th1/Th2 differentiation, development and/or functions directly or indirectly (Supplementary Table B). A total of 354 SNPs were selected for genotyping using Golden Gate Assay on Illumina platform. Detailed description of gene and SNP selection and OPA (Oligonucleotide Pool All) design has been provided in the Supplementary methods.

Genotyping and data cleaning

Samples were genotyped with Illumina Bead Array system in accordance with manufacturer's protocol[14] and as described in Supplementary methods. GenCall module of BeadStudio package v3 was used to generate genotype calls. All the genotype clusters were inspected and corrected manually; the threshold for GenTrain score of >0.25 was set to call a SNP successfully genotyped.[14] We retained markers for further analysis if the call rate was above 90%, maximum of one reproducibility error (5% replicates were used), maximum of five Mendelian errors and showed consistency with Hardy–Weinberg equilibrium at the level of $P<0.01$.

Data/statistical analyses

A detailed description of the statistical methods is provided in Supplementary methods. In brief, Hardy–Weinberg calculations were performed using PLINK (http://pngu.mgh.harvard.edu/~purcell/plink/).[15] Pairwise linkage disequilibrium (LD) calculations and TagSNP selection was done using Haploview v 4.2.[16] Single marker and haplotypic association studies were done using PLINK and FBAT.[15,17,18]

RESULTS

Genotyping and data cleaning

After applying the standard quality control filters to the 354 SNPs, we retained 295 SNPs for further analysis. Of the 1368 samples, 17 samples had call rate below 95% and were therefore removed. Furthermore, eight triads were consistently showing transmission errors and were discarded. In total, therefore, 1326 individuals (239 pedigrees with 993 individuals and case–control cohort (139 cases+194 controls)) finally passed the quality control. After data cleaning, at most one Mendelian error was observed for 99% of the SNPs. Furthermore, ~98% of the markers had call rate above 97%.

LD plots and TagSNPs

The SNPs that passed the quality control criteria were used for gene-wise LD calculation and TagSNP selection. We used the LD values (r^2) to identify tag SNPs using the tagger as implemented in Haploview. Using tagger (gene-wise), we selected 229 tag SNPs for our further association analysis (Supplementary Table C).

Association analysis with asthma

As mentioned in Supplementary methods family based and case–control association analyses have been performed. As the individuals in the families and case–control cohorts were non-overlapping but from the same ethnic background and therefore constituted two independent observations, Fisher's combined probability test, which is a technique, as data fusion was used to aid our decision making. Fisher's combination of probability test is a robust test in the sense that distribution of the underlying test statistic does not have any effect on the performance of the test. It only exploits the property of the *P*-value obtained using any statistics. Four novel SNPs (previously unreported to be associated with asthma or related phenotypes); rs3733475A/C (*IRF2*), rs2069832A/G (*IL6*), rs2012075G/A (*IFNGR2*)

Table 1 Characteristics of the patient and the control groups (case–control study) and the probands (family based study)

	Patients (N = 147)	*Controls (N = 199)*	*Probands (N = 247)*
Native place	North/northwest India	North/northwest India	North/northwest India
Mean age (years):	38.5 (±15)	37.0 (±13.5)	15.6 (±11.2)
Sex ratio (F vs M):	48:52	50:50	44:56
Familial history of *asthma/atopy*[a]	All	None	All
Smoking history[b]	None	None	None
% reversibility from baseline FEV1 (after β_2-agonist usage)	>15%	ND	>15%
Log_{10}-mean serum total IgE (IU ml^{-1})	2.86 (±0.57)	2.44 (±0.6)	2.84 (±0.63)
Atopy/self-reported history of allergies and SPT[c] positivity	All	None	All (probands)

