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Original Article

Association of single nucleotide polymorphisms of *MD-1* gene with pediatric and adult asthma in the Taiwanese population

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Background and Purpose: *MD-1* (myeloid differentiation 1; also known as Ly86, lymphocyte antigen 86), interacting with radioprotective 105, plays an important role in the Toll-like receptor 4 signaling pathway. It has been suggested that *MD-1* is involved in the development of inflammation and atopic diseases. The purpose of this study was to investigate the genetic association of single nucleotide polymorphisms (SNPs) of *MD-1* and asthma in the Taiwanese population.

Methods: Genotyping 34 SNPs in the *MD-1* gene region was performed in a case-control study involving 281 children with asthma and 237 controls. A further 309 children and adults were genotyped for the SNP rs7740529 only, for validation of an identified association.

Results: In intron 1, we identified an SNP, rs7740529, which is associated with asthma in Taiwanese children (p=0.0358). Inclusion of a further 309 subjects increased the significance of the association (p=0.0093), and also demonstrated that rs7740529 is associated with adult asthma. The TT genotype confers risk of both pediatric and adult asthma.

Conclusion: These results suggest that *MD-1* could be a susceptible gene for asthma in the Taiwanese population.

Key words: Adult; Antigens, *Dermatophagoides*; Asthma; Child; Polymorphism, single nucleotide

Introduction

Chronic asthma is a complex disease affecting nearly 300 million individuals worldwide [1]. The rising incidence of asthma and atopic disorders over the past few decades attests to the importance of environmental and lifestyle factors in disease risk assessment [2-4]. Strong genetic components associated with asthma are supported by family and twin studies [5,6], and many genes have been identified or are suspected to be involved in the pathogenesis of asthma [7].

In Taiwan, there are slight differences in the reported symptoms of allergic diseases, but the prevalence of allergic diseases is rising [8-10]. More than 90% of asthmatic children have allergic asthma. These patients are characterized by elevated levels of total serum immunoglobulin E (IgE) and allergen-specific IgE antibodies against a wide range of allergens [11]. In Taiwan, asthmatic children had 75% and 74% prevalence rates of sensitization to *Dermatophagoides pteronyssinus* (Der p) and *Dermatophagoides farinae* (Der f), respectively, as reported by the Mackay Memorial Hospital in Taipei [12], and 82% and 82.5%, respectively, as reported by the National Cheng-Kung University in Tainan [13].

Recently, we reported that the promoter polymorphism of *MD-1* (myeloid differentiation 1; also known as Ly86, lymphocyte antigen 86) is associated with mite-sensitive allergy [14]. Although we did not find an association between *MD-1* polymorphism and asthma in the previous study [14], the association

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was found in this study when a larger sample size in intron 1 polymorphism was applied. Furthermore, the polymorphism was also found to be associated with adult asthma. This study investigated the genetic association of single nucleotide polymorphisms (SNPs) of *MD-1* and asthma in a Taiwanese population.

Methods

Subjects

The study subjects comprised asthmatic patients and non-asthmatic controls, including children aged 3 to 12 years and adults older than 30 years. The study protocol was approved by the Ethical and Clinical Trial Committee of the National Cheng-Kung University Hospital, Tainan, and Taoyuan General Hospital, Taoyuan, Taiwan. All participants and their guardians, if applicable, were informed about the study protocol and signed consent forms. They answered a modified British Medical Society respiratory questionnaire, which is the same as the European Community Respiratory Health Survey [15,16]. These surveys have similar validity as the International Study of Asthma and Allergies in Childhood (ISAAC) for the diagnosis and assessment of asthma [15,16]. Pulmonary function was tested using standard methods, including spirometry, before and after the administration of 2 puffs of inhaled salbutamol (200 μg/puff). The definition of asthma was as follows:

- history of wheezing and shortness of breath during or without concurrent respiratory infections;
- chronic cough for more than 1 month and diagnosis of wheezing episode(s); and
- bronchodilator test showing a positive response of 15% increase in forced expiratory volume in 1 second.

Allergy evaluations included skin prick tests for responsiveness to 6 common aeroallergens of Der p, Der f, house dust, cockroach, *Alternaria*, and egg yolk. Other evaluations included a differential blood count (including total eosinophil count), measurement of total serum IgE, and 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 a reaction with a wheal diameter of 5 mm. Total serum IgE was measured with solid-phase immunoassay (Pharmacia IgE EIA; Pharmacia Diagnostics).

