

ORIGINAL PAPER

Distinctive immunoglobulin E anti-house dust allergen-binding specificities in a tropical Australian Aboriginal community

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Clinical and Experimental Allergy

Summary

Background There is evidence that the specificity of the IgE binding in allergy tests can vary for different populations.

Objective We aimed to examine the allergenic specificity of IgE binding in sera from house dust mite (HDM)-atopic subjects in a tropical Australian Aboriginal community.

Methods Sera shown to contain IgE antibodies to an HDM extract of *Dermatophagoides pteronyssinus* were examined for IgE binding to a panel of nine purified HDM allergens from this mite species by quantitative microtitre assays. IgG antibody binding (IgG1 and IgG4) was also measured.

Results The IgE-binding activity in the sera from the Aboriginal community was not directed to the expected major groups 1 and 2 HDM allergens but instead to the group 4 amylase allergen. There was also little IgE binding to the potentially cross-reactive tropomyosin (Der p 10) or arginine kinase (Der p 20) allergens. The IgG4 antibody was rarely detected and limited to the Der p 4 allergen. IgG1 antibody binding was frequently measured to all the allergens regardless of an individual's atopic status, whereas in urban communities it is restricted to the major allergens and to atopic subjects.

Conclusion The high IgE anti-HDM response of Australian Aboriginals predominantly bound Der p 4 and not the Der p 1 and 2 allergens, showing a distinctive allergy that could affect the disease outcome and diagnosis.

Keywords allergens, component-resolved diagnosis, *Dermatophagoides pteronyssinus*, IgE, IgG

Submitted 1 February 2007; revised 7 May 2007; accepted 20 June 2007

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Introduction

The increase in the prevalence of asthma over the last three decades has created a need for more health resources [1]. The increase, once the preserve of Western cultures, is, however, spreading to developing societies with an expected high socio-economic burden [2–4]. Early medication can minimize hospital admissions and so early monitoring of the problem is desirable. One of the major risk factors for asthma is atopy, particularly sensitization to prevailing aeroallergens. Atopy could accordingly predict the risk of asthma and identify environmental triggers. It is, however, a concern that ill-defined allergen extracts are being used to measure atopy when purified

allergens that can identify major and cross-reacting allergens are available. The possible impact of this has already been shown in at least two studies. Firstly, a school-based study of children in Venezuela showed that an IgE antibody bound by a major mite allergen, but not IgE measured by mite extract, predicted disease [5]. Secondly, a comparison of the IgE antibody with different house mite allergens in sera from Europe and Zimbabwe found high-frequency binding to the cross-reactive tropomyosin allergen in the African population [6].

Isolated communities of Australian Aboriginals have been investigated for asthma and allergy where there is considerable interest in comparing disease patterns and allergy in their varying environments [7]. Members of one

community in the tropical northwest have shown very high levels of anti-house dust mite (anti-HDM) IgE as measured by serology (65%) [7] but the prevalence of asthma, at a level of 15%, was similar to urban Australia [8]. It was proposed that endemic parasitism once found in the community could ameliorate the disease [7]. These results prompted this investigation with the use of component-resolved diagnosis for HDM allergy using a panel of purified allergens. The results revealed that the IgE binding was not directed to the classical major mite allergens and so a close association with disease and sensitivity would not necessarily be expected. This shows that tests that define the IgE-reacting specificities need to be used to investigate allergies in previously unstudied communities.

Methods

Study population

Sera from 83 subjects (52 atopic; 31 non-atopic, age range 3.96–70 years) from an Aboriginal population in the tropical north of Western Australia were collected in 1995 and frozen until assayed. Sera from a random selection of 28 mite-atopic subjects (age range 4.5–49.5 years) from Perth, the capital city of Western Australia, were also assayed and included sera from three Aboriginal subjects. Atopy was defined by mite-specific IgE measured using the CAP system (Pharmacia Diagnostics AB, Uppsala, Sweden) performed either in-house or by the Immunology Department at Princess Margaret Hospital (Subiaco, Perth, Western Australia). The CAP score is measured in kU/L where 1 kU/L is equal to 2.24 ng/mL. The mite-specific CAP score had a median of 22.6, with an interquartile range from 10.9 to 61.4 kU/L for the mite-atopic Aboriginal population. The median total IgE of the atopic subjects was 3540 kU/L (interquartile range 2300–8040 kU/L) and 434 kU/L (242–873 kU/L) for the non-atopic subjects. The mite-specific CAP score had a median of 68.4 kU/L (33.0–150 kU/L) for the atopic Perth population. The median total IgE for the Perth subjects was 354 kU/L (198–665). Ethics approval was obtained from the Sir Charles Gardiner Hospital Research Institutional Ethics Committee, the Health Department of WA Aboriginal Ethics Committee and The Princess Margaret Hospital Human Ethics Committee. Support for the study was obtained from the community councils, and informed personal and parental consent was obtained with the assistance of local community health workers. A previous study of the Aboriginal community has been reported [7].

