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ASTHMA AND GENETICS

Genetic Polymorphism of Manganese Superoxide Dismutase is Associated with Childhood Asthma

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Objective. Cellular defenses against allergens and reactive oxygen species (ROS) exposure are critical in the pathogenesis of asthma. CD14 is a receptor for various bacterial products, such as lipopolysaccharides (LPS), and is also a mediator of inflammatory processes. Manganese superoxide dismutase (MnSOD) is an ROS scavenger, and myeloperoxidase (MPO) can convert hydrogen peroxide into hypochlorous acid; thus, they are considered to be involved in inflammatory defense. The authors conducted a case-control study to evaluate the susceptibility to childhood asthma based on *CD14*, *MnSOD*, and *MPO* genes. *Methods*. The *CD14* −260, *MnSOD* −9, and *MPO* −463 genotypes were identified by polymerase chain reactions for 116 asthmatic children and 232 healthy controls. Questionnaires were administered to obtain demographic characteristics. Allergen testing used common Taiwanese aeroallergens. *Results*. A higher level of parental education, family history of asthma, incense burning at home, allergen-test positive, and the *MnSOD Val-Ala/Ala-Ala* genotypes (matched relative risk = 2.0; 95% confidence interval = 1.0–4.2) were significantly associated with childhood asthma. Interactions between *CD14*, *MnSOD*, *MPO* genotypes and allergy status were significantly associated with asthma risk in these children (all $p < .001$). Furthermore, atopic cases with *MnSOD Val-Ala/Ala-Ala* (log eosinophil 2.66/mm^3, log total serum immunoglobulin E [IgE] 2.48 IU/ml) or *Val-Val* (log eosinophil 2.61/mm^3, log total serum IgE 2.63 IU/ml) genotypes had elevated eosinophil counts and total serum IgE levels as compared to nonatopic cases with *MnSOD Val-Val* genotype (log eosinophil 2.27/mm^3, log total serum IgE 1.83 IU/ml). *Conclusions*. Susceptible *MnSOD* genotypes might modulate the development of asthma in Taiwanese children.

Keywords allergen; *CD14*; childhood asthma; environmental tobacco smoke; *MnSOD*; *MPO*

Introduction

Asthma is a complex bronchial disorder either triggered or worsened by numerous genetic and environmental factors. Possible associated factors include exposure to allergens (1), viral and bacterial infections (2, 3), or airway irritants like cigarette smoke (environmental tobacco smoke [ETS]) (4) and other environmental pollutants (5). Importantly, the prevalence of asthma continues to increase globally. However, the pathogenesis of pediatric asthma remains unclear.

Microbial products can provide activation signals for Th1 maturation, thereby suppressing Th2-type responses and protect against development of atopy (6). CD14 is a high-affinity receptor for various bacterial products, such as lipopolysaccharides (LPS), and is also an important mediator of inflammatory processes (7). It is constitutively expressed on monocytes and macrophages as membrane CD14 (mCD14); its soluble form (sCD14) is secreted or enzymatically cleaved from CD14 protein in serum (8). It is known that sCD14 dramatically limits LPS binding to monocytes by transferring LPS to plasma lipoproteins, rather than the Toll-like receptor 4 (TLR4)/myeloid differentiation 2 (MD-2) complex, and subsequently reduces cytokine responses (9).

Reactive oxygen species (ROS) are generated both internally and externally; their formation in lungs takes place after inhalation of ozone, dust particles, and cigarette smoke, resulting in the depletion of endogenous antioxidants in the epithelial lining fluid (10). Manganese superoxide dismutase (superoxide dismutase 2 [SOD2/MnSOD]) is a known ROS scavenger in mitochondria, which is the major source of ROS in cells (11). It catalyzes the dismutation of superoxide radicals, thereby producing hydrogen peroxide and oxygen (12). However, hydrogen peroxide may react with ferrous iron to form more cytotoxic hydroxyl radicals (13). Myeloperoxidase (MPO) is a lysosomal hemoprotein released from the primary azurophilic granules of activated inflammatory cells, and is part of the host defense system responsible for microbicidal activity against a wide range of organisms (14). MPO is responsible for converting the hydrogen peroxide produced by the ROS scavenging process into hypochlorous acid, which can cause cellular damage in adjacent epithelial cells (14).