Abbreviations: IgE, immunoglobulin E; ND, not done; SPT, skin prick test. Parenthesis contains the values for s.d.
Patients and controls were recruited from Delhi, Lucknow (UP), Jaipur (Rajasthan), Shimla (HP), Chandigarh (Punjab) and Mumbai (Maharastra).
[a]Control individuals were subjected to a questionnaire so as to eliminate all individuals having atopic disorders or family history of atopic disorders.
[b]Patients and controls known to have experienced smoking in the past, or suffering from parasitic infections were excluded from the study.
[c]Fifteen common environmental allergens (house dust mite, *Amaranthus spinosus*, *Brassica campestris*, *Cynodon dactylon*, *Parthenium hysterophorus*, *Proposis juliflora*, *Ricinus communis*, *Alternaria tenuis*, *Aspergillus fumigatus*, cockroach, mosquito, moth, grain dust rice, hay dust and house dust) were used for the SPT.

Table 2 Results of allelic association analyses with asthma in families and case–control cohorts together with combined *P*-values

			Family based analysis (n = *239 families*)					*Case-control analysis (Cases = 139; controls = 194)*						
GENE	*SNP*	*ALL 1*	*A_FRQ*	N	*Z'*	P-*value*	*OR (95% CI)*	*F_A*	*F_U*	*CHI*	P-*value*	*OR (95% CI)*	*Com_P*	*Corr_P*
IL6	rs2069832	A	0.21	153	−3.7	0.00025	0.62 (0.48−0.79)	0.13	0.22	8.3	0.004	0.54 (0.35−0.82)	0.00001	0.001
IRF2	rs3733475	A	0.40	211	−3.7	0.00025	0.68 (0.56−0.83)	0.39	0.49	7.5	0.006	0.65 (0.47−0.88)	0.00002	0.002
STAT4	rs1400656	G	0.11	75	−2.9	0.004	0.57 (0.39−0.85)	0.06	0.14	10.8	0.001	0.39 (0.22−0.69)	0.00005	0.003
IFNGR2	rs2012075	G	0.043	52	−3.9	0.0001	0.37 (0.22−0.62)	0.04	0.07	3.1	0.077	0.53 (0.26−1.08)	0.00008	0.004
IL4RA	rs1805011	C	0.045	44	−5	0.000001	0.23 (0.12−0.43)	0.08	0.06	0.9	0.32	1.36 (0.73−2.52)	<0.0001	0.001

Abbreviations: A_FRQ, allele frequency (minor allele); ALL 1, minor or polymorphic allele; CHI, χ^2 statistic; CI, confidence interval; F_A, frequency affected (cases); F_U, frequency unaffected (normal controls); *N*, number of informative families; OR, odds ratio; SNP, single-nucleotide polymorphism; Z', Z' statistics.
For the family based analysis, FBAT software has been used except for the odds ratio calculation that was done using PLINK.
For description on Com_P, combined *P*-values and Corr_P, corrected *P*-value, refer Supplementary methods.

and rs1400656G/A (*STAT4*) showed statistically significant association with asthma in families as well as case–control analyses (Table 2). The combined *P*-values for these SNPs remains significant after correction for multiple testing ($P=0.002$, $P=0.001$, $P=0.004$ and $P=0.003$, respectively) (Table 2). Among the SNPs, which have been previously shown to be associated with asthma or related phenotypes, we found evidence of association only for rs1805011C/A (*IL4RA*) as the combined *P*-value ($P=0.001$) was statistically significant (Table 2). The minor alleles at all the loci show a trend toward negative association (odd ratio <1; Table 2).

