Polymorphic variants of interleukin-13 R130Q, interleukin-4 T589C, interleukin-4RA I50V, and interleukin-4RA Q576R in allergic rhinitis: A pilot study

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ABSTRACT

The development of allergic rhinitis is considered to be caused by the complex interactions between genetic predisposition and environmental factors. Polymorphisms in the interleukin (IL)-13/4/4RA pathway have previously been shown to be associated with atopic diseases. The purpose of this study was to determine the association between IL-13 R130Q, IL-4 T589C, IL4 receptor alpha (IL-4RA) I50V, or IL-4RA Q576R polymorphisms and risk of allergic rhinitis in a hospital-based Malaysian population. A case-control pilot study was undertaken and genotyping of these polymorphisms was performed using polymerase chain reaction–restriction fragment length polymorphism on 54 allergic rhinitis patients and 45 healthy individuals. Polymorphism of IL-13 R130Q showed significant difference in genotype ($p = 0.048$) and allele ($p = 0.002$) frequencies in allergic rhinitis when compared with healthy controls. Individuals who were GA heterozygotes (adjusted odds ratio [OR^{adj}] = 3.567; 95% CI, 1.211–10.509), and carriers of A allele genotype ($OR^{adj} = 3.686$; 95% CI, 1.300–10.451) and A allele ($OR^{adj} = 3.071$; 95% CI, 1.514−6.232) had an elevated risk of developing allergic rhinitis. The genotype and allele frequencies of IL-4 T589C, IL-4RA I50V, and IL-4RA Q576R polymorphisms were not significantly different between the allergic rhinitis patients and normal healthy individuals and did not show an associated risk with allergic rhinitis. Our findings indicate that polymorphic variant of IL-13 R130Q appears to be associated with increased risk for development of allergic rhinitis in a hospital-based Malaysian population but not IL-4 T589C, IL-4RA I50V, and IL-4RA Q576 polymorphisms. Additional studies using larger sample size are required to confirm our findings and its exact role in allergic rhinitis.

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The development of allergic rhinitis is considered to be caused by the complex interaction between genetic predisposition and environmental factors. Elevated levels of serum IgE are known to play a central role in mediating allergic responses. Linkage studies indicate that genes located on chromosome 5q31-33 have a major influence in regulating basal serum IgE levels in Asian populations.[1,2] Atopy is currently immunologically understood to be Th2 regulated *via* interleukin (IL)-13 and IL-4 cytokines. Therefore, IL-13 and IL-4 genes coded in this region are implicated in atopic diseases. IL-13 and IL-4 exert their respective biological activities by binding to the commonly shared IL-4 receptor alpha (IL-4RA).[3,4] IL-4RA deficient mice have been shown unable to produce IgE and diminished Th2 reactions,[5] thereby marking its importance in allergic reactions.

The G to A nucleotide transition at position +2044 of the IL-13 protein causes an amino acid change from arginine to glutamine. It was found that individuals with the A variant of the polymorphism had a significantly higher IgE level when compared with individuals with the G variant.[6] Functional studies have further indicated that the polymorphic substitution of arginine for glutamine brings about a gain of function in the IL-13 physiological activity[7] and increased modulation in the airway smooth muscle cells of asthmatic patients.[8,9] Subsequent studies have shown that the IL-13 R130Q polymorphic allele and genotype are significantly associated with asthma,[10] allergic rhinitis,[11] and atopic dermatitis.[12] Additionally, IL-4 gene polymorphisms have previously been associated with increased total IgE serum[13] and asthma.[14,15] It is possible that the IL-4 T589C polymorphism located in the promoter region could modify IL-4 gene transcription.

