

Brief report

Allergen micro-array detection of specific IgE-reactivity in Chinese allergy patients

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Keywords: *specific immunoglobulin E; micro-array; Dermatophagoides pteronyssinus; Dermatophagoides farinae; cross-reactivity*

Background Allergen micro-arrays are powerful tools for screening of serum IgE-reactivity. In this study allergen micro-arrays were used to identify dominating IgE-binding allergens and cross-reactivity patterns among selected Chinese allergy patients.

Methods The study was conducted using patient sera from the cities of Guangzhou, Nanjing, Chengdu and Shenyang. In total 100 sera with Der p specific IgE-levels higher than 50 kU/L were selected for testing against 103 individual allergens.

Results Among 100 selected patients, 95% showed IgE-reactivity towards house-dust mite allergens Der f 1, Der f 2 and Der p 2 and 94% were IgE positive against Der p 1, and 60% of sera contained IgE reacting against allergen Eur m 2. IgE against Cat allergen, Fel d 1, was seen in 20%. Only 2% showed specific IgE-reactivity to Der p 10, a panallergen belonging to the tropomyosin family. Serum IgE-reactivity towards other allergens was in general low. IgE-reactivity against pollen allergens showed geographic differences.

Conclusions This study clearly confirms that group 1 and group 2 are major allergens of house dust mites. These selected house-dust mite allergy patients are close to being mono-sensitized. Der p 10 is not an important allergen for cross-reactivity. Specific IgE-sensitization towards pollen allergens is low in southern China compared to other regions. The prevalence of food and stinging insect allergens known to give rise to IgE-mediated cross-reactivity are 2% or less.

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Allergen micro-arrays are powerful tools for screening of serum IgE-reactivity against a large number of purified single allergens using a small amount of sera. In populations where the specific IgE and IgE-mediated cross-reactivity patterns need to be defined, allergen micro-arrays are convenient to use since a very large number of allergens representing inhaled, food and stinging insect allergenic sources can be arrayed together with allergens known to induce serum IgE-mediated cross-reactivity. Thus, allergen micro-arrays can help to identify dominating allergenic sources and allergens and indicate patterns of poly-sensitizations and IgE-mediated cross-reactivity among various allergenic sources. Several studies have also suggested that allergen micro-arrays may play an important role in future specific allergy diagnosis in combination with clinical history and *in vivo* testing.¹⁻⁴

In China, house-dust mites of the species *Dermatophagoides pteronyssinus* (Der p) and *Dermatophagoides farinae* (Der f) have been identified to be the dominating indoor allergenic sources with major allergens belonging to group 1 and group 2; following the nomenclature of the World Health Organization-International Union of Immunological Societies (WHO-IUIS) (www.allergen.org).^{5,6} However, due to lack of specific well-characterized reagents, it is not known if these patients are mono-sensitized to Der p/Der f or also

are they sensitive to other allergens; e.g. pollens and foods. Sensitization against cockroaches, animal dander, moulds and storage mites have been shown to have a relatively high prevalence based on skin prick test (SPT) reactivity in Chinese allergy patients.⁶ Several pollen sources have also been suggested to cause allergic sensitization in China; including mugwort, ragweed, and Japanese hops.⁶⁻⁹ However, a complete picture of the allergenic landscape in China is probably not yet fully developed due to lack of reagents. In addition, China is a very large area covering three different climate zones

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with clear differences in allergen exposure levels and panels of allergy sensitizations. In addition, very little is known about the prevalence and sources of stinging insect allergy and food allergy in China.

In this study we have conducted an allergen micro-array analyses of sera collected from allergic patients originating from four different geographical regions of China. The allergen arrays used contain 103 single allergens representing 46 different inhalant, food and venom allergenic sources. This is to, our knowledge, the first time such allergen micro-array analysis has been performed in China.

METHODS

The allergen micro-array study was conducted using sera collected in the SPT prevalence China Alliance of Research on Respiratory Allergic Disease (CARRAD) study reported elsewhere.⁶ In brief, the CARRAD study was conducted in 24 hospitals located in northern, eastern and southern China from February 2006 to June 2007. A total of 6304 (3456 males and 2848 females) outpatients aged 5–65 years with bronchial asthma and/or rhinitis from the clinics for respiratory diseases, pediatrics or allergic diseases participated in the study.

