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Cockroach is a major cross-reactive allergen source in shrimp-sensitized rural children in southern China

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Abstract

Background: Little is known about the prevalence of food allergy (FA) in China.

Objective: To investigate the prevalence of FA and its disparity between urban and rural areas in southern China.

Methods: Europrevall questionnaire responses were obtained from 5,542 school-age-children in urban Guangzhou and 5,319 in rural Shaoguan. A case-control study enrolled 190 children with adverse reactions (ARs) after food intake as cases, and 212 controls in Guangzhou; 116 cases and 233 controls in Shaoguan. These subjects underwent skin-prick test (SPT) and serum IgE measurements to food and inhalant allergens. Allergen

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extracts from shrimp, house dust mite (HDM), and cockroach were prepared for IgE cross-reactivity testing in 23 Guangzhou and 20 Shaoguan shrimp-sensitized subjects.

Results: The prevalence of ARs to shrimp was higher in Guangzhou than Shaoguan children (3.5% vs. 1.4%, $p<0.001$). However, sensitization rate to shrimp (SPT: 3.7% vs. 11.2%, $p=0.015$; IgE: 12.6% vs. 36.2%, $p<0.001$) and cockroach (SPT: 5.3% vs. 33.5%; IgE: 2.6% vs. 27.6%, $p<0.001$) were lower in Guangzhou. A significant correlation between shrimp and HDM/cockroach IgE was found in Shaoguan children. The proportions of positive IgE to tropomyosin (Pen a 1, Der p 10) were lower than 7.4% in both areas. Cockroach allergen has a significantly higher inhibition rate of binding to IgE to house-dust-mite allergens in Shaoguan sera.

Conclusions: Shrimp is a common allergic food in southern China. Higher proportion of shrimp sensitization in rural subjects could be explained by cross-reactive to cockroach. Tropomyosin was not a major allergen responding for the cross-reactivity.

Key words: food allergy; cross-reactivity; shrimp; cockroach; tropomyosin

Abbreviations

AR, adverse reactions; FA, food allergy; HDM, house dust mite; nsLTP, non-specific lipid transfer proteins; sIgE, specific IgE; SPT, skin-prick test.

Introduction

Food allergy (FA) consists of adverse responses to food allergens, resulting from IgE-mediated and non-IgE-mediated immune processes(1). Prevalence of FA is often difficult to determine. Through food challenge testing, researchers in Western countries have shown the prevalence of FA in adults and children to be 1–3% and 3–8%, respectively(2). There is very little published epidemiology data regarding FA in China. Our previous work revealed that the prevalence of FA in children in the Chinese urban and rural areas were 4.0% and 3.5%, respectively(3). Interestingly, an unselected rural Chinese cohort of twins in northern China demonstrated a low prevalence of self-reported FA (less than 1%) but high rate of atopic sensitization (47.2%) to shell fish, peanut, dust mite and cockroach(4). Due to safety constraints, most studies have not included food challenge test for the determination of the prevalence of FA in China. Recent FA evidence-based guidelines issued by the European Academy of Allergy and Clinical Immunology present that IgE-mediated FA frequently involves common cross-reacting allergens(5). This cross-reactivity has geographic variations. For example, our team reported that in patients in northern China, the non-specific lipid transfer proteins (nsLTP) of *Artemisia* pollen initiate peach allergy, while in the Mediterranean region, peach nsLTP is the major allergen of peach allergy(6).

In the case of shrimp allergy, tropomyosin is a common cross-allergen of shrimp (Pen a 1), *Dermatophagoides pteronyssinus* (dust mite, Der p 10) and *Blattella germanica* (cockroach, Bla g 7). Cross reactive immune sensitization is found in populations without

exposure to shrimp(7). A recent study demonstrated that inner-city children in the United States have high exposure to cockroach allergens that can contribute to high shrimp-specific IgE levels(8).

The EuroPrevall-INCO study compared the prevalence of FA in China, India, and Russia (9). Our data from China showed that shrimp was the main food associated with adverse reactions in children in southern China(3), and that dust mite and cockroach allergy are common in this population (with higher exposure of cockroach allergens in rural households)(10). We speculated that dust mite and cockroach might be associated with shrimp allergy, and that this correlation might vary between urban and rural areas. This current study aims to clarify the prevalence of IgE-mediated FA in children in southern China and to analyze the differences in shrimp allergy in the context of different inhalant allergens in urban and rural areas.

Methods

Study design

Figure 1 shows the outline of our study protocol including the questionnaires and methods, which have been published previously(9). In this study, a random sample of 10 elementary schools in Guangzhou cities and Shaoguan rural areas was selected to participate. 5,878 and 5,598 in the urban and rural areas were screened by the Europrevall FA screening questionnaires. According to the questionnaire responses, children with reported adverse reactions within two hours after the consumption of a specific food were

considered as cases while age-matched healthy children without adverse food reactions were recruited as controls. A detailed questionnaire was distributed to each of the children in the control and case groups, followed by skin-prick testing (SPT) and serum-specific IgE analysis. Sera from children with shrimp-specific IgE and concomitant dust mite or cockroach-specific IgE were tested by IgE inhibition assay. The study protocol was approved by the Ethics Committee of the First Affiliated Hospital of Guangzhou Medical University and was performed with the written informed consent of the parents of enrolled subjects.

