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Molecules and Cells

MYLK Polymorphism Associated with Blood Eosinophil Level among Asthmatic Patients in a Korean Population

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The myosin light chain kinase (*MYLK*) gene encodes both smooth muscle and nonmuscle cell isoforms. Recently, polymorphisms in *MYLK* have been reported to be associated with several diseases. To examine the genetic effects of polymorphisms on the risk of asthma and related phenotypes, we scrutinized *MYLK* by re-sequencing/genotyping and statistical analysis in Korean population (n = 1,015). Seventeen common polymorphisms located in or near exons, having pairwise r^2 values less than 0.25, were genotyped. Our statistical analysis did not replicate the associations with the risk of asthma and log-transformed total IgE levels observed among African descendant populations. However, two SNPs in intron 16 (*+89872C* > *G* and *+92263T* > *C*), which were in tight LD (|D'| = 0.99), revealed significant association with log-transformed blood eosinophil level even after correction multiple testing (P = 0.002/P^{corr} = 0.01 and P = 0.002/P^{corr} = 0.01, respectively). The log-transformed blood eosinophil levels were higher in individuals bearing the minor alleles for *+89872C* > *G* and *+92263T* > *C,* than in those bearing other allele. In additional subgroup analysis, the genetic effects of both SNPs were much more apparent among asthmatic patients and atopic asthma patients. Among atopic asthma patients, the log-transformed blood eosinophil levels were proportionally increased by gene-dose dependent manner of in both *+89872C* > *G* and *+92263T* > *C* (P = 0.0002 and P = 0.00007, respectively). These findings suggest that *MYLK* polymorphisms might be among the genetic factors underlying differential increases of blood eosinophil levels among asthmatic patients. Further biological and/or functional studies are needed to confirm our results.

INTRODUCTION

Asthma is a heterogeneous respiratory disease characterized by intermittent airway obstruction and inflammation, bronchial hyperresponsiveness, atopy, and elevated IgE levels. Further, many environmental variables such as hygiene (Sublett, 2005), smoking (Chen et al., 2005), activity levels, and childhood infection histories (Tattersfield et al., 2002) have been implicated in the etiology of this disease. It is generally accepted that asthma is a complex, multifactorial phenotype resulting from various genetic factors interacting with a suite of environmental variables. Over time, many genes have been identified that are thought to influence this condition, including both susceptibility and resistance factors (Ober, 2005).

The myosin light chain kinase (*MYLK*) gene is a member of the immunoglobin superfamily. The full length of the human MYLK gene is about 217 kb on chromosome 3q21.1. The gene contains 32 exons and encodes 3 different proteins, including the nonmuscle isoform (nmMYLK, 1914 amino acids), the smooth muscle isoform (smMYLK, 1147 amino acids), and telokin, resulting from different promoters with tissue-specific transcription (Burrows et al., 1989; Watterson et al., 1999). The encoded protein is a calcium-/calmodulin-dependent enzyme that regulates smooth muscle contraction via phosphorylation of a serine residue in the N-terminus of the myosin light chain. This enables interaction between myosin and actin filaments to initiate contractile activity (Kamm and Stull, 2001). *MYLK* is a multifunctional protein that participates in multiple aspects of the inflammatory response, including apoptosis, vascular barrier regulation and permeability, and leukocyte diapedesis (Dudek and Garcia, 2001; Garcia et al., 1995; 1998; Petrache et al., 2001).

MYLK is a key enzyme determining the velocity of smooth muscle contraction. One study showed that increased contractility of bronchial smooth muscle cells from asthmatic subjects was accompanied by increased abundance of MYLK mRNA (Ma et al., 2002). MYLK levels were significantly higher in the human airway smooth muscle of sensitized compared to nonsensitized bronchi. Moreover, the contractile reactivity of airway preparations depended upon the sensitization status (Ammit et al., 2000).