Dust sampling

Exposure to *Dermatophagoides pteronyssinus* in the Aboriginal community was confirmed by the detection of

Der p 1 in dust samples using reagents available from Indoor Biotechnologies Ltd. (Cardiff, UK). Briefly, dust samples were collected from homes in the community and frozen until extracted and assayed. A 5% extract in borate-buffered saline was prepared from the dust samples and assayed for Der p 1 using the method of Luczynska et al. [9].

Antibody assays

A panel of nine purified or recombinant HDM (*D. pteronyssinus*) allergens were used to measure IgE and IgG antibody-binding activity [10]. The allergens used were Der p 1 and 2, which are the major allergens, and Der p 3, 4, 5, 7 and 8. Der p 10 (tropomyosin) and Der p 20 (arginine kinase) were included because it has been reported that antibodies induced by insects and parasites can cross-react with these specificities. Possible IgE reactivity to midge and to the storage mite *Blomia tropicalis* was examined by IgE binding to an extract of *Culicoides* species (Greer Laboratories, Lenoir, NC, USA) and to the major *Blomia* allergen Blo t 5, respectively. The quantities of IgE, IgG1 and IgG4 antibodies to the specific allergens were measured in allergen-coated microtitre plates using DELFIA™ (Wallac, Turku, Finland) as performed previously [10]. The quantities of antibody were interpolated from titration curves created for each allergen with serum standardized for antibody binding by an assay that used monoclonal antibodies on the microtitre plates to standardize capture of allergen and comparison with a titration of isotypic anti-Der p 2 standards. The anti-Der p 2 standards were humanized monoclonal antibodies made for this purpose by Indoor Biotechnologies Ltd. by incorporating the constant regions of human IgE, IgG1 or IgG4. The quantities of these antibodies were known. The use of this assay in ELISA format and its comparison with the CAP method are presented by Trombone et al. [11]. For the IgE assays, sera with high IgE antibody titres to non-mite allergens were included as controls on every plate and for the IgG assays, human myeloma IgG1 and IgG4 proteins (Bioscience International, Saco, ME, USA) were used. The percentage of IgE binding to each allergen was calculated as per the procedures in Hales et al. [10]. The summation of IgE binding (ng/mL) to each of the nine allergens for each subject was initially obtained to provide a measure of their total IgE binding to the specificities, the sum of the specificities (SOS). IgE binding to the individual allergens was then calculated as a percent of the SOS.

Results

High IgE binding to mite allergens for the Aboriginal subjects was mostly found in the samples previously shown to contain antibody using the Pharmacia CAP assay (Fig. 1). The pattern of binding to the purified

allergens was, however, extremely unusual. IgE antibody to the major allergens Der p 1 and 2 was almost absent, rarely being more than 5 ng/mL, whereas allergic subjects from the Perth population have an average of approximately 40 and 70 ng/mL of antibody to these proteins, respectively. The major IgE binding in the sera from the tropical Aboriginal subjects was directed to the Der p 4 allergen, which is an amylase enzyme. Der p 4 bound on average 55% of the IgE antibody in each sera, with a mean titre of 45 ng/mL. Der p 4 is a minor mite allergen for the Perth population, including for the Aboriginal subjects from Perth, where Der p 4 accounted for <10% of the IgE titre. IgE antibody binding to most of the other allergens was also generally low in the sera from the tropical Aboriginal subjects and this included the known potentially cross-reactive tropomyosin (Der p 10) and arginine kinase (Der p 20) allergens (Fig. 1). Der p 3 and 5 had similar low-titre levels of IgE binding in sera from atopic and non-atopic Aboriginal subjects. The Der p 1 allergen was readily detected in dust samples collected from homes in the Aboriginal community, confirming that the subjects are exposed to *D. pteronyssinus* despite the lack of sensitization to the major mite allergens. The median Der p 1 concentration was 6.04 µg/g dust (interquartile range 0.44–25.4 µg/g).