SPT and serum specific IgE (sIgE) measurement

The eighteen food allergens (ALK, Horsholm, Denmark) used for SPT included mango, crab, egg, date, mussel, orange, milk, soybean, hazelnut, shrimp, wheat, peanut, fish, peach, beef, apple, lemon, and tomato. Serum-specific IgE measurement by ImmunoCAP™ as described previously(3) was used to test 27 food allergens [crab, mango, hazelnut, potato, carrot, celery, walnut, shrimp, mustard, poppy, lentils, sunflower, apple, kiwifruit, lemon, banana, peach, egg, milk, fish, peanut, soybean, wheat, maize, rice, sesame, and buckwheat] as well as seven inhalant allergens [i.e., dust mite, cockroach, cat hair, birch, *Artemisia*, *Achillea millefolium*, and *Parietaria* pollen]. Subjects with positive sIgE for dust mite and cockroach allergens underwent further tests for specific allergens, Der p 1, Der p 2, and tropomyosin (i.e., Der p 10 and Pen a 1, but not for Bla g 7 due to the lack of availability). sIgE ≥ 0.35 kU_A/L was considered to be a positive result.

Definition of health outcomes

Children with more than one adverse reaction within two hours after eating specific food(s), to which evidence of relevant sensitization was confirmed with positive results of SPT and/or sIgE measurement, were defined as having IgE-mediated FA. Since the food challenge is both time and labor intensive and carries the risk of severe allergic reactions, IgE-mediated food allergy in our study were not confirmed further by either blinded or open oral challenges.

IgE Inhibition assay

Shrimp (RMF34P), dust mite (*Dermatophagoides pteronyssinus*, RMB84M), and German cockroach (*Blattella germanica*, RMB46P) allergens were purchased from Greer labs (Lenoir, NC). Allergens were added to Coca's solution(11) at a mass ratio of 1:10 and incubated at room temperature for an hour, followed by centrifuging at $\geq 10,000 \times g$, 4°C for 10 min to collect the allergen crude extracts. The amount of extract and serum used in the inhibition assay was identified using a titration assay. Specifically, each serum sample was diluted to a specific IgE concentration of approximately 10 kU_A/L for the inhibition assay (Supplementary-Figure 2). The inhibition assays were performed by ImmunoCAP™ with reference to the published protocol(6) by mixing 1:1 diluted serum and allergen crude extract and incubating at room temperature for an hour. Specific IgE concentrations were determined before and after the immunoassay to calculate the IgE inhibition rate according to the following formula: IgE inhibition rate (%) = $[(\text{IgE}_{\text{PBS}} - \text{IgE}_{\text{allergen}}) / (\text{IgE}_{\text{PBS}} - \text{IgE}_{\text{negative control}})] \times 100\%$, where IgE_{negative control} represent the corresponding sIgE value of the serum

pool of healthy children without allergy.

Statistical analysis

Data were presented as rate, mean $\pm$ standard deviation, or quartile. Data were compared using Chi-square test, student's t-test, Mann-Whitney U test, or Pearson correlation coefficient where appropriate. Serum shrimp-, dust mite-, and cockroach-sIgE concentration underwent linear correlation analysis after log conversion. P values < 0.05 were considered to be statistically significant. All statistical analyses were performed using SPSS software (SPSS Inc., Chicago, IL). Flow chart and figures were prepared using Office Visio and GraphPad Prism software version 5.0 (San Diego, California, USA).

Experimental results

Baseline characteristics of populations, FA cases, and controls

Of 5,878 and 5,598 screening questionnaires distributed to the tested subjects, there was a response rate 5,542 (94.3%) and 5,139 (91.8%), respectively, in Guangzhou and Shaoguan. The average age of the reporting children from Shaoguan was significantly higher than children from Guangzhou (9.9 ± 2.1 vs. 8.9 ± 1.7 years, $p < 0.001$). Prevalence of reported adverse reaction to food (9.7 % vs. 9.2 %, $p = 0.448$) was not significantly different between children from Guangzhou and Shaoguan. Based on questionnaire results, crustacean shellfish (shrimp and crab) were the most common food in the two regions to cause adverse reactions with significantly higher percentage of affected children in Guangzhou than in Shaoguan (5.1% vs. 1.7%, $p < 0.001$); other common foods causing adverse reactions

included milk, mango, fish, egg, wheat, and pork. The percentages of FA diagnosed by physicians among the children in Guangzhou and Shaoguan were 4.0% and 3.5%, respectively, with no significant difference between two regions (Table 1).