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Table 1. Clinical profiles of the study subjects

Clinical profile	All subjects	Normal controls	Asthmatics
Number of subject	1,015	243	772
Age [mean(range)]	45.83(5.09-80.20)	43.62(5.21-80.08)	46.53(5.09-80.20)
Sex (male/female)	379/636	103/140	276/496
Current Smokers (%)*	32.02%	29.63%	32.78%
BMI (kg/m2) [mean ± SD]*	24.19 ± 3.36	23.68 ± 3.11	24.33 ± 3.41
Log transformed Blood eosinophil (%) [mean ± SD]*	1.49 ± 0.74	1.09 ± 0.51	1.60 ± 0.75
FVC %, predicted [mean ± SD]*	85.69 ± 17.52	92.92 ± 12.35	83.41 ± 18.28
FEV1 %, predicted [mean ± SD]*	85.09 ± 23.05	102.52 ± 14.78	79.61 ± 22.45
Log transformed PC20, methacholine (mg/ml) [mean ± SD]*	0.52 ± 0.80	1.35 ± 0.18	0.24 ± 0.72
Log transformed Total IgE (IU/ml) [mean ± SD]*	2.03 ± 0.67	1.70 ± 0.65	2.13 ± 0.64
Positive rate of skin test (%)*	51.43%	34.98%	43.39%
Positive rate of specific IgE (D.f., %)*	30.05%	18.52%	33.68%
Positive rate of specific IgE (D.p., %)*	38.62%	26.75%	42.36%

Values are mean ± SD. *P* values are obtained using *t*-tests or χ^2 tests between subjects with asthma and normal control subjects.
*P value < 0.005 for difference between subjects with asthma and normal controls

MYLK activation is a critical step in the cytoskeletal rearrangements leading to eosinophil migration, a characteristic feature of several allergic diseases, including asthma (Adachi et al., 2003). MYLK is a molecular target in ventilator-associated lung injury (Parker, 2000), and *in vivo* studies in *MYLK* mutant mice demonstrate an essential role for *MYLK* in inflammation disease, suggesting the gene as a potential drug-discovery target (Wainwright et al., 2003).

Recently, polymorphisms in *MYLK* have been reported to be associated with several diseases, including acute lung injury (Gao et al., 2006), sepsis (Flores et al., 2007), IgE levels among asthmatics and risk of asthma (Flores et al., 2007; Gao et al., 2007). In an attempt to replicate the genetic effects of the polymorphisms, we examined *MYLK* by re-sequencing and statistical analysis in a Korean asthma study (n = 1,015). In the present study, we identified 23 polymorphisms in the MYLK gene by re-sequencing. Among them, 17 polymorphisms were genotyped in a larger case-control study to assess their influence on the risk of asthma and related phenotypes in a Korean population.

MATERIALS AND METHODS

Subjects

Subjects were recruited from the Asthma Genome Research Center that consists of four tertiary hospitals in Korea (Soonchunhyang University, Seoul; Bucheon, Chunan, and Gumi Hospitals). Ethical approvals were obtained from the institutional review board of each hospital. All patients had clinical symptoms and physical examination results compatible with asthma. Each patient showed airway reversibility as documented by an inhalant bronchodilator-induced improvement of more than 15% of FEV1 and/or an airway hyperreactivity of less than 10 mg/ml of methacholine. Normal subjects were spouses of the patients or members of the general population who answered negatively to a screening questionnaire for respiratory symptoms and had FEV1 greater than 75% predicted, the provocation concentration causing a fall in the FEV1 of 20% (PC_{20}) by methacholine greater than 10 mg/ml, and normal finding on a simple chest radiogram. DNA extractions were performed using Qiagen (USA) DNA extraction kit. Total IgE (IU/ml) and specific IgE to *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* were measured using the UniCAP system (Pharmacia Diagnostics, Sweden). Atopy was defined as having wheal reaction to an allergen extract equal to or greater than a reaction to histamine (1 mg/ml), or 3 mm in diameter, and/or positive response of specific IgE to D.f. and D.p. The clinical parameters are summarized in Table 1.

