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An association between IL-9 and IL-9 receptor gene polymorphisms and atopic dermatitis in a Korean population

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ABSTRACT

Background: The genes encoding IL-9 and IL-9R have recently been implicated in the genetic basis of asthma and allergy. Although studies performed on transgenic and knockout mice have shown conflicting results, no evidence of skin changes has ever been reported in these animals.

Objective: To find association of the SNPs in IL-9 and IL-9R genes and interaction of these genes in atopic dermatitis.

Method: We genotyped 5 SNPs from the IL-9 and IL-9R genes of 1090 subject samples (631 AD patients and 459 normal controls). A luciferase assay was then performed for the rs31563 (–4091G/A) SNP located in the IL-9 gene promoter region.

Result: The rs31563 (–4091G/A) SNP in the IL-9 gene was significantly associated with the AD phenotype, especially allergic-type AD. In the luciferase assay, the rs31563 G construct was observed to have 1.54 times higher activity than the rs31563 A construct. Although no association was found between SNPs in IL-9R gene and AD, the rs3093467 SNP showed association with non-allergic AD. In the gene–gene interaction analysis, we found that IL-9/IL-9R genotype rs31563 GG/rs3093467 TT conveyed a greater risk for AD phenotype development.

Conclusion: Significant evidence exists to suggest that the rs31563 SNP (–4091G/A) located in the IL-9 gene is associated with an increased susceptibility to AD. Similarly, the rs3093467 SNP in IL-9R gene seems to be associated with an increased risk for developing non-allergic AD. In a subsequent gene–gene interaction analysis, the rs31563 GG/rs3093467 TT genotype combination (IL-9/IL-9R) was found to exert a synergistic effect in the development of the AD phenotype. As the classes of helper T cells are diverse and the function of IL-9 cytokine has not been fully described, the cutaneous function of IL-9 needs to be further explored in future studies.

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1. Introduction

Atopic dermatitis (AD) is an inflammatory skin disease characterized by severe pruritis, a chronic relapsing clinical course, and characteristically distributed xerotic lesions with overlying excoriations and lichenification [1,2]. The pathophysiology underlying AD consists of a complex series of interactions between local and infiltrating inflammatory cells, primarily orchestrated by pro-inflammatory cytokines and chemokines. Historically, two different subsets of helper T cells (Th) – Th1 and Th2 – were believed to

play an important role in allergic diseases. However, since the recognition of the IL-17 producing Th17 and regulatory T cells (Treg), the pathophysiologic basis for the etiology of allergic diseases, such as asthma and AD, has grown substantially more complicated.

IL-9 is a Th2 type cytokine first described in a murine model as a potent growth factor for T cells and mast cells. Later, IL-9 was discovered to also have an inhibitory effect on IFN γ -mediated CD4⁺ cell lymphokine production and promote CD8⁺ cell proliferation. Moreover, IL-9 is also known to induce bronchial epithelial cell mucus and chemokine secretion, promote mast cell proliferation, and increase IgE production in B cells. In mice, the protein sequence for IL-9 consists of 144 amino acid residues, with the typical signal peptide 18 amino acids long. Human IL-9 is also comprised by 144 amino acids and has 4 potential N-linked

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glycosylation sites. In humans, the gene for IL-9 is located on chromosome 5 (5q31–35) between the sequences for IL-3 and early growth response (EGR)-1. Both human and murine IL-9 genes have 5 exons and 4 introns stretching over about 4 kb. The receptor for IL-9, IL-9 receptor (IL-9R), was initially identified on several murine hematopoietic cells, including T cells, mast cells and macrophages. Human IL-9R cDNA has been shown to encode 522 amino acids, and have a 53% homology to murine IL-9R [3,4].

Cytokines encoded on chromosome 5q31 are believed to play a critical role in the allergic response through their regulation of B cell isotype switching from IgM to IgE synthesis (IL-4, IL-13). Moreover, these cytokines also promote the growth and maturation of mast cells, basophils (IL-3, IL-9, IL-10), and eosinophils (IL-3, IL-5, GM-CSF). Further underscoring this relationship, linkage between markers on chromosome 5q31–33 and total serum IgE has also been reported [5,6].