Haplotype-based association tests are thought to be more powerful than single SNP-based tests.[19] We performed haplotypic association analyses to further validate the results of single marker association analyses to assess whether the significant SNP(s) are present in the significant haplotypes. Also, as explained in Supplementary Methods, we have performed sliding window haplotypic analysis (with fixed window of 5 for genes with more than five SNPs) in case controls to identify region of highest significance. In families, haplotype-based association tests were performed with region (marker combinations) that were identified as regions of high significance. In the sliding window haplotypic analysis of *IRF2* gene, in the region encompassing 5′ untranslated region, the five locus haplotypes, showed statistically significant association with asthma ($P<0.001$) (Table 3A and B). The rs3733475C/A SNP, as mentioned previously, showed significant association with asthma in single marker association analysis, whereas the rs1863314G/A SNP showed weak association signal ($P=0.062$) in families but not in case–control cohort ($P>0.05$). Interestingly, T**AA**CG, the haplotype that has the protective alleles of rs3733475C/A as well as rs1863314G/A, is significantly under-transmitted to asthmatic offsprings in family analysis ($P=1.1\times10^{-6}$); while present with significantly higher frequency in normal controls (>7%) than cases (<1%) in case–control analysis ($P=0.01$) (Table 3A). The permutation procedure with one million iterations also shows statistically significant results ($P<0.001$). In *IL6* gene as well haplotypes CAG with protective allele A was significantly under-transmitted to asthmatics in families ($P=0.0008$) and present with higher frequency of occurrence in normal controls ($P=0.06$), whereas haplotype CGG was over transmitted to asthmatics in families and present with significantly higher frequency ($P=0.06$) in normal controls. In other genes as well, a number of haplotypes showed association but statistical significance was lost owing to multiple testing corrections particularly in case–control analyses (Table 3A). A number of haplotypes showed association in family based analyses, however, further investigation and/or meta analysis with large sample size in future would be required to confirm these findings (Table 3A).

Association analysis with log10 total serum IgE

Total serum IgE levels follow a log normal distribution and when cases and controls were compared with respect to the log serum IgE levels, a statistically significant difference was obtained ($P<0.0001$) (data not shown). In association analysis with log serum IgE, SNPs that showed association with asthma did provide minor or suggestive association signals, although none of them was significant after multiple testing corrections ($P>0.05$) (Table 4). In the haplotypic analysis, haplotypes TAACG and AAGAA that were negatively associated with asthma showed strong association signals with lower $\log_{10}$ total serum IgE levels in family based and case–control studies ($P<0.001$) (data not shown).

DISCUSSION

We have undertaken genetic association studies with 33 genes, most of which are candidates modulating Th1/Th2 differentiation/development and/or function, whereas four differentially expressed genes from microarray data regulating cellular stress pathways, such as cell cycle, apoptosis and so on, have also been included (Supplementary Table B). To the best of our knowledge, this is the first candidate gene association study, focused on a number of genes from Th1/Th2 pathway and asthma. The gene selection in this study is based on strong evidences of their functional role in modulating asthma pathogenesis, creating high prior probabilities for the existence of genetic effects, thereby strengthening the posterior probabilities of the noted effects.

We identified four novel SNPs (genes) and one previously reported SNP to be associated with asthma (Tables 2 and 3) and suggestive association with serum total IgE (Table 4). IL6 is a proinflammatory cytokine that is known to promote allergic inflammation together with IL4 and IL13, IL6 is also involved in terminal differentiation of B lymphocytes and generation of specific allergen IgE by B cells.[20] IL6 could also induce the production of IL3, IL4 and IL5 by T lymphocytes, which in turn promotes differentiation of eosinophils and basophils.[21,22] Despite important role played by IL6, reports of association of this gene with atopic asthma are lacking. Interestingly, a joint effect of atopy/asthma and an *IL6* polymorphism on risk of lung cancer has been previously observed.[23] Also, genetic variants in *IL6* and *IL6R* together have been shown to have interaction effect in modifying response to bronchodilators.[24] Furthermore, joint effect of *IL6* and *TGFB1* polymorphism in atopic sensitization was noted in childhood asthmatics.[25] IL6 levels are likely influenced by genetic polymorphism[26] and our results further support studies that indicate that *IL6* gene polymorphisms could modify the risk of developing asthma. IL4RA is the common subunit required for both IL4 and IL13

Table 3 Result of haplotypic analyses (with asthma) in families and case–control cohort; (A) haplotype wise association analyses and (B) global (Omnibus) haplotypic association analyses

