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Component-Resolved Diagnostic Study of *Dermatophagoides Pteronyssinus* Major Allergen Molecules in a Southern Chinese Cohort

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Abstract

Background and Objective: Little is known about component-resolved diagnosis (CRD) for *Dermatophagoides pteronyssinus* (Der p) sensitization in the Chinese population. We aimed to evaluate sensitization to Der p components in southern China.
Methods: Two-hundred immunotherapy-naïve patients with asthma and/or rhinitis positive to specific IgE (sIgE) against Der p extract, along with 20 Der p-negative nonallergic healthy controls, were tested for sIgE against Der p 1, Der p 2, and Der p 10 using ImmunoCAP 100. Seventy-five were further examined with the ImmunoCAP Immuno Solid-phase Allergen Chip (ISAC). Der p 10-positive patients were also tested for sIgE against crude extracts of cockroach, moth, and shrimp.
Results: In total, 183 (91.5%) of the 200 patients were sensitized to Der p 1 and/or Der p 2. The proportion of positive results and the median level of sIgE against Der p 1 were higher in children than in adults. Der p 1 and Der p 2 correlated with Der p in sIgE levels. ImmunoCAP ISAC demonstrated 100% specificity and 84% sensitivity in detecting Der p 1, Der p 2, and Der p10 compared with ImmunoCAP 100. Sensitization to Der p 10 correlated well with sIgE to shrimp, moths, cockroaches, Pen m 1, Bla g 7, and Ani s 3.
Conclusions: The detection of Der p 1 and Der p 2 provided a good reflection of atopy to Der p in a Chinese cohort. Sensitization to Der p 10 may result from cross-reactivity with seafood and cockroaches in coastal southern China. ImmunoCAP ISAC may be a useful tool for CRD, with comparable performance to ImmunoCAP 100.

Key words: Component-resolved diagnosis. Asthma. Allergic rhinitis. Der p 1. Der p 2. Der p 10. Tropomyosin. Microarray-based specific IgE detection assay.

Resumen

Introducción y Objetivo: El diagnóstico por componentes en pacientes sensibilizados a *Dermatophagoides pteronyssinus* (Der p) en la población china es un tema poco estudiado.
El objetivo de este estudio fue evaluar la sensibilización a componentes de Der p en el sur de China.
Método: Para ello se estudiaron 200 pacientes con asma y /o rinitis con IgE específica positiva frente a extracto completo de Der p (d 1) no sometidos a inmunoterapia y 20 controles sanos no alérgicos (Der p-negativos) con IgEesp negativa frente a Der p 1, Der p 2, y Der p 10 mediante ImmunoCAP 100. Setenta y cinco fueron analizados mediante ISAC (ImmunoCAP Immuno Solid-Phase Allergen Chip). Los sujetos positivos a Der p 10 fueron, además, analizados mediante IgEesp frente a extracto de cucaracha, polilla y gamba.
Resultados: En cuanto a los resultados obtenidos, 183/200 (91.5%) pacientes estaban sensibilizados a Der p 1 y /o Der p 2. La proporción positiva y la mediana de IgEesp frente a Der p 1 fue mayor en niños que en adultos. La IgEesp frente a Der p 1 y Der p 2 se correlacionaba con los niveles de IgEesp frente a extracto completo. El ISAC mostró una especificidad del 100% y una sensibilidad del 84% para Der p 1, Der p 2 y Der p 10. La sensibilización a Der p 10 se correlacionó bien con la IgEesp frente a gamba, polilla y cucaracha, Pen m 1, Bla g 7, Ani s 3.
Conclusiones: La detección de Der p 1 y Der p 2 refleja adecuadamente la sensibilización a *Dermatophagoides pteronyssinus* en la población

china. La sensibilización a Der p 10 puede ser el resultado de la reactividad cruzada a marisco y cucaracha en la población del sur de China. El ISAC puede considerarse una herramienta útil para realizar el diagnóstico por componentes comparable al Immuno Cap-100.

Palabras clave: Diagnóstico basado en componentes. Asma. Rinitis alérgica. Der p 1. Der p 2. Der p 10. Tropomiosina. Ensayo de detección de IgEesp basado en micromatrices.

