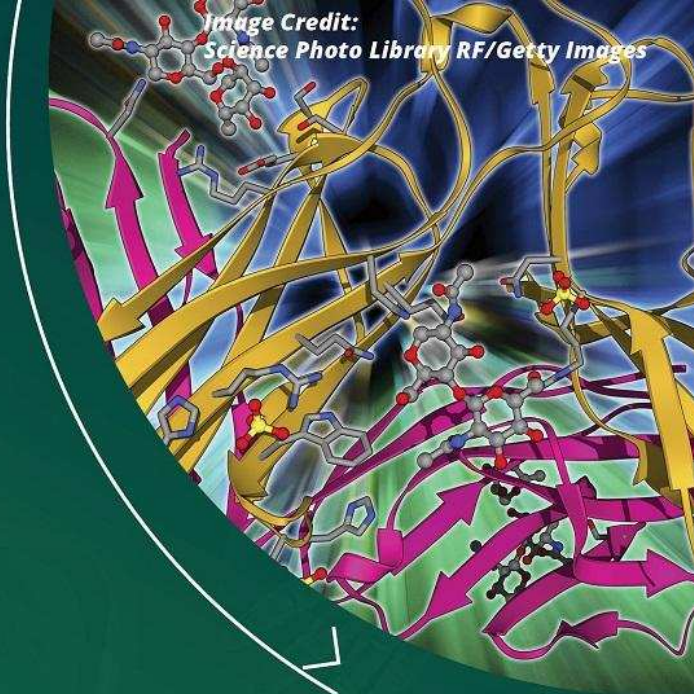
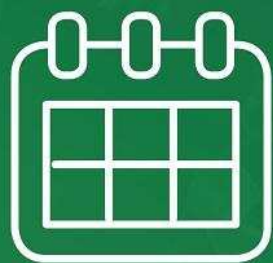


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FcεR1α gene polymorphism shows association with high IgE and anti-FcεR1α in Chronic Rhinosinusitis with Nasal Polyposis[†]

Running head: FcεR1α polymorphism is associated with CRSwNP

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Abstract

Despite large number of investigations, the etiology of chronic rhinosinusitis (CRS) remains unclear. Several factors are likely involved in its onset. The genetic susceptibility of IgE-responsiveness likely caused by polymorphism(s) in high affinity receptor for IgE (FcεR1α) gene can help in understanding the pathophysiology of CRS with nasal polyposis (CRSwNP). A population-based case-control association analysis was conducted to assess the risk of CRSwNP conferred by single nucleotide polymorphisms (SNPs) in FcεR1α gene in a North Indian cohort. Two promoter and three exonic regions of FcεR1α gene were amplified and sequenced to investigate five SNPs - rs2427827, rs2251746, rs2298804, rs2298805, and rs2269718. BLAST analysis and subsequent multiple alignments, with known sequences available in the NCBI database, were performed. Total serum IgE and FcεR1α antibody levels were estimated. Patient IgE level of 461.22 ± 436.43 in comparison to 83.62 ± 58.043 IU/mL in controls ($p < 0.0001$), and FcεR1α antibody level of 292.38 ± 115.27 in comparison to 160.56 ± 105.9 in controls ($p < 0.0001$), depicts their highly significant associations with CRSwNP disease. However, no SNP showed evidence of association with CRSwNP; although relatively higher Odds ratios were observed with rs2427827, rs2251746 and rs2298804. Patient stratification revealed a significant association ($p < 0.05$) of rs2427827 SNP with high IgE level CRSwNP patients. Nonetheless, we found no SNP associated with low serum IgE level patients. SNP (rs2427827) in the FcεR1α gene region and high IgE levels may confer susceptibility to CRSwNP in north Indian population. However, further studies including larger sample size, gene-gene and gene-environment interactions are required for its elucidation. This article is protected by copyright. All rights reserved

Keywords:

- Chronic Rhinosinusitis
- Nasal Polyposis
- FcεR1α
- Single Nucleotide Polymorphism
- IgE

Introduction

Chronic rhinosinusitis (CRS) is one of the most common heterogeneous inflammations in adults persisting for more than 3 months. It involves the sinus mucosa and its fluids (mucous membranes of the nasal cavity and paranasal sinuses), and/or underlying bone imparting a big personal and economic burden in terms of compromised quality of life and productivity [Christodoulopoulos et al., 2000; Meltzer et al., 2006; Yao et al., 2009; Fokkens et al., 2012; Keswani et al., 2012].

