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Associations between sensitization to allergens and allergic diseases: a hospital-based case-control study in China

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Associations between sensitization to allergens and allergic diseases: a hospital-based case-control study in China

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Abstract

Objectives To assess the independent and combined associations of common allergens with atopic dermatitis, allergic rhinitis allergic and asthma, and to explore the dose-response relations between levels of allergen-specific IgE and the prevalence of allergic diseases in Harbin in northeastern China.   **Methods** In our case-control, 687 participants aged eighteen years or older were included: 2,631 with atopic dermatitis, 1,320 with allergic rhinitis, 1,160 with allergic asthma and 2,576 non-allergic controls. Tests of allergen-specific serum immunoglobulin E to 16 allergens were measured in allergic cases and controls. The associations of allergens with allergic diseases were assessed using logistic regressions.   **Results** We found that atopic dermatitis had something to do with food allergens while allergic rhinitis and allergic asthma were in connection with both indoor and outdoor allergens. Between levels of allergen-specific IgE and the allergic diseases' prevalence, there is a dose-response relevance. The prevalence of allergic diseases increased in pace with the increase of specific IgE levels to allergens (*P* trend <0.0001) and also increased with increasing number of allergens (*P* trend <0.0001). **Conclusion** There was a dose-response relevance between levels of allergen-specific IgE and allergic diseases' prevalence, and multiple sensitization to diverse allergens increased the risk of allergy-related diseases. This study provided evidence for the prophylaxis and treatment of three allergy-related diseases.

Keywords: Allergy-related diseases; allergens; association; case-control study

Strengths and limitations of this study

- This study assess the independent and combined associations of common allergens with atopic dermatitis, allergic rhinitis allergic and asthma, and to explore the dose-response relations between levels of allergen-specific IgE and the prevalence of allergic diseases in Harbin in northeastern China.

- Our findings could provide evidence for control and management of allergic diseases.

- We could not analyze the impact of seasonal factors on allergic diseases, and the universality of our results for the whole China still needs to be further explored.

Introduction

Allergy-related diseases are global and can profoundly affect the quality of life of patients[1]. The incidence of allergy-related diseases, including atopic dermatitis, allergic rhinitis, and allergic asthma, has been increasing rapidly around the world, especially in China and other low-and middle-income countries [2-4]. As triggering factors, allergens stimulate the body to produce abnormal immune responses, which eventually lead to allergic diseases[5]. The type and dose of allergens determine the type of allergic disease and its severity[6]. Therefore, it is crucial to clarify the distributions of positive allergens among patients with different allergic diseases.

Currently, most studies are limited by focusing on a small number of allergens or a single allergic disease when exploring the association between sensitization to allergens and allergic diseases. A study reported the prevalence of egg allergy in South African

children with atopic dermatitis and the predictive values of egg allergy screening tests [7].

A Japanese study showed that there is a causal relevance between pollen counts, social

media users' posting behavior, and the number of patients suffer from seasonal allergic

rhinitis[8]. Pomés et al. reviewed the relevance between indoor allergens and allergy-

related diseases[9]. Considering that allergy sufferers usually have different levels of

specific IgE to an allergen and those allergens are frequently co-existent[10], it is critical

to understand associations among multiple allergens and allergic diseases. Previous

studies revealed that the incidence of allergic diseases varies among different geographic

locations and subpopulations[11, 12], which suggest that genetic and environmental features

may play important roles in allergic diseases, and it is interesting to understand the

underlying causes of those variations. Currently, few such studies have been conducted

in northeastern China with its distinctive environmental and climate conditions [13-15].

In present study, we assessed the associations between sensitization to 16 allergens

and three common allergic diseases in northeastern China and explored if there is a dose-

response connection between the specific IgE levels to an allergen and of allergy-related

diseases' prevalence. We also examined if there are combined effects of multiple

allergens on a specific allergic disease. Our study aimed to supply the basis for the

prevention and management of allergy-related diseases.

Materials and methods

Patients and Public involvement

Harbin is located in Northeastern China with a longitude of 125°42'-130°10' E, and a latitude 44°04'-46°40' N, which is the capital of Heilongjiang Province. The winter of Harbin is long and cold, and the annual average temperature is relatively low (3.6℃), which ranges from - 15 ℃ to - 30 ℃ in January. We recruited the participants into our research from Harbin. Only those who signed the informed consent form were recruited into our study, and this project was authorized by the Institutional Review Board of Harbin Medical University. Patients with one of the three common allergy-related diseases (atopic dermatitis, allergic asthma or allergic rhinitis), who visited the Department of Allergy of the First Affiliated Hospital of Harbin Medical University during March 2009 and December 2017 were collected as the candidate cases. Healthy adults who visited the same hospital for a physical examination were recruited as the candidate controls during the same period. Under this condition, 7,000 candidate cases and 3,500 candidate controls were initially identified. Excluding those without written informed consents, 6,647 candidate cases and 3,303 candidate controls remained. Eligible cases and controls carried on allergen-specific IgE tests to 16 kinds of allergens and a questionnaire investigation. After excluding those with missing values and logic errors, and some special groups (sportsmans, pregnant or lactating women), we had 5,111 cases (2,631 atopic dermatitis, 1,320 allergic rhinitis and 1,160 allergic asthma) and 2,576 non-allergic controls for our final analysis. The flow diagram of study participants is summarized in Figure 1.

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Allergen-specific IgE testing

Serum allergen specific IgE concentrations of 16 common allergens were measured in all eligible participants using the AllergyScreen system (Mediwise Analytic GmbH, Germany). The 16 allergens are shown in Supplementary information, which were defined as three types: indoor allergens, outdoor allergens and food allergens[16], as shown in Table 2a, Table 2b and Table 2c. Based on previous research, allergens-specific IgE > 0.35 was considered as allergens sensitization in this study, and specific IgE concentration with 0.35-0.70 kU/l was defined as class 1, 0.70-3.50 kU/l as class 2, and > 3.50 kU/l as class 3[17].

Allergic-diseases definition

We focused on three common allergy-related diseases (atopic dermatitis, allergic asthma and allergic rhinitis) in Harbin. Experienced doctors take advantage of the existing benchmarks for atopic dermatitis[18], allergic rhinitis[19] and allergic asthma[20] to diagnose three allergic diseases. They also accord with the following criteria:

(1) At least have one positive outcome of the 16 allergen-specific IgE results;

(2) Had been diagnosed with one of the allergic diseases using current guidelines mentioned above.

Demographic, lifestyle characteristics and family history

All of the participants in this study conducted a questionnaire investigation to collect data

of demographic, lifestyle characteristics and family history. Sex, age, level of education, residential region and marital status were analyzed as demographic characteristics. Somking, drinking, and physical exercise were contained in lifestyle factors. We also collected the information of patients' family history with allergy-related diseases (atopic dermatitis, allergic rhinitis and allergic asthma). The definition of those factors was contained in the Supplementary Information.

Statistical analysis

To assess the dose-response relationship between levels of allergen-specific IgE to 16 different allergens and prevalence of allergic diseases, we apply logistic regressions to evaluate odds ratios (*OR*) and their 95% confidence intervals (*CI*). Those significantly associated with at least one of allergic diseases in univariate analysis or previously suggested in the literature were chosen as confounding factors. They included sex, age, BMI, level of education, smoking, drinking, living region, marital status, family history of allergic diseases, physical exercise and other 15 allergens specific-IgE responses. In order to inspect the combined effects of multiple allergens on allergic diseases, multiple logistic regressions were conducted with different numbers for sensitization with one allergen, two allergens, and three or more allergens. The analysis software of this study is SAS 9.1 (SAS Institute Inc., Cary, NC, USA).

Results

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Characteristics in cases and controls

There are statistical differences between participants with atopic dermatitis and controls in terms of sex, age, height, weight, BMI, residential region, smoking, education and family history ($P<0.05$). Patients with allergic rhinitis were significantly different from controls in sex, height, weight, BMI, education, and family history ($P<0.05$). Sex, age, weight, BMI, residential region, cigarette smoking, levels of education, marital status, and physical exercise were different between those with allergic asthma and controls ($P<0.05$). Detail demographic characteristics are summarized in Table 1.

Prevalence of allergen sensitization in atopic dermatitis, allergic rhinitis and allergic asthma

Participants with different allergic diseases had different prevalence of allergens sensitization. Among patients with atopic dermatitis, prevalence of food allergens sensitization was higher than other allergens. The prevalence of outdoor allergens was higher in those with allergic rhinitis while that of indoor allergens was more higher in patients with allergic asthma (Figure 2).

Adjusted for sex, age, BMI, level of education, smoking, drinking, living region, family history, physical exercise and other 15 allergens specific-IgE responses, specific IgE to common ragweed and mugwort, Dermatophagoides pteronyssinus, dog fur and cat fur were significantly related to the risk of allergic asthma (Table 2a and Table 2b). Allergic rhinitis was significantly associated with common ragweed and mugwort, mould mixture,

tree pollen mixture and Hop, while atopic dermatitis was significantly associated with egg white/egg yolk, crab, blue mussel, milk, fish and shrimp (Table 2b and Table 2c). There was a dose-response relationship between specific IgE levels, from class 1 to class 3, and the prevalence of allergy-related diseases, the prevalence of allergy-related diseases increased along with the increasing levels of allergen-specific IgE to allergens (*P* trend <0.0001).

Combined effects of sensitization to multiple allergens on allergic diseases

*OR*s and their 95% *CI*s of allergic diseases with different number of allergens shown in Table 3. Although the three allergic diseases had different *OR*s and 95% *CI*, the patterns of the relationships between allergic diseases and the number of allergens were similar. *OR*s increased as the number of sensitive allergens increased (*P* trend <0.0001). The associations between two or more allergens and atopic dermatitis were strong, adjusted *OR*=4.28 (95% *CI*: 2.57, 7.11). The combination of specific IgE to three or more sensitive allergens possess the highest *OR* for allergic rhinitis (adjusted *OR*=13.00, 95% *CI*: 3.76, 45.00), while the combination of specific IgE to two or more allergens with the highest *OR* for allergic asthma (adjusted *OR*=2.37, 95% *CI*: 1.67, 3.37).

Discussion

In our present hospital-based case-control research, we found that different allergens were significantly related to different allergy-related diseases, and there was a dose-response

relationship between levels of allergen-specific IgE and the prevalence of allergic diseases, and combined effects of sensitization to multiple allergens on allergy-related diseases. The incidences of allergy-related diseases had increased rapidly worldwide[2-4]. The World Allergy Organization (WAO) reported that, among 1.39 billion people in 33 countries, 22 percent of the population suffered allergic diseases[21]. The world health organization (WHO) had clearly stipulated that allergic disease was an extremely considerable disease that the whole world should attach importance to[22]. Identifying specific allergens that trigger allergic diseases is particularly significant for the prophylaxis and treatment of these allergy-related diseases.

Our findings indicated that atopic dermatitis was significantly related to food allergens while allergic rhinitis and allergic asthma were significantly associated with both indoor and outdoor allergens. Our discoveries are identical with some previous studies. It was reported that indoor allergens act as important factors in patients with allergic rhinitis and allergic asthma[9, 23, 24]. Some studies also stated that there was a relevance between sensitization to outdoor allergens and allergic rhinitis and allergic asthma[25-27]. Food allergens were found to crucial in the development of atopic dermatitis in some observations[27, 28]. The interaction between different allergens and human cells may be different, and some allergens may be more likely to induce certain allergic diseases than others. However, some studies showed inconsistent findings. Several studies showed that indoor allergens have a bearing on the prevalence of atopic dermatitis in some individuals[29, 30]. Other studies showed a relationship between sensitization to

food allergens and allergic rhinitis and allergic asthma[31]. The inconsistency of those studies may be related to regional variation and different climate characteristics, which affected the protein ingredients of allergens. In addition, genetic susceptibility and socio-economic status of populations may also have an influence on the occurrence of allergy-related diseases.

This study revealed that there was a dose-response relationship between specific IgE levels of allergens and prevalence of allergic diseases, and the *OR*s for allergic diseases with specific IgE to allergens increased along with the increase of allergen-specific IgE levels. This evidence suggested that participants would have a higher risk of suffering from allergic diseases if they faced higher concentrations of allergens. Some previous studies support our findings. A study in America revealed a dose-response relationship between the exposure to mouse and the morbidity of allergic asthma among urban children and adolescents[32]. Another research evaluated that whether TPI ASM8 induced a dose-dependent reduction in the inflammatory and physiological changes following inhaled allergen challenge[33]. It is worth noting that there was a combined effect of sensitization to different allergens on allergic diseases. Multiple sensitization to several allergens had a relatively stronger association with allergic diseases than single sensitization to one allergen. Some research results are similar to our findings. Carlsten et al. reported that in a birth cohort, simultaneous exposure to dog and indoor pollution made the risk of incident asthma increased[34]. In a cross-sectional study, researchers in Italy revealed that the severity of symptoms was more serious in polysensitized patients

with allergic rhinitis than in monosensitized[35]. In addition, for most allergens, they were significantly associated with allergic diseases only when their IgE level classes ≥ 2. Such findings may be of more clinical value than a dichotomous designation of sensitized or not sensitized to allergens, and can potentially be used for improving diagnosis of allergic diseases.

It is easy to think that allergens avoidance is the most effective means of prevention for allergy sufferers considering that allergic diseases are triggered by those allergens. However, this may not be true in some cases. Completely avoiding contact with some indoor and outdoor allergens is not always feasible. Moreover, early exposure to food allergens has been turned out to be a successful method for the prevention of peanut allergy[36]. Many studies reported that allergen immunotherapy was an effective treatment strategy for allergic diseases[37, 38]. However, for some patients with severe symptoms, allergen avoidance and treatment of symptoms may be necessary. Our findings could provide some evidence for clinicians about avoidance or specific immunotherapy treatment for patients with allergic diseases.

There are several strengths in this study. First, the sample size of this study is relatively large, and enabled us to divided specific IgE levels of 16 common allergens into three levels: class 1, class 2 and class 3. This enabled us to analyze the dose-response relationship between specific IgE levels and the prevalence of allergic diseases, and the combined effect of sensitization to multiple allergens of allergic diseases. Second, the 16 allergens were categorized into three categories: indoor allergens, outdoor allergens, and

food allergens, and the associations between each type of allergens and different allergic diseases were analyzed. Our study also suffers from several limitations.However, there are some limitations in this study. Firstly, we cannot determine when the patients was first sensitized to allergens or when they first developed an allergic disease. We did not collect the specific date of the patient's visit to the hospital, so we could not analyze the impact of seasonal factors on allergic diseases. Secondly, this study was performed only in one hospital in Harbin, the universality of our results for the whole China still needs to be further explored.

In conclusion, atopic dermatitis was associated with food allergens while allergic rhinitis and allergic asthma were related to both indoor and outdoor allergens sensitization in northeastern China. There was a dose-response relationship between levels of specific-allergic IgE levels and the prevalence of allergic diseases, and combined effects of multiple sensitizations to several allergens increased the risk of allergic diseases. Our findings could provide evidence to the control and management of allergiy-related diseases, and may have important public health implications.

Ethical approval

This study was authorized by the Institutional Review Board of Harbin Medical University.

Patient and Public Involvement

We recruited the participants into our research. Only those who signed the informed consent form were recruited into our study, and this project was authorized by the Institutional Review Board of Harbin Medical University. Patients with one of the three

common allergy-related diseases (atopic dermatitis, allergic asthma or allergic rhinitis), who visited the Department of Allergy of the First Affiliated Hospital of Harbin Medical University during March 2009 and December 2017 were collected as the candidate cases. Healthy adults who visited the same hospital for a physical examination were recruited as the candidate controls during the same period.

Funding

Data sharing statement

Data in this study were all collected by our own study team, and we may provide our data if there are reasonable requests.

Authors' contributions

W.Z. and B.X. designed the process of this study and the structure of this article. Y.W. and M.L. designed the questionnaire. W.Z. and B.X. participated in case collection and questionnaire investigation, and analyzed the data. W.Z. wrote the manuscript, and all other authors read and confirmed the final version.

Competing interests

None declared

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Table 1. Characteristics of atopic dermatitis, allergic rhinitis, allergic asthma and controls.

	Control	Atopic dermatitis	P[1]	Allergic rhinitis	P[a]	Allergic asthma	P[b]
Number	2576	2631		1320		1160	
Men, n (%)	732(28.42)	684(26.00)	0.0499	532(40.30)	<.0001	398(34.31)	0.0003
Age, years[2]	34.96(16.13)	36.70(15.33)	<.0001	34.59(13.76)	0.4868	41.60(17.25)	<.0001
Height, cm	160.40(14.71)	163.00(11.14)	<.0001	164.90(11.07)	<.0001	160.90(14.59)	0.3205
Weight, kg	58.43(15.95)	60.34(13.70)	<.0001	62.49(15.22)	<.0001	60.02(15.20)	0.0041
BMI, kg/m^{2}	22.26(4.17)	22.51(3.95)	0.0268	22.71(3.98)	0.0009	22.79(4.04)	0.0003
Rural, n (%)	750(29.12)	1020(38.75)	<.0001	374(28.32)	0.6533	416(35.88)	0.0003
Married, n (%)	1661(64.49)	1708(64.92)	0.8370	875(66.31)	0.4722	891(76.85)	<.0001
Cigarette smoking, n (%)	338(13.12)	437(16.61)	0.0004	194(14.70)	0.1752	296(25.52)	<.0001
Alcohol drinking, n (%)	317(12.31)	349(13.26)	0.3002	192(14.55)	0.0496	156(13.45)	0.3313
Physical exercise, n (%)	893(34.68)	897(34.08)	0.7735	490(37.15)	0.3281	319(27.52)	0.0015
Education, n (%)							
Junior high school or lower	1253(48.64)	1084(41.20)	<.0001	459(34.77)	<.0001	610(52.59)	0.0220
Senior high school	618(23.99)	734(27.90)		315(23.86)		280(24.14)	
University or higher	705(27.37)	813(30.90)		546(41.36)		270(23.28)	
Family history, n (%)	452(17.55)	1937(26.38)	<.0001	379(28.71)	<.0001	202(17.41)	0.9213

[a] P values comparing cases with controls.

[b] The quantitative variables were expressed as means and their standard deviation.

Table 2a ORs for allergic diseases with specific IgE class to indoor allergens.