Sequence analysis in unaffected controls

All 32 *MYLK* exons and their flanking regions, including 1.5 kb of the promoter region, were re-sequenced in 24 unrelated Korean DNA samples using an Applied Biosystems (ABI) PRISM 3730 DNA analyzer (Min et al., 2008). Thirty-five primer sets were designed from the GenBank reference sequence (NT_086640). Primer sequences are given in Supplementary Table 1 (http://www.snp-genetics.com/user/additional_content.asp).

Genotyping

Primer Express software (ABI) was used to design PCR primers and MGB probes for TaqMan® genotyping. One probe was labeled with FAM and the other with VIC (Supplementary Table 2). PCR was run in the TaqMan Universal Master mix without UNG (ABI) at primer concentrations of 900 nM and probe concentrations of 200 nM. Reactions were performed in a 384-well format in a total volume of 5 ul using 20 ng of genomic DNA. The plate was then placed in a PE 9700 thermal cycler (ABI) and heated for 2 min at 50°C and for 10 min at 95°C, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. The plate was then transferred to a Prism 7900HT instrument (ABI) where the fluorescence intensity of each well was read. Fluorescence data files from each plate were analyzed using automated software (SDS 2.1). For two SNPs, the SNaPshot method (Makridakis and Reichardt, 2001) was used for genotyping.

Statistics

Two statistics measured linkage disequilibrium between pairs of biallelic loci, Lewontin's D' (|D'|) (Hedrick, 1987) and r^2. Comparisons of genotype distributions between asthmatics and normal subjects were carried out with logistic regression controlling for age (continuous value), sex (male or female), and smoking status (non-smoker = 0, ex-smoker = 1, smoker = 2) as covariates. Blood eosinophil and log-transformed total serum IgE levels were compared using multiple linear regressions. Statistical analysis was performed by SAS statistical software

A Haplotypes of *MYLK*

	Block1			Block2									Block3					
	-37897C>T	-37890A>T		+89872G>C	+92263T>C	+92907C>T	+92956C>T	+93116C>A	+96908G>A	+101167C>G			+144676C>T	+172855T>C	+175275C>G	+179461C>G		
Hap.			Freq.	Hap.								Freq.	Hap.					Freq.
ht1	C	A	0.449	*ht1*	G	T	C	C	A	G	G	0.400	*ht1*	T	T	G	C	0.359
ht2	C	T	0.420	*ht2*	C	C	C	T	C	G	C	0.394	*ht2*	C	C	C	C	0.354
ht3	T	A	0.130	*ht3*	G	T	C	C	A	G	C	0.080	*ht3*	C	T	C	G	0.249
Others†	.	.	0.001	*Others*†	.	.	.	.	.	.	.	0.125	*Others*†	.	.	.	.	0.038

B LDs among *MYLK* polymorphisms

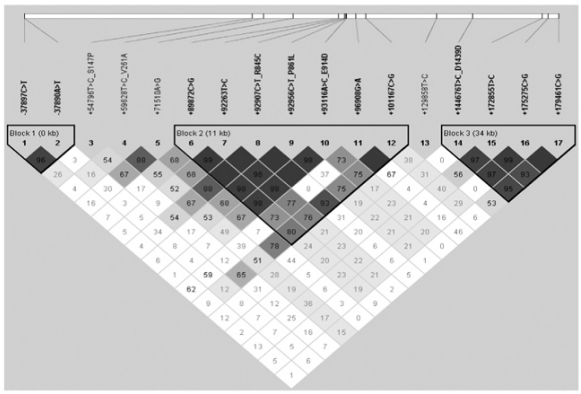

Fig. 1. Haplotypes and linkage disequilibrium of *MYLK* (myosin, light polypeptide kinase) on chromosome 3q21. (A) Haplotypes of *MYLK*. †Other contains rare haplotypes (frequencies < 0.05). (B) LDs among *MYLK* polymorphisms.