Recent analyses of linkages [7] and polymorphisms [8] in the promoter region of the IL-9 gene have shown associations between IgE levels and IL-9 bronchial hyperresponsiveness in a mouse model, suggesting that certain genetic variations in the IL-9 gene may predispose to asthma [9]. Such evidence of a correlation between polymorphisms in DXYS154 (Xq28 and Yq12) and bronchial hyperresponsiveness and/or asthma also implicates the IL-9R gene in asthma pathogenesis [10].

Transgenic mice (TG) overexpressing IL-9 have been shown to face an increased susceptibility to multiple pathologic states, including lymphoma [11,12], intestinal mastocytosis [13], B-1 lymphocyte population expansion [14], bronchial hyperresponsiveness [15,16] and airway eosinophilia [17]. Conversely, IL-9 deficient (knockout:KO) mice are generally healthy, displaying no overt phenotypic abnormalities. IL-9 deficiency has also not been associated with significant reductions in IgE production [18] or bone marrow mast cell populations, as determined by c-kit staining [19]. Notably, several IL-9-associated conditions are suppressed to a variable degree in IL-13 and IL-4/5/9/13 KO mice, including airway hyperresponsiveness (AHR), eosinophilic inflammation, airway smooth muscle (ASM) hyperplasia, goblet cell hyperplasia and sensitization-mediated increases in serum IgE. Specifically, AHR, ASM hyperplasia and allergy-associated increases in serum IgE are completely inhibited in both KO lines, while goblet cell hyperplasia and eosinophilic inflammation are completely suppressed in IL-4/5/9/13 KO mice but only partially inhibited in IL-13 KO mice [20].

Recently, oral antigen-induced intestinal and systemic anaphylaxis associated with IL-9/IL-9R signalling in an animal study [21] and the IL-9 gene polymorphism associated to lung function and to a marker of polysensitization [22] were reported.

Here, we performed extensive genotypic analysis to evaluate whether SNPs from IL-9 and IL-9R genes were associated with an increased risk of AD, additionally looking for any effect of interactions between these genes. We found that the rs31563 SNP in the IL-9 gene, and the rs3093467 SNP and in the IL-9R gene, are associated with AD. We also found that the IL-9 and IL-9R genes interact to develop the phenotype of AD.

2. Methods and materials

2.1. Subjects

In total, 1090 subjects were enrolled in this study, including samples from 631 patients with AD and 459 normal control subjects (NR). AD samples were obtained from non-asthmatic atopic patients examined at Samsung Medical Center, in Seoul, Korea. The diagnosis criteria for AD, the classification method for subdividing individuals with AD into ADe (allergic type of AD or extrinsic type of AD) and ADi (non-allergic type of AD or intrinsic type of AD), and the methodology for all allergen blood and prick testing is as described in our previous report [23]. All 631 AD patients (M:F = 351:280, age range: 13.42 ± 9.92 years) meet the criteria described in our previous reports [23,24]. To make our study design simple, we have excluded AD patients with asthma or allergic rhinitis that may exert AD as secondary symptom of those allergic diseases. Thus, our study results present the role of genetic variants of IL9 and IL9R in development of AD without asthma or allergic rhinitis. To select representative patient samples, AD patients were divided into ADe and ADi subgroups: 417 were classified as ADe (M:F = 250:167, age range: 15.6 ± 9.3 years), and 214 were classified as ADi (M:F = 101:113, age range: 9.2 ± 9.7 years). The 459 NR subjects were recruited locally, primarily from medical students and volunteers (M:F = 238:221, age range: 23.33 ± 2.19 years) with no prior history of AD, allergic rhinitis or asthma. Demographic information for all study subjects has been summarized in Table 1a.

This study was approved by The Samsung Medical Center Ethics Committee and was conducted according to the Declaration of Helsinki Principles, with written informed consents obtained from all participants.

2.2. Marker selection

All SNP information was retrieved from the dbSNP (build 119, <http://www.ncbi.nlm.nih.gov/SNP>; accessed 31 May 2007). Polymorphic markers were selected from the region 5 kb upstream and downstream of each gene. 12 SNPs were selected from IL-9 gene and 20 from the IL-9R gene. All SNPs were genotyped for 48

Table 1

(a) Demographic features of the three subject groups, including total number of subjects, mean and standard deviation for age, IgE level, serum eosinophil count, and SCORAD index. (b) SNP markers genotyped for the case–control samples (dbSNP build 126).