		Atopic dermatitis (n=2631)			Allergic rhinitis (n=1320)			Allergic asthma (n=1160)		
Allergen category	Control (n=2576)	n	cOR[b] (95% CI)	aOR[b] (95% CI)	n	cOR[b] (95% CI)	aOR[b] (95% CI)	n	cOR[b] (95% CI)	aOR[b] (95% CI)
Dermatophagoides pteronyssinus										
No	2098	2107	1.00 (ref)	1.00 (ref)	1038	1.00 (ref)	1.00 (ref)	852	1.00 (ref)	1.00 (ref)

Class 1	177	203	1.14 (0.93-1.41)	1.17 (0.93-1.47)	90	1.03 (0.79-1.34)	0.89 (0.63-1.24)	70	0.97 (0.73-1.30)	1.19 (0.85-1.66)
Class 2	228	243	1.06 (0.88-1.28)	1.11 (0.91-1.36)	155	1.37 (1.11-1.71)	1.16 (0.90-1.51)	153	1.65 (1.33-2.06)	1.40 (1.07-1.83)
Class 3	73	78	1.06 (0.77-1.47)	1.19 (0.84-1.69)	37	1.02 (0.69-1.53)	0.80 (0.51-1.26)	85	2.87 (2.08-3.96)	3.68 (2.50-5.40)
P trend			0.3415			0.0306			<.0001	
Cat and dog fur										
No	2513	2539	1.00 (ref)	1.00 (ref)	1287	1.00 (ref)	1.00 (ref)	1089	1.00 (ref)	1.00 (ref)
Class 1	35	54	0.95 (0.59-1.53)	0.90 (0.54-1.49)	18	1.00 (0.57-1.78)	1.08 (0.58-2.01)	20	1.32 (0.76-2.30)	1.08 (0.58-1.99)
Class 2	14	20	1.40 (0.71-2.78)	1.67 (0.78-3.59)	8	1.12 (0.47-2.67)	1.26 (0.46-3.44)	16	2.64 (1.28-5.42)	4.10 (1.67-9.91)
Class 3	14	18	1.26 (0.63-2.54)	1.29 (0.62-2.69)	7	0.98 (0.39-2.43)	1.17 (0.46-3.82)	35	5.77 (3.09-10.77)	7.13 (3.44-14.79)
P trend			0.3498			0.9260			<.0001	
Mould mixture										
No	2205	2252	1.00 (ref)	1.00 (ref)	1059	1.00 (ref)	1.00 (ref)	990	1.00 (ref)	1.00 (ref)
Class 1	234	245	1.03 (0.85-1.24)	1.05 (0.86-1.29)	123	1.09 (0.87-1.38)	0.81 (0.61-1.08)	106	1.01 (0.79-1.28)	0.98 (0.74-1.29)
Class 2	100	97	0.95 (0.71-1.26)	1.07 (0.79-1.44)	80	1.67 (1.23-2.26)	1.58 (1.12-2.22)	46	1.03 (0.72-1.46)	0.67 (0.44-1.01)
Class 3	37	37	0.98 (0.62-1.55)	1.04 (0.65-1.67)	58	3.26 (2.15-4.96)	3.32 (2.14-5.14)	18	1.09 (0.62-1.91)	1.01 (0.55-1.86)
P trend			0.8736			<.0001			0.7814	

b, OR values of three allergic disease compared with controls.

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a OR, adjusted odds ratio.

c OR, crude odds ratio.

CI, confidence interval.

Table 2b ORs for allergic diseases with specific IgE class to outdoor allergens.

Allergen category	Control (n=2576)	Atopic dermatitis (n=2631)			Allergic rhinitis (n=1320)			Allergic asthma (n=1160)		
		n	cOR[b] (95% CI)	aOR[b] (95% CI)	n	cOR[b] (95% CI)	aOR[b] (95% CI)	n	cOR[b] (95% CI)	aOR[b] (95% CI)
Common ragweed and mugwort										
No	2325	2378	1.00 (ref)	1.00 (ref)	1130	1.00 (ref)	1.00 (ref)	1020	1.00 (ref)	1.00 (ref)
Class 1	113	114	0.99 (0.76-1.29)	0.97 (0.72-1.30)	58	1.06 (0.76-1.46)	0.95 (0.64-1.40)	38	0.77 (0.53-1.12)	0.75 (0.49-1.15)
Class 2	72	73	0.99 (0.71-1.38)	1.09 (0.74-1.60)	52	1.49 (1.03-2.14)	1.92 (1.23-2.99)	41	1.30 (0.88-1.92)	1.95 (1.17-3.27)
Class 3	66	66	0.98 (0.69-1.38)	0.75 (0.50-1.13)	80	2.49 (1.79-3.48)	1.87 (1.24-2.81)	61	2.11 (1.48-3.01)	2.26 (1.43-3.56)
P trend			0.8767			<.0001			0.0002	
German cockroach										
No	2391	2438	1.00 (ref)	1.00 (ref)	1225	1.00 (ref)	1.00 (ref)	1073	1.00 (ref)	1.00 (ref)
Class 1	113	114	0.99 (0.76-1.29)	0.79 (0.58-1.09)	58	1.00 (0.73-1.39)	0.75 (0.47-1.19)	52	1.03 (0.73-1.44)	1.35 (0.89-2.06)
Class 2	61	59	0.95 (0.66-1.36)	0.69 (0.44-1.09)	31	0.99 (0.64-1.54)	0.59 (0.32-1.10)	27	0.99 (0.62-1.56)	0.70 (0.37-1.31)
Class 3	11	20	1.78 (0.85-3.73)	1.32 (0.58-3.04)	6	1.07 (0.39-2.89)	0.57 (0.17-1.84)	8	1.62 (0.65-4.04)	1.84 (0.67-5.08)
P trend			0.5522			0.9707			0.5848	
Tree pollen mixture										
No	2249	2297	1.00 (ref)	1.00 (ref)	1078	1.00 (ref)	1.00 (ref)	1003	1.00 (ref)	1.00 (ref)

	Control	n	cOR (95% CI)	aOR (95% CI)	n	cOR (95% CI)	aOR (95% CI)	n	cOR (95% CI)	aOR (95% CI)
Class 1	169	174	1.01 (0.81-1.26)	0.99 (0.77-1.27)	76	0.94 (0.71-1.24)	0.86 (0.61-1.22)	74	0.98 (0.74-1.30)	0.96 (0.67-1.38)
Class 2	129	132	1.00 (0.78-1.29)	1.09 (0.81-1.46)	102	1.65 (1.26-2.16)	1.50 (1.06-2.14)	73	1.27 (0.94-1.71)	0.78 (0.51-1.18)
Class 3	29	28	0.95 (0.56-1.59)	0.82 (0.45-1.51)	64	4.60 (2.95-7.18)	3.79 (2.28-6.29)	10	0.77 (0.38-1.59)	0.51 (0.21-1.23)
P trend			0.9430			<.0001			0.4338	
Hop										
No	2412	2461	1.00 (ref)	1.00 (ref)	1150	1.00 (ref)	1.00 (ref)	1076	1.00 (ref)	1.00 (ref)
Class 1	90	89	0.97 (0.72-1.31)	0.89 (0.63-1.27)	46	1.07 (0.75-1.54)	1.28 (0.81-2.03)	50	1.25 (0.88-1.77)	1.15 (0.74-1.79)
Class 2	45	52	1.13 (0.76-1.69)	1.21 (0.77-1.91)	50	2.33 (1.55-3.51)	3.71 (2.24-6.13)	20	1.00 (0.59-1.70)	0.70 (0.36-1.35)
Class 3	29	29	0.98 (0.58-1.65)	0.85 (0.45-1.61)	74	5.35 (3.46-8.27)	6.89 (4.05-11.73)	14	1.08 (0.57-2.06)	0.68 (0.31-1.53)
P trend			0.8221			<.0001			0.5204	

b, OR values of three allergic disease compared with controls.

a OR, adjusted odds ratio.

c OR, crude odds ratio.

CI, confidence interval.

Table 2c ORs for allergic diseases with specific IgE class to food allergens.

Allergen category	Control (n=2576)	Atopic dermatitis (n=2631)			Allergic rhinitis (n=1320)			Allergic asthma (n=1160)		
		n	cOR[b] (95% CI)	aOR[b] (95% CI)	n	cOR[b] (95% CI)	aOR[b] (95% CI)	n	cOR[b] (95% CI)	aOR[b] (95% CI)

Egg white/egg yolk										
No	2461	2415	1.00 (ref)	1.00 (ref)	1267	1.00 (ref)	1.00 (ref)	1106	1.00 (ref)	1.00 (ref)
Class 1	78	79	1.03 (0.75-1.42)	1.12 (0.78-1.61)	32	0.80 (0.53-1.21)	0.64 (0.38-1.08)	35	1.00 (0.67-1.50)	0.90 (0.55-1.46)
Class 2	17	42	2.52 (1.43-4.44)	2.32 (1.28-4.19)	10	1.14 (0.52-2.50)	1.39 (0.55-3.55)	9	1.18 (0.52-2.65)	0.72 (0.29-1.81)
Class 3	20	95	4.84 (2.98-7.86)	2.65 (1.53-4.61)	11	1.07 (0.51-2.24)	1.55 (0.67-3.57)	10	1.11 (0.52-2.39)	0.75 (0.30-1.85)
P trend			<.0001			0.8415			0.7029	
Blue mussel										
No	2521	2503	1.00 (ref)	1.00 (ref)	1289	1.00 (ref)	1.00 (ref)	1126	1.00 (ref)	1.00 (ref)
Class 1	40	38	0.96 (0.61-1.50)	0.57 (0.33-0.98)	20	0.98 (0.57-1.68)	1.29 (0.64-2.60)	18	1.01 (0.58-1.77)	0.64 (0.30-1.36)
Class 2	7	29	4.17 (1.82-9.54)	3.27 (1.40-7.65)	6	1.68 (0.56-5.00)	0.94 (0.22-4.03)	8	2.56 (0.93-7.07)	0.80 (0.23-2.84)
Class 3	8	61	7.66 (3.66-16.04)	5.98 (2.79-12.80)	5	1.22 (0.40-3.74)	1.09 (0.31-3.80)	8	2.24 (0.84-5.98)	0.98 (0.28-3.35)
P trend			<.0001			0.5126			0.0335	
Fish										
No	2378	2354	1.00 (ref)	1.00 (ref)	1215	1.00 (ref)	1.00 (ref)	1068	1.00 (ref)	1.00 (ref)
Class 1	155	163	1.06 (0.85-1.33)	1.09 (0.83-1.42)	80	1.01 (0.77-1.34)	0.94 (0.64-1.39)	74	1.06 (0.80-1.42)	1.28 (0.88-1.86)
Class 2	27	53	1.98 (1.24-3.16)	2.13 (1.28-3.55)	16	1.16 (0.62-2.16)	0.74 (0.35-1.59)	8	0.66 (0.30-1.46)	0.53 (0.22-1.28)
Class 3	16	61	3.85	2.24	9	1.10	1.01	10	1.39	1.14

			(2.21-6.70)	(1.18-4.25)		(0.49-2.50)	(0.38-2.71)		(0.63-3.08)	(0.43-3.03)
P trend			<.0001			0.6747			0.7889	
Crab										
No	2423	2409	1.00 (ref)	1.00 (ref)	1235	1.00 (ref)	1.00 (ref)	1094	1.00 (ref)	1.00 (ref)
Class 1	120	122	1.02 (0.79-1.32)	0.95 (0.70-1.28)	62	1.01 (0.74-1.39)	0.92 (0.61-1.38)	44	0.81 (0.57-1.16)	0.79 (0.52-1.20)
Class 2	22	46	2.10 (1.26-3.51)	1.80 (1.04-3.12)	13	1.16 (0.58-2.31)	0.55 (0.25-1.20)	12	1.21 (0.60-2.45)	0.72 (0.31-1.66)
Class 3	11	54	4.94 (2.58-9.46)	4.75 (2.27-9.91)	10	1.78 (0.76-4.21)	0.66 (0.23-1.87)	10	2.01 (0.85-4.76)	1.45 (0.55-3.82)
P trend			<.0001			0.2796			0.5212	
Mutton										
No	2408	2450	1.00 (ref)	1.00 (ref)	1234	1.00 (ref)	1.00 (ref)	1078	1.00 (ref)	1.00 (ref)
Class 1	118	123	1.03 (0.79-1.33)	1.12 (0.85-1.48)	60	0.99 (0.72-1.36)	0.93 (0.63-1.36)	52	0.98 (0.71-1.38)	1.04 (0.72-1.51)
Class 2	31	39	1.24 (0.77-1.99)	1.28 (0.75-2.19)	16	1.01 (0.55-1.85)	0.74 (0.33-1.68)	15	1.08 (0.58-2.01)	0.48 (0.22-1.04)
Class 3	19	19	0.98 (0.52-1.86)	0.54 (0.25-1.14)	10	1.03 (0.48-2.22)	1.29 (0.50-3.35)	15	1.76 (0.89-3.48)	1.63 (0.72-3.66)
P trend			0.5808			0.9746			0.2266	
Milk										
No	2424	2398	1.00 (ref)	1.00 (ref)	1243	1.00 (ref)	1.00 (ref)	1090	1.00 (ref)	1.00 (ref)
Class 1	126	119	0.96 (0.74-1.23)	1.00 (0.76-1.32)	64	0.99 (0.73-1.35)	0.93 (0.65-1.33)	50	0.88 (0.63-1.23)	0.95 (0.66-1.37)

Class 2	16	51	3.22 (1.83-5.67)	3.61 (1.96-6.65)	7	0.85 (0.35-2.08)	1.28 (0.42-3.93)	10	1.39 (0.63-3.07)	1.36 (0.55-3.40)
Class 3	10	63	6.36 (3.26-12.43)	5.84 (2.73-12.50)	6	1.17 (0.42-3.23)	1.02 (0.26-4.07)	10	2.22 (0.92-5.36)	1.40 (0.48-4.09)
P trend			<.0001			0.9814			0.2746	
Beef										
No	2443	2498	1.00 (ref)	1.00 (ref)	1250	1.00 (ref)	1.00 (ref)	1097	1.00 (ref)	1.00 (ref)
Class 1	94	91	0.95 (0.71-1.27)	0.91 (0.66-1.26)	48	1.00 (0.70-1.42)	0.94 (0.62-1.41)	34	0.81 (0.54-1.20)	0.86 (0.55-1.33)
Class 2	19	21	1.08 (0.58-2.02)	0.22 (0.09-0.51)	11	1.13 (0.54-2.39)	1.07 (0.42-2.76)	14	1.64 (0.82-3.29)	1.04 (0.45-2.38)
Class 3	20	21	1.03 (0.56-1.90)	0.53 (0.25-1.14)	11	1.08 (0.51-2.25)	1.56 (0.55-4.44)	15	1.67 (0.85-3.28)	1.31 (0.57-3.03)
P trend			0.9970			0.7771			0.1923	
Shrimp										
No	2455	2434	1.00 (ref)	1.00 (ref)	1270	1.00 (ref)	1.00 (ref)	1098	1.00 (ref)	1.00 (ref)
Class 1	102	102	1.01 (0.76-1.34)	0.92 (0.66-1.28)	39	0.74 (0.51-1.08)	0.66 (0.40-1.08)	46	1.01 (0.71-1.44)	0.87 (0.56-1.37)
Class 2	9	24	2.69 (1.25-5.80)	4.07 (1.62-10.22)	5	1.07 (0.36-3.21)	0.58 (0.15-2.18)	8	1.99 (0.77-5.17)	2.03 (0.65-6.34)
Class 3	10	71	7.15 (3.68-13.89)	5.18 (2.49-10.75)	6	1.16 (0.42-3.20)	0.85 (0.25-2.82)	8	1.79 (0.70-4.55)	1.92 (0.69-5.35)
P trend			<.0001			0.4474			0.1490	
Wheat										
No	2354	2400	1.00 (ref)	1.00 (ref)	1195	1.00 (ref)	1.00 (ref)	1054	1.00 (ref)	1.00 (ref)

Class 1	175	183	1.03 (0.83-1.27)	0.95 (0.74-1.22)	100	1.13 (0.87-1.45)	0.73 (0.53-1.02)	79	1.01 (0.77-1.33)	0.91 (0.66-1.27)
Class 2	26	26	0.98 (0.57-1.69)	1.00 (0.52-1.94)	13	0.99 (0.50-1.92)	0.61 (0.27-1.38)	14	1.20 (0.63-2.31)	0.89 (0.37-2.18)
Class 3	21	22	1.03 (0.56-1.87)	0.86 (0.44-1.68)	12	1.13 (0.55-2.30)	0.66 (0.27-1.60)	13	1.38 (0.69-2.77)	1.36 (0.59-3.11)
P trend			0.8730			0.4715			0.3747	

b, OR values of three allergic disease compared with controls.

a OR, adjusted odds ratio.

c OR, crude odds ratio.

CI, confidence interval.

Table 3 ORs for allergic diseases in relation to number of allergens.

	Control (n=2576)	n	cOR[d] (95% CI)	aOR[d] (95% CI)
		Atopic dermatitis (n=2631)		
Number of allergens [b1]				
No	2422	2142	1.00 (ref)	1.00 (ref)
1	135	375	3.14(2.56-3.86)	3.00(2.44-3.69)
2	19	79	4.70(2.84-7.78)	4.28(2.57-7.11)
≥3	0	35	-	-
P trend			<.0001	
		Allergic rhinitis (n=1320)		
Number of allergens [b2]				
No	2181	893	1.00 (ref)	1.00 (ref)
1	286	312	2.66(2.23-3.19)	2.64(2.20-3.18)
2	106	97	2.24(1.68-2.98)	2.13(1.58-2.87)
≥3	3	18	14.65(4.31-49.87)	13.00(3.76-45.00)
P trend			<.0001	
		Allergic asthma (n=1160)		
Number of allergens [b3]				
No	2183	845	1.00 (ref)	1.00 (ref)
1	319	243	1.97(1.64-2.37)	2.06(1.70-2.49)
2	74	68	2.37(1.69-3.33)	2.37(1.67-3.37)
≥3	0	4	-	-
P trend			<.0001	

d, OR values of three allergic disease compared with controls.

a OR, adjusted odds ratio.

c OR, crude odds ratio.

CI, confidence interval.

