

Association between genetic variations of vascular endothelial growth factor receptor 2 and atopy in the Korean population

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Background: Vascular endothelial growth factor (VEGF) has been suggested to be a key mediator in the development of atopy and T_H2 inflammation.

Objective: We sought to evaluate the effects of variations in the gene coding VEGF receptor (VEGFR) 2 on intermediate phenotypes of asthma in the Korean population.

Methods: A cohort of 2055 children and adolescents responded to a questionnaire concerning asthma symptoms and risk factors and underwent methacholine bronchial challenge and skin tests. The *VEGFR2* gene, including the promoter area, was sequenced on 24 healthy subjects to discover informative single nucleotide polymorphisms (SNPs; minor allele frequency >2%). After haplotype reconstruction, 4 tagging SNPs (IVS6+54A>G, +889G>A, +1416T>A, and IVS25-92G>A) were scored. These SNPs were also scored in 480 adult asthmatic patients to verify the above genetic association study. **Results:** The prevalence of atopy was associated with a single SNP (+889G>A) of *VEGFR2* with borderline significance ($P = .048$; relative risk, 1.13; 95% CI, 1.00-1.28). However, haplotype analysis showed that the atopy prevalence was strongly associated with a haplotype (AGAG) of *VEGFR2* ($P = .002$; relative risk, 1.25; 95% CI, 1.09-1.42). As for airway hyperresponsiveness, neither individual SNPs nor haplotypes were found to be associated. Interestingly, the significant association was also found between atopy and the AGAG haplotype among adult asthmatic patients ($P = .008$; odds ratio, 1.66; 95% CI, 1.14-2.44).

Conclusions: The present study demonstrated that genetic variations of *VEGFR2* are significantly associated with atopy in the Korean population. (J Allergy Clin Immunol 2006;117:774-9.)

Key words: Vascular endothelial growth factor, kinase insert domain-containing receptor, single nucleotide polymorphism, Korean population, atopy

Asthma is a chronic inflammatory disease of the airways that is associated with airway hyperresponsiveness (AHR) to a number of stimuli and is phenotypically a complex genetic disorder.¹ However, the development of asthma is significantly associated with intermediate phenotypes, such as atopy and AHR, that have provided useful objective alternatives in genetic and epidemiologic studies.² Atopy is defined as a genetic predisposition to an enhanced IgE response to common environmental allergens and is considered to be the factor that most strongly predisposes an individual to asthma development.³ On the other hand, AHR is believed to be an essential component of asthma development and can be used to differentiate asthma and other airway diseases.⁴

Vascular endothelial growth factor (VEGF) was originally described as a vascular permeability factor because of its ability to generate tissue edema.⁵ Subsequently, it was appreciated to be a multifunctional angiogenic regulator that stimulates epithelial cell proliferation, blood vessel formation, and endothelial cell survival.^{6,7} Interestingly, increased VEGF levels were detected in tissues and biologic samples from asthmatic patients⁸⁻¹¹ and have been correlated directly with disease activity.¹² A colocalization study conducted on asthmatic patients demonstrated that macrophages, eosinophils, and $CD34^+$ cells are the major sources of VEGF and that $CD34^+$ cells, macrophages, and T cells express VEGF receptors (VEGFRs).⁹ In addition, our recent study with lung-targeted VEGF transgenic mice demonstrated that VEGF induces AHR and the airway inflammation and remodeling that characterize asthma.¹³ This study also demonstrated that VEGF enhances T_H2 sensitization to allergens by promoting dendritic cell maturation.¹³

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Abbreviations used

AHR: Airway hyperresponsiveness
KDR: Kinase insert domain-containing receptor
RR: Relative risk
SNP: Single nucleotide polymorphism
VEGF: Vascular endothelial growth factor
VEGFR: Vascular endothelial growth factor receptor

Thus far, 3 VEGFRs have been identified: Flt-1 (the fms-like tyrosine kinase, murine and human; VEGFR1); Flk-1 (the murine fetal liver kinase; VEGFR2) or its human homolog, the kinase insert domain-containing receptor (KDR; VEGFR2); and Flt-4 (the fms-like tyrosine kinase, murine and human; VEGFR3).⁷ Flt-1 has the highest affinity to VEGF ($K_d = 1\text{--}10\text{ pM}$) among the VEGFRs, but its tyrosine kinase activity is very weak.¹⁴ On the other hand, VEGFR2 carries one-order weaker affinity to VEGF ($K_d = \text{about } 200\text{ pM}$); however, its kinase activity is as strong as that of other representative tyrosine kinase receptors, such as epidermal growth factor receptor.^{15,16} These findings imply that KDR plays a major role in VEGF signaling pathways by mediating biologic effects of VEGF.