(a)								
Group	Number of subjects (F/M)	Age	IgE	Eosinophil	ECP	SCORAD		
ADe	417 (167/250)	15.6 (±9.3)	1909.2 (±3284.6)	610.2 (±651.6)	79 (±266.2)	33.6 (±19.7)		
ADi	214 (113/101)	9.2 (±9.7)	51.9 (±46.2)	386.6 (±362.7)	39 (±48.6)	22.5 (±16.3)		
NR	459 (221/238)	23.33 (±2.19)	241.5 (±415.8)	–	–	–		
(b)								
Gene	ID	RS number	Chr	Position	Function	MAF ^a	HWE ^a	Success rate
IL9	–4091G/A	rs31563	chr5	135263505	Promoter	0.198	0.871	0.978
IL9	IVS3 – 413C/A	rs31564	chr5	135258152	Intron 3	0.27	1	0.962
IL9R	–3720G/T	rs7051412	chrX/Y	154787409	Promoter	0.41	0.806	0.975
IL9R	IVS1 + 1737C/T	rs3093467	chrX/Y	154792893	Intron 1	0.399	0.255	0.978
IL9R	IVS2 + 154A/C	rs3093493	chrX/Y	154796542	Intron 2	0.438	0.925	0.974

HWE: *p*-values from chi-square testing for Hardy–Weinberg equilibrium.

The MAF and HWE are calculated from 459 normal control samples.

^a MAF: minor allele frequency.

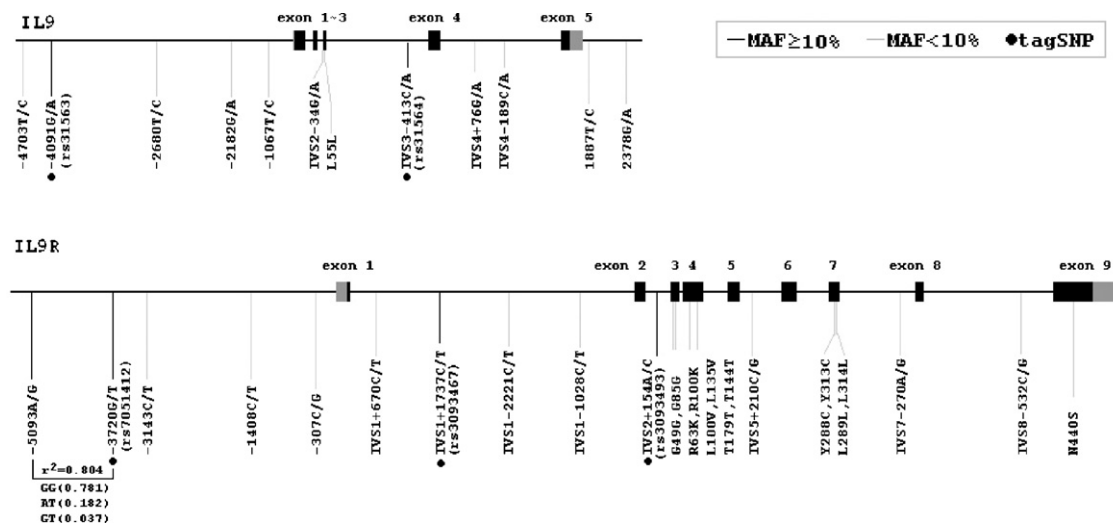


Fig. 1. Maps of IL9 on chromosome 5q31.1 (10.1 kb) and IL19R on Xq28 and Yq12 (17.5 kb). Coding exons are indicated by black blocks, and 5' and 3' UTRs by gray blocks. The first base of the translation start site has been termed nucleotide +1. Genotyped polymorphisms in all two genes are indicated by vertical bars. Minor allele frequencies for polymorphisms in 48 samples are distinguished as over 10% or less. Dots signify the polymorphisms genotyped in a larger population ($n = 1090$). LD for the tag SNPs and the corresponding tagged SNPs are presented as r^2 , with all haplotypes listed.

independent samples from the general Korean population (data not shown). Based on these results, we selected SNPs with minor allele frequencies >0.1 . Next, tag SNPs were determined from the chosen SNPs through the linkage disequilibrium (LD) bin approach implemented in the Tagger program (<http://www.broad.mit.edu/mpg/tagger>; accessed 31 May 2007). This approach initially defines the SNP bins that are in very strong LD with a specific r^2 threshold, then selects one SNP representative of the remaining SNPs in each bin [25]. Here, we used an r^2 threshold of 0.8, and 2 SNPs from the IL-9 gene and 3 SNPs from IL-9R gene were selected as markers for the association analysis (Fig. 1).