The unusual pattern of IgE reactivity for the tropical Aboriginal population prompted an investigation of possible allergy to *B. tropicalis*, which is a mite found in tropical Australia, or allergy to the common *Culicoides* biting midge found in this region. No IgE binding was found to the major Blo t 5 allergen from *B. tropicalis* or to the *Culicoides* extract.

IgG4 antibody was mostly absent in the atopic Aboriginal population, although antibody to the Der p 4 allergen was occasionally detected (Fig. 2). In contrast, IgG1 antibody to all the mite allergens was found in high titre in the sera of tropical Aboriginal subjects regardless of their atopic status (Fig. 3). Sera from the Perth population showed similar patterns of binding for IgG4 (Fig. 2) and IgE, whereas IgG1 antibody binding was restricted to the Der p 1, 2 and 4 allergens (Fig. 3). As published before, IgG antibody was rarely produced in the absence of IgE for the Perth population (data not shown) [10].

Discussion

People allergic to the HDM *D. pteronyssinus* typically produce high titres of IgE antibody to the Der p 1 and 2 allergens [10–12]. Instead of binding these allergens, the IgE in the sera from the Aboriginal community bound the minor Der p 4 amylase allergen as a major specificity. The sera also did not readily bind to the Der p 5 and 7 allergens that constitute the next most important specificities in the Perth population [10]. Both Der p 1 and 2 are produced in more abundance than amylase [13] and so this or other properties could affect the development of disease. The study was performed with the expectation that, by analogy to responses from rural Africa [6], high IgE binding to tropomyosin (Der p 10) would be found, consistent with its cross-reactivity [14]. Arginine kinase (Der p 20) was also examined because its potential cross-reactivity has been reported [15]. In contrast to these expectations, few Aboriginal subjects showed IgE binding to these allergens. Given the dominance of the

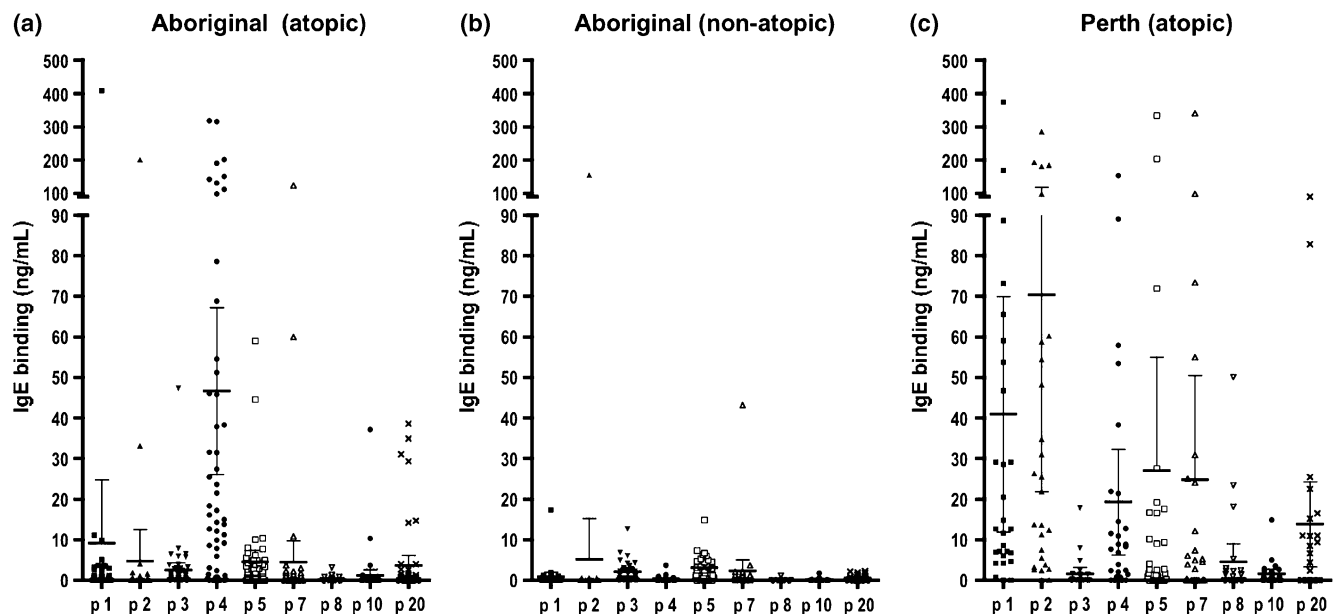


Fig. 1. Quantification of IgE binding (ng/mL) to the mite allergens for the atopic ($n = 52$; a) and non-atopic ($n = 31$; b) tropical Aboriginal subjects and atopic Perth individuals ($n = 28$; c). The mean and 95% CI are indicated. CI, confidence interval.

