Individuals using assistive technology may not be able to fully access the information contained in this file. For assistance, please call 800-835-4709 or 240-402-8010, extension 1. CBER Consumer Affairs Branch or send an e-mail to: ocod@fda.hhs.gov and include 508 Accommodation and the title of the document in the subject line of your e-mail.

Approaches to Allergen Standardization Related to Dust Mites

I. House dust extract prior to 1985

II. Cultured dust mites; Voorhoorst ,Amsterdam ; Bencard, UK ; Hollister. Spokane,

a. Isolated mite bodies
b. Whole culture
c. Mite Feces

III. To provide a method suitable for comparing batches of product to an established standard: FDA, EMA, or In House Reference Preparations (IHRP)

a. Skin testing: by end point titration
b. In vitro methods of measuring potency of an extract.
c. Immunoassays for: Der p 1, Der f 1 (group 1)*, Der p 2, Der f 2 (group 2)*

IV. Several forms of Mass Spectroscopy.
Cross Radio Immunoelectrophoresis (CRIE).
Proteomics.

SOURCE MATERIALS FOR DIAGNOSIS OR TREATMENT OF PATIENTS WHO ARE ALLERGIC TO DUST MITES.

House dust was in use in the USA up to 1985 and it was estimated that it was being used as part of SCIT for several million patients.

House dust was abandoned once the major elements of the dust were available as extracts and there were assays to measure some of the relevant allergens in house dust

The first mite allergens were purified in 1980 (Der p 1) and 1989 (Der p 2). Der p 1 assays lead to the measurement of this mite allergen in bodies, eggs, and fecal pellets (Tovey et al, 1981 and 1982).

Mite feces can be “purified” by sieving while mite bodies were/are enriched by using a light gradient or by sieving.

Chapman and Platts-Mills, J Immunol 1980
Heyman et al, JACI 1989

Mite faeces are a major source of house dust allergens

E. R. Tovey, M. D. Chapman & T. A. E. Platts-Mills*

Division of Immunology, Clinical Research Centre, Watford Road, Harrow, Middlesex HA1 3UJ, UK

The association between house dust allergy and asthma has long been recognized, and it has been demonstrated that a major allergen in house dust is related to the presence of mites of the genus *Dermatophagoides*[1]. Using extracts of mite culture for skin testing, as many as 10% of the population and up to 90% of allergic asthmatics give positive immediate reactions[2]. Although mites may occasionally become airborne during bed-making[3], it

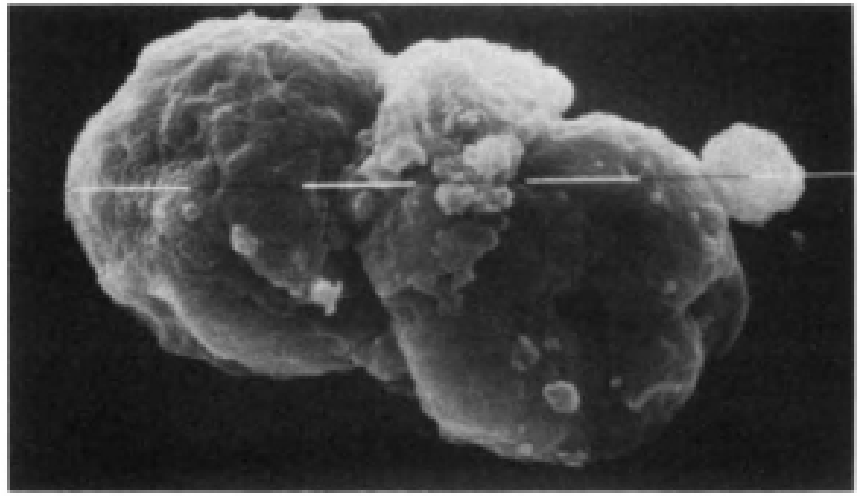


Fig. 1 Electron scanning micrograph of two mite faecal balls. Mite faeces range from 10 to 40 μm in diameter with a mean of

Table 1 Quantity of antigen P_1 in mite culture components

	Culture components	ng antigen P_1 per 100 components	% Of total antigen P_1
Mites	Female	185	0.6
	Male	105	
	Immature	75	
Cuticles		29	0.4
Eggs		0.4	–
Faeces	Mixed size (22 ± 6 μm)	12	99
	Large (31 ± 8 μm)	17	
	Small (17 ± 4 μm)	3	

Antigen P_1 content of separated components of mite culture. Live

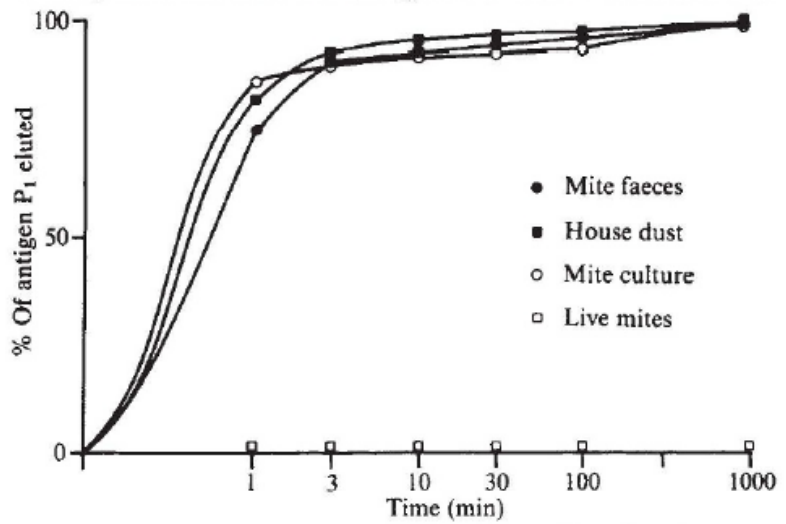


Fig. 2 The elution of antigen P_1 from faeces, live mites, house dust and culture material. Mite faeces or live mites mounted on Cellotape (1 × 5 mm) were contained in a section of capillary tubing and eluted with saline using a peristaltic pump (25 μl min^{-1}). At

The Distribution of Dust Mite Allergen in the Houses of Patients with Asthma

Euan R. Tovey , Martin D. Chapman , Clive W. Wells , and Thomas A. E. Platts-Mills

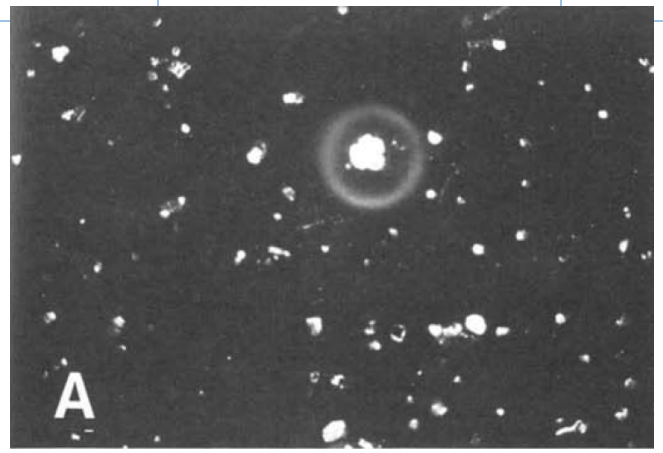
Am Reviews of Resp Disease Vol 124 ,, 1981

AIRBORNE ANTIGEN P_1 COLLECTED WITH A CASCADE IMPACTOR*						
Impactor Stages	**Approximate Diameter of Particles Collected †**					
		Bedrooms Studied During Domestic Activity‡				
	Microns (μ)	5	6	14	4, 12, 13	Mean %
1	>20-6	28	8.1	17.2	<0.3	88.8
2	15-2	5.1	<0.3	1.84	<0.3	6.7
3	5-1	1.48	<0.3	1.12	<0.3	1.8
4	2.5-0.3	0.78	<0.3	1.04	<0.3	1.4
Final	<0.5	<0.3	<0.3	1.48	<0.3	1.3

