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Effect of gene polymorphisms in *ADAM33*, *TGFβ1*, *VEGFA*, and *PLAUR* on asthma in Thai population

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Abstract

Background: Most of the asthma susceptibility genes have demonstrated moderate effect. Gene-gene interaction may play a role in asthma.

Objectives: To investigate the genetic and gene-gene interaction effects of single nucleotide polymorphisms (SNPs) in the *ADAM33*, *TGFβ1*, *VEGFA*, and *PLAUR* genes on asthma in Thai population.

Methods: Two hundred and fifty control and 250 asthmatic Thai subjects were recruited. Asthma was diagnosed based on symptoms and spirometry assessments using criteria outlined by the American Thoracic Society. Degrees of asthma severity were determined according to guidelines provided by the Global Initiative for Asthma. Asthmatic subjects were subcategorized into the low-severity (n = 106) and high-severity (n = 144) groups. Eleven SNPs in four genes were genotyped, including *ADAM33* SNPs (rs528557/S2, rs598418, rs44707/ST+4), *TGFβ1* SNPs (rs2241715, rs11466345), *VEGFA* SNPs (rs833069, rs3025010), and *PLAUR* SNPs (rs344781, rs344787, rs2239374, rs2239372). Association analyses between SNPs and asthma, and tests for gene-gene interaction were performed.

Results: The *ADAM33* rs528557/S2 SNP was found to be associated with asthma according to the additive and dominant models. Comparison between the low-severity group and controls showed the *VEGFA* rs833069 SNP to be significantly associated with the low-severity group. No gene-gene interactions were observed in this study.

Conclusions: The *ADAM33* rs528557/S2 and the *VEGFA* rs833069 SNPs were associated with Thai asthmatics, as well as with other populations worldwide. Further studies are warranted to investigate the use these SNPs as biomarkers for establishing early diagnosis or for predicting future risk of asthma.

Key words: Gene polymorphisms, gene interactions, *PLAUR*, *VEGFA*, *TGFβ1*, *ADAM33*, asthma, Thai population

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Introduction

Airway structural changes or remodeling occurred in adults and children with asthma.[1] In most asthma phenotypes, chronic airway inflammation plays a causal role in airway remodeling. However, airway remodeling was reported in some young children who subsequently developed asthma, which suggests that remodeling can occur early in the disease course.[2] Although inhaled corticosteroid, which is the mainstay of asthma treatment, provides asthma control and reduces airway hyperresponsiveness, it does not affect the natural course of the disease or the decline in lung function.[3]

Improved understanding of the mechanisms of the remodeling process may yield other therapeutic targets that could potentially change the natural course of the disease.

Given the structural components of airway remodeling, factors of interest should include those involving tissue fibrosis, angiogenesis, and the repair process. The genes identified as having a role in these processes include a disintegrin and metalloprotease 33 (*ADAM33*), transforming growth factor β1 (*TGFβ1*), vascular endothelial growth factor (*VEGFA*), and urokinase plasminogen activator receptor (*PLAUR*). Ito, *et al.* investigated *ADAM33* expression in airway smooth muscle (ASM) cells, and found *ADAM33* mRNA and protein expression to be significantly higher in asthmatic subjects than in controls.[4] Genetic research by positional cloning method revealed the *ADAM33* gene to be significantly associated with the prevalence of asthma and airway hyperresponsiveness.[5] Thongngarm, *et al.* found single nucleotide polymorphisms (SNPs) and haplotypes of *ADAM33* to be associated with not only the prevalence, but also the severity of asthma in Thai population.[6] *ADAM33* SNPs were also found to be associated with decline in lung function and airway remodeling.[7] Evidence was reported that the *ADAM33* gene teamed up with other genes (i.e., *TGFβ1*, *VEGFA*, and *PLAUR*), which affected the proteolytic pathway and resulted in airway remodeling.[8] *TGFβ1* expressed on inflammatory cells in submucosal and smooth muscle cell layers in the airways is associated with the severity of asthma.[9] *TGFβ1* SNPs were shown to be associated with asthma and its severity in many different ethnic populations.[10] *VEGFA* enhances *ADAM33* expression and ASM cell proliferation by activating the VEGFR2/ERK1/2 signaling pathway, which might involve in the pathogenesis of airway remodeling.[11] Inhibition of VEGF, which increased in the lung tissues and sputum of asthmatic patients, significantly reduced goblet cell hyperplasia and basement membrane thickness.[12] *PLAUR* activation is mediated by ADAM proteins, which suggests that there might be a functional relationship underlying the association between *ADAM33* and *PLAUR*.[13] *PLAUR* mediating *VEGF*-induced migration of vascular endothelial cells was also reported.[14] Previous studies identified an association between multiple *PLAUR* SNPs and asthma, decline in FEV_1, and *PLAUR* serum levels.[15]