A

		TDT						Case-control				
Gene	*Markers*	*Haplotype*	*T*	*U*	*CHISQ*	P-*value*	*Corr_P*	*F_A*	*F_U*	*CHISQ*	P-*value*	*Corr_P*
IRF2	rs1863316T/A, rs3733475C/A, rs1863314G/A, rs1059492C/A, rs7682813A/G	**TAACG**	**2.543**	**35.08**	**28.14**	**1.1×10^{-7}**	**1.1×10^{-6}**	**0.00939**	**0.07655**	**15.26**	**9.4×10^{-5}**	**0.01**
		TCACG	93.46	77.92	1.409	0.2352	0.37	0.2312	0.1714	3.545	0.05971	0.93
		TCGCG	18.32	13	0.9042	0.3417	0.38	0.02085	0.02005	0.00508	0.9432	0.99
		AAGAA	**1.669**	**24.37**	**19.79**	**8.6×10^{-6}**	**4.3×10^{-5}**	0.02457	0.07094	6.872	0.00876	0.56
		TAGAA	8.041	13.22	1.261	0.2614	0.37	0.02048	0.02401	0.08859	0.766	0.99
		ACGAA	147.4	98.1	9.886	0.00167	0.006	0.2848	0.2783	0.03264	0.8566	0.99
		TCGAA	19.15	20.11	0.02347	0.8782	0.87	0.01308	0.018	0.2414	0.6232	0.99
		ACACA	22	14.82	1.4	0.2367	0.37	0.03099	0.0207	0.6804	0.4095	0.99
		TAGCA	140.6	124.8	0.9404	0.3322	0.38	0.3224	0.2936	0.6135	0.4335	0.99
		TCGCA	5.015	12.03	2.889	0.08917	0.22	0.02015	0.00263	4.901	0.02684	0.93
IL6	rs2069827C/A, rs2069832G/A, rs2069840G/C	**CGC**	**103.2**	**72.37**	**5.408**	**0.02004**	**0.04**	0.1989	0.1846	0.2125	0.6448	0.64
		AAG	21	26	0.5319	0.4658	0.46	**0.01806**	**0.05664**	**6.106**	**0.01347**	**0.05**
		CAG	**69.08**	**120.3**	**13.84**	**0.0002**	0.0008	**0.1006**	**0.1544**	**4.037**	**0.04451**	**0.06**
		CGG	**180.8**	**141.6**	**4.782**	**0.02877**	0.04	**0.6824**	**0.6043**	**4.213**	**0.04013**	**0.06**
STAT4	rs7574608A/T, rs16833224G/C, rs3024861A/T, rs3024851T/A, rs1400656A/G	TGTAG	36.79	55.36	3.744	0.05299	0.13	0.0579	0.1223	7.638	0.00571	0.59
		TGTAA	13.46	11.84	0.1036	0.7476	0.75	0.02427	0.00338	5.749	0.0165	0.59
		AGTTA	108.8	75.97	5.841	0.01566	0.09	0.2005	0.2378	1.28	0.2579	0.76
		ACATA	35	51	2.977	0.08447	0.13	0.06927	0.05407	0.6454	0.4218	0.79
		TGATA	4.006	11.09	3.324	0.06828	0.13	0.02916	0.01335	2.021	0.1551	0.69
		AGATA	137.4	120	1.177	0.278	0.33	0.6189	0.5691	1.627	0.2021	0.72
IFNGR2	rs2070386C/A, rs2834211A/G, rs2012075A/G, rs1059293A/G, rs8131980G/A	CAAGA	125	103	2.123	0.1451	0.36	0.2662	0.1888	5.584	0.01813	0.09
		CGAAG	56.13	52.13	0.1478	0.7007	0.82	0.09038	0.1141	0.9717	0.3242	0.46
		AAAGG	91.92	94.96	0.04941	0.8241	0.82	0.2169	0.2191	0.00459	0.946	0.95
		CAGAG	**20.1**	**52.34**	**14.34**	**0.00015**	**0.001**	0.03479	0.06097	2.318	0.1279	0.32
		CAAAG	172.2	154	1.019	0.3127	0.52	0.3592	0.3935	0.8026	0.3703	0.46
IL4RA	rs1805011A/C rs1805015A/G rs1801275A/G rs2074570A/G rs8832G/A	AGGAA	20.77	20.26	0.00641	0.9362	0.94	0.03442	0.02837	0.1925	0.6608	0.98
		AAAAA	**177.5**	**133.7**	**6.148**	**0.01316**	**0.04**	0.3323	0.3938	2.556	0.1099	0.98
		AAGGG	41.99	40.9	0.01431	0.9048	0.94	0.1146	0.1084	0.0613	0.8045	0.98
		CGGAG	**11.81**	**29.26**	**7.42**	**0.00645**	**0.04**	0.05689	0.04739	0.291	0.5896	0.98
		AAGAG	33.9	40.05	0.5106	0.4749	0.86	0.05083	0.04656	0.06227	0.8029	0.98
		AAAAG	163.1	173.4	0.3152	0.5745	0.86	0.411	0.3755	0.8316	0.3618	0.98