[b1] allergens excluding those allergens which were not related with atopic dermatitis in Table 2a, Table 2b and Table 2c. [b2] allergens excluding those allergens which were not related with allergic rhinitis in Table 2a, Table 2b and Table 2c. [b3] allergens excluding those allergens which were not related with allergic asthma in Table 2a, Table 2b and Table 2c.
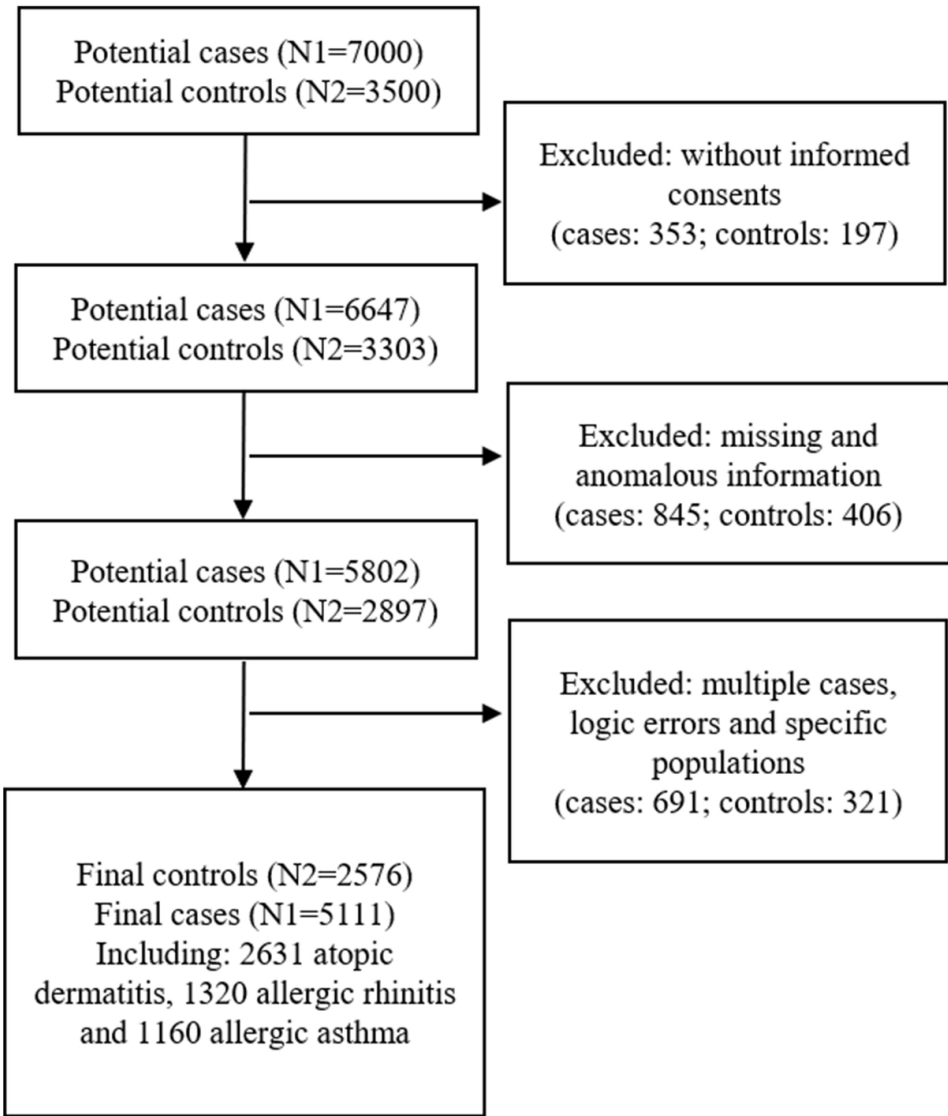
Figure 1 The flow diagram of study participants.
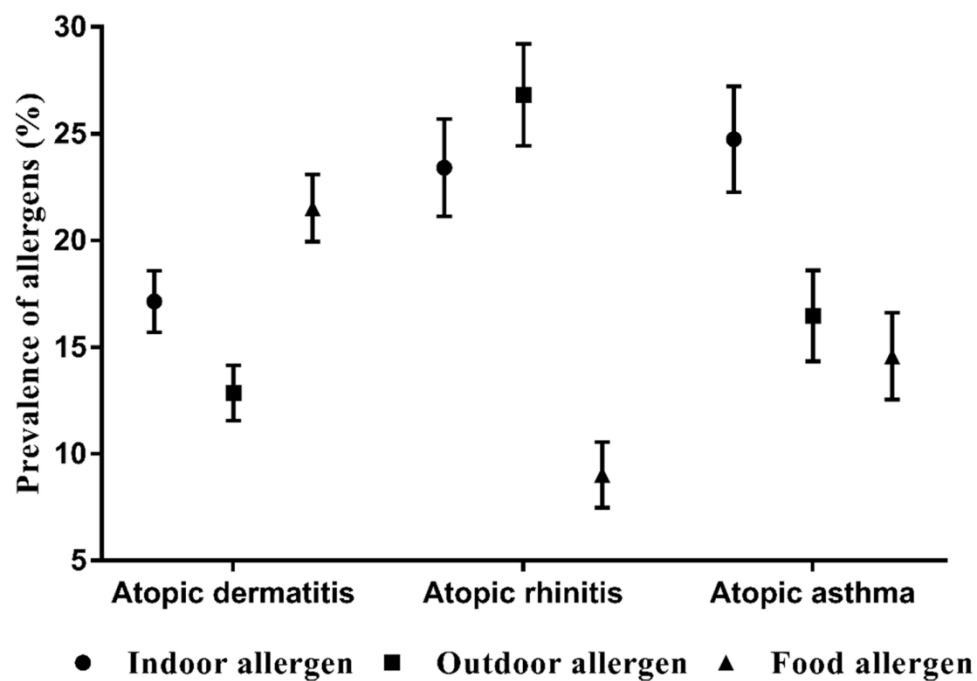
107x124mm (300 x 300 DPI)

Figure 2. Prevalence of allergens sensitization among atopic dermatitis, allergic rhinitis and allergic asthma in Chinese adults

129x92mm (300 x 300 DPI)

Associations between sensitization to allergens and allergic diseases: a hospital-based case-control study in China

Supplementary Method:

The definition of demographic, family history and lifestyle factors

Age was calculated as the difference between the year of birth and the year of interview. Educational status was categorized into three levels: junior high school or lower, senior high school, and university or higher. Residential region was categorized into two groups: urban and rural. Marital status was also categorized into two groups: unmarried and married. Current smokers were defined on the basis of the World Health Organization criteria, as those who self-reported smoking every day for at least 6 months[9]. Regular alcohol drinkers were defined as drinking more than twice per week for at least one year. Classification of physical exercise (PE) was defined as followed:

We estimated PE condition of study participants using three variables of structured questionnaire, exercise intensity (EI), exercise time (ET) and exercise frequency (EF) [1-4].

EI of study participants were classified into three groups, mild, moderate and strenuous exercise according to their exercise types. Value 1, 2 and 3 were assigned to the three groups respectively [5-8]. ET was measured by the hour. EF referred to how many times a week. We defined PE as

$$PE = \sum_{i=1}^{N} EI \times ET$$

Where N represented EF. If PE $\geq$ 2, PE was positive; PE was negative when PE < 2. Therefore, as for a subject jogging (moderate exercise) 15 minutes 5 times a week, his/her PE was positive because PE $\geq$ 2 (PE $= 5 \times 0.25 \times 2 = 2.5$).

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Supplementary Table:

Supplementary Table S1. Sixteen common allergens used in this study.

Dermatophagoides pteronyssinus	mould mixture [a]	fish	beef

egg white/egg yolk	German cockroach	crab	shrimp
common ragweed and mugwort	blue mussel	mutton	wheat
cat and dog fur	tree pollen mixture [b]	milk	Hop

[a] Mould mixture is composed of *Penicillium notatum, Cladosporium herbarum, Aspergillus fumigatus* and *Alternaria alternate.*

[b] Tree pollen mixture is composed of *Robur, Elm, London Plane, Willow* and *cottonwood.*

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Associations between sensitization to allergens and allergic diseases: a hospital-based case-control study in China

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Manuscripts

1   **Associations between sensitization to allergens and allergic**

2   **diseases: a hospital-based case-control study in China**

3

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5

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Abstract

Objectives: To assess the associations of sensitization to independent and combined common allergens with atopic dermatitis, allergic rhinitis allergic, and asthma in Harbin, China, and to provide evidence for the prevention and treatment of the three allergic diseases.

Design: Case-control study.

Setting: Data was collected from the First Affiliated Hospital of Harbin Medical University in Harbin, China.

Participants: 5111 patients (2631 with atopic dermatitis, 1320 with allergic asthma, and 1160 with allergic rhinitis) were recruited from the Department of Allergy from March 2009 to December 2017, and 2576 controls were from the same hospital for a physical examination during the same period.

Main outcome measures: Odds ratios (*ORs*) and 95% confidence intervals (95% *CIs*) for the association between specific IgE levels of 16 different allergens and prevalence of allergic disease, and ORs and 95% CIs for examining the combined effects of sensitization to multivariate allergens on allergic diseases by multivariate logistic regression models.

Results: The prevalence of sensitization to food allergens, outdoor allergens, indoor allergens were higher in patients with atopic dermatitis, allergic rhinitis, allergic asthma, respectively (atopic dermatitis: indoor=17.14%, outdoor=12.85%, food=21.44%; allergic rhinitis: indoor=23.18%, outdoor=26.81%, food=8.94%; allergic asthma: indoor=24.65%, outdoor=16.46%, food=14.31%). After adjusting for potential confounding variables, there was a dose-response relevance between levels of allergen-specific IgE and the allergic diseases (*P* trend <0.0001). The associations of allergic diseases with the number of allergens were significant (atopic dermatitis: highest adjusted OR=4.28,

2

1 95% CI 2.57 to 7.11; allergic rhinitis: highest adjusted OR=13.00, 95% CI 3.76 to 45.00; allergic

2 asthma: OR=2.37, 95% CI 1.67 to 3.37).

3 **Conclusion** There was a dose-response relevance between levels of allergen-specific IgE and allergic

4 diseases' prevalence, and multiple sensitizations to diverse allergens increased the risk of allergic

5 diseases. This study provided evidence for the prophylaxis and treatment of the three allergic diseases.

6 **Keywords:** allergic diseases; allergens; association; case-control study

7

8 **Strengths and limitations of this study**

9 • This study not only assessed the dose-response relations between independent allergens and

10 allergic diseases but also explored the associations between the number of allergens and allergic

11 diseases.

12 • Various types of allergens were considered and categorized into indoor, outdoor and food

13 allergens.

14 • Large size of participants.

15 • Potential confounding variables were taken into consideration.

16 • Multicenter studies in more regions of China need to be further explored.

17

18 **Introduction**

19 Allergic diseases are global and can profoundly affect the quality of life of patients[1]. The incidence of

20 allergic diseases, including atopic dermatitis, allergic rhinitis, and allergic asthma, has been increasing

21 rapidly around the world, especially in China and other low-and middle-income countries [2-4]. As

3

1   triggering factors, allergens stimulate the body to produce abnormal immune responses, which

2   eventually lead to allergic diseases[5]. The type and dose of allergens determine the type of allergic

3   disease and its severity[6]. Therefore, it is crucial to clarify the distributions of positive allergens among

4   patients with different allergic diseases.

5   Currently, many studies have been devoted to exploring the association between sensitization to

6   allergens and allergic diseases. Gray CL et al. randomly enrolled 100 children aged 6 months to 10

7   years with atopic dermatitis and found that a high prevalence of egg sensitization of 54%, and SPT to

8   fresh egg white showed a high predictive value for egg allergy[7]. A Japanese study explored the causal

9   relationships among pollen counts, tweet numbers, and patient numbers for seasonal allergic rhinitis,

10   and reported a positive correlation between pollen counts and the patient numbers[8]. Sensitization and

11   exposure to indoor allergens (such as dust mites, cockroaches, wild rodents, pets, and fungi) have been

12   reported to be associated with allergic diseases, especially the development of asthma[9]. Patients with

13   allergies usually have different levels of specific IgE to a certain allergen and allergens are often co-

14   existent, so it is critical to understand associations among multiple allergens and allergic diseases[10].

15   According to previous studies, the incidence of allergic diseases varies among different geographic

16   locations and subpopulations. For Koreans, high sensitizations to various types of pollen were in the

17   Gangwon region, whereas sensitization to Japanese cedar pollen was unique in the Jeju region. Among

18   the individuals from the Americas, grass pollen and animal dander allergies were relatively common,

19   while weed and grass pollen allergies were common in people from Central Asia. Therefore, it is

20   suggested that genetic and environmental features may play important roles in allergic diseases, and

21   it is interesting to understand the underlying causes of those variations[11,12]. At present, most relevant

4

1   studies have a limited sample size, and few such studies have been conducted in northeastern China

2   with its distinctive environmental and climate conditions[13-15].

3   In the present study, we assessed the associations between sensitization to 16 allergens and three

4   common allergic diseases in northeastern China and explored if there is a dose-response connection

5   between the specific IgE levels to an allergen and of allergic diseases' prevalence. We also examined

6   the combined effects of multiple allergens on a specific allergic disease. Our study aimed to supply

7   basis for the prevention and management of allergic diseases.

8   **Materials and methods**

9   **Participants and Public involvement**

10   Harbin is located in northeast of China with longitude spanning 125°42'-130°10' E, and latitude

11   44°04'-46°40' N, which is the provincial capital of Heilongjiang Province. Under the direct influence

12   of the Siberian Anticyclone, the average daily temperature is -19.7℃ (-3.5℉) in winter. Annual low

13   temperatures below -35.0℃ (-31.0℉) are not uncommon. Nicknamed "Ice City" due to its freezingly

14   cold winter. We recruited the participants into our research from Harbin. Only those who signed the

15   informed consent form were recruited into our study. This project was authorized by the Institutional

16   Review Board of Harbin Medical University. Adult patients aged 18 years or older with one of the

17   three common allergic diseases (atopic dermatitis, allergic asthma, or allergic rhinitis), who visited the

18   Department of Allergy of the First Affiliated Hospital of Harbin Medical University during March

19   2009 and December 2017 were collected as the candidate cases. Healthy adults without any clinical

20   symptoms and diseases who underwent physical examination at the same hospital during the same

21   period were recruited as the candidate controls. All participants carried on allergen-specific IgE tests

5

1 to 16 kinds of allergens and a questionnaire investigation.

2 **Allergen-specific IgE testing**

3 Serum allergen-specific IgE concentrations of 16 common allergens were measured in all eligible

4 participants using the AllergyScreen system (Mediwise Analytic GmbH, Germany). As shown in

5 Supplementary Information, the 16 allergens were defined as three types: indoor allergens, outdoor

6 allergens, and food allergens[16]. Based on previous research, allergens-specific IgE > 0.35 kU/l was

7 considered as allergens sensitization in this study, and specific IgE concentration of 0.35-0.70 kU/l

8 was defined as class 1, 0.70-3.50 kU/l as class 2, and > 3.50 kU/l as class 3[17].

9

10 **Allergic diseases definition**

11 We focused on three common allergic diseases (atopic dermatitis, allergic asthma, and allergic rhinitis)

12 in Harbin. The three allergic diseases are diagnosed by experienced physicians using existing

13 benchmarks for atopic dermatitis[18], allergic rhinitis[19,] and allergic asthma[20]. The detailed diagnostic

14 criteria were shown in Supplementary Information. Besides, the following criteria should be obeyed:

15 (1) There were at least have one positive outcome of the 16 allergen-specific IgE results;

16 (2) The patients had been diagnosed with one of the allergic diseases using current guidelines

17   mentioned above.

18

19 **Demographic, lifestyle characteristics, and family history**

20 All of the participants in this study answered the questionnaire face to face to collect data on

21 demographic, lifestyle characteristics and family history. Gender, age, level of education, residential

6

1   region, and marital status were analyzed as demographic characteristics. Smoking, drinking, and

2   physical exercise were included in lifestyle factors. We also collected the family history information

3   of patients with allergic diseases (atopic dermatitis, allergic rhinitis, and allergic asthma). The

4   definition of those factors was shown in the Supplementary Information.

5

6   **Statistical analysis**

7   To assess the dose-response relationship between levels of allergen-specific IgE to 16 different

8   allergens and prevalence of allergic diseases, we apply logistic regressions to evaluate odds ratios

9   (*OR*) and their 95% confidence intervals (*CI*). Those significantly associated with at least one of

10   allergic diseases in univariate analysis or previously suggested in the literature were chosen as

11   confounding factors. They included gender, age, BMI, level of education, smoking, drinking, living

12   region, marital status, family history of allergic diseases, physical exercise, and other 15 allergens

13   specific-IgE responses. To inspect the combined effects of multiple allergens on allergic diseases,

14   multivariate logistic regressions were conducted with different numbers for sensitization with one

15   allergen, two allergens, and three or more allergens. The statistical analysis software of this study was

16   SAS 9.1 (SAS Institute Inc., Cary, NC, USA).

17

18   **Results**

19   **Participants**

20   In the present study, 7000 candidate cases and 3500 candidate controls were initially recruited.

21   Excluding those without written informed consents, 6647 candidate cases and 3303 candidate controls

7

1 remained. After excluding those with missing values and logic errors, and some special populations

2 (sportsmen, pregnant or lactating women), we had 5111 cases (2631 atopic dermatitis, 1320 allergic

3 rhinitis, and 1160 allergic asthma) and 2576 controls for our final analysis. The flow diagram of the

4 screening process of study participants is summarized in Figure 1.

5

6 **Characteristics in cases and controls**

7 There are statistical differences between patients with atopic dermatitis and controls in terms of gender,

8 age, height, weight, BMI, residential region, smoking, education, and family history ($P<0.05$). Patients

9 with allergic rhinitis were significantly different from controls in gender, height, weight, BMI,

10 education, and family history ($P<0.05$). Gender, age, weight, BMI, residential region, smoking, levels

11 of education, marital status, and physical exercise were different between those with allergic asthma

12 and controls ($P<0.05$). Detail demographic characteristics were summarized in Table 1.

13

8

1   **Table 1. Characteristics of atopic dermatitis, allergic rhinitis, allergic asthma and controls.**

	Control	Atopic dermatitis	P [a]	Allergic rhinitis	P [a]	Allergic asthma	P [a]
Number	2576	2631		1320		1160	
Men, n (%)	732(28.42)	684(26.00)	0.0499	532(40.30)	<.0001	398(34.31)	0.0003
Age, years [b]	34.96(16.13)	36.70(15.33)	<.0001	34.59(13.76)	0.4868	41.60(17.25)	<.0001
Height, cm [b]	160.40(14.71)	163.00(11.14)	<.0001	164.90(11.07)	<.0001	160.90(14.59)	0.3205
Weight, kg [b]	58.43(15.95)	60.34(13.70)	<.0001	62.49(15.22)	<.0001	60.02(15.20)	0.0041
BMI, kg/m^2 [b]	22.26(4.17)	22.51(3.95)	0.0268	22.71(3.98)	0.0009	22.79(4.04)	0.0003
Rural, n (%)	750(29.12)	1020(38.75)	<.0001	374(28.32)	0.6533	416(35.88)	0.0003
Married, n (%)	1661(64.49)	1708(64.92)	0.8370	875(66.31)	0.4722	891(76.85)	<.0001
smoking, n (%)	338(13.12)	437(16.61)	0.0004	194(14.70)	0.1752	296(25.52)	<.0001
Alcohol drinking, n (%)	317(12.31)	349(13.26)	0.3002	192(14.55)	0.0496	156(13.45)	0.3313
Physical exercise, n (%)	893(34.68)	897(34.08)	0.7735	490(37.15)	0.3281	319(27.52)	0.0015
Education, n (%)							
Junior high school or lower	1253(48.64)	1084(41.20)	<.0001	459(34.77)	<.0001	610(52.59)	0.0220
Senior high school	618(23.99)	734(27.90)		315(23.86)		280(24.14)	
University or higher	705(27.37)	813(30.90)		546(41.36)		270(23.28)	
Family history, n (%)	452(17.55)	1937(26.38)	<.0001	379(28.71)	<.0001	202(17.41)	0.9213

2   [a] P values comparing cases with controls.

3   [b] The quantitative variables were expressed as means and their standard deviation.

4

5   **Prevalence of allergen sensitization in atopic dermatitis, allergic rhinitis, and allergic asthma**

6   Patients with different allergic diseases had different prevalence of allergens sensitization. Among

7   patients with atopic dermatitis, the prevalence of food allergens sensitization was higher than other

8   allergens. The prevalence of outdoor allergens was higher in those with allergic rhinitis while that of

9   indoor allergens was higher in patients with allergic asthma (Figure 2).

10   Adjusted for gender, age, BMI, level of education, smoking, drinking, living region, family history,

11   physical exercise, and other 15 allergens specific-IgE responses, specific IgE to common ragweed and

12   mugwort, Dermatophagoides pteronyssinus, dog fur, and cat fur were significantly related to the risk

13   of allergic asthma (Table 2 and Table 3). Allergic rhinitis was significantly associated with common

14   ragweed and mugwort, mould mixture, tree pollen mixture, and Hop, while atopic dermatitis was

15   significantly associated with egg white/egg yolk, crab, blue mussel, milk, fish, and shrimp (Table 3

9

1   2   3   4   5   6   7   8   9   10   11   12   13   14   15   16   17   18   19   20   21   22   23   24   25   26   27   28   29   30   31   32   33   34   35   36   37   38   39   40   41   42   43   44   45   46   47   48   49   50   51   52   53   54   55   56   57   58   59   60

1   and Table 4). There was a dose-response relationship between specific IgE levels, from class 1 to class

2   3, and the prevalence of allergic diseases, the prevalence of allergic diseases increased along with the

3   increasing levels of allergen-specific IgE to allergens (*P* trend <0.0001).

4

10

1

Table 2. ORs for allergic diseases with specific IgE class to indoor allergens.

