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ORIGINAL ARTICLE

Polymorphisms of *EHF-ELF5* genomic region and its association with pediatric asthma in the Taiwanese population

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Background: The *EHF* and *ELF5* genes, located on chromosome 11p and linked to asthma phenotypes, are high-potential candidate genes conferring asthma susceptibility. The purpose of this study was to investigate the genetic association among single nucleotide polymorphisms (SNPs) of *EHF* and *ELF5*, and their relationship with asthma in the Taiwanese population.
Methods: We selected and performed genotyping on 16 SNPs that encompass the genomic region of *EHF* and *ELF5* in Taiwanese children with or without asthma. A total of 1983 children, 523 in the test group and 619 and 842 in two validation groups, were recruited for this study.
Results: The SNP rs3910901, located in the 5′ upstream region of *ELF5*, was found to have a weak association ($p = 0.043$) with asthma in the odds ratio analysis. The genotype distribution was similar in all comparison groups, but the CC genotype was more frequent in asthma patients. Logistic regression adjusted allergy comorbidity showed obviously diluted association.
Conclusion: The results indicated that SNP rs3910901 may have a minor impact on pediatric asthma in the Taiwanese population.

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Introduction

Chronic asthma is a complex disease that affects approximately 300 million individuals worldwide.[1] The rising incidence of asthma and atopic disorders over recent decades attests to the importance of environmental and lifestyle factors in risk assessment.[2–4] Strong genetic components associated with asthma were supported by family and twin studies.[5,6] Many genes have been identified or are suspected of being involved the pathogenesis of asthma.[7] In Taiwan, there are some slight differences in the reported symptoms of allergic diseases; nevertheless, the prevalence of allergic diseases is rising.[8–10]

E74-like factor 5 (*ELF5*; also known as *ESE-2*) and ETS homologous factor (*EHF*; also known as *ESE-3*) are members of the epithelium-specific ETS transcription factor subfamily.[11–14] *ELF5* and *EHF* are thought to involve the induction or repression of epithelium-specific genes in the context of an inflammatory microenvironment and oncogenes of epithelium-derived tumors in tubulogenesis, as well as a branch of morphogenesis glandular organs, such as the lung.[11–14] EHF and another member of the ETS family transcription factors, ESE-1, are upregulated by the inflammatory cytokines interleukin-1® and tumor necrosis factor-α in bronchial epithelial cell lines. The results indicated that EHF should play an important role in airway inflammation.[15]

ELF5 and *EHF*, located on chromosome 11p12–15, were identified as candidate genes for asthma in a genome-wide linkage analysis performed in a population from Tristan de Cunha.[16–18] The *ELF5* polymorphism was found to be associated with a weak effect on adults (aged 18–45 years) with asthma in the Caucasian population.[19] Our previous study found that the microsatellite marker in chromosome 11p13, near *ELF5* and *EHF*, was associated with the immunoglobulin E (IgE) level in asthmatic children.[20] The limitations of the previous genetic study on the relation of *EHF* and *ELF5* with asthma were the restriction of sample size, fewer single nucleotide polymorphism (SNP) targets, and lack of adjustment by allergy comorbidity.

In the present study, we investigated the association between selected SNPs of the *EHF-ELF5* genomic region and asthma. The results were validated in independently recruited participants.