Most of the asthma susceptibility genes identified thus far have demonstrated only moderate effect, and the results are sometimes poorly replicated. Gene-gene interaction, which is defined as the phenotypic effect of a variant at one genetic locus depending on variants at other loci, as opposed to single genetic variant, may play a role in complex genetic diseases like asthma. Given the close relationship and overlapping functions among these four genes and asthma, we aimed to investigate: 1) the genetic effects of SNPs in the *ADAM33*, *TGFβ1*, *VEGFA*, and *PLAUR* genes on asthma susceptibility, and 2) the effect of gene-gene interactions among these genes on asthma in Thai pediatric and adult population.

Methods

Subjects

Two hundred and fifty each of asthmatic and control Thai subjects were recruited from pediatric and adult allergy and immunology clinics from two tertiary care centers located in Bangkok, Thailand. Asthma was diagnosed based on symptoms and spirometry assessments using criteria outlined by the American Thoracic Society. Degree of asthma severity was determined according to guidelines provided by the Global Initiative for Asthma. Asthmatic subjects were subcategorized into the low-severity group (n = 106) and the high-severity group (n = 144). Control subjects were asymptomatic for asthma and were devoid of atopic or pulmonary diseases. Pregnant or lactating female subjects were excluded. This study was conducted in accordance with the principles set forth in the Declaration of Helsinki and all of its subsequent amendments. The protocol for this study was approved by the institutional review board (IRB) of both participating centers, and written informed consent was obtained from all enrolled subjects.

Polymorphism genotyping

As the four genes (*TGFβ1*, *VEGFA*, *PLAUR* and *ADAM33*) with overlapping functions have a close relationship with asthma via airway remodeling involving tissue fibrosis, angiogenesis, and the repair process. We thus hypothesized that they are associated with the development of asthma and gene interactions among them may be possible. In the present study, we selected 11 SNPs from them to verify the hypothesis in Thai population.

All the three SNPs in *ADAM33* gene (rs528557/S2, rs598418 and rs44707/ST+4) were selected because they have been identified as validated SNPs from our previous study.(6) The other eight SNPs including the two in *TGFβ1* (rs2241715 and rs11466345), two in *VEGFA* (rs833069 and rs3025010), and four in *PLAUR* (rs344781, rs344787, rs2239374 and rs2239372) were chosen based on their validation status, minor allele frequency (MAF) higher than 0.2 and heterozygosity between 0.4-0.5 using the data from Pan-Asian SNP (http://www4a.biotec.or.th/PASNP), Tag SNP (https://www.genome.gov/10001688/international-hapmap-project), SNPedia (https://www.snpedia.com/index.php/SNPedia) and ThaiSNP2 database (http://www4a.biotec.or.th/thaisnp2/). These eight SNPs are haplotype-tagging SNPs regarding haplotype structures in Thai population.

Blood samples were collected in EDTA-containing tubes. Genomic DNA was extracted and used as a template. Eleven SNPs in four genes were genotyped in this study, as described in **Table 1**.

Three sets of multiplex polymerase chain reaction (multiplex PCR) were performed, using newly designed primers by Oligo 6 Primer Analysis Software (Molecular Biology Insights, Inc., Colorado Springs, CO, USA). Each multiplex PCR set was amplified in 25 μL reaction volume containing 1x Immobuffer, 3.0 mM $MgCl_2$, 1.0 mM dNTPs (Promega Corporation, Madison, WI, USA), 10 pmol of each primer, 1.0 U Immolase™ DNA Polymerase (Bioline, London, United Kingdom), and 50 ng of genomic DNA. The PCR condition consisted of predenaturation at 95°C for 15 min, followed by 40 cycles of denaturation at 95°C for 30 s, annealing at the optimal melting temperature for 30 s, extension at 65°C for 2 min, and then final extension at 65°C for 10 min on a Mastercycler® Pro S PCR system (Eppendorf, Hamburg, Germany). Multiplex PCR products were cleaned up using Exo-SAP-IT®