2.3. Genotyping with fluorescence polarization detection

Genomic DNA was extracted from 5 ml of the whole blood by using a DNA isolation kit (Gentra Genomic DNA purification kit, Minneapolis, MN, USA) per protocol. All genotype identification was performed by GenomeLab SNPstream system (Ultra-high throughput; UHT system [26]), which uses multiplexed PCR in combination with both tag array single base extension genotyping technology (Beckman Coulter, Fullerton, CA, USA) and the accompanying SNPstream software, as previously described by Denomme and Van Oene [27].

2.4. Statistical analysis

The chi-square test was used to determine whether individual variants were in Hardy-Weinberg equilibrium (HWE) for each locus from the samples. The allelic (additive allelic effect) and the genotypic effects of the individual SNPs were evaluated by logistic regression model, in which gender and age were defined as the adjusting covariates. We have presented nominal p -values and after accounting for multiple testing on five SNPs values <0.01 considered significant in all cases. Odds ratios were also estimated using the logistic regression model. Haplotype associations were determined using the R (<http://www.r-project.org>) function `haplo.glm` [28], which estimates the significance and relative effect of each haplotype on the trait, as compared to the effect of the baseline haplotype. The baseline haplotype used here was comprised of a combination of the major alleles from each locus. Total serum IgE levels were stratified into five consecutive groups determined by data quantile: <40 U/ml, 40–200 U/ml, 200–500 U/

ml, 500–2000 U/ml, and >2000 U/ml. A cumulative logistic regression analysis was then conducted to assess for any correlations between genotypes and total IgE level. Similarly, a linear regression model was used to evaluate the genetic effect on blood eosinophil count and eosinophilic cationic protein (ECP) level among individuals with AD, with both variables log-transformed prior to regression analysis and age, gender, and the scoring of atopic dermatitis (SCORAD) index used as adjusting covariates. All statistical analyses were performed with SAS 9.1 (SAS Institute Inc., Cary, NC, USA) and R statistical language (<http://www.r-project.org>).

2.5. Gene-gene interaction analysis

In order to determine whether interactions between the rs31563 SNP and the rs3093467 SNP affect disease susceptibility, association test was conducted using genotypic combinations of these two SNPs. A logistic regression model was used to evaluate for statistical significance, with gender and age included as adjusting covariates. The ORs for each genotypic combination were also obtained from the logistic regression analysis, after the genotypes were subdivided into two groups for each locus (GG and others for rs31563, and TT and others for rs3093467).

2.6. Luciferase assay

Synthesized double-stranded oligonucleotides were cloned into a pGL3-promoter vector (Promega, Madison, WI, USA) that included the Simian virus-40 (SV40) promoter, and *KpnI* and *BglII* at the 5' and 3'-ends. Additionally, all such oligonucleotides contained three concatenated copies of the G or A alleles of rs31563 with their 21-bp flanking sequence centered around the polymorphism.

HEK293 cells were transfected with 1 μ g of the reporter constructs and 0.1 μ g of a pRL-TK *Renilla* luciferase vector (Promega) using lipofectamine 2000 reagents (Invitrogen) per manufacturer protocol. Transfection efficiency was normalized by *Renilla* luciferase activity. The medium then was replaced with growth medium 18 h after transfection. At 24 h, cells were harvested after the medium was changed and the luciferase activity was measured via Dual-Luciferase Reporter Assay System (Promega).

Table 2

Comparison of allelic and genotypic frequencies between the two AD subtypes and normal control groups: (a) the rs31563 SNP from the IL9 gene and (b) the rs3093467 SNP from the IL9R gene. Odds ratios and corresponding *p* values for the effects were obtained from the analysis using a logistic regression model where age and gender were used as adjusting covariates.