CRS through multiple disease processes leads to sino-nasal blockage and mucosal inflammation. The histological characteristics of the disorder include thickening of the mucosal membrane, goblet cell hyperplasia, sub-epithelial fibrosis, and persistent inflammation involving lymphocytes and plasma cells and/or eosinophils [Christodoulopoulos et al., 2000]. It is commonly classified into 2 types as CRS with nasal polyposis (CRSwNP) or CRS without nasal polyposis (CRSsNP) based on the presence of nasal polyps [Meltzer et al., 2006; Fokkens et al., 2012; Keswani et al., 2012]. Patients with nasal polyposis (NPs) exhibit proliferation of the epithelial layer, significant bilateral mucosal thickening, glandular hyperplasia, edema, focal fibrosis and persistent nasal mucosal inflammation [Yao et al., 2009]. Despite being actively investigated for more than past two decades, the etiology of CRS remains unclear. Several factors are likely involved in its onset like allergy, defective mucociliary clearance, impaired immune function, bacterial and fungal infections and impaired epithelial defense, along with an important genetic component [Schubert, 2001; Schubert, 2006; Van Cauwenberge et al., 2006; Kern et al., 2008; Hsu and Peters, 2011].

A significant increase in local IgE antibodies in nasal polyps in CRSwNP patients also indicates an association between high levels of total IgE and eosinophilic inflammation leading to worsening of symptoms [Bachert et al., 2001; Riechelmann et al., 2005; Van Zele et al., 2006]. Additionally, genetic factors influence basal IgE production, which is independent of specific responses to allergens. However, it remains to be elucidated that whether the underlying inflammation is an IgE mediated hypersensitivity phenomena with host mucosal damage due to the abundant eosinophils found in the tissue or is it a probable consequence of an autoimmune phenomena with formation of auto-antibodies against high affinity receptor for IgE (FcεR1α) [Marsh et al., 1994; Palmer et al., 2000; Jacobsen et al., 2001; Strachan et al., 2001; Haukim et al., 2002; Erbek et al., 2007; Otto and Wenzel, 2008]. In an allergic disease, IgE receptor

facilitates coupling of allergen with mast cell triggering the inflammatory and immediate hypersensitivity responses. The allergic response occurs when two or more high affinity IgE receptors are cross linked via IgE molecule that in turn are bound to an allergen molecule [Galli and Tsai, 2012].

FcεR1α encodes the ligand-binding subunit of the high-affinity IgE receptor (FcεR1), the α-chain [Zhang et al., 2007]. The genetic susceptibility of IgE-responsiveness which is likely to be caused by a pattern of polymorphisms in FcεR1α gene can help in understanding the pathophysiology of CRS and pathogenesis of NP [Hasegawa et al., 2003a; Potaczek et al., 2006; Bae et al., 2007; Potaczek et al., 2007; Kanada et al., 2008]. The role of atopy, autoantibodies to IgE receptor -FcεR1α on mast cell leading to dimerization and degranulation may have functional relevance in understanding the outcome of these patients.

We hypothesized that FcεR1α gene affects the production of total serum IgE and that single nucleotide polymorphisms (SNPs) in the FcεR1α gene region may influence the risk of developing CRSwNP. Therefore, to investigate the effects of polymorphisms in the FcεR1α gene with CRSwNP risk and on the value of total IgE in CRSwNP patients, we conducted a population-based case-control association analysis to assess the risk of CRSwNP conferred by SNPs in the FcεR1α gene region in a North Indian cohort.