(SAS Institute, USA). The haplo.score (Schaid et al., 2002) approach tested for associations between statistically inferred haplotypes and asthma or related phenotypes. For correction for multiple testing, the effective number of independent markers in *MYLK* was calculated using the software SNPSpD (http://genepi.qimr.edu.au/general/daleN/SNPSpD), which is based on the spectral decomposition (SpD) of matrices of pairwise LD between SNPs (Nyholt, 2004). The number of independent marker loci in *MYLK* was calculated as 7.1514, and this was applied to correct for multiple testing (P-value X 7.1514).

RESULTS

Polymorphism discovery and linkage disequilibrium

Direct DNA sequencing of 24 unrelated Korean DNA samples identified 23 single nucleotide polymorphisms (SNPs), nine within coding regions of exons and 14 in introns of the MYLK gene. Among polymorphisms that were identified in this study and previous studies (Gao et al., 2007), 17 common polymorphisms were selected based on the allele frequency and LDs for genotyping in the larger asthma case-control study population. Polymorphisms with over 5% of minor allele frequencies in Korean and/or other Asian population as well as one among polymorphisms with tight LDs were primarily selected. Their minor allele frequencies (MAFs) are shown in Table 2 and supplementary Table 3. The comparison of genotype frequencies among different ethnic groups is listed in supplementary Table 3. The overall pattern among the Korean population was similar to that observed in other ethnic groups (Gao et al., 2007), e.g., 17 SNPs spanning *MYLK* could be grouped into three LD blocks (Fig. 1B). Common (freq. > 0.05) haplotypes of each LD block are shown in Fig. 1A. Only common haplotypes (freq. > 0.05) in each LD block were used in subsequent association analyses (Fig. 1A).

Disease association analysis

First, we attempted to replicate the genetic effects of *MYLK* polymorphisms reported in other studies. The significant associations with a lower risk of asthma observed in family studies among African populations (Flores et al., 2007; Gao et al., 2007) could not be replicated in the Korean population, although weak signals were detected at intron 2 and in one common (freq. > 0.4) haplotype in the first LD block *(rs41533145[-37890A>T]* and *BL1_ht2;* not significant after correction for multiple testing) (Table 2). Similarly, the significant associations with total IgE level observed in African populations (Gao et al., 2007) were not replicated in this study either (see supplementary Table 4). However, two polymorphisms in LD block 1 *(rs41533145[-37890A > T]* and *BL1_ht2)* revealed significant associations with both risk of asthma and log-transformed total IgE level among asthmatic patients in the Korean population, although the significances did not withstand the correction for multiple testing.

Next, as one of important phenotypes in asthma, we analyzed genetic effects on the blood eosinophil level among asthmatic patients. Among 17 *MYLK* polymorphisms, two SNPs in intron 16 (*+89872C > G* and *+92263T > C*), which were in tight LD (|D'| = 0.99, Fig. 1B), revealed significant association with log-transformed blood eosinophil level even after correction for multiple testing (P = 0.002/P^{corr} = 0.01 and P = 0.002/ P^{corr} = 0.01, respectively) (Table 3). The log-transformed blood eosinophil levels were higher in individuals bearing the "G" allele (one or two copies) in *+89872C > G* and the "C" allele (one or two copies) in *+92263T > C* than in those who do not possess these allele (Table 4). In addition, the genetic effect was analyzed in subgroup patients stratified by status of disease and atopy. Interestingly, the genetic effects of *+89872C > G* and *+92263T > C* on log-transformed blood eosinophil level were much more apparent among asthmatic patients and atopic asthma patients. Among atopic asthma patients, the log-transformed blood eosinophil levels were proportionally increased by gene-dose dependent manner of in both *+89872C > G* and *+92263T > C* (P = 0.0002 and P = 0.00007, respectively) (Table 4).