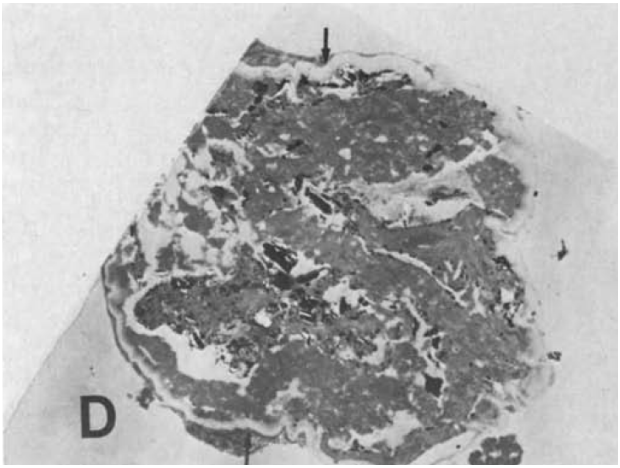
*Nanograms (ng) of P_1 (Der p 1) collected on stages or final filter

‡ Mean of values from 8 households collected over 40 mins

† In 3 houses no allergens were detected on the Impactor or impact stages



A. Particles detected on stage one of the impactor in agar
B. plus rabbit ab to mite allergen



B. EM of Fecal Particle

Fractionation of Source Materials Leads to a High Reproducibility of the SQ House Dust Mite SLIT-Tablets

Helene Henmar[a] Sofie Marie Toft Frisenette[a] Karin Grosch[a] Kim Nielsen[a]
Gary Smith[a, b] Susanne Sønderkær[a] Jørgen Nedergaard Larsen[a]

[a]ALK A/S Research and Development, Hørsholm, Denmark; [b]ALK A/S Research and Development, Post Falls, Idaho, USA

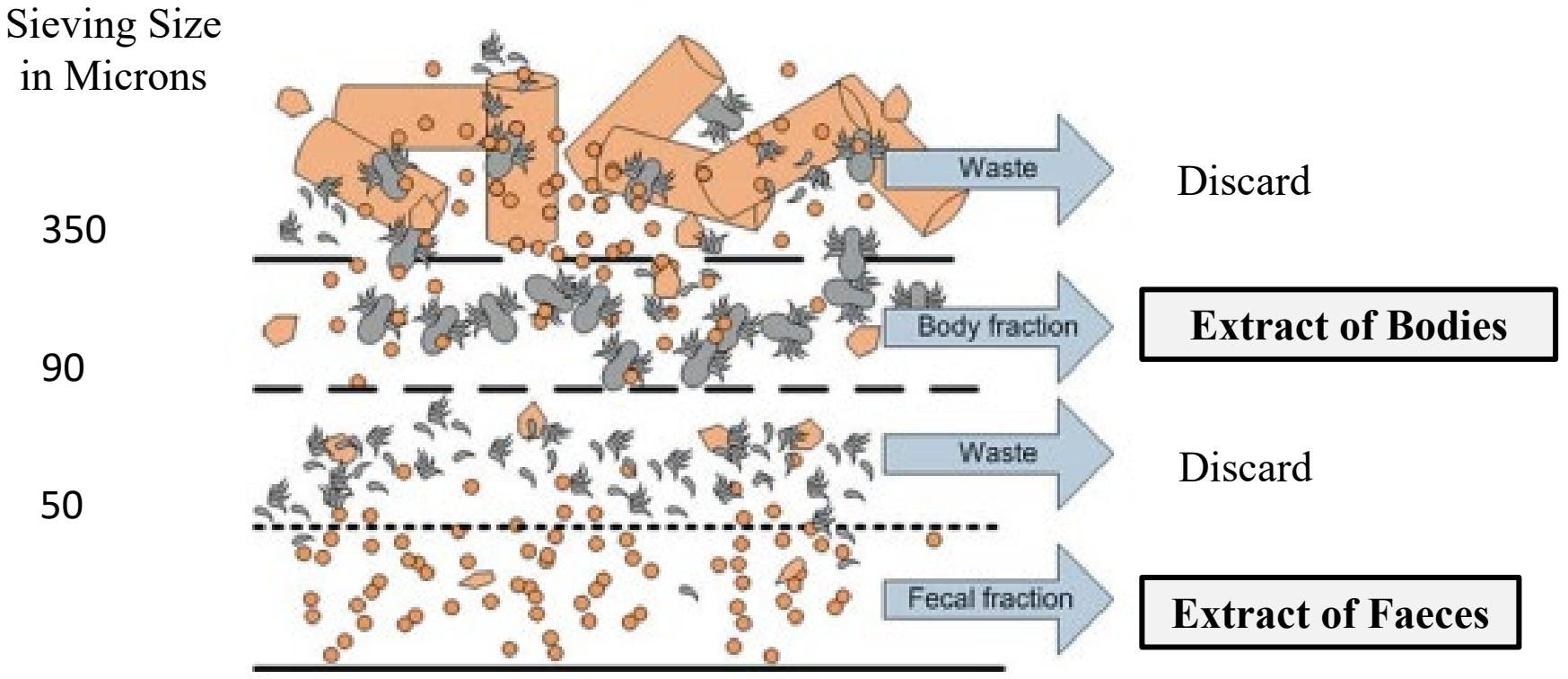
Primary cultures of *D. pteronyssinus* and *D. farinae* were dried and sieved, creating group1 rich, fecal pellet extracts and group 2 rich, whole body extracts, which are mixed to create a I:I:I:I, Der p 1; Der f 1; Der p 2; & Der f 2. This provides ~15 µg group I and ~15 µg group 2 allergens per tablet.

In house assays for Der p 1, Der f 1, and also Der p 2 and Der f 2 in the sieved fractions from each species. Followed by CIE & CRIE which are used to identify a wide range of allergens in the extracts to compare batch samples to an in house reference preparation (IHRP).

Int Arch Allergy Immunol 2016;169:23–32

C.I.E Crossed Immuno Electrophoresis
C.R.I.E. Crossed Radioimmunoelectrophoresis

SIEVING PROCESS TO OBTAIN TWO FRACTIONS FROM *D. PTERONYSSIUS* AND *D. FARINAE* CULTURES



*Cultures are killed by freezing at -20°C and then dried to below 15% moisture content

Henmar H, Frisenette SM, Grosch K, Nielsen K, Smith G, Sønderkaer S, Larsen JN. Fractionation of source materials leads to a high reproducibility of the SQ house dust mite SLIT-tablets. Int Arch Allergy Immunol 2016; 169: 23-32.

Accepted: 25 June 2017
DOI: 10.1111/pai.12747
Pediatric Allergy Immunol. 2017;28:661–667
WILEY
ORIGINAL ARTICLE
Upper Airways

Pooled efficacy and safety data for house dust mite sublingual immunotherapy tablets in adolescents

Tomokazu Matsuoka[1] | David I. Bernstein[2] | Keisuke Masuyama[1] | Hendrik Nolte[3] | Kazuhiro Okamiya[4] | Dorthe Seitzberg[3] | Harold S. Nelson[5]

Two DBPC studies on adolescents over age 12 years using sublingual tablets were carried out in North America and Japan & concluded that the tablets labelled 12 SQ were safe and effective.

In the published report of the trials, the allergen content of the tablets is described in detail. Source Materials: Mixture of mite bodies and mite feces from both D.pteronyssinus and D. farinae designed to produce tablets with 15 µg of group I allergens (Der p 1 & Der f 1 combined) as well as 15 µg of group 2 allergens (Der p 2 & Der f 2 combined). In addition, they state the tablets contained "the broadest possible spectrum of major and minor allergens from these mite species."

This description implies that ALK is using ELISA assays to measure Group I and Group 2 allergens, and also mass spectroscopy or CRIE to identify the presence of other allergens.