Introduction

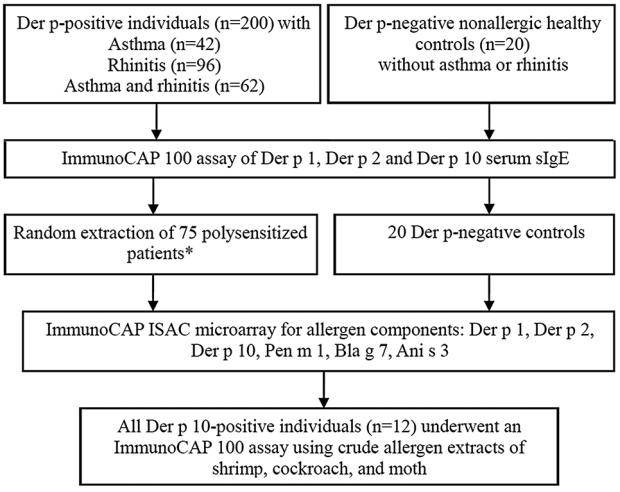
Sensitization to house dust mites (HDMs), an important source of inhalant allergens worldwide, can trigger a wide range of airway allergies [1,2]. HDMs, and *Dermatophagoides pteronyssinus* (Der p) in particular, are also the most prevalent allergens in patients with asthma or rhinitis in China [3]. Until recently, diagnostic approaches for HDM-related atopy were based on serum specific IgE (sIgE) measurements or skin prick tests to crude mite allergen extracts [4]. However, crude mite extracts contain a mixture of at least 23 allergen components with varying individual concentrations [5-7]. For diagnosis and immunotherapy, well-defined allergen components are a promising alternative to crude allergen extracts, as they can be easily quantified and characterized [8].

The advent of component-resolved diagnosis (CRD), which uses allergen components, heralds a new epoch in the study of allergies. Several HDM components are drawing research interest. These include group 1 (Der p 1), 2 (Der p 2), and 10 (Der p 10) allergens of Der p. Der p 10 is a member of a large family of tropomyosins, as is Ani s 3 from anisakis, Bla g 7 from cockroach, and Pen m 1 from shrimp. In previous studies by our group, cockroach, moth, and shrimp were shown to be major inhalant or food allergens in southern China [9-11], and may be cross-reactive with Der p 10 allergens (tropomyosins) [12]. It would therefore be important to analyze associations between sensitization to Der p 10, Ani s 3, Bla g 7, and Pen m 1. While a growing body of investigational data on these allergen components as used in CRD techniques compared with the established extract-based sIgE test is available in western countries, such information is lacking in China. Here, we present a study on the prevalence of sensitization to Der p allergen components in a southern Chinese cohort.

Methods

Study Population

The allergy registry at our institution has a large dataset on allergic patients referred for allergen tests from 51 hospitals in the Guangdong Province in southern China. Analysis of the registry data from January 2008 through June 2014 identified 200 allergic patients who had been serially followed and had positive results (≥0.35 kU/L) for Der p extract (d1) confirmed by ImmunoCAP 100 (Thermo Fisher Scientific) and fulfilled the following criteria: *1*) a physician diagnosis of asthma, rhinitis, or both; *2*) positive sIgE reactivity to Der p (≥0.35 kU/L) as shown by previous ImmunoCAP 100 results; and *3*) no history of specific immunotherapy (SIT). These 200 Der p-positive patients comprised 99 children (aged 2-14 years) and 101 adults (aged 15-66 years). A contemporary cohort of 20 Der p-negative nonallergic healthy controls (12 adults and 8 children) was also included. Five milliliters of venous blood were drawn from all individuals to be used for the relevant study tests.



*Those who tested positive to Der p and at least 2 other allergens.

Figure 1. Study design and flow chart.

We performed in vitro tests in 3 steps, as shown in Figure 1. In the first step, sera from the study population were tested for Der p 1, Der p 2, and Der p 10 sIgE using ImmunoCAP 100. In the second step, 75 patients who were sIgE-positive to Der p and at least 2 other whole allergens (ie, ≥3 whole allergens including Der p, as recorded in our registry), were selected from the 200 Der p-positive individuals. As indicated by data in the registry, the sensitizing whole allergens in these patients (apart from Der p) were cockroaches, molds, pollens, moths, and cat and dog dander. Sera from these 75 polysensitized patients and the 20 Der p-negative controls underwent ImmunoCAP Immuno Solid-phase Allergen Chip (ISAC) microarray testing (Thermo Fisher Scientific) for serum sIgE against 112 allergen components, including Der p 1, Der p 2, Der p 10, Ani s 3 (anisakis), Bla g (cockroach), and Pen m 1 (shrimp). In the third and final step, all individuals with Der p 10-positive sIgE identified by ImmunoCAP 100 in step 1 were investigated further for sIgE to crude extracts of cockroach, moth, and shrimp using ImmunoCAP 100.

Ethical Approval

The present study was approved by the ethics committee of the First Affiliated Hospital of Guangzhou Medical University. All participants gave written informed consent. The study was registered in the Chinese Clinical Trial Registry (http://www.chictr.org/cn/, registration number: ChiCTR-DCC-13004003).