Allergen category	Control (n=2576)	Atopic dermatitis (n=2631)			Allergic rhinitis (n=1320)			Allergic asthma (n=1160)		
		n	cOR[b] (95% CI)	aOR[b] (95% CI)	n	cOR[b] (95% CI)	aOR[b] (95% CI)	n	cOR[b] (95% CI)	aOR[b] (95% CI)
Dermatophagoides pteronyssinus										
No	2098	2107	1.00 (ref)	1.00 (ref)	1038	1.00 (ref)	1.00 (ref)	852	1.00 (ref)	1.00 (ref)
Class 1	177	203	1.14 (0.93-1.41)	1.17 (0.93-1.47)	90	1.03 (0.79-1.34)	0.89 (0.63-1.24)	70	0.97 (0.73-1.30)	1.19 (0.85-1.66)
Class 2	228	243	1.06 (0.88-1.28)	1.11 (0.91-1.36)	155	1.37 (1.11-1.71)	1.16 (0.90-1.51)	153	1.65 (1.33-2.06)	1.40 (1.07-1.83)
Class 3	73	78	1.06 (0.77-1.47)	1.19 (0.84-1.69)	37	1.02 (0.69-1.53)	0.80 (0.51-1.26)	85	2.87 (2.08-3.96)	3.68 (2.50-5.40)
P trend			0.3415			0.0306			<.0001	
Cat and dog fur										
No	2513	2539	1.00 (ref)	1.00 (ref)	1287	1.00 (ref)	1.00 (ref)	1089	1.00 (ref)	1.00 (ref)
Class 1	35	54	0.95 (0.59-1.53)	0.90 (0.54-1.49)	18	1.00 (0.57-1.78)	1.08 (0.58-2.01)	20	1.32 (0.76-2.30)	1.08 (0.58-1.99)
Class 2	14	20	1.40 (0.71-2.78)	1.67 (0.78-3.59)	8	1.12 (0.47-2.67)	1.26 (0.46-3.44)	16	2.64 (1.28-5.42)	4.10 (1.67-9.91)
Class 3	14	18	1.26 (0.63-2.54)	1.29 (0.62-2.69)	7	0.98 (0.39-2.43)	1.17 (0.46-3.02)	35	5.77 (3.09-10.77)	7.13 (3.44-14.79)
P trend			0.3498			0.9260			<.0001	
Mould mixture										
No	2205	2252	1.00 (ref)	1.00 (ref)	1059	1.00 (ref)	1.00 (ref)	990	1.00 (ref)	1.00 (ref)
Class 1	234	245	1.03 (0.85-1.24)	1.05 (0.86-1.29)	123	1.09 (0.87-1.38)	0.81 (0.61-1.08)	106	1.01 (0.79-1.28)	0.98 (0.74-1.29)

11

Class 2	100	97	0.95 (0.71-1.26)	1.07 (0.79-1.44)	80	1.67 (1.23-2.26)	1.58 (1.12-2.22)	46	1.03 (0.72-1.46)	0.67 (0.44-1.01)
Class 3	37	37	0.98 (0.62-1.55)	1.04 (0.65-1.67)	58	3.26 (2.15-4.96)	3.32 (2.14-5.14)	18	1.09 (0.62-1.91)	1.01 (0.55-1.86)
P trend			0.8736			<.0001			0.7814	

1 b, OR values of three allergic disease compared with controls.

2 a OR, adjusted odds ratio.

3 c OR, crude odds ratio.

4 CI, confidence interval.

5

6

Table 3. ORs for allergic diseases with specific IgE class to outdoor allergens.

Allergen category	Control (n=2576)	Atopic dermatitis (n=2631)			Allergic rhinitis (n=1320)			Allergic asthma (n=1160)		
		n	cOR[b] (95% CI)	aOR[b] (95% CI)	n	cOR[b] (95% CI)	aOR[b] (95% CI)	n	cOR[b] (95% CI)	aOR[b] (95% CI)
Common ragweed and mugwort										
No	2325	2378	1.00 (ref)	1.00 (ref)	1130	1.00 (ref)	1.00 (ref)	1020	1.00 (ref)	1.00 (ref)
Class 1	113	114	0.99 (0.76-1.29)	0.97 (0.72-1.30)	58	1.06 (0.76-1.46)	0.95 (0.64-1.40)	38	0.77 (0.53-1.12)	0.75 (0.49-1.15)
Class 2	72	73	0.99 (0.71-1.38)	1.09 (0.74-1.60)	52	1.49 (1.03-2.14)	1.92 (1.23-2.99)	41	1.30 (0.88-1.92)	1.95 (1.17-3.27)
Class 3	66	66	0.98 (0.69-1.38)	0.75 (0.50-1.13)	80	2.49 (1.79-3.48)	1.87 (1.24-2.81)	61	2.11 (1.48-3.01)	2.26 (1.43-3.56)
P trend			0.8767			<.0001			0.0002	
German cockroach										

12

No	2391	2438	1.00 (ref)	1.00 (ref)	1225	1.00 (ref)	1.00 (ref)	2073	1.00 (ref)	1.00 (ref)
Class 1	113	114	0.99 (0.76-1.29)	0.79 (0.58-1.09)	58	1.00 (0.73-1.39)	0.75 (0.47-1.19)	152	1.03 (0.73-1.44)	1.35 (0.89-2.06)
Class 2	61	59	0.95 (0.66-1.36)	0.69 (0.44-1.09)	31	0.99 (0.64-1.54)	0.59 (0.32-1.10)	127	0.99 (0.62-1.56)	0.70 (0.37-1.31)
Class 3	11	20	1.78 (0.85-3.73)	1.32 (0.58-3.04)	6	1.07 (0.39-2.89)	0.57 (0.17-1.84)	8	1.62 (0.65-4.04)	1.84 (0.67-5.08)
P trend			0.5522			0.9707			0.5848	
Tree pollen mixture										
No	2249	2297	1.00 (ref)	1.00 (ref)	1078	1.00 (ref)	1.00 (ref)	2003	1.00 (ref)	1.00 (ref)
Class 1	169	174	1.01 (0.81-1.26)	0.99 (0.77-1.27)	76	0.94 (0.71-1.24)	0.86 (0.61-1.22)	174	0.98 (0.74-1.30)	0.96 (0.67-1.38)
Class 2	129	132	1.00 (0.78-1.29)	1.09 (0.81-1.46)	102	1.65 (1.26-2.16)	1.50 (1.06-2.14)	173	1.27 (0.94-1.71)	0.78 (0.51-1.18)
Class 3	29	28	0.95 (0.56-1.59)	0.82 (0.45-1.51)	64	4.60 (2.95-7.18)	3.79 (2.28-6.29)	10	0.77 (0.38-1.59)	0.51 (0.21-1.23)
P trend			0.9430			<.0001			0.4338	
Hop										
No	2412	2461	1.00 (ref)	1.00 (ref)	1150	1.00 (ref)	1.00 (ref)	2076	1.00 (ref)	1.00 (ref)
Class 1	90	89	0.97 (0.72-1.31)	0.89 (0.63-1.27)	46	1.07 (0.75-1.54)	1.28 (0.81-2.03)	250	1.25 (0.88-1.77)	1.15 (0.74-1.79)
Class 2	45	52	1.13 (0.76-1.69)	1.21 (0.77-1.91)	50	2.33 (1.55-3.51)	3.71 (2.24-6.13)	20	1.00 (0.59-1.70)	0.70 (0.36-1.35)
Class 3	29	29	0.98 (0.58-1.65)	0.85 (0.45-1.61)	74	5.35 (3.46-8.27)	6.89 (4.05-11.73)	14	1.08 (0.57-2.06)	0.68 (0.31-1.53)

13

P trend			0.8221			<.0001			0.5204	

b, OR values of three allergic disease compared with controls.

a OR, adjusted odds ratio.

c OR, crude odds ratio.

CI, confidence interval.

Table 4. ORs for allergic diseases with specific IgE class to food allergens.

		Atopic dermatitis (n=2631)			Allergic rhinitis (n=1320)			Allergic asthma (n=1160)		
Allergen category	Control (n=2576)	n	cOR[b] (95% CI)	aOR[b] (95% CI)	n	cOR[b] (95% CI)	aOR[b] (95% CI)	n	cOR[b] (95% CI)	aOR[b] (95% CI)
Egg white/egg yolk										
No	2461	2415	1.00 (ref)	1.00 (ref)	1267	1.00 (ref)	1.00 (ref)	1106	1.00 (ref)	1.00 (ref)
Class 1	78	79	1.03 (0.75-1.42)	1.12 (0.78-1.61)	32	0.80 (0.53-1.21)	0.64 (0.38-1.08)	35	1.00 (0.67-1.50)	0.90 (0.55-1.46)
Class 2	17	42	2.52 (1.43-4.44)	2.32 (1.28-4.19)	10	1.14 (0.52-2.50)	1.39 (0.55-3.55)	9	1.18 (0.52-2.65)	0.72 (0.29-1.81)
Class 3	20	95	4.84 (2.98-7.86)	2.65 (1.53-4.61)	11	1.07 (0.51-2.24)	1.55 (0.67-3.57)	10	1.11 (0.52-2.39)	0.75 (0.30-1.85)
P trend			<.0001			0.8415			0.7029	
Blue mussel										
No	2521	2503	1.00 (ref)	1.00 (ref)	1289	1.00 (ref)	1.00 (ref)	1126	1.00 (ref)	1.00 (ref)
Class 1	40	38	0.96 (0.61-1.50)	0.57 (0.33-0.98)	20	0.98 (0.57-1.68)	1.29 (0.64-2.60)	18	1.01 (0.58-1.77)	0.64 (0.30-1.36)
Class 2	7	29	4.17	3.27	6	1.68	0.94	8	2.56	0.80

14

			(1.82-9.54)	(1.40-7.65)		(0.56-5.00)	(0.22-4.03)		(0.93-7.07)	(0.23-2.84)
Class 3	8	61	7.66	5.98	5	1.22	1.09	8	2.24	0.98
			(3.66-16.04)	(2.79-12.80)		(0.40-3.74)	(0.31-3.80)		(0.84-5.98)	(0.28-3.35)
P trend			<.0001			0.5126			0.0335	
Fish										
No	2378	2354	1.00 (ref)	1.00 (ref)	1215	1.00 (ref)	1.00 (ref)	1068	1.00 (ref)	1.00 (ref)
Class 1	155	163	1.06	1.09	80	1.01	0.94	74	1.06	1.28
			(0.85-1.33)	(0.83-1.42)		(0.77-1.34)	(0.64-1.39)		(0.80-1.42)	(0.88-1.86)
Class 2	27	53	1.98	2.13	16	1.16	0.74	8	0.66	0.53
			(1.24-3.16)	(1.28-3.55)		(0.62-2.16)	(0.35-1.59)		(0.30-1.46)	(0.22-1.28)
Class 3	16	61	3.85	2.24	9	1.10	1.01	10	1.39	1.14
			(2.21-6.70)	(1.18-4.25)		(0.49-2.50)	(0.38-2.71)		(0.63-3.08)	(0.43-3.03)
P trend			<.0001			0.6747			0.7889	
Crab										
No	2423	2409	1.00 (ref)	1.00 (ref)	1235	1.00 (ref)	1.00 (ref)	1094	1.00 (ref)	1.00 (ref)
Class 1	120	122	1.02	0.95	62	1.01	0.92	44	0.81	0.79
			(0.79-1.32)	(0.70-1.28)		(0.74-1.39)	(0.61-1.38)		(0.57-1.16)	(0.52-1.20)
Class 2	22	46	2.10	1.80	13	1.16	0.55	12	1.21	0.72
			(1.26-3.51)	(1.04-3.12)		(0.58-2.31)	(0.25-1.20)		(0.60-2.45)	(0.31-1.66)
Class 3	11	54	4.94	4.75	10	1.78	0.66	10	2.01	1.45
			(2.58-9.46)	(2.27-9.91)		(0.76-4.21)	(0.23-1.87)		(0.85-4.76)	(0.55-3.82)
P trend			<.0001			0.2796			0.5212	
Mutton										
No	2408	2450	1.00 (ref)	1.00 (ref)	1234	1.00 (ref)	1.00 (ref)	1078	1.00 (ref)	1.00 (ref)
Class 1	118	123	1.03	1.12	60	0.99	0.93	52	0.98	1.04

15

			(0.79-1.33)	(0.85-1.48)		(0.72-1.36)	(0.63-1.36)		(0.71-1.38)	(0.72-1.51)
Class 2	31	39	1.24	1.28	16	1.01	0.74	15	1.08	0.48
			(0.77-1.99)	(0.75-2.19)		(0.55-1.85)	(0.33-1.68)		(0.58-2.01)	(0.22-1.04)
Class 3	19	19	0.98	0.54	10	1.03	1.29	15	1.76	1.63
			(0.52-1.86)	(0.25-1.14)		(0.48-2.22)	(0.50-3.35)		(0.89-3.48)	(0.72-3.66)
P trend			0.5808			0.9746			0.2266	
Milk										
No	2424	2398	1.00 (ref)	1.00 (ref)	1243	1.00 (ref)	1.00 (ref)	1090	1.00 (ref)	1.00 (ref)
Class 1	126	119	0.96	1.00	64	0.99	0.93	50	0.88	0.95
			(0.74-1.23)	(0.76-1.32)		(0.73-1.35)	(0.65-1.33)		(0.63-1.23)	(0.66-1.37)
Class 2	16	51	3.22	3.61	7	0.85	1.28	10	1.39	1.36
			(1.83-5.67)	(1.96-6.65)		(0.35-2.08)	(0.42-3.93)		(0.63-3.07)	(0.55-3.40)
Class 3	10	63	6.36	5.84	6	1.17	1.02	10	2.22	1.40
			(3.26-12.43)	(2.73-12.50)		(0.42-3.23)	(0.26-4.07)		(0.92-5.36)	(0.48-4.09)
P trend			<.0001			0.9814			0.2746	
Beef										
No	2443	2498	1.00 (ref)	1.00 (ref)	1250	1.00 (ref)	1.00 (ref)	1097	1.00 (ref)	1.00 (ref)
Class 1	94	91	0.95	0.91	48	1.00	0.94	34	0.81	0.86
			(0.71-1.27)	(0.66-1.26)		(0.70-1.42)	(0.62-1.41)		(0.54-1.20)	(0.55-1.33)
Class 2	19	21	1.08	0.22	11	1.13	1.07	14	1.64	1.04
			(0.58-2.02)	(0.09-0.51)		(0.54-2.39)	(0.42-2.76)		(0.82-3.29)	(0.45-2.38)
Class 3	20	21	1.03	0.53	11	1.08	1.56	15	1.67	1.31
			(0.56-1.90)	(0.25-1.14)		(0.51-2.25)	(0.55-4.44)		(0.85-3.28)	(0.57-3.03)
P trend			0.9970			0.7771			0.1923	
Shrimp										

16

			cOR	aOR		cOR	aOR		cOR	aOR
No	2455	2434	1.00 (ref)	1.00 (ref)	1270	1.00 (ref)	1.00 (ref)	1098	1.00 (ref)	1.00 (ref)
Class 1	102	102	1.01 (0.76-1.34)	0.92 (0.66-1.28)	39	0.74 (0.51-1.08)	0.66 (0.40-1.08)	46	1.01 (0.71-1.44)	0.87 (0.56-1.37)
Class 2	9	24	2.69 (1.25-5.80)	4.07 (1.62-10.22)	5	1.07 (0.36-3.21)	0.58 (0.15-2.18)	8	1.99 (0.77-5.17)	2.03 (0.65-6.34)
Class 3	10	71	7.15 (3.68-13.89)	5.18 (2.49-10.75)	6	1.16 (0.42-3.20)	0.85 (0.25-2.82)	8	1.79 (0.70-4.55)	1.92 (0.69-5.35)
P trend			<.0001			0.4474			0.1490	
Wheat										
No	2354	2400	1.00 (ref)	1.00 (ref)	1195	1.00 (ref)	1.00 (ref)	1054	1.00 (ref)	1.00 (ref)
Class 1	175	183	1.03 (0.83-1.27)	0.95 (0.74-1.22)	100	1.13 (0.87-1.45)	0.73 (0.53-1.02)	79	1.01 (0.77-1.33)	0.91 (0.66-1.27)
Class 2	26	26	0.98 (0.57-1.69)	1.00 (0.52-1.94)	13	0.99 (0.50-1.92)	0.61 (0.27-1.38)	14	1.20 (0.63-2.31)	0.89 (0.37-2.18)
Class 3	21	22	1.03 (0.56-1.87)	0.86 (0.44-1.68)	12	1.13 (0.55-2.30)	0.66 (0.27-1.60)	13	1.38 (0.69-2.77)	1.36 (0.59-3.11)
P trend			0.8730			0.4715			0.3747	

1 b, OR values of three allergic disease compared with controls.

2 a OR, adjusted odds ratio.

3 c OR, crude odds ratio.

4 CI, confidence interval.

17

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60

1 **Combined effects of sensitization to multiple allergens on allergic diseases**

2 *OR*s and their 95% *CI*s of allergic diseases with the different number of allergens were shown in Table

3 5. Although the three allergic diseases had different *OR*s and 95% *CI*, the patterns of the relationships

4 between allergic diseases and the number of allergens were similar. *OR*s increased as the number of

5 sensitive allergens increased (*P* trend <0.0001). The associations between two or more allergens and

6 atopic dermatitis were strong, adjusted *OR*=4.28 (95% *CI*: 2.57, 7.11). The combination of specific

7 IgE to three or more sensitive allergens possessed the highest *OR* for allergic rhinitis (adjusted

8 *OR*=13.00, 95% *CI*: 3.76, 45.00), while the combination of specific IgE to two or more allergens

9 showed the highest *OR* for allergic asthma (adjusted *OR*=2.37, 95% *CI*: 1.67, 3.37).

10 **Table 5. ORs for allergic diseases in relation to number of allergens.**

	Control (n=2576)	n	cOR[d] (95% CI)	aOR[d] (95% CI)
			Atopic dermatitis (n=2631)	
Number of allergens [b1]				
No	2422	2142	1.00 (ref)	1.00 (ref)
1	135	375	3.14(2.56-3.86)	3.00(2.44-3.69)
2	19	79	4.70(2.84-7.78)	4.28(2.57-7.11)
≥3	0	35	-	-
P trend			<.0001	
			Allergic rhinitis (n=1320)	
Number of allergens [b2]				
No	2181	893	1.00 (ref)	1.00 (ref)
1	286	312	2.66(2.23-3.19)	2.64(2.20-3.18)
2	106	97	2.24(1.68-2.98)	2.13(1.58-2.87)
≥3	3	18	14.65(4.31-49.87)	13.00(3.76-45.00)
P trend			<.0001	
			Allergic asthma (n=1160)	
Number of allergens [b3]				
No	2183	845	1.00 (ref)	1.00 (ref)
1	319	243	1.97(1.64-2.37)	2.06(1.70-2.49)
2	74	68	2.37(1.69-3.33)	2.37(1.67-3.37)
≥3	0	4	-	-
P trend			<.0001	

18

1 d, OR values of three allergic disease compared with controls.

2 a OR, adjusted odds ratio.

3 c OR, crude odds ratio.

4 CI, confidence interval.

5 [b1] allergens excluding those allergens which were not related with atopic dermatitis in Table 2, Table

6 3 and Table 4. [b2] allergens excluding those allergens which were not related with allergic rhinitis in

7 Table 2, Table 3 and Table 4. [b3] allergens excluding those allergens which were not related with

8 allergic asthma in Table 2, Table 3 and Table 4.

9

10 **Discussion**

11 In our present hospital-based case-control research, we found that different allergens were

12 significantly related to different allergic diseases, and that was a dose-response relationship between

13 levels of allergen-specific IgE and the prevalence of allergic diseases, and combined effects of

14 sensitization to multiple allergens on allergic diseases were also found. The incidences of allergic

15 diseases had increased rapidly worldwide[2-4]. The World Allergy Organization (WAO) reported that,

16 among 1.39 billion people in 33 countries, 22 percent of the population suffered from allergic

17 diseases[21]. The world health organization (WHO) had clearly stipulated that allergic disease was an

18 extremely considerable disease that the whole world should attach importance to[22]. Identifying specific

19 allergens that trigger allergic diseases was particularly significant for the prophylaxis and treatment of

20 these allergic diseases.