Allergy EUROPEAN JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY 2016; 71: 967-976

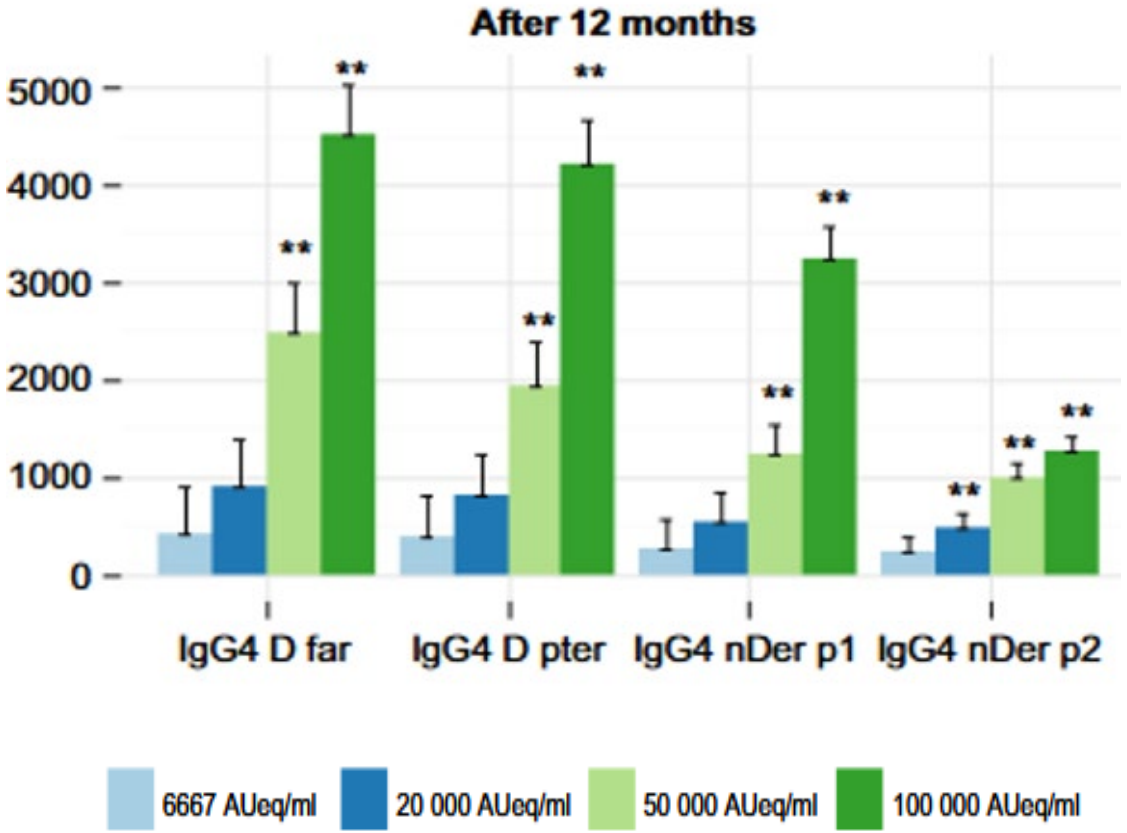
Allergy

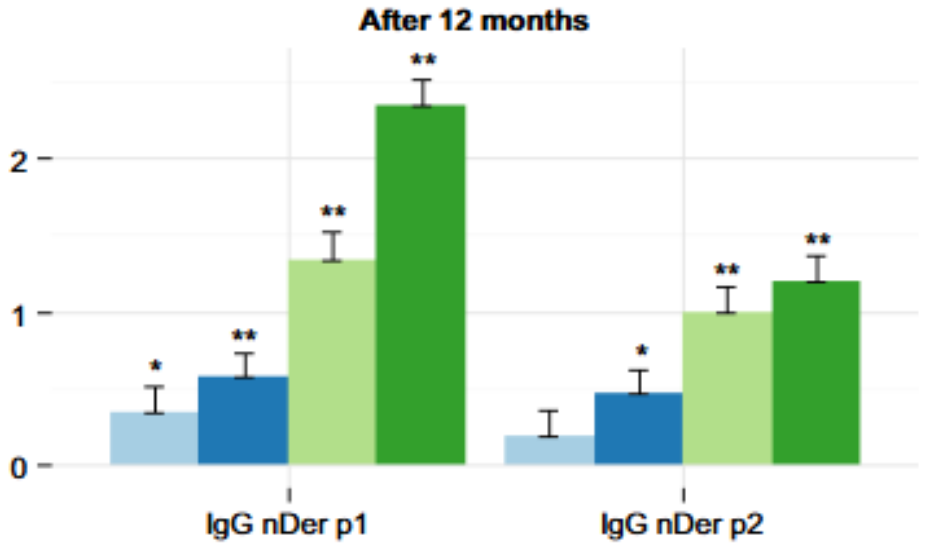
ORIGINAL ARTICLE EXPERIMENTAL ALLERGY AND IMMUNOLOGY

A randomized, 5-arm dose finding study with a mite allergoid SCIT in allergic rhinoconjunctivitis patients

O. Pfaar[1,2], M. J. Nell[3], J. D. Boot[3], S. A. Versteeg[4], R. van Ree[4,5], A. Roger[6], H. Riechelmann[7], A. Sperl[1], J. N. G. Oude Elberink[8], Z. Diamant[9,10] & C. Bachert[11]

- Dosage of allergoid ranged from 6,660, AU eq/mL to 100,000 AU eq/mL in 4 doses. Evaluated at 6 months and 12 months: Dose range favored 20,000 or 50,000 for clinical response.
- Immune response measured as IgG and IgG4 specific for Der p 1, Der p 2 in cohorts treated with 6,000, 20,000, 50,000, or 100,000 doses. The specific IgG4 response to Der p 1 increased dramatically compared to minimal effect on sIgG4 to Der p 2.
- Previous studies suggested a dose of Der p 1 and Der p 2 of 15 µg and 13 µg.
- Funded by HAL, Allergy BV; Leiden





Allergy
EUROPEAN JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY
EAACI
Allergy 2016 71:967-976.
Allergy

ORIGINAL ARTICLE EXPERIMENTAL ALLERGY AND IMMUNOLOGY

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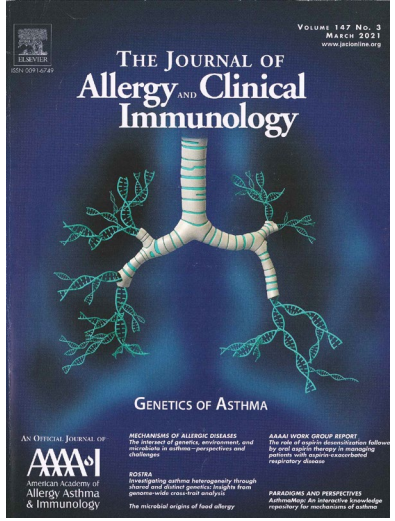
Allergoids for Sub Cutaneous Immunotherapy

Two forms have been developed in the United States

- Glutaraldehyde-modified [David Marsh]
- Polyethylene glycol [Roy Patterson]
  - Alum absorbed
  - Objective, efficacy and safety.
- Major problem that it is difficult, if not impossible, to measure the allergen content of allergoids with immunoassays for allergens.
- Can mass spec measure allergens in allergoids? In all probability, yes.

Reference: Spiric J., Schulenborg T,…Vieths S., Mahler V., and Reuter A. Quality control of allergen products with mass spectrometry part I: Positioning within the EU regulatory framework. Allergy 2024;00:1-9



J ALLERGY CLIN IMMUNOL 2021; 147:1020-1030

A 300 IR sublingual tablet is an effective, safe treatment for house dust mite–induced allergic rhinitis: An international, double-blind, placebo-controlled, randomized phase III clinical trial

Pascal Demoly, MD, PhD,[a,b] Jonathan Corren, MD,[c] Peter Creticos, MD,[d,e] Frédéric De Blay, MD, PhD,[f] Philippe Gevaert, MD, PhD,[g] Peter Hellings, MD, PhD,[h] Krzysztof Kowal, MD, PhD,[i] Martine Le Gall, MD,[j] Natalia Nenasheva, MD, PhD,[k] Giovanni Passalacqua, MD,[l] Oliver Pfaar, MD,[m] Miguel Tortajada-Girbés, MD, PhD,[n,o,p] Carmen Vidal, MD, PhD,[q] Margitta Worm, MD,[r] and Thomas B. Casale, MD[s]

Montpellier, Paris, Strasbourg, and Antony, France; Los Angeles, Calif; Baltimore, Md; Charleston, SC; Ghent and Leuven, Belgium; Bialystok, Poland; Moscow, Russia; Genoa, Italy; Marburg and Berlin, Germany; Valencia and Santiago de Compostela, Spain; and Tampa, Fla

Units given as IR, meaning index of reactivity, but no clear description of method.

Allergen content 14- 17 µg Der p 1 and 53-68 µg Der p 2.

(Methods) Extract includes Der p 3-11, 14, 15, 18, 20, 21, 23, 36.
Presumably identified by MS

Study of 1,476 subjects with allergic rhinitis of whom 555 had concomitant asthma.

Response to treatment with allergen tablet or placebo, analyzed over 12 months.