On the basis of these findings, we focused on KDR and performed association studies between genetic variations of KDR and intermediate phenotypes of asthma, such as atopy and AHR, in a cohort of 2055 general children and adolescents. We found that genetic variations of KDR were significantly associated with the prevalence of atopy in this cohort. Interestingly, the significant association was also found among adult asthmatic patients. To the best of our knowledge, this is the first population-based genetic association study demonstrating the importance of genetic variations of KDR on the predisposition to atopy.

METHODS

Study subjects and clinical phenotyping

All the subjects enrolled in this study provided written informed consent, and the study protocol was approved by the Ethics Committee of Seoul National University Hospital. A cohort of 2055 ethnically identical subjects aged from 10 to 18 years living in rural areas on Jeju Island, Korea, was randomly recruited. All subjects responded to a questionnaire concerning asthma symptoms and risk factors. A questionnaire developed by the International Study of Asthma and Allergic Disease in Children was translated into Korean following the guidelines laid down by the International Study of Asthma and Allergic Disease in Children, as previously described.¹⁷ Those questions concentrated on recurrent wheezing and a nocturnal cough in the absence of respiratory tract infections over the prior 12-month period. Questions on risk factors covered items on a family history of allergic disease, a history of passive smoking, and vaccination history, which included measles, *Mycobacterium tuberculosis*, and hepatitis B virus.

None of the subjects had been treated or had used oral or inhaled bronchodilators for the 5 days preceding the methacholine bronchial provocation testing. Subjects who had contracted an upper respiratory tract infection during the 2-week period before the study were also

TABLE I. Demographic and clinical characteristics of the study population

Characteristics	Cohort (n = 2055)	Asthmatic patients (n = 480)
Age (y), mean (range)	14.6 (10-18)	51.8 (20-87)
Male sex	1004 (48.9%)	255 (53.1%)
Atopy*	767 (37.3%)	240 (50.0%)
AHR†	525 (25.6%)	—
Nocturnal cough	246 (12.0%)	—
Current wheezing	147 (7.2%)	—
Asthma‡	133 (6.5%)	—

*Positive skin test responses to one or more common aeroallergens.

†PC₂₀ methacholine <16 mg/mL.

‡A subject with a current wheeze or nocturnal cough by questionnaire showed a positive AHR.

excluded from the methacholine challenge. The methacholine bronchial provocation test was performed as previously described.¹⁷ Methacholine AHR was expressed as PC₂₀ and was regarded as positive if the PC₂₀ was less than 16 mg/mL. Skin prick testing to 11 common aeroallergens (Allergopharma, Reinbek, Germany) was performed, as previously described,³ to evaluate T_H2 sensitization to allergens. Subjects who had received oral antihistamines during the 5 days before skin prick testing or had dermographism were excluded from the skin prick testing. Atopy was defined as a positive skin prick test response (allergen/histamine ratio >1.0 plus a mean wheal size >4 mm) to one or more allergens. Asthma was defined when a subject with current wheeze or nocturnal cough by questionnaire had a positive AHR.

A total of 480 adult asthmatic patients were also enrolled from those attending the Allergy Clinic of Seoul National University Hospital located in the capital city of Korea to verify the significance of association results in the cohort. Asthma was diagnosed if a subject with dyspnea, wheezing, or recurrent cough had reversible airways obstruction or positive methacholine AHR. All of them underwent skin prick tests as described above. The characteristics of study populations are shown in Table I.

