

Promoter Polymorphism in the *MS4A2* Gene and Asthma in the Indian Population

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Key Words

Asthma · *FcεRIβ* · *MS4A2*, promoter polymorphism

Abstract

Background: A previous study from our laboratory identified 3-locus risk and protective haplotypes of the *MS4A2* gene, a prime candidate for asthma and atopy, that were associated with differential histamine release profiles from basophils and *FcεRIβ* transcript levels in the Indian population.

Methods: To explore the role of promoter polymorphisms in the observed association, 4 additional promoter polymorphisms (–752C/T, –654C/T, –426T/C and –109T/C) were investigated in 240 nuclear families with atopic asthma, 237 atopic asthmatics and 221 unrelated controls, all of whom were clinically well characterized. The β-subunit transcript levels were measured for 15 individuals and were correlated with the associated promoter polymorphisms. **Results:** We observed a significant association of the –752C/T and –109T/C polymorphisms with asthma, in addition to the already reported association for the INT2 G/A, intron 5 (CA)_n and 3'-UTR C/T polymorphisms. Additionally, the allele T of the –109T/C polymorphism was associated with a reduced risk of asthma and lower percent peripheral blood eosinophils, thereby pointing towards a protective role for this allele in asthma. Further, the 7-locus haplotypes C_C_T_C_A_16_C and T_C_T_T_G_18_T were identified as the major risk/susceptibility and protective haplotypes, respectively ($p < 0.05$). Three-locus sliding-window haplotype analysis also identi-

fied the –426T/C, –109T/C and INT2 G/A polymorphisms to be in regions of high priority ($p < 0.00001$). Indeed, the –109T allele was found to be associated with reduced expression levels for *FcεRIβ*. **Conclusions:** A promoter-dependent mechanism with altered transcriptional regulation of *FcεRIβ* may be involved for its association with asthma. These results, therefore, could be useful in predicting the genetic susceptibility of individuals for developing asthma.

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Introduction

Atopic disorders, including atopic asthma, are characterized by enhanced immunoglobulin E (IgE) production, increased mediator release from inflammatory cells and hypersensitivity of target organs [1]. Several cells and molecules are involved in the production of these mediators, including mast cells, basophils, IgE, the high-affinity IgE receptor (*FcεRI*) and many signal transduction molecules [2].

The β subunit of the high affinity receptor for IgE (*FcεRIβ*) plays an important role in IgE-mediated allergic reactions as an amplifier of cell surface expression and signal transduction of *FcεRI*. In addition, *FcεRIβ* (*MS4A2*) has been presumed to be one of the most important genes linked with atopic diseases and bronchial hyper-responsiveness in several studies [2] (genetic location: chromosome 11q13). Polymorphisms in and around

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

	Patients (n = 237)	Controls (n = 221)	Probands (n = 240)
Mean $\pm$ SD age, years	34.4 $\pm$ 12.5	35.0 $\pm$ 10.6	15.3 $\pm$ 10.5
Sex ratio (M:F)	0.48:0.52	0.47:0.53	0.44:0.56
Family history of asthma/atopy	All	None	All
% reversibility from baseline FEV ₁ (after β_2 -agonist usage)	>15	Not done	>15
Log mean $\pm$ SD total serum IgE, IU/ml	2.85 $\pm$ 0.47	2.32 $\pm$ 0.83	2.84 $\pm$ 0.63
Self-reported history of allergies	All	None	All

All patients and controls were recruited from Delhi, Lucknow (Uttar Pradesh), Haryana, Chandigarh (Punjab) and Rajasthan, which are all in north or northwest India. Individuals known to have smoked in the past 3 years or who were suffering from parasitic infections were excluded from the study. Control individuals were subjected to a questionnaire so as to eliminate those who had atopic disorders or a family history of atopic disorders.

the gene have been extensively studied using both case-control and family-based studies in populations of diverse origins. Studies carried out in several populations have identified various polymorphisms in the *MS4A2* gene, including 3 nonsynonymous SNPs in the coding region of the gene [3–6]. Of these, the 2 coding SNPs (cSNPs) in exon 6 (I181V and V183L) were often absent in certain populations, including the Japanese population, whereas another SNP in exon 7 (E237G) was the more common SNP in this gene [7, 8]. Although a few reports have presented a positive association between the E237G polymorphism and atopic disorders [7, 9], it has been demonstrated by in vitro studies that amino acid variations in the β -chain, including E237G, do not influence the function or the expression of Fc ϵ RI [10, 11]. In contrast, another study carried out in the Japanese population identified –426T/C and –654C/T SNPs in the promoter region, in close linkage disequilibrium (LD) with E237G, to directly affect transcriptional activity of the gene [9].

Our earlier study was carried out in 2 cohorts, 1 each from the northern and western Indian populations, and it identified 2 major haplotypes, A₁₆C and G₁₈T [INT2(CA)_n3'UTR], to be positively and negatively associated with asthma, respectively [12]. Furthermore, the risk haplotype A₁₆C was associated with increased histamine release (IgE receptor mediated) from basophils and higher β -subunit transcript levels [13]. These observations raised the possibility that unidentified polymorphisms in the promoter region of *Fc ϵ RI β* could be responsible for the observed effect.