IR= Index of Reactivity

Funded by Stallergens Greer France

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 NOVEMBER 22, 2018 VOL. 379 NO. 21

AR101 Oral Immunotherapy for Peanut Allergy

The members of the writing committee (Brian P. Vickery, M.D., Andrea Vereda, M.D., Ph.D., Thomas B. Casale, M.D., Kirsten Beyer, M.D., George Du Toit, M.B., B.Ch., Jonathan O. Hourihane, M.D., Stacie M. Jones, M.D., Wayne G. Shreffler, M.D., Daniel C. Adelman, M.D., et al

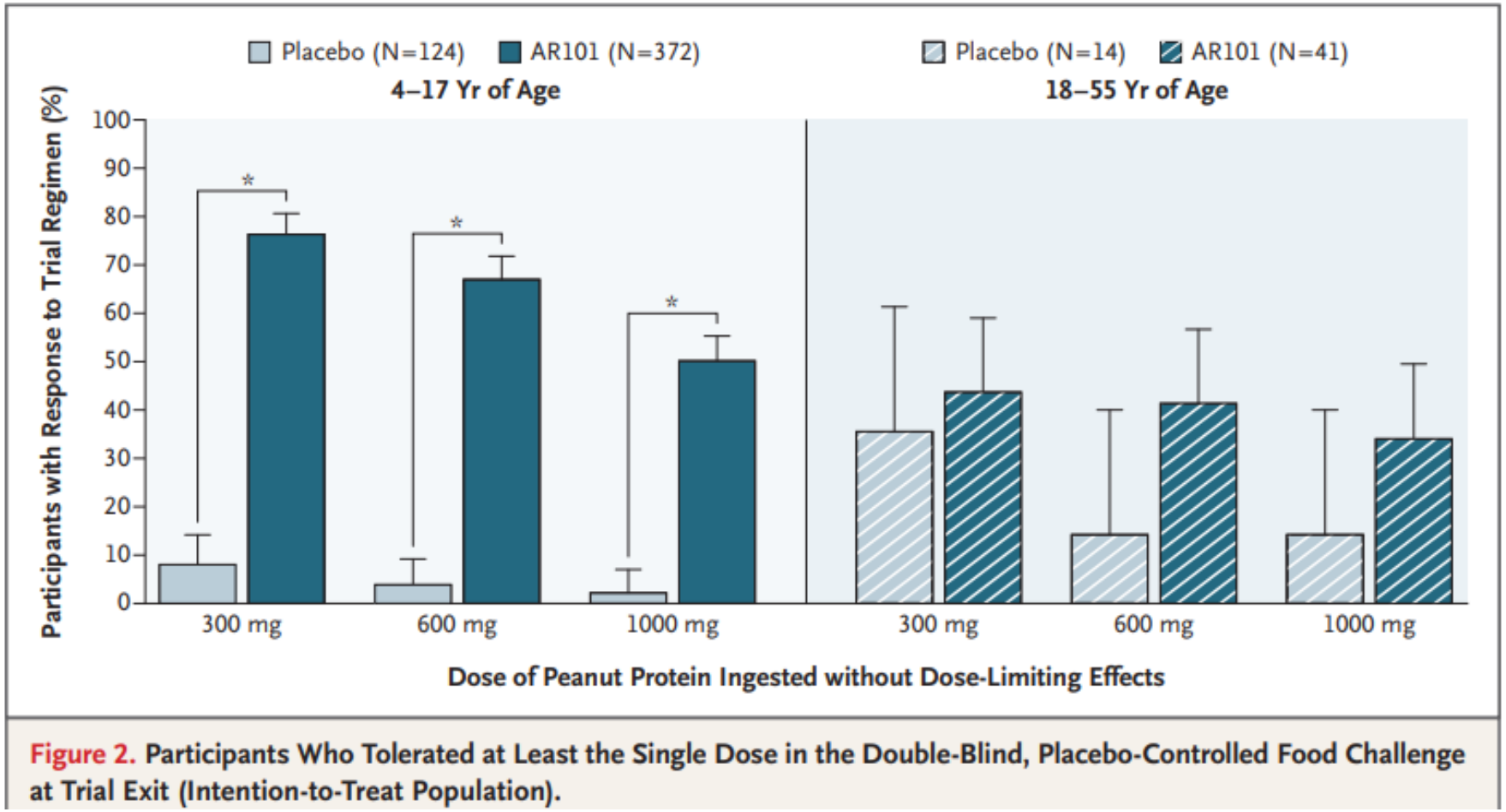
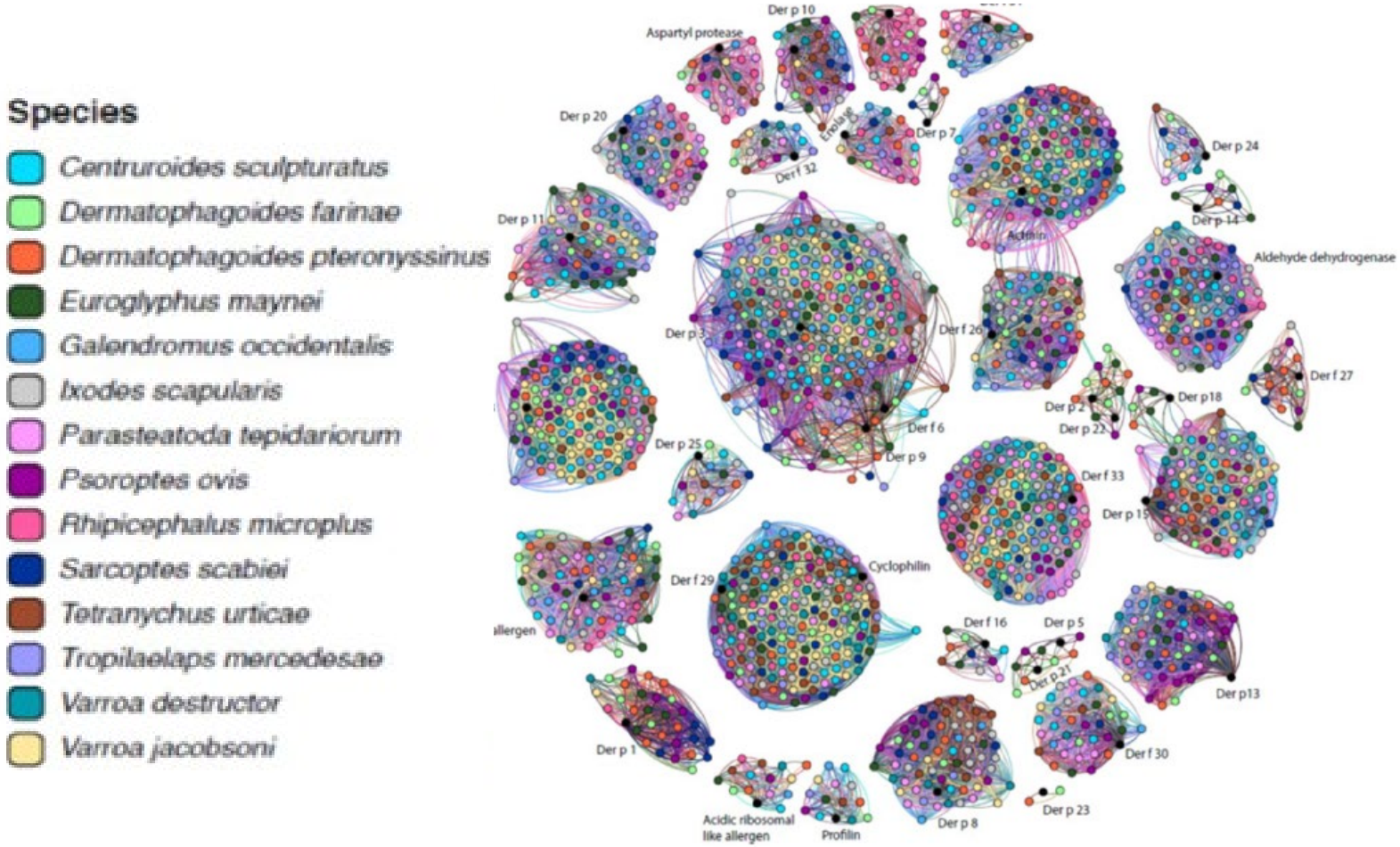


Figure 2. Participants Who Tolerated at Least the Single Dose in the Double-Blind, Placebo-Controlled Food Challenge at Trial Exit (Intention-to-Treat Population).