For allergen micro-array analyses, CARRAD sera from each of the cities of Guangzhou, Nanjing, Chengdu and Shenyang, covering four regions of mainland of China, were tested for specific IgE-levels against 16 allergenic extracts including Der p and Der f using ADVIA Centaur (Bayer Healthcare LLC, USA). Twenty-five sera from each city, in total 100 sera, with Der p specific IgE-levels higher than 50 kU/L (class 5) were selected for testing on allergen ISAC 103 micro-array (VBC-Genomics, Vienna, Austria).

Allergen micro-array tests were performed according to the manufacture's recommendations.¹⁻⁴ Briefly, the ISAC micro-array slide was loaded with 20 µl of serum and incubated for 120 minutes in a humid chamber. Excess serum was removed by washing using MilliQ water followed by washing with TBS-T buffer (150 mmol/L sodiumchloride, 10 mmol/L Tris base, and 0.5% Tween 20, pH 8.0) for 15 minutes. After additional washing with MilliQ water, the micro-array slide was dried in air for 15 minutes followed by incubation at room temperature with 20 µl of an Alexa Fluor 546 fluorescence-labelled (Alexa Fluor 546 protein labelling kit, Molecular Probes, Leiden, Netherlands) goat anti-human IgE antibody (Pharmingen, San Diego, CA, USA) diluted 1:1000 in TBS-T containing 5% milk powder. Afterwards, slides were washed twice for 10 minutes with TBS-T, rinsed with deionised water, dried, and stored in the dark until scanning.

Images were acquired by scanning allergen chips with a ScanArrayGx Microarray Analysis System (PerkinElmer

Life and Analytical Sciences, Shelton, CT, USA) with two different laser power settings. In the first scan, 70% of the laser power (50 db) was employed to obtain the highest possible signal intensities with acceptable background noise. In a second scanning step, laser power was reduced to 35 db in order to prevent saturated signals (65.535). Both images of each spot were analysed using the ScanArray Express 3.0 (PerkinElmer, USA). Captured images were then imported into the ISAC software (VBC-Genomics, USA) which specifically process the fluorescence in each spot. From the three fluorescence values obtained for each allergen on triplicate spots, the mean value was calculated and used for further analysis. IgE values were expressed as kU/L by interpolating the mean fluorescence value with a previously established reference curve.

Allergens spotted on the micro-arrays were Act d 1, Act d 2, Act d 5, Act d 11 (*Actinidia deliciosa*, Kiwi); Aln g 1 (*Alnus glutinosa*, Alder); Alt a 1, Alt a 6 (*Alternaria alternata*, alternaria rot fungus); Amb a 1 (*Ambrosia artemisiifolia*, short ragweed); Ana c 2 (*Ananascomosus*, pineapple), Ana o 2 (*Anacardium occidentale*, Cashew); Ani s 1, Ani s 3 (*Anisakis simplex*, herring worm); Api g 1 (*Apium graveolens*, celery); Api m 1, Api m 4 (*Apis mellifera*, honeybee venom); Ara h 1, Ara h 2, Ara h 3, Ara h 8 (*Arachis hypogaea*, peanut); Art v 1, Art v 3 (*Artemisia vulgaris*, mugwort); Asp f 1, Asp f 2, Asp f 3, Asp f 4, Asp f 6 (*Aspergillus fumigatus*, *Sartoryafumigata*); Ber e 1 (*Bertholletia excelsa*, Brazil nut); Bet v 1, Bet v 2, Bet v 4 (*Betula verrucosa*, white birch); Bla g 1, Bla g 2, Bla g 4, Bla g 5, Bla g 7 (*Blattella germanica*, German cockroach); Bos d 4, Bos d 5, Bos d 6, Bos d 8, Bos d Lactoferrin (*Bos taurus*, cow's milk); Can f 1, Can f 2, Can f 3 (*Canis familiaris*, dog hair); Cla h 8 (*Cladosporium herbarum*, *Davidiellatassiana*); Cor a 1.0101, Cor a 1.0401, Cor a 8, Cor a 9 (*Corylus avellana*, European hazel); Cry j 1 (*Cryptomeria japonica*, Japanese cedar); Cup a 1 (*Cupressus arzonica*, Arizona cypress); Cyn d 1 (*Cynodondactylon*, Bermuda grass); Cyp c 1 (*Cyprinus carpio*, carp fish); Dau c 1 (*Daucus carota*, carrot); Der f 1, Der f 2 (*Dermatophagoides farinae*, house dust mite); Der p 1, Der p 2, Der p 10 (*Dermatophagoidepteronysinus*, house dust mite); Equ c 3 (*Equuscaballus*, horse); Eur m 2 (*Euroglyphus maynei*, storage mite); Fel d 1, Fel d 2, Fel d 4 (*Felisdomesticus*, cat); Gad c 1 (*Gadus callarias*, Baltic cod); Gal d 1, Gal d 2, Gal d 3, Gal d 5 (*Gallus domesticus*, chicken's egg); Gly m 4, Gly m 5, Gly m 6 (*Glycine max*, soybean); Hev b 1, Hev b 3, Hev b 5, Hev b 6, Hev b 8 (*Hevea brasiliensis*, latex from rubber tree); Mal d 1 (*Malusdomestica*, apple); Mer a 1 (*Mercurialisannua*, annual mercury); Mus m 1 (*Mus musculus*, mouse); Ole e 1, Ole e 2 (*Olea europaea*, common olive); Par j 2 (*Parietariajudaica*); Pen a 1 (*Penaeusztecus*, shrimp); Pen i 1 (*Penaeus indicus*, India prawn); Pen m 1 (*Penaeus monodon*, black tiger shrimp); Phl p 1, Phl p 11, Phl p 12, Phl p 2, Phl p 4, Phl p 5, Phl p 6, Phl p 7