ImmunoCAP 100 Detection

Serum sIgE to Der p 1, Der p 2, Der p 10, shrimp, cockroach, and moth was measured with ImmunoCAP 100 according to the manufacturer's instructions. The concentration of sIgE to Der p components was measured between 0.10 and 100 kU/L. Any measurement above the upper limit of the measurement range was assigned a value of 100 kU/L. With a cutoff value of 0.35 kU/L, tests with an sIgE level of over 0.35 kU/L were defined as sIgE-positive. sIgE-positive tests were categorized into 6 classes: class 1 (≥0.35 to <0.70 kU/L), class 2 (≥0.70 to <3.50 kU/L), class 3 (≥3.50 to <17.50 kU/L), class 4 (≥17.50 to <50.00 kU/L), class 5 (≥50.00 to <100.00 kU/L), and class 6 (≥100.00 kU/L).

Microarray-Based Specific IgE Detection

ImmunoCAP ISAC is a microarray-based specific IgE assay that detects a panel of sIgE antibodies against allergen components immobilized on solid substrate according to the manufacturer's instructions. The immobilized components react with the sIgE in the patient serum. Once nonspecific IgE has been washed away, fluorescence-labeled anti-human IgE antibody is added to form a complex. After incubation, unbound fluorescence-labeled antihuman IgE antibodies are removed by washing, followed by fluorescence measurement using a microarray scanner. The test results are expressed in ImmunoCAP standardized units (ISUs). In our study, individuals were considered to be sensitized to an allergen component if the sIgE level was 0.3 ISU or greater.

Statistical Analysis

Statistical analyses were performed using SPSS version 16.0 for Windows. Levels of sIgE were expressed as medians and 25% to 75% interquartile ranges (IQRs). Comparisons of the prevalence of sIgE reactivity were performed with the χ^2 or Fisher exact tests. Between-group comparisons of numerical data were performed using the Mann-Whitney U-test or the Kruskal-Wallis Test. Kappa tests were used to evaluate agreement in positive sIgE tests between ImmunoCAP ISAC and ImmunoCAP 100. The diagnostic efficiency of the ISAC test was assessed by receiver operating characteristic (ROC) curves. Correlation analyses of serological measurements were performed by calculating the Spearman correlation coefficient (r_s). *P* values of less than .05 were considered significant.

Results

Prevalence of Sensitization and sIgE Levels of Der p Components Detected by ImmunoCAP 100

Of the 200 Der p-positive patients, 186 (93.0%) tested positive to at least 1 Der p component (Table 1). Specifically, 183 (91.5%) were sensitized to Der p 1 and/or Der p 2. Only 12 patients (6.0%)—8 children and 4 adults—tested positive for Der p 10. Of these 12 Der p 10-positive patients, only 3 were positive to Der p 10 alone; the remaining 9 were concurrently positive to Der p 1 and Der p 2. The proportion of positive IgE reactivity to Der p 1 was higher in children (93.9%) than in adults (84.2%) (χ^2, 4.88; *P*<.05), but this difference was not

Table 1. Prevalence of Sensitization to Der p Allergen Components in Der p-Positive Patients (n=200)

Age, y (Mean, Range)	No. (Male)	Der p 1 No. (%)	Der p 2 No. (%)	Der p 10 No. (%)	Der p 1 and/or Der p 2 No. (%)	At least One of Der p 1, Der p 2, and Der p 10 No. (%)
All (19.7, 2-66)	200 (139)	178 (89.0)	167 (83.5)	12 (6.0)	183 (91.5)	186 (93.0)
Children[a] (10.5, 2-14)	99 (77)	93 (93.9)	85 (85.8)	8 (8.1)	95 (96.0)	97 (98.0)
Adults[a] (28.9, 15-66)	101 (62)	85 (84.2)	82 (81.2)	4 (3.9)	88 (87.1)	89 (88.1)

[a]Children were defined as 14 years or younger and adults as over 14 years.

Table 2. Prevalence of Sensitization to Der p Components for Different Der p Specific IgE (sIgE) Levels (n=200)

Der p sIgE Levels	No. of Patients	Der p 1 No. (%)	Der p 2 No. (%)	Der p 10 No. (%)
Class 1 (≥0.35 to <0.70 kU/L)	1	0 (0.0)	0 (0.0)	0 (0.0)
Class 2 (≥0.70 to <3.50 kU/L)	10	2 (20.0)	3 (30.0)	1 (10.0)
Class 3 (≥3.50 to <17.50 kU/L)	26	16 (61.5)	16 (61.5)	2 (7.7)
Class 4 (≥17.50 to <50.00 kU/L)	56	53 (94.6)	43 (76.8)	1 (1.8)
Class 5 (≥50.00 to <100.00 kU/L)	45	45 (100.0)	44 (97.8)	2 (4.4)
Class 6 (≥100.00 kU/L)	62	62 (100.0)	61 (98.4)	6 (9.7)