Proteome and allergenome of the European house dust mite *Dermatophagoides pteronyssinus*

Rose Waldron[1,2], Jamie McGowan[1,3], Natasha Gordon[2], Charley McCarthy[1,3], E. Bruce Mitchell[2], David A. Fitzpatrick[1,3]*

1 Department of Biology, National University of Ireland Maynooth, Co. Kildare, Ireland, **2** Airmid Healthgroup Ltd., Trinity Enterprise Campus, Dublin, Ireland, **3** Human Health Research Institute, Maynooth University, Maynooth, Co. Kildare, Ireland



TOLERANCE TO CAT AND OTHER INHALANT ALLERGENS INDUCED BY HIGH EXPOSURE

There is abundant evidence that children who live in a house with a cat can become clinically and immunologically tolerant to cat allergens.

In most studies on population-based cohorts the subjects with the highest exposure to mite allergens have the highest prevalence of sensitization and asthma.

However, occasional reports from areas with high exposure to mite allergens have found lower levels of sensitization among children with the higher levels of exposure. The best example of this comes from Sydney Australia.[1] The most likely explanation of the effect is the production of high quantities of sIgG4 to Der p 1. [2]

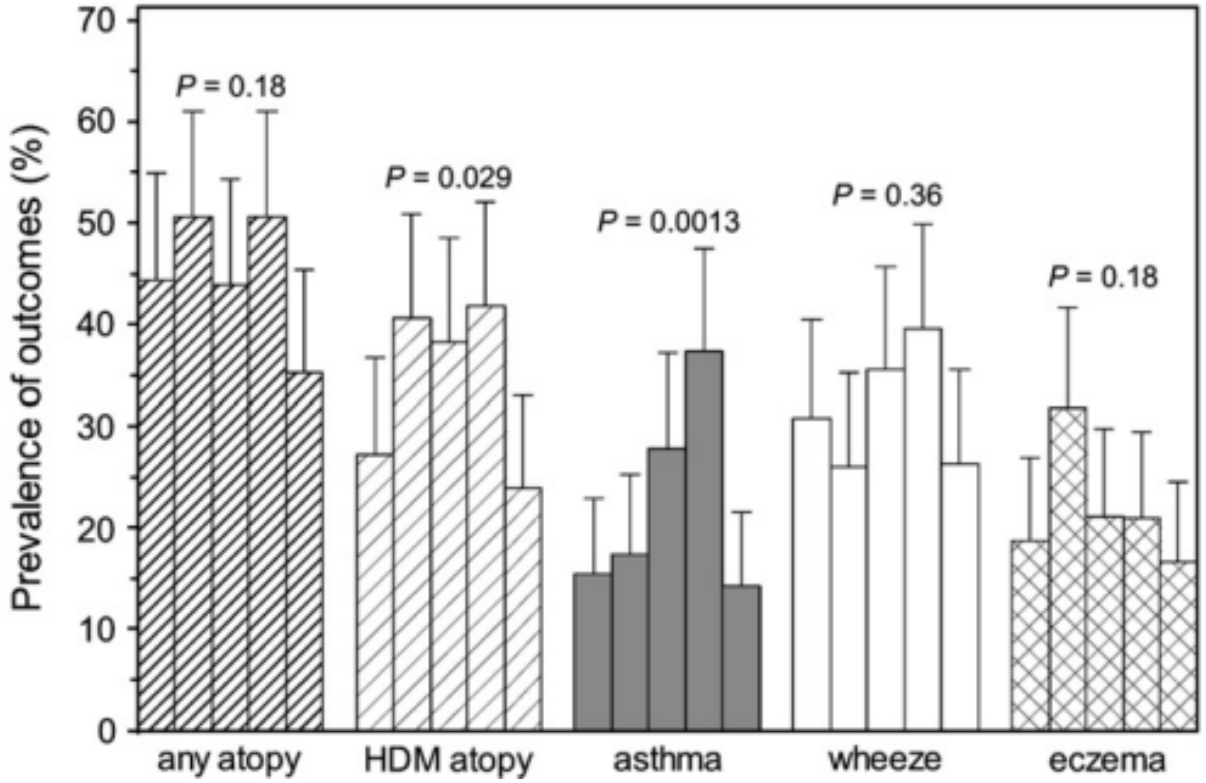
[1] *Nonlinear relationship of mite allergen exposure to mite sensitization and asthma in a birth cohort.Tovey et al. J Allergy Clin Immunol 2008; 122:114-8*

[2] *High risk of asthma among early teens is associated with quantitative differences in mite and cat allergen specific IgE and IgG4. Platts-Mills, Keshavarz B, Wilson, et al. eBiomedicine Lancet Science 2025; 112; 1-15*

Nonlinear relationship of mite allergen exposure to mite sensitization and asthma in a birth cohort [Sidney, Australia]

Euan R. Tovey, PhD,[a,b] Catarina Almqvist, PhD,[a,b,c,d] Qiang Li, BSc,[a,e] Daniele Crisafulli, BSc,[a,b,c] and Guy B. Marks, PhD[a,b,c] *Sydney, Australia, and Stockholm, Sweden*

J Allergy Clin Immunol 2008;122:114-8



The prevalence of the 5 clinical outcomes at age 5 years of age is shown for each quintile of time-weighted average mite allergen concentration over the period of birth to 5 years. The quintiles are: (i) 0 to 3.48, (ii) 3.48 to 7.78, (iii) 7.78 to 13.51, (iv) 13.51 to 23.40, & > 23.40 µg of Der p 1/g of dust. The bars show the 95% CIs.

Available values for exposure to Cat and mite allergens.
Fel d 4 and Der p 23 results from Indoor Biotechnologies

Box 1: Estimates of Exposure to Allergens from Cat or Dust Mite: Airborne and Floor Dust			
	Component	**Airborne**	**Floor Dust / gram of dust**
Cat	Fel d 1	2 µg/m^3	Up to 300 µg/g
	Fel d 4	No Measurements	~ 4 µg/g
Mite	Der p 1	≤ 0.068 µg/m^3	0.3 – 30 µg/g
	Der p 2	≤ 0.026 µg/m^3	0.2 – 5 µg/g
	Der p 23	Too Low to Measure	≤ 1 µg/g

Dust Mite and Mite component specific IgE: in 13 year olds from the *VIVA* cohort.
Data from all 84 subjects with asthma and 199 subjects without current asthma.

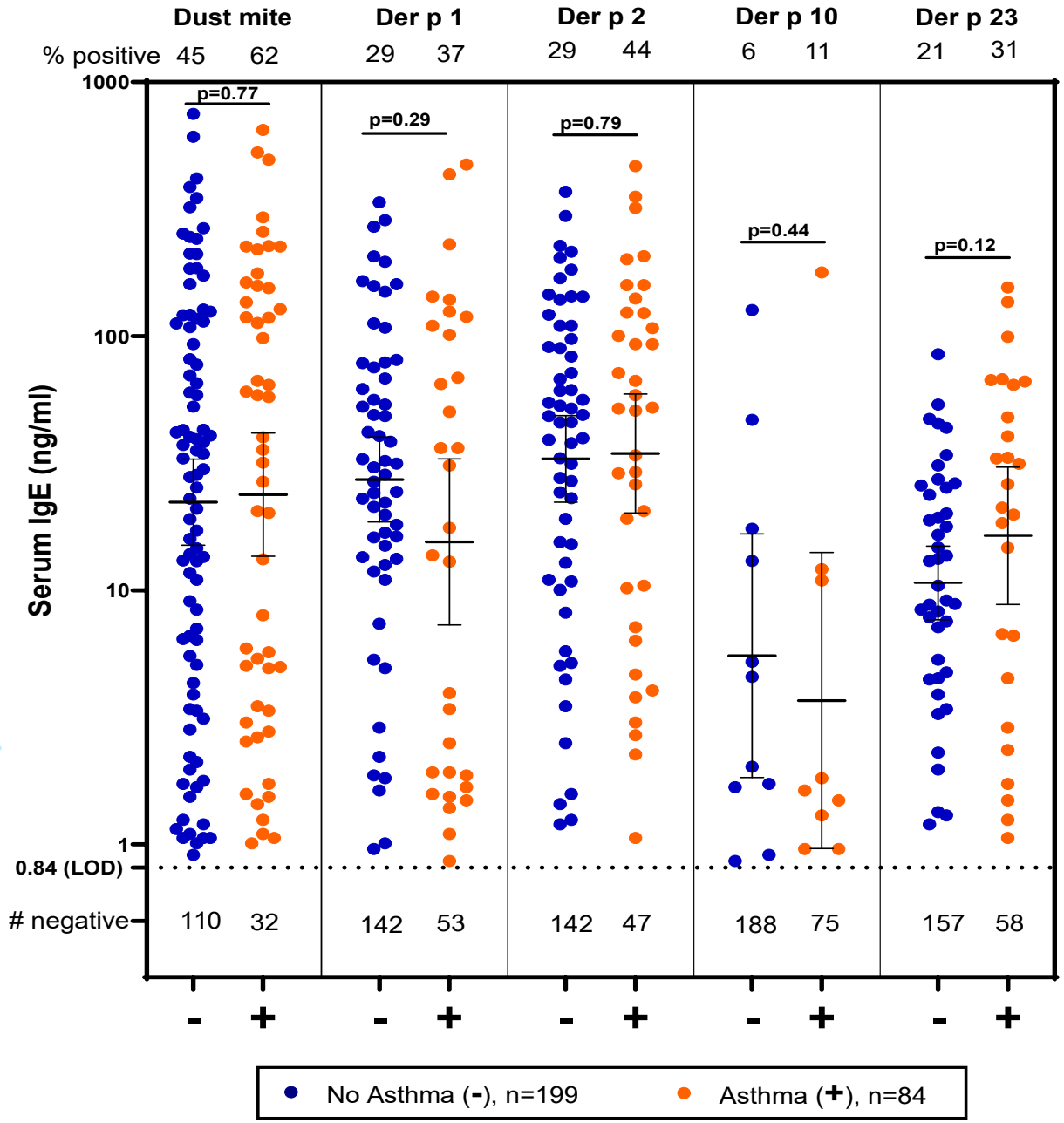
High risk of asthma among early teens is associated with quantitative differences in mite and cat allergen specific IgE and IgG4: a modified Th2 related antibody response revisited