Genotyping

Single nucleotide polymorphism discovery. On the basis of the genomic sequence available in GenBank (accession no. NT_022853), primers to amplify all exons and juxtaposing intronic sequences of the KDR gene were designed. In addition, we designed primers to amplify approximately 10 kb upstream of the first exon. All regions were amplified on genomic DNA from 24 healthy subjects. All PCR products were sequenced by using Big Dye Terminator sequencing chemistry (Applied Biosystems, Foster City, Calif) in both directions, according to standard protocols. After sequencing of the KDR gene, 12 informative single nucleotide polymorphisms (SNPs), in which minor allele frequencies were higher than 2%, were discovered. A graphic overview of the structure of the gene and the location of the informative SNPs are shown in Fig 1.

SNP scoring. Priority in the SNP selection for scoring was given to nonsynonymous coding SNPs and SNPs that tag most of the remaining variants after determining the linkage disequilibrium pattern (Fig 2). The selected SNPs were scored by using the high-throughput single-base-pair extension method (SNP-IT assay) with an SNPstream25K system, which was customized to automatically genotype DNA samples in 384-well plates and provide a colorimetric readout (Orchid Biosciences, Princeton, NJ), as previously described.¹⁸

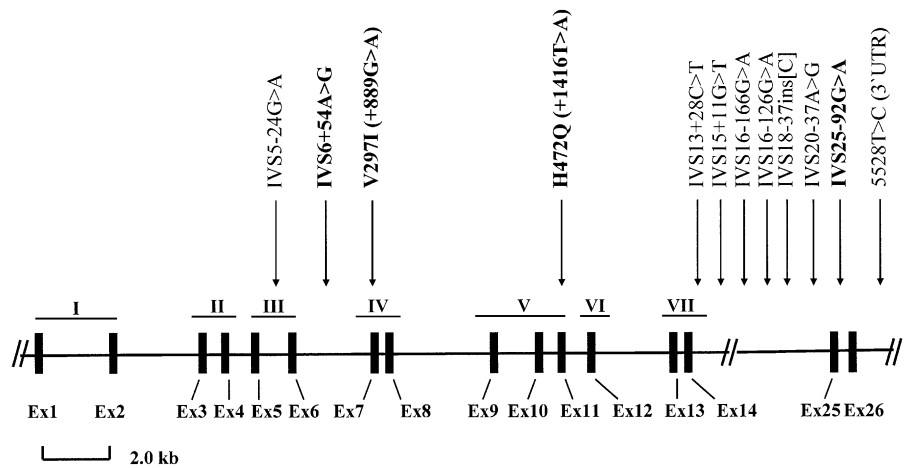


FIG 1. Genomic organization of the KDR gene region on human chromosome 4q11-12. Boxes and arrows indicate exons and the approximate locations of SNPs, respectively. SNPs selected for scoring are shown in bold. The structural domains encoded by each exon are indicated; immunoglobulin-like domains are marked with roman numbers.

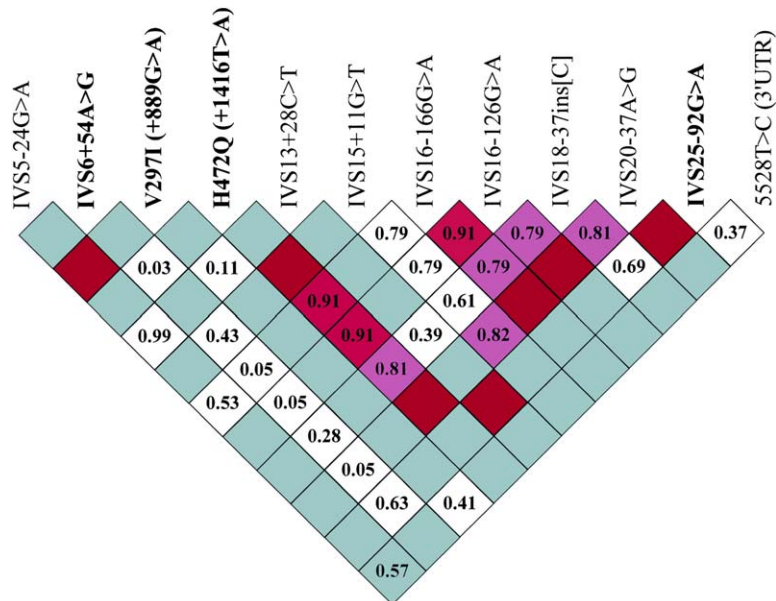


FIG 2. Pairwise linkage disequilibrium (D') in the study population. SNPs selected for scoring are shown in bold.