Table 3. Levels of sIgE Against Der p, Der p 1, Der p 2, and Der p 10 in Der p sIgE–Positive Patients (n=200)[a]

Components	Total	Children	Adults
Der p	54.8 (22.3-100.0)	60.2 (28.4-100.0)#	39.3 (15.0-98.4)
Der p 1	34.8 (9.6-80.1)†*	49.0 (16.8-87.4)##	23.6 (2.7-58.5)
Der p 2	40.6 (9.4-88.2)†*	49.0 (18.6-98.7)	31.3 (2.1-81.0)
Der p 10	0.03 (0.01-0.04)	0.03 (0.01-0.04)	0.02 (0.01-0.04)

Abbreviation: sIgE, specific IgE.
[a]Data are shown as median serum IgE in kU/L (interquartile range). As shown by the Mann-Whitney U test, the levels of Der p 1 and Der p 2 sIgE were comparable to each other†, but significantly higher than Der p 10 levels*. sIgE levels of Der p# and Der p 1## were higher in children than in adults. #*P*=.005; †*P*>.05; **P*<.01; ##*P*=.001.

found for Der p 2 (χ^2, 0.06; *P*>.05) or Der p 10 (χ^2, 0.50; *P*>.05). There were no differences between the positive proportions of Der p 1 and Der p 2 in children (χ^2, 3.56; *P*>.05) or in adults (χ^2, 0.31; *P*>.05). The proportion of Der p 1 and Der p 2 detection in patients with a class 5 or 6 sIgE response to Der p was approximately 98.0% compared with 30.0% in those with a class 3 or lower response (Table 2).

In the group of 200 Der p-positive patients, the median (IQR) sIgE levels were 54.8 kU/L (22.3-100.0 kU/L) for Der p (as recorded in the registry), 34.8 kU/L (9.8-80.1 kU/L) for Der p 1, 40.6 kU/L (9.4-88.2 kU/L) for Der p 2, and 0.03 kU/L (0.01-0.04 kU/L) for Der p 10 (Table 3). As shown by the Mann-Whitney U test, the levels of Der p 1 and Der p 2 sIgE were comparable to each other (*P*>.05) but significantly higher than the level of Der p 10 (*P*<.01). sIgE levels of Der p (*P*=.005) and Der p 1 (*P*=.001), but not of Der p 2 (*P*=.055) or Der p 10 (*P*=.279), were higher in children than in adults. With the Kruskal-Wallis test, Der p and Der p 1 sIgE levels were significantly different among patients with asthma, patients with rhinitis, and those with concomitant asthma and rhinitis (both *P*<.05). No differences were detected in this case for Der p 2 (*P*=.265). Between-group comparisons showed that patients with asthma alone and patients with concomitant asthma and rhinitis had higher serum levels of Der p (*P*=.02 and *P*=.002, respectively) and Der p 1 sIgE (*P*=.04 and *P*=.02, respectively) than those with rhinitis alone (Figure 2).

The concentration of sIgE against Der p was significantly correlated with Der p 1 (r_s, 0.862; *P*<.001; Figure 3A), Der p 2 (r_s, 0.799; *P*<.001; Figure 3B), and Der p 10 (r_s, 0.209, *P*<.01, Figure 3C). The sIgE responses to Der p 1, Der p 2, and Der p 10 were all negative in the 20 Der p-negative controls.

Diagnostic Efficiency of ImmunoCAP ISAC in Detecting HDM Allergen Components Compared With ImmunoCAP 100

Seventy-five polysensitized patients and 20 Der p-negative subjects underwent both ImmunoCAP 100 and ImmunoCAP ISAC detection of serum Der p components. The 75 polysensitized patients included all Der p 10-positive patients

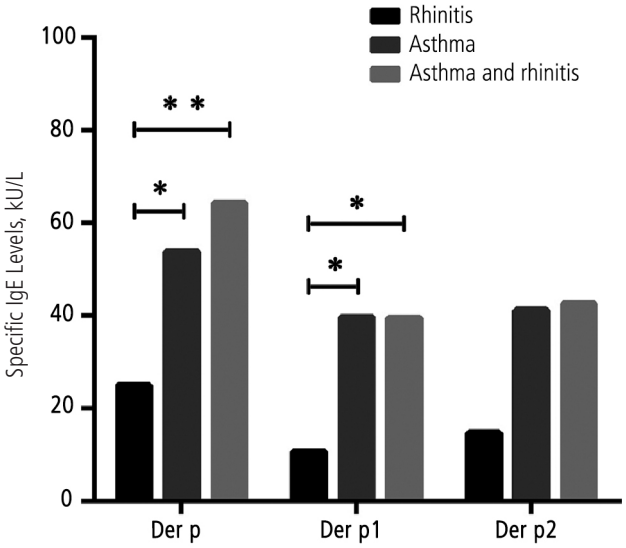


Figure 2. Levels of Der p, Der p 1, and Der p 2 specific IgE (sIgE) in patients (n=200) according to clinical status. Patients with asthma alone or with asthma and rhinitis had higher serum levels of Der p (*P*<.01) and Der p 1 sIgE (*P*<.05), but not Der p 2 sIgE (*P*=.265), than patients with rhinitis alone. **P*<.05; ***P*<.01.