Thomas A. Platts-Mills,[a,*] Behnam Keshavarz,[a] Jeffrey M. Wilson,[a] Sheryl L. Rifas-Shiman,[b] Samuel M. Ailsworth,[a] Joanne E. Sordillo,[b] Lisa Workman,[a] Martin Chapman,[c] Jonas Lidholm,[d] Emily Oken,[b] and Diane R. Gold[e,f]

Platts-Mills, Kesharvarz, Wilson et al.
EbioMedicine, Lancet Science: Feb '25

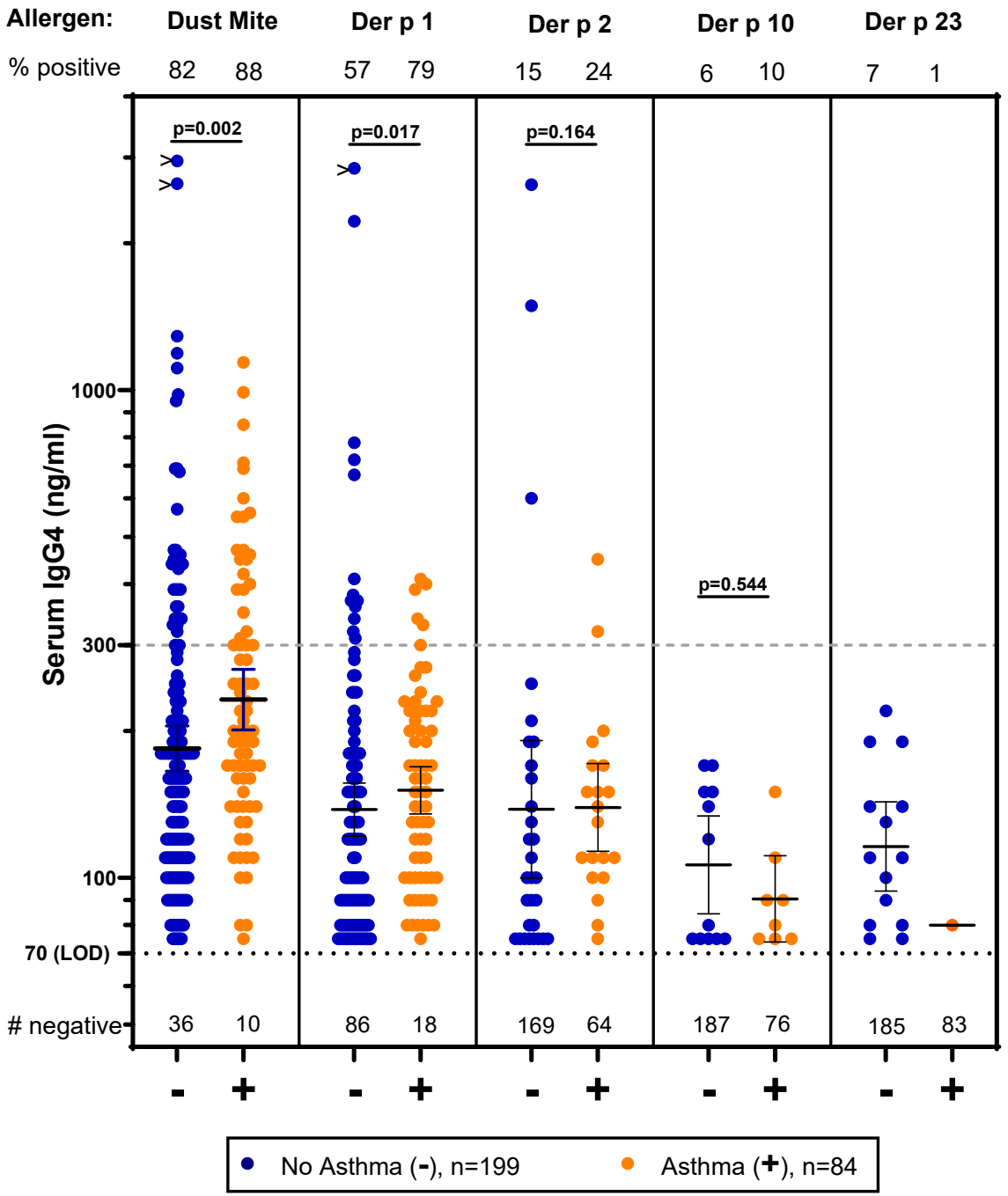
www.thelancet.com Vol 112 February, 2025