(*Phleumpratense*, common timothy); Pla a 1, Pla a 2 (*Platanusacerifolia*, London plane tree); Pru p 1, Pru p 3 (*Prunus persica*, peach); Sal k 1 (*Salsola kali*, Russian thistle); Ses i 1 (*Sesamum indicum*, white sesame); Tri a 18, Tri a Gliadin, Tri a 19.0101, Tri a 30 (*Triticum aestivum*, wheat).

RESULTS

Characteristics of the patients participating in the allergen micro-array study are shown in Table 1. The serum specific IgE-reactivity as determined by ADVIA Centaur is shown in Figure, expressed as IgE-levels >0.35 kU/L and >3.5 kU/L. Apart from high levels of specific IgE against Der p and Der f, sera also contained IgE specific for storage mite *Blomiatropicalis* and cat extract.

Table 1. Characteristics of the 100 selected patients

Variables	Guangzhou	Chengdu	Nanjing	Shenyang	Overall
Patients No.	25	25	25	25*	100
Age (median) (years)	6–60/10.9	5–38/8.9	5–48/12.6	7–57/35	5–60/14.7
Gender (n,Female/Male)	11/14	9/16	9/16	11/14	40/60
Diagnosis (n)	A: 21 R: 20 A+R: 16	A: 22 R: 15 A+R: 13	A: 22 R: 15 A+R: 13	A: 17 R: 20 A+R: 13	A: 82 R: 70 A+R: 55

*including 4 sera from Beijing and 3 from Hebei province. A: asthma; R: rhinitis.

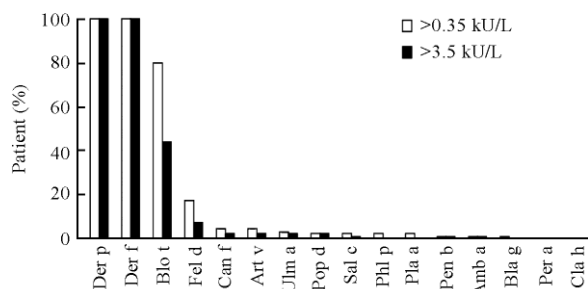


Figure. Prevalence of specific IgE in sera from 100 selected patients determined by ADVIA Centaur against 16 allergenic sources (Der p: *Dermatophagoides pteronyssinus*; Der f: *D. farinae*; Blo t: *Blomiatropicalis*; Fel d: *Felisdomesticus*; Can f: *Canis familiaris*; Art v: *Artemisia vulgaris*; Ulm a: *Ulmusamericana*; Pop d: *Populusdeltoides*; Sal c: *Salix caprea*; Phl p: *Phleumpratense*; Pla a: *Platanusacerifolia*; Pen b: *Penicillium brevicompactum*; Amb a: *Ambrosia artemisiifolia*; Bla g: *Blattella germanica*; Per a: *Periplaneta americana*; Cla h: *Cladosporium herbarum*).