Table 1. Eleven selected SNPs in four genes for genotyping

Gene	SNP	HGVS name	Observed allele	1000 Genomes allele frequency		Position
				CDX	KHV	
TGFβ1	rs2241715	NM_000660.6:c.355+1709T>G	G/T	T = 0.6183 G = 0.3817	T = 0.5909 G = 0.4091	Intron 1
	rs11466345	NM_000660.6:c.860+4327A>G	A/G	A = 0.6344 G = 0.3656	A = 0.7475 G = 0.2525	Intron 5
VEGFA	rs833069	NM_003376.5:c.658+450T>G	T/C	T = 0.6129 C = 0.3871	T = 0.6111 C = 0.3889	Intron 2
	rs3025010	NM_003376.5:c.963-892T>C	C/T	T = 0.7097 C = 0.2903	T = 0.6364 C = 0.3636	Intron 5
PLAUR	rs344781	NM_002659.3:c.-516G>A	G/A	C = 0.6022 T = 0.3978	C = 0.5152 T = 0.4848	Promoter
	rs344787	NM_002659.3:c.167-975T>A	A/T	A = 0.6183 T = 0.3817	A = 0.5404 T = 0.4596	Intron 2
	rs2239374	NM_002659.3:c.310+1187G>A	G/A	C = 0.5860 T = 0.4140	C = 0.4848 T = 0.5152	Intron 3
	rs2239372	NM_002659.3:c.311-2162T>C	T/C	A = 0.3656 G = 0.6344	A = 0.3586 G = 0.6414	Intron 3
ADAM33	rs528557/S2	NM_025220.4:c.2151G>C	G/C	C = 0.7312 G = 0.2688	C = 0.7374 G = 0.2626	Exon 19
	rs598418	NM_025220.4:c.2240+384C>T	C/T	G = 0.5968 A = 0.4032	G = 0.5859 A = 0.4141	Intron 19
	rs44707/ST+4	NM_025220.4:c.2240+427C>A	C/A	G = 0.6989 T = 0.3011	G = 0.6919 T = 0.3081	Intron 19

CDX = Chinese Dai in Xishuangbanna, China; KHV = Kinh in Ho Chi Minh City, Vietnam

PCR Product Cleanup Reagent (Affymetrix, Santa Clara, CA, USA). The purified PCR products were used as templates for genotyping using the single-base extension (SBE) method.

Each set of SBE product was directly detected by a fully-denaturing mode of denaturing high-performance liquid chromatography (DHPLC) using a WAVE™ Nucleic Acid Fragment Analysis System equipped with an OLIGOSep™ cartridge (Transgenomic, Inc., Omaha, NE, USA). The elution profile of each SNP was confirmed by direct DNA sequencing.

Direct DNA sequencing was performed using a BigDye® Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific, Inc., Waltham, MA, USA). The sequencing reaction was prepared in 10 µL volume consisting of 1 µL of BigDye® Terminator v3.1 Solution, 6.4 pmol of primer, and 30 ng of PCR product, which was subjected to predenaturation at 96°C for 1 min followed by 35 cycles of denaturation at 96°C for 1 min, annealing 50°C for 10 s, and extension at 60°C for 4 min on a Mastercycler® Pro S PCR system (Eppendorf).

Statistical analysis

Single-locus analysis

The genotype distribution of each SNP within the control, case, low-severity, high-severity, combined case-control, combined low-severity-control, and combined high-severity-control groups was tested for departure from Hardy-Weinberg equilibrium using χ^2 test ($\alpha = 0.05$). Association between each SNP and the disease or the severity level was determined by single-locus analysis. The following four genetic models were considered: additive, dominant, multiplicative, and recessive models. Bonferroni correction was subsequently performed to compensate for multiple hypothesis testing in association analysis.

Sample size calculation

After the single-locus analysis was performed, the number of samples required to detect genetic association was calculated using a Power for Genetic Association Analyses (PGA) package.[16] This process was performed to ensure that this study has sufficient statistical power to identify significant associations, without the risk of identifying false-positive associations ($\beta = 0.2$).