Genetic polymorphisms of human *CD14* have been identified (15). A *C*-to-*T* transition polymorphism at position −260 results in increase of both CD14 mRNA expression and serum level of sCD14. In addition, one important single-nucleotide variation at position −9 of human *MnSOD* has also been identified: GTT (valine) to GCT (alanine) (16). This variation can alter the secondary structure of the protein from a β-pleated sheet to an α-helical conformation

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(13), and might affect its cellular allocation along with its transportation into mitochondria. Inefficient targeting of Mn-SOD could leave mitochondria with an incomplete defense against superoxide radicals. A single-base substitution at position −463 (*G*-to-*A*) of the *MPO* gene was also found to be associated with a decreased transcriptional activity due to the disruption of an SP1-binding site in an *Alu* element (17).

Cellular defenses against allergens and ROS exposure are critical in the pathogenesis of asthma. We conducted a case-control study to assess associations of asthma with polymorphisms of *CD14*, *MnSOD*, and *MPO* genes in Taiwanese children.

Methods

Identification of Study Subjects

Our study subjects were collected from two populations. Asthmatic children were recruited from Chung-Shan Medical University Hospital in central Taiwan. The hospital was accessible to patients from all socioeconomic classes. Cases that fulfilled the American Thoracic Society's criteria (18) for reversible airway diseases were selected from the outpatient clinic of Chung-Shan Medical University Hospital by a pediatrician. In addition, control subjects were recruited from an elementary school in central Taiwan based on the following criteria: (1) no physician-diagnosed asthma or persistent wheeze; (2) no chronic phlegm; and (3) no shortness of breath for the past 12 months. Cases were matched to controls in a 1:2 ratio on age (± 5 years) and gender. The case and control groups were aged from 5 to 12 years. All the biological parents of the participating children were of Chinese descent. The subjects in our study were assumed to have the same ethnogeographic origin. In total, 116 asthmatic cases and 232 healthy controls were included for analysis. Among the asthmatic cases, 89 (76.7%) were also diagnosed as having allergic rhinitis, and 74 (63.8%) had suffered from concurrent wheezing for the past year. At study entry, a parent of each participating child provided written informed consent. All study subjects also had to have been able to provide a clinical history. The study protocol was approved by the ethics committee of Chung-Shan Medical University Hospital.

Epidemiologic Information

Independent interviews with parents were conducted during the study period by research staffs using the standard International Study of Asthma and Allergies in Childhood—Chinese version questionnaire (19). We opted to add several questions about environmental risk factors of asthma to explore possible connections with asthma. The questions covered demographic characteristics, lifestyles (including cigarette-smoking habits of family members, incense burning at home, textile work at home, pet keeping, home dampness, and appearance of cockroaches in the bedroom during the past year), as well as family histories of asthma. Household smoking was defined as daily smoking inside the house by anyone living there. Information on subjects' parents included the number of cigarettes smoked daily and the duration that the child was exposed to environmental tobacco smoke. Home dampness was defined as the presence of visible surface mold or mildew growth inside the home, standing water within the home, water damage, or leakage of water into the home (20). Family history of asthma was defined as disease among the first-degree relatives of the study subjects.

Clinical Evaluations of Asthma, Allergy, and Pulmonary Function

Asthma was defined by clinical history and reconfirmed by interviews with our recruited participants. Briefly, a clinical test was performed to validate the asthmatic phenotype by assessing the presence of bronchial hyperresponsiveness, defined as the ratio of forced expiratory volume in one second (FEV_1) to forced vital capacity (FVC) <80% at the time of the episode and improvement with a bronchodilator. Asthma was also defined by an affirmative response to at least one of the following questions in the past year: (a) Have you ever had episodes of breathlessness and wheezing while at rest? (b) Have you ever had an asthma attack at night? (c) Were you ever admitted to the hospital for asthma? (d) Are you taking any medications for asthma treatment?

