A pilot study on the allergen-specific IgE to molecular components on polysensitized mite allergic asthmatic patients in Guangzhou, China

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**Objective:** Using multiplex microarray-based component resolved diagnosis (CRD) to investigate the allergen sensitization profile of allergic asthma patients in southern China.

**Method:** Serum samples from 57 polysensitized mite allergic asthmatic patients in a tertiary referral centre of southern China were tested with multiplex CRD (ISAC) for specific immunoglobulin E (sIgE) against 112 single allergen and components. Result was then compared with those from singleplex ImmunoCAP.

**Results:** With ISAC, the highest sensitization was seen for nDer f 1 (71.9%), rDer f 2 (73.7%), nDer p 1 (70.2%) and rDer p 2 (66.7%), whereas rDer p 10 and other storage mites' components only showed 10% positivity. rFel d 1 and rCan f 1 were found positive in 29.8% and 14.0% samples respectively. Other epithelia components had less than 7.0% positive rate. Sensitization to pollen components was dominated by nCyn d 1 (17.5%) and nPhl p 4 (12.3%). Carbohydrate cross-reactive determinants (CCD) was positive in 4 patients who were also positive to nPhl p 4, nCyn d 1 and rPla a 2, and all of them have combined asthma and rhinitis. The sensitivity to mold (rAsp f 3), cockroach (nBla g 7) and Anisakis simplex component (rAni s 3) were all the same at 8.8%. 93.0% patients were sensitive to more than one component, with more than half of them (57.9%) positive to five or more components.

Patients with combined asthma and rhinitis (AA + AR) were sensitive to more components than those with asthma only (AA). Positive rate to nPhl p 4 was significantly higher in patients with AA + AR than with AA only ($\chi^2 = 4.31$, $P = 0.038$).

Compared with ImmunoCAP, ISAC showed a similar high detection rate for D. pteronyssinus and D. farinae, but only 10.0% of B. tropicalis sensitive patients were positive to rBlo t 5. Optimal scale analysis on correlation of allergens components showed rDer p 10 was associated to food allergy.

**Conclusion:** Being the first multiplex microarray based CRD study on southern Chinese, ISAC showed house dust mites components were the major allergen components led to sensitization in asthmatic patients. Patients with combined AA + AR were sensitive to more components than those with AA only. Other components with higher positive rate include pollen components nCyn d 1, nPhl P 4 and animal dander components rFel d 1 and rCan f 1. For B. tropicalis, the rBlo t 5 in ISAC may not represent the major Blomia component in southern Chinese patients.

1. Introduction

Traditionally, the diagnosis of allergic asthma is based on patients’ clinical history, bronchial provocation test, and quantitative determination of specific immunoglobulin E (sIgE) to allergens (Casset et al., 2012). The commercially available sIgE kits used in common practice are made from natural allergen extracts which consist of heterogeneous mixture of not just major allergens, but also cross-reactive proteins, non-allergenic antigens, as well as interfering substances (Pomés et al., 2015). These affect the sensitivity and specificity of the test and create cross-reactivity problem for the diagnosis of polysensitized patients. To complement the potential bias, allergen component-resolved diagnosis
(CRD) based on natural, recombinant or synthetic allergen molecules was introduced to target on detecting the allergen components which are involved in the specific immune response to allergens (Valenta et al., 1999). With careful interpretation, this methodology can help to exclude false reactivity to pan-allergens and predict the risk for food anaphylaxis (Usaf et al., 2015), as well as direct the use of specific allergen immunotherapy (Sastre et al., 2012).

Asthma is a lung disease characterized by chronic inflammation and recurrent exacerbations. It has become a major public health concern globally since the 1960s, with global prevalence ranges from 1% to 18% among different population in different countries (Anderson et al., 1986; Moone et al., 2006). Several studies in Europe have revealed the prevalence of asthma is doubling every 10 years. In the US, 60% more asthmatic patients have been found since 1980s (Song et al., 2014). Among these patients, 50% of adults and 80% of children have a sensitivity history to different allergens (Keglowich et al., 2014) and most of them are polysensitized to more than one allergen (Santoso et al., 2015). In China, around 30 million people are suffering from asthma. Lately, the asthma prevalence in southern China has been found to be 1.13% (Lin et al., 2018). Guangzhou is the capital city of Guangdong province in southern China. It houses a population of 13 million and resides in the subtropical monsoon area with high temperature and humidity (Tong et al., 2016). This makes the city very suitable for proliferation of dust mites and fungi and gives rise to polysensitization in asthma patients (Jeong et al., 2012; Tanimoto et al., 2015). As the potential wide spread cross-reactivity may make traditional sIgE determination methods fail to accurately identify the sensitivity of these patients, CRD seems to be a promising method to find out the IgE reactivity pattern of the patients (Valenta et al., 1999).