Linkage disequilibrium analysis

Linkage disequilibrium between loci in or near the same gene was measured and described as D' and r^2. Linkage disequilibrium is an indicator for deciding whether the association result is influenced by a haplotype effect.[17] A Java LINkage disequilibrium plotter (JLIN) was used to display D' and r^2.[18]

Multi-locus analysis

Statistical epistasis was detected using a multifactor dimensionality reduction (MDR) technique.[19] Statistical epistasis detection was performed to increase the possibility of detecting susceptibility loci.[20]

Haplotype association and interaction analysis

In addition to the genetic association analysis that uses genotype information, analysis that uses haplotype information was also performed. Haplotypes were inferred from every combination of SNPs in or near the same gene. All configurations of haplotypes and combinations of haplotypes from different genes were analyzed for possible association with the disease or severity level. The consideration of haplotype combinations facilitates the detection of haplotype interaction. The selected configuration of haplotypes or haplotype combinations for association explanation was the one with the lowest unadjusted *p*-value obtained through a permutation test. If two or more configurations had the lowest unadjusted *p*-value, then the most parsimonious configuration, the configuration with the least number of SNPs, was the one selected. The global null hypothesis that none of configurations of haplotypes and haplotype combinations is associated with the disease or severity level was also tested. Haplotype association and interaction analysis was performed using FAMHAP software.[21]

Results

Clinical characteristics of high and low severity groups are shown in **Table 2**. Subjects in the high severity group were significantly older and suffered from asthma for longer duration than those in the low severity group. The mean FEV_1 was significantly lower in the high severity group as compared to those of the low severity group. Allele frequencies of eleven SNPs in four genes including *TGFβ1*, *VEFGA*, *PLAUR* and *ADAM33* are shown in **Table 3**.

Table 2. Clinical Characteristics of high and low severity groups

Characteristics	Control (n = 250)	Case		*p*-value
		Low severity group (n = 106)	High severity group (n = 144)	
Age (years)	29.91	25.90 (19.28)	40.42 (17.59)	< 0.001
Gender (M/F ratio)	0.44	0.93	0.97	NS
Duration of asthma (years)	NA	8.86 (6.92)	12.49 (8.43)	< 0.001
FVC (% of predicted)	NA	82.71 (20.79)	79.17 (15.91)	NS
FEV_1 (% of predicted)	NA	74.64 (17.25)	62.69 (16.99)	< 0.001
Bronchodilator response (% change in FEV_1)	NA	18.79 (9.59)	18.36 (14.72)	NS

* For age, duration of asthma, FVC, FEV_1, and bronchodilator response, the displayed values are means while the numbers in brackets are SDs. Statistical analysis was performed to compare the low- and high-severity groups. For gender, the displayed values are the ratios between male and females and *p*-value is calculated using χ^2 test. For age and duration of asthma, *p*-values are obtained from Mann Whitney U test while FVC, FEV_1 and bronchodilator response are obtained from Student t test. FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 second; NS, non-significant.

Table 3. Allele frequency of 11 selected SNPs in cases and controls

Gene	SNP	Observed allele	Allele frequency				Position
			Control (n = 250)		Case (n = 250)		
					Low-severity (n = 106)	High-severity (n = 144)	
TGFβ1	rs2241715	G/T	GG = 29 GT = 135 G = 0.386 TT = 86 T = 0.614		GG = 14 GT = 44 G = 0.340 TT = 48 T = 0.660	GG = 26 GT = 68 G = 0.417 TT = 50 T = 0.583	Intron 1
	rs11466345	A/G	AA = 126 AG = 107 A = 0.718 GG = 17 G = 0.282		AA = 58 AG = 44 A = 0.755 GG = 4 G = 0.245	AA = 83 AG = 54 A = 0.764 GG = 7 G = 0.236	Intron 5
VEGFA	rs833069	T/C	TT = 82 TC = 132 T = 0.592 CC = 36 C = 0.408		TT = 37 TC = 40 T = 0.538 CC = 29 C = 0.462	TT = 47 TC = 74 T = 0.583 CC = 23 C = 0.417	Intron 2
	rs3025010	C/T	CC = 29 CT = 111 C = 0.338 TT = 110 T = 0.662		CC = 12 CT = 51 C = 0.354 TT = 43 T = 0.646	CC = 22 CT = 53 C = 0.337 TT = 69 T = 0.663	Intron 5
PLAUR	rs344781	G/A	GG = 47 GA = 130 G = 0.448 AA = 73 A = 0.552		GG = 23 GA = 48 G = 0.443 AA = 35 A = 0.557	GG = 27 GA = 71 G = 0.434 AA = 46 A = 0.566	Promoter