Statistical analysis

Individual SNPs and haplotypes were examined as 3-component variables (eg, AA, AB, and BB, where A is the major frequency allele or the haplotype of concern and B is the minor frequency allele or the other haplotype). Haplotypes and their frequencies were estimated by using an expectation-maximization algorithm. A contingency table was drafted according to the analysis model: a dominant model (AA vs AB + BB), a recessive model (BB vs AB + AA), and a codominant model (AA vs AB vs BB). Then P values were obtained by using logistic analysis controlling for age, sex, a family history of allergic disease, smoking history, and vaccination history. The relative risks (RRs) in the cohort of general children and adolescents

and odds ratios in adult asthmatic patients were calculated. The Hardy-Weinberg equilibrium was tested for with χ^2 tests. All statistical analyses were performed with SAS genetics software (version 8.1; SAS, Cary, NC). P values of less than .05 were regarded as significant.

RESULTS

SNP discovery and haplotype reconstruction

After sequencing of the KDR gene, 12 SNPs were found to be informative in terms of minor allele frequency higher than 2%. All the SNPs were in Hardy-Weinberg

equilibrium and had minor allele frequencies between 6.2% and 43.8%. Among them, 4 SNPs were selected for scoring according to the criteria mentioned above (shown in bold in Fig 1: IVS6+54A>G, +889G>A [V297I], +1416T>A [H472Q], and IVS25-92G>A). Seven haplotypes with a frequency of greater than 5% in at least one of the 2 study populations were identified across the genomic region by using the SNPs selected. The frequencies of individual SNPs and haplotype are shown in Table II.

Genetic effects of individual SNPs and of haplotypes of the KDR gene on the atopy and AHR in the cohort of general children and adolescents

The prevalence of atopy was associated with +889G>A (V297I) polymorphism with borderline significance ($P = .048$; RR, 1.13; 95% CI, 1.00-1.28 in a dominant model and $P = .029$ in a codominant model). However, haplotype analysis showed that the atopy prevalence was strongly associated with Ht2 (AGAG) of the KDR gene ($P = .002$; RR, 1.25; 95% CI, 1.09-1.42 in a dominant model for Ht2 [AGAG] and $P < .001$ in a codominant model for Ht2 [AGAG]; Table III).

As for methacholine AHR, the present study showed that a positive rate of AHR was associated with the +889G>A (V297I) polymorphism of the KDR gene with borderline significance ($P = .043$ in a codominant model). However, haplotype analysis found no significant association between AHR and any haplotypes of the KDR gene (Table IV).

The present study showed that 133 (6.5%) subjects of the cohort were given diagnoses of asthma according to our criteria. However, neither individual SNPs nor haplotypes of the KDR gene showed significant association with the prevalence of asthma (data not shown).

Genetic effects of individual SNPs and of haplotypes of the KDR gene on atopy among adult asthmatic patients

In terms of association between individual SNPs of the KDR gene and the prevalence of atopy among adult asthmatic patients, there are no significant associations (data not shown). However, the present study showed that atopy prevalence among adult asthmatic patients was significantly associated with the haplotype Ht2 (AGAG), which showed a significant association with atopy in the general population ($P = .008$; odds ratio, 1.66; 95% CI, 1.14-2.44 in a dominant model and $P = .021$ in a codominant model; Table V).