Materials and Methods

Sample composition and clinical evaluation

Our study population consisted of asthmatic children aged 5–12 years. The study protocol was approved by the Ethical and Clinical Trial Committee of National Cheng-Kung University Hospital and Mackay Memorial Hospital, Taiwan. An informed consent form was required for all participants or their guardians after answering a modified British Medical Society respiratory questionnaire, which is identical to the European Community Respiratory Health Survey (ERCHS). These surveys showed similar results as those of International Study of Asthma and Allergies in Childhood (ISAAC) and ERCHS, pertinent to the diagnosis and assessment of asthma.[21,22] Pulmonary function was evaluated using standard methods including spirometry before and after the administration of two puffs of inhaled salbutamol (200 μg/puff). The definition of asthma must meet the following criteria: (1) a history of wheezing and experiencing shortness of breath during or without concurrent respiratory infections; (2) chronic coughing for >1 month, as well as the presence of wheezing, as observed by a physician; and (3) a bronchodilator test confirms a 15% increase in FEV1 (forced expiratory volume in 1 second). Nonasthma controls were defined as neither having asthma history as in criterion (1) above nor being diagnosed as in criterion (2). Other evaluations included skin prick tests for responsiveness to six common aeroallergens, a differential blood count (including total eosinophil count), levels of total serum IgE, as well as IgE specific to house dust and mixed pollens using the Unicap system (Pharmacia Diagnostics, Uppsala, Sweden). A positive skin test was defined as the presence of $\geq$ 1 reaction with a wheal diameter $\geq$ 5 mm. Total serum IgE was measured using solid-phase immunoassay (Pharmacia IgE EIA; Pharmacia Diagnostics). Nonallergy patients were defined as having a total serum IgE < 200 IU/mL and a negative skin test. All participants were Han Taiwanese and living in Taiwan.

DNA preparation

Genomic DNA was extracted from the blood samples of the study participants using a QIAamp DNA Blood kit (Qiagen, Valencia, CA, USA). The extracted genomic DNA was analyzed by agarose gel electrophoresis, quantified by spectrophotometer, and stored at −80°C until use.

SNP selection and genotyping

The *EHF-ELF5* genomic region encompasses two genes spanning 183.5 kb. We selected 16 SNPs within and near the *EHF* and *ELF5* genes to determine the genotyping association results. All SNP genotyping tests were performed using the Taqman SNP genotyping assays (Applied Biosystems, Foster City, CA, USA). The primers and probes of the selected SNPs were from the Applied Biosystems Assay on Demand kit. The probe fluorescence signal was detected using the ABI Prism 7900 Real-Time PCR System (Applied Biosystems).

Statistical analysis

The quality of the genotype data was evaluated using Hardy–Weinberg equilibrium (HWE) proportion tests. Intermarker linkage disequilibrium measures, r^2 and D', were estimated and haplotype blocks were defined using the Haploview program.[23] All of the single-point association analyses were carried out using the SAS/genetics package (SAS, Cary, NC, USA). SNPs showed significant association ($p \leq 0.05$) in the tests. The odds ratio (OR) was evaluated using logistic regression to demonstrate which genotype was adjusted to allergy.

Results

Characteristics of the study participants

Five hundred and twenty-three DNA samples extracted from 239 nonasthmatic children and 284 asthmatic children

were collected as Group 1 and genotyping was performed on all selected SNPs. There were 619 participants (including 258 asthmatic children and 361 controls) in Group 2 that were recruited from the same hospital as Group 1 at a different time. Group 3 included 670 asthmatic children and 172 controls that were recruited from two different hospitals. There were significant differences in the distribution of sex and allergy comorbidity between participants with and without asthma in the three groups (Table 1).

rs3910901 SNP in the genomic region between EHF and ELF5 associated with asthma

Sixteen SNPs randomly selected from the *EHF* and *ELF5* genomic region were genotyped for every participant. The genotype distributions of the SNPs did not deviate from the HWE in either asthmatic or nonasthmatic participants. Linkage disequilibrium values of the polymorphic SNPs in the *EHF* and *ELF5* genes are listed in Fig. 1. Two and one haplo-blocks were identified in the *ELF5* and *EHF* genes, respectively.

The strengths of associations and genotype frequencies of all selected SNPs with asthma are summarized in Table 2. None of the SNPs appeared to be significantly associated with asthma. The rs3901901SNP located upstream of the *ELF5* gene showed weak significance in the OR analysis ($p = 0.043$). There was not a single SNP displaying a statistically significant difference after Bonferroni correction.