(a)									
Group	Allele					OR (95% CI)		p value	
	G		A						
NR	739 (0.819)			163 (0.181)					
AD	1059 (0.861)			171 (0.139)		0.667 (0.505, 0.88)		0.004	
ADe	693 (0.86)			113 (0.14)		0.673 (0.498, 0.909)		0.01	
ADi	366 (0.863)			58 (0.137)		0.654 (0.389, 1.1)		0.109	
Group	Genotype			AA vs. GG		AG vs. GG		p value ^a	
	GG	AG	AA	OR (95% CI)	p value	OR (95% CI)	p value		
NR	303 (0.672)	133 (0.295)	15 (0.033)						
AD	458 (0.745)	143 (0.233)	14 (0.023)	0.618 (0.441, 0.866)		0.574 (0.248, 1.329)		0.195	
ADe	301 (0.747)	91 (0.226)	11 (0.027)	0.6 (0.415, 0.868)		0.652 (0.269, 1.579)		0.343	
ADi	157 (0.741)	52 (0.245)	3 (0.014)	0.67 (0.367, 1.223)		0.381 (0.059, 2.455)		0.31	
(b)									
Group	Allele					OR (95% CI)		p value	
	C		T						
NR	574 (0.643)			318 (0.357)					
AD	787 (0.635)			453 (0.365)		1.049 (0.844, 1.303)		0.669	
ADe	532 (0.65)			286 (0.35)		0.93 (0.735, 1.177)		0.548	
ADi	255 (0.604)			167 (0.396)		1.602 (1.087, 2.361)		0.017	
Group	Genotype			TT vs. CC		CT vs. CC		p value ^a	
	CC	CT	TT	OR (95% CI)	p value	OR (95% CI)	p value		
NR	179 (0.401)	216 (0.484)	51 (0.114)						
AD	251 (0.405)	285 (0.46)	84 (0.135)	0.909 (0.665, 1.242)		1.24 (0.772, 1.993)		0.373	
ADe	180 (0.44)	172 (0.421)	57 (0.139)	0.79 (0.565, 1.105)		1.004 (0.6, 1.677)		0.989	
ADi	71 (0.336)	113 (0.536)	27 (0.128)	1.504 (0.844, 2.68)		2.647 (1.193, 5.87)		0.017	

p-values that are significant at the 0.01 level are written in bold type.

^a *p*-value of the type III effect of the genotype.

3. Results

A total of five SNPs from the IL-9 and IL-9R genes were genotyped from the 1090 subject samples. Information from the SNPs is presented in Table 1b, including genomic function, chromosomal position, dbSNP id, and minor allele frequency. All genotyped SNPs were in HWE at a significance level of 0.01, with an average genotyping success rate of 97.4%.

The distributions of the allelic and genotypic frequencies for the five SNPs were compared among the AD and normal groups (data not shown). Statistical significance was determined by logistic regression analysis using age and gender as adjusting covariates.

3.1. Difference in allelic distribution of IL-9 and IL-9R polymorphisms between individuals with AD and normal controls

Two SNPs were genotyped from the IL-9 gene, with the rs31563 (−4091G/A) SNP showing significantly different allelic distributions between the AD and normal groups (*p*-value = 0.004). For the allelic test, the resulting odd ratio was 0.66 (95% confidence interval (CI) = 0.505–0.88) (Table 2a).

When allelic and genotypic distributions were compared between the two AD subgroups (ADe and ADi) and normal controls, the rs31563 SNP was found to occur at a marginally significantly different frequency between ADe patients and normal controls, with *p*-values of 0.01 from allelic effect tests (Table 3a). The A allele to G allele odds ratio was 0.673 (95% CI: 0.505–0.924). However, the rs31563 SNP was not significantly associated with ADi.

No significant allelic or genotypic associations were found between AD and any of the three SNPs from the IL-9R gene or the

rs3093467 SNP. However, a marginally significant association was observed between the rs3093467 SNP and the ADi subtype by allelic comparison (*p*-value = 0.017) (Table 2b).

Using a regression analysis, polymorphisms in the two genes were also compared with total IgE level, eosinophil count and ECP level to identify any significant correlations, with age, gender and SCORAD index used as adjusting covariates. None of the SNPs in the IL-9 or IL-9R genes were observed to have significant associations with any of these three variables.