21 According to the previous studies, genetic and environmental factors play important roles in

22 sensitization to allergens[19,23-26]. As presented in Table 1, the proportions of family history in atopic

23 dermatitis, allergic rhinitis, allergic asthma, controls were 26.38%, 28.71, 17.41%, and 17.55%

24 respectively. The family history of atopic dermatitis and allergic rhinitis were significantly different

25 compared to the controls, while there was no difference in family history between allergic asthma and

19

1 controls. In studying environmental factors, we considered the location of residence. The proportions

2 of living in rural in atopic dermatitis, allergic rhinitis, allergic asthma, controls were 38.75%, 28.32%,

3 35.88%, and 29.12%, respectively. According to the results, we found that the proportion living in

4 rural were significantly higher in patients with atopic dermatitis and allergic asthma than controls,

5 while there was no difference in location of residence between allergic rhinitis and controls. We can

6 investigate the family history and location of residence and combine clinical symptoms to speculate

7 whether the patients are likely to be sensitive to allergens and recommend further appropriate testing.

8 Regrettably, we just conducted this study in Harbin, a city in northeast China, without considering the

9 differences between different geographical environments across China. Previously, a multicentre

10 cross-sectional study was conducted to assess the prevalence of sensitizations in patients with asthma

11 and/or rhinitis, which contained 17 cities from 4 regions of China[27]. This is also a target of our further

12 research to collaborate with hospitals in other regions of China for more in-depth study.

13 Our findings indicated that atopic dermatitis was significantly related to food allergens, while allergic

14 rhinitis and allergic asthma were significantly associated with both indoor and outdoor allergens. The

15 recognition to food allergens through antigen-presenting cells in eczematous skin plays an important

16 role in the link between food allergy and allergic dermatitis. In addition, the dysfunction of filaggrin

17 or Th2-relateed cytokines or rare genetic syndromes may also are involved in the mechanism[28].

18 Different types of allergens induce different allergic diseases, which may due to the properties or size

19 of allergens. Allergic rhinitis is associated with outdoor allergens, mainly pollen, probably because

20 pollen particles are slightly larger than indoor dust and are more likely to stay in the nose[29]. Inhalation

21 of indoor dust, mites and fungi can increase the airway reactivity and causes allergic asthma[30]. Our

20

1   discoveries were identical to some previous studies. It was reported that indoor allergens act as

2   important factors in patients with allergic rhinitis and allergic asthma[9,31,32]. Some studies also stated

3   that there was a relevance between sensitization to outdoor allergens and allergic rhinitis and allergic

4   asthma[33-35]. Food allergens were found to be crucial in the development of atopic dermatitis in some

5   observations[35,36]. Allergens, commonly proteins, induce allergic diseases through complex

6   interactions with the immune system. Due to the presence of epitopes with allergenic potential,

7   glycosylation status, resistance to proteolysis, and enzymatic activity, some proteins are more

8   allergenic than others and different allergens may cause different allergic diseases through various

9   underlying mechanisms[37]. However, some studies showed inconsistent findings. Several studies

10   showed that indoor allergens have a bearing on the prevalence of atopic dermatitis in some

11   individuals[38,39]. Other studies showed a relationship between sensitization to food allergens and

12   allergic rhinitis and allergic asthma[40]. The inconsistency of those studies may be related to regional

13   variation and different climate characteristics, which affected the protein ingredients of allergens. In

14   addition, genetic susceptibility and socio-economic status of populations may also influence the

15   occurrence of allergic diseases.

16   This study revealed that there was a dose-response relationship between specific IgE levels of

17   allergens and prevalence of allergic diseases, and the *OR*s for allergic diseases with specific IgE to

18   allergens increased along with the increase of allergen-specific IgE levels. This evidence suggested

19   that participants would have a higher risk of suffering from allergic diseases if they faced higher

20   concentrations of allergens. Some previous studies supported our findings. A study in America

21   revealed a dose-response relationship between exposure to mice and the morbidity of allergic asthma

21

1   among urban children and adolescents[41]. Another research evaluated that whether TPI ASM8 induced

2   a dose-dependent reduction in the inflammatory and physiological changes following inhaled allergen

3   challenge[42]. It is worth noting that there was a combined effect of sensitization to different allergens

4   on allergic diseases. Multiple sensitizations to several allergens had a relatively stronger association

5   with allergic diseases than single sensitization to one allergen. Some research results were similar to

6   our findings. Carlsten et al. reported that in a birth cohort, simultaneously exposure to dog and indoor

7   pollution made the risk of incident asthma increased[43]. In a cross-sectional study, researchers in Italy

8   revealed that the severity of symptoms was more serious in polysensitized patients with allergic

9   rhinitis than in monosensitized patients[44]. In addition, most allergens were significantly associated

10   with allergic diseases only when their IgE level classes $\geq$ 2. Such findings may be of more clinical

11   value than a dichotomous designation of sensitized or not sensitized to allergens, and can potentially

12   be used for improving the diagnosis of allergic diseases.

13   It is obviously that allergens avoidance is the most effective means of prevention for allergy sufferers

14   considering that allergic diseases are triggered by those allergens. However, this may not be true in

15   some cases. Completely avoiding contact with some indoor and outdoor allergens is not always

16   feasible. Moreover, early exposure to food allergens has been turned out to be a successful method for

17   the prevention of peanut allergy[45]. Many studies reported that allergen immunotherapy was an

18   effective treatment strategy for allergic diseases[46,47]. However, for some patients with severe

19   symptoms, allergen avoidance and treatment of symptoms may be necessary. Our findings could

20   provide some evidence for clinicians about avoidance or specific immunotherapy treatment for

21   patients with allergic diseases.

22

1   There are several strengths in this study. Firstly, the sample size of this study was relatively large

2   which enabled us to divided specific IgE levels of 16 common allergens into three levels: class 1, class

3   2, and class 3. This enabled us to analyze the dose-response relationship between specific IgE levels

4   and the prevalence of allergic diseases, and the combined effect of sensitization to multiple allergens

5   of allergic diseases. Secondly, the 16 allergens were categorized into three categories: indoor allergens,

6   outdoor allergens, and food allergens, and the associations between each type of allergens and different

7   allergic diseases were analyzed. However, there are some limitations to this study. Firstly, we cannot

8   determine when the patients were first sensitized to allergens or when they first developed an allergic

9   disease. We did not collect the specific date of the patient's visit to the hospital, so we could not analyze

10   the impact of seasonal factors on allergic diseases. Secondly, this study was performed only in one

11   hospital in Harbin, the universality of our results for the whole of China still needs to be further

12   explored.

13   In conclusion, atopic dermatitis was associated with food allergens while allergic rhinitis and allergic

14   asthma were related to both indoor and outdoor allergens sensitization in northeastern China. There

15   was a dose-response relationship between levels of specific-allergic IgE levels and the prevalence of

16   allergic diseases, and combined effects of multiple sensitizations to several allergens increased the risk

17   of allergic diseases. Our findings could provide evidence to the control and management of allergic

18   diseases, and may have important public health implications.

19

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23

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Ethics approval statement

Participants were not subjected to intervention and gave informed consent to participate in the study

before taking part, so our Ethics Committee exempted our study according to the regulations of our

school.

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Data sharing statement

Not applicable.

Authors' contributions

W.Z. and B.X. designed the process of this study and the structure of this article. Y.W. and M.L.

designed the questionnaire. W.Z. and B.X. participated in case collection and questionnaire

investigation, and analyzed the data. W.Z. wrote the manuscript, and all other authors read and

confirmed the final version.

Competing interests

None declared

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Figure legends

Figure 1. The flow diagram of study participants.

Figure 2. Prevalence of allergens sensitization among atopic dermatitis, allergic rhinitis and allergic
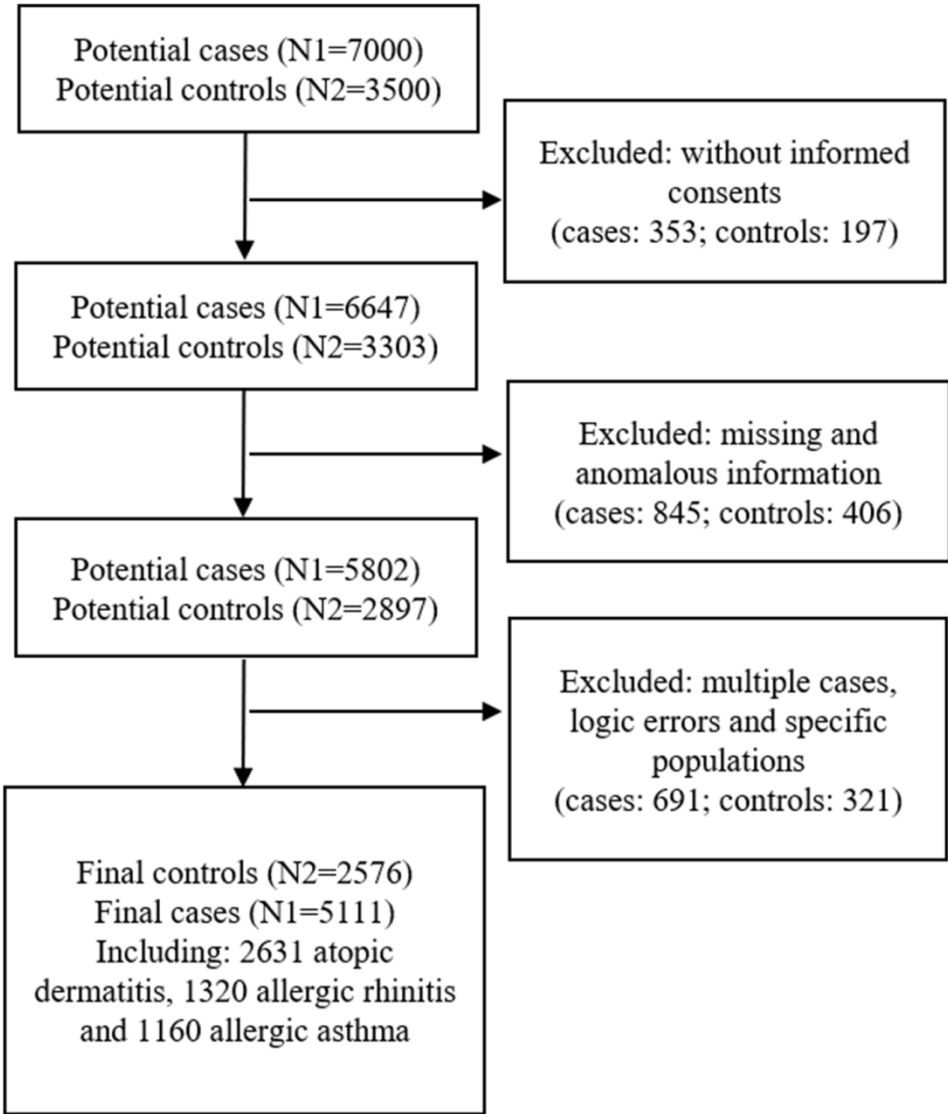
asthma in Chinese adults.

31

Figure 1 The flow diagram of study participants.
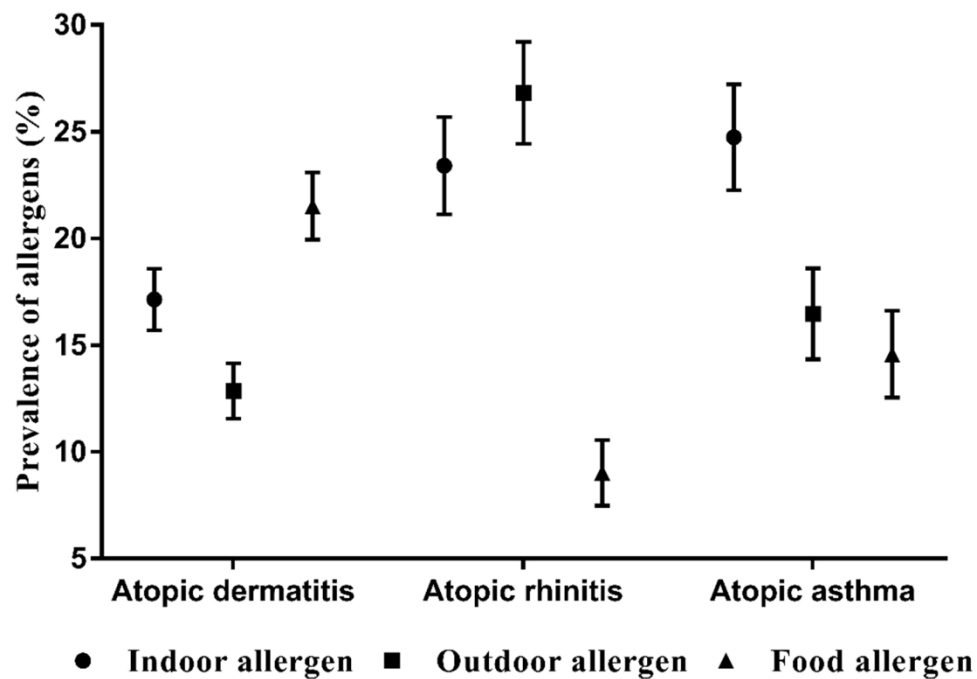
107x124mm (350 x 350 DPI)

Figure 2. Prevalence of allergens sensitization among atopic dermatitis, allergic rhinitis and allergic asthma in Chinese adults

129x92mm (350 x 350 DPI)

Associations between sensitization to allergens and allergic diseases: a hospital-based case-control study in China

Supplementary Method:

1. The definition of demographic, family history, and lifestyle factors

Age was calculated as the difference between the year of birth and the year of interview. Educational status was categorized into three levels: junior high school or lower, senior high school, and university or higher. The residential region was categorized into two groups: urban and rural. Marital status was also categorized into two groups: unmarried and married. Current smokers were defined on the basis of the World Health Organization criteria, as those who self-reported smoking every day for at least 6 months[9]. Regular alcohol drinkers were defined as drinking more than twice per week for at least one year. Classification of physical exercise (PE) was defined as followed:

We estimated PE condition of study participants using three variables of a structured questionnaire, exercise intensity (EI), exercise time (ET), and exercise frequency (EF) [1-4].

EI of study participants was classified into three groups, mild, moderate and strenuous exercise according to their exercise types. Value 1, 2 and 3 were assigned to the three groups respectively [5-8]. ET was measured by the hour. EF referred to how many times a week. We defined PE as

$$PE = \sum_{i=1}^{N} EI \times ET$$

Where N represented EF. If PE $\geq$ 2, PE was positive; PE was negative when PE < 2. Therefore, as for a subject jogging (moderate exercise) 15 minutes 5 times a week, his/her PE was positive because PE $\geq$ 2 (PE = 5$\times$ 0.25 $\times$ 2 = 2.5).

2. The diagnostic criteria of atopic dermatitis, allergic asthma, and allergic rhinitis

2.1 Atopic dermatitis

The following diagnostic criteria for atopic dermatitis was developed by the American

Academy of Dermatology (AAD), which is based on age-specific clinical criteria that include pruritus and chronic or relapsing spongiotic dermatitis involving the face, trunk, and/or extensor extremities in infants, flexural surfaces like the wrists/ankles and antecubital/popliteal fossae in children, or the hands in adults[10].

Essential Features (both must be present):

    1) Pruritus

    2) Eczema (acute, subacute, chronic)

        a. Chronic or relapsing history

        b. Typical morphology and age-specific patterns

            Infants: face, trunk (except "diaper area"), extensor extremities

            Children: flexors (wrists, ankles, antecubital/popliteal fossae)

            Adults: hands

            All ages: sparing of the groin and axillary regions

Important Features (support the diagnosis and are observed in most cases of atopic dermatitis):

    1) Early age of onset

    2) Atopy

        a. personal and/or family history; or

        b. IgE reactivity

    3) Xerosis

Associated Features (suggestive of atopic dermatitis, but too nonspecific to define or detect atopic dermatitis in research or epidemiologic studies):

    1) Atypical vascular responses (eg, facial pallor, white dermatographism, delayed blanch response)

    2) Keratosis pilaris, pityriasis alba, hyperlinear palms/ichthyosis

    3) Ocular/periorbital changes (fissures, infraorbital folds)

    4) Other regional findings (eg, perioral changes/periauricular lesions)

    5) Perifollicular accentuation/lichenification/prurigo lesions

2.2 Allergic rhinitis

    The diagnosis criteria for allergic rhinitis are as follows[11]:

    1) Clinical history

Clinical history is essential for the accurate diagnosis of allergic rhinitis and the assessment of its severity as well as its response to treatment. The most frequent symptoms include sneezing, anterior rhinorrhea, bilateral nasal obstruction, and nasal pruritus in patients with allergic rhinitis. In addition, most patients with pollen-induced rhinitis have eye symptoms. It is also important to distinguish between allergy and nonallergy symptoms. Subjective assessment of symptoms of allergic rhinitis is generally based on 4 nasal symptoms (sneezing, rhinorrhoea, nasal itching, and nasal obstruction) and 2 ocular symptoms (ocular itching/grittiness/redness and ocular tearing). In China, VAS is most commonly used to quantify the above-mentioned assessments. VAS was used to quantify the above-mentioned assessments.

2) Nasal examinations

Anterior rhinoscopy and nasal endoscopy are the widely used approaches. The nasal examination should describe: 1) the anatomical situation in the nose (e.g. the septum, the size of the inferior turbinate, and if possible the structures in the middle meatus); 2) the color of the mucosa; and 3) the amount and aspect of the mucus. Endoscopic images of nasal mucosa in patients suffering from allergic rhinitis generally demonstrate pale and edematous nasal mucosa, watery nasal discharge, and swollen inferior turbinates.

3) Skin tests

Two methods of skin testing, including intradermal skin tests and skin prick tests (SPTs), are available in China. Skin tests should be read at the peak of their reaction by measuring the wheal and the flare approximately 15 minutes after the performance of the tests. For prick tests, when the control site is completely negative, wheals of >3 mm represent a positive skin response.

4) Serum specific IgE measurements

Enzyme-labeled anti-IgE measurement has been widely used in China. Results are expressed in terms of units of IgE (IU/mL, KU/L). The IgE level above 0.35 KU/L is usually testified as a positive result.

5) Differential diagnosis

In addition, it is necessary to differentiate allergic rhinitis from other diseases, such as vasomotor rhinitis, nonallergic rhinitis with eosinophilia syndrome (NARES), infectious rhinitis, hormonal rhinitis, medicamentous rhinitis (rhinitis medicamentosa), aspirin intolerance triad, cerebrospinal fluid rhinorrhea, and so on.