(n=12) identified by ImmunoCAP 100 in the first step of the study. For this subgroup, the positive proportions of Der p 1 (χ^2, 9.09; P<.01) and Der p 2 (χ^2, 9.09; *P*<.01) were lower with ImmunoCAP ISAC than with ImmunoCAP 100, whereas the positive proportion of Der p 10 did not differ between the 2 test methods (χ^2, 0.50; *P*=.48). Based on the positive results detected by ImmunoCAP 100, ImmunoCAP ISAC missed 11 cases of Der p 1 (10 with a class ≤3 and 1 with a class 4 response), 11 cases of Der p 2 (also 10 with a class ≤3 and 1 with a class 4 response), and 2 cases of Der p 10. Despite these false negatives, we found acceptable consistencies between ImmunoCAP ISAC and ImmunoCAP 100 for the detection of Der p 1 (κ=0.76), Der p 2 (κ=0.77), and Der p 10 (κ=0.95) (all *P*<.01). Using ImmunoCAP 100 as the reference (Table 4), ImmunoCAP ISAC yielded 100.0% specificity and over 80.0% sensitivity (Der p 1, 82.2%; Der p 2, 81.7%; Der p 10, 91.7%) in the detection of Der p 1 (area under curve [AUC], 0.911), Der p 2 (AUC, 0.908), and Der p 10 (AUC, 0.917). Remarkably, sIgE levels of Der p 1 (r_s= 0.831), Der p 2 (r_s= 0.804), and Der p 10 (r_s= 0.574) correlated well between ImmunoCAP ISAC and ImmunoCAP 100 (all *P*<.01) (Figure 3D-F).

Table 4. Diagnostic Efficiency of ImmunoCAP ISAC Microarray Compared With ImmunoCAP 100

	Der p 1	Der p 2	Der p 10
Sensitivity, %	82.2	81.7	91.7
Specificity, %	100.0	100.0	100.0
AUC (95% CI)	0.911 (0.849-0.973)	0.908 (0.845-0.972)	0.917 (0.791-1.042)

Abbreviation: AUC, area under curve.