Materials and Methods

Subjects

One hundred patients of CRSwNP were admitted to a tertiary care hospital, East Delhi with a diagnosis of unilateral or bilateral nasal polyps for functional endoscopic sinus surgery (FESS) during the period of January, 2013 to January, 2016. The diagnosis of CRSwNP was done according to definition by the European Position Paper on Chronic Rhinosinusitis and Nasal Polyps [Fokkens et al., 2012]. The following data were collected from each subject: age, sex, CT scan findings, IgE level, operative details and final diagnosis based on histopathological findings of the removed tissue specimens. The CT score for each patient was calculated on basis of study by Lund and Kennedy, 1997. Age and sex matched 50 healthy adult volunteers without any history of allergy or any previous nasal or sinus surgery were also enrolled as controls. The study was approved by Institutional Ethical Committee for Human Research (IEC-HR).

Sample collection and processing

Venous blood samples (4ml each) were collected from all the patients of CRSwNP and healthy volunteers in plain vacutainer (for serum isolation) and in EDTA vial. Sera were stored at -80°C until IgE & FcεR1α antibody levels were estimated. Blood in EDTA vials was used to extract genomic DNA using HiYield Genomic DNA Mini Kit (RBC, Germany).

Amplification of FcεR1α genomic regions by PCR

The extracted genomic DNA was subjected to PCR amplification using intronic forward and reverse primers of promoter, and exonic regions of FcεR1α gene (**Table 1**) [Kim et al., 2006; Bae et al., 2007; Weidinger et al., 2008]. The PCR products were sent to 1st Base DNA Sequencing, Malaysia for sequencing. The mutation site and SNP reference IDs are shown in **Table 2**. Briefly, the PCR amplification was performed in a Mastercycler personal (Eppendorf, Germany) with initial denaturation at 95°C for 5 min followed by 40 cycles of amplification, comprising of denaturation step at 95°C for 30 sec, annealing step at 58°C for 30 sec, extension at 72°C for 45 sec, and a final extension at 72°C for 10 min. The PCR amplicon were resolved on 2% agarose gel with 0.5 mM ethidium bromide and visualized on UV trans-illuminator.

BLAST was used to analyze sequences and subsequent multiple alignment with the known sequences available in the database.

Estimation of serum level of FcεR1α antibody and Immunoglobulin E

Total serum IgE (Calbiotech, USA) and FcεR1α antibodies (MyBiosource, USA) levels were estimated using ELISA kits following manufacturer's instructions.

Statistical analysis

Statistical analysis was carried out using SPSS software (SPSS Inc., Chicago, IL, USA; version 20.0). Independent *t*- test was performed to compare the serum levels of IgE and FcεR1α antibody among cases versus control subjects. Data were expressed as mean (±SD). All the tests were two sided with the significance level at probability below 0.05. Genotype frequency, minor allele frequency and Hardy Weinberg equilibrium at each polymorphic site was tested using chi-square test.

Results

Patients' profile

The patient's profile including age, sex, clinical details and relevant laboratory investigations are described in **Table 3**. CRSwNP patients and healthy volunteers were compared in terms of age,

male/female ratio and the presence and absence of allergy (based on serum level of total IgE and absolute eosinophil count). The numbers of males affected were more than females among the study groups. Mean duration of disease was 10.56 ± 8.60 months while as mean CT score was recorded to be 8.16 ± 1.60 .

Analysis of IgE and FcεR1α antibodies level

Total IgE levels and FcεR1α antibody in serum of CRSwNP patients and controls were estimated and expressed as mean ($\pm$ SD). The IgE level was 461.22 ($\pm$ 436.43) IU/mL for patients and 83.62 ($\pm$ 58.043) IU/mL for controls. An independent sample test showed a highly significant ($p < 0.0001$) association of high IgE level in CRSwNP cases as compared to controls. Also, high FcεR1α antibody level (292.38 ± 115.27) in CRSwNP cases as compared to controls (160.56 ± 105.9) showed the substantial significant ($p < 0.0001$) association (**Table 4**). Further, the CRSwNP cases were grouped as high IgE (above 500) and low IgE level (below 100) and FcεR1α antibody levels were analyzed in high IgE and low IgE group of CRSwNP cases and found their levels as 283.54 ± 110.89 and 243.14 ± 107.03 , respectively but no significant association could be established among these groups (**Table 4**).