2.2 Allergic asthma

The diagnosis criteria for allergic asthma are as follows[12]:

1) Clinical history

Wheezing, coughing, chest tightness

May only be present or worsened with exertion, upper respiratory infection, seasonal or perennial allergies

Nocturnal cough, particularly 2 AM to 4 AM

Need for short-acting β_2-agonist inhaler for relief of symptoms

Personal or family history of atopy

2) Spirometry

Airway obstruction evidenced by FEV_1: FVC ratio < lower limit of normal

Demonstrated reversibility of obstruction by increase in $FEV_1 \geq 200mL$ and $\geq 12\%$ from baseline measure after inhalation of 2-4 puffs of short-acting β_2-agonist

Normal spirometry findings are not inconsistent with asthma

3) Bronchoprovocation with methacholine

20% or more decrease in FEV_1 with after inhalation of low concentration (< 4 mg/mL) of methacholine; used principally in patients with symptoms consistent with asthma but who exhabit normal pulmonary function tests

4) Impedance oscillometry

Elevated airway resistance at 5 HZ, elevated area of reactance, increased resonant frequency (Fres), reactance at 5 HZ more negative than predicted

5) Chest radiograph or CT scan of thorax

Usually normal but can exclude other diagnoses such as emphysema, lung cancer, infiltrative diseases, pneumonia

6) CBC

Eosinophilia, particularly > 300/μL; results can inform selection for mepolizumab or reslizumab therapy

7) Serum total IgE

Elevated in atopic asthma, not in nonatopic asthma; can inform selection of omalizumab therapy

8) Skin prick testing or serum-specific IgE for aeroallergens

Positive, particularly for perennial allergens, or seasonal allergens with corresponding seasonal variation in asthma symptoms; may be negative in nonatopic asthma; can inform omalizumab therapy

Positive testing can guide allergen avoidance strategies

9) Fractional excretion of nitric oxide

Intermediate level: 25-50 ppb in patients aged $\geq$ 12 y

High level: > 50ppb in patients aged $\geq$ 12 y

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Supplementary Table:

Supplementary Table S1. Sixteen common allergens used in this study.

category	allergens			
indoor allergens	Dermatophagoides pteronyssinus	Cat and dog fur	Mould mixture [a]	
outdoor allergens	common ragweed and mugwort	German cockroach	Tree pollen mixture [b]	Hop
food allergens	Egg white/egg yolk	Blue mussel	Fish	Crab
	Mutton	Milk	Beef	Shrimp
	Wheat			

[a] Mould mixture is composed of Penicillium notatum, Cladosporium herbarum, Aspergillus fumigatus and Alternaria alternate.

[b] Tree pollen mixture is composed of Robur, Elm, London Plane, Willow and cottonwood.

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *case-control studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2,3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3,4
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5,6,7
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls	5,7
		(*b*) For matched studies, give matching criteria and the number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	5,6,7
Bias	9	Describe any efforts to address potential sources of bias	
Study size	10	Explain how the study size was arrived at	
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7,8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7
		(*b*) Describe any methods used to examine subgroups and interactions	
		(*c*) Explain how missing data were addressed	
		(*d*) If applicable, explain how matching of cases and controls was addressed	
		(*e*) Describe any sensitivity analyses	
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	7,8
		(b) Give reasons for non-participation at each stage	7,8
		(c) Consider use of a flow diagram	7,8
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8
		(b) Indicate number of participants with missing data for each variable of interest	
Outcome data	15*	Report numbers in each exposure category, or summary measures of exposure	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9-18
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
Discussion			
Key results	18	Summarise key results with reference to study objectives	19
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	23
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	19-22
Generalisability	21	Discuss the generalisability (external validity) of the study results	23
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	24

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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1 **Associations between sensitization to allergens and allergic**

2 **diseases: a hospital-based case-control study in China**

3

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16 Word count: 2667

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1

1 **Abstract**

2 **Objectives:** To assess the associations of sensitization to common allergens with atopic dermatitis,

3 allergic rhinitis, and allergic asthma in adults.

4 **Design:** Case-control study.

5 **Setting:** Data was collected from the First Affiliated Hospital of Harbin Medical University in Harbin,

6 China.

7 **Participants:** Case were 5111 patients with physician-diagnosed atopic dermatitis (n = 2631), allergic

8 asthma (n = 1320), and allergic rhinitis (n = 1160) recruited from the Department of Allergy from

9 March 2009 to December 2017. Controls were 2576 healthy adults who underwent physical

10 examination at the same hospital during the same period.

11 **Main outcome measures:** Specific IgE levels to 16 common food, indoor, and outdoor allergens were

12 assessed in all participants. Adjusted Odds ratios (ORs) and 95% confidence intervals (95% CIs) for

13 the association between allergen sensitization and allergic diseases was estimated using multivariate

14 logistic regression.

15 **Results:** The prevalence of allergen sensitization was higher in patients with atopic dermatitis

16 (indoor=17.14%, outdoor=12.85%, food=21.44%), allergic rhinitis (indoor=23.18%, outdoor=26.81%,

17 food=8.94%), and allergic asthma (indoor=24.65%, outdoor=16.46%, food=14.31%) compared to

18 controls (indoor=11.03%, outdoor=6.84%, food=5.83%). After adjustment for potential confounding

19 variables, there was a dose-response relevance between the levels of allergen-specific IgE and allergic

20 diseases (P trend <0.0001). The number of allergens to which a patient was sensitized increased the

21 risk of allergic diseases (atopic dermatitis: highest adjusted OR=4.28, 95% CI 2.57 to 7.11; allergic

1 rhinitis: highest adjusted OR=13.00, 95% CI 3.76 to 45.00; allergic asthma: OR=2.37, 95% CI 1.67 to

2 3.37).

3 **Conclusion:** There was a dose-response relevance between levels of allergen-specific IgE and allergic

4 diseases' prevalence, and multiple sensitizations increased the risk of allergic diseases. This study

5 provides evidence for the prophylaxis of allergic diseases.

6 **Keywords:** allergic diseases; allergens; association; case-control study

7

8 **Strengths and limitations of this study**

9 • We assessed the dose-response relationship between allergen-specific IgE levels to 16 different

10 allergens and prevalence of allergic diseases using logistic regressions.

11 • Various types of allergens were considered and categorized into indoor, outdoor and food

12 allergens.

13 • Adjusted Odds ratios (ORs) and 95% confidence intervals (95% CIs) for the association between

14 allergen sensitization and allergic diseases was estimated using multivariate logistic regression.

15 • The combined effects of multiple allergens on allergic diseases were inspected through

16 multivariate logistic regression analyses, which were conducted with different number of

17 sensitizing allergens (1, 2, ≥3 allergens).

18 • Although this was not a multicenter study, it was a population-based study with a large sample

19 size.

20

21 **Introduction**

3

1 Allergic diseases are global and can profoundly affect the quality of life of patients[1]. The incidence of

2 allergic diseases, including atopic dermatitis, allergic rhinitis, and allergic asthma, has been increasing

3 rapidly around the world, especially in China and other low-and middle-income countries [2-4]. As

4 triggering factors, allergens stimulate the body to produce abnormal immune responses, which

5 eventually lead to allergic diseases[5]. The type and dose of allergens determine the type of allergic

6 disease and its severity[6]. Therefore, it is crucial to clarify the distributions of positive allergens among

7 patients with different allergic diseases.

8 Currently, many studies have been devoted to explore the association between sensitization to

9 allergens and allergic diseases. Gray CL et al. randomly enrolled 100 children aged 6 months to 10

10 years with atopic dermatitis and found that a high prevalence of egg sensitization of 54%, and SPT to

11 fresh egg white showed a high predictive value for egg allergy[7]. A Japanese study explored the causal

12 relationships among pollen counts, tweet numbers, and patient numbers for seasonal allergic rhinitis,

13 and reported a positive correlation between pollen counts and the patient numbers[8]. Sensitization and

14 exposure to indoor allergens (such as dust mites, cockroaches, wild rodents, pets, and fungi) have been

15 reported to be associated with allergic diseases, especially the development of asthma[9]. Patients with

16 allergies usually have different levels of specific IgE to a certain allergen and allergens are often co-

17 existent, so it is critical to understand the associations between multiple allergens sensitization and

18 allergic diseases[10]. According to previous studies, the incidence of allergic diseases varies among

19 different geographic locations and subpopulations. For Koreans, high sensitizations to various types

20 of pollen were in the Gangwon region, whereas sensitization to Japanese cedar pollen was unique in

21 the Jeju region[11]. Among the individuals from the Americas, grass pollen and animal dander allergies

4

1   were relatively common, while weed and grass pollen allergies were common in people from Central

2   Asia[11]. Therefore, it is suggested that genetic and environmental features may play important roles in

3   allergic diseases, and it is interesting to understand the underlying causes of those variations. At

4   present, most relevant studies have a limited sample size, and few such studies have been conducted

5   in northeastern China with its distinctive environmental and climate conditions[12-14].

6   In the present study, we assessed the associations between sensitization to 16 allergens and three

7   common allergic diseases in northeastern China and explored if there is a dose-response connection

8   between the specific IgE levels to an allergen and of allergic diseases' prevalence. We also examined

9   the combined effects of multiple allergens on a specific allergic disease. Our study aimed to supply

10   basis for the prevention and management of allergic diseases.

11   **Materials and methods**

12   **Participants and Public involvement**

13   Harbin is located in northeast of China with longitude spanning 125°42'-130°10' E, and latitude

14   44°04'-46°40' N, which is the provincial capital of Heilongjiang Province. Under the direct influence

15   of the Siberian Anticyclone, the average daily temperature is -19.7℃ (-3.5℉) in winter. Annual low

16   temperatures below -35.0℃ (-31.0℉) are not uncommon. Nicknamed "Ice City" due to its freezingly

17   cold winter. We recruited the participants into our research from Harbin. Only those who signed the

18   informed consent form were recruited into our study. This project was authorized by the Institutional

19   Review Board of Harbin Medical University. Adult patients aged 18 years or older with one of the

20   three common allergic diseases (atopic dermatitis, allergic asthma, or allergic rhinitis), who visited the

21   Department of Allergy of the First Affiliated Hospital of Harbin Medical University during March

5

1   2009 and December 2017 were collected as the candidate cases. Healthy adults without any clinical

2   symptoms and diseases who underwent physical examination at the same hospital during the same

3   period were recruited as the candidate controls. All participants carried on allergen-specific IgE tests

4   to 16 kinds of allergens and a questionnaire investigation.

5   **Allergen-specific IgE testing**

6   Serum allergen-specific IgE concentrations of 16 common allergens were measured in all eligible

7   participants using the AllergyScreen system (Mediwise Analytic GmbH, Germany). As shown in

8   Supplementary Information, the 16 allergens were defined as three types: indoor allergens, outdoor

9   allergens, and food allergens[15]. Based on previous research, allergens-specific IgE > 0.35 kU/l was

10   considered as allergens sensitization in this study, and specific IgE concentration of 0.35-0.70 kU/l

11   was defined as class 1, 0.70-3.50 kU/l as class 2, and > 3.50 kU/l as class 3[16].

12

13   **Allergic diseases definition**

14   We focused on three common allergic diseases (atopic dermatitis, allergic asthma, and allergic rhinitis)

15   in Harbin. The three allergic diseases are diagnosed by experienced physicians using existing

16   benchmarks for atopic dermatitis[17], allergic rhinitis[18,] and allergic asthma[19]. The detailed diagnostic

17   criteria were shown in Supplementary Information. Besides, the following criteria should be obeyed:

18   (1) There were at least have one positive outcome of the 16 allergen-specific IgE results;

19   (2) The patients had been diagnosed with one of the allergic diseases using current guidelines

20   mentioned above.

21

6

1   **Demographic, lifestyle characteristics, and family history**

2   All of the participants in this study answered the questionnaire face to face to collect data on

3   demographic, lifestyle characteristics and family history. Gender, age, level of education, residential

4   region, and marital status were analyzed as demographic characteristics. Smoking, drinking, and

5   physical exercise were included in lifestyle factors. The definition of those factors was shown in the

6   Supplementary Information.

7

8   **Statistical analysis**

9   To assess the dose-response relationship between levels of allergen-specific IgE to 16 different

10   allergens and prevalence of allergic diseases, we applied logistic regressions to evaluate the odds ratios

11   (*OR*) and their 95% confidence intervals (*CI*). Multivariate logistic regression models were adjusted

12   for potential confounding variables significantly associated with allergic diseases identified from

13   univariate analysis or as suggested in previous literature. They included gender, age, BMI, level of

14   education, smoking, drinking, living region, marital status, family history of allergic diseases, physical

15   exercise, and other 15 allergens specific-IgE responses. To inspect the combined effects of multiple

16   allergens on allergic diseases, multivariate logistic regression analyses were conducted with different

17   number of sensitizing allergens (1, 2, ≥3 allergens). The statistical analysis software of this study was

18   SAS 9.1 (SAS Institute Inc., Cary, NC, USA).

19

20   **Results**

21   **Participants**

7

1   In the present study, 7000 candidate cases and 3500 candidate controls were initially recruited.

2   Excluding those without written informed consents, 6647 candidate cases and 3303 candidate controls

3   remained. After excluding those with missing values and logic errors, and some special populations

4   (sportsmen, pregnant or lactating women), we had 5111 cases (2631 atopic dermatitis, 1320 allergic

5   rhinitis, and 1160 allergic asthma) and 2576 controls for our final analysis. The flow diagram of the

6   screening process of study participants is summarized in Figure 1.

7

8   **Characteristics in cases and controls**

9   There are statistical differences between patients with atopic dermatitis and controls in terms of gender,

10   age, height, weight, BMI, residential region, smoking, education, and family history (P<0.05). Patients

11   with allergic rhinitis were significantly different from controls in gender, height, weight, BMI,

12   education, and family history (P<0.05). Gender, age, weight, BMI, residential region, smoking, levels

13   of education, marital status, and physical exercise were different between those with allergic asthma

14   and controls (P<0.05). Detail demographic characteristics were summarized in Table 1.

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Table 1. Characteristics of patients with atopic dermatitis, allergic rhinitis, allergic asthma and controls.

	Control	Atopic dermatitis	P [a]	Allergic rhinitis	P [a]	Allergic asthma	P [a]
Number	2576	2631		1320		1160	
Men, n (%)	732(28.42)	684(26.00)	0.0499	532(40.30)	<.0001	398(34.31)	0.0003
Age, years [b]	34.96(16.13)	36.70(15.33)	<.0001	34.59(13.76)	0.4868	41.60(17.25)	<.0001
Height, cm [b]	160.40(14.71)	163.00(11.14)	<.0001	164.90(11.07)	<.0001	160.90(14.59)	0.3205
Weight, kg [b]	58.43(15.95)	60.34(13.70)	<.0001	62.49(15.22)	<.0001	60.02(15.20)	0.0041
BMI, kg/m^2 [b]	22.26(4.17)	22.51(3.95)	0.0268	22.71(3.98)	0.0009	22.79(4.04)	0.0003
Rural, n (%)	750(29.12)	1020(38.75)	<.0001	374(28.32)	0.6533	416(35.88)	0.0003
Married, n (%)	1661(64.49)	1708(64.92)	0.8370	875(66.31)	0.4722	891(76.85)	<.0001
smoking, n (%)	338(13.12)	437(16.61)	0.0004	194(14.70)	0.1752	296(25.52)	<.0001
Alcohol drinking, n (%)	317(12.31)	349(13.26)	0.3002	192(14.55)	0.0496	156(13.45)	0.3313
Physical exercise, n (%)	893(34.68)	897(34.08)	0.7735	490(37.15)	0.3281	319(27.52)	0.0015
Education, n (%)							
Junior high school or lower	1253(48.64)	1084(41.20)	<.0001	459(34.77)	<.0001	610(52.59)	0.0220
Senior high school	618(23.99)	734(27.90)		315(23.86)		280(24.14)	
University or higher	705(27.37)	813(30.90)		546(41.36)		270(23.28)	
Family history, n (%)	452(17.55)	1937(26.38)	<.0001	379(28.71)	<.0001	202(17.41)	0.9213

3   [a] P values comparing cases with controls.

4   [b] The quantitative variables were expressed as means and their standard deviation.

5

6   **Prevalence of allergen sensitization in patients with atopic dermatitis, allergic rhinitis, and**

7   **allergic asthma**

8   Patients with different allergic diseases had different prevalence of allergens sensitization. Among

9   patients with atopic dermatitis, the prevalence of food allergens sensitization was higher than other

10   allergens. The prevalence of outdoor allergens sensitization was higher in those with allergic rhinitis

11   while that of indoor allergens was higher in patients with allergic asthma (Figure 2).

12   Adjusted for gender, age, BMI, level of education, smoking, drinking, living region, family history,

13   physical exercise, and other 15 allergens specific-IgE responses, specific IgE to common ragweed and

14   mugwort, Dermatophagoides pteronyssinus, dog fur, and cat fur were significantly related to the risk

15   of allergic asthma (Table 2 and Table 3). Allergic rhinitis was significantly associated with common

16   ragweed and mugwort, mould mixture, tree pollen mixture, and Hop (Table 3), while atopic dermatitis

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1   was significantly associated with egg white/egg yolk, crab, blue mussel, milk, fish, and shrimp (Table

2   4 and Table 5). There was a dose-response relationship between specific IgE levels, from class 1 to

3   class 3, and the prevalence of allergic diseases, the prevalence of allergic diseases increased along

4   with the increasing levels of allergen-specific IgE to allergens (*P* trend <0.0001).

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1 **Table 2. ORs for allergic diseases with specific IgE class to indoor allergens.**

Allergen category	Control (n=2576)	Atopic dermatitis (n=2631)			Allergic rhinitis (n=1320)			Allergic asthma (n=1160)		
		n	cOR[b] (95% CI)	aOR[b] (95% CI)	n	cOR[b] (95% CI)	aOR[b] (95% CI)	n	cOR[b] (95% CI)	aOR[b] (95% CI)
Dermatophagoides pteronyssinus										
No	2098	2107	1.00 (ref)	1.00 (ref)	1038	1.00 (ref)	1.00 (ref)	852	1.00 (ref)	1.00 (ref)
Class 1	177	203	1.14 (0.93-1.41)	1.17 (0.93-1.47)	90	1.03 (0.79-1.34)	0.89 (0.63-1.24)	70	0.97 (0.73-1.30)	1.19 (0.85-1.66)
Class 2	228	243	1.06 (0.88-1.28)	1.11 (0.91-1.36)	155	1.37 (1.11-1.71)	1.16 (0.90-1.51)	153	1.65 (1.33-2.06)	1.40 (1.07-1.83)
Class 3	73	78	1.06 (0.77-1.47)	1.19 (0.84-1.69)	37	1.02 (0.69-1.53)	0.80 (0.51-1.26)	85	2.87 (2.08-3.96)	3.68 (2.50-5.40)
P trend			0.3415			0.0306			<.0001	
Cat and dog fur										
No	2513	2539	1.00 (ref)	1.00 (ref)	1287	1.00 (ref)	1.00 (ref)	1089	1.00 (ref)	1.00 (ref)
Class 1	35	54	0.95 (0.59-1.53)	0.90 (0.54-1.49)	18	1.00 (0.57-1.78)	1.08 (0.58-2.01)	20	1.32 (0.76-2.30)	1.08 (0.58-1.99)
Class 2	14	20	1.40 (0.71-2.78)	1.67 (0.78-3.59)	8	1.12 (0.47-2.67)	1.26 (0.46-3.44)	16	2.64 (1.28-5.42)	4.10 (1.67-9.91)
Class 3	14	18	1.26 (0.63-2.54)	1.29 (0.62-2.69)	7	0.98 (0.39-2.43)	1.17 (0.46-3.02)	35	5.77 (3.09-10.77)	7.13 (3.44-14.79)
P trend			0.3498			0.9260			<.0001	
Mould mixture										
No	2205	2252	1.00 (ref)	1.00 (ref)	1059	1.00 (ref)	1.00 (ref)	990	1.00 (ref)	1.00 (ref)
Class 1	234	245	1.03 (0.85-1.24)	1.05 (0.86-1.29)	123	1.09 (0.87-1.38)	0.81 (0.61-1.08)	106	1.01 (0.79-1.28)	0.98 (0.74-1.29)
Class 2	100	97	0.95 (0.71-1.26)	1.07 (0.79-1.44)	80	1.67 (1.23-2.26)	1.58 (1.12-2.22)	46	1.03 (0.72-1.46)	0.67 (0.44-1.01)
Class 3	37	37	0.98 (0.62-1.55)	1.04 (0.65-1.67)	58	3.26 (2.15-4.96)	3.32 (2.14-5.14)	18	1.09 (0.62-1.91)	1.01 (0.55-1.86)
P trend			0.8736			<.0001			0.7814	

2   aOR, adjusted odds ratio.

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1   b, OR values of three allergic disease compared with controls.

2   cOR, crude odds ratio.