From the screening questionnaire, we identified 223 and 179 children from Guangzhou and Shaoguan, respectively, with both physician-diagnosed FA and a history of adverse reactions after food consumption (Table 2). A detailed questionnaire was administered to each of these children to eventually include case subjects from 223 originally to 190 children from urban areas (response rate of 85.2%) and from 179 originally to 116 children from rural areas (response rate of 64.8%). In addition, for the control group, we invited 300 healthy children from each of the two regions who had reported no FA history and had no history of adverse reaction after eating, including 212 children from urban areas (response rate of 70.7%) and 233 children from rural areas (response rate of 77.7%) (Figure 1). Case and control groups did not differ in regard to gender and body weight. However, The mean ages of rural subjects were significantly higher than urban subjects in both case group (10.7 ± 1.5 vs. 10.2 ± 1.5 years, $p=0.005$) and control group (10.6 ± 1.4 vs. 10.3 ± 1.5 , $p=0.027$).

Food and inhalant allergies

As expected, results of SPT and sIgE measurement revealed that the percentages of sensitization of the case groups from urban and rural areas were significantly higher than the control groups from urban and rural areas ($p<0.001$). Significant within group differences between urban and rural areas was not seen in either case groups or the control groups. Shrimp and crab were the dominant implicated food allergens of the cases from

both regions. SPT and sIgE measurement demonstrated that shrimp sensitization rates of the case subjects from rural areas were significantly higher than the case subjects from urban areas (SPT: 11.2% vs. 3.7%, $p = 0.015$; sIgE: 36.2% vs. 12.6%, $p < 0.001$). In addition, the milk and egg sensitization rates by specific IgE determination of the case subjects from urban areas were significantly higher than the case subjects from rural areas whereas no differences were found by skin-prick test (Supplementary-Figure 1).

Inhalant allergen sensitivity in the case subjects from urban and rural areas tested by SPT and sIgE measurement were significantly more common than in the control subjects; however, no significant difference between urban and rural areas was found within the cases or the controls. The most common inhalant allergens of the two regions were dust mite and cockroach. Among the cases, cockroach SPT (33.6% vs. 5.3%) and sIgE (27.6% vs. 2.6%) rates from rural areas were significantly higher than the cases from urban areas ($p < 0.001$). In contrast, the cat hair sensitization rate of the cases from urban areas was significantly higher than the cases from the rural areas (Supplementary-Figure 1).

Component-sIgE measurement showed that Der p 1 and Der p 2 were the two dominant allergens in our cases, with the positive rates of the cases from urban areas significantly higher than both cases and controls from rural areas. In addition, dust mite Der p 10 and shrimp Pen a 1, belonging to the same tropomyosin family, had the highest positive rates in the case subjects from urban areas (14/108 and 9/24, respectively) although they were not the dominant allergens. The serum sIgE levels of the 29 shrimp-sensitized subjects from the urban areas correlated significantly with presence of cockroach sensitization ($p = 0.014$).

Shrimp sIgE of the 75 shrimp-sensitized subjects from rural areas was associated with sensitization to either dust mite or cockroach ($p < 0.001$, Figure 2).

Prevalence of IgE-mediated FA

In this study, 21 urban and 19 rural case subjects were IgE-mediated FA patients, accounting for 5.2% and 5.4% of the subjects in the corresponding areas. Seven kinds of FA were involved (Table 3). Shrimp was the primary allergen that caused IgE-mediated FA in urban and rural areas, accounting for 4.5% ($n = 18$) and 3.7% ($n = 13$), respectively.

IgE Inhibition assay

Tropomyosin was not a major allergen of our population. This study employed shrimp, dust mite, and cockroach allergen extracts as the inhibitors. Results of IgE-inhibition-assay showed that all three extracts completely inhibited respective serum sIgE (with the inhibition rate of $> 95\%$, Supplementary-Figure 2). Subjects co-sensitized to shrimp and dust mite or cockroach, including 23 cases from the urban area and 20 from the rural area with sufficient available serum, were included for IgE-inhibition assay (Figure 1). The sIgE-inhibitory effect of cockroach extract on Der p binding in the serum of case subjects from rural areas was significantly higher than case subjects from urban areas (Figure 3A). The sIgE-inhibitory effect of shrimp extract on Bla-g binding in the serum of cases from rural areas was significantly lower than cases from urban areas (Figure 3B). No significant difference in the inhibitory effect of cockroach and dust mite extracts on binding Shrimp-sIgE was found. Despite non-significant difference, dust mite inhibitors almost completely (80%) inhibited the shrimp-sIgE of children from Guangzhou; however, shrimp

inhibitors could not completely (<50%) inhibit dust mite-sIgE (Figure 3A, 3C). In addition, cockroach inhibitors highly inhibited Der p and Shrimp-sIgE in the case subjects from rural areas (with the inhibition rates > 85%, Figure 3A, 3C).