Table 3. (Continued)

Gene	SNP	Observed allele	Allele frequency				Position
			Control (n = 250)		Case (n = 250)		
					Low-severity (n = 106)	High-severity (n = 144)	
PLAUR (Continued)	rs344787	A/T	AA = 37 AT = 147 A = 0.442 TT = 66 T = 0.558		AA = 23 AT = 54 A = 0.472 TT = 29 T = 0.528	AA = 31 AT = 80 A = 0.493 TT = 33 T = 0.507	Intron 2
	rs2239374	G/A	GG = 61 GA = 122 G = 0.488 AA = 67 A = 0.512		GG = 26 GA = 57 G = 0.514 AA = 23 A = 0.486	GG = 37 GA = 73 G = 0.510 AA = 34 A = 0.490	Intron 3
	rs2239372	T/C	TT = 27 TC = 99 T = 0.306 CC = 124 C = 0.694		TT = 8 TC = 47 T = 0.297 CC = 51 C = 0.703	TT = 15 TC = 64 T = 0.326 CC = 65 C = 0.674	Intron 3
ADAM33	rs528557/S2	G/C	GG = 148 GC = 82 G = 0.756 CC = 20 C = 0.244		GG = 86 GC = 18 G = 0.896 CC = 2 C = 0.104	GG = 96 GC = 42 G = 0.813 CC = 6 C = 0.187	Exon 19
	rs598418	C/T	CC = 83 CT = 121 C = 0.574 TT = 46 T = 0.426		CC = 35 CT = 58 C = 0.604 TT = 13 T = 0.396	CC = 37 CT = 75 C = 0.517 TT = 32 T = 0.483	Intron 19
	rs44707/ST+4	C/A	CC = 92 CA = 118 C = 0.604 AA = 40 A = 0.396		CC = 35 CA = 50 C = 0.566 AA = 21 A = 0.434	CC = 42 CA = 78 C = 0.563 AA = 24 A = 0.437	Intron 19

Single-locus analysis

The genotype distribution calculated by using χ^2 test of two SNPs, the *TGFβ1* rs2241715 and *PLAUR* rs344787, in the control group departed from Hardy-Weinberg equilibrium. As a result, these two SNPs were excluded from further association analysis. As a result of the departure from Hardy-Weinberg equilibrium, a multiplicative model was not considered in the case-control association analysis of the *ADAM33* rs528557/S2 SNP, in the low-severity group-control association analysis of the *VEGFA* rs833069 SNP and *ADAM33* rs528557/S2 SNP, and in the high-severity group-control association analysis of *VEGFA* rs3025010 SNP.[22] The *ADAM33* rs528557/S2 SNP was found to be associated with asthma according to the additive and dominant models. In addition, the *VEGFA* rs833069 SNP was associated with the low-severity group of disease according to a recessive model, while the *ADAM33* rs528557/S2 SNP was also associated with the low-severity group of disease according to the additive and dominant models. No SNPs were associated with the high-severity group of asthma. Therefore, no further association analysis involving the high-severity group was performed. The association results are summarized in **Table 4**.