Here, using both family-based and case-control study designs, we genotyped polymorphisms in the promoter region of the *MS4A2* gene, in addition to the widely stud-

ied repeat polymorphism and SNPs within the gene. We analyzed the association of these polymorphisms independently and at the level of the haplotype with asthma, total serum IgE (TsIgE) level, percent peripheral blood eosinophil count and β -subunit transcript level.

Subjects and Methods

Subjects

In a multicenter asthma genetics study, 240 nuclear families of Indo-Aryan origin, ascertained through asthmatic probands (age range 3–48 years, mean 15.3 $\pm$ 10.5 years) and an independent case-control cohort (237 patients, 221 controls) were recruited from various collaborating hospitals in north and northwest India (table 1). Ethical approval was obtained from the review board of each collaborating hospital. Written informed consent was obtained from all individuals (parents in the case of minors) participating in the study. The study design was such that at least 1 child with asthma along with both the parents were recruited. The families were further extended whenever other members gave consent to participate. Thus, a total of 955 individuals were recruited with an average family size of 3.98 (3–9 individuals per family).

Asthma in the recruited study population was defined by clinical history and was later validated by interview questions, as described previously [14]. The diagnosis of asthma was based on physician assessment, which followed the guidelines of the National Asthma Education and Prevention Program (Expert Panel Report) [15]. The patients met the following criteria: 2 or more episodes of wheezing and shortness of breath during the past year; FEV₁/FVC of less than 70% at the time of attack and improvement by a bronchodilator; positive skin prick test to at least 1 of the 15 common environmental allergens, and undergoing asthma therapy.

Healthy volunteers were recruited from the general population (geographically matched with the patients) on the basis of normal pulmonary function test, negative skin prick test and lacking any family history of asthma and/or atopy. TsIgE was es-

timated using ELISA. All individuals recruited to the study were nonsmokers who had had no parasitic/helminthic infestation in the past 3 years.

PCR Amplification and Genotyping

DNA was isolated from peripheral white blood cells and genotyped for polymorphisms within the *MS4A2* gene. The promoter region was amplified using the primer pair (FP: 5'-CCA CCA ATT CCT GAA GAC-3' and RP: 5'-CAC CGT GAC TAT GAC TTC-3') while the polymorphisms were studied using primers detailed in suppl. table 1 (see www.karger.com/doi/10.1159/000199716). SNaPshot primer extension reactions were carried out (Applied Biosystems, Foster City, Calif., USA) as per the manufacturer's instructions. To clean up the primer extension reaction, 1U of calf intestinal phosphatase (CIP, New England Biolabs) was added to the reaction mixture and the mixture was incubated at 37°C for 1 h, followed by 15 min at 72°C for enzyme inactivation. These samples were subsequently electrophoresed using the ABI Prism 3100 Genetic Analyzer (Applied Biosystems) and the results were analyzed using the ABI Prism GeneScan and Genotyper (Applied Biosystems). The intron 2 and 3'-UTR polymorphisms were assessed using *RsaI* restriction endonuclease digestion, as described previously [12]. The genotyping was confirmed by sequencing genomic DNA from representative samples ($n = 20$) corresponding to the respective genotypes for all the loci investigated.

The genotyping for the microsatellite (CA)_n repeat polymorphism in intron 5 was performed as described previously [12]. The fragment lengths were determined using the GeneMapper v. 3.5 (Applied Biosystems).

Estimation of *FcεRIβ* mRNA using Real-Time PCR

Total RNA was extracted from the leukocyte suspensions obtained for asthmatic individuals recruited for an earlier study ($n = 15$) [13] and converted to cDNA (cDNA archive kit, Applied Biosystems). The amount of *FcεRIβ* mRNA was quantified using a 7900 Real-Time PCR System (Applied Biosystems) with an Assays-on-Demand gene expression product (no. Hs00175091_m1# for human *FcεRIβ*) and TaqMan Universal PCR Mastermix (Applied Biosystems).

Statistical Analysis

Each SNP was evaluated for Hardy-Weinberg equilibrium and pair-wise LD using De Finetti (<http://ihg2.helmholtz-muenchen.de/cgi-bin/hw/hwa2.pl>) and EMLD (<https://cge.mdanderson.org/~quang/Software/pub.htm>) software. For the family-based analysis, family-based association test (FBAT; www.biostat.harvard.edu/~fbat) was used to evaluate the association in the presence of linkage [16, 17] for the different polymorphisms with asthma. Excessive transmissions of the multilocus haplotypes were tested using the *hbat* package using the additive model with bi-allelic (individual haplotype based) and multi-allelic (global test of association) modes. A quantitative transmission disequilibrium test (v. 2.4.6) [18] was carried out to test the influence of the polymorphisms on the log TstgE levels in the families with asthma (www.sph.umich.edu/csg/abecasis/QTDT). The orthogonal model of association was considered, including variance components (individual and nuclear family environment and a polygenic component), wherein global p values were calculated for all the polymorphisms investigated using the multi-allelic option.