Discussion

This study revealed that shrimp was one of the most frequent causes of food allergy in the urban and rural areas in southern China based on clinical history, skin prick testing, and blood testing for specific IgE. A significant correlation between shrimp and cockroach sensitization was found. Higher rate of shrimp sensitization but lower clinical manifestation after shrimp ingestion in rural children could be explained by cross-reactivity to cockroach allergen. In addition, tropomyosin was not the dominant allergen responsible for such cross-reactivity.

IgE-mediated FA prevalence in our study is low and shrimp was the most common food resulting in FA. Because of different definitions, comparing FA prevalence studies is generally difficult(12). The addition of food challenge would result in a much lower rate than the rate estimated by questionnaires only(12). FA in China has rarely been reported, perhaps reflecting a generally low prevalence. The EuroPrevall-INCO study showed that the prevalence of IgE-mediated FA in Indian adults was low (1.2%) and shrimp was the most common allergen (sensitization rate of 15.5% in the population)(14). Unlike our pediatric Chinese population, shrimp allergy is rare in Indian children, yet milk and apple allergy were commonly encountered in clinical practice(13).

It is well documented that patterns of FA vary greatly in different regions. FA prevalence in Europe ranges from 0.1% to 6.0%, with the major food allergens being milk and egg among European children(14). Prevalence of shrimp sensitization in American children has been declining(15). Prevalence of seafood allergy is 5.2% in Singapore, which is higher than the United States at 2.0%(16). Seafood allergy are common FAs in the Asia-Pacific region, especially in South Asia, Hong Kong, and Taiwan(12). This study was carried out geographically close to Hong Kong, and so it is not surprising that shrimp allergy was also the major food allergen of the children in this study(17).

Our study showed shrimp sensitization in rural subjects in our region of China is more common and closely related to cockroach sensitization than that in urban subjects; yet no significant difference in frequency of seafood consumption has been reported between the two regions by analyzing their food frequency data(3). This finding is supported by strong correlations of seafood sensitization and inhalant allergen sensitization, mainly to dust mite and cockroach(18). Fernandes et al. reported that certain Orthodox Jews who avoid seafood in their diet were sensitized to shrimp and may have clinical symptoms, with dust mite or cockroach being their primary sensitizer(7). In contrast, the Reykjavik region of Iceland has almost no dust mite exposure indoors. Of those who are sensitized to dust mite, 58% and 33% are also sensitized to shrimp- and cockroach-allergens, respectively(19). Gamez et al. conducted a study in Spain with patients co-sensitized to shrimp and mite in Madrid (dry climate) and Lugo (humid climate) and found that shrimp was the initiating allergen of the patients in Madrid, and dust mite was the initiating allergen of the patients in Lugo(20).

Tropomyosin is considered to be responsible for cross-reactivity between shellfish and arthropods such as dust mite and cockroach(18,21-24). Tropomyosin from shrimp (Genebank accession number AAZ76743) has a high sequence homology to dust mite (CAA75141, 80.3% identity) and cockroach (AAF72534, 82.4%) tropomyosins. This may explain this frequent cross-reactivity. However, in our study, positive responses to dust mite and shrimp tropomyosin were relatively low, suggesting that tropomyosin is not the major cross-reactive allergen responsible in our population. Consistent with our findings, the positive rate of Der p 10 in patients with dust mite sensitization is only 2% in four cities of China(25), 6% in other coastal cities(26) and <20% in European populations(22,23), but higher in central Africa (55%)(24). Besides, only a minority of (16%) of cockroach-sensitized subjects exhibited tropomyosin cross-reactivity in inner-city Atlanta, Georgia(27), raising the possibility that a variety of allergens might play roles in cross-reactivity(18,28). In this regards, recent European studies have demonstrated several novel allergens particularly relevant to this cross-reactivity(29,30). Among them, Arginine kinase (Pen m 2, Bla g 9, Der p 20), Troponin C (Pen m 6, Bla g 6), Sarcoplasmic calcium-binding protein (Pen m 4, Bla g 8) and Hemocyanin (Bla g 3) are the most relevant (for the latest review, see reference(31)) We used ImmunoCAP assay to show that cockroach and shrimp inhibitors distinguished the sensitization patterns in urban vs rural regions, suggesting that cockroach might be the primary sensitizer for some rural children with shrimp sensitization. In this regard, we previously reported that house dust of rural households contains significantly more cockroach allergens than urban households(10).

Similar to our findings, a recent study of inner-city children in the United States revealed that shrimp-allergen sIgE level was closely correlated with cockroach sIgE level and indoor cockroach-allergen exposure(8), suggesting that analysis of shrimp sIgE should be carried out based on the exposure to inhalant allergens.