3   CI, confidence interval.

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Table 3. ORs for allergic diseases with specific IgE class to outdoor allergens.

Allergen category	Control (n=2576)	Atopic dermatitis (n=2631)			Allergic rhinitis (n=1320)			Allergic asthma (n=1160)		
		n	cOR[b] (95% CI)	aOR[b] (95% CI)	n	cOR[b] (95% CI)	aOR[b] (95% CI)	n	cOR[b] (95% CI)	aOR[b] (95% CI)
Common ragweed and mugwort										
No	2325	2378	1.00 (ref)	1.00 (ref)	1130	1.00 (ref)	1.00 (ref)	1020	1.00 (ref)	1.00 (ref)
Class 1	113	114	0.99 (0.76-1.29)	0.97 (0.72-1.30)	58	1.06 (0.76-1.46)	0.95 (0.64-1.40)	38	0.77 (0.53-1.12)	0.75 (0.49-1.15)
Class 2	72	73	0.99 (0.71-1.38)	1.09 (0.74-1.60)	52	1.49 (1.03-2.14)	1.92 (1.23-2.99)	41	1.30 (0.88-1.92)	1.95 (1.17-3.27)
Class 3	66	66	0.98 (0.69-1.38)	0.75 (0.50-1.13)	80	2.49 (1.79-3.48)	1.87 (1.24-2.81)	61	2.11 (1.48-3.01)	2.26 (1.43-3.56)
P trend			0.8767			<.0001			0.0002	
German cockroach										
No	2391	2438	1.00 (ref)	1.00 (ref)	1225	1.00 (ref)	1.00 (ref)	1073	1.00 (ref)	1.00 (ref)
Class 1	113	114	0.99 (0.76-1.29)	0.79 (0.58-1.09)	58	1.00 (0.73-1.39)	0.75 (0.47-1.19)	52	1.03 (0.73-1.44)	1.35 (0.89-2.06)
Class 2	61	59	0.95 (0.66-1.36)	0.69 (0.44-1.09)	31	0.99 (0.64-1.54)	0.59 (0.32-1.10)	27	0.99 (0.62-1.56)	0.70 (0.37-1.31)
Class 3	11	20	1.78 (0.85-3.73)	1.32 (0.58-3.04)	6	1.07 (0.39-2.89)	0.57 (0.17-1.84)	8	1.62 (0.65-4.04)	1.84 (0.67-5.08)
P trend			0.5522			0.9707			0.5848	
Tree pollen mixture										
No	2249	2297	1.00 (ref)	1.00 (ref)	1078	1.00 (ref)	1.00 (ref)	1003	1.00 (ref)	1.00 (ref)
Class 1	169	174	1.01 (0.81-1.26)	0.99 (0.77-1.27)	76	0.94 (0.71-1.24)	0.86 (0.61-1.22)	74	0.98 (0.74-1.30)	0.96 (0.67-1.38)
Class 2	129	132	1.00 (0.78-1.29)	1.09 (0.81-1.46)	102	1.65 (1.26-2.16)	1.50 (1.06-2.14)	73	1.27 (0.94-1.71)	0.78 (0.51-1.18)
Class 3	29	28	0.95 (0.56-1.59)	0.82 (0.45-1.51)	64	4.60 (2.95-7.18)	3.79 (2.28-6.29)	10	0.77 (0.38-1.59)	0.51 (0.21-1.23)
P trend			0.9430			<.0001			0.4338	
Hop										

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No	2412	2461	1.00 (ref)	1.00 (ref)	1150	1.00 (ref)	1.00 (ref)	1076	1.00 (ref)	1.00 (ref)
Class 1	90	89	0.97 (0.72-1.31)	0.89 (0.63-1.27)	46	1.07 (0.75-1.54)	1.28 (0.81-2.03)	50	1.25 (0.88-1.77)	1.15 (0.74-1.79)
Class 2	45	52	1.13 (0.76-1.69)	1.21 (0.77-1.91)	50	2.33 (1.55-3.51)	3.71 (2.24-6.13)	20	1.00 (0.59-1.70)	0.70 (0.36-1.35)
Class 3	29	29	0.98 (0.58-1.65)	0.85 (0.45-1.61)	74	5.35 (3.46-8.27)	6.89 (4.05-11.73)	14	1.08 (0.57-2.06)	0.68 (0.31-1.53)
P trend			0.8221			<.0001			0.5204	

1 aOR, adjusted odds ratio.

2 b, OR values of three allergic disease compared with controls.

3 cOR, crude odds ratio.

4 CI, confidence interval.

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Table 4. ORs for allergic diseases with specific IgE class to food allergens (fish and seafood).

Allergen category	Control (n=2576)	Atopic dermatitis (n=2631)			Allergic rhinitis (n=1320)			Allergic asthma (n=1160)		
		n	cOR[b] (95% CI)	aOR[b] (95% CI)	n	cOR[b] (95% CI)	aOR[b] (95% CI)	n	cOR[b] (95% CI)	aOR[b] (95% CI)
Blue mussel										
No	2521	2503	1.00 (ref)	1.00 (ref)	1289	1.00 (ref)	1.00 (ref)	1126	1.00 (ref)	1.00 (ref)
Class 1	40	38	0.96 (0.61-1.50)	0.57 (0.33-0.98)	20	0.98 (0.57-1.68)	1.29 (0.64-2.60)	18	1.01 (0.58-1.77)	0.64 (0.30-1.36)
Class 2	7	29	4.17 (1.82-9.54)	3.27 (1.40-7.65)	6	1.68 (0.56-5.00)	0.94 (0.22-4.03)	8	2.56 (0.93-7.07)	0.80 (0.23-2.84)
Class 3	8	61	7.66 (3.66-16.04)	5.98 (2.79-12.80)	5	1.22 (0.40-3.74)	1.09 (0.31-3.80)	8	2.24 (0.84-5.98)	0.98 (0.28-3.35)
P trend			<.0001			0.5126			0.0335	
Fish										
No	2378	2354	1.00 (ref)	1.00 (ref)	1215	1.00 (ref)	1.00 (ref)	1068	1.00 (ref)	1.00 (ref)
Class 1	155	163	1.06 (0.85-1.33)	1.09 (0.83-1.42)	80	1.01 (0.77-1.34)	0.94 (0.64-1.39)	74	1.06 (0.80-1.42)	1.28 (0.88-1.86)
Class 2	27	53	1.98 (1.24-3.16)	2.13 (1.28-3.55)	16	1.16 (0.62-2.16)	0.74 (0.35-1.59)	8	0.66 (0.30-1.46)	0.53 (0.22-1.28)
Class 3	16	61	3.85 (2.21-6.70)	2.24 (1.18-4.25)	9	1.10 (0.49-2.50)	1.01 (0.38-2.71)	10	1.39 (0.63-3.08)	1.14 (0.43-3.03)
P trend			<.0001			0.6747			0.7889	
Crab										
No	2423	2409	1.00 (ref)	1.00 (ref)	1235	1.00 (ref)	1.00 (ref)	1094	1.00 (ref)	1.00 (ref)
Class 1	120	122	1.02 (0.79-1.32)	0.95 (0.70-1.28)	62	1.01 (0.74-1.39)	0.92 (0.61-1.38)	44	0.81 (0.57-1.16)	0.79 (0.52-1.20)
Class 2	22	46	2.10 (1.26-3.51)	1.80 (1.04-3.12)	13	1.16 (0.58-2.31)	0.55 (0.25-1.20)	12	1.21 (0.60-2.45)	0.72 (0.31-1.66)
Class 3	11	54	4.94 (2.58-9.46)	4.75 (2.27-9.91)	10	1.78 (0.76-4.21)	0.66 (0.23-1.87)	10	2.01 (0.85-4.76)	1.45 (0.55-3.82)
P trend			<.0001			0.2796			0.5212	

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Shrimp										
No	2455	2434	1.00 (ref)	1.00 (ref)	1270	1.00 (ref)	1.00 (ref)	1098	1.00 (ref)	1.00 (ref)
Class 1	102	102	1.01 (0.76-1.34)	0.92 (0.66-1.28)	39	0.74 (0.51-1.08)	0.66 (0.40-1.08)	46	1.01 (0.71-1.44)	0.87 (0.56-1.37)
Class 2	9	24	2.69 (1.25-5.80)	4.07 (1.62-10.22)	5	1.07 (0.36-3.21)	0.58 (0.15-2.18)	8	1.99 (0.77-5.17)	2.03 (0.65-6.34)
Class 3	10	71	7.15 (3.68-13.89)	5.18 (2.49-10.75)	6	1.16 (0.42-3.20)	0.85 (0.25-2.82)	8	1.79 (0.70-4.55)	1.92 (0.69-5.35)
P trend			<.0001			0.4474			0.1490	

1 aOR, adjusted odds ratio.

2 b, OR values of three allergic disease compared with controls.

3 cOR, crude odds ratio.

4 CI, confidence interval.

16

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Table 5. ORs for allergic diseases with specific IgE class to food allergens (non-fish and seafood).

Allergen category	Control (n=2576)	Atopic dermatitis (n=2631)			Allergic rhinitis (n=1320)			Allergic asthma (n=1160)		
		n	cOR[b] (95% CI)	aOR[b] (95% CI)	n	cOR[b] (95% CI)	aOR[b] (95% CI)	n	cOR[b] (95% CI)	aOR[b] (95% CI)
Egg white/egg yolk										
No	2461	2415	1.00 (ref)	1.00 (ref)	1267	1.00 (ref)	1.00 (ref)	1106	1.00 (ref)	1.00 (ref)
Class 1	78	79	1.03 (0.75-1.42)	1.12 (0.78-1.61)	32	0.80 (0.53-1.21)	0.64 (0.38-1.08)	35	1.00 (0.67-1.50)	0.90 (0.55-1.46)
Class 2	17	42	2.52 (1.43-4.44)	2.32 (1.28-4.19)	10	1.14 (0.52-2.50)	1.39 (0.55-3.55)	9	1.18 (0.52-2.65)	0.72 (0.29-1.81)
Class 3	20	95	4.84 (2.98-7.86)	2.65 (1.53-4.61)	11	1.07 (0.51-2.24)	1.55 (0.67-3.57)	10	1.11 (0.52-2.39)	0.75 (0.30-1.85)
P trend			<.0001			0.8415			0.7029	
Mutton										
No	2408	2450	1.00 (ref)	1.00 (ref)	1234	1.00 (ref)	1.00 (ref)	1078	1.00 (ref)	1.00 (ref)
Class 1	118	123	1.03 (0.79-1.33)	1.12 (0.85-1.48)	60	0.99 (0.72-1.36)	0.93 (0.63-1.36)	52	0.98 (0.71-1.38)	1.04 (0.72-1.51)
Class 2	31	39	1.24 (0.77-1.99)	1.28 (0.75-2.19)	16	1.01 (0.55-1.85)	0.74 (0.33-1.68)	15	1.08 (0.58-2.01)	0.48 (0.22-1.04)
Class 3	19	19	0.98 (0.52-1.86)	0.54 (0.25-1.14)	10	1.03 (0.48-2.22)	1.29 (0.50-3.35)	15	1.76 (0.89-3.48)	1.63 (0.72-3.66)
P trend			0.5808			0.9746			0.2266	
Milk										
No	2424	2398	1.00 (ref)	1.00 (ref)	1243	1.00 (ref)	1.00 (ref)	1090	1.00 (ref)	1.00 (ref)
Class 1	126	119	0.96 (0.74-1.23)	1.00 (0.76-1.32)	64	0.99 (0.73-1.35)	0.93 (0.65-1.33)	50	0.88 (0.63-1.23)	0.95 (0.66-1.37)
Class 2	16	51	3.22 (1.83-5.67)	3.61 (1.96-6.65)	7	0.85 (0.35-2.08)	1.28 (0.42-3.93)	10	1.39 (0.63-3.07)	1.36 (0.55-3.40)
Class 3	10	63	6.36 (3.26-12.43)	5.84 (2.73-12.50)	6	1.17 (0.42-3.23)	1.02 (0.26-4.07)	10	2.22 (0.92-5.36)	1.40 (0.48-4.09)
P trend			<.0001			0.9814			0.2746	
Beef										

17

No	2443	2498	1.00 (ref)	1.00 (ref)	1250	1.00 (ref)	1.00 (ref)	1097	1.00 (ref)	1.00 (ref)
Class 1	94	91	0.95 (0.71-1.27)	0.91 (0.66-1.26)	48	1.00 (0.70-1.42)	0.94 (0.62-1.41)	34	0.81 (0.54-1.20)	0.86 (0.55-1.33)
Class 2	19	21	1.08 (0.58-2.02)	0.22 (0.09-0.51)	11	1.13 (0.54-2.39)	1.07 (0.42-2.76)	14	1.64 (0.82-3.29)	1.04 (0.45-2.38)
Class 3	20	21	1.03 (0.56-1.90)	0.53 (0.25-1.14)	11	1.08 (0.51-2.25)	1.56 (0.55-4.44)	15	1.67 (0.85-3.28)	1.31 (0.57-3.03)
P trend			0.9970			0.7771			0.1923	
Wheat										
No	2354	2400	1.00 (ref)	1.00 (ref)	1195	1.00 (ref)	1.00 (ref)	1054	1.00 (ref)	1.00 (ref)
Class 1	175	183	1.03 (0.83-1.27)	0.95 (0.74-1.22)	100	1.13 (0.87-1.45)	0.73 (0.53-1.02)	79	1.01 (0.77-1.33)	0.91 (0.66-1.27)
Class 2	26	26	0.98 (0.57-1.69)	1.00 (0.52-1.94)	13	0.99 (0.50-1.92)	0.61 (0.27-1.38)	14	1.20 (0.63-2.31)	0.89 (0.37-2.18)
Class 3	21	22	1.03 (0.56-1.87)	0.86 (0.44-1.68)	12	1.13 (0.55-2.30)	0.66 (0.27-1.60)	13	1.38 (0.69-2.77)	1.36 (0.59-3.11)
P trend			0.8730			0.4715			0.3747	

1 aOR, adjusted odds ratio.

2 b, OR values of three allergic disease compared with controls.

3 cOR, crude odds ratio.

4 CI, confidence interval.

5

18

1 **Combined effects of sensitization to multiple allergens on allergic diseases**

2 *OR*s and their 95% *CI*s of allergic diseases with the different number of allergens are shown in Table

3 6. Although the three allergic diseases had different *OR*s and 95% *CI*, the patterns of the relationships

4 between allergic diseases and the number of allergens were similar. *OR*s increased as the number of

5 sensitive allergens increased (*P* trend <0.0001). The associations between two or more allergens and

6 atopic dermatitis were strong, adjusted *OR*=4.28 (95% *CI*: 2.57, 7.11). The combination of specific

7 IgE to three or more sensitive allergens possessed the highest *OR* for allergic rhinitis (adjusted

8 *OR*=13.00, 95% *CI*: 3.76, 45.00), while the combination of specific IgE to two or more allergens

9 showed the highest *OR* for allergic asthma (adjusted *OR*=2.37, 95% *CI*: 1.67, 3.37).

10 **Table 6. ORs for allergic diseases in relation to number of allergens.**

	Control (n=2576)	n	cOR[b] (95% CI)	aOR[b] (95% CI)
			Atopic dermatitis (n=2631)	
Number of allergens[d1]				
No	2422	2142	1.00 (ref)	1.00 (ref)
1	135	375	3.14(2.56-3.86)	3.00(2.44-3.69)
2	19	79	4.70(2.84-7.78)	4.28(2.57-7.11)
≥3	0	35	-	-
P trend			<.0001	
			Allergic rhinitis (n=1320)	
Number of allergens [d2]				
No	2181	893	1.00 (ref)	1.00 (ref)
1	286	312	2.66(2.23-3.19)	2.64(2.20-3.18)
2	106	97	2.24(1.68-2.98)	2.13(1.58-2.87)
≥3	3	18	14.65(4.31-49.87)	13.00(3.76-45.00)
P trend			<.0001	
			Allergic asthma (n=1160)	
Number of allergens [d3]				
No	2183	845	1.00 (ref)	1.00 (ref)
1	319	243	1.97(1.64-2.37)	2.06(1.70-2.49)
2	74	68	2.37(1.69-3.33)	2.37(1.67-3.37)
≥3	0	4	-	-
P trend			<.0001	

11 aOR, adjusted odds ratio.

12 b, OR values of three allergic disease compared with controls.

13 cOR, crude odds ratio.

14 CI, confidence interval.

15 [d1] allergens excluding those allergens which were not related with atopic dermatitis in Table 2, Table

16 3, Table 4 and Table 5. [d2] allergens excluding those allergens which were not related with allergic

19

1   rhinitis in Table 2, Table 3, Table 4 and Table 5. [d3] allergens excluding those allergens which were not

2   related with allergic asthma in Table 2, Table 3, Table 4 and Table 5.

3

Discussion

5   In our present hospital-based case-control research, we found that different allergens are significantly

6   related to different allergic diseases. There is a dose-response relationship between allergen-specific

7   IgE levels and allergic diseases, and multiple sensitizations increase the risk of allergic diseases. The

8   incidence of allergic diseases has increased rapidly worldwide[2-4]. The World Allergy Organization

9   (WAO) reported that, among 1.39 billion people in 33 countries, 22 percent of the population suffered

10   from allergic diseases[20]. The World Health Organization (WHO) has clearly stipulated that allergic

11   disease is an extremely considerable disease that the whole world should attach importance to[21].

12   Identifying specific allergens that trigger allergic diseases is particularly significant for the prophylaxis

13   and treatment of allergic diseases.

14   Our findings indicated that atopic dermatitis was significantly related to food allergens sensitization,

15   while allergic rhinitis and allergic asthma were significantly associated with both indoor and outdoor

16   allergens sensitization. The recognition to food allergens through antigen-presenting cells in

17   eczematous skin plays an important role in the link between food allergy and allergic dermatitis. In

18   addition, the dysfunction of filaggrin or Th2-relateed cytokines or rare genetic syndromes may also

19   involve in the mechanism[22]. Different types of allergens induce different allergic diseases, which may

20   due to the properties or size of allergens. Allergic rhinitis is associated with outdoor allergens, mainly

21   pollen, probably because pollen particles are slightly larger than indoor dust and are more likely to

22   stay in the nose[23]. Inhalation of indoor dust, mites and fungi can increase the airway reactivity and

20

1   causes allergic asthma[24]. Our findings were consistent with previous studies that sensitization to indoor

2   allergens are important risk factors in patients with allergic rhinitis and allergic asthma[9,25,26]. Some

3   studies also stated that there was a relevance between sensitization to outdoor allergens and allergic

4   rhinitis and allergic asthma[27-29]. Food allergens were found to be crucial in the development of atopic

5   dermatitis in some observations[29,30]. Allergens, commonly proteins, induce allergic diseases through

6   complex interactions with the immune system. Due to the presence of epitopes with allergenic

7   potential, glycosylation status, resistance to proteolysis, and enzymatic activity, some proteins are

8   more allergenic than others and different allergens may cause different allergic diseases through

9   various underlying mechanisms[31]. However, some studies showed inconsistent findings. Several

10   studies showed that indoor allergens have a bearing on the prevalence of atopic dermatitis in some

11   individuals[32,33]. Other studies showed a relationship between sensitization to food allergens and

12   allergic rhinitis and allergic asthma[34]. The inconsistency of those studies may be related to regional

13   variation and different climate characteristics, which affect the morphology of the allergen-carrying

14   agents and modify their allergenic potential[35,36]. In addition, genetic susceptibility and socio-economic

15   status of the populations may influence the occurrence of allergic diseases[3,37,38].