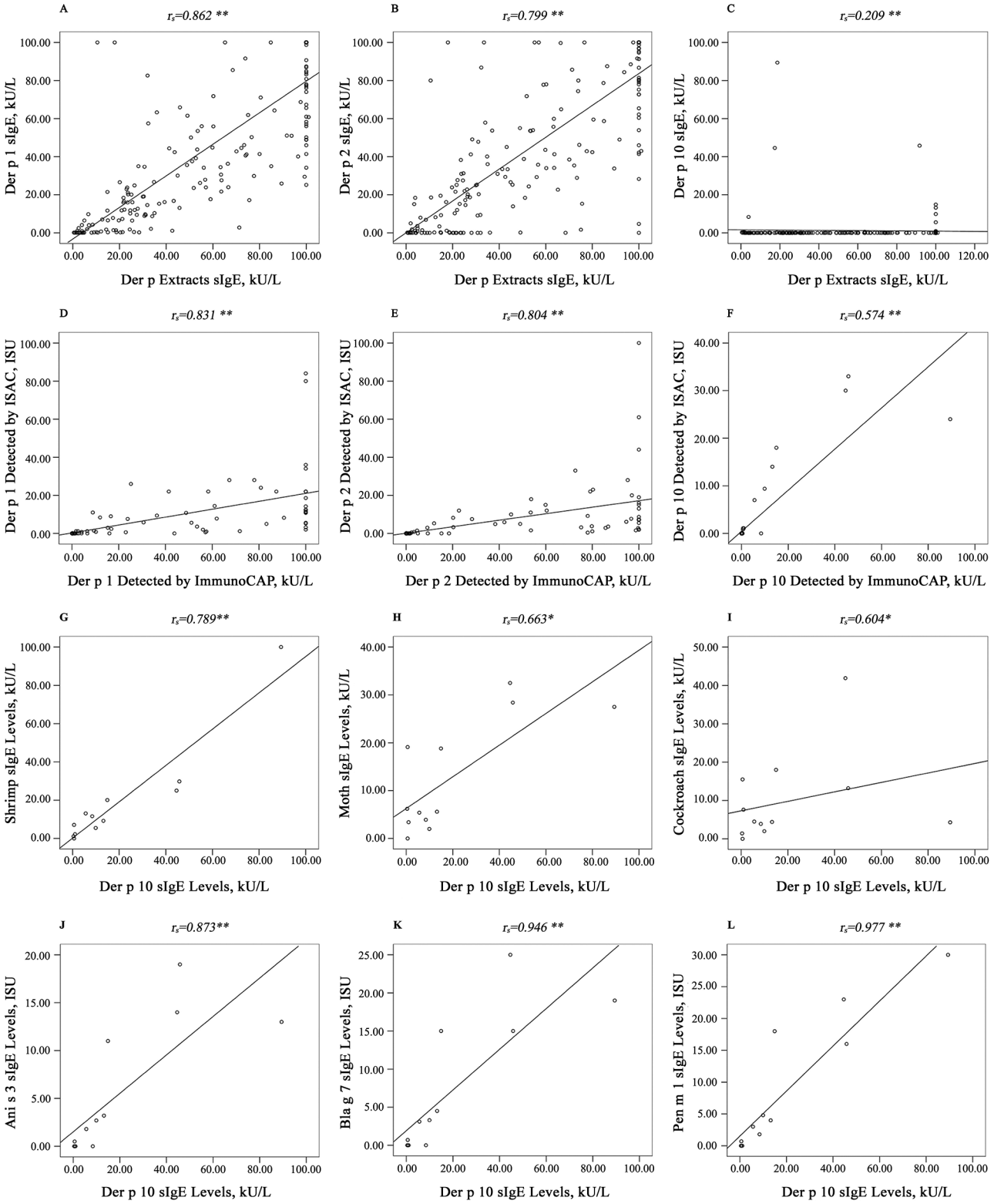


Figure 3. Spearman correlation analysis of serological measurements. Der p specific IgE (sIgE) levels correlated well with Der p 1 (A) and Der p 2 (B), but not with Der p 10 (C) (n=200). The correlation between Der p 1 (D), Der p 2 (E), and Der p 10 (F) detected by ImmunoCAP ISAC and ImmunoCAP 100 was significantly high (n=95). Der p 10 sIgE showed good association with shrimp (G), Pen m 1 (H), Bla g 7 (I), Ani s 3 (J), moth (K), and cockroach (L) (n=12). rs indicates Spearman's correlation coefficient. *P<.05; **P<.01.

Table 5. Additional ImmunoCAP 100 Profiles in Patients With Positive Der p 10 Reactivity Shown by ImmunoCAP ISAC (n=12)

Patient	Sex (M/F)	Age, y	ImmunoCAP 100, kU/L							ImmunoCAP ISAC, ISU			
			Der p	Der p 1	Der p 2	Der p 10	Shrimp	Moth	Cockroach	Der p 10	Ani s 3	Bla g 7	Pen m 1
1	M	14	18.7	0.0	0.0	89.4	100.0	27.5	4.3	24.0	13.0	19.0	30.0
2	M	14	91.7	51.1	49.0	45.8	29.8	28.4	13.2	33.0	19.0	15.0	16.0
3	M	52	17.4	1.4	1.2	44.6	25.0	32.5	41.9	30.0	14.0	25.0	23.0
4	M	12	100.0	58.3	97.0	14.9	20.1	18.8	18.0	18.0	11.0	15.0	18.0
5	F	12	100.0	100.0	80.3	13.2	9.2	5.6	4.4	14.0	3.2	4.5	4.0
6	M	7	100.0	100.0	4.7	9.9	5.5	2.0	2.0	9.4	2.7	3.3	4.8
7	F	13	100.0	100.0	100.0	5.6	13.1	5.4	4.5	7.0	1.8	3.1	3.0
8	M	4	3.9	0.0	0.0	8.4	11.6	3.9	3.9	0.0	0.0	0.0	1.8
9	M	23	100.0	78.0	79.8	0.6	7.1	19.1	15.5	1.1	0.5	0.7	0.7
10	M	62	1.4	0.0	0.0	0.4	1.1	6.2	1.4	0.0	0.0	0.0	0.0
11	M	18	100.0	61.0	75.2	1.0	2.2	3.4	7.6	1.1	0.0	0.0	0.0
12	F	12	63.5	30.6	59.9	0.6	0.0	0.0	0.0	0.8	0.0	0.0	0.0

Abbreviations: F, female; M, male.