DISCUSSION

As noted above, several recent studies have reported that polymorphisms in *MYLK* were associated with a number of diseases and disease related phenotypes, including acute lung injury (Gao et al., 2006), sepsis (Gao et al., 2007), risk of asthma (Flores et al., 2007; Gao et al., 2007), and elevated IgE levels among asthmatics (Gao et al., 2007). In terms of asthma-related phenotypes, Gao et al. (2007) reported that *MYLK* SNPs were associated with the risk of asthma and total serum IgE concentrations in African Caribbean families. In their study, the promoter SNP (*rs936170*) and a haplotype including

Table 2. Logistic analysis of *MYLK* polymorphisms while controlling for age, sex, smoking status, and atopy status as covariates

Loci (From **A**TG: NM_053025.3)	rs#	Position	AA change	MAF		OR (95%CI)	*P*	P^{corr*}
				Asthmatics (n = 772)	NC (n = 243)			
-37897C > T	*rs17302008*	Intron2	-	0.124	0.142	0.81 (0.60-1.09)	0.16	1
-37890A > T	*rs41533145*	Intron2	-	0.431	0.379	1.29 (1.04-1.60)	**0.02**	0.16
BL1_ht1		-	-	0.443	0.479	0.86 (0.70-1.07)	0.17	1
BL1_ht2		-	-	0.432	0.379	1.29 (1.04-1.60)	**0.02**	0.17
BL1_ht3		-	-	0.124	0.142	0.81 (0.60-1.10)	0.17	1
+54796T > C	*rs9840993*	Exon7	S147P	0.050	0.044	1.19 (0.71-1.98)	0.52	1
+59628T > C	*rs3796164*	Exon10	V261A	0.425	0.462	0.87 (0.70-1.07)	0.18	1
+71510A > G	*rs820356*	Intron12	-	0.044	0.035	1.31 (0.75-2.29)	0.34	1
+89872C > G	*rs936170*	Intron16	-	0.456	0.477	0.91 (0.74-1.12)	0.38	1
+92263T > C	novel	Intron16	-	0.471	0.479	0.96 (0.78-1.18)	0.68	1
+92907C > T	*rs3732485*	Exon18	R845C	0.039	0.034	1.20 (0.67-2.14)	0.54	1
+92956C > T	*rs3732486*	Exon18	P861L	0.464	0.472	0.95 (0.77-1.17)	0.64	1
+93116A > C	*rs3732487*	Exon18	E914D	0.501	0.479	1.10 (0.88-1.38)	0.41	1
+96908G > A	*rs820336*	Intron18	-	0.033	0.025	1.26 (0.71-2.24)	0.42	1
+101167C > G	*rs2305631*	Intron19	-	0.463	0.473	0.98 (0.80-1.21)	0.86	1
BL2_ht1		-	-	0.395	0.420	0.93 (0.75-1.15)	0.49	1
BL2_ht2		-	-	0.385	0.418	0.86 (0.70-1.07)	0.17	1
BL2_ht3		-	-	0.088	0.062	1.41 (0.94-2.13)	0.10	0.70
+129858T > C	*rs820324*	Intron23	-	0.394	0.416	0.89 (0.72-1.11)	0.31	1
+144676T > C	*rs1254392*	Exon25	D1439D	0.370	0.366	1.05 (0.84-1.32)	0.67	1
+172855T > C	*rs3845915*	Intron31	-	0.360	0.362	0.99 (0.80-1.23)	0.95	1
+175275C > G	*rs860224 (ss65824112)*	Intron33	-	0.385	0.387	1.03 (0.82-1.30)	0.77	1
+179461C > G	novel	Intron33	-	0.258	0.249	1.00 (0.78-1.29)	0.99	1
BL3_ht1		-	-	0.361	0.366	1.02 (0.81-1.27)	0.90	1
BL3_ht2		-	-	0.349	0.357	0.97 (0.78-1.21)	0.78	1
BL3_ht3		-	-	0.250	0.246	0.97 (0.76-1.26)	0.84	1