ImmunO Solid-phase Allergen Chip (ISAC) (Thermo Fisher, Uppsala, Sweden) is a multiplex microarray based CRD system for determining specific IgE antibodies against recombinant and natural allergens (Martínez-Aranguren et al., 2014). In contrast to the singleplex ImmunoCAP, it can simultaneously detect 112 allergenic proteins with a small volume of serum (30 μl) (Martínez-Aranguren et al., 2014). In addition, ISAC-identified allergen components in 57 polysensitized asthmatic patients

2. Materials and methods

2.1. Serum samples

57 asthma patients were randomly taken from the serum bank of Allergy Information Repository (AIR-SKLRD) in Guangzhou, China, which was established from January 2013 to June 2017 with sera from 8000 patients aged 1–85 years old recruited in Guangzhou city. All these 8000 patients have been confirmed to have asthma with or without rhinitis based on the GINA (Bateman et al., 2008) and ARIA (Pawankar et al., 2012) guideline and have the following characteristics: (a) have contact history with one or more common inhalants or food allergens in China (such as mites, pollens, animal dander and food); (b) show a positive result in bronchial provocation test or bronchial dilation test; (c) have a positive ImmunoCAP serum specific IgE titer against D. pteronyssinus and at least one more common inhalant allergen (≥0.35 kU/L). None of these patients has taken specific allergen immunotherapy, with no autoimmune diseases, parasitic infections, obstructive pulmonary diseases such as chronic obstructive pneumonia, bronchiectasis, COPD, lung cancer nor systemic blood diseases.

2.2. Testing method

All 57 Sera were tested with ImmunoCAP Immuno-Solid phase Allergy Chip (ISAC) (Thermo Fisher Scientific, Uppsala, Sweden), which can detect IgE antibodies to 112 components from 51 allergen sources (Martínez-Aranguren et al., 2014). The tests were performed according to the manufacturer’s recommendations with 30 μl of serum as previously reported (Zeng et al., 2015). The resulting fluorescent signals were measured with a confocal laser scanner and the microarray image data was analyzed with ImmunoCAP ISAC Xplain® software (Phadia). Results were reported in ISAC Standardized Units (ISU) and categorized based on the manufacturer’s cutoff levels (<0.30 ISU = Class 0; ≥0.30 to <1.00 ISU = Class 1; ≥1.00 to <15.00 ISU = Class 2; >15.00 ISU = Class 3). sIgE titers ≥0.30 ISU was classified as positive. Singleplex specific IgE determination to China common allergens (Dermatophagoides farinae, Blomia tropicalis, Felis domesticus, Canis familiaris, Blatella germanica and Aspergillus fumigatus) were performed with UniCAP 1000 platform. Results were recorded with respect to manufacturer’s guideline: those with a result of ≥0.35 kU/L were considered positive. The two types of results were compared to review the patient’s sensitization pattern to molecular components.

2.3. Ethical approval

The study was approved by the Institutional Review Board of First Affiliated Hospital (GYFY-2013-47) of Guangzhou Medical University. Written informed consent was obtained from all adults and legal guardians of the participating children.

2.4. Data analysis

Statistical studies were conducted with SPSS 22.0 (SPSS, Chicago, IL). Parametric quantitative data was presented as the mean ± standard deviation. Non-parametric quantitative data was presented as a median (interquartile range). Categorical data was reported as a percentage showing the proportion of positive results. Proportions were compared between groups with chi-square tests (χ2). F-tests were used to compare the variance of data amongst the groups. Correlation analyses between non-parametric data were performed using Spearman’s tests, with the correlation coefficients presented as “rs”. The correlation between components was calculated with optimal scale analysis. P-value <0.05 was considered to be statistically significant.