Shrimp sensitization as a result of the cross-reactivity between shellfish and arthropods in unexposed subjects has been documented(7). Hypothesis of the extraintestinal (respiratory and/or cutaneous) origin of food allergy has been proposed(29), which seems also to occur in our co-sensitized subjects. Dust mites are partially responsible for shrimp sensitization in our urban subjects. Unlike the rural environment, dust mites are the major airborne allergens for children in urban areas, where the positive rate of cockroach sensitization is low. In this context, dust mite inhibitor could inhibit the shrimp sIgE of most subjects, whereas shrimp inhibitor could not fully inhibit dust mite sIgE in urban area, indicating that shrimp-sIgE-positivity in the children in urban areas was partially caused by the cross-reactivity with dust mite allergy and that the effect of cockroach-allergen was small. Resulting from the complexity in the preparation of crude allergen extracts, the degree of cross-reactivity between shrimp and dust mite allergy should be further validated using allergen component(s) in the ImmunoCAP assay.

Our study has the following shortcomings. First, we did not conduct food challenge testing to confirm the diagnosis of FA in the patients, but it should not bias our conclusion because previous study has showed history of shrimp allergy and positive SPT and sIgE demonstrated a positive predictive value (87%)(32). Second, we did not use

natural/recombinant allergens, which, unlike the inhibitors in the ImmunoCAP assay, are not commercially available. However, tropomyosin from shrimp, dust mite and cockroach may be useful to confirm our hypothesis. Thirdly, we did not include the assessment of inhalant allergen exposures from household dust, although our previous report has shown a higher exposure of cockroach-allergen in the children from rural areas than urban areas(10).

In conclusion, this study provides new data of FA prevalence among the children from southern China and suggests that shrimp is the major food allergen relevant to children. Tropomyosin was not a major allergen in our subjects responsible for the cross-reactivity observed using extracts from three different sources. In addition, clinical significance of shrimp allergy in the rural areas might be affected by cockroach allergy. Further studies will be necessary to evaluate the impacts of cockroach exposure in the living environment on the clinical manifestations of shrimp allergy.

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Author contributions

J. L. and G.W.K.W. mainly designed the study, conducted the survey, collected the data and performed the statistical analysis. Z.W.Y. had responsibility for experiments, performed the statistical analysis and drafted the first version of the manuscript. All authors were involved in the subsequent revision of the manuscript. J.F.Z. and N.L.W conducted the survey and collected the data. F.M., M.X., X.S., Z.Z. and Q.S. were involved in data collection.

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Conflict of interest

All the authors have no conflict of interest to declare.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Supplementary Figure 1: Detail information of skin-prick test and specific IgE measurement in cases and controls between Guangzhou and Shaoguan subjects.

Supplementary Figure 2: The inhibition potential of house dust mite (A), cockroach (B) and shrimp (C) extracts were test with sera binding to corresponding allergens after dilution with PBS buffer to a level of 10.0 kUA/L.

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Figure 1. Study design and flow chart.

Figure 2. Correlation between specific IgE to shrimp and Der p or Bla g in shrimp-sensitized subjects co-sensitized to house dust mite (A/B) or cockroach (C/D) in Guangzhou (n = 29) and Shaoguan (n = 75).

Figure 3. Pre-incubation of serum samples from urban and rural shrimp-sensitized subjects with HDM, cockroach and shrimp extracts, respectively, and inhibition of IgE binding measured by Der p- (A), Bla g- (B) and shrimp- (C) specific IgE ImmunoCAP™. Significant difference ($p < 0.05$) of inhibition percentage (*) between Guangzhou and Shaoguan subjects are noted.

Table 1 Comparison of the Prevalence of Doctor-diagnosed Food Allergy in Children from Urban Guangzhou and Rural Shaoguan

Variables	Urban Guangzhou (N = 5878)	Rural Shaoguan (N = 5598)	<i>P</i> value
Subjects, response rate, No. (%)	5542 (94.3)	5139 (91.8)	<0.001
Ethnicity, No. (%)			
Han Chinese	5542 (100)	5139 (100)	-
Sex, No. (%)			
Female	2704 (48.8)	2523 (49.1)	0.781
Age, y	8.9 ± 1.7	9.9 ± 2.1	<0.001
Adverse reactions to food, No. (%) [†]	538 (9.7)	473 (9.2)	0.448
Crustacean shellfish	285 (5.1)	87 (1.7)	<0.001
Shrimp	191 (3.5)	72 (1.4)	<0.001
Crab	94 (1.7)	15 (0.3)	<0.001
Milk	73 (1.3)	43 (0.8)	0.020
Mango	49 (0.9)	72 (1.4)	0.013
Fish	37 (0.7)	47 (0.9)	0.155
Egg	33 (0.6)	27 (0.5)	0.697
Wheat	31 (0.6)	53 (1.0)	0.006
Poultry	29 (0.5)	23 (0.4)	0.581
Self-reported and doctor-diagnosed food allergy, No. (%)	223 (4.0)	179 (3.5)	0.154

[†]Only the eight most frequent foods that trigger adverse reactions were listed.