Genotype distributions, odds ratio (OR) (95% confidential interval), and *P* values for logistic analyses of co-dominant models controlling for age, sex, smoking status, and atopy as covariates are shown. To achieve the optimal correction for multiple testing of single-nucleotide polymorphisms (SNPs) in linkage disequilibrium (LD) with each other, the effective number of independent marker loci (7.1514) in *MYLK* was calculated using the software SNPSpD (http://genepi.qimr.edu.au/general/daleN/SNPSpD/), on the basis of the spectral decomposition (SpD) of matrices of pair-wise LD between SNPs. (Nyholt, 2004)

P^{corr*}: Corrected *P* value

rs936170 corresponding to the actin-binding activity of the nonmuscle and smooth muscle forms were associated with the risk of asthma. In addition, other groups also reported one non-synonymous polymorphism in *MYLK* was related to the severe asthma phenotype in African Americans (Flores et al., 2007).

In this study, we identified additional polymorphisms in *MYLK* and found that log-transformed blood eosinophil levels were associated with two intronic SNP (intron 16). Furthermore, the genetic effects on log-transformed eosinophil level were much more apparent among asthmatic patients and atopic asthma patients, which suggest that the increased blood eosinophil levels among asthmatic patients might be alternatively controlled by polymorphisms in *MYLK*.

Introns are the noncoding DNA sections, located between coding regions (exons), and they are spliced out from mRNA before translation. Although intronic large portions of human genome comprises about 37% of the human genome, they were generally thought of as junk DNA regions with no function after their recognition (Gilbert, 1978). However, recent evidence has indicated that introns contain important gene regulatory sequences that have a variety of functional roles, such as alternative intronic promoters enhancers, noncoding RNAs, RNA editing, nested genes, and transacting elements. Sometimes, intronic mutations may even lead to diseases due to the modification of the mRNA splicing process (Amsellem et al., 2002; King et al., 2002). Therefore, further investigations would be needed to elucidate the association of those intronic SNPs from this study.

Table 3. Regression analyses of log-transformed blood Eosinophil level (%) with *MYLK* polymorphisms among Asthmatic patients adjusted by age, sex, smoking, and atopy status