The control sera used was positive for all allergens on the micro-array (results not shown) indicating that the different purified allergens retains their antibody-binding properties after immobilization to the micro-array support. Micro-array tests in general gave the same results as ADVIA Centaur but with individual allergen IgE levels. Among the sera collected from 100 Chinese allergy patients (Table 2), 95% showed IgE-specific for house-dust mite allergens Der f 1, Der f 2 and Der p 2 and 94% were IgE positive against Der p 1. In addition, 60% of sera contained IgE reacting against allergen Eur m 2 from the house-dust mite, *Euroglyphus maynei* and were always positive when the other group 2 mite allergens

Table 2. Distribution of serum IgE-reactivity for 100 selected house dust mite allergic patients (n)

Allergen	Allergen source	Guangzhou (n=25)	Chengdu (n=25)	Nanjing (n=25)	Shenyang (n=25)	Total (n=100)
Der f 1	House dust mite	24	25	23	23	95
Der f 2	House dust mite	24	25	24	22	95
Der p 1	House dust mite	24	25	23	22	94
Der p 2	House dust mite	24	25	24	22	95
Der p 10	House dust mite	0	1	1	0	2
Eur m 2	Housedust mite	13	21	14	12	60
Fel d 1	Cat	8	5	3	4	20
Fel d 2	Cat	0	1	1	1	3
Fel d 4	Cat	0	1	0	1	2
Can f 1	Dog	1	1	0	0	2
Can f 3	Dog	1	1	0	0	2
Equ c 3	Horse	0	1	1	0	2
Mus m 1	Mouse	1	0	1	0	2
Art v 1	Mugwort	0	0	1	3	4
Art v 3	Mugwort	0	0	0	3	3
Amb a 1	Short ragweed	0	0	0	1	1
Sal k 1	Russian thistle	0	0	1	1	2
Mer a 1	Annual mercury	0	2	1	0	3
Phl p 4	Common timothy	0	0	1	0	1
Phl p 12	Common timothy	0	1	1	0	2
Cyn d 1	Bermuda grass	1	0	1	0	2
Bet v 2	White birch	0	1	1	0	2
Cor a	European hazel	0	0	0	1	1
1.0401						
Ole e 1	Common olive	0	0	1	0	1
Ole e 2	Common olive	0	1	1	0	2
Cry j 1	Japanese cedar	0	1	1	0	2
Pla a 2	London plane tree	0	0	1	0	1
Cup a 1	Arizona cypress	0	3	1	0	4
Par j 2	Parietariapollen	0	1	0	0	1
Asp f 1	Sartoryafumigata	0	0	0	1	1
Alt a 1	Alternaria rot fungus	0	5	2	5	12
Ara h 1	Peanut	1	0	0	0	1
Pru p 3	Peach	0	0	1	2	3
Dau c 1	Carrot	0	1	3	0	4
Sesi i	White sesame	0	0	1	0	1
Bos d 6	Cow's milk	0	1	1	0	2
Bos d 8	Cow's milk	0	0	1	0	1
Bos d	Cow's milk	1	0	0	0	1
lactoferrin						
Hev b 1	Latex rubber tree	0	0	1	0	1
Hev b 6	Latex rubber tree	0	1	0	0	1
Hev b 8	Latex rubber tree	0	1	1	0	2
Api m 1	Honeybee venom	0	0	1	0	1

Negative results (61 single allergens) are not shown. Shading presents different allergen sources.

were positive. Only 2% showed specific IgE-reactivity to Der p 10, a panallergen belonging to the tropomyosin family. Tropomyosin was considered the reason for cross-reactivity among house dust mites, storage mites, cockroach and shrimp/crab.

Serum IgE specificity for other allergens displayed on the micro-arrays was in general low compared to house-dust mite allergens. Specific IgE-binding to allergens Fel d 1 and Alt a 1 was found in 20% and 12% of sera, respectively. Allergens Art v 1, Cup a 1 and Dau c 1 bound IgE in 4% of the sera. Allergens Art v 3, Fel d 2, Mer a1 and Pru p 3 bound serum IgE in 3% of sera. Serum IgE-binding to Bet v 2, Bos d 6, Can f 1, Can f 3, Cry j 1, Cyn d 1, Der p 10, Equ c 3, Fel d 4, Hev b 8, Mus

m 1, Ole e 2, Phl p 12 and Sal k 1 were detected in 2% of sera and only 1% of sera contained IgE specific for Amb a 1, Api m 1, Ara h 1, Asp f 1, Bos d 8, Cor a 1.0401, Hev b 1, Hev b 6, Ole e 1, Par j 2, Phl p 4, Pla a 2, and Sesj 1. For the remaining 61 individual allergens displayed on the micro-array, no specific IgE could be detected.

In Table 3, the IgE reactivity against all pollen allergens displayed on the micro-array is shown for the different regions of China. Almost 50% of the sera from Nanjing contained specific IgE against pollen allergens, whereas sera from the southern city of Guangzhou showed very low IgE that recognized pollen allergens.