B

		FBAT		Case-control	
Gene	*Markers*	*Global CHISQ*	P-*values*	*Global CHISQ*	P-*values*
IRF2	rs1863316T/A,rs3733475C/A,rs1863314G/A,rs1059492C/A,rs7682813A/G	62.7	2.9×10^{-9}	34.7	0.0001
IL6	rs2069827C/A,rs2069832G/A,rs2069840G/C	21.986	0.0005	11.06	0.01
STAT4	rs7574608A/T, rs16833224G/C,rs3024861A/T,rs3024851T/A,rs1400656A/G	22.31	0.001	16.84	0.005
IFNGR2	rs2070386C/A,rs2834211A/G,rs2012075A/G,rs1059293A/G,rs8131980G/A	22.6	0.0004	8.65	0.2
STAT6	rs1059513A/G, rs324015G/A, rs324011G/A, rs2598483G/A	15.2	0.018	14.6	0.02
IL4RA	rs1805011A/C, rs1805015A/G, rs1801275A/G, rs2074570A/G, rs8832G/A	18.9	0.0043	2.7	0.75

Abbreviations: CHISQ, χ^2 statistic; Corr_P, corrected *P*-value; F_A, frequency affected; F_U, frequency unaffected (normal controls); FBAT, family based association test; T, transmission to affected offsprings; TDT, transmission disequllibrium test; U, non-transmission to affected offspring.
Strategies used for performing haplotypic association analyses have been detailed in Supplementary methods. The bold lettering indicates significant association.

signaling, both of which are critical for Th2 differentiation and/or isotype switching IgE that have a central role in allergic inflammation.[27] Several polymorphisms in this gene have been reported to be associated with asthma in different populations.[27] The rs1805011 in *IL4RA* gene is a coding region polymorphism[28] and its association in our study further highlights the critical role played by IL4/IL13 signaling pathway genes in genetic predisposition to asthma.

The other three genes (*IRF2, IFNGR2* and *STAT4*) in which novel asthma-associated polymorphisms have been identified are essential for the differentiation and/or function of Th1 cells. The genes in this pathway have consistently been associated with autoimmune diseases such as celiac disease, multiple sclerosis and so on.[29] Also, there are previous reports of associations of this cytokine signaling pathway genes with asthma or related phenotype, although these reports are scanty and inconsistent.[29–34] The same polymorphisms could not be directly tested in this study either because of technical problems of multiplexing, genotyping or since the reports came after our study was designed. Nevertheless, it is notable that our study supports the involvement of these genes in asthma susceptibility. It should be taken

Table 4 Results of allelic association analysis with log_{10} serum total IgE in families and in case–control cohort together with combined *P*-values

			Families (FBAT)				Case-control cohort (PLINK)			
GENE	*SNP*	*ALL 1*	*A_FRQ*	N	*Z'*	*P*	*T*	*P*	*Com_P*	*Corr_P*
IL6	rs2069832	A	0.208	118	−3.056	0.002243	−2.248	0.02533	0.00054	0.057
IRF2	rs3733475	A	0.402	158	−2.981	0.003	−1.377	0.17	0.004	0.17
STAT4	rs1400656	G	0.109	68	−2.143	0.03	−2.205	0.03	0.009	0.25
IFNGR2	rs2012075	G	0.043	44	−3.396	0.0007	−1.362	0.1743	0.001	0.06
IL4RA	rs1805011	C	0.045	37	−4.135	0.000035	0.04374	0.9651	0.0004	0.057