For the case-control cohort, the differences in the allele frequencies for individual markers in the patients and controls were analyzed using $2 \times 2 \chi^2$ tests with 1 degree of freedom, and the OR were calculated with 95% CIs. Analysis of variance (ANOVA) was carried out to test the effect of these polymorphisms on percent peripheral blood eosinophil counts and *FcεRIβ* transcript levels. Haplotypes were generated in patients and unrelated controls using Phase v. 2.1 [19]. The difference in the haplotype frequencies in the patients and the controls was compared using the Monte Carlo approach, by performing 1 million simulations, as implemented in Clump v. 2.2 [20]. $p < 0.008$ (Bonferroni-adjusted $\alpha = 0.05/6$) was considered significant.

Results

The genotype frequencies for the -752C/T, -654C/T, -426T/C, -109T/C, intron 2 G/A (INT2) and the 3'-UTR C/T polymorphisms and the allele frequencies for the (CA)_n repeat in intron 5 were as per the Hardy-Weinberg expectations in the study population. In the initial genotyping, the exon 6 polymorphisms (I181V and V183L) were found to be nonpolymorphic while the E237G variant was in complete LD with the -654C/T polymorphism. Thus, for all subsequent genotyping, the -654C/T polymorphism was used. In addition, a moderate-to-very-high intermarker LD was observed amongst the 6 SNPs investigated in the study population (fig. 1). The SNP pairs -654C/T and -426T/C were in almost complete LD with each other ($D' = 0.97$, $r^2 = 0.90$).

Association of *MS4A2* Gene Polymorphisms with Asthma

In the family-based study, the -752C/T, -109T/C, INT2 G/A and the 3'-UTR C/T were found to be associated with asthma using the multi-allelic additive model in FBAT ($p = 0.0017$, 0.0011 , 0.0004 and < 0.00001 , respectively; table 2). Importantly, the allele C of -752C/T ($p = 0.0017$), allele C of -109T/C ($p = 0.0011$), allele A of INT2 G/A ($p = 0.0004$), allele 16 of (CA)_n in intron 5 ($p = 0.0014$) and allele C of 3'-UTR C/T ($p < 0.00001$) were significantly over-transmitted in the asthmatic probands, as indicated by the positive Z values in the FBAT analysis in the bi-allelic mode (table 2). On the other hand, the allele T of -752C/T, allele T of -109T/C, allele G of INT2 G/A, allele 18 of the (CA)_n repeat in intron 5 ($p = 0.0579$) and allele T of 3'-UTR polymorphism were significantly under-transmitted in the asthmatic probands. The -426T/C and -654C/T polymorphisms failed to show any significant association with asthma ($p > 0.05$; table 2). Similar results were obtained in the case-control studies (table 3).

Table 2. Bi-allelic and multi-allelic results using FBAT (additive model)

Polymorphism	Allele	Allele frequency	Families, n	Z	p value	Global χ^2	Global p value (d.f.)
<i>FC_-752 (rs573790)</i>	C	0.757	131	3.138	0.0017	9.846	0.0017 (1)
	T	0.243		-3.138			
<i>FC_-654 (rs574700)</i>	C	0.966	38	0.258	0.7963	0.067	0.7963 (1)
	T	0.034		-0.258			
<i>FC_-426 (rs1441585)</i>	C	0.033	36	-0.267	0.7893	0.071	0.7893 (1)
	T	0.967		0.267			
<i>FC_-109 (rs1441586)</i>	C	0.544	170	3.275	0.0011	10.723	0.0011 (1)
	T	0.456		-3.275			
<i>FC_INT2 (rs2583476)</i>	G	0.497	164	-3.56	0.0004	12.676	0.0004 (1)
	A	0.503		3.56			
<i>FC_(CA)_n (UniSTS_155830)</i>	15	0.002	3	–		13.712	0.0175 (5)
	16	0.47	167	3.204	0.0014		
	17	0.029	23	0.539	0.5900		
	18	0.175	115	-1.896	0.0579		
	19	0.272	149	-1.302	0.1930		
	20	0.008	8	–			
	22	0.004	3	–			
	23	0.007	6	–			
	24	0.008	9	–			
	25	0.012	16	-2.065	0.0389		
	26	0.008	9	–			
<i>FC_3'-UTR (rs569108)</i>	C	0.434	156	4.108	<0.0000	16.876	<0.00001 (1)
	T	0.566		-4.108			

The significantly associated polymorphisms are marked in italics. The p values are nominal and have not been corrected for multiple testing (Bonferroni-adjusted $\alpha = 0.05/6 = 0.008$). Z = Association statistic, where a positive value indicates over-transmission and a negative value under-transmission; Families = the number of informative families (i.e. those with a non-zero contribution to the test statistic Z).

d.f. = 53, $p = 0.00001$; maximum (T4) $\chi^2 = 65.35$, $p = 0.00001$]. Similar to the family-based study, the haplotype C_C_T_C_A_16_C was over-represented in the patients (OR = 1.97, 95% CI 1.49–2.60, $p < 0.00001$) and was identified as the risk haplotype (fig. 2).