DNA preparation

Genomic DNAs were obtained from blood samples of 824 unrelated Taiwanese children and 334 adults using QIAamp DNA blood kit (QIAGEN, Valencia, CA, USA) according to the manufacturer's instructions. The isolated genomic DNAs were checked by agarose gel electrophoresis to ensure quality. The DNA quantity was determined using spectrophotometry. The processed DNA samples were stored at –80°C until use.

Single nucleotide polymorphism genotyping

DNA fragments in the exon regions of the *MD-1* gene were amplified using an ABI 9700 (ABI; Applied Biosystems Inc., Foster City, CA, USA) polymerase chain reaction (PCR) instrument employing 2 pairs of forward and reverse primers. The sequences and related information of the primers are described in our previous work [14]. The fragments of PCR products were sequenced by an ABI 3700 automatic sequencer, according to the manufacturer's protocol. The sequence data were analyzed by PolyPhred software (University of Washington, Seattle, WA, USA) to identify the potential candidate SNPs. The identified potential SNPs were manually checked to ensure the presence of a true SNP and the allele of each individual. Three independent manual confirmations were performed for all sequence data and only those confirmed were subjected to statistical analysis.

SNP genotyping in other polymorphism sites was performed by use of the TaqMan® SNP genotyping assays (Table 1). Reactions were performed according to the manufacturer's protocol. Detection of probe fluorescence signal was conducted using the ABI Prism 7900 Real-Time PCR System.

Genotyping with all relevant *MD-1* SNPs was conducted on only 518 children. The other 309 children and adults were only genotyped to the SNP rs7740529 only.

Statistical analysis

The primary dichotomous outcome variables of association analyses were either control or case status for asthma. The principal explanatory variables were the genotyped polymorphisms. All statistical analyses were performed using SAS software (SAS Institute Inc., Cary, NC, USA).

Results

Pediatric asthma

Thirty four SNPs were identified and used to analyze the region of *MD-1* gene in the children. The locations

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Table 1. Genotype frequencies from *MD-1* single nucleotide polymorphism (SNP) testing according to asthma status in children

SNP ID	Position	Allele 1/2	Asthma 11/12/22	Non-asthma 11/12/22	p^b
rs1334710[a]	6,523,720	A/G	260/21/0	217/19/1	0.5358
rs9328372	6,526,604	A/G	132/115/31	106/101/22	0.7613
rs4959389	6,526,834	C/T	0/20/259	1/19/217	0.5164
rs2876016	6,526,960	A/G	4/60/215	4/49/184	0.9509
rs481924	6,527,057	C/T	48/135/96	40/117/80	0.9757
rs1334711	6,527,193	C/T	130/128/20	107/108/20	0.8500
rs2233116	6,528,493	G/T	1/51/210	4/35/191	0.1644
rs977785	6,528,880	A/C	22/104/124	19/101/107	0.8161
rs2233120	6,529,164	C/T	257/12/0	219/12/0	0.7019
rs3765285	6,529,276	C/G	127/115/32	106/99/22	0.7648
rs3804465[a]	6,531,303	G/T	96/136/46	82/117/38	0.9871
rs2294459[a]	6,537,325	C/G	2/50/229	1/56/180	0.2434
rs3789765[a]	6,539,587	A/G	10/52/219	3/46/188	0.2490
rs7740529[a]	6,550,608	C/T	148/98/33	122/100/14	0.0358
rs909790[a]	6,552,196	A/C	256/22/1	203/32/1	0.1101
rs932365[a]	6,556,779	A/G	195/62/19	152/62/22	0.2955
rs763405[a]	6,559,697	G/T	199/72/8	174/57/5	0.7689
rs2233124	6,565,417	A/G	192/80/7	169/62/4	0.6688
rs3804476[a]	6,565,703	A/G	156/101/20	123/85/26	0.2921
rs11961398[a]	6,571,673	A/C	0/18/259	0/22/212	0.2234
rs3804483[a]	6,577,676	C/T	127/124/30	96/107/34	0.3520
rs3804485[a]	6,577,860	A/C	35/130/116	38/107/92	0.4973
rs5743650	6,589,690	G/T	275/2/0	228/6/0	0.0947
rs4960227	6,589,795	A/G	35/109/134	35/88/112	0.7386
rs1005384[a]	6,593,344	A/G	12/76/188	10/68/157	0.9404
rs3765282	6,594,516	A/C	27/99/148	24/72/121	0.7634
rs5743654	6,594,639	G/T	259/12/0	209/6/0	0.3425
rs2233128	6,594,898	A/G	0/16/256	1/4/219	0.0388
rs760894[a]	6,596,197	A/G	73/128/73	71/93/71	0.2690
rs201006[a]	6,599,373	C/T	152/107/22	130/84/22	0.7507
rs201007[a]	6,600,500	A/G	16/100/161	20/83/133	0.7697
rs201008[a]	6,600,534	A/G	163/100/18	134/83/19	0.7691
rs201119[a]	6,600,880	A/G	158/102/18	125/89/20	0.5872
rs201010[a]	6,602,285	C/T	238/41/2	196/38/2	0.8765