DISCUSSION

The present study shows that genetic variations of KDR are significantly associated with atopy in the general population living in rural areas of Korea. Moreover, the genetic association is also reproducible among adult

TABLE II. Frequencies of individual SNPs and haplotypes of the KDR gene in the general population (cohort) and among adult asthmatic patients

Genotype	Minor allele frequency (%)	
	Cohort	Asthmatic patients
Individual SNPs		
IVS6+54A>G	34.6	41.3
+889G>A (V297I)	14.2	12.3
+1416T>A (H472Q)	42.6	40.0
IVS25-92G>A	21.0	18.9
Haplotypes*		
Ht1 (GGTG)	22.6	28.2
Ht2 (AGAG)	19.9	22.5
Ht3 (AGTA)	17.4	12.1
Ht4 (AAAG)	13.4	11.3
Ht5 (AGTG)	13.3	12.7
Ht6 (GGAG)	9.2	6.0
Ht7 (GGTA)	2.9	5.8

*IVS6+54A>G_+889G>A (V297I)_+1416T>A (H472Q)_IVS25-92G>A.

asthmatic patients. To the best of our knowledge, this is the first study to demonstrate the importance of genetic variations in the KDR gene on the predisposition to atopy.

VEGF is known to be a potent mediator of vascular remodeling and angiogenesis in the lung.¹³ Exaggerated levels of VEGF in asthma might contribute to the proclivity of asthmatic patients to become sensitized to respiratory antigens. The ability of cockroach antigen to directly stimulate epithelial VEGF elaboration might also account for the impressive levels of sensitization that are caused by even low-level exposure to this antigen.^{19,20} Moreover, our previous study showed that VEGF augments T_H2 antigen sensitization through the respiratory tract, partly by enhancing the maturation of pulmonary dendritic cells.¹³ It is clear that T_H2 sensitization to common environmental aeroallergens is a primary factor in the development of asthma, especially atopic asthma.¹⁷ Taken together, these findings imply that VEGF plays an important role in the development of atopy and subsequent atopic asthma. Indeed, the present study showed that atopy was more prevalent both in the general population and in asthmatic patients with a common haplotype of the KDR gene, which suggests that variations in the KDR gene partly determined genetic predisposition to T_H2 sensitization to allergens.

Questions will remain concerning the functional roles of nonsynonymous coding SNPs that are responsible for the association results of the present study. Thus far, there have been no reports demonstrating the functional consequences of any nonsynonymous coding SNPs in the KDR gene. Interestingly, 2 nonsynonymous coding SNPs analyzed in this study (V297I and H472Q) are located in regions coding for the extracellular fourth and fifth immunoglobulin-like domains, as shown in Fig 1. These domains are known to be essential for maintaining the high association rate with VEGF and retention of the VEGF on the receptor.²¹ Therefore we can speculate

TABLE III. Genetic effects of individual SNPs and of haplotypes in the KDR gene on atopy in the cohort

Genotype frequency				Dominant		Recessive		Codominant
				P value*	RR (95% CI)	P value*	RR (95% CI)	P value*
IVS6+54A>G								
	AA	AG	GG					
Case	314 (36.5%)	355 (38.0%)	98 (37.5%)	.554	1.03 (0.92-1.16)	.896	0.98 (0.83-1.17)	.792
Control	547 (73.5%)	578 (62.0%)	163 (63.5%)					
+889G>A (V297I)								
	GG	GA	AA					
Case	543 (35.7%)	206 (41.6%)	12 (27.98%)	.048	1.13 (1.00-1.28)	.212	0.75 (0.46-1.21)	.029
Control	976 (64.3%)	289 (58.4%)	31 (72.1%)					
+1416T>A (H472Q)								
	TT	TA	AA					
Case	256 (28.9%)	355 (38.6%)	148 (57.4%)	.559	1.03 (0.91-1.17)	.698	1.03 (0.89-1.19)	.827
Control	628 (71.1%)	565 (61.4%)	110 (42.6%)					
IVS25-92G>A								
	GG	GA	AA					
Case	492 (38.1%)	238 (35.6%)	37 (39.4%)	.291	0.94 (0.83-1.05)	.397	1.01 (0.77-1.32)	.524
Control	800 (61.9%)	431 (64.4%)	57 (60.6%)					
Ht2 (AGAG)†								
	Ht2/Ht2	Ht2/others	Others/others					
Case	125 (52.7%)	143 (35.6%)	499 (34.9%)	.002	1.25 (1.09-1.42)	<.001	1.87 (1.47-2.38)	<.001
Control	112 (47.3%)	248 (64.4%)	928 (65.1%)					

*P values from logistic analysis after controlling for age, sex, a family history of allergic diseases, passive smoking history, and vaccination history.