Allergen testing used either intracutaneous skin testing or MAST (Multiple Antigen Simultaneous Test) with common Taiwanese aeroallergens, including house dust, American cockroach, and dust mites (standardized mite *Dermatophagoides farinae* and standardized mite *Dermatophagoides pteronyssinus*), as suggested previously (21). Skin tests were considered positive if the largest wheal diameter was ≥3 mm. Atopy was defined by at least one positive test reaction result. Total serum immunoglobulin E (IgE) levels were measured using fluorescent enzyme immunoassay (AutoCAP System; Pharmacia Diagnostics AB, Uppsala, Sweden). Eosinophil counts from peripheral blood were expressed as absolute numbers per mm^3. For pulmonary function tests, standard spirometry was used for the analysis of vital capacity (VC), FVC, and FEV_1. All measurements were carried out according to spirometric standards by a physician and well-trained research staff.

Genotyping

Venous blood samples were collected into heparinized tubes and stored at −80°C after plasma and DNA separation. Genotyping was performed on genomic DNA extracted from peripheral blood lymphocytes. For the analysis of *CD14* −260 *C→T* (rs2569190) (22), *MnSOD* −9 *Val→Ala* (rs4880) (23), and *MPO* −463 *G→A* (rs2333227) (24) genetic polymorphisms, we used polymerase chain reaction (PCR)-based restriction fragment length polymorphisms (RFLPs). Primers used for the amplification of the *CD14* gene were 5′-GTG CCA ACA GAT GAG GTT CAC-3′ and 5′-GCC TCT GAC AGT TTA TGT AAT C-3′. DNA (0.5 μl) was added to a PCR buffer containing 200 ng of primers, 1.5 mM $MgCl_2$, 0.2 mM of dNTPs, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), and 0.1% of bovine serum albumin (BSA) in a final volume of 50 μl. The PCR products were digested with *Ava*II. Homozygous *CC* individuals had a product fragment of 497 bp, homozygous *TT* individuals had 353-bp and 144-bp fragments, and heterozygous *CT* individuals had all three fragments. Primers used for the amplification of the *MnSOD* gene were 5′-ACC AGC AGG CAG CTG GCG CCG G-3′ and 5′-GCG TTG ATG TGA GGT TCC AG-3′. The PCR products were also digested with *Ngo*MIV. Homozygous

Val-Val individuals had a product fragment of 107 bp, homozygous *Ala-Ala* individuals had 89-bp and 18-bp fragments, and heterozygous *Val-Ala* individuals had all three fragments. Primers used for the amplification of the *MPO* gene were 5′-CGG TAT AGG CAC ACA ATG GTG AG-3′ and 5′-GCA ATG GTT CAA GCG ATT CTT C-3′. The PCR products were digested with *Aci*I. Homozygous *AA* individuals had two product fragments of 289 and 61 bp, homozygous *GG* individuals had three product fragments of 168 bp, 121 bp, and 61 bp, and heterozygous *GA* individuals had all four fragments.

All genotype reading was done blinded to case or control groups. Rigorous quality control procedures were applied throughout the entire process. To avoid PCR contamination, reagents were carefully aliquoted, and each aliquot was used no more than three times. For each assay, a nontemplate control was also added to monitor reagent contamination. After genotyping each of the genetic polymorphisms, around 20% of the samples in each genotype group were randomly selected for repeated assays to validate the results.

Statistical Analysis

Matched relative risks (RR_m) and corresponding 95% confidence intervals (CIs) on childhood asthma were evaluated for the *CD14* −260 *C→T*, *MnSOD* −9 *Val→Ala*, and *MPO* −463 *G→A* genotypes, as well as family history of asthma, passive smoking exposure, incense burning, textile work at home, pet keeping, home dampness, appearance of cockroaches during the past year, and atopic status using a conditional logistic regression model. Additionally, atopic status and susceptible *CD14* −260 *C→T*, *MnSOD* −9 *Val→Ala*, and *MPO* −463 *G→A* genotypes were taken into multiple conditional logistic regression models to determine their interactions on asthma development. Finally, a least-squares mean was performed to predict the adjusted serum total IgE levels and eosinophil counts for asthmatic children with different combinations of atopic status and susceptible genotypes. All data were analyzed using SAS program version 9.1 (SAS Institute, Cary, NC, USA). Statistical tests were two-sided, with $p < .05$ considered statistically significant.

TABLE 1.—Demographic and clinical characteristics of study subjects.