Table 2 Baseline and Sensitization Characteristics between Case Subjects and Control Subjects in Urban Guangzhou and Rural Shaoguan

	Case Subjects		<i>P</i> value ^a	Control Subjects		<i>P</i> value ^b	<i>P</i> value ^c	<i>P</i> value ^d
	Guangzhou n=223	Shaoguan n=179		Guangzhou n=300	Shaoguan n=300			
Subjects, response rate, No. (%)	190 (85.2)	116 (64.8)		212 (70.7)	233 (77.7%)			
Demographics								
Sex, No. (%)								
Female	82 (43.2)	54 (46.6)	0.635	110 (51.9)	110 (47.2)	0.343	0.089	0.908
Age, y	10.2 ± 1.5	10.7 ± 1.5	0.005	10.3 ± 1.5	10.6 ± 1.4	0.027	0.351	0.638
BMI (kg/m ²)	17.9 ± 3.6	17.4 ± 3.1	0.171	17.3 ± 3.1	17.0 ± 2.6	0.397	0.047	0.279
Symptoms (%)								
Adverse food reactions	104 (54.7)	36 (31.0)	<0.001	20 (9.4)	21 (9.0)	0.878	<0.001	<0.001
Doctor-diagnosed food allergy	27 (14.2)	14 (12.1)	0.730	3 (1.4)	1 (0.4)	0.351	-	-
Sensitization (%)								
Skin prick test (≥3 mm) [¶]								
Any allergens tested	101 (53.2)	66 (56.9)	0.555	70 (33.0)	87 (37.3)	0.372	<0.001	0.001
Food allergens	15 (7.9)	24 (20.7)	0.002	6 (2.8)	18 (7.7)	0.034	0.026	0.001
Crab	12 (6.3)	14 (12.1)	0.093	2 (0.9)	11 (4.7)	0.022	0.005	0.016
Shrimp	7 (3.7)	13 (11.2)	0.015	2 (0.9)	9 (3.9)	0.065	0.091	0.010
Environmental allergens	100 (52.6)	61 (52.6)	0.994	69 (32.5)	86 (36.9)	0.370	<0.001	0.006
<i>Dermatophagoides pteronyssinus</i>	98 (51.6)	57 (49.1)	0.724	65 (30.7)	71 (30.5)	0.996	<0.001	0.001
<i>Blatella germanica</i>	10 (5.3)	39 (33.6)	<0.001	7 (3.3)	43 (18.5)	<0.001	0.458	0.002
Specific IgE (≥0.35 kU _A /L) [¶]								
Any allergens tested	121 (63.7)	67 (57.8)	0.334	87 (41.0)	90 (38.6)	0.629	<0.001	0.001
Food allergens	62 (32.6)	50 (43.1)	0.068	36 (17.0)	59 (25.3)	0.037	<0.001	0.001
Shrimp	24 (12.6)	42 (36.2)	<0.001	5 (2.4)	33 (14.2)	<0.001	<0.001	<0.001

Pen a 1	9 (4.7)	8 (6.9)	0.448	1 (0.5)	3 (1.3)	0.625	0.008	0.008
Crab	15 (7.8)	22 (19.0)	0.006	2 (0.9)	11 (4.7)	0.022	0.001	<0.001
Environmental allergens	108 (56.8)	66 (56.9)	0.993	72 (34.0)	78 (33.5)	0.920	<0.001	<0.001
Der p	108 (56.8)	60 (51.7)	0.408	72 (34.0)	73 (31.3)	0.613	<0.001	<0.001
Der p 1	86 (45.3)	19 (16.4)	<0.001	54 (25.5)	21 (9.0)	<0.001	<0.001	0.050
Der p 2	77 (40.5)	12 (10.3)	<0.001	51 (24.1)	10 (4.3)	<0.001	0.001	0.036
Der p 10	14 (7.4)	8 (6.9)	0.876	1 (0.5)	4 (1.7)	0.375	<0.001	0.024
<i>Blatella germanica</i>	5 (2.6)	32 (27.6)	<0.001	2 (0.9)	26 (11.2)	<0.001	0.263	<0.001