Loci	C/C*	C/R*	R/R*	*P*	P^{corr*}	*Power*[a]
-37897C > T	580 (1.57 ± 0.75)	144 (1.68 ± 0.76)	22 (1.83 ± 0.62)	**0.01**	0.10	0.45
-37890A > T	244 (1.55 ± 0.77)	329 (1.65 ± 0.74)	143 (1.59 ± 0.76)	0.43	1	0.17
BL1_ht1	236 (1.65 ± 0.76)	320 (1.63 ± 0.74)	155 (1.48 ± 0.77)	**0.01**	0.10	0.6
BL1_ht2	242 (1.56 ± 0.77)	327 (1.65 ± 0.74)	142 (1.59 ± 0.76)	0.44	1	0.17
BL1_ht3	553 (1.58 ± 0.76)	136 (1.68 ± 0.77)	22 (1.83 ± 0.62)	**0.01**	0.11	0.43
+54796T > C	661 (1.60 ± 0.75)	70 (1.54 ± 0.76)	2 (1.93 ± 0.76)	0.84	1	0.06
+59628T > C	249 (1.58 ± 0.77)	345 (1.62 ± 0.75)	138 (1.57 ± 0.72)	0.88	1	0.07
+71510A > G	687 (1.62 ± 0.75)	61 (1.43 ± 0.77)	2 (1.24 ± 0.20)	0.06	0.42	0.21
+89872C > G	217 (1.45 ± 0.76)	359 (1.65 ± 0.75)	152 (1.68 ± 0.73)	**0.002**	**0.01**	0.39
+92263T > C	210 (1.44 ± 0.75)	367 (1.67 ± 0.74)	166 (1.67 ± 0.75)	**0.002**	**0.01**	0.46
+92907C > T	676 (1.61 ± 0.76)	56 (1.46 ± 0.72)	1 (1.39)	0.14	0.98	0.27
+92956C > T	213 (1.47 ± 0.76)	362 (1.66 ± 0.74)	159 (1.64 ± 0.76)	**0.02**	0.14	0.29
+93116A > C	162 (1.65 ± 0.75)	369 (1.65 ± 0.74)	166 (1.45 ± 0.75)	**0.009**	0.07	0.36
+96908G > A	703 (1.59 ± 0.76)	36 (1.66 ± 0.65)	7 (1.83 ± 0.62)	0.29	1	0.05
+101167C > G	217 (1.65 ± 0.75)	355 (1.61 ± 0.76)	162 (1.51 ± 0.73)	0.06	0.42	0.12
BL2_ht1	276 (1.62 ± 0.75)	348 (1.64 ± 0.75)	119 (1.44 ± 0.74)	0.06	0.43	0.12
BL2_ht2	287 (1.51 ± 0.73)	343 (1.64 ± 0.76)	113 (1.69 ± 0.76)	**0.01**	0.09	0.37
BL2_ht3	618 (1.61 ± 0.75)	118 (1.53 ± 0.79)	7 (1.96 ± 0.58)	0.80	1	0.07
+129858T > C	269 (1.59 ± 0.79)	351 (1.61 ± 0.74)	114 (1.61 ± 0.74)	0.90	1	0.05
+144676T > C	285 (1.63 ± 0.79)	355 (1.60 ± 0.74)	95 (1.49 ± 0.70)	0.13	0.92	0.27
+172855T > C	300 (1.58 ± 0.71)	343 (1.58 ± 0.79)	94 (1.71 ± 0.75)	0.29	1	0.31
+175275C > G	265 (1.64 ± 0.80)	372 (1.59 ± 0.74)	97 (1.53 ± 0.68)	0.19	1	0.38
+179461C > G	403 (1.61 ± 0.73)	303 (1.60 ± 0.79)	41 (1.58 ± 0.75)	0.91	1	0.05
BL3_ht1	295 (1.62 ± 0.79)	355 (1.60 ± 0.74)	90 (1.52 ± 0.69)	0.25	1	0.28
BL3_ht2	314 (1.57 ± 0.72)	335 (1.59 ± 0.78)	91 (1.71 ± 0.75)	0.22	1	0.38
BL3_ht3	406 (1.60 ± 0.74)	299 (1.59 ± 0.78)	35 (1.61 ± 0.75)	0.92	1	0.05

Genotype distributions, means, standard deviation (SD) of log-transformed blood eosinophil percentage, and *P* values for regression analysis of co-dominant models were adjusted for age, sex, smoking, and atopy status.

*C/C, C/R and R/R represent homozygotes for the common allele, and heterozygotes and homozygotes for the minor allele, respectively.

[a]Statistical powers calculated based on partial correlation between the tested predictors and the response and total number.

P^{corr*}: Corrected *P* value

Eosinophil migration in the tissue is one characteristic feature of allergic diseases. The blood level of eosinophils is clearly associated with the pathogenesis of allergies, specifically allergic asthma (Bochner and Busse, 2005). In response to the diverse stimuli, eosinophils are recruited from the circulation into inflammatory foci where they modulate immune responses through an array of mechanisms (Weller, 1994). They can accumulate either for repairing or for destroying the bronchial epithelium. In addition, eosinophils are multifunctional leukocytes involved in the defense system of the mucosal surface (Gonlugur and Gonlugur, 2006). There have been several studies that show genetic polymorphisms to be associated with blood eosinophil levels in asthmatics (Chae et al., 2004; Cheong et al., 2006; Karjalainen et al., 2003; Lee et al., 2007; Namkung et al., 2007). At least two different linkage studies have implicated chromosome 3q21 (where *MYLK* resides) as containing a risk factor for asthma and atopy (Haagerup et al., 2002; Kurz et al., 2005).