Mutation analysis

The polymorphisms of functional genetic variants of FcεR1α gene were analyzed by BLAST and multiple alignment methods. The sequences were multiple aligned with the known sequences using MEGA5 software and polymorphism of FcεR1α gene namely rs2427827, rs2251746, rs2298804, rs2298805, and rs2269718 were analyzed among CRSwNP cases and healthy volunteers.

The analysis of allele frequencies for the 5 SNPs tested in the FcεR1α gene revealed no significant associations ($p > 0.05$) with CRSwNP outcome. Although none of the SNPs showed evidence of association, rs2427827, rs2251746 and rs2298804 showed relatively higher Odds ratios among the selected SNPs (**Table 5**).

Further, CRSwNP cases were stratified in 2 groups according to the value of IgE serum level. The analysis of allele frequency for rs2427827 SNP tested in the FcεR1α gene revealed a significant association ($p < 0.05$) with CRSwNP outcome with an OR of 2.24 in cases having high IgE serum level (**Table 6**). However, none of the analyzed SNPs in the FcεR1α gene was associated with CRSwNP outcome in cases having low serum IgE level (**Table 7**).

Discussion

The role of genetic predisposition in the development of allergic diseases has led to many genomic studies to elucidate underlying processes [Young et al., 1992; Cox et al., 1998; Cookson, 2002]. Although an allergic disease has a high genetic and phenotypic heterogeneity, but the study of total IgE and the factors influencing its production may provide important clues about the development and progression of atopic reactions [Jacobsen et al., 2001; Strachan et al., 2001; Gieger et al., 2008; Gibson, 2009].

A recent large-scale population-based GWAS showed that functional variants of the *FcεR1α* gene encoding the alpha chain of the high affinity receptor for IgE may regulate total IgE production [Weidinger et al., 2008]. Allergic response is largely characterized by production of IgE and raised total IgE levels can lead to atopy and diseases like asthma, rhinitis and atopic dermatitis [Kay, 2001].

Although many metabolic networks contribute to IgE production but serum IgE levels are genetically controlled with heritability estimates of 50–80% [Hanson et al., 1991; Marsh et al., 1994; Palmer et al., 2000; Jacobsen et al., 2001; Strachan et al., 2001; Geha et al., 2003].

Stochastically, interaction of allergen specific IgE bound to *FcεR1* plays a central role in allergic disease, through degranulation of mast cells initiating the inflammatory and immediate hypersensitivity responses and eventual manifestation of allergic symptoms [Pawankar et al., 1997]. The cross-linking of human IgE to *FcεR1* is located in the extra cytoplasmic region of the receptor [Hakimi et al., 1990], complex encoded by alpha, beta, gamma subunits. The α -chain of high-affinity receptor for IgE, responsible for binding of IgE with *FcεR1α* is encoded by gene representing the alpha subunit. Few reports suggest that gene polymorphisms in the β -chain of IgE receptor modulates IgE responsiveness [Cookson et al., 1992]. However, majority of reports show an association of functional polymorphism in *FcεR1α*, an important immunity-related gene, with the development of allergic diseases [Weidinger et al., 2008; Palikhe et al., 2008; von Berg et al., 2008]. The observation have led to many gene-variability studies in human [Shikanai et al., 2002; Hasegawa et al., 2003a; Potaczek et al., 2006; Bae et al., 2007; Potaczek et al., 2007; Kanada et al., 2008; Weidinger et al., 2008; Potaczek et al., 2008] and other mammals [Musilova et al., 2005; Vychodilova-Krenkova et al., 2005].