The IL-4RA Q576R polymorphism is caused by an A to G transition leading to substitution of glutamine with arginine. The IL-4RA Q576R site is within the intracytoplasmic portion of the receptor and has previously been associated with allergic asthma.[16] The IL-4RA I50V, due to an A to G transition, results in a

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change of amino acid from isoleucine to valine. Its extracellular domain site is believed to play an important role in atopy and has been associated with atopic asthma and enhanced IgE synthesis.[17,18]

Although the role of IL-13, IL-4, and IL-4RA genes have been implicated in atopic disease, very little data are currently available on their role in allergic rhinitis. In the present case-control pilot study we aim to investigate whether IL-13 R130Q, IL-4 T589C, IL-4RA Q576R, and IL-4RA I50V polymorphisms are associated with risk of developing allergic rhinitis in a hospital-based Malaysian population. The genotypic and allelic distribution of these polymorphic variants will be determined in allergic rhinitis patients and normal healthy individuals.

MATERIALS AND METHODS

Subjects

After approval from the University Malaya Medical Center Ethical Review Board and obtaining written consent from participants, a total of 99 adults including 54 allergic rhinitis patients (23 male and 31 female patients) and 45 healthy individuals (25 male and 20 female patients) as controls, matched for gender and age, were prospectively recruited at the Otorhinolaryngology Department, University Malaya Medical Center. The ethnic composition of the rhinitis patients and control groups were as follows: 21 of 54 (38.8%) cases were Malays; 23 (42.6%) were Chinese; 9 (16.7%) were Indians; 1 (1.9%) was classified as others, and 12 of 45 (26.7%) were Malays; 22 (48.9%) were Chinese; 8 (17.8%) were Indians; and 3 (6.7%) were classified as others, respectively. Mean age of allergic rhinitis patients was 46 years and for the control group participants mean age was 39 years. Rhinitis patients were clinically evaluated by the attending ear, nose, and throat specialist based on symptoms such as rhinorrhoea, sneezing, itchiness and nasal congestion. A panel of 18 allergens, including *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Blomia tropicalis*, cockroach, egg, soybean, cow's milk, shrimp, crab, cod fish, peanut, chicken, garlic, ciku, candida spore, dog dander, coconut pollen and papaya pollen, were then tested by skin-prick test. Only clinically diagnosed allergic rhinitis patients testing positive on the skin-prick test were recruited for the study. Strict selection criteria were applied to the control group including no history of atopic disorders, no family history of atopy, and a negative skin-prick test.

Genotyping of IL-13 R130Q, IL-4 T589C, IL-4RA I50V, and IL-4RA Q576R Polymorphisms

High molecular weight genomic DNA was extracted from the peripheral blood samples using a commercially available Puregene Genomic DNA Purification kit (Gentra Systems, Minneapolis, MN). The DNA was extracted according to the method provided by the manufacturer with some modification. The red blood cells were lysed using red blood cell lysis solution followed by lysis of the white blood cells in cell lysis solution. The proteins in the cell lysate were then precipitated using protein precipitation solution. The DNA was precipitated using 100% isopropanol. The DNA pellet was washed with 70% ethanol, air-dried. and hydrated using DNA hydration solution overnight at room temperature and stored at 4°C. The purity and concentration of extracted DNA was measured and a working concentration of 100 ng/mL was prepared from the stock solution to be used as a template for polymerase chain reaction (PCR).

The presence of IL-13 R130Q, IL-4 T589C, IL-4RA I50V, and IL-4RA Q576R polymorphisms were detected using PCR-restriction fragment length polymorphism method as previously described by Graves,[18] Hegab,[19] Faffe,[20] and Isidoro-Garcia,[21] respectively. The details of the primer sequences, cycling parameters, and restriction fragment length polymorphism analysis for IL-13 R130Q, IL-4 T589C, IL-4RA I50V, and IL-4RA Q576R are shown in Table 1. Each PCR amplification was performed in a 50-μL reaction mixture containing 100 ng of genomic DNA; 1× PCR buffer (50 mM of KCl, 10 mM of Tris-HCl [pH 8.3], and 1.5 mM of $MgCl_2$); 250 mM each of dGTP, dCTP, dTTP, and dATP; 0.2 mM of each primer; and 2.5 U of Ampli*Taq* DNA polymerase (Fermentas, Vilnius, Lithuania). The samples were amplified in a thermocycler (Eppendorf, Hamburg, Germany).