3.2. Luciferase assay in IL-9 gene

The allelic effect of the rs31563 SNP on transcriptional activity was quantified by luciferase reporter assays. Specifically, the luciferase gene constructs three concatenated copies of either G or A alleles in a 21-bp region centered around the SNP (p3X4091G and p3X4091A) (Fig. 2a). In HEK193 cells, the transfection of the p3X4091G construct resulted in an increase in luciferase activity when compared with p3X4091A (1.54 ± 0.09 fold induction) (Fig. 2b). These results suggest that the IL-9 rs31563 SNP may affect the transcriptional activity of the gene. To examine the putative transcription factors binding to the rs31563 SNP, we used matinspector (<http://www.genomatix.de>) to analyze the flanking sequences of the SNP 'G' alleles. The rs31563 SNP is located in close proximity to the sequence for several putative transcription factor binding sites, including Sp-1, Pax-3, sterol regulatory element-binding protein (SREBP), and spermatogenic leucine zipper 1 (Spz1). Of these, the Spz1 binding site has the highest core match value with the SNP allele (core-match = 1, matrix-match = 0.967). Although there has been no conclusive reporting regarding the relevance of Spz1 and other putative transcription factors in AD or other inflammatory diseases,

Table 3

Logistic regression analysis evaluating the effect of genotypic combinations of the rs31563 and rs3093467 SNPs. Age and gender were included in the model as adjusting covariates.

rs31563/rs3093467	AD vs. NR		ADe vs. NR		ADi vs. NR	
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
GG/TT	2.541 (1.381, 4.674)	0.003	2.155 (1.127, 4.12)	0.02	4.066 (1.54, 10.735)	0.005
GG/C-	1.475 (1.034, 2.105)	0.032	1.484 (1.014, 2.173)	0.042	1.495 (0.78, 2.867)	0.226
A-/TT	0.543 (0.235, 1.255)	0.153	0.479 (0.183, 1.259)	0.136	0.956 (0.233, 3.927)	0.951
A-/C-						

p-values that are significant at the 0.01 level are written in bold type.

the proximity of several transcription factor binding sites to SNP allele indicates that this region is likely transcriptionally relevant and potentially genetically imprinted.

3.3. Gene–gene interaction between IL-9 and IL-9R

In order to investigate whether interactions between the rs31563 and rs3093467 SNPs affect the disease susceptibility, an association test was conducted using genotype combinations of the two SNPs. After analysis, these data showed that the rs31563 GG/rs3093467 TT genotype combination most significantly increased the risk of AD. These results suggest that the combination of the two risk genotypes – rs31563 GG from the IL-9 gene and rs3093467 TT from the IL-9R gene – synergistically increase disease susceptibility (Table 3).

4. Discussion

Here, the rs31563 (–4091G) SNP in IL-9 gene was found to be significantly associated with AD, especially allergic-type AD (ADe), with the results from the rs31563 (–4091G) luciferase assay confirming this finding. Although none of the SNPs from the IL-9R gene were significantly associated with AD, the rs3093467 SNP was correlated with non-allergic AD (ADi) with marginal significance. Additionally, the rs31563 GG/rs3093467 TT combination between the IL-9/IL-9R genes was found to significantly increase the risk of developing the AD phenotype. Although allelic

association between IL-9 and total serum IgE levels have previously been demonstrated [7], no correlations between SNPs from IL-9 and IL-9R genes and total serum IgE, blood eosinophil count, and ECP were observed.

IL-9 and IL-9R genes have been implicated in asthma pathogenesis [7–10], while the –351A/C in IL-9 was also reported to be significantly associated with asthma via single-strand conformation polymorphism (SSCP) assay in 20 asthmatic families not homozygous at this site [8]. Notably, we did not genotype this SNP, as it has not been reported to be polymorphic in Asian populations (http://www.ncbi.nlm.nih.gov/sites/entrez?Db=pubmed&DbFrom=snp&Cmd=Link&LinkName=snp_pubmed_cite-d&IdsFromResult=1799962). Furthermore, previous evidence from TG and KO murine studies of the IL-9 gene did not identify any significant phenotypic changes in the skin or any differences in cutaneous mast cell number or location [13,18].

Due to its involvement in host immune response against helminthes as well as allergy [4,29], IL-9 is generally associated with Th2 cells, although one recent study has suggested that Treg cells produce larger quantities of IL-9 than Th2 cells [30]. Furthermore, IL-9 production does not increase after exposing cultures of naïve T cells with cytokines known to promote Th1, Th2, iTreg and Th17 differentiation. Conversely, placing naïve T cells in TGF- β and IL-4 rich environments upregulates IL-9 production and secretion. Although CD4+ T cells producing IL-9 do not express any of the transcription factors that define the other T cell subsets, specifically T-bet, GATA-3, Foxp3, and/or ROR γ t, it was reported recently that transcription factor interferon-regulatory factor 4 [31] and PU.1 [32] are essential for the development of IL-9 producing T cells. As IL-9 producing T cells are seemingly distinct from Th1, Th2, Treg and Th17 cells, Veldhoen et al. [33] have provisionally termed this population ‘Th-9’ cells.