Table 4. Association results from single-locus analysis

SNP	Gene	Genetic model	Genotype	Bonferroni-corrected χ^2's p-value
Case-control				
rs528557/S2	*ADAM33*	Additive	GG GC CC	0.00468
		Dominant	GG GC + CC	0.01197
Low-severity-control				
rs833069	*VEGFA*	Recessive	AA + AG GG	0.03420
rs528557/S2	*ADAM33*	Additive	GG GC CC	0.00054
		Dominant	GG GC + CC	0.00063

Sample size

Dejsomritrutai, *et al.* reported a prevalence of asthma in Thailand of 2.91%.[23] With the availability of prevalence, it is possible to estimate the penetrance of each SNP genotype that is associated with asthma or asthma severity level. As a result, the required number of samples for association studies can be calculated. The minimum required number of balanced case-control samples was calculated to be 468; so, the number of case-control samples in this study was sufficient. The minimum required numbers of low-severity samples and control samples based on the detected association at the *VEGFA* rs833069 SNP were 103 and 242, respectively; as such, these numbers of samples were also sufficient. In contrast, the required minimum numbers of low-severity samples and control samples based on the detected association at the *ADAM33* rs528557/S2 SNP were 115 and 270, respectively. These minimum required numbers exceeded the numbers of low-severity samples and control samples in this study. Therefore, it cannot be definitely concluded that *ADAM33* rs528557/S2 SNP was associated with the low-severity group of disease.

Linkage disequilibrium

Seven SNPs shown in **Figure 1** exhibited pairwise linkage disequilibrium. These SNPs can be divided into three groups of linked SNPs according to their locations in the genome.

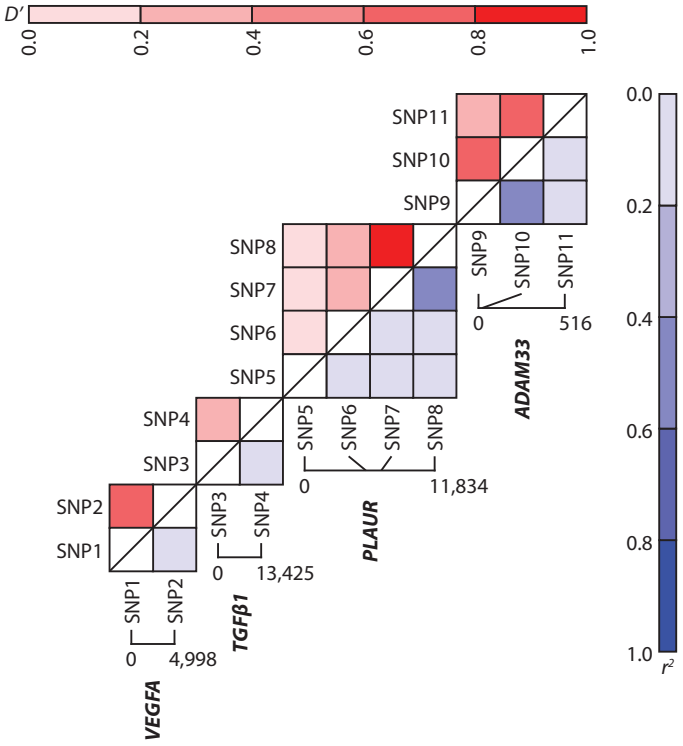

Figure 1. Linkage disequilibrium patterns of SNPs in or near *VEGFA*, *TGFβ1*, *PLAUR*, and *ADAM33*. *D′* is displayed in upper triangles, while r^2 is displayed in lower triangles. Dark colors denote high values, and pale colors denote low values. The distance between SNPs indicates the number of base pairs. SNP1 = rs833069, SNP2 = rs3025010, SNP3 = rs11466345, SNP4 = rs2241715, SNP5 = rs2239372, SNP6 = rs2239374, SNP7 = rs344787, SNP8 = rs344781, SNP9 = rs44707/ST+4, SNP10 = rs598418, and SNP11 = rs528557/S2.

The first group consisted of the *VEGFA* rs833069 and rs3025010 SNPs. The second group consisted of the *PLAUR* rs344787 SNP, and the rs344781 SNP located near *PLAUR*. The third group consisted of the *ADAM33* rs44707/ST+4, rs598418, and rs528557/S2 SNPs. Haplotype association and interaction analysis was, therefore, deemed appropriate.

Multi-locus analysis

MDR selected the *ADAM33* rs528557/S2 SNP as the most informative SNP for both case-control and low-severity group-control classification. Nonetheless, a permutation test with 1,000 randomized replicates revealed that the null hypothesis of no association cannot be rejected in either setting ($p > 0.05$). As a result, multi-locus analysis did not provide additional information regarding genetic association.