Variables	Cases ($n = 116$)	Controls ($n = 232$)	RR_m (95% CI)*
Male gender	76 (65.5%)	152 (65.5%)	Matched
Age	7.9 ± 2.2	10.6 ± 0.8	Matched
Parental education			
Above junior high school	58 (50.0%)	60 (25.9%)	2.8 (1.8–4.5)†
Below junior high school	58 (50.0%)	172 (74.1%)	1.0 (reference)
Family history of asthma			
Yes	23 (19.8%)	16 (6.9%)	3.3 (1.7–6.5)†
No	93 (80.2%)	216 (93.1%)	1.0 (reference)
FEV_1% predicted			
<80%	69 (59.5%)	44 (19.0%)	6.4 (3.9–10.5)†
≥80%	47 (40.5%)	188 (81.0%)	1.0 (reference)
Serum total IgE level >100 IU/ml	95 (81.9%)		
Q1–Q3	159.0–725.5		
Log (total IgE)	2.4 ± 0.6		
Eosinophil count >100/mm^3	104 (89.7%)		
Q1–Q3	230.0–690.0		
Log (eosinophil count)	2.6 ± 0.4		

Note. Results are either number (percentage) or mean ± standard deviation.
*Controls were matched to cases on age and gender.
†Significantly different from controls, $p < .01$.

TABLE 2.—Matched relative risks for asthma development based on various environmental factors.

Variables	Cases ($n = 116$)	Controls ($n = 232$)	RR_m (95% CI)*
Parental smoking			
Yes	33(28.4%)	68(29.3%)	0.8(0.5–1.4)
With household smoking restrictions	19(16.4%)	55(23.7%)	0.6(0.3–1.1)
No	64(55.2%)	109(47.0%)	1.0 (reference)
Incense burning at home			
Yes	34(29.3%)	146(62.9%)	0.3(0.2–0.4)†
No	82(70.7%)	86(37.1%)	1.0 (reference)
Textile work at home			
Yes	11(9.5%)	12(5.2%)	1.9(0.8–4.5)
No	105(90.5%)	220(94.8%)	1.0 (reference)
Pet keeping			
Yes	17(14.7%)	48(20.7%)	0.7(0.4–1.2)
No	99(85.3%)	184(79.3%)	1.0 (reference)
Home dampness			
Yes	14(12.1%)	20(8.6%)	1.5(0.7–3.0)
No	102(87.9%)	212(91.4%)	1.0 (reference)
Observed cockroaches in bedroom			
Yes	48(41.4%)	99(42.7%)	0.9(0.6–1.5)
No	68(58.6%)	133(57.3%)	1.0 (reference)
Allergen test			
Positive	94(81.0%)	102(44.0%)	5.6(3.3–9.6)†
Negative	22(19.0%)	130(56.0%)	1.0 (reference)

*Controls were matched to cases on age and gender.
†Significantly different from controls, $p < .01$.

RESULTS

A total of 348 children (228 boys and 120 girls) were included with a mean age of 9.7 years (range, 4–12 years). Their demographic characteristics, family histories of asthma, pulmonary function test results, serum total IgE levels, and eosinophil counts are listed in Table 1. Children with parents who had more than a junior high school education had a significantly higher risk of asthma than children with parents who had less than a junior high school education ($RR_m = 2.8$, 95% CI = 1.8–4.5). A significantly higher percentage of family history of asthma was observed for cases than for controls (19.8% versus 6.9%, $RR_m = 3.3$; 95% CI = 1.7–6.5). A significantly higher proportion of children who had predicted $FEV_1\% < 80\%$ was also found in the asthma case group than in controls (59.5% versus 19.0%). In addition, 81.9% and 89.7%, respectively, of the asthmatic children had elevated serum total IgE levels and eosinophil counts compared to the cut-off levels (100 IU/ml and 100/mm^3).

Matched relative risks for asthma development based on various environmental factors are shown in Table 2. An inverse association was found between incense burning at home with childhood asthma development ($RR_m = 0.3$; 95% CI = 0.2–0.4). An increased childhood asthma risk was also associated with positive allergen test results ($RR_m = 5.6$; 95% CI = 3.3–9.6). No significant associations were found between childhood asthma and parental smoking, textile work at home, pets keeping, home dampness, or cockroaches observed in the bedroom.

Matched relative risks of childhood asthma stratified by *CD14* −260 *C→T*, *MnSOD* −9 *Val→Ala*, and *MPO* −463 *G→A* genotypes were calculated (Table 3). Because only a

TABLE 3.—Genetic polymorphisms of *CD14* −260, *MnSOD* −9, and *MPO* −463 and corresponding relative risks of childhood asthma.