In addition to the 7-locus haplotypes, analysis was also performed in 3-marker sliding windows. Such analysis has been previously performed in association studies to identify regions in genes that may carry particularly strong associations [21–23]. The results of this analysis for the family-based and the case-control study are shown in tables 7 and 8, respectively. As can be seen from the tables, almost all the marker combinations tested showed global significance, while a few of the 3-locus sliding window haplotypes could be positively and negatively associated with asthma. This helped us to identify the region containing the polymorphisms, namely, -426T/C, -109T/C and INT2 G/A, to be regions of high priority (as evident

by the highest significance; global $\chi^2 = 32.538$, d.f. = 5, $p < 0.00001$ in the family-based study; T1 $\chi^2 = 50.776$, d.f. = 5, $p < 0.0000001$ in the case-control study).

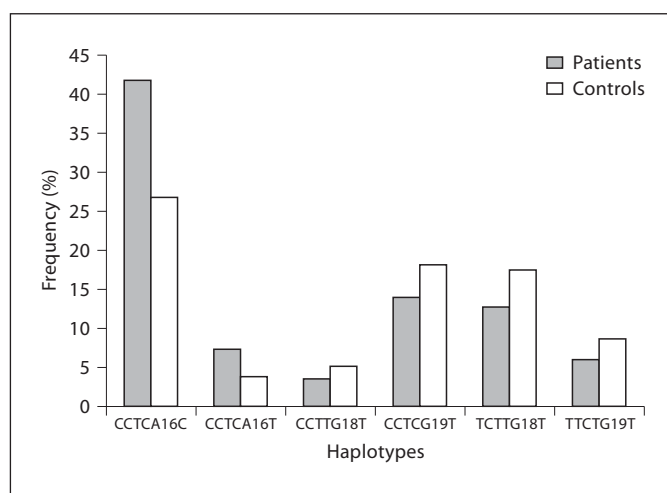
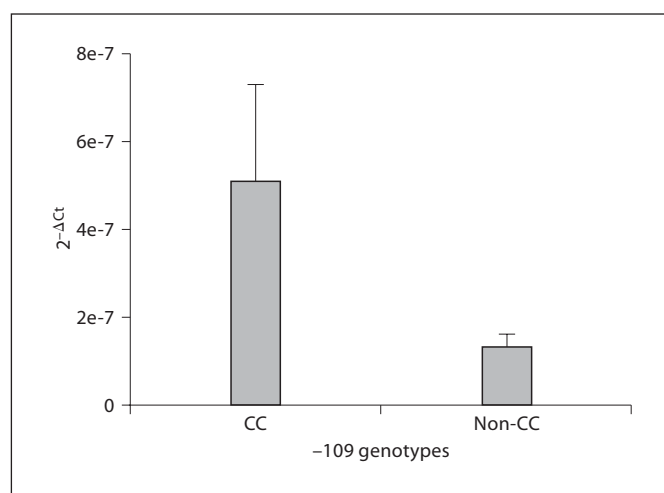
Functional Correlation of -109T/C Polymorphism with the FcεRIβ Expression Profile

To find any functional correlation of the -109T/C promoter polymorphism, identified in the high priority region using sliding-window analysis, the expression of FcεRIβ encoding mRNA was compared in individuals with different allele combinations. As can be seen in figure 3, the individuals homozygous for the risk allele C (i.e. CC genotype individuals) had higher levels of the FcεRIβ transcripts as compared to those subjects who lacked it (i.e. non-CC genotype; F ratio = 5.91, d.f. = 2, $p = 0.03$).

Table 3. Allele distribution of the *MS4A2* polymorphisms in the case-control cohort

Alleles		Patients, n (%)	Controls, n (%)	OR (95% CI)	p value (LR χ^2)
<i>FC</i> ₋₇₅₂	C	367 (77.43)	308 (69.68)	1.50 (1.11–2.01)	0.0078 (7.10)
	T	107 (22.57)	134 (30.32)		
<i>FC</i> ₋₆₅₄	C	454 (95.78)	425 (96.15)		NS
	T	20 (4.22)	17 (3.85)		
<i>FC</i> ₋₄₂₆	C	18 (3.80)	17 (3.85)		NS
	T	456 (96.20)	425 (96.15)		
<i>FC</i> ₋₁₀₉	C	286 (60.34)	186 (42.08)	2.09 (1.61–2.73)	<0.0001 (30.69)
	T	188 (39.66)	256 (57.92)		
<i>FC</i> _{INT2}	A	274 (57.81)	158 (35.75)	2.46 (1.89–3.21)	<0.0001 (44.66)
	G	200 (42.19)	284 (64.25)		
<i>FC</i> _{(CA)_n}	16	253 (53.38)	154 (34.84)	2.01 (1.54–2.62)	<0.0001 (26.27)
	18	83 (17.51)	104 (23.53)		
<i>FC</i> _{3'UTR}	C	233 (49.16)	153 (34.62)	1.83 (1.40–2.38)	<0.0001 (19.94)
	T	241 (50.84)	289 (65.38)		

Significantly associated polymorphisms are marked in italics. The p values are nominal and have not been corrected for multiple testing (Bonferroni-adjusted $\alpha = 0.05/6 = 0.008$). LR = Likelihood ratio; NS = not significant.

**Fig. 2.** The major 7-locus haplotypes (frequency $\geq 5\%$) of the *MS4A2* gene identified from the case-control study.**Fig. 3.** TaqMan real-time quantitative RT-PCR analysis for *FcεRIβ* transcripts in individuals with CC and non-CC genotypes for the locus $-109T/C$. Bars are means $\pm$ SEM. The ΔC_t values ($C_{t_{FcεRIβ}} - C_{t_{18S}}$) were calculated from duplicate samples normalized to human 18S rRNA and were converted to a linear scale by using the formula $2^{-\Delta C_t}$.