[a]Genotyping by TaqMan.

[b]Chi-squared test.

of these SNPs and the analysis results are shown in Table 1. Two SNPs, rs7740529 and rs2233128, were observed to have significantly different frequencies between asthma and non-asthma children for the tested genotype (Table 1). However, the *p* values for the difference were not significant after Bonferroni correction.

To confirm the weak association, the number of children was increased to 824 and genotyping for rs7740529 was performed with this larger sample, the *p* value associated with genotype testing reached 0.0093 (Table 2). The TT genotype of rs7740529 was found to be more frequent in the asthma group than in the non-asthma group (Table 2).

The SNP rs2233128 showed low minor allele frequencies in this study population and therefore further analysis was not conducted.

Adult asthma

We extended the association finding from the pediatric asthma population to the adult asthma population. The *p* value for genotype testing in the adult population was marginal significance (p=0.0915). The TT genotype was more frequent in asthma subjects than non-asthma subjects (Table 2). Combining the data from children and adults, the results indicated that rs7740529 was associated with asthma phenotype

Table 2. Genotype frequencies and odds ratio analysis for rs7740529 in pediatric and adult asthma groups

Genotype	Frequency		*p*	OR (95% CI)
	Case	Control		
Pediatric asthma (n = 515)				
CC/CT/TT	148/98/33	122/100/14	0.0358	
TT/CC+CT	33/246	14/222	0.0206	2.1272 (1.1094-4.0786)
Pediatric asthma (n = 824)				
CC/CT/TT	232/142/41	218/170/21	0.0093	
TT/CC+CT	41/374	21/388	0.0098	2.0255 (1.1747-3.4924)
Adult asthma (n = 334)				
CC/CT/TT	124/68/19	71/48/4	0.0915	
TT/CC+CT	19/192	4/119	0.0452	2.9440 (0.9778-8.8639)
Total (n = 1158)				
CC/CT/TT	356/210/60	289/218/25	0.0009	
TT/CC+CT	60/566	25/507	0.0015	2.1498 (1.3280-3.4803)

Abbreviations: OR = odds ratio; CI = confidence interval

and the TT genotype of the SNP was a risk factor for asthma in all individuals (Table 2).

Discussion

In this study, we found that the SNP rs7740529 in the intron 1 region of *MD-1* is associated with pediatric and adult asthma in the Taiwanese population. The results suggest that *MD-1* could be a susceptible gene for asthma in this study population.

There are physiological rationales for studying the polymorphisms of the *MD-1* gene in relation to allergy. It is known that *MD-1*, as the molecular helper of radioprotective (RP) 105, interacts with the Toll-like receptor 4 (TLR4) signaling complex. In the mouse model and human embryonic kidney 293 cell line, TLR4/*MD-2* signaling was negatively regulated or inhibited by RP105/*MD-1* complex [17,18]. It has been reported that TLR4 signaling is involved in many disease processes, including allergy and asthma [19,20], inflammatory bowel disease [21], increased susceptibility to infection [22], experimental autoimmune encephalomyelitis, and multiple sclerosis in animal models [23]. Therefore, it is reasonable to believe that *MD-1* could be a modifier gene candidate for a variety of inflammatory and atopic diseases.

From an epidemiologic viewpoint, the association of the polymorphism of the *MD-1* gene with both pediatric and adult asthma suggests that adult asthma could result from pathophysiologic processes that begin in early childhood [24]. Despite many investigations, accurate prediction in childhood of the risk of asthma as an adult is still not feasible [25]. The association of *MD-1* polymorphism is more significant in pediatric than in adult asthma. However, more intensive studies of association need to be done for *MD-1* to confirm the functions of this gene in asthma or allergy pathophysiology.

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