Genotypes	Cases ($n = 116$)	Controls ($n = 232$)	Adjusted RR_m (95% CI)*
CD14 −260 *C→T*			
TT	35 (30.2%)	69 (29.7%)	1.8 (0.7–4.7)
CT	64 (55.2%)	118 (50.9%)	1.7 (0.7–4.1)
CC	17 (14.6%)	45 (19.4%)	1.0 (reference)
MnSOD −9 *Val→Ala*			
Ala-Ala	1 (0.9%)	5 (2.1%)	0.2 (0.0–3.8)
Val-Ala	33 (28.4%)	47 (20.3%)	2.3 (1.1–4.9)†
Val-Val	82 (70.7%)	180 (77.6%)	1.0 (reference)
Val-Ala/Ala-Ala	34 (29.3%)	52 (22.4%)	2.0 (1.0–4.2)‡
Val-Val	82 (70.7%)	180 (77.6%)	1.0 (reference)
MPO −463 *G→A*			
AA	12 (10.3%)	32 (13.8%)	0.7 (0.2–1.9)
GA	29 (25.0%)	45 (19.4%)	1.7 (0.8–3.9)
GG	75 (64.7%)	155 (66.8%)	1.0 (reference)

*Controls were matched to cases on age and gender, and adjusted for the effects of parental education status, family history of asthma, incense burning at home, and allergen test results.
†Significantly different from controls, $p = .04$.
‡Significantly different from controls, $p = .05$.

few of our study subjects had the *MnSOD Ala-Ala* genotype, and because individuals with at least one *MnSOD Ala* allele have a higher enzyme activity than those with the *MnSOD Val* allele (13), the *MnSOD Ala-Ala* and *Val-Ala* genotypes were combined for further analysis. The frequency for *MnSOD Val-Ala/Ala-Ala* genotypes in our asthmatic cases was higher than those in controls (29.3% versus 22.4%). These children had a 2.0-fold higher risk of asthma development compared to subjects carrying the *Val-Val* genotype (95% CI = 1.0–4.2; $p = .05$), after adjusting the effects of parental education status, family history of asthma, incense burning at home, and allergen test results. However, no significant associations were observed between childhood asthma development and *CD14* −260 *C→T* and *MPO* −463 *G→A* genotypes.

In our current observations, atopy was a strong determinant for childhood asthma (Table 2). Therefore, we also evaluated the interactions of different inherited genotypes and allergen burden on childhood asthma (Table 4). For statistical power considerations, subjects possessing the *CD14* −260 *TT* and *CT* genotypes were combined for this analysis. Subjects possessing *MPO* −463 *AA* and *GA* were also combined. Taking the nonatopic children with the *CD14* −260 *CC* genotype as a reference, atopic children carrying the *TT*/*CT* genotypes had a significantly higher risk for asthma development ($RR_m = 3.8$; 95% CI = 1.4–10.2), whereas atopic children carrying the *CC* genotype had a higher, although not significant, risk for asthma development ($RR_m = 2.7$; 95% CI = 0.8–8.7). Compared to nonatopic subjects carrying the *MnSOD* −9 *Val-Val* genotype, atopic children carrying the *Val-Ala*/*Ala-Ala* and *Val-Val* genotypes had, respectively, a 6.9-fold (95% CI = 3.0–15.7) and a 4.1-fold (95% CI = 2.1–7.8) higher asthma risk. For the consideration of *MPO* −463 *G→A* genetic variations, nonatopic children with the *MPO* −463 *AA*/*GA* genotype were taken as a reference; atopic subjects possessing *MPO* −463 *AA*/*GA* ($RR_m = 4.3$; 95% CI = 2.0–8.8) and *GG* ($RR_m = 3.8$; 95% CI = 2.0–7.4) genotypes also had increased risks for childhood asthma. Furthermore, interactions between *CD14* −260, *MnSOD* −9, and *MPO* −463 genotypes and allergy status on the risks of childhood asthma were significant (all $p < .01$).