Abbreviations: A_FRQ, minor allele frequency; ALL 1, minor allele; *N*, number of informative families; P, probability value; SNP, single-nucleotide polymorphism; T, Wald test statistics (based on T distribution); Z', Z statistics.
For description on Com_P, combined *P*-values and Corr_P, corrected *P*-value, refer Supplementary methods.

into account that in our study the marker selection was very dense with uniform representation across the gene; therefore, it is highly unlikely that we might have missed any significant information. The results of the sliding window haplotypic association analyses of *IRF2* gene (Table 3 A and B), where a protective haplotype TAACG is identified, suggests important role for 5′ untranslated region in *IRF2* gene regulation and/or in asthma.

A number of previously associated (other studies/populations) SNPs (for example, rs2069812 (*IL5*), rs20541 (*IL13*), rs2243250 (*IL4*) and so on (Supplementary Table C)) were not found to be associated with asthma in our study. In this regard, it would also be valuable to add that in our study design we focused only on allergic asthma, which is the dominant clinical subtype for which heritability is well established, however, this may not represent asthma in its entirety. The other reasons for non association in our study could be ethinc differences between our study population and previous studies. Furthermore, our study may be underpowered to draw conclusive inferences for some of the genes, which might confer only modest risk. The 239 trios included in our study would have ~80% power to detect an odds ratio ranging from 1.9 to 2.2; however, these odds ratios are very high for complex trait genetics. Further studies with increased sample size and/or replication studies in another cohort might be required for further validation of our results.

The bias in our results owing to population stratification is highly unlikely. The genetic homogeneity of our asthmatics and normal controls has been verified in our laboratory by genotyping loci (microsatellite markers) that are yet unlinked to asthma.[12,13] Also, recently, subsets of individuals from the cases and controls have been genotyped on genome wide association studies and no evidence of stratification (based on analysis of randomly selected 10 000 markers) was observed (unpublished data). Family based and case–control studies have their own pros and cons; family based studies are robust against population stratification but they are prone to type II or false-negative associations, whereas case–control studies are more sensitive to population stratification and prone to false-positive associations or type I error.[13] Therefore, family based studies segregate genes with modest effect while case–control studies are powered for common disease common variant approach. A positive association signal in both families and case–control analyses, as seen in our study, is more likely to reject the null hypothesis. Our decision making is further strengthened by the combined probability testing method as it provides a way to integrate information from more samples and resultant increase in power.

The associated SNPs may either themselves have an independent functional role or might be in LD with neighboring functional polymorphism. The rs1805011 (*IL4RA*) is a coding region polymorphism, leading to a missense mutation and amino-acid change from glutamine to alanine.[28] Although, the functional role of this non-synonymous mutation is yet to be discovered, the functional role of other missense mutations such as rs1805010 A/G (Ile50Val) and rs1801275 A/G (Gln551Arg) in close vicinity of this SNP have been demonstrated.[35] Understanding the functional significance of the associated SNPs and/or identification of functional polymorphisms in LD with these should be the logical step forward, in unraveling further, their relevance in asthma pathogenesis or molecular mechanisms governing asthma. Of particular interest would to understand how these variations confer protection with respect to asthma susceptibility.

In summary, a number of Th1/Th2 candidate genes show association with atopic asthma in family based and case–control analyses. We identify the important role played by 5′ untranslated region gene polymorphism in *IRF2* gene in asthma susceptibility. To the best of our knowledge, this is first report of independent association of *IL6* gene polymorphism with atopic asthma. Understanding the functional significance of the asthma-associated polymorphisms might provide further insight into the roles of these genes in asthma pathogenesis.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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