In this study, we observed significantly high IgE and *FcεR1α* antibody levels in allergic rhinosinusitis patients indicating the role of *FcεR1α* as a candidate receptor for influencing total

serum IgE. Early studies show that IgE levels are strongly associated with common variants in FcεR1α gene [Hasegawa et al., 2003a; Weidinger et al., 2008; Chen et al., 2009; Mahachie et al., 2010]. Many SNPs associated with atopy localized in the regulatory sequences of the 5'-flanking region and 5'-untranslated region (5'-UTR) influence transcriptional activity of the FcεR1α gene and the subsequent receptor expression along with serum IgE levels [Shikanai et al., 2002; Hasegawa et al., 2003a; Bae et al., 2007; Potaczek et al., 2007; Kanada et al., 2008; Potaczek et al., 2009].

We examined five single nucleotide polymorphisms (SNPs) in the FcεR1α gene region for associations with the risk of CRSwNP outcome, and found significant association of SNP rs2427827 with high serum IgE levels in CRSwNP cases which is concordant with many previous reports showing rs2427827 polymorphism association with atopy and with serum IgE levels in allergic patients [Shikanai et al., 2002; Potaczek et al., 2006; Bae et al., 2007; Potaczek et al., 2007]. This SNP has been found to be associated with higher proximal promoter activity in mast cell line, creating binding motif for MAZ transcriptional factor and higher histamine release from peripheral basophils obtained from aspirin-induced chronic urticaria patients [Kim et al., 2006; Bae et al., 2007].

Another polymorphism analyzed in the current study was rs2251746, genomic neighbor of rs2427827, which has an association with increased proximal promoter activity in cultured mast cells. Resultant enhanced FcεR1α expression play an important role in the development of atopy [Hasegawa et al., 2003a]. Early studies showed that SNP rs2427827 and rs2251746 strongly influence FcεR1α expression in mast cells and/or basophils in synergistic manner [Hasegawa et al., 2003a; Bae et al., 2007; Kanada et al., 2008].

Presence of T allele at -95 locus hinted at an association of SNP rs2251746 with the development of CRSwNP in the subjects having high IgE levels in our study. However association failed to reach significance. The reason for this inconsistency may lie in the ethnic difference as the previous studies were conducted in Caucasian and east Asian races.

The SNP rs2298804 that has been shown to have a protective effect against SLE in Chinese female patients, along with rs2298805 and rs2269718 also suggested an association of SNP with the development of CRSwNP in the subjects having high IgE levels in our study but the association failed to reach statistical significance [Shikanai et al., 2002].

Early reports analyzing autoimmune background of allergic diseases show the presence of autoantibodies against IgE and/or FcεR1α in allergic disorders, like chronic idiopathic urticarial and asthma [Sabroe et al., 1999; Platzer et al., 2005; Jang et al., 2007]. We also observed significantly high FcεR1α antibodies in the study group. However, FcεR1α promoter polymorphisms analysis for association with the autoimmune phenomena reported no association of rs2427827 and rs2251746 with the presence of autoantibodies against FcεR1α in a heterogeneous group of asthma or urticarial subjects [Potaczek et al., 2006; Bae et al., 2007]. These two SNPs were also not associated with the presence of common markers for autoimmunity as antithyroglobulin, antimicrosomal or antinuclear autoantibodies in aspirin-induced chronic urticarial (AICU) patients [Bae et al., 2007].

The high circulating autoantibodies to high affinity IgE receptor present predominantly on mast cells and basophils indicate an underlying autoimmune mechanism due to molecular mimicry between the fungal antigen and self-protein (FcεR1α receptor). These autoantibodies may be the mechanism of bystander sensitization to self-proteins by epitope spreading that remains to be elucidated.