Discussion

Our earlier studies in the Indian population identified risk and protective haplotypes in the *MS4A2* gene, which were associated with differential histamine release profiles and β -subunit transcript levels [12, 13]. As the earlier study [13] provided evidence for the transcriptional

regulation of the gene, we focused on the promoter region in addition to the polymorphisms shown to be associated with atopic asthma in the Indian population [12].

Here we found a significant association of the $-752C/T$ and $-109T/C$ promoter polymorphism with asthma in

Table 4. Q-TDT orthogonal tests of association with variance components, to assess the effect of each polymorphism in the *MS4A2* gene with log TsIgE levels

Polymorphism	Allele	F	p value	Global F	Global p value
FC ₋₇₅₂	C	4.66	0.0312	4.66	0.0312
	T	4.66	0.0312		
FC ₋₆₅₄	C	5.45	0.0198	5.45	0.0198
	T	5.45	0.0198		
FC ₋₄₂₆	C	5.85	0.0158	5.85	0.0158
	T	5.85	0.0158		
FC ₋₁₀₉	C	3.2	0.074	3.2	0.074
	T	3.2	0.074		
FC _{INT2}	G	0	0.9696	0	0.9696
	A	0	0.9696		
FC _{(CA)_n}	15	NT	–	3.32	0.0193
	16	0.01	0.9236		
	17	0	0.9824		
	18	5.11	0.0241		
	19	0.9	0.3427		
	20	NT	–		
	22	NT	–		
	23	NT	–		
	24	NT	–		
	25	NT	–		
	26	NT	–		
FC _{3'-UTR}	C	0.33	0.563	0.33	0.563
	T	0.33	0.563		

The p values are nominal and have not been corrected for multiple testing (Bonferroni-adjusted $\alpha = 0.05/6 = 0.008$). NT = Not tested.

addition to the already reported association for the INT2 G/A, intron 5 (CA)_n and 3'-UTR C/T polymorphisms [12] in both the family-based and case-control study. The allele T of the –109T/C polymorphism was associated with a reduced risk of asthma and lower percent peripheral blood eosinophils, thereby pointing towards a protective role for this allele in asthma. However, no association was observed for this polymorphism with log TsIgE levels. Our results are supported by a recent study by Hizawa et al. [24], who also showed that the T-109 (and –654 CC in haplotype analysis) is negatively associated with asthma. However, contrary to our findings, an earlier study conducted in the Japanese population found a significant association of the TT genotype at –109T/C locus with higher TsIgE levels in patients with asthma, although this polymorphism was not associated with asthma [25]. Another study reported the association of –109T allele with

Table 5. Percent peripheral blood eosinophil levels with respect to different alleles and genotypes in the *MS4A2* gene

Allele/genotype	Number	Mean $\pm$ SD	F ratio	p value
-109C/T				
CC	25	8.72 $\pm$ 2.95	6.42	0.0025
CT	44	8.17 $\pm$ 3.76		
TT	21	5.39 $\pm$ 3.04		
Intron 5 (CA) _n				
16	83	8.57 $\pm$ 3.68	3.58	0.0151
17	5	6.03 $\pm$ 4.34		
18	36	6.50 $\pm$ 3.35		
19	50	7.26 $\pm$ 3.48		
Significant results are shown in italics.				

high IgE to staphylococcal enterotoxin in aspirin-sensitive asthmatics [26]. The inconsistencies in the results obtained could be due to ethnic variations in different populations, study design and patient selection criteria. For example, our study included families and asthma patients (for the case-control study) with a history of atopic asthma, with patients in the age group of 3–48 years and who were of Indo-Aryan origin. In addition, all the individuals recruited (including the normal controls) were nonsmokers and had no history of parasitic/helminthic infestation in the past 3 years, thereby excluding all intrinsic asthmatics from the study.

The –426T/C and –654C/T polymorphisms failed to show any association with asthma, TsIgE and percent peripheral blood eosinophils in the present study. The C→T at –654 position augments the YY-1 transcription factor binding site with preferential binding of the T allele to YY-1 and a significantly higher transcriptional activity [9]. These 2 SNPs showed a high degree of LD with each other in the Indian population. Our results are supported by studies conducted by Traherne et al. [27] and Nishiyama et al. [9] which studied the LD pattern in the *MS4A2* gene and found a high degree of LD between the SNPs between positions –654 and +14050 (labeled as –756 to +13948 in [27]). As observed in the earlier study [12], the A allele of INT2 G/A, allele 16 of intron 5 (CA)_n and allele C of the 3'-UTR C/T polymorphism showed a positive association with asthma while the G allele of INT2 G/A, allele 18 of intron 5 (CA)_n and allele T of the 3'-UTR C/T polymorphism showed a negative association with asthma in both the study designs. A modest degree of LD was also observed in these poly-

Table 6. Family-based association analysis (model: additive, mode: bi-allelic, multi-allelic) of FcεRIβ haplotypes with asthma