Statistical Analysis

The significance of association between the observed and expected number of the genotypes for a population in the Hardy-Weinberg equilibrium was analyzed using the Pearson's two-sided chi-square test. The chi-square test was also used to show the significant difference of genotype and allele frequencies between rhinitis cases and normal controls. The odds ratio (OR) and its 95% confidence interval (CI) were analyzed using the logistic regression method to determine the correlation between the genotypes or alleles of IL-13 R130Q, IL-4 T589C, IL-4RA I50V, and IL-4RA Q576R polymorphisms and allergic rhinitis risk. The crude OR was determined through univariate logistic regression with only the genotype or allele factor taken into consideration. The adjusted OR (OR^{adj}) was determined using the multivariate logistic regression method with an adjustment for age and gender. Statistical analysis was performed using SPSS (Version 18; SPSS, Inc., Chicago, IL). A 0.05 (5%) level of significance was used throughout the statistical test.

Table 1 **PCR-RFLP parameters and analysis of IL-13 R130Q, IL-4 T589C, IL-4RA I50V, and IL-4RA Q576R polymorphisms in allergic rhinitis and control groups**

Polymorphism	Primer Sequences	Cycling Temperature	Restriction Enzyme	RFLP Analysis
IL-13 R130Q	5′-CTTCCGTGAGGACTGAAT GAGACGGTC-3′ (forward) 5′-GCAAATAATGATGCTTTCGA AGTTTCAGTGGA-3′ (reverse)	Denaturation: 94°C for 4 min. 33 cycles of 94°C for 50 s, 59°C for 45 s, 72°C for 45 s, and final cycle of 72°C for 7 min	*Nla IV* at 37°C for 18 hr	178 bp (GG) 210 bp (AA) 210 and 178 bps (GA)
IL-4 T589C	5′-ACTAGGCCTCACCTGA TACG-3′ (forward) 5′-AGGTGTCGATTTGC AGTGAC-3′ (reverse)	94°C for 4 min, 33 cycles of 94°C for 1 min, 56°C for 1 min, 72°C for 1 min, and final cycle of 72°C for 7 min	*BsmF1* at 65°C for 6 hr	45 bp, 601 bp (CC) 646 bp (TT), 45, 601 and 646 bps (CT)
IL-4RA I50V	5′-TGCATGTGGTAAGAG GCTGTGG-3′ (forward) 5′-TCAGCCCAGGCAGCT GTG-3′ (reverse)	94°C for 4 min, 33 cycles of 94°C for 50 s, 50°C for 50 s, 72°C for 50 s, and final cycle of 72°C for 7 min	*Nla IV* at 37°C for 6 hr	221bp (AA) 198bp (GG) 221 and 198 bps (AG)
IL-4RA Q576R	5′-CCCCCACCACCAGTGG CTACC-3′ (forward) 5′-CCAGGAATGAGGTC TTGGAA-3′ (reverse)	94°C for 4 min, 33 cycles of 94°C for 50 s, 55°C for 50 s, 72°C for 50 s, and final cycle of 72°C for 7 min	*Msp 1* at 37°C for 8 hr	292 and 135 bps (AA) 209, 135 and 83 bps (GG) 292, 209, 135 and 83 bps (AG)

PCR-RFLP = polymerase chain reaction–restriction fragment length polymorphism; IL = interleukin.

RESULTS

The restriction analysis of IL-13 R130Q and IL-4 T589C are shown in Table 2 and IL-4RA I50V and IL-4RA Q576R are shown in Table 3. The distribution of each genotype in the control and allergic rhinitis individuals were in accordance with Hardy-Weinberg equilibrium.