Next, we performed a least-squares mean analysis among asthmatic children to assess blood eosinophil counts and total serum IgE levels with a combination of allergen reactions and *MnSOD* genotypes, adjusted for the cofounding effects of age, gender, parental education, family history of asthma, and incense burning at home (Table 5). Atopic cases with *MnSOD Val-Ala/Ala-Ala* (log eosinophil 2.66/mm^3, log total serum IgE 2.48 IU/ml) or *Val-Val* (log eosinophil 2.61/mm^3, log total serum IgE 2.63 IU/ml) genotypes had elevated eosinophil counts and total serum IgE levels compared to nonatopic cases with the *MnSOD Val-Val* genotype (log eosinophil 2.27/mm^3, log total serum IgE 1.83 IU/ml). However, nonatopic cases with *MnSOD Val-Ala/Ala-Ala* genotypes did not have obviously increased eosinophil counts or total serum IgE levels.

DISCUSSION

Genetic modifications that cause increases in asthma risks may have more profound influences on individuals with certain types of environmental exposures (25). In the present study, ROS scavenger *MnSOD* −9 *Val-Ala/Ala-Ala* genotypes were found to be significantly associated with an increased risk of asthma in children.

Importantly, MnSOD is the major scavenger of ROS (11). However, the exact role of MnSOD is not well understood,

TABLE 4.—Interactions of allergy burden and *CD14* −260, *MnSOD* −9, and *MPO* −463 genotypes on childhood asthma.

Genotypes	Atopic children			Nonatopic children		
	CA $n = 94$	CN $n = 102$	RR_m (95% CI)*	CA $n = 22$	CN $n = 130$	RR_m (95% CI)*
CD14 −260 *C→T*						
TT/CT	80	80	3.8 (1.4–10.2)†	18	107	0.7 (0.3–2.1)
CC	14	22	2.7 (0.8–8.7)	4	23	1.0 (reference)
			p for interaction < .01			
MnSOD −9 *Val→>Ala*						
Val-Ala/Ala-Ala	28	22	6.9 (3.0–15.7)†	6	30	1.0 (0.4–3.0)
Val-Val	66	80	4.1 (2.1–7.8)†	16	100	1.0 (reference)
			p for interaction < .01			
MPO −463 *G→A*						
AA/GA	35	37	4.3 (2.0–8.8)†	6	40	0.6 (0.2–1.8)
GG	59	65	3.8 (2.0–7.4)†	16	90	1.0 (reference)
			p for interaction < .01			

*Controls were matched to cases on gender and age, and relative risks were adjusted for parental education status, family history of asthma, and incense burning at home.
†Significantly different from reference, $p < .01$.

TABLE 5.—Adjusted mean blood eosinophil counts (mm^3) and total serum IgE levels (IU/ml) in asthmatic children ($n = 116$) according to allergen test status and *MnSOD* genotype.

			log Eosinophil counts (mm^3)		log total serum IgE levels (IU/ml)	
Atopic	*MnSOD −9 Val→Ala* genotype	*n*	Regression coefficient (standard error)	*p* value	Regression coefficient (standard error)	*p* value
Yes	*Val-Ala/Ala-Ala*	28	2.66 (0.07)	<.01	2.48 (0.10)	<.01
Yes	*Val-Val*	66	2.61 (0.04)	<.01	2.63 (0.07)	<.01
No	*Val-Ala/Ala-Ala*	6	2.46 (0.14)	.24	1.65 (0.22)	.50
No	*Val-Val*	16	2.27 (0.09)	Reference	1.83 (0.14)	Reference

Note. Data were adjusted for effects of age (per 1-year increment), gender, parental education, family history of asthma, and incense burning at home.