3. Result

3.1. The distribution of ISAC-identified allergen components in 57 polysensitized asthmatic patients

Among the 57 polysensitized asthmatic patients, dust mite components have the highest sensitization rate, followed by animal dander including cat and dog. The highest positive rate was seen in rDer f 2 (73.7%), nDer f 1 (71.9%), nDer p 1 (70.1%) and rDer p 2 (66.7%), whereas rDer p 10 and the other two storage mites’ components (rBlO t 5 and rLep d 2) had only 10.0% positive rate. Among the animal dander group, most patients were sensitive to rFel d 1 (29.8%) and rCan f 1 (66.7%), whereas rCan f 1 (14.0%), with only 4.1%–7.3% of them were sensitive to other dog and cat components respectively. Both horse (rEq u c 1, nEq u c 3) and mouse (nMus m 1) dander had a low positive rate. Sensitization to pollen components was dominated by nPhl p 4 (12.3%) and nCyn d 1 (17.5%) (Fig. 1). CCD (Carbohydrate cross-reactive determinants, nMus f 3) was positive in 4 patients (7.0%). All patients sensitive to CCRD were positive for nPhl p 4, nCyn d 1 and rPla a 2, and all of them have combined asthma and rhinitis (Table 1).

Only 12 patients (21.1%) were sensitive to food components, with the highest positivity seen in nPen m 1 (8.8%), nJug r 2 (8.8%) and nPen m 2 (5.3%). 12 patients (21.1%) were sensitive to mold
components. Their positive rate from high to low were rAsp f 3 (8.8%), rAsp f 1 (7.0%), rAlt a 1 (5.3%), rAsp f 6 (5.3%) and rAlt a 6 (3.5%). The sensitivity to cockroach (nBla g 7) and Anisakis simplex component (rAni s 3) were the same at 8.8% (Fig. 1).

3.2. Components sensitization profile of asthmatic patients with or without rhinitis

As seen in Fig. 2a, 93.0% patients were sensitive to more than one component. While more than half of them (57.9%) were positive to five or more components, 7.0% didn’t show sensitization to any tested components. Moreover, patients with combined asthma and rhinitis (AA + AR) were sensitive to more components than those with asthma only (AA). For example, 81.1% AA + AR patients were sensitive to four or more components. 13.5% were sensitive to ten or more components. It contrasts with 75.0% and 5.0% respectively in AR group.

While house dust mites’ components (nDer f 1, rDer f 2, nDer p 1, rDer p 2) and cat component rFel d 1 have the highest sensitization in both groups. The positive rate of nPhl p 4 were significantly higher in AA + AR than in AA only ($\chi^2 = 4.31, P = 0.038$). In the animal dander group, AA patients were only sensitive to rFel d 1 and rCan f 1 and sensitization to other dander’s components were only found in AA + AR group. The number of patients sensitive to food and mold components were all below 8.8% (Fig. 2b, c).

3.3. Positive rate between ISAC and ImmunoCAP sIgE results

The Venn diagram in Fig. 4a showed among those positive to ImmunoCAP for D. farinae, 33 (78.6%) and 34 (81.0%) were positive to nDer f 1 and rDer f 2 respectively. The dominant sensitization pattern was similar to D. pteronyssinus which the positivity to nDer p 1 and rDer p 2 were 40 (70.2%) and 38 (66.7%) respectively (Fig. 4b). Only 7 (12.3%) D. pteronyssinus sensitive patients were positive to rDer p 10, which was much lower than nDer f 1 (41), rDer f 2 (42), nDer p 1 (40) and rDer p 2 (38). For the 30 patients sensitized to B. tropicalis, only 3 (10.0%) of them were positive to rBlo t 5 (Fig. 4c). The highest positive component for German cockroach was nBla g 7, but it only accounted for 20.8% and no sensitization was found in other cockroach components (Fig. 4d). There were 29 patients sensitive to dog dander. Their positive rate to rCan f 1, rCan f 2, nCan f 3, rCan f 5 were 14.0%, 5.3%, 7.0% and 7.0% (Fig. 4e). This contrasts with cat dander which the majority was positive to rFel d 1 (24.6%) and a much lower positive