Haplotype association and interaction analysis

Haplotype association and interaction analysis revealed two sets of SNP pair to be associated with asthma. Both sets of SNP pair had the lowest unadjusted *p*-values ($p < 0.0001$) and contained the least number of SNPs. The first set consisted of the *ADAM33* rs528557/S2 and rs598418 SNPs, while the second set consisted of the *ADAM33* rs528557/S2 and rs44707/ST+4 SNPs. All three SNPs were in linkage disequilibrium, and all three are in *ADAM33*. No haplotype interactions were detected in the case-control analysis. These results are summarized in **Table 5A**.

Table 5A. Association results from case-control haplotype association and interaction analysis using FAMHAP

Haplotype and gene	Haplotype frequency		Odds ratio (95% CI)
	Control	Case	
rs528557/S2 and rs598418 (*ADAM33*)			
GC	0.505	0.547	1.18 (0.92-1.51)
GT	0.251	0.301	1.29 (0.98-1.70)
CC	0.069	0.007	0.10 (0.03-0.30)
CT	0.175	0.145	0.80 (0.57-1.12)
rs528557/S2 and rs44707/ST+4 (*ADAM33*)			
GC	0.511	0.503	0.97 (0.76-1.24)
GA	0.245	0.345	1.62 (1.23-2.14)
CC	0.093	0.061	0.63 (0.39-1.02)
CA	0.151	0.091	0.56 (0.38-0.83)

Global *p*-value = 0.0048

Table 5B. Association results from low severity-control haplotype association and interaction analysis using FAMHAP

Allele of rs528557/S2 (*ADAM33*)	Allele frequency		Odds ratio (95% CI)
	Control	Case	
G	0.756	0.896	2.79 (1.71-4.53)
C	0.244	0.104	0.36 (0.22-0.58)

Global *p*-value = 0.0058

Haplotype association and interaction analysis also revealed *ADAM33* rs528557/S2 SNP to be associated with low-severity asthma (unadjusted $p < 0.0001$), and no haplotype interactions were detected in the low-severity group-control analysis. These results are given in **Table 5B**. However, since the sample size calculation required a higher number of samples than the number of samples that we had in this study for detecting association between *ADAM33* rs528557/S2 SNP and low-severity group, a conclusion regarding the aforementioned low-severity group-control association cannot be conclusively established.

Discussion

Gene segments covering 11 SNPs in four genes, including *ADAM33*, *TGFβ1*, *VEGFA*, and *PLAUR*, were amplified. Single locus analysis revealed the *ADAM33* rs528557/S2 SNP and *VEGFA* rs833069 SNP to be associated with asthma susceptibility. The *ADAM33* rs528557/S2 SNP exhibited significant association with asthma compared to controls in both additive and dominant model ($p = 0.00468$ and $p = 0.01197$, respectively). This *ADAM33* rs528557/S2 SNP was previously shown to be associated with asthma in Thai population,[6] as well as in other populations worldwide.[24] We also identified the *VEGFA* rs833069 SNP as being significantly associated with low-severity asthmatics compared to controls in recessive model ($p = 0.03420$). The frequency of haplotype CC at the *ADAM33* rs528557/S2 and rs598418 SNPs of asthmatic subjects was significantly lower than that of controls (odds ratio [OR]: 0.1, 95% confidence interval [CI]: 0.03-0.3). At *ADAM33* rs528557/S2 and rs44707/ST+4 SNPs, a significantly higher frequency of haplotype GA in asthmatic subjects was observed (OR: 1.62, 95% CI: 1.23-2.14); whereas, haplotype CA was shown to be a protective variant (OR: 0.56, 95% CI: 0.38-0.83). No gene-gene interactions were observed in this study.