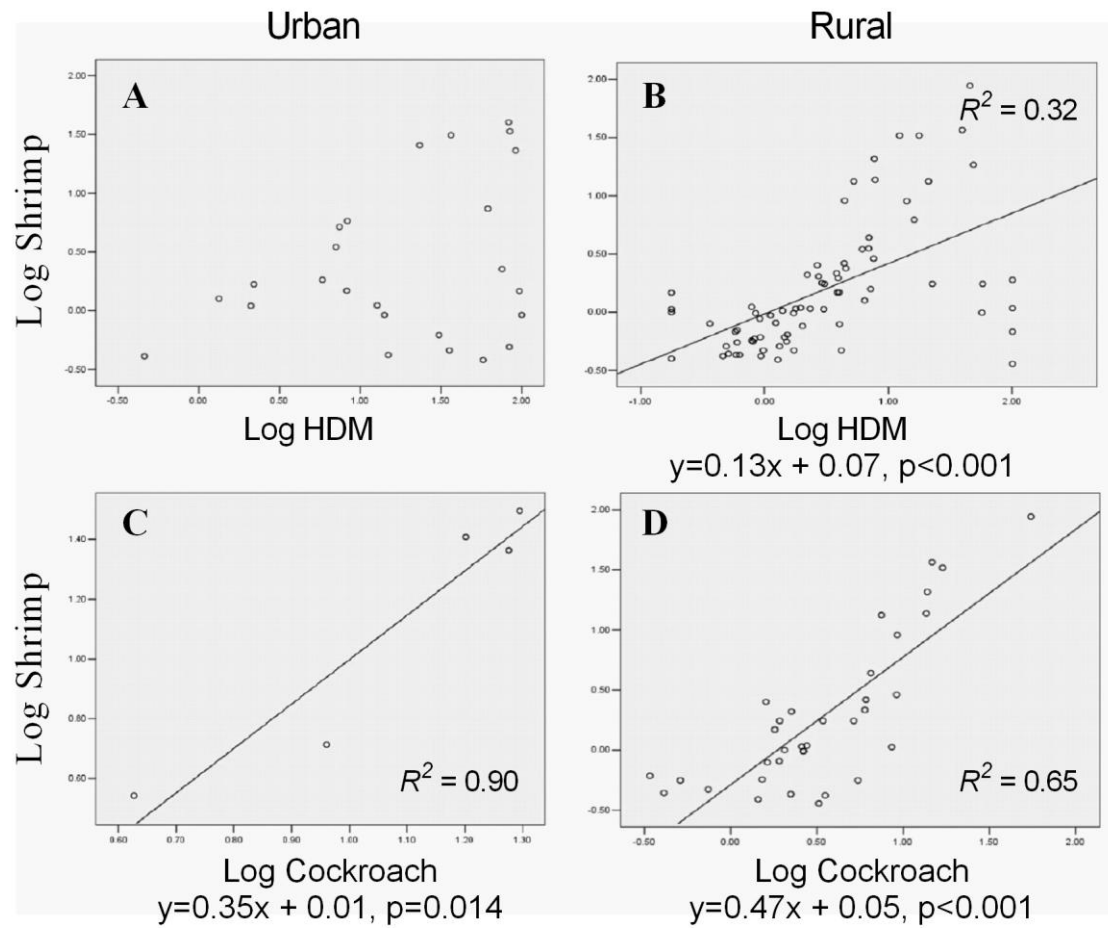
[†]Only the most frequent food and environmental allergens were listed for skin prick test and specific IgE measurement. For detail information in regard to the complete list (18 food and 5 environmental allergens for SPT and 27 food and 7 environmental allergens), see supplementary figure 1.

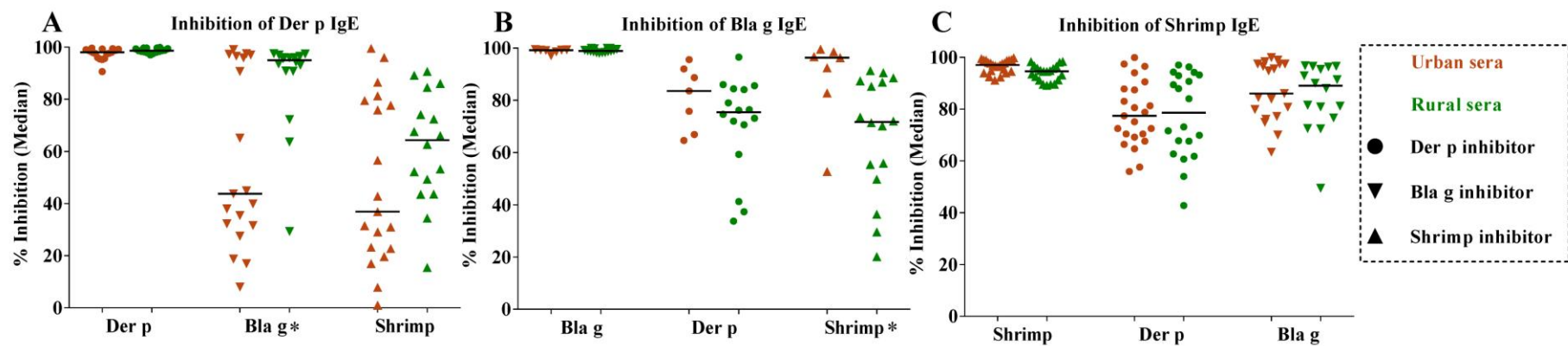
P values were compared between Guangzhou and Shaoguan in case subjects (*P* value^a) and control subjects (*P* value^b) and difference between case subjects and control subjects in Guangzhou (*P* value^c) or Shaoguan (*P* value^d) subjects.

Table 3 Proportion of IgE-mediated Food Allergy in Guangzhou and Shaoguan Case-control Subjects.