because it also produces hydrogen peroxide that, in turn, may react with ferrous iron to form more cytotoxic hydroxyl radicals (13). It has been reported that the −9 *Ala* allele of a mitochondrial targeting sequence of the *MnSOD* gene has an α-helix structure, whereas the −9 *Val* allele does not (13). Because an amphiphilic helix structure is an essential requirement for efficient mitochondrial transport, the MnSOD precursor protein with the −9 Ala-type signal peptide may be more easily transported into mitochondria than the precursor with the −9 Val-type signal peptide. Therefore, the difference in the signal peptide sequence may affect the amount of MnSOD expressed within mitochondria (13). A previous association study also found a significantly higher frequency of the −9 *Ala* allele of *MnSOD* in patients with Parkinson's disease than in healthy controls (13). The relationship between structural variations in the *MnSOD* gene and asthma development is still unknown. Most of the previous reports indicated that the *Ala*-9*Val* polymorphism of *MnSOD* was not associated with asthma (26–28). However, Mak and colleagues (29) reported a protective effect of the *Ala* allele against asthma among atopic cases and control subjects who were never-smokers, yet they did not observe any overall association between this polymorphism and asthma. In our study, we also observed that children who carried *MnSOD Ala-Ala* genotype had a lower risk of asthma (Table 3), although our statistical power was limited. It is possible that the *MnSOD* gene may only be a susceptibility gene for pediatric asthma in certain ethnic populations. Because only a few of our study subjects had the *MnSOD Ala-Ala* genotype, the *MnSOD Ala-Ala* and *Val-Ala* genotypes were combined for further analysis. Further, we observed that subjects carrying the *MnSOD* −9 *Val-Ala/Ala-Ala* genotypes were at greater risks of asthma than those carrying the *Val-Val* genotype. This may be due to the *MnSOD* −9 *Ala* allele in our asthmatic children results in a potential to form an amphiphilic helix, and higher MnSOD activity might play a negative role for protecting the airway epithelium. Increased formation of cytotoxic hydroxyl radicals by MnSOD may be a mechanism for this negative role during the pathogenesis of asthma. It has also been proposed that the *MnSOD* −9 *Ala* allele may affect the cleavage process of the mitochondrial targeting sequence, which may, in turn, influence the MnSOD activity. Further studies will be required to test these hypotheses.

Atopy is an immune hypersensitivity disorder to some agents, such as house dust, dust mites, and cockroaches (21). It is also the leading cause of childhood asthma (1, 2). In our study, atopic children also showed a significantly higher risk for asthma development. A crucial control mechanism lies in the balance between Th1- and Th2-type cytokines (30). Thl lymphocyte development is primed by interleukin (IL)-12 and is associated with interferon (IFN)-γ and IL-2 cytokine production; these cytokines play crucial roles in the development of cell-mediated immunity. Th2 lymphocyte development is primed by IL-4, and it is associated with IL-5 and IL-13 cytokine production, which promotes the secretion of IgE and the recruitment of eosinophils. Th2 mechanisms are central to the development of hypersensitivity to environmental antigens in atopy.

In our study, atopic children with *MnSOD Val-Ala/Ala-Ala* genotypes had more pronounced risks of asthma development than did nonatopic children with the *MnSOD Val-Val* genotype. Interaction between the *MnSOD* genotype and allergy status on the risk of childhood asthma was significant. Atopic children with either *MnSOD Val-Ala/Ala-Ala* or *Val-Val* genotypes also had higher total serum IgE levels and eosinophils counts. However, significant differences were not observed for total serum IgE levels and eosinophil counts between the atopic children with the *MnSOD Val-Ala/Ala-Ala* and with the *Val-Val* genotypes. Th2 cells that are responsive to environmental allergen stimuli could be substantially increased, at least up to some plateau levels. Th2 mechanisms are central to the development of hypersensitivity to environmental antigens in atopy. It is well established that IgE responses are regulated by inhibitory signals from Th1-type cells and by stimulatory signals from Th2-type cells (30). Our findings suggest that *MnSOD* −9 *Val→Ala* genetic variations might results in defective mitochondrial defenses against ROS, and thus might regulate the proportions of Th1-Th2 cells that are responsive to environmental allergen stimuli. This could increase the subsequent development of asthma. Future epidemiological studies on the relationships between allergen exposure and asthma/allergy must take into account the genetic variants of *MnSOD*.

To date, the −260 *C→>T* is the one single-nucleotide polymorphism (SNP) found to have functional significance in the *CD14* gene (16). It was associated with increases of both CD14 mRNA expressions and sCD14 levels in vitro (9), and was related to increased asthma risks, as well as lowered serum total IgE levels, in children carrying the T allele in several ethnic groups (31, 32). However, other studies have indicated that the *CD14* −260 *T* allele was more common among patients with nonatopic asthma and food allergy than control subjects (33), and that lowered serum total IgE levels were only observed among radioallergosorbent test-negative children carrying the *TT*/*CT* genotypes (34). A recent review article concluded that ethnicity, population admixture,

gene-gene interactions, gene-environment interactions, linkage disequilibrium, and study designs may be responsible for these inconsistencies (35). In our study, no significant association was observed between childhood asthma development and *CD14* −260 *C→T* genotypes. However, atopic children possessing *CD14* −260 *TT*/*CT* genotypes had an elevated risk of asthma as compared to nonatopic children carrying the CC genotype; interaction between the *CD14* −260 genotypes and allergy status on the risk of childhood asthma was significant. Our findings suggest that the *CD14* −260 C→T polymorphism may modulate susceptibility to atopic asthma in Taiwanese children.