Table 3. Microarray IgE-reactivity against pollen allergens (n)

Items	Guangzhou (n=25)	Chengdu (n=25)	Nanjing (n=25)	Shenyang (n=25)
Pollen sensitized patients*	1	10	12	9
Pollen sensitized percentage (%)	4	40	48	36

*Pollen allergens: Art v 1, Art v 3, Amb a 1, Sal k 1, Mer a 1, Phl p 4, Phl p 12, Cyn d 1, Bet v 2, Cor a 1.0401, Ole e 1, Ole e 2, Cry j 1, Pla a 2, Cup a 1, Par j 2.

DISCUSSION

The allergen micro-array results in this study clearly confirm that group 1 and group 2 are major allergens from house dust mites. Furthermore, very little serum IgE-reactivity towards other indoor and outdoor allergens suggests that house-dust mite allergic patients in China are close to being mono-sensitized. It may indicate that patients with high specific IgE levels against house dust mite do not require to be screened with other allergens in diagnosis. The allergen with the second highest prevalence of micro-array IgE-binding is the major cat allergen, Fel d 1 and to a lesser extent, Fel d 2 and Fel d 4. The detection of specific IgEs by ADVIA Centaur and the micro-array were in general agreement, however, it should be noted that no allergens representing storage mites, like *Blomiatropicalis*, are present on the micro-arrays used in this study. There is a relatively low prevalence of IgE-reactivity towards the venom and food allergens present on the micro-array. Since there are very little data in the literature regarding prevalence of food and stinging insect allergy in China, it is difficult to put these results in context. No IgE-reactivity was detected against the five German cockroach allergens present on the micro-array; which are somewhat surprising considering the relatively high SPT cockroach prevalence reported elsewhere.⁶ These data should be carefully evaluated on the basis of exposure to different cockroach species, sensitization to other cockroach allergens, or the presence of contaminants in extract preparations used for SPT. There was also limited specific IgE micro-array reactivity towards timothy and Bermuda grass pollen allergens, however, this agrees with previous SPT data on grass pollen allergy prevalence.⁶

The geographical distribution of the patients is partly reflected in the micro-array IgE-reactivity pattern.

Relatively low prevalence of IgE-reactivity towards pollen allergens were detected in sera derived from patients originating from the city of Guangzhou in agreement with a higher prevalence of pollen sensitization in northern China compared to southern China as previously reported.⁶

The selection of patients participating in the study could influence the micro-array results; patients were selected on the basis of high *in vitro* and *in vivo* IgE-reactivity towards Der p and Der f and they also represent subjects who had actively and voluntarily chosen to seek health care due to their allergic condition and symptoms. In addition, although patients participating in this study live in different parts of China they all come from large cities and very few from rural areas. Other parameters to consider are the level of detection of IgE-binding on the micro-arrays and the selection of allergens immobilized to the micro-array. For recombinant allergens the complete spectra of naturally occurring allergen isoforms are not represented on the micro-arrays which may influence the binding of specific IgE.

One objective in performing the micro-array study was to detect any IgE-reactivity towards pollen allergens that represented many different pollen sources and that are known to give rise to cross-reactive serum IgE. Serum IgE-reactivity towards such panallergens could indicate cross-reactive patterns among Chinese patients that may be sensitized towards pollen sources specific for China and for which we today lack well-characterized allergenic extracts. However, very little IgE-reactivity could be detected towards any of the known cross-reactive allergens displayed on the micro-arrays; including pollen allergens profilin and polcalcins as well as other allergens known to induce IgE-mediated cross-reactivity in foods, such as lipid-transfer proteins (LTP)¹⁰ or tropomyosin. Any statistical correlation of IgE-reactivity between different pollen sources was hampered by the too few sera contained IgE specific for these antigens and relative low tested samples.

In conclusion, this first allergen micro-array analysis of sera from Chinese allergy patients clearly confirmed that group 1 and group 2 are the major allergens for house dust mites. For these selected patients, the allergen with the second highest prevalence of micro-array IgE-binding was the major cat allergen, Fel d 1. Specific IgE-reactivity towards pollen allergens was low in southern China compared to other regions. The prevalence of allergen micro-array IgE-reactivity towards food and stinging insect allergens and towards allergens known to give rise to IgE-mediated cross-reactivity, were in the order of 2% or less.

Further studies are needed to avoid the bias of low sample number tested in the current study. A broader number of purified natural or recombinant allergens, including all those relevant for the Chinese regions, and a routine

testing of all suspected allergic patients would increase our knowledge on specific sensitization patterns among Chinese allergy sufferers.

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