Fig 5a

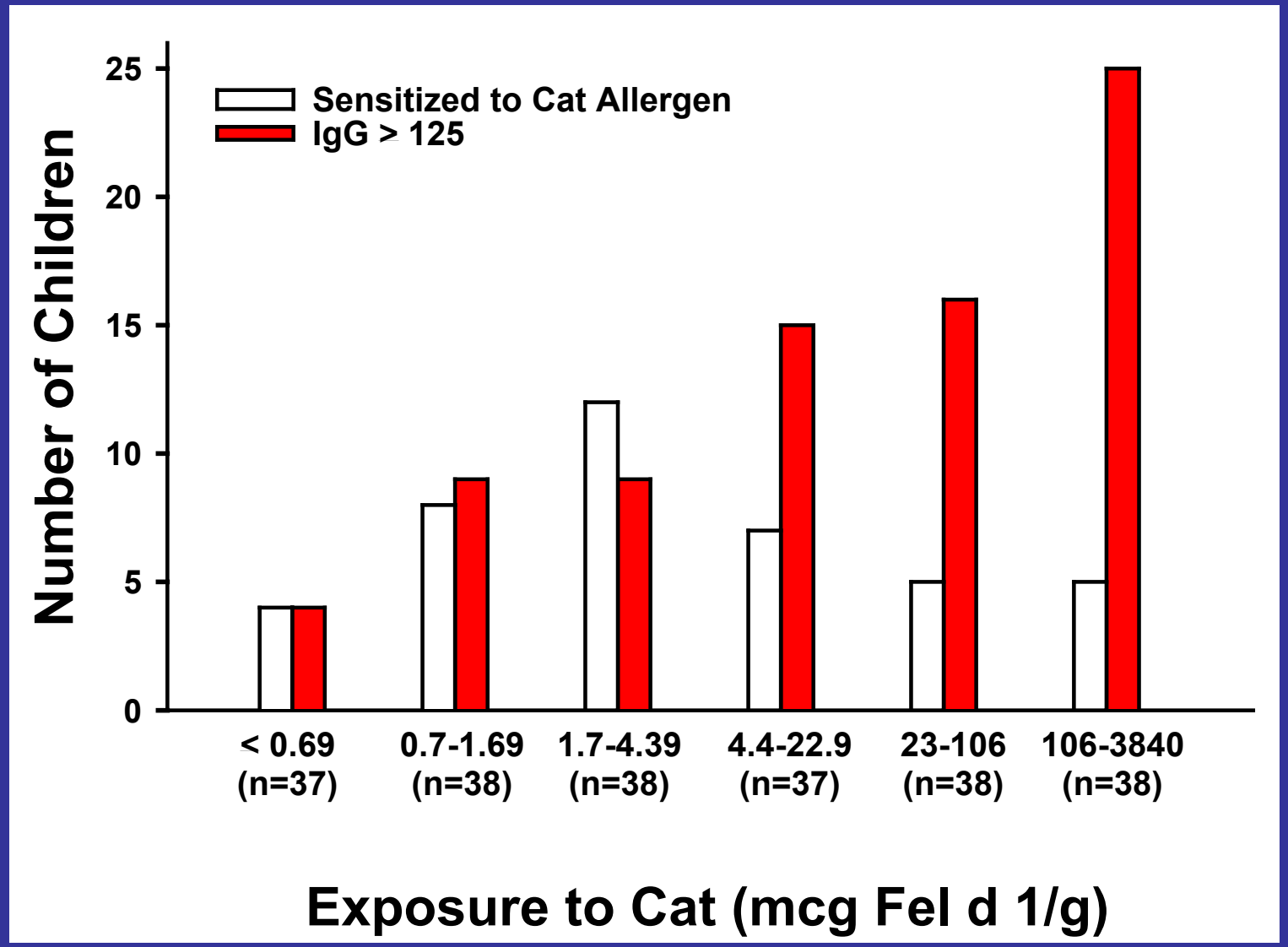


Specific IgG4 to Cat and cat components in sera from 84 subjects with asthma and 199 controls from the Viva Birth cohort at age 13.