16   This study revealed that there was a dose-response relationship between specific IgE levels of

17   allergens and prevalence of allergic diseases, in which the odds of allergic diseases increase with

18   increasing allergen-specific IgE levels. Our findings are in line with a study in America revealing a

19   dose-response relationship between exposure to mouse allergen and the morbidity of allergic asthma

20   in urban children and adolescents[39]. Another research evaluated that whether TPI ASM8 induced a

21   dose-dependent reduction in the inflammatory and physiological changes following inhaled allergen

21

1   challenge[40]. It is worth noting that there was a combined effect of sensitization to different allergens

2   on allergic diseases. Multiple sensitizations to several allergens had a relatively stronger association

3   with allergic diseases than single sensitization to one allergen. Some research results were similar to

4   our findings. Kumar et al. reported that simultaneous sensitization to food and inhalant allergens

5   (insect and pollen) may increase the risk of asthma and rhinitis or exacerbate symptoms[41]. A cross-

6   sectional population-based case-control study in Finland adults found that the pathomechanisms of

7   sensitization to one allergen are different from those of sensitization to several allergen types, and the

8   latter are more likely to induce asthma[42]. In addition, most allergens were significantly associated with

9   allergic diseases only when their IgE level classes $\geq$ 2 according to our study. Such findings may be

10   of more clinical value than a dichotomous designation of sensitized or not sensitized to allergens, and

11   can potentially be used for improving the diagnosis of allergic diseases.

12   According to the previous studies, genetic and environmental factors play important roles in

13   sensitization to allergens[18,43-46]. In our study, the family history of atopic dermatitis and allergic rhinitis

14   were significantly different compared to the controls, while there was no difference in family history

15   between allergic asthma and controls. The proportion living in rural were significantly higher in

16   patients with atopic dermatitis and allergic asthma than controls, while there was no difference in

17   location of residence between allergic rhinitis and controls. We can investigate the family history and

18   location of residence and combine clinical symptoms to speculate whether the patients are likely to be

19   sensitive to allergens and recommend further appropriate testing.

20   It is obviously that allergens avoidance is the most effective means of prevention for allergy sufferers

21   considering that allergic diseases are triggered by those allergens. However, this may not be true in

22

1   some cases. Completely avoiding contact with some indoor and outdoor allergens is not always

2   feasible. Moreover, early exposure to food allergens has been turned out to be a successful method for

3   the prevention of peanut allergy[47]. Many studies reported that allergen immunotherapy was an

4   effective treatment strategy for allergic diseases[48,49]. However, for some patients with severe

5   symptoms, allergen avoidance and treatment of symptoms may be necessary. Our findings could

6   provide some evidence for clinicians about avoidance or specific immunotherapy treatment for

7   patients with allergic diseases.

8   There are several strengths in this study. Firstly, the sample size of this study was relatively large

9   which enabled us to classify specific IgE levels of 16 common allergens into three levels: class 1, class

10   2, and class 3. This enabled us to analyze the dose-response relationship between specific IgE levels

11   and the prevalence of allergic diseases, and the combined effect of sensitization to multiple allergens

12   with allergic diseases. Secondly, the 16 allergens were categorized into three categories: indoor

13   allergens, outdoor allergens, and food allergens, and the associations between each type of allergens

14   and different allergic diseases were analyzed. However, there are some limitations in this study. Firstly,

15   we cannot determine when the patients were first sensitized to allergens or when they first developed

16   an allergic disease. We did not collect the specific date of the patient's visit to the hospital, so we could

17   not analyze the impact of seasonal factors on allergic diseases. Secondly, this study was performed

18   only in one hospital in Harbin, the generalizability of our results to the whole of China still needs to

19   be further explored.

20   In conclusion, food allergens sensitization is associated with atopic dermatitis, while indoor and

21   outdoor allergens sensitization are related to allergic rhinitis and asthma in northeastern China. There

23

1   is a dose-response relationship between allergen-specific IgE levels and allergic diseases, and multiple

2   sensitizations increase the risk of allergic diseases. Our findings could provide evidence for the control

3   and management of allergic diseases, and may have important public health implications.

4

5   **Ethics approval statement**

6   Participants were not subjected to intervention and gave informed consent to participate in the study

7   before taking part, so the Institutional Review Board (IRB) of Harbin Medical University exempted

8   our study according to the regulations of our school.

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13   **Data sharing statement**

14   Not applicable.

15   **Authors' contributions**

16   W.Z. and B.X. designed the process of this study and the structure of this article. Y.W. and M.L.

17   designed the questionnaire. W.Z. and B.X. participated in case collection and questionnaire

18   investigation, and analyzed the data. W.Z. wrote the manuscript, and all other authors read and

19   confirmed the final version.

20   **Competing interests**

21   None declared

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Figure legends

Figure 1. The flow diagram of study participants.

Figure 2. Prevalence of allergens sensitization among patients with atopic dermatitis, allergic rhinitis,

and allergic asthma.

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Potential cases (N1=7000)
Potential controls (N2=3500)

Excluded: without informed consents
(cases: 353; controls: 197)

Potential cases (N1=6647)
Potential controls (N2=3303)

Excluded: missing and anomalous information
(cases: 845; controls: 406)

Potential cases (N1=5802)
Potential controls (N2=2897)

Excluded: multiple cases, logic errors and specific populations
(cases: 691; controls: 321)

Final controls (N2=2576)
Final cases (N1=5111)
Including: 2631 atopic dermatitis, 1320 allergic rhinitis and 1160 allergic asthma
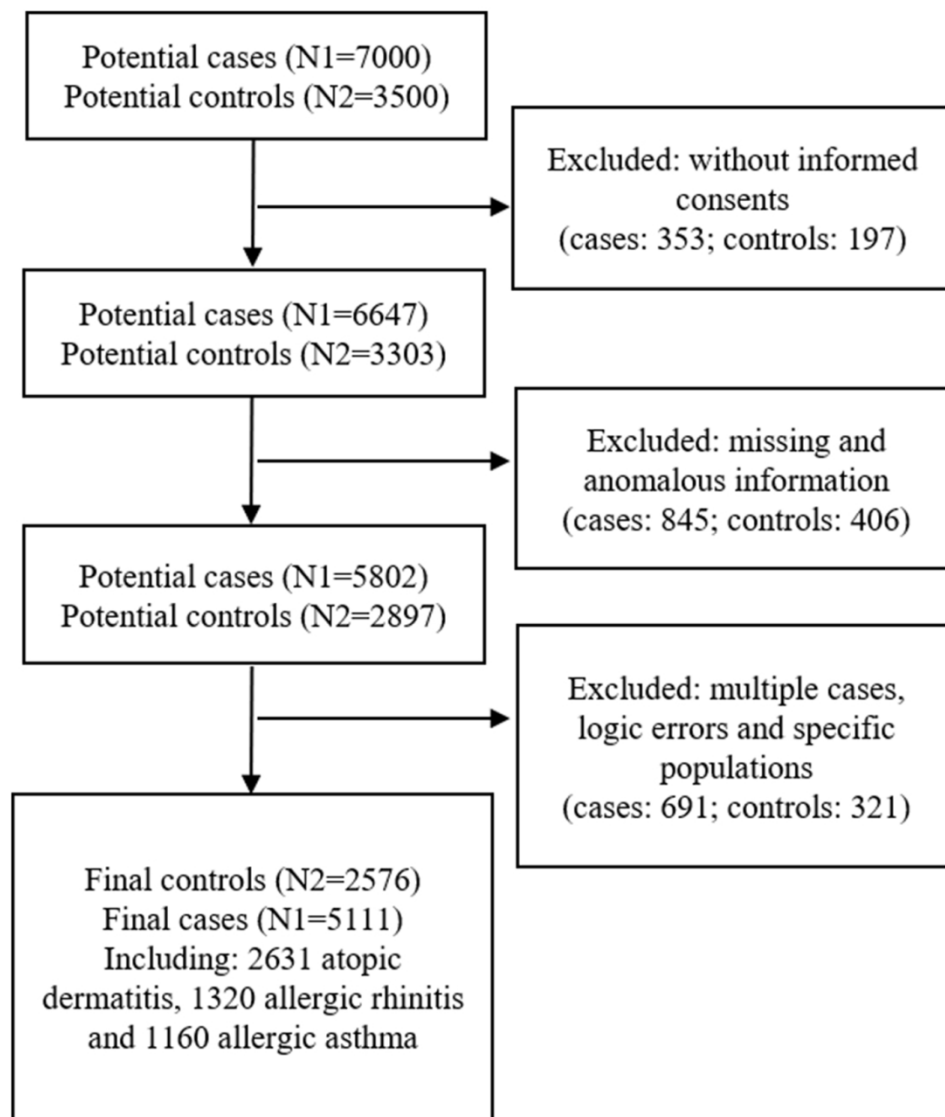
Figure 1. The flow diagram of study participants.
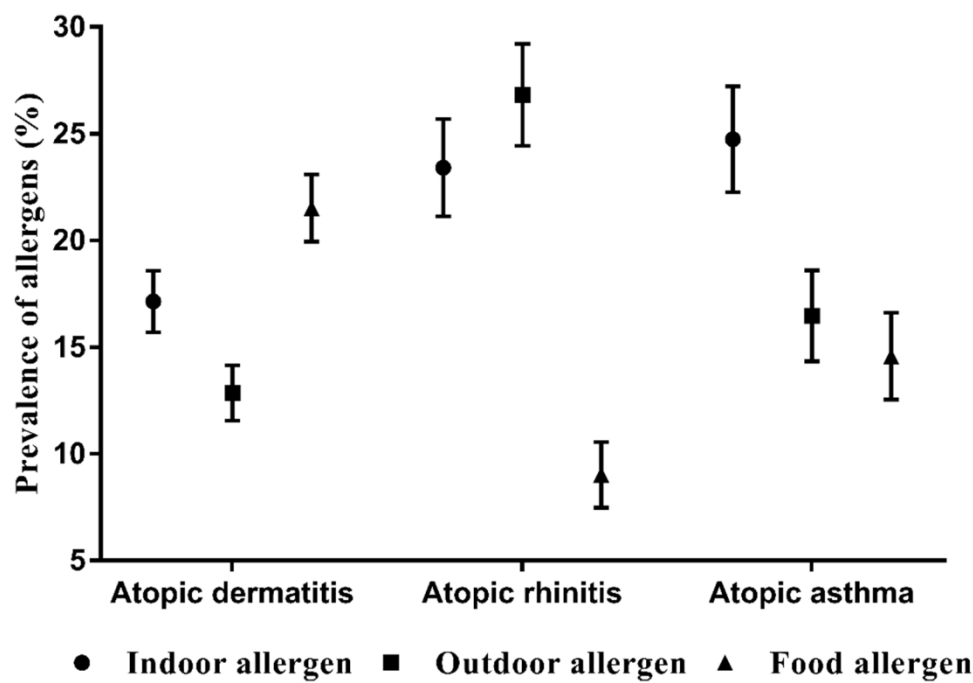
107x124mm (350 x 350 DPI)

Figure 2. Prevalence of allergens sensitization among patients with atopic dermatitis, allergic rhinitis, and allergic asthma.

129x92mm (350 x 350 DPI)

Associations between sensitization to allergens and allergic diseases: a hospital-based case-control study in China

Supplementary Method:

1. The definition of demographic, family history, and lifestyle factors

Age was calculated as the difference between the year of birth and the year of interview. Educational status was categorized into three levels: junior high school or lower, senior high school, and university or higher. The residential region was categorized into two groups: urban and rural. Marital status was also categorized into two groups: unmarried and married. Current smokers were defined on the basis of the World Health Organization criteria, as those who self-reported smoking every day for at least 6 months[9]. Regular alcohol drinkers were defined as drinking more than twice per week for at least one year. Classification of physical exercise (PE) was defined as followed:

We estimated PE condition of study participants using three variables of a structured questionnaire, exercise intensity (EI), exercise time (ET), and exercise frequency (EF) [1-4].

EI of study participants was classified into three groups, mild, moderate and strenuous exercise according to their exercise types. Value 1, 2 and 3 were assigned to the three groups respectively [5-8]. ET was measured by the hour. EF referred to how many times a week. We defined PE as

$$PE = \sum_{i=1}^{N} EI \times ET$$

Where N represented EF. If PE $\geq$ 2, PE was positive; PE was negative when PE < 2. Therefore, as for a subject jogging (moderate exercise) 15 minutes 5 times a week, his/her PE was positive because PE $\geq$ 2 (PE = $5 \times 0.25 \times 2 = 2.5$).

2. The diagnostic criteria of atopic dermatitis, allergic asthma, and allergic rhinitis

2.1 Atopic dermatitis

The following diagnostic criteria for atopic dermatitis was developed by the American

Academy of Dermatology (AAD), which is based on age-specific clinical criteria that include pruritus and chronic or relapsing spongiotic dermatitis involving the face, trunk, and/or extensor extremities in infants, flexural surfaces like the wrists/ankles and antecubital/popliteal fossae in children, or the hands in adults[10].

Essential Features (both must be present):

    1) Pruritus

    2) Eczema (acute, subacute, chronic)

        a. Chronic or relapsing history

        b. Typical morphology and age-specific patterns

            Infants: face, trunk (except "diaper area"), extensor extremities

            Children: flexors (wrists, ankles, antecubital/popliteal fossae)

            Adults: hands

            All ages: sparing of the groin and axillary regions

Important Features (support the diagnosis and are observed in most cases of atopic dermatitis):

    1) Early age of onset

    2) Atopy

        a. personal and/or family history; or

        b. IgE reactivity

    3) Xerosis

Associated Features (suggestive of atopic dermatitis, but too nonspecific to define or detect atopic dermatitis in research or epidemiologic studies):

    1) Atypical vascular responses (eg, facial pallor, white dermatographism, delayed blanch response)

    2) Keratosis pilaris, pityriasis alba, hyperlinear palms/ichthyosis

    3) Ocular/periorbital changes (fissures, infraorbital folds)

    4) Other regional findings (eg, perioral changes/periauricular lesions)

    5) Perifollicular accentuation/lichenification/prurigo lesions

2.2 Allergic rhinitis

The diagnosis criteria for allergic rhinitis are as follows[11]:

1) Clinical history

Clinical history is essential for the accurate diagnosis of allergic rhinitis and the assessment of its severity as well as its response to treatment. The most frequent symptoms include sneezing, anterior rhinorrhea, bilateral nasal obstruction, and nasal pruritus in patients with allergic rhinitis. In addition, most patients with pollen-induced rhinitis have eye symptoms. It is also important to distinguish between allergy and nonallergy symptoms. Subjective assessment of symptoms of allergic rhinitis is generally based on 4 nasal symptoms (sneezing, rhinorrhoea, nasal itching, and nasal obstruction) and 2 ocular symptoms (ocular itching/grittiness/redness and ocular tearing). In China, VAS is most commonly used to quantify the above-mentioned assessments. VAS was used to quantify the above-mentioned assessments.

2) Nasal examinations

Anterior rhinoscopy and nasal endoscopy are the widely used approaches. The nasal examination should describe: 1) the anatomical situation in the nose (e.g. the septum, the size of the inferior turbinate, and if possible the structures in the middle meatus); 2) the color of the mucosa; and 3) the amount and aspect of the mucus. Endoscopic images of nasal mucosa in patients suffering from allergic rhinitis generally demonstrate pale and edematous nasal mucosa, watery nasal discharge, and swollen inferior turbinates.

3) Skin tests

Two methods of skin testing, including intradermal skin tests and skin prick tests (SPTs), are available in China. Skin tests should be read at the peak of their reaction by measuring the wheal and the flare approximately 15 minutes after the performance of the tests. For prick tests, when the control site is completely negative, wheals of >3 mm represent a positive skin response.

4) Serum specific IgE measurements

Enzyme-labeled anti-IgE measurement has been widely used in China. Results are expressed in terms of units of IgE (IU/mL, KU/L). The IgE level above 0.35 KU/L is usually testified as a positive result.

5) Differential diagnosis

In addition, it is necessary to differentiate allergic rhinitis from other diseases, such as vasomotor rhinitis, nonallergic rhinitis with eosinophilia syndrome (NARES), infectious rhinitis, hormonal rhinitis, medicamentous rhinitis (rhinitis medicamentosa), aspirin intolerance triad, cerebrospinal fluid rhinorrhea, and so on.

2.2 Allergic asthma

The diagnosis criteria for allergic asthma are as follows[12]:

1) Clinical history

Wheezing, coughing, chest tightness

May only be present or worsened with exertion, upper respiratory infection, seasonal or perennial allergies

Nocturnal cough, particularly 2 AM to 4 AM

Need for short-acting β_2-agonist inhaler for relief of symptoms

Personal or family history of atopy

2) Spirometry

Airway obstruction evidenced by FEV_1: FVC ratio < lower limit of normal

Demonstrated reversibility of obstruction by increase in $FEV_1 \geq 200mL$ and $\geq 12\%$ from baseline measure after inhalation of 2-4 puffs of short-acting β_2-agonist

Normal spirometry findings are not inconsistent with asthma

3) Bronchoprovocation with methacholine

20% or more decrease in FEV_1 with after inhalation of low concentration (< 4 mg/mL) of methacholine; used principally in patients with symptoms consistent with asthma but who exhabit normal pulmonary function tests

4) Impedance oscillometry

Elevated airway resistance at 5 HZ, elevated area of reactance, increased resonant frequency (Fres), reactance at 5 HZ more negative than predicted

5) Chest radiograph or CT scan of thorax

Usually normal but can exclude other diagnoses such as emphysema, lung cancer, infiltrative diseases, pneumonia

6) CBC

Eosinophilia, particularly > 300/μL; results can inform selection for mepolizumab or reslizumab therapy

7) Serum total IgE

Elevated in atopic asthma, not in nonatopic asthma; can inform selection of omalizumab therapy

8) Skin prick testing or serum-specific IgE for aeroallergens

Positive, particularly for perennial allergens, or seasonal allergens with corresponding seasonal variation in asthma symptoms; may be negative in nonatopic asthma; can inform omalizumab therapy

Positive testing can guide allergen avoidance strategies

9) Fractional excretion of nitric oxide

Intermediate level: 25-50 ppb in patients aged $\geq$ 12 y

High level: > 50ppb in patients aged $\geq$ 12 y

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Supplementary Table:

Supplementary Table S1. Sixteen common allergens used in this study.

category	allergens			
indoor allergens	Dermatophagoides pteronyssinus	Cat and dog fur	Mould mixture [a]	
outdoor allergens	common ragweed and mugwort	German cockroach	Tree pollen mixture [b]	Hop
food allergens	Egg white/egg yolk	Blue mussel	Fish	Crab
	Mutton	Milk	Beef	Shrimp
	Wheat			

[a] Mould mixture is composed of Penicillium notatum, Cladosporium herbarum, Aspergillus fumigatus and Alternaria alternate.

[b] Tree pollen mixture is composed of Robur, Elm, London Plane, Willow and cottonwood.

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *case-control studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2,3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3,4
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5,6,7
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls	5,7
		(*b*) For matched studies, give matching criteria and the number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	5,6,7
Bias	9	Describe any efforts to address potential sources of bias	
Study size	10	Explain how the study size was arrived at	
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7,8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7
		(*b*) Describe any methods used to examine subgroups and interactions	
		(*c*) Explain how missing data were addressed	
		(*d*) If applicable, explain how matching of cases and controls was addressed	
		(*e*) Describe any sensitivity analyses	
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	7,8
		(b) Give reasons for non-participation at each stage	7,8
		(c) Consider use of a flow diagram	7,8
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8
		(b) Indicate number of participants with missing data for each variable of interest	
Outcome data	15*	Report numbers in each exposure category, or summary measures of exposure	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9-18
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
Discussion			
Key results	18	Summarise key results with reference to study objectives	19
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	23
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	19-22
Generalisability	21	Discuss the generalisability (external validity) of the study results	23
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	24

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.