The distribution of IL-13 R130Q genotypes (p = 0.048) and A allele (p = 0.002) were significantly different between the allergic rhinitis and control groups. The frequencies of GA (66.7%), AA (14.8%), A allele genotype (GA+AA; 81.5%), and A allele (48.1%) were elevated in allergic rhinitis patients when compared with GA (55.6%), AA (4.4%), A allele genotype (60.0%), and A allele (32.2%) in healthy individuals. Individuals who were having GA genotypes (OR^{adj} = 3.567; 95% CI, 1.211–10.509) and carriers of A allele genotype (OR^{adj} = 3.686; 95% CI, 1.300–10.451) or A allele (OR^{adj} = 3.071; 95% CI, 1.514–6.232) showed a significant increased risk of developing allergic rhinitis. However, AA homozygotes (OR^{adj} = 0.113; 95% CI, 0.705–26.541) showed lack of association with allergic rhinitis risk.

The distributions of IL-4 T589C genotypes (p = 0.745) and allele (p = 0.585) were not significantly different between allergic rhinitis and healthy individuals. The data shows that frequencies of the CC genotype and C allele were higher in allergic rhinitis patients (14.8% versus 30.6%) compared with healthy individuals (13.6% versus 29.5%), respectively. Individuals who were CC homozygotes (OR^{adj} = 1.074; 95% CI, 0.385–2.996) or TC heterozygotes (OR^{adj} = 0.627; 95% CI, 0.162–2.431) and carriers of C allele genotype (OR^{adj} = 0.915; 95% CI, 0.363–2.310) or C allele (OR^{adj} = 0.823; 95% CI, 0.409–1.657) were not associated with risk of allergic rhinitis.

Genotypic (p = 0.411) and allelic (p = 0.301) distributions of IL-4RA I50V did not significantly differ between allergic rhinitis and healthy individuals. No significant association was noted between AG (OR^{adj} = 0.336; 95% CI, 0.066–1.721), GG (OR^{adj} = 0.419; 95% CI, 0.095–1.847), G allele genotype (OR^{adj} = 1.472; 95% CI, 0.506–4.282), G allele (OR^{adj} = 1.406; 95% CI, 0.737–2.685), and the risk of allergic rhinitis.

Genotype frequency of IL-4RA Q576R showed no significant difference in distribution (p = 0.641) be-

Table 2 **Distribution of IL-13 R130Q and IL-4 T589C genotype and allele frequencies in allergic rhinitis and control groups**

	Control No. (%)	Allergic Rhinitis No. (%)	χ^2 p Value*	OR (95% CI) Adjusted for Age and Gender	χ^2 p Value*	Crude OR (95% CI)
IL-13 R130Q genotype	(n = 45)	(n = 54)				
GG	18 (40.0)	10 (18.5)	0.048#	1.00 (reference)	0.037#	1.00 (reference)
GA	25 (55.6)	36 (66.7)	0.021	3.567 (1.211–10.509)	0.044	2.592 (1.027–6.545)
AA	2 (4.4)	8 (14.8)	0.113	4.327 (0.705–26.541)	0.025	7.200 (1.274–40.678)
A-carrier (GA+AA)	27 (60.0)	44 (81.5)	0.014	3.686 (1.300–10.451)	0.020	2.933 (1.181–7.284)
Alleles	(n = 90)	(n = 108)				
G	61 (67.8)	56 (51.9)		1.00 (reference)		1.00 (reference)
A	29 (32.2)	52 (48.1)	0.002	3.071 (1.514–6.232)	0.016	2.083 (1.148–3.780)
IL-4 T589C genotype	(n = 44)	(n = 54)				
TT	24 (54.5)	29 (53.7)	0.745#	1.00 (reference)	0.986#	1.00 (reference)
TC	14 (31.8)	17 (31.5)	0.500	0.627 (0.162–2.431)	0.871	1.103 (0.336–3.622)
CC	6 (13.6)	8 (14.8)	0.891	1.074 (0.385–2.996)	0.991	1.005 (0.412–2.448)
C-carrier (CC+TC)	20 (45.5)	25 (46.3)	0.851	0.915 (0.363–2.310)	0.934	1.034 (0.465–2.300)
Alleles	(n = 88)	(n = 108)				
T	62 (70.5)	75 (69.4)		1.00 (reference)		1.00 (reference)
C	26 (29.5)	33 (30.6)	0.585	0.823 (0.409–1.657)	0.878	1.049 (0.568–1.939)