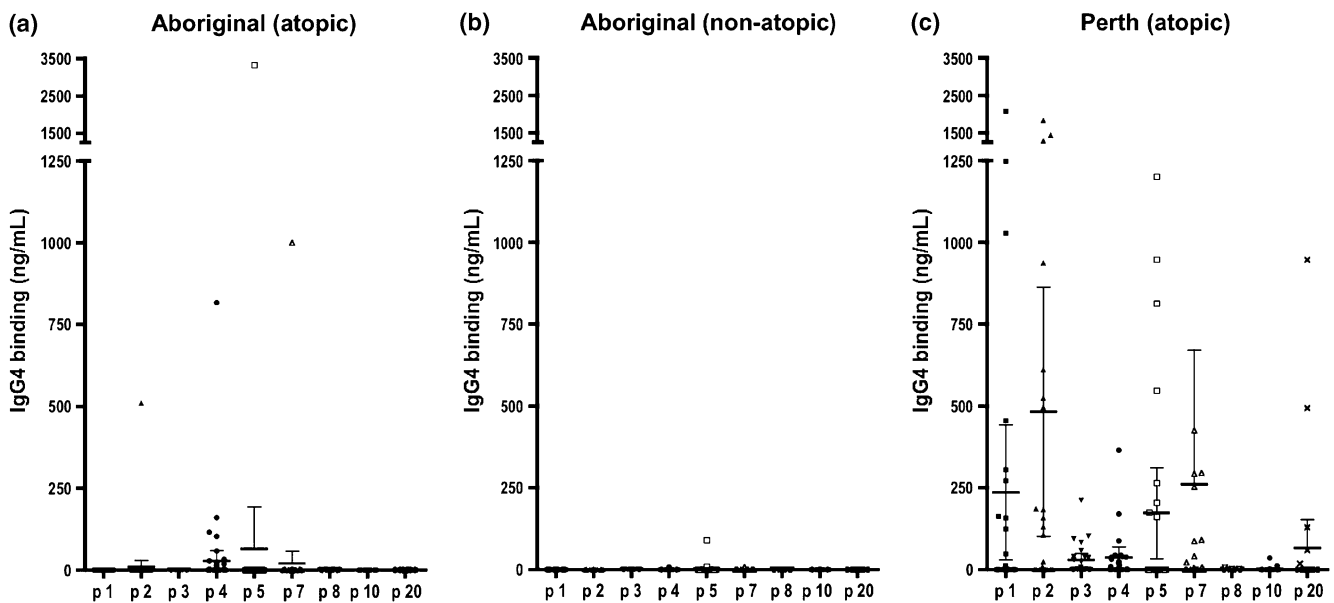


Fig. 2. Quantification of IgG4 binding (ng/mL) to the mite allergens for the (a) atopic and (b) non-atopic tropical Aboriginal subjects and (c) atopic Perth individuals. The mean and 95% CI are indicated. CI, confidence interval.