FC_-752	FC_-654	FC_-426	FC_-109	FC_INT2	FC_(CA) _n	FC_3'-UTR	Fre- quency	Families n	Z	p value	Global χ^2	Global p value (d.f.)
Haplotype												
C	C	T	C	A	16	C	0.345	110.2	2.63	0.0085	21.984	0.0012 (6)
C	C	T	T	G	19	T	0.157	81.1	-1.97	0.0488		
T	C	T	T	G	18	T	0.134	60	-2.593	0.0095		
T	C	T	T	G	19	T	0.073	45	-1.6	0.1096		
C	C	T	T	G	18	T	0.057	30	-0.3	0.7642		
C	C	T	C	A	16	T	0.051	25	0.894	0.3715		
Others							0.183	-	-	-		

The significantly associated haplotypes are marked in italics. The p values are nominal and have not been corrected for multiple testing (Bonferroni-adjusted $\alpha = 0.05/6 = 0.008$). Z = Association statistic, where a positive value indicates over-transmission and a negative value under-transmission; Families = number of informative families (i.e. those with a non-zero contribution to the test statistic Z).

Table 7. Family-based association analysis of FcεRIβ haplotypes (in 3-marker sliding windows) with asthma

FC_-752	FC_-654	FC_-426	FC_-109	FC_INT2	FC_(CA) _n	FC_3'-UTR	Fre- quency	Families n	Z	p value	Global χ^2	Global p value (d.f.)
Haplotype												
C	C	T					0.71	140	2.799	0.0051	11.272	0.0103 (3)
T	C	T					0.252	127	-3.193	0.0014		
C	T	C					0.035	25.8	0.191	0.8486		
	C	T	C				0.488	170	3.497	0.0005	16.321	0.0010 (3)
	C	T	T				0.471	163.8	-3.582	0.0003		
	T	C	C				0.036	30.8	-0.57	0.5689		
		T	T	G			0.445	126.9	-4.258	<0.0000	32.538	<0.00001 (5)
		T	C	A			0.443	142.8	2.756	0.0059		
		T	C	G			0.041	29.1	1.877	0.0605		
		T	T	A			0.034	24.2	2.582	0.0098		
		C	C	G			0.033	24	-1.424	0.1545		
			C	A	16		0.408	136	2.564	0.0104	29.444	0.0003 (8)
			T	G	19		0.238	119	-2.068	0.0387		
			T	G	18		0.184	94	-2.053	0.0401		
			C	G	16		0.027	13	-0.01	0.9920		
			C	A	17		0.017	12	1	0.3173		
			C	G	25		0.015	13	-2.5	0.0124		
			C	G	19		0.015	11	1.729	0.0838		
			T	A	16		0.015	12	2.427	0.0152		
				A	16	C	0.361	123.5	3.04	0.0024	12.547	0.0508 (6)
				G	19	T	0.24	114.8	-1.887	0.0592		
				G	18	T	0.19	93	-1.983	0.0473		
				A	16	T	0.067	37	0.347	0.7287		
				G	16	T	0.022	13	-0.003	0.9975		
				A	17	C	0.018	13	1	0.3173		

The results of the significantly associated haplotypes are marked in italics. The p values are nominal and have not been corrected for multiple testing. Z = Association statistic, where a positive value indicates over-transmission and a negative value under-transmission; Families = number of informative families (i.e. those with a non-zero contribution to the test statistic Z).

Table 8. Association analysis of FcεRIβ haplotypes (in 3-marker sliding windows) with asthma in the case-control cohort

FC_-752	FC_-654	FC_426	FC_-109	FC_INT2	FC_(CA) _n	FC_3'-UTR	Patients	Controls	Likelihood ratio χ^2	p value	Global χ^2 (T1)	Global p value (d.f.)
Haplotype												
C	C	T					0.73	0.658	5.535	0.019	10.669	0.0158 (4)
T	C	T					0.226	0.303	7.007	0.008		
C	T	C					0.036	0.038	0.043	0.835		
	C	T	C				0.559	0.385	28.072	<0.0000	34.835	0.000002 (5)
	C	T	T				0.397	0.577	29.929	<0.0000		
	T	C	C				0.036	0.036	0.001	0.978		
		T	T	G			0.367	0.552	31.7	<0.0000	50.776	<0.0000001 (5)
		T	C	A			0.548	0.333	43.616	<0.0000		
		T	C	G			0.017	0.052	8.951	0.0027		
		T	T	A			0.030	0.025	0.187	0.666		
		C	C	G			0.038	0.036	0.02	0.887		
			C	A	16		0.511	0.312	37.412	<0.0000	77.012	<0.000001 (23)
			T	G	19		0.205	0.285	8.04	0.0045		
			T	G	18		0.156	0.226	7.32	0.0068		
			C	G	16		0.011	0.018	0.937	0.333		
			C	A	17		0.021	0.018	0.107	0.745		
			C	G	25		0.019	0.016	0.133	0.716		
			C	G	19		0.00	0.018	–	–		
			T	A	16		0.015	0.014	0.023	0.879		
				A	16	C	0.435	0.290	20.79	<0.0000	84.406	<0.00001 (25)
				G	19	T	0.20	0.287	9.408	0.0021		
				G	18	T	0.158	0.224	6.433	0.0112		
				A	16	T	0.095	0.036	12.695	0.0002		
				G	16	T	0.00	0.020	–	–		
				A	17	C	0.019	0.018	0.01	0.9207		

The results of the significantly associated haplotypes are marked in italics. The global p values were calculated using Clump v. 2.2 with 1 million Monte Carlo simulations. The p values are nominal and have not been corrected for multiple testing.

morphisms (fig. 1). Thus, replication of our earlier results in a more robust family-based study have further confirmed our findings and have made it very unlikely that our results are due to stratification and/or an inherent statistical bias.