Table 1

| Serum specific IgE level (ISU) of allergen component in CCD positive patients. |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| **CCD**         | Phleum pratense | Cynodon dactylon | Trichium acerisum | Plantus acerifolia | Cryptomeria japonica | Cupressus arvernica | Salvia hali |              |              |              |              |              |
| nMax f 1        | 8.90            | 17.00            | 13.00            | 11.00            | 6.30            | 0.00            | 0.00            | 2.00            | 0.00            | 0.00            | 0.00            | 0.00            |
| nPhl p 4        | 1.80            | 5.40            | 6.30            | 2.90            | 4.80            | 0.00            | 0.00            | 1.50            | 3.40            | 0.00            | 0.00            | 0.00            |
| nPsy d 1        | 1.20            | 3.30            | 2.30            | 2.70            | 2.20            | 0.00            | 0.00            | 0.00            | 0.00            | 0.00            | 0.00            | 0.00            |
| nPsy r 2        | 0.50            | 0.50            | 1.00            | 1.50            | 1.30            | 0.70            | 3.90            | 0.00            | 1.00            | 0.00            | 0.00            | 0.00            |

*light gray: Class 1; gray: Class 2; dark grey: Class 3 (≥ 0.30 to < 1.00 ISU = Class 1; ≥ 1.00 to < 15.00 = Class 2; ≥ 15.00 ISU = Class 3).

CCD: Carbohydrate cross-reactive determinants.
rate was seen in rFel d 4 (5.3%) and nFel d 2 (3.5%) (Fig. 4f). Among the 13 patients positive to Aspergillus fumigatus, only 1 (7.7%) of them was positive to rAsp f 1, rAsp f 3 and rAsp f 6 (Fig. 4g).

Optimal scale analysis on correlation of allergens components showed nDer f 1 sensitivity correlated to rDer p 2. Positive correlation was also found among rDer p 10, rBla g 2, and rAni a 3 (Fig. 5a). rDer p 10 was found to be associated with food components (Fig 5b).

4. Discussion

Our study showed that more than 93.0% of the subjects were sensitive to more than one allergen component (Fig. 2a). Dust mites were perceived to be the most important allergens for asthmatics in subtropical areas with high humidity (Keglowich et al., 2014). Our data also confirmed nDer f 1, rDer f 2, nDer p 1 and rDer p 2 had the highest sensitization rate. This is similar to our previous component analysis study with singleplex ImmunoCAP for allergic patients (Zeng et al., 2015). As Southern China is in the subtropical climate zone where is generally warm and humid, house dust mites can grow quickly and their fecal excretions can readily enter the human respiratory tract to induce asthma (Scala et al., 2010). Our result showed that like singleplex ImmunoCAP, ISAC can identify the patients who are suitable for immunotherapy with a Der p/Der f extract (Zeng et al., 2016).

Another mite component rDer p 10 was found to be positive in 12.3% of the patients. It is higher than the 2.0% found in previous study (Zheng et al., 2011). Moreover, all rDer p 10 positive patients were also sensitive to nPen m 1, nBla g 7 and rAni a 3. This agrees with the previous finding that elevated sIgE to shrimp, mite, tropomyosin and Der p 10 might be the relevant risk factors for mite-induced asthma (De Weger et al., 2011; Farioli et al., 2017).

As what have been found in other parts of the world (Sookrung et al., 2016; Wang et al., 2015), Southern Chinese always show a polysensitized pattern (Zeng et al., 2018). The current study identified Der p 1, Der p 2, Der f 1, Der f 2 and Fel d 1 as the major sensitized components (Zheng et al., 2011). Due to their high correlation to the respective sIgE titers against the crude allergen extracts, Chinese doctor may take ISAC result as an alternative to confirm patients’ allergic status.

For pet’s dander, rFel d 1 (24.7%) and rCan f 1 (14.0%) were found to be the most sensitized component for cat and dog respectively. Since they were also identified as the major allergenic components in European studies (Arbes et al., 2004; Zheng et al., 2011), we might conclude that the two components could serve as the specific component marker for the respective pets. Nevertheless, since there is vast difference in positive rate between them, rFel d 1 seems to have a better association with cat’s sensitivity than rCan f 1 with dog in Chinese population. More studies should be done to confirm this.

Bl a g 7 and Bl o t 5 have been found to be the major sensitized components in B. germanica and B. tropicalis sensitive patients respectively (Jeong et al., 2003; Naik et al., 2008). Their positive rates were only found to be 8.8% (nBla g 7) and 10.0% (rBl o t 5) in our study. Further studies are needed to verify whether Chinese asthmatics have a different major allergenic profile to the two arthropods.