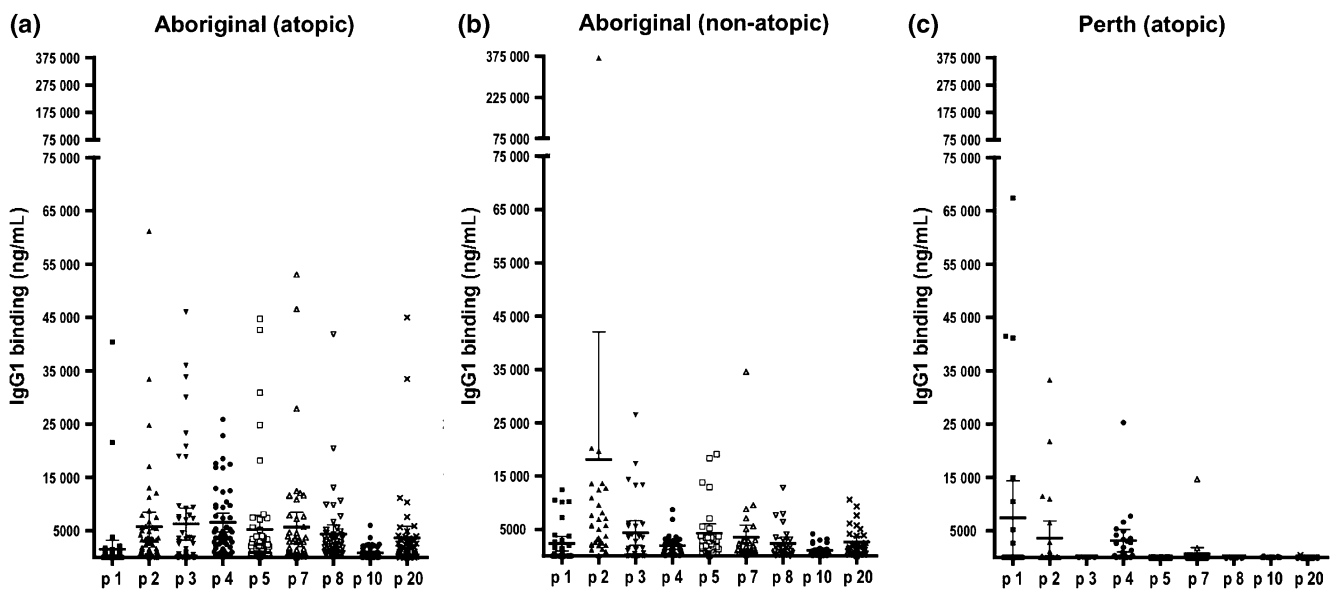


Fig. 3. Quantification of IgG1 binding (ng/mL) to the mite allergens for the (a) atopic and (b) non-atopic tropical Aboriginal subjects and (c) atopic Perth individuals. The mean and 95% CI are indicated. CI, confidence interval.

anti-Der p 4 response in the tropical population, the absence of a similar response in Aboriginals from the Perth region indicates that the specificity is not a pan association. The regions are, however, climatically very different, with Perth being more than 2000 km to the south. Australian Aboriginals are also genetically diverse and have differences in genes such as the HLA-DR that can affect the immune response [16].

The possibility exists that the IgE binding to Der p 4 is a cross-reactivity to another amylase. Binding to *Culicoides* extract was tested because biting midges are common in

the north west of Western Australia and their saliva is rich in amylase [17] but this was negative. IgE binding to the major allergen of *B. tropicalis*, a prevalent mite in the tropics [18], was also negative. The *B. tropicalis* information also shows that the sensitization is also unlikely to be to another glycyphagid mite such as *Lepidoglyphus destructor*. The glycyphagid allergens cross-react with each other but show minimal reactivity to *D. pteronyssinus* [19]. A detailed study of *L. destructor* sensitization due to exposure from farming or from flour contaminants in baking showed no reactivity to *D. pteronyssinus* [20].

Exposure to flour itself can induce sensitivity to amylase, especially the fungal amylase additive in baking. However, the wheat (P08117) and fungal amylase (CAA29252) have minimal sequence identity (below 20%) to Der p 4 and so would not be cross-reactive, especially at the high titre shown here. *B. tropicalis* has been shown to be a HDM in tropical Australia, but *L. destructor* was absent [21]. It is unlikely that the antibody was induced by parasites because parasitic infections also induce IgG4 antibody [22–25] and this was notably absent. While novel infections cannot be excluded, the parasitic diseases found in Aboriginal subjects in northern Australia with sufficient prevalence to be associated with the anti-mite responses are the intestinal nematodes *Strongyloides stercoralis*, *Trichuris trichiura* and hookworm *Ancylostoma duodenale* [22]. The IgG4 responses to *Trichuris* antigens are well documented [23] as are the IgG4 responses to *Strongyloides* [24] and hookworm [25]. Reactivity to tropomyosin (Der p 10), which is a major cross-reacting allergen of parasites [26], was additionally not present. Scabies mites are also found in the region but this infestation induces IgE antibodies to glutathione-S-transferase and tropomyosin [27, 28] and this was missing as shown by the lack of reactivity to the biochemically homologous allergens Der p 8 and 10, respectively. Molluscs also contain amylases that could potentially induce sensitization but molluscs are rarely consumed in the community and therefore would not account for the high prevalence of sensitization seen here. The sequence identity between mollusc amylases and Der p 4 is also relatively low (50%) and cross-reactivity between the amylases is unlikely. Mills et al. [29] found that although the Der p 4 and Eur m 4 mite allergens had 90% amino acid sequence identity, IgE binding in sera from a small panel of subjects only had IgE binding to Der p 4. Given that the anti-amylase titres were in fact very high, the most plausible explanation of the titres is sensitivity to the mite allergen itself. The mean antibody titres of 45 ng/mL are not only similar to the titres found to the groups 1 and 2 HDM allergens in other populations but are also higher than those published for other allergens such as pollen, cat and cockroach.