High levels of IgE in Indian population owing to underlying parasitic infections probably leads to chronic allergen stimulation which when coupled with the genetic variation of FcεR1α gene affects its expression in CRSwNP patients that may eventually influence the levels of IgE and receptor autoantibodies (FcεR1α). FcεR1 expression on the cell surface is greatly modulated by IgE [MacGlashan et al., 1998; Macglashan, 2005] and in turn serum IgE levels are associated with FcεR1 expression on different cell types [Sihra et al., 1997; Foster et al., 2003]. Thus, the presence of a positive feedback loop involving IgE upregulation of FcεR1 on basophils and mast cells, greater IL-4 secretion induced by upregulated FcεR1, and higher IgE production resulting from IL-4-dependent B-cell switch for IgE synthesis is speculated [Sihra et al., 1997; Sanak et al., 2007].

Furthermore, FcεR1 is also constitutively expressed in dendritic cells (DCs) and monocytes in addition to mast cells and basophils in humans. A recent study propose that human FcεR1 is intrinsically endocytosed and moved to the lysosomes in BDCA1⁺ DCs and monocytes, mediating cellular entry of circulating IgE leading to serum IgE clearance. Our observation of significantly high FcεR1α and total IgE antibodies suggest alteration in FcεR1 endolysosomal trafficking in the DCs or monocytes, resulting in an accumulation of IgE in circulation and/or an

increase in IgE available to bind to mast cells or basophils, leading to exacerbation of atopic reactions in CRSwNP patients [Waldmann et al., 1976; Dreskin et al., 1987; Greer et al., 2014]. CRSwNP onset and progression is likely the result of a complicated network of numerous players affecting an additive or synergistic effect [Nishiyama et al., 2002; Hasegawa et al., 2003b].

Conclusion: SNPs in the FcεR1α gene region probably affect a smaller risk of developing CRSwNP. However, a larger sample size is required to elucidate the role of FcεR1α SNPs in CRSwNP risk/ outcome. Our report, first from India, suggests the involvement of rs2427827 SNP, elevated levels of IgE and FcεR1α autoantibodies in CRSwNP risk. In conclusion, rs2427827 polymorphisms in the FcεR1α gene region and high IgE levels may confer susceptibility to CRSwNP in a north Indian population. Further studies including gene-gene and gene-environment interactions in CRSwNP cohorts are required to elucidate the influence of FcεR1α SNPs on CRSwNP.

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Table 1: List of primer sequences used to amplify the promoter and exonic regions of FcεR1α gene.

Target Gene	Location	Primers	References
FcεR1α	Promoter	Forward: tggcatatgtttggtattcagt Reverse: aatctgtcaatctgtgtacaactatttag	Kim et al., 2006; Bae et al., 2007
FcεR1α	Promoter	Forward: agaagcaaaaccaggcacag Reverse: cagctgctcccaaattc	Kim et al., 2006; Bae et al., 2007
FcεR1α	Exon	Forward: tgattgtcagaatattgcttcg Reverse: tctggtgtcctaacccttgg	Weidinger et al., 2008
FcεR1α	Exon	Forward: tgattgtcagaatattgcttcg Reverse: tctggtgtcctaacccttgg	Weidinger et al., 2008
FcεR1α	Exon	Forward: gacacatgctctatgcgtgg Reverse: agctgcaactgctcaggc	Weidinger et al., 2008

Table 2: List of SNPs of FcεR1α gene and their mutation site.

Reference SNP	Mutation site
rs2427827	ATTAGTTGCTGCTGTTTTATTCTGC[C]CTCCCTTGCATCCCACCTTTTCTAAG ATTAGTTGCTGCTGCTGTTTTATTCTGC[T]CTCCCTTGCATCCCACCTTTTCTAAG
rs2251746	CAGCTGATGGGTTAACCAGATATGA[C]ACAGAAAACATTTCTTCTGCTTTT CAGCTGATGGGTTAACCAGATATGA[T]ACAGAAAACATTTCTTCTGCTTTT
rs2298804	TCAAGTTTGAATATTGTGAATGCCA[A]ATTTGAAGACAGTGGAGAATACAAA TCAAGTTTGAATATTGTGAATGCCA[G]ATTTGAAGACAGTGGAGAATACAAA
rs2298805	TGTCAGCACCAACAAGTTAATGAGA[A]TGAACCTGTGTACCTGGAAGTCTTC TGTCAGCACCAACAAGTTAATGAGA[G]TGAACCTGTGTACCTGGAAGTCTTC
rs2269718	GTTTCTGACACATGCTCTATGCGTG[A]CTCTCTTTTCTCTATTCAATTCTCTC GTTTCTGACACATGCTCTATGCGTG[G]CTCTCTTTTCTCTATTCAATTCTCTC

Table 3: Profile of CRSwNP patients and healthy controls.