Recently, TGF- β – a cytokine influencing fate ‘decisions’ in Th-17 and Treg cell development, was identified as having a crucial role in ‘reprogramming’ committed Th2 cells to adopt a Th-9 phenotype [33,34]. Most evidence indicates that the physiological function of IL-9 is somehow connected to the Th2 response: IL-9 is not only important in host defense against helminthic infections but also contributes to allergic reactions in the lung, both of which result from increased Th2 signalling [29]. As IL-9 receptors are also expressed on naturally occurring Treg cells, IL-9 may mediate the activity of both T cells and Treg cells [34].

Although Th2 cells secrete IL-9 on activation, Treg cells have been proven as superior in IL-9 production on a per-cell basis. However, despite the capability of Treg cells to secrete high concentrations the cytokine, IL-9 has no apparent autocrine function and does not promote Treg cell growth or suppression *in vitro* [30].

We also found the relatively weak association of IL-9R polymorphism with ADi. Previously, IL-9R gene polymorphism was reported to be associated with rheumatoid arthritis (RA) and the RA patients with homozygous SNP in IL-9R gene in man are 3 times more affected than female. The explanation for this SNP was either different IL-9R splice variants influencing in IL-9 binding

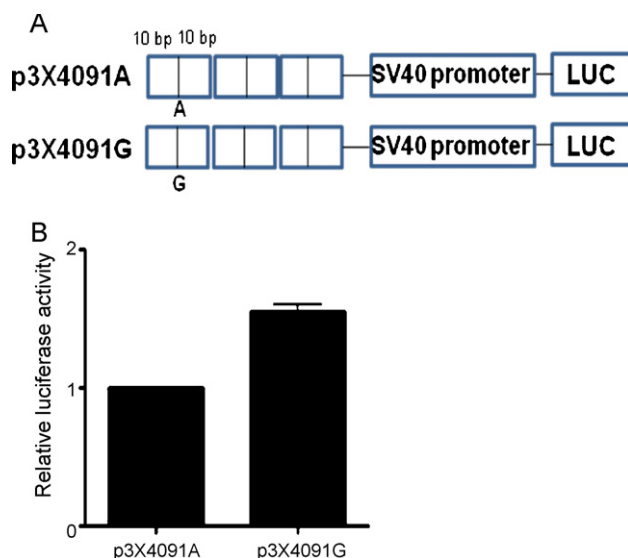


Fig. 2. Luciferase assay of the IL-9 gene. (a) Luciferase reporter constructs containing either a G or A allele in a 21-bp region centered around the SNPs (p3X4091G and p3X4091A) are shown. (b) The mean $\pm$ standard deviation of the relative luciferase activities of p3X4091G compared to p3X4091A is shown, comprising the results from three duplicate, independent experiments. The differential expression by the rs31563 SNP allelic effect in HEK193 cells, after the transfection of the p3X4091G construct.

ability or IL-9R gene involvement in early T cell development [35]. In our case, as the number of ADi patients was not big enough, the possibility for a false positive result does exist.

In conclusion, our data identifies an association between the rs31563 SNP in the IL-9 gene and the AD phenotype. In particular, this SNP was especially predictive of ADe traits, while the combination of rs31563 GG/rs3093467 TT in the IL-9/IL-9R genes conveyed a significantly higher risk for developing AD. Additionally, we examined the effect of variations in rs31563 on IL-9 transcription via luciferase assay, ultimately found that the AA genotype not only exerts a significant protective effect against developing allergic-type AD, but also results in significantly lower levels of IL-9 transcription.

However, our study has limitation of that the sample size is not large when the patient samples are divided into subtype groups. Especially for the ADi subtype, our study may have missed some association signals.

Today, the classification of Th cells is more diverse and complex than ever before. As the exact physiologic function of IL-9 in the skin has yet to be fully described with IL-9 TG and KO mice not exhibiting any cutaneous changes, discovering the complete role of this cytokine will contribute greatly to our understanding of dermatologic and allergic disease.

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