Sensitization to Additional Allergens in Der p 10-Positive Patients

Eleven of the 12 Der p 10-positive patients tested positive to shrimp, cockroach, and moth in an additional ImmunoCAP 100 test, and 1 was negative to all 3 allergens (Table 5). According to ImmunoCAP ISAC, 8 of these 12 patients were triple positive, 3 were triple negative to Pen m 1, Bla g 7, and Ani s 3, and 1 was positive to Pen m 1 only. There were significant correlations in sIgE levels between Der p 10 and shrimp (r_s= 0.789), Pen m 1 (r_s= 0.977), Bla g 7 (r_s= 0.946), and Ani s 3 (r_s= 0.873) (all P<.01) and also between Der p 10 and moth (r_s= 0.663) and cockroach (r_s= 0.604) (both P<.05) (Figure 3G-L).

Discussion

HDM sensitization has been reported in over 50% of atopic patients and in approximately 80% of asthmatic children [13]. Der p is one of the most common HDM allergens in China. Although crude HDM extracts are currently in use for diagnostic and therapeutic purposes worldwide, they represent mixtures of allergenic and nonallergenic components [6,7], which may cause new sensitizations and complicate clinical interpretation [14].The introduction of microarrays with purified allergen components has enabled panel examinations of allergen components in suspect individuals [15]. ImmunoCAP ISAC has been recognized for CRD for nearly a decade without validated clinical value [16]. CRD data on HDM components so far have been limited in Chinese patients.

In our study, 89% and 84% of Der p-positive patients were sensitized to Der p 1 and Der p 2, respectively. The rate of Der p 1 sensitization is comparable to rates reported for European cohorts (80%-100%) [7,17,18]. Levels of sIgE to Der p 1 and Der p 2 correlated well with Der p sIgE levels. Patients with records of higher Der p levels were more likely to be positive for Der p 1 and Der p 2. Although at least 23 HDM allergen components have been identified, several studies have demonstrated that clinically Der p 1 and p 2 are the most important HDM allergens [5-7], and this was reflected in our Der p-sensitized individuals. Only 8.5% (17/200) of patients tested negative to both Der p 1 and Der p 2. Therefore, the present study shows that Der p 1 and Der p 2 are the major allergenic HDM components in Chinese patients. The findings also echo data from an Australian study showing that Der p components in groups other than groups 1 and 2 account for a small percentage of IgE reactivity [19]. In fact, it has been shown that assessment of Der p 1 and Der p 2 allows for diagnosis of HDM allergy in over 95% of patients [20].

The pattern of sensitization to Der p 1 and Der p 2 in Chinese patients may be clinically relevant in guiding SIT for HDM allergy. Pittner et al [18] suggested that only patients sensitized to Der p 1 and/or Der p 2 are eligible for HDM immunotherapy, pointing to the importance of properly selecting SIT candidates using CRD as a prelude to effective treatment. Unfortunately, such treatment has not been routinely practiced in China, where SIT for HDM allergy is usually performed in unselected HDM-sensitized patients and conceivably yields variable outcomes. The good correlation of Der p 1 and Der p 2 with Der p sIgE suggests that these allergen components could possibly be used as a substitute for Der p crude extract in clinical practice [21]. Importantly, we found a higher positive proportion of Der p 1, but not of Der p 2 or Der p 10, in children than in adults. In consistence with published data [17], children also showed a higher median level of Der p 1 sIgE than adults. These results for Der p 1 sensitization suggest a relatively higher intensity of immune response in children, and may be partly explained by the enhanced T-cell proliferation observed after a Der p 1 challenge in HDM-sensitized children but not adults [22]. The levels of Der p and Der p 1 (but not Der p 2) were higher in patients with either asthma alone or concomitant asthma and allergic rhinitis than in those with allergic rhinitis alone. Inflammatory markers of asthma, including nitrate in exhaled breath condensate and exhaled nitric oxide, have been found

to correlate with the concentration of the HDM allergen Der p 1 [23], indicating that this allergen may play a major role in asthma patients with HDM allergies.

Tropomyosin has been proposed as a cross-reacting allergen between foods and aeroallergens of animal origin, such as HDM or cockroaches. Reese et al [24] suggested that sensitization to shrimp tropomyosin may cause allergy to HDM and *Blattella germanica*. In the present study, Der p 10 sIgE was detectable in 12 Der p-positive patients (6%). Although close to the lower end of the range from European studies (6%-28%) [6,22,25], this rate is higher than figures previously reported for China [26]. Using similar microarray tests, Zheng and colleagues [26], who studied a smaller cohort of 100 Der p-sensitized patients from 4 separate Chinese cities, found that only 2% showed specific IgE reactivity to Der p 10. The higher positive proportion of Der p 10 in Chinese southerners shown in our study may be explained by the frequent intake of seafood (a source of major food allergens) in the population of the coastal regions of southern China [10] and consequently a greater likelihood of sensitization to tropomyosin.