†The other haplotypes showed no significant association with the prevalence of atopy.

TABLE IV. Genetic effects of individual SNPs in the KDR gene on AHR in the cohort

Genotype frequency				Dominant		Recessive		Codominant
				P value*	RR (95% CI)	P value*	RR (95% CI)	P value*
IVS6+54A>G								
	AA	AG	GG					
Case	220 (25.6%)	245 (26.4%)	60 (22.3%)	.888	0.99 (0.85-1.16)	.130	0.83 (0.65-1.06)	.297
Control	638 (74.4%)	683 (75.6%)	209 (77.7%)					
+889G>A (V297I)								
	GG	GA	AA					
Case	367 (24.4%)	136 (27.9%)	15 (34.6%)	.066	1.17 (0.99-1.37)	.183	1.35 (0.89-2.05)	.043
Control	1137 (75.6%)	351 (72.1%)	29 (65.4%)					
+1416T>A (H472Q)								
	TT	TA	AA					
Case	174 (24.7%)	247 (26.2%)	96 (24.2%)	.610	1.04 (0.89-1.22)	.638	0.95 (0.78-1.16)	.689
Control	531 (75.3%)	694 (73.8%)	297 (75.8%)					
IVS25-92G>A								
	GG	GA	AA					
Case	320 (25.0%)	180 (26.9%)	21 (22.8%)	.474	1.05 (0.90-1.23)	.539	0.88 (0.60-1.30)	.540
Control	959 (75.0%)	488 (73.1%)	71 (77.2%)					

*P values from logistic analysis after controlling for age, sex, a family history of allergic diseases, passive smoking history, and vaccination history.

TABLE V. Genetic effects of haplotypes in the KDR gene on atopy among adult asthmatic patients

Haplotype frequency				Dominant		Recessive		Codominant
				P value*	OR (95% CI)	P value*	OR (95% CI)	P value*
Ht2 (AGAG)†								
	Ht2/Ht2	Ht2/others	Others/others					
Atopic	14 (66.7%)	88 (56.0%)	121 (44.6%)	.008	1.66 (1.14-2.44)	.110	2.09 (0.82-5.29)	.021
Nonatopic‡	7 (33.3%)	69 (44.0%)	150 (55.4%)					

OR, Odds ratio.

*P values from logistic analysis after controlling for age, sex, a family history of allergic diseases, passive smoking history, and vaccination history.

†The other haplotypes showed no significant association with the prevalence of atopy.

‡Nonatopic subjects among asthmatic patients.

that these nonsynonymous coding SNPs possibly alter VEGF signaling pathways. However, statistical power of the associations between these nonsynonymous SNPs and the prevalence of atopy were weak or insignificant both in the general population and among asthmatic patients enrolled in this study, whereas the haplotype constructed with adjacent SNPs showed stronger and significant association with atopy prevalence. There are 2 possible explanations. First, a single nonsynonymous SNP alone might not alter VEGF signaling pathways sufficiently, and its functional consequences become evident only when additional regulatory elements harboring in intronic regions are added. Second, the significant association might be derived from linkage disequilibrium between the tagging SNPs scored and the other functionally important SNPs, which were discovered but not scored in the present study.

In the present study more than 2000 children and adolescents were randomly selected for the association study. This type of cohort study has the advantage of being able to yield estimates of relative risk, whereas case-control designs cannot estimate it. Moreover, the present study showed that the significant association between atopy and genetic variations of KDR were also reproduced among asthmatic patients, which made it possible to generalize our results in the Korean population. We are sure that the present study provides new and interesting information regarding the possible role of VEGF signaling pathways on the predisposition to atopy, although additional studies will be needed to confirm the our association results on other ethnic groups.

In summary, the present study shows that genetic variations in the KDR gene are associated with atopy in the Korean population. These results suggest that VEGF might play an important role in the T_H2 sensitization to allergen and that genetic variations in VEGF signaling pathways provide genetic markers predictive of the genetic susceptibility to atopy.

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