**Significance at $p < 0.05$.*
#Represents χ^2-analysis between allergic rhinitis cases and normal controls for the genotypes.
CI = confidence interval; OR = odds ratio; IL = interleukin.

tween allergic rhinitis and healthy subjects. Individuals with allergic rhinitis when compared with healthy individuals had higher frequency of AG (42.6% versus 35.6%) and G allele genotype (46.3% versus 40%) but lower GG (3.7% versus 4.4%) genotype, respectively. No apparent risk was observed between allergic rhinitis and AG (OR^{adj} = 2.508; 95% CI, 0.235–26.712), GG (OR^{adj} = 3.069; 95% CI, 0.279–33.803) and G allele genotype (OR^{adj} = 1.113; 95% CI, 0.453–2.732). The mutant G allele frequency in healthy individuals (22.2%) was lower than allergic rhinitis (25.9%) and not significantly different (p = 0.949) between the two groups. The G allele was not associated with risk of allergic rhinitis (OR^{adj} = 0.976; 95% CI, 0.466–2.045).

DISCUSSION

In this study, significant genotypic and allelic difference of IL-13 R130Q distribution was observed in allergic rhinitis patients when compared with healthy individuals. The GA heterozygotes, carriers of A allele genotype, and A allele had an increased risk for developing allergic rhinitis. The frequencies of AA and GA genotypes in Korean (11.1% versus 53.4%)[22] and Chinese (9.0% versus 45.7%)[23] allergic rhinitis patients were lower than Malaysian patients. Additionally, A allele frequency in allergic rhinitis patients of our population (48.1%) was higher than Korean (37.8%)[22] and Chinese (31.9%)[23] patients. Although Kim and coworkers found no significant genotype difference in allergic rhinitis subjects when compared with healthy individuals, the A allele was significantly elevated in Korean allergic rhinitis subjects.[22] This tends to differ with our observation because in addition to allele, we also noticed significant difference in genotype distribution with allergic rhinitis. Further analysis on associated risk of IL-13 R130Q polymorphism was not performed in the Korean study to enable comparison. Conversely, Wang and colleagues reported no significant difference in distributions of genotype or allele and there was no associated risk for allergic rhinitis, although a significantly higher serum IgE level was detected in AA genotype Chinese allergic rhinitis patients.[23] In a Japanese study, no association between IL-13 R130Q and the risk for cedar pollen allergic rhinitis or serum IgE levels was noted.[24] Malaysia is a tropical region, hence the type of allergic rhinitis is primarily perennial, unlike other populations experiencing seasonal allergic rhinitis. Environmental factors could therefore influence this varied behavior of IL-13 R130Q across diverse Asian populations.

The distribution of IL-4 T589C allele and genotype frequencies between both the studied groups was not significant; and there was no associated risk for allergic rhinitis. To the best of our knowledge, this is the first study on IL-4 polymorphism in allergic rhinitis. Although IL-4 T589C has previously been implicated

Table 3 **Distribution of IL-4RA I50V and IL-4RA Q576R genotype and allele frequencies in allergic rhinitis and control groups**