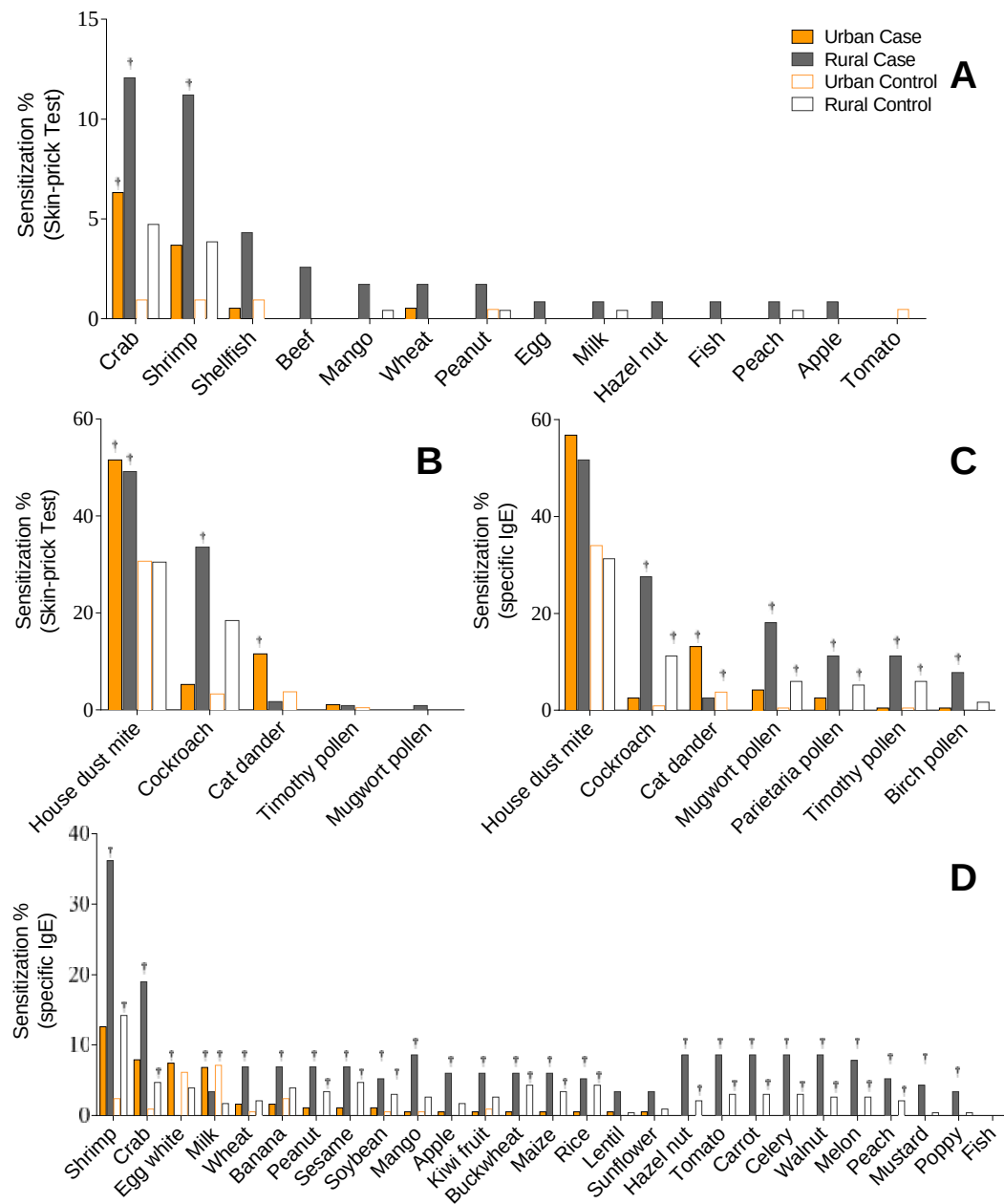
Probable food allergy	Guangzhou	Shaoguan	<i>P</i> value
	(n=402)	(n=349)	
Food allergy, No. (%)	21 (5.2)	19 (5.4)	0.893
Shrimp allergy	18 (4.5)	13 (3.7)	0.714
Crab allergy	1 (0.2)	2 (0.6)	0.600
Egg allergy	1 (0.2)	2 (0.6)	0.600
Milk allergy	1 (0.2)	1 (0.3)	0.920
Fish allergy	1 (0.2)	1 (0.3)	0.920
Peach allergy	0	1 (0.3)	-
Mango allergy	0	1 (0.3)	-







Supplementary Figure 1 Detail information of skin-prick test and specific IgE measurement in cases and controls between Guangzhou and Shaoguan subjects. Significant difference between urban Guangzhou and rural Shaoguan († , $P < 0.05$) in case and control subjects are indicated. For skin-prick test results, only allergens with positive results were listed thus four other allergens (jujube, melon, orange, and soybean) were not listed.



Supplementary Figure 2 The inhibition potential of house dust mite (A), cockroach (B) and shrimp (C) extracts were tested with sera binding to corresponding allergens after dilution with PBS buffer to a level of 10.0 kU_A/L. Ten-times gradient dilution was used and the highest concentration of allergen extract (2.0 mg/ml for Der p and shrimp extract, 6.0 mg/ml for cockroach extract) was chosen for allergen inhibition test.