Parameters	CRSwNP	Healthy
Patients (n)	100	50
Age (years)	29.93 ± 9.7	27.4 ± 4.18
Sex (Male: Female)	63:37	28:22
Duration of disease (months)	10.56 ± 8.60	0
CT Score	8.16 ± 1.60	0

Table 4: Serum level of total IgE and FcER1 α antibody in CRSwNP patients versus controls.

	Total IgE	<i>p</i> value	FcER1 α	<i>p</i> value
Total cases (CRSwNP) (N=100)	461.22±436.43	<0.0001	292.38±115.27	<0.0001
Healthy controls (N=50)	83.62±58.043		160.56±105.9	
High IgE cases (N=64)	697.88±334.009	<0.0001	283.54±110.89	0.281
Low IgE cases (N=36)	62.3±32.24		243.14±107.03	

Table 5: Allelic frequency and Odds ratio of SNPs in FcεR1α gene.

Reference SNP	Allele	Cases (N=100)	Controls (N=50)	Minor Allele Frequency (MAF)		Odds Ratio	Chi square (<i>p</i> value)	
				Cases	Controls		Yates	Pearson
rs2427827	C	162	88	0.12	0.19	1.72	0.17	0.12
	T	38	12					
rs2251746	T	182	94	0.06	0.09	1.54	0.49	0.36
	C	18	6					
rs2298804	A	188	96	0.04	0.06	1.53	0.64	0.46
	G	12	4					
rs2298805	A	200	100	0.00	0.00	NA	NA	NA
	G	0	0					
rs2269718	G	186	92	0.08	0.07	0.86	0.92	0.75
	A	14	8					

Table 6: Allelic frequency and Odds ratio of SNPs in FcεR1α gene in cases having high IgE serum level.

Reference SNP	Allele	Cases (N=64)	Controls (N=50)	Allele Frequency (MAF)		Odds Ratio	Chi square (<i>p</i> value)	
				Cases	Controls		Yates	Pearson
rs2427827	C	98	88	0.23	0.19	2.24	0.05*	0.02*
	T	30	12					
rs2251746	T	116	94	0.09	0.09	1.62	0.4	0.34
	C	12	6					
rs2298804	A	120	96	0.06	0.06	1.6	0.64	0.45
	G	8	4					
rs2298805	A	128	100	0.00	0.00	NA	NA	NA
	G	0	0					
rs2269718	G	120	92	0.08	0.07	0.95	0.86	0.92
	A	10	8					

* Statistically significant

Table 7: Allelic frequency and Odds ratio of SNPs in FcεR1α gene in cases having low IgE serum level.

Reference SNP	Allele	Cases (N=36)	Controls (N=50)	Allele Frequency (MAF)		Odds Ratio	Chi square (<i>p</i> value)	
				Cases	Controls		Yates	Pearson
rs2427827	C	64	88	0.11	0.19	0.91	1	0.86
	T	8	12					
rs2251746	T	66	94	0.08	0.09	1.42	0.77	0.55
	C	6	6					
rs2298804	A	68	96	0.06	0.06	1.4	*	*
	G	4	4					
rs2298805	A	72	100	0.00	0.00	NA	NA	NA
	G	0	0					
rs2269718	G	66	92	0.08	0.07	1.04	0.84	0.92
	A	6	8					

* Chi square is calculated only if all expected cell frequencies are ≥ 5 . In this case they are 3.35 and 4.65 in cases and controls, respectively.