	Control No. (%)	Allergic Rhinitis No. (%)	χ^2 p Value*	OR (95% CI) Adjusted for Age and Gender	χ^2 p Value*	Crude OR (95% CI)
IL-4RA I50V genotype	(n = 45)	(n = 54)				
AA	12 (26.7)	16 (29.6)	0.411#	1.00 (reference)	0.772#	1.00 (reference)
AG	28 (62.2)	30 (55.6)	0.191	0.336 (0.066–1.721)	0.790	0.833 (0.217–3.198)
GG	5 (11.1)	8 (14.8)	0.250	0.419 (0.095–1.847)	0.523	0.670 (0.196–2.292)
G-carrier (GG+AG)	33 (73.3)	38 (70.4)	0.478	1.472 (0.506–4.282)	0.745	0.864 (0.358–2.086)
Alleles	(n = 90)	(n = 108)				
A	52 (57.8)	62 (57.4)		1.00 (reference)		1.00 (reference)
G	38 (42.2)	46 (42.6)	0.301	1.406 (0.737–2.685)	0.958	1.015 (0.576–1.788)
IL-4RA Q576R genotype	(n = 45)	(n = 54)				
AA	27 (60.0)	29 (53.7)	0.641#	1.00 (reference)	0.774#	1.00 (reference)
AG	16 (35.6)	23 (42.6)	0.446	2.508 (0.235–26.712)	0.945	1.074 (0.141–8.169)
GG	2 (4.4)	2 (3.7)	0.360	3.069 (0.279–33.803)	0.730	1.437 (0.183–11.292)
G-carrier (GG+AG)	18 (40.0)	25 (46.3)	0.816	1.113 (0.453–2.732)	0.529	1.293 (0.580–2.881)
Allele	(n =90)	(n = 108)				
A	70 (77.8)	81 (75.0)		1.00 (reference)		1.00 (reference)
G	20 (22.2)	27 (25.0)	0.949	0.976 (0.466–2.045)	0.648	1.167 (0.603–2.259)

**Significance at $p < 0.05$.*
#Represents χ^2-analysis between allergic rhinitis cases and normal controls for the genotypes.
CI = confidence interval; OR = odds ratio; IL = interleukin.

with asthma, other studies have failed to establish this.[25–27] A recent meta-analysis, however, reported an association of IL-4 T589C with atopic asthma,[28] underlying the need for continued interest in allergic rhinitis.

No associated risk for allergic rhinitis with IL-4RA I50V polymorphism was detected, and there was no significant difference in frequencies of genotype and allele, when compared with healthy individuals. These findings concur with Nakamura and colleagues in a population of Japanese cedar pollinosis patients.[29] Interestingly, a meta-analysis concluded that IL-4RA I50V variant singly was not associated with asthma risk.[30] A growing body of evidence does seem to suggest that IL-4RA I50V may impart a modifying effect in asthma when paired with other polymorphic variants, particularly IL-4 RA Q576R.[31–34] Larger studies would be warranted to further elucidate this relationship in allergic rhinitis.

In the current study, IL-4RA Q576R was not a risk factor for allergic rhinitis. Genotype frequencies of 192 Korean allergic rhinitis patients was AG (31.3%), GG (5.2%) and G allele genotype (36.5%).[22] Allergic rhinitis patients of our population had a higher AG (44.4%) and G allele genotype (48.1%) but lower GG (3.7%) genotype. The G allele frequency was lower in Korean allergic rhinitis (20.8%) patients than our patient population (25.9%). In agreement with our findings, no significant difference of genotype and allele distribution was established between allergic rhinitis and normal Korean subjects. Perhaps there is a need for IL-4RA Q576R to be studied in view of other polymorphic variants, as previously described.

In conclusion, IL-13 R130Q variant appears to impart an elevated risk for development of allergic rhinitis in a hospital-based Malaysian population, but not IL-4 T589C, IL-4RA I50V, and IL-4RA Q576 polymorphisms. Additional investigations using larger sample sizes are needed to confirm these findings and to define its exact role in allergic rhinitis across the context of ethnic group, environmental factors, and relationship to other genes.

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