We did not find a strong correlation between levels of Der p 10 and Der p sIgE. Nevertheless, ImmunoCAP ISAC indicated that the 12 Der p 10-positive patients also tested positive for combinations of other cross-reactive tropomyosins, namely Ani s 3 (67%, 8/12), Bla g 7 (67%, 8/12), and Pen m 1 (75.3%, 9/12). In the third step of our study, additional ImmunoCAP tests in these Der p 10-positive patients using crude allergen extracts confirmed that 92% also tested positive for several common whole allergens in the region, including shrimp, moth, and cockroach. In particular, shrimp was the sensitizing allergen in 11 (91.7%) of these 12 patients. In this group, 3 Der p-sensitized patients were double negative for Der p 1 and Der p 2 and positive for Der p 10, shrimp, cockroach, and moth. Studies have demonstrated cross-reactivity between Der p 10 and components of shrimp (Pen m 1) and anisakis, (Ani s 3) [17,27]. Our findings suggest that, in southern China, there may be a higher proportion of HDM sensitization attributable to cross-reactions with other allergens rather than to a primary sIgE response to major HDM components, but further investigations are needed for clarification. We acknowledge that, although a few researchers have reported on the cross-reactivity between HDM and other arthropods or invertebrates (which contain allergenic tropomyosin), it would not be sufficiently robust to conclude that tropomyosin is the only probable allergen contributing to the cross-reaction in our study [28,29]. Nevertheless, the present study provides valuable information on Der p 10 allergens in southern Chinese patients and also suggests the need to routinely include Der p 10 detection in patient selection for SIT. Patients positive to Der p 1 and Der p 2 and negative to Der p 10 are most likely to benefit from SIT; alternatively, a positive reaction to Der p 10 alone would not be indicative for therapy.

The clinical relevance and performance of component-based microarray allergen tests in the background of classical ImmunoCAP needs to be evaluated. A number of studies have calculated the sensitivity and specificity of tests for different allergenic sources by comparing the sum of IgE levels against all microarrayed components belonging to the given source with the same values obtained using a conventional technique such as the fluorescence enzyme immunoassay (ImmunoCAP) of the whole allergenic extract [15,30,31]. It has been noted that the microarray test and whole allergen ImmunoCAP showed equally high sensitivity and specificity in diagnosing grass and cypress pollen allergy [32]. However, a similar comparison between the microarray and classical ImmunoCAP for Der p components remains to be fully clarified. According to the protocol of the present study, 75 Der p-positive patients and 20 Der p-negative patients underwent both ImmunoCAP 100 and ImmunoCAP ISAC tests for Der p 1, Der p 2, and Der p 10. Compared with ImmunoCAP 100, the gold standard for determining allergen-specific IgE [33], ImmunoCAP ISAC showed good performance in sensitivity and specificity in detecting sensitization to Der p 1, Der p 2, and Der p 10. In the ROC analysis, ImmunoCAP ISAC yielded favorable AUCs for the detection of Der p 1 (0.911), Der p 2 (0.908), and Der p 10 (0.917) sIgE. ImmunoCAP ISAC correlated well with ImmunoCAP 100 in the determination of sIgE levels of Der p 1 (κ=0.76), Der p 2 (κ=0.77), and particularly Der p 10 (κ=0.95). Although the overall sensitivity of ImmunoCAP ISAC was slightly lower than ImmunoCAP 100 in detecting sensitization to HDM allergen components, 90.9% (10/11) of the missed cases were with class 3 or lower Der p 1 and Der p 2 response by ImmunoCAP 100; the sensitivity of ImmunoCAP ISAC appeared to be satisfactory in patients with a higher-class sIgE response to Der p components. In addition, both approaches were comparable in specificity.

It has been suggested that CRD or component IgE follow-up are best carried out by classical ImmunoCAP testing [21]. However, this is an expensive technique and requires greater amounts of test serum, which would compromise its use in cases of multiple allergen sensitization in developing countries. By contrast, ImmunoCAP ISAC enables simultaneous detection of up to 112 allergens using a modest volume of serum (30 μL). This advantage may be helpful in identifying polysensitized patients or patients with cross-reactivity to multiple allergens. In the present study, quantitative tests of Der p 1, Der p 2, and Der p 10 sIgE with ImmunoCAP ISAC showed good correlation with ImmunoCAP 100 (r_s= 0.835, 0.810, and 0.915, respectively). Accordingly, the measurements may accurately reflect the sIgE levels of these allergen components.

In summary, the present study has shown that Der p 1 and Der p 2 are the major HDM allergen components in southern Chinese individuals. Combined detection of Der p 1 and Der p 2 may help to identify patients who are "primarily" HDM-sensitized. Patients with positive Der p 10 sIgE alone should be further investigated for sensitization to other tropomyosin allergens to provide information for a comprehensive diagnosis. ImmunoCAP ISAC may be a useful CRD tool for detecting these Der p components, and appears to offer comparable performance to ImmunoCAP 100.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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