With skin prick test, Zheng et al. (Zheng et al., 2011) reported pollen sensitization was only found in 2.0% of the allergic patients in southern China. This contrasts with our findings that sensitization to nPhl p 4 and nCyn d 1 were 12.3% and 15.8% respectively. As Phleum pratense and Cynodon dactylon can commonly be found in both Europe and western Asia (Scala et al., 2010), it seems reasonable to assume Chinese patients have a similar susceptibility to the two pollens as in the western world. The discrepancy between the two studies may due to different compositions of the extract used (Scala et al., 2010).

CCD was found positive in 4 patients (6.3%), and all of them were sensitive to more than 6 components (from 6 to 16), including rPla a 2, nPhl p 4 and nCyn d 1. It implies cross-reactivity might exist among these pollens. As there were studies showed CCD might be positive in about 20% of atopic patients and gave rise to different cross-reactivity (Holzweber et al., 2013), more investigations are needed to confirm it for southern Chinese.

Sensitization to mold components were only found in around 5.0% of the samples, mainly belonged to components from Aspergillus fumigatus (rAsp f 1 and rAsp f 3). The prevalence of broncho-pulmonary aspergillosis (ABPA) among asthmatics was estimated to be around 2.5% (Medina et al., 2017). Tanimoto H et al. have also found that patient sensitizes to rAsp f 1 and rAsp f 3 might indicates ABPA (Tanimoto et al., 2015). More studies should be done to investigate whether these components can serve as biomarker of the disease in China.

For comparison between ISAC and ImmunoCAP, our study showed high positive concordance in both D. pteronyssinus and D. farinae. This implies measuring the group 1 and group 2 house dust mites’ components might be a good marker to those allergic to D. pteronyssinus and D. farinae. However, the same principle cannot apply to B. tropicalis whereas rBl o t 5 was only found in 10.0% of the storage mite sensitive

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**Fig. 3.** Comparison of whole extract’s positive rate between ISAC and ImmunoCAP for major inhalant allergens.

*ISAC: Positive result was defined as specific IgE titers ≥ 0.30 ISU.*

*ImmunoCAP: Positive result was defined as specific IgE titers ≥ 0.35 kUA/L.*
patients. This big difference might reflect rBlo t 5 was not the major allergenic component for *Blomia tropicalis* in Chinese patients or the rBlo t 5 test in ISAC was not sensitive enough for the Chinese patients. In fact, we also couldn’t find any mite components sensitivity in 14.0% of the house dust mites sensitive patients detected by ImmunoCAP. This might confirm ISAC test was not as sensitive as ImmunoCAP, which has also been reported by Zeng et al. (Zeng et al., 2015), or some important mite components in the extract-based ImmunoCAP were missing in ISAC such as Der p 23 (Banerjee et al., 2014). More investigation is needed with bigger sample size and a singleplex Der p 23 should be added into the ISAC analysis.

5. Conclusion

This was the first ISAC study on mite sensitized asthma patients in southern China and showed nDer p 1, rDer p 2, nDer f 1 and rDer f 2 were the major dust mite components led to sensitization. As these components are the major allergenic molecules included in therapeutic allergen extracts by manufacturers, ISAC can help to accurately identify the right group of Chinese patients for mite immunotherapy. It can also help to sort out those patients who are sensitized to rDer p 10 only which the house dust mite immunotherapy will have less effect on them. For patients with combined asthma and rhinitis, they were sensitive to more allergens than those with asthma only. This poly-sensitization pattern was not just seen in dust mite species only, but also among the pollen components nPhl p 4 and nCyn d 1, which were not considered to be important in southern China in previous study (Luo et al., 2016).

This pilot study concludes that ISAC might be a reliable alternative to singleplex test for diagnosing mite allergic asthma in southern China. More funding and support are needed to verify this as well as confirming its usefulness in complementing or even replacing other singleplex specific IgE test and skin prick test.
Fig. 5. Optimal scale analysis of connection between components.

a) Optimal scale analysis of connection between dust mites’ components and tropomyosin components, Cronbach’s Alpha – 95.5%.
b) Optimal analysis of connection between food allergy and dust mites’ components, Cronbach’s Alpha – 92.0%.
Optimal Scale Analysis is a “Dimensional Reduction” analytical method. Using sGE concentration as the continuous variable and food allergy as the categorical variable, the closer of the two points, the higher is the correlation between the two factors.

Conflicts of interest
The authors declare that they have no conflict of interest.

Author contributions
Conceived and designed the experiments: SBQ. Performed the experiments: LWT, WZH. Analyzed the data: HHS, CCX, HHM. Wrote the paper: HHS, LWT, HHM.

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