The significant associations with risk of asthma and total IgE levels observed in family-based (Gao et al., 2007) and population-based studies (Flores et al., 2007) among African descendant populations were not replicated (single SNPs and haplotypes) in the Korean population. Genetic association studies provide a potentially powerful tool for identifying genetic variations that influence susceptibility to common diseases. However, there are many cases of associations that are not replicated afterward, which has led to skepticism about genetic epidemiology studies of complex diseases (Cardon and Bell, 2001; Lazar and Garcia, 1999; Lohmueller et al., 2003). At the present time, the discrepancy among studies cannot be explained clearly. However, it is well-known that ethnicity is one of the most important factors for evaluating genetic effects on common complex traits. In addition to the different ethnicities in the various studies, other plausible explanations might include differences in sample size and type of study (fam- ily-based versus population-based).

Table 4. Regression analyses of log-transformed blood Eosinophil level (percentage) with *MYLK* intronic polymorphism (*+92263T > C*) in subgroup patients stratified by status of Asthma and Atopy

Loci	Subgroup		C/C*	C/R*	R/R*	*Pa*	*Pb*	*Pc*
+ 89872C > G	All		282 (1.38 ± 0.72)	453 (1.53 ± 0.75)	204 (1.53 ± 0.73)	**0.01**	**0.002**	0.39
	BA	ALL	217 (1.45 ± 0.76)	359 (1.65 ± 0.75)	152 (1.68 ± 0.73)	**0.002**	**0.0003**	0.15
		Atopy	123 (1.44 ± 0.79)	202 (1.68 ± 0.73)	87 (1.83 ± 0.69)	**0.0002**	**0.0007**	**0.009**
		Non-Atopy	94 (1.46 ± 0.71)	157 (1.62 ± 0.77)	65 (1.47 ± 0.75)	0.45	0.08	0.52
	NC	ALL	65 (1.15 ± 0.54)	94 (1.06 ± 0.50)	52 (1.08 ± 0.50)	0.47	0.42	0.70
		Atopy	26 (1.11 ± 0.60)	28 (1.18 ± 0.58)	15 (1.26 ± 0.55)	0.40	0.21	0.94
		Non-Atopy	39 (1.18 ± 0.50)	66 (1.01 ± 0.46)	37 (1.01 ± 0.47)	0.15	0.10	0.47
+92263T > C	All		273 (1.38 ± 0.71)	462 (1.54 ± 0.74)	218 (1.53 ± 0.74)	**0.01**	**0.002**	0.36
	BA	ALL	210 (1.44 ± 0.75)	367 (1.67 ± 0.74)	166 (1.67 ± 0.75)	**0.002**	**0.0001**	0.21
		Atopy	120 (1.43 ± 0.76)	209 (1.69 ± 0.74)	91 (1.85 ± 0.67)	**0.00007**	**0.0002**	**0.005**
		Non-Atopy	90 (1.46 ± 0.72)	158 (1.64 ± 0.74)	75 (1.45 ± 0.79)	0.60	0.06	0.29
	NC	ALL	63 (1.17 ± 0.53)	95 (1.03 ± 0.50)	52 (1.09 ± 0.50)	0.32	0.20	0.73
		Atopy	24 (1.15 ± 0.58)	30 (1.11 ± 0.60)	15 (1.27 ± 0.55)	0.70	0.53	0.98
		Non-Atopy	39 (1.18 ± 0.50)	65 (1.00 ± 0.45)	37 (1.01 ± 0.47)	0.16	0.08	0.57

BA: Bronchial asthmatics, NC: Normal controls

*C/C, C/R and R/R represent homozygotes for the common allele, and heterozygotes and homozygotes for the minor allele, respectively. Genotype distributions, means, standard deviation (SD) of blood eosinophil percentage, and *P* values for regression analyses of three alternative models (co-dominant [*Pa*], dominant [*Pb*], recessive models [*Pc*]) adjusted for age, sex, smoking, and atopy status were shown.

In summary, the genetic effects of MYLK polymorphisms reported in African populations were not replicated in a Korean population. However, statistically significant associations with log-transformed blood eosinophil levels among asthmatic patients were observed. Further biological and/or functional studies are needed to confirm our results.

Note: Supplementary information is available on the Molecules and Cells website (www.molcells.org).

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