Over the last 15 years, the effect of the *ADAM33* gene on asthma has been replicated in different populations worldwide, particularly in Asian population. Previous studies in adult and pediatric Indian population found the *ADAM33* rs528557/S2 and rs44707/ST+4 SNPs to associate with asthma and its severity.[25] Regarding association studies, some positive results might not be replicated possibly due to a small sample size and/or the heterogeneity of samples. Therefore, meta-analyses might be better for identifying causative polymorphisms with better consistency, and this would result in accurate analysis of genetic risks. Two large meta-analyses investigated *ADAM33* polymorphisms, particularly the rs528557/S2 and rs44707/ST+4 SNPs. Song, *et al.* recruited 13 studies (4,942 asthmatics and 7,933 controls) in five *ADAM33* SNPs, and found that *ADAM33* rs528557/S2 SNP conferred susceptibility to asthma in European population, whereas *ADAM33* rs44707/ST+4 did so in Asian population.[26] Tripathi, *et al.* reviewed 13 studies (3,270 asthmatics and 2,922 controls) in 12 *ADAM33* SNPs, and found rs597980/ST+5, rs528557/S2 and rs2280091/T1 SNPs to be associated with asthma with an odds ratio ranging from 1.67-4.34.[27] A recent meta-analysis of eight studies found *ADAM33* rs2280091/T1, rs2280090/T2, rs2787094/V4, rs44707/ST+4, and rs528557/S2 SNPs to be significantly associated with a high risk of childhood asthma.[28] However, different variants, including the rs2280091/T1, rs2787094/V4, rs511898/F+1, rs2280090/T2, and rs2280089/T+1 polymorphisms on 14 *ADAM33* SNPs, identified in a huge meta-analysis were significantly associated with asthma, especially in Asian population.[29] As a result of the expression of *ADAM33* on airway epithelium, fibroblasts, myofibroblasts, and smooth muscle cells, it was postulated that *ADAM33* possibly associates with airway responsiveness and remodeling, although the exact mechanism remains unknown. Of interest, airway remodeling was reported to be reversible after inhibiting human soluble ADAM33, which was found to increase in the airways and cause remodeling independent of inflammation.[30]

Genetic variants of *VEGFA* have only rarely been reported as an asthma susceptibility gene, especially in Asian population. To our knowledge, the only study in Asia was performed in Chinese Han population, and that study found significant association between the *VEGFA* rs3025020 and rs3025039 SNPs and asthma compared to controls.[31] A previous study that analyzed 10 *VEGFA* SNPs found the rs833058 to be associated with asthma and increased airway responsiveness.[32] In addition, the *VEGFA* rs4711750 SNP and its haplotype were found to be associated with FEV_1/FVC and decline in pulmonary function.[32] *VEGFA* SNPs demonstrated association with lung function parameters in childhood independent of asthma.[33] This supports the results of a previous study that showed that airway inflammation and remodeling can occur in parallel.[34] The effect of the *VEGFA* gene on lung function parameters, independent of the degree of inflammation, may explain why we found significant association between the *VEGFA* SNP and the low-severity asthma group, but not the high-severity asthma group.

Regarding the observed associations between the *ADAM33* rs528557/S2 SNP with asthma according to the additive and dominant models, the SNP (NM_025220.4:c.2151G>C, p.G717=) is merely a silent variant which does not cause an amino acid substitution. While the *VEGFA* rs833069 SNP (NM_003376.5:c.658+450T>C) which was significantly associated with the low-severity asthma group compared with the controls is only a deep intronic variant. Its effect is thus not directly related to the protein structural change. However, the two non-coding SNPs possibly induce aberrant splicing or only being in linkage disequilibrium (LD) with the actual functional SNPs elsewhere on the same chromosome. Furthermore, the functional consequences of both SNPs as expression quantitative trait loci (eQTL) influencing the abundance of specific transcripts would be also possible.[35] For biological link between these SNPs and asthma, we postulate that airway epithelial injury by allergens and irritants together with these SNPs induces inflammatory cytokine release leading to activation of dendritic cells, T-helper cell type 2 and innate lymphoid cell type 2 which causes aberrant expression of mRNA and protein expression in mesenchymal cells, ASM cells and fibroblasts. However, the exact role of these SNPs in asthma pathogenesis and severity remain to be elucidated.

This study has some limitations. First, allele frequencies may be altered by different environmental factors that interact with the selected genetic variants. Second and last, the modest size of our study population may have given our study insufficient statistical power to detect interactions between SNPs with low minor allele frequencies.

In conclusions, the results of this study revealed the *ADAM33* rs528557/S2 SNP and *VEGFA* rs833069 SNP to be associated with Thai pediatric and adult asthma, as well as with other populations worldwide. No gene-gene interactions were observed in this study. Further studies are warranted to investigate the use these SNPs as biomarkers for establishing early diagnosis or for predicting future risk of asthma.

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