Platts-Mills, Kesharvarz, Wilson et al
E'Biomedicine: Lancet Science. Feb '25

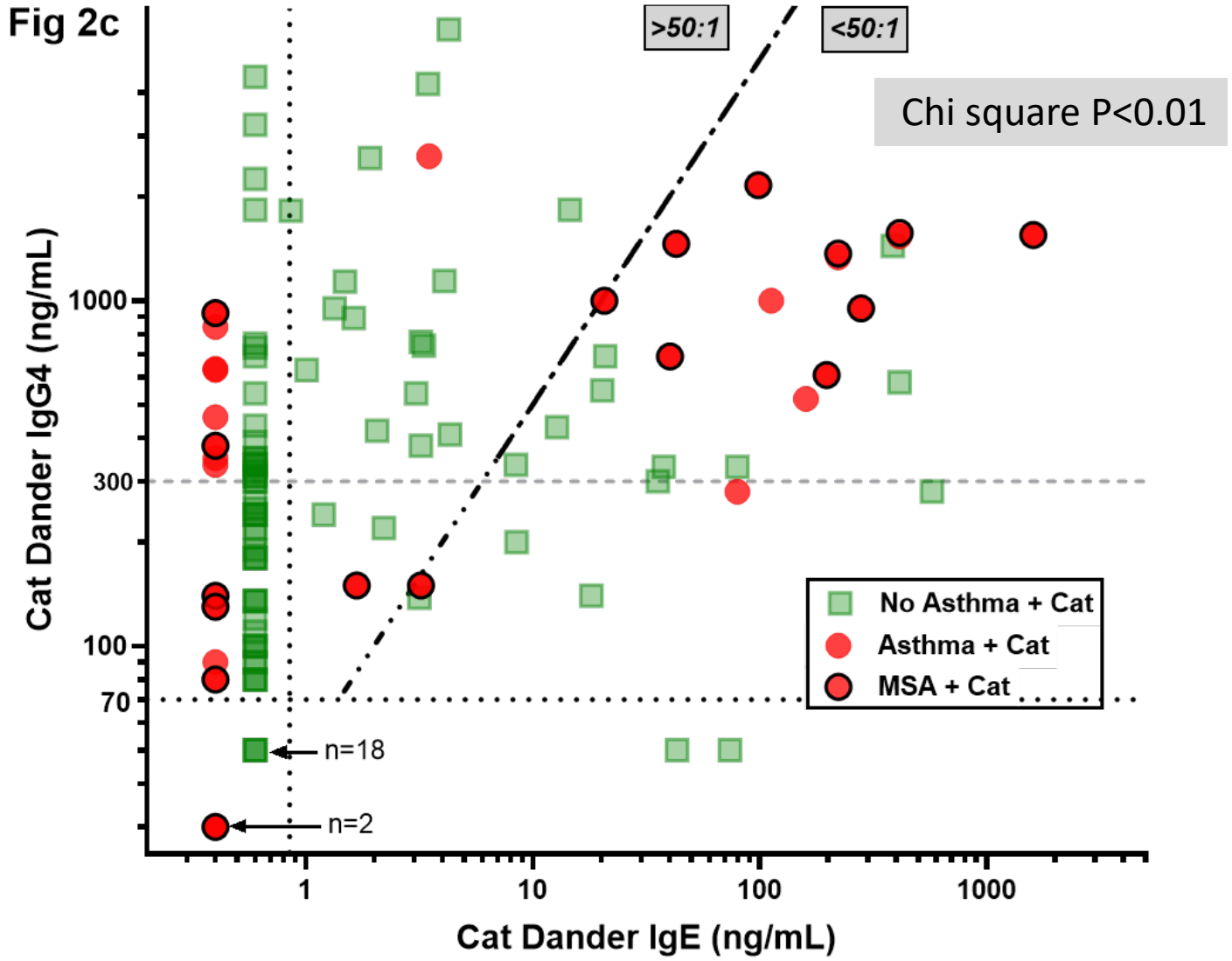


Exposure and Immune Response to Cat Allergen in 226 Middle School Children

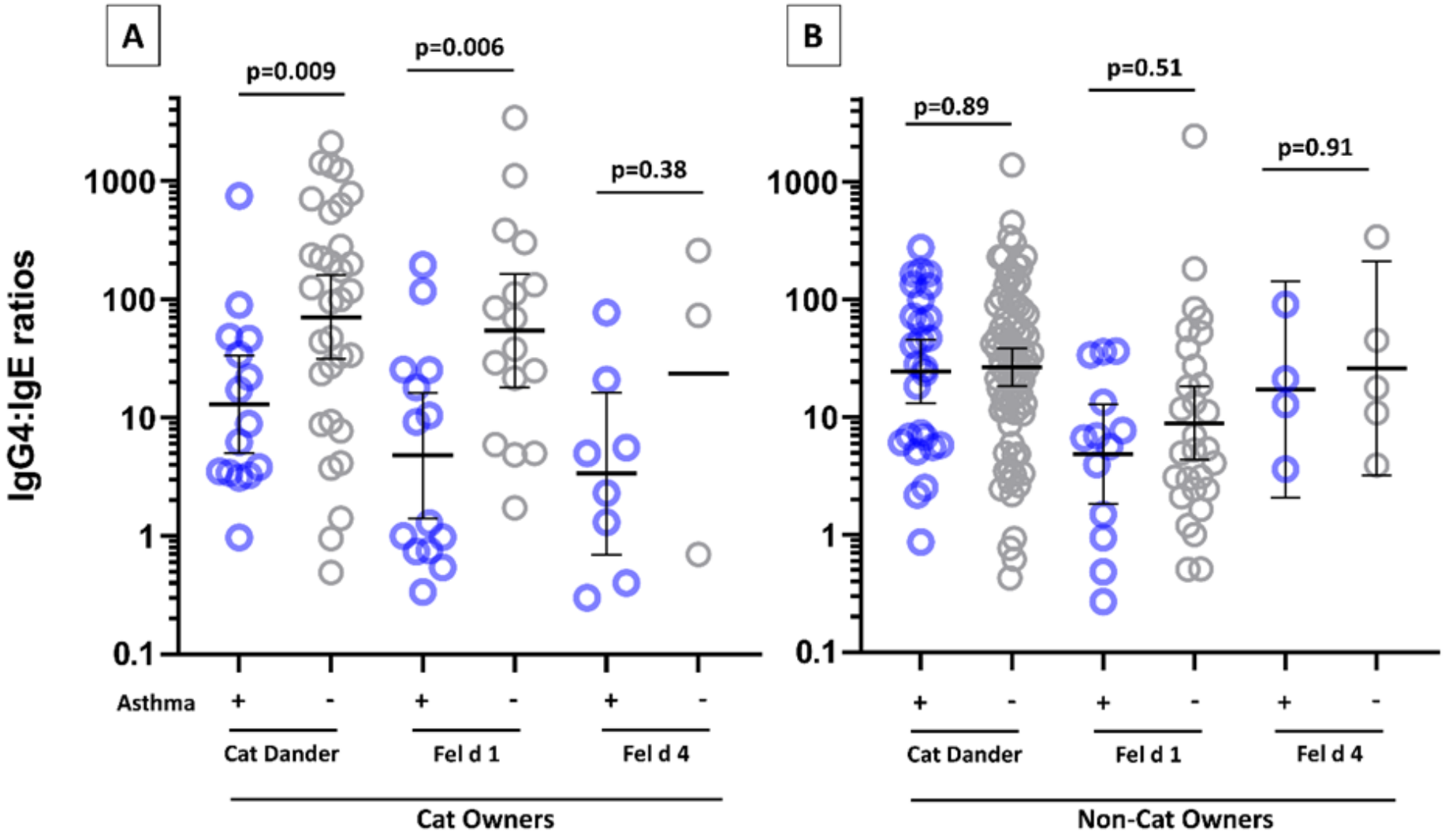


Platts-Mills et al., Lancet 2001;357:752

IgG4 and IgE antibodies to cat extract in sera from 135 subjects in the Viva Cohort age 13 living in a house with a cat & analysed according to current asthma (n=30) and moderate severe asthma (MSA) (n=17).



Ratios of IgG4: IgE antibodies to Cat Dander, Fel d 1 and Fel d 4 in ng/ml among individuals with a cat at home or not; and with or without asthma.



eBioMedicine- LANCET discovery Science. Feb 2025

Intra- and Inter- Molecular Epitope Spreading

There is extensive evidence that clinically relevant IgE responses include IgE to multiple epitopes on “major” allergens: involving **Intra-molecular epitope spreading: certainly true for Fel d 1 and Der p 1.**

Epitope spreading from one protein to others from the same source may be equally important; that is **Inter-molecular epitope spreading**

What we appear to be looking at here is a major difference in **inter-molecular epitope spreading** between IgE and IgG4 antibodies to cat or mite components.

Hypothesis: The low or absent IgG4 response to some protein allergens (eg. Der p 2, Der p 23, and Fel d 4) could be the reason why some of these allergens have a greater role on symptoms including asthma.

SUMMARY

- The ability to separate fractions of dust mite cultures has been available for many years. Over the last 20 years there has been a progressive move to defined extracts, particularly in the production of tablets used for oral or sublingual treatment.

- Assays from Group I and Group II mite allergens have been possible/available for at least 30 years. However, there is no simple basis for defining a ratio apart from 1:1 because the relative importance of these or other mite allergens can change with different levels of exposure.

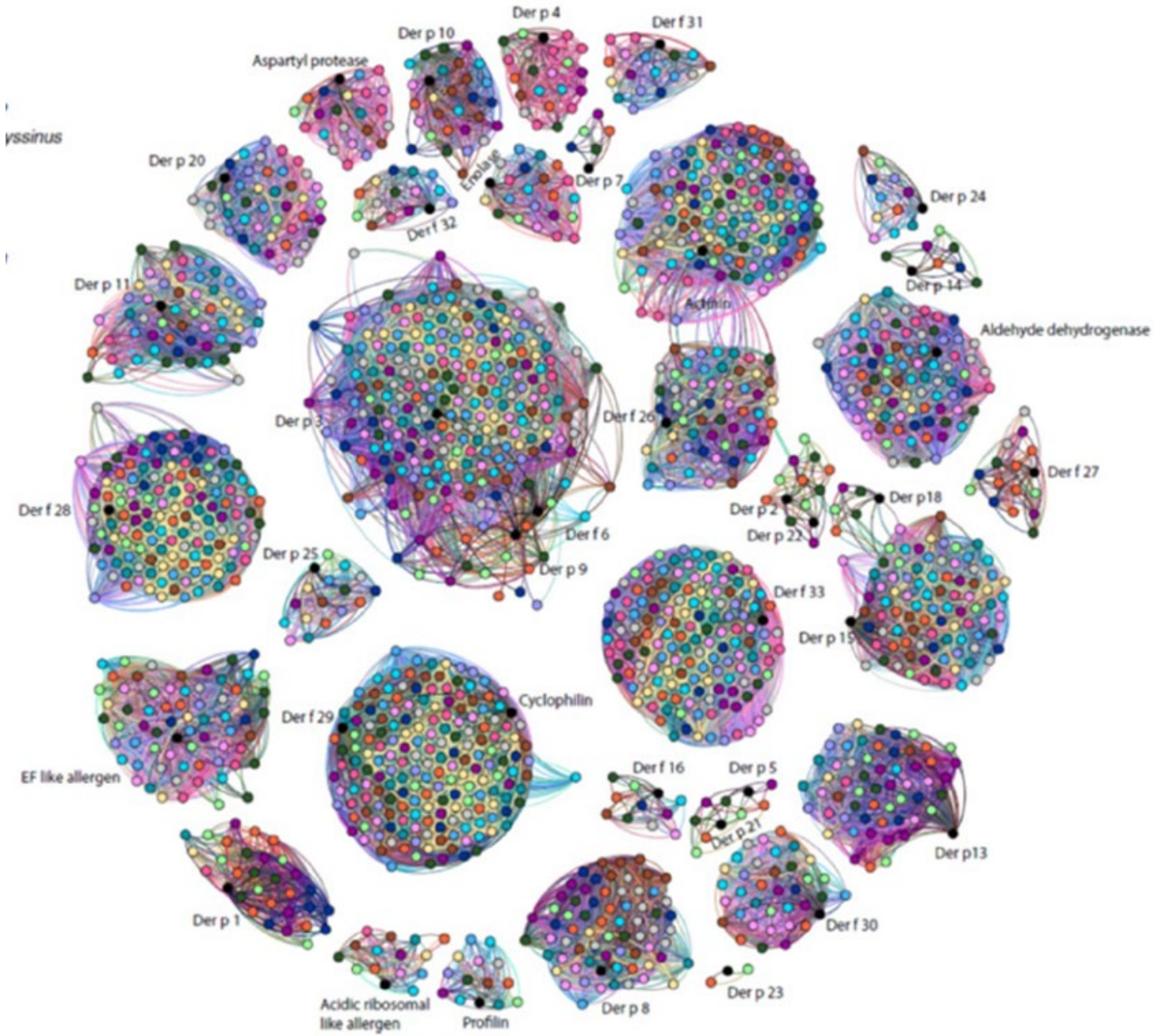
- By selecting or sieving different sources of mites it is possible to enrich whole bodies or feces and to create extracts with defined quantities of Group I and Group II allergens. It is be difficult to extend this to other allergens because their quantities in extracts or the environment are lower.

CONCLUSIONS RE THE PROCESS FOR STANDARDIZING MITE ALLERGEN EXTRACTS

I. Skin tests are essential to be sure that the product is clinically active, but no form of skin testing can define the strength of an extract because it depends on the choice of subjects tested.

II. Assays for Group I and Group II mite allergens to assess the consistency of products should be used to relate batches to local, national, of international standards: Der p 1 and Der p 2, but also Fel d 1, Bla g 2 & Bla g 5.

I. Assessment of the quality of extracts by either CRIE or Mass Spec should be encouraged to evaluate the diversity of allergens: with a focus on standardization of the Mass Spec techniques used.


yssinus
Der p 4
Der f 31
Der p 10
Aspartyl protease
Der p 20
Enolase
Der p 7
Der f 32
Der p 24
Der p 14
Der p 11
Actinin
Aldehyde dehydrogenase
Der p 3
Der f 26
Der f 28
Der f 6
Der f 27
Der p 2
Der p18
Der p 22
Der p 25
Der p 9
Der f 33
Der p 15
Cyclophilin
Der f 29
EF like allergen
Der f 16
Der p 5
Der p 21
Der p13
Der p 1
Der f 30
Der p 23
Der p 8
Acidic ribosomal
like allergen
Profilin