Component-resolved diagnosis has been promoted as a better strategy for examining atopy than immunoabsorbent tests conducted with extracts [30–32]. Arrays of the specific purified allergens from the different sources are tested individually as has been performed here with a panel of nine HDM allergens. The advantage of this has been demonstrated in the European setting where cross-reactivities among pollen and food allergens, such as apple and birch pollen, can be identified and the allergies correctly attributed. With the advent of microarray technologies, component-resolved diagnosis is imminently feasible on a custom or proprietary basis. A universal chip with panels of all the common allergens is not only

practical but could have an economic advantage both with respect to manufacture and for diagnosis because only one test would be required. There are logistical hurdles that need to be overcome to change the routine use of extracts in the diagnostic arena but from a technical perspective, the performance of chips has been reported to be similar to the current immunoabsorbent assays [30, 32]. Comparison between *in vivo* tests and the *in vitro* IgE binding to specific purified proteins would be of interest, especially with respect to the activity of the amylase and any possible activity to the groups 1 and 2 allergens. It could also be of interest in other populations. The studies of Trombone et al. [11] found that about 20% of people with allergic symptoms to mites had undetectable titres of antibody to the groups 1 and 2 allergens. It has also been shown that skin test reactivity can be found in the absence of measurable IgE antibody as for example shown in some subjects for shrimp and HDM cross-reactivities [14].

The spread of the increase of asthma prevalence in developing societies has led to a number of studies comparing the prevalence of atopy and asthma in previously unstudied locations. There are numerous reports of an increase in atopy in rural communities without a corresponding increase in asthma, and these have not been explained by confounders such as parasitism. These include studies of rural populations from New Guinea [33], Ethiopia [34], South Africa [35], Kenya [36], Gambia [37] and Ecuador [38]. None have examined the specificity of the sensitization and only cursory quantitative information has been obtained. It is predicted that much of the IgE binding or skin test responses reported will not be directed to the major allergens.

The interaction between parasitic infections and allergic disease is an unresolved and multifaceted issue. There are examples where parasitism has been proposed to explain decreased atopy or decreased disease in atopic people [39] but the finding is not universal [40]. The study group here had previously been exposed to parasites and had high IgE levels across the whole population [7]. The atopic subjects had significantly higher total IgE titres ($P < 0.0001$) that may just result from the added allergy or some unknown relationship, for example with infection. Detailed investigations have now shown that high levels of IgE antibody do not block IgE antibody function on mast cells and therefore have little effects on allergic responses [41]. The community here had a prevalence of asthma similar to the Australian population in general, thus showing no evidence of protection [7].

In conclusion, atopy is a major risk factor for asthma and so the ability to monitor changes in the pattern of allergic sensitization could be used to plan health resources. Early medical intervention in the treatment of asthma can greatly decrease hospital admissions and the cost of the health systems [42–44]. There is, however, a need to investigate the specificity of the responses in new

populations and how this relates to disease and diagnosis. The use of defined and the most relevant specificities will be important for developing better prognostic tests that can be used not only for IgE binding but also for *in vivo* challenge tests and cell-based assays.

Acknowledgements

This work was supported by the National Health and Medical Research Council of Australia. Ingrid A. Laing was supported by the Australian Respiratory Council Ann Woolcock Research Fellowship. We thank the people of the Aboriginal community for their generous participation and help in the study. We also thank the members of the Perth Respiratory Epidemiology Team (PREG) who were involved with recruiting and collecting the samples.

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