Neutrophils and their secreted MPO proteins might also be involved in asthmatic airway inflammation. However, serum MPO levels of asthmatic children were found to be increased only during inflammation (36). *MPO* −463 *G→A* genetic variation results in disrupted SP1-binding site, and a lowered gene transcriptional level (17). In the present study, no significantly different risk for asthma was observed among children with different *MPO* −463 genotypes. This may be due to an insufficient sample size to detect small increases in risk. Similarly, a significant interaction between the *MPO* −463 genotypes and allergy status on children's risk was revealed. This may suggest that the *MPO* gene plays a minor, modifier role in childhood asthma.

Familial aggregation has been commonly reported for asthma, and has been considered to represent complex gene-environment interactions by inherited genetic factors and shared environmental factors (37). This was also observed in our study. In the current study, the parental education level (a proxy for socioeconomic status) was also significantly associated with children's asthmatic development. Parental education levels may convey information regarding the patterns of potential environmental exposures, as well as health care for children. Parents with higher education levels might strive to improve living conditions and reduce exposures to respiratory infections in early childhood.

Children spend most of their time at home. Thus, considerations of household environmental allergens are essential for the assessment of childhood asthma. Household exposures to mites (1) and cigarette smoke (4) have been identified as risk factors for asthma development. A previous meta-analysis indicated that children exposed to ETS during early childhood had approximately twice the risk of developing serious respiratory problems than children of nonsmokers; however, the risk diminished with an increase in age (38). In particular, risk of ETS exposure on childhood respiratory problems was concluded to be around 1.0 for children older than 5 years of age. Similarly, our results indicated that ETS exposure did not substantially contribute to asthma risk among our children (mean age 9.7 years). Maternal smoking is considered a major risk factor of childhood asthma (4). However, only six of the mothers of our controls (and none of the cases) in our study smoked; therefore, no association could be observed. This suggests that Taiwanese asthmatic children may not have regular exposure to tobacco smoke from their family members, and thus the effects of passive smoking on asthma in children are less likely to appear in our case-control study. In Taiwan, about half of the population burns incense daily at home to worship god and ancestors, based on their Buddhist and Taoist religious practices. Although no adverse effects of incense smoke on health have yet been reported, several of its components have been suggested to affect pulmonary function and even asthma exacerbations, such as particulate matter, SO_2, NO_2, volatile organic compounds, and aldehydes (39). However, an inverse association was found between incense burning at home with childhood asthma in our study. This may be explained by more intense protection performed for children by more educated parents; a similar tendency had been reported previously in Taiwan (19). In addition, in our study, these indicators were self-reported and were, therefore, subjective, so misclassifications for exposure assessment may have occurred. Moreover, there may be other environmental factors that we did not account for in the current study that contributed to the pathogenesis of childhood asthma.

In our study, atopy was a strong determinant for childhood asthma. We thus further examined the distribution of genotype frequencies among atopic and nonatopic children. The prevalence of *MnSOD* −9 *Val→Ala* ($p = .68$, χ^2-test), *CD14* −260 *C→T* ($p = .58$), and *MPO* −463 *G→A* ($p = .45$) genotypes among the atopic and nonatopic children groups did not differ significantly. Ideally, an allergic, nonasthmatic control group should be selected for indentifying the susceptible effect of interest genes in our study. However, more than half (56%) of our 232 controls were nonatopic. There is a concern regarding the small sample size in our study. Small numbers of subjects in each subgroup limits the statistical power to detect a small increase in risk. Additional studies including more subjects would be necessary to provide further evidence regarding our findings.

In conclusion, we report here that *MnSOD* −9 *Val-Ala*/*Ala-Ala* genotypes may be related to asthma risk among Taiwanese children.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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