Further, 7-locus haplotype analysis using *hbat* identified C_C_T_C_A_16_C as the major risk/susceptibility haplotype that is positively associated with asthma, and T_C_T_T_G_18_T as the major protective haplotype that is negatively associated with asthma. Furthermore, when analysis was performed in 3-locus sliding windows, we identified the 3-locus [INT2_(CA)_n3'UTR] haplotype A_16_C and G_18_T to be positively and negatively associated with asthma, respectively, thereby confirming our earlier results [12]. This analysis also

helped us to identify the –426T/C, –109T/C and INT2 G/A polymorphisms to be in regions of highest priority as evident by the highest significance obtained ($p < 0.00001$).

The common haplotype consisting of –752T, –654C, –426T and –109T has previously been associated with decreased cell expression of FcεRI on peripheral blood basophils and with a lower promoter activity in vitro [9]. Our earlier study also found the protective haplotype G_18_T to be associated with lower histamine release (IgE receptor mediated) from basophils and decreased transcript levels of the β subunit [13]. This haplotype constitutes a subpart of the 7-locus protective haplotype T_C_T_T_G_18_T identified from this study. Additionally, the –109C allele was associated with higher levels of the FcεRI

β -chain transcripts (fig. 3). Importantly, the –109T allele has been shown to up-regulate Fc ϵ RI β through enhanced promoter activity in transfection experiments [26], while the *MS4A2* promoter haplotype that includes the T alleles for –752C/T and –109T/C has recently been shown to be associated with decreased levels of Fc ϵ RI on the surface of peripheral blood basophils and with lower transcriptional activity in vitro [9].

In the light of these observations, our present results suggest that the genetic association obtained between the *MS4A2* polymorphisms and asthma could be justified on the basis of a possible promoter-dependent functional mechanism. Thus, we speculate that the –109T/C polymorphism could play an important role in the transcriptional regulation of the *MS4A2* gene. Although no transcription-factor binding to the site containing the –109T/C polymorphism has been identified [9, 26] to date in the transcription factor database (TRANSFAC), we cannot exclude the possibility of an unidentified transcription-factor binding site being present at this point, resulting in altered expression in the 2 alleles for this variation. Nevertheless, the possibility of the presence of any other functional polymorphisms in linkage

disequilibrium with the –109T allele or on the background of T_C_T_T_G_18_T haplotype should not be overlooked.

In conclusion, our study has identified that the –109T/C polymorphism differentially regulates Fc ϵ RI β mRNA levels in the Indian population. Thus, a possible promoter-dependent mechanism may explain the earlier association observed with differential IgE receptor-mediated histamine release from basophils as well as β -subunit transcript levels. These results could be useful in predicting the genetic susceptibility of individuals towards developing asthma.