Determination and validation of the results were described above; genotyping of the associated SNPs for Group 2 and Group 3 was performed. The genotype distributions of rs3901901 in Group 2 and Group 3 did not deviate from the HWE either in asthmatic or nonasthmatic participants. The rs3901901 SNP displayed marginal significance in both groups (genotypic and OR analysis), and was statistically significant when the total number of participants was considered (Table 3). The OR analysis showed that the CC genotype of the rs3901901SNP was associated with the risk of asthma, and this association was consistent among the three groups and the total number of participants (Table 3). Although the results were not significant or strongly associated with asthma, the trend in the genotype distribution was similar across all comparison groups. Genotype CC was found more frequently in asthma patients in all comparison groups. The effects of rs3901901 on asthma were further adjusted to the influence of sex and allergy comorbidity by logistic regression. As shown in Table 3, the strength of associations was not observed to be diluted by sex, but was obviously affected by allergy comorbidity.

The OR analysis of rs3901901 and allergy were also performed for the total number of participants. The CC genotype appeared to be significantly associated with the risk of allergy with an OR of 1.72, 95% confidence interval of 1.06—2.80, and a p value $= 0.026$. The significance was dramatically diluted by adjustment with asthma ($p = 0.347$) using logistic regression.

Discussion

In the current study, we investigated the association between the *EHF* and *ELF5* polymorphisms and pediatric asthma. We found that association between the asthma phenotype and *ELF5* polymorphism rs3901901 was marginally significant in Group 1. The association between asthma phenotype and rs3901901 was further validated in Group 2 and Group 3. The results of this study indicated that polymorphisms of the *ELF5* gene confer a risk for allergy but with minor impact on pediatric asthma in the Taiwanese population. The main limitation of this study was the low number of SNPs in the investigated genomic region that were selected for genotyping. The negative association between the candidate genes regions (or haplotype) and asthma may be due to the low density of SNP markers that were genotyped.

The rs3901901SNP is located at position −18 kb from the starting site of the *ELF5* mRNA. Several SNPs in the 5′ upstream region of *ELF5* are in linkage disequilibrium ($r^2 \geq 0.8$) with rs3901901 (according to the Seattle SNPs website http://pga.mbt.washington.edu/education.html; Han-Chinese Beijing population). The observation

Table 1 Sex and allergy information of the study participants

	Group 1		Group 2		Group 3	
	Case [a]	Control [b]	Case	Control	Case	Control
Sex						
Male	196	105	167	176	466	87
Female	88	134	91	185	204	85
p value	<0.0001		0.0001		<0.0001	
OR (95% CI)	2.84 (1.99—4.07)		1.93 (1.39—2.68)		2.23 (1.59—3.14)	
Allergy						
Yes	203	80	215	187	663	80
No	81	159	43	174	7	92
p value	<0.0001		<0.0001		<0.0001	
OR (95% CI)	4.98 (3.43, 7.23)		4.65 (3.16, 6.85)		108.92 (48.81, 243.07)	

[a] Asthma patients.
[b] Nonasthma participants.
Allergy defined as total IgE > 250 IU/mL and positive skin test to six common allergens.
CI = confidence interval; OR = odds ratio.