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References

- Marone G, Poto S, Celestino D, Bonini S: Human basophil releasability. III. Genetic control of human basophil releasability. *J Immunol* 1986;137:3588–3592.
- Kinet JP: The high-affinity IgE receptor (Fc epsilon RI): from physiology to pathology. *Annu Rev Immunol* 1999;17:931–972.
- Hill MR, Cookson WO: A new variant of the beta subunit of the high-affinity receptor for immunoglobulin E (Fc epsilon RI-beta E237G): associations with measures of atopy and bronchial hyper-responsiveness. *Hum Mol Genet* 1996;5:959–962.
- Kim YK, Cho SH, Koh YY, Son JW, Lee BJ, Min KU, Kim YY: Linkage between IgE receptor-mediated histamine releasability from basophils and gene marker of chromosome 11q13. *J Allergy Clin Immunol* 1999;104:618–622.
- Kim YK, Oh SY, Oh HB, Chun SY, Cho SH, Koh YY, Min KU, Kim YY: Coding single nucleotide polymorphism in the high-affinity immunoglobulin E receptor b chain (Fc epsilon RI-beta) gene is associated with immunoglobulin E receptor-mediated histamine release from basophils. *Clin Exp Allergy* 2002;32:751–755.
- Shirakawa T, Li A, Dubowitz M, Dekker JW, Shaw AE, Faux JA, Ra C, Cookson WO, Hopkin JM: Association between atopy and variants of the beta subunit of the high-affinity immunoglobulin E receptor. *Nat Genet* 1994;7:125–129.
- Kim YK, Park HW, Yang JS, Oh SY, Chang YS, Shin ES, Lee JE, Kim S, Gho YS, Cho SH, Min KU, Kim YY: Association and functional relevance of E237G, a polymorphism of the high-affinity immunoglobulin E-receptor beta chain gene, to airway hyper-responsiveness. *Clin Exp Allergy* 2007;37:592–598.
- Shirakawa T, Mao XQ, Sasaki S, Enomoto T, Kawai M, Morimoto K, Hopkin J: Association between atopic asthma and a coding variant of Fc epsilon RI beta in a Japanese population. *Hum Mol Genet* 1996;5:1129–1130.
- Nishiyama C, Akizawa Y, Nishiyama M, Tokura T, Kawada H, Mitsuishi K, Hasegawa M, Ito T, Nakano N, Okamoto A, Takagi A, Yagita H, Okumura K, Ogawa H: Polymorphisms in the Fc epsilon RI beta promoter region affecting transcription activity: a possible promoter-dependent mechanism for association between Fc epsilon RI beta and atopy. *J Immunol* 2004;173:6458–6464.
- Donnadieu E, Cookson WO, Jouvin MH, Kinet JP: Allergy-associated polymorphisms of the Fc epsilon RI beta subunit do not impact its two amplification functions. *J Immunol* 2000;165:3917–3922.
- Furumoto Y, Hiraoka S, Kawamoto K, Sasaki S, Kitamura T, Okumura K, Ra C: Polymorphisms in Fc epsilon RI beta chain do not affect IgE-mediated mast cell activation. *Biochem Biophys Res Commun* 2000;273:765–771.
- Sharma S, Nagarkatti R, Rao C, Niphadkar PV, Vijayan V, Sharma SK, Ghosh B: A_16_C haplotype in the Fc epsilon RIbeta gene confers a higher risk for atopic asthma in the Indian population. *Clin Genet* 2004;66:417–425.
- Sharma S, Nagpal K, Mabalirajan U, Kumar A, Ghosh B: Correlation of FCER1B gene haplotypes with histamine release from basophils in atopic asthma. *J Allergy Clin Immunol* 2006;118:960–963.
- Nagpal K, Sharma S, Rao C, Nahid S, Niphadkar PV, Sharma SK, Ghosh B: TGFbeta1 haplotypes and asthma in Indian populations. *J Allergy Clin Immunol* 2005;115:527–533.

- 15 National Asthma Education and Prevention Program Expert Panel Report: Guidelines for the diagnosis and management of asthma update on selected topics: 2002. *J Allergy Clin Immunol* 2002;110:S141–S219.
- 16 Laird NM, Horvath S, Xu X: Implementing a unified approach to family-based tests of association. *Genet Epidemiol* 2000;19(suppl 1): S36–S42.
- 17 Rabinowitz D, Laird N: A unified approach to adjusting association tests for population admixture with arbitrary pedigree structure and arbitrary missing marker information. *Hum Hered* 2000;50:211–223.
- 18 Abecasis GR, Cardon LR, Cookson WO: A general test of association for quantitative traits in nuclear families. *Am J Hum Genet* 2000;66:279–292.
- 19 Stephens M, Donnelly P: A comparison of bayesian methods for haplotype reconstruction from population genotype data. *Am J Hum Genet* 2003;73:1162–1169.
- 20 Sham PC, Curtis D: Monte Carlo tests for associations between disease and alleles at highly polymorphic loci. *Ann Hum Genet* 1995;59:97–105.
- 21 Zabolli G, Gizatullin R, Nilsson A, Wilczek A, Jonsson EG, Ahnemark E, Asberg M, Leopardi R: Tryptophan hydroxylase-1 gene variants associate with a group of suicidal borderline women. *Neuropsychopharmacology* 2006;31:1982–1990.
- 22 Zabolli G, Jonsson EG, Gizatullin R, Asberg M, Leopardi R: Tryptophan hydroxylase-1 gene variants associated with schizophrenia. *Biol Psychiatry* 2006;60:563–569.
- 23 Hawkins GA, Tantisira K, Meyers DA, Ampleford EJ, Moore WC, Klanderman B, Liggett SB, Peters SP, Weiss ST, Bleeker ER: Sequence, haplotype, and association analysis of ADRbeta2 in a multiethnic asthma case-control study. *Am J Respir Crit Care Med* 2006;174:1101–1109.
- 24 Hizawa N, Maeda Y, Konno S, Fukui Y, Takahashi D, Nishimura M: Genetic polymorphisms at FCER1B and PAI-1 and asthma susceptibility. *Clin Exp Allergy* 2006;36: 872–876.
- 25 Hizawa N, Yamaguchi E, Jinushi E, Kawakami Y: A common FCER1B gene promoter polymorphism influences total serum IgE levels in a Japanese population. *Am J Respir Crit Care Med* 2000;161:906–909.
- 26 Kim SH, Bae JS, Holloway JW, Lee JT, Suh CH, Nahm DH, Park HS: A polymorphism of MS4A2 (–109T>C) encoding the beta-chain of the high-affinity immunoglobulin E receptor (Fc epsilon R1beta) is associated with a susceptibility to aspirin-intolerant asthma. *Clin Exp Allergy* 2006;36:877–883.
- 27 Traherne JA, Hill MR, Hysi P, D'Amato M, Broxholme J, Mott R, Moffatt MF, Cookson WO: LD mapping of maternally and non-maternally derived alleles and atopy in Fc epsilon RI-beta. *Hum Mol Genet* 2003;12: 2577–2585.