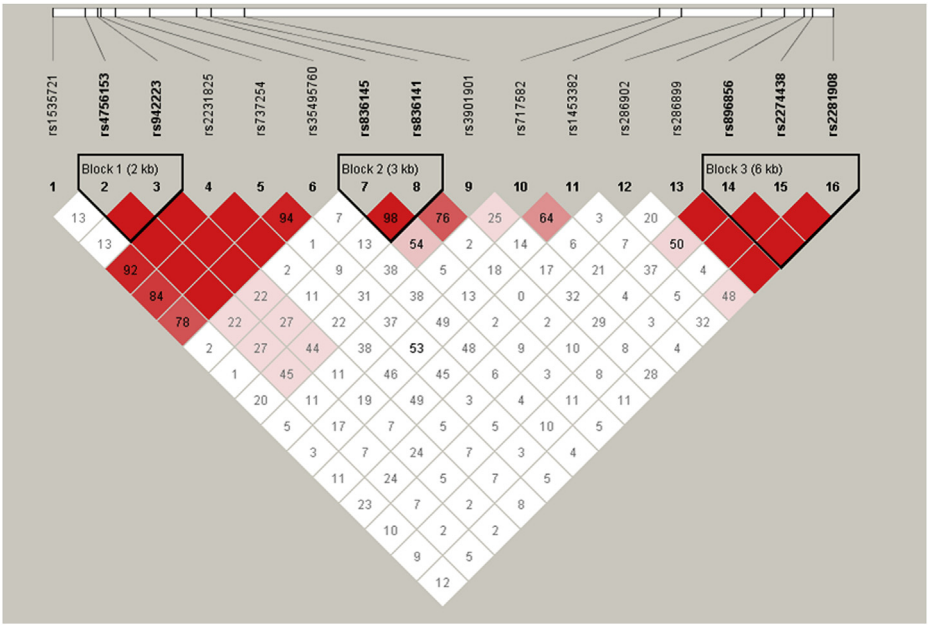

Figure 1. Linkage disequilibrium plot in D′ demonstrating adjacent strength between single nucleotide polymorphism pairs at the *EHF* and *ELF5* genes. D′ values are multiplied by 100, for example, 76 in the square at the bottom means a D′ of 0.76. A square without a number indicates a value of 100 that equals a D′ of 1.

suggested that the *ELF5* promoter or its 5′ upstream region may contain polymorphic loci conferring asthma susceptibility in our study population.

In Groups 1—3, the rs3901901 SNP only showed marginally significant association with asthma. The minimal sample size for the detection of a significant association was calculated using Power for Association With Errors (PAWE; http://linkage.rockefeller.edu/pawe/).[24,25] Under the same ratio of condition and allele frequencies of rs3901901 in participants of the case and control groups from the total number of participants, and assuming that the genotyping data are without error, 1398 cases and 889 controls are necessary to obtain enough statistical power ($p = 0.05$, power = 0.8). The calculation suggested that our sample size in Group 1—3 may not have been large enough to detect a significant association between rs3901901 and asthma. After pooling all study groups, the sample size consisted of 1212 cases and 771 controls; however, the

Table 2 χ^2 test for *ELF5-EHF* SNP genotyping

SNP ID	Location	Allele	Case	Control	*p*
		1/2	11/12/22	11/12/22	
ELF5					
rs1535721	3′ near gene	A/G	76/150/57	70/112/57	0.356
rs4756153	Intron 4	A/G	55/133/94	49/104/86	0.703
rs942223	Intron 4	C/T	93/133/54	86/102/49	0.596
rs2231825	Intron 4	A/C	0/22/261	0/19/220	0.941
rs737254	Intron 3	G/T	0/22/261	0/21/218	0.675
rs35295760	Intron 2	A/G	0/26/257	0/19/220	0.616
rs836145	Intron 1	A/C	50/131/100	46/115/75	0.648
rs836141	5′ near gene	A/G	112/119/50	87/112/38	0.532
rs3901901	Intergenic	A/G	21/91/171	8/84/147	0.120
EHF					
rs717582	Intron 1	C/T	79/150/54	58/118/61	0.181
rs1453382	Intron 1	A/T	17/99/167	13/102/124	0.197
rs286902	Intron 2	C/T	35/120/128	24/118/97	0.266
rs286899	Intron 4	A/G	3/41/239	2/32/204	0.910
rs896856	Intron 6	G/T	222/56/5	173/61/5	0.272
rs2274438	Intron 7	A/G	58/141/83	41/114/83	0.355
rs2281908	3′ near gene	A/G	5/53/222	5/62/172	0.147

1: allele 1; 2: allele 2; 11: homozygous of allele 1; 12: heterozygous; 22: homozygous of allele 2.
SNP = single nucleotide polymorphism.

Table 3 Genotyping frequencies, proportion, and odds ratio analyses of SNP rs3910901 in three independent groups

Genotype	Group 1		Group 2		Group 3		Total	
	Case [a]	Control [b]	Case	Control	Case	Control	Case	Control
CC	21 (7%)	8 (3%)	20 (8%)	15 (4%)	45 (7%)	5 (3%)	87 (7%)	28 (4%)
CG	91 (32%)	84 (35%)	86 (33%)	124 (34%)	227 (34%)	50 (29%)	404 (33%)	257 (33%)
GG	171 (60%)	147 (62%)	152 (59%)	222 (62%)	398 (59%)	117 (68%)	721 (60%)	486 (63%)
χ^2	4.2402		3.6543		5.9703		11.1944	
p value	0.120		0.161		0.051		0.004	
CC	21	8	20	15	45	5	87	28
CG+GG (ref)	262	231	238	346	625	167	1125	743
OR (95% CI)	2.31 (1.01, 5.33)		1.94 (0.97, 3.86)		2.40 (0.94, 6.15)		2.05 (1.33, 3.17)	
p value	0.043		0.056		0.059		0.001	
OR (95% CI) adjust with sex[c]	2.51 (1.01, 5.92)		1.87 (0.93, 3.76)		2.49 (0.97, 6.42)		2.07 (1.33, 3.23)	
p value	0.035		0.079		0.059		0.001	
OR (95% CI) adjust with allergy[c]	1.97 (0.81, 4.77)		1.52 (0.72, 3.18)		3.22 (0.85, 12.13)		1.76 (1.08, 2.87)	
p value	0.135		0.271		0.084		0.024	
OR (95% CI) adjust with sex and allergy [c]	2.21 (0.86, 5.58)		1.50 (0.72, 3.15)		3.18 (0.87, 11.67)		1.79 (1.09, 2.92)	
p value	0.094		0.282		0.081		0.021	

[a] Asthma patients.
[b] Nonasthma participants.
[c] SNP was coded as a categorical variable, 1 for CC, and 0 for others.
CI = confidence interval; OR = odds ratio; ref = reference genotypes.

power was still insufficient to detect an association between rs3901901 and asthma. Furthermore, the allele frequencies of rs3901901 are different between the Caucasian and Asian (Han Chinese) populations. The allele frequencies of A (risk allele in this study) are 0.513 and 0.198 in the Caucasian and Han Chinese Beijing populations, respectively. The risk allele is less frequent in our study population and thus a bigger sample size may be required to identify an association between asthma and the *EHF* and *ELF5* genomic region in the Asian population. The risk genotype AA, a minor genotype of rs3901901, was also was consistently less frequent in the case group compared to the controls (Table 3). The observation also suggested that polymorphisms of the *ELF5* gene may confer risk but with minor impact on pediatric asthma in our study population.

In a mouse model, the level of *Elf5* regulated the specification and differentiation of epithelial cells in the lung.[26] *Elf5* is dynamically expressed during lung development and regulated by fibroblast growth factor through the phosphatidylinositol 3-kinase/Akt pathway.[27] Elf5 regulates the expression of key mediators of the PrlR/Jak2/Stat5 signaling pathway and plays an important role in mammary gland development.[28] ELF5 is expressed in some organs such as the lung, stomach, kidney, prostate, bladder and mammary gland and is suggested to play roles in mammary, lung, prostate and/or kidney functions and possibly also in tumorigenesis.[29] In breast cancer, ELF5 provides a key transcriptional determinant of molecular subtype by suppression of estrogen sensitivity in luminal breast cancer cells and promotion of basal characteristics in basal breast cancer cells; an action that may be utilized to acquire antiestrogen resistance.[30] The loss-of-function mutations or polymorphisms of *ELF5* are unlikely to be associated with chronic diseases such as asthma. It is suggested that the regulatory polymorphisms of *ELF5* play a role in the pathophysiological processes of asthma.

The results of this study suggest that *ELF5* is a candidate gene that confers genetic susceptibility for pediatric asthma in the Taiwanese population. Furthermore, the results demonstrated a genetic basis in the pathogenesis of asthma. Further investigations are required to understand the functions and mechanisms of the associated SNPs in the regulation of *ELF5* expression.

Conflicts of interest

The authors declare that they have no financial or non-financial conflicts related to the subject matter or materials discussed in the manuscript.

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