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The molecular allergen sensitization profile of an Algerian cohort



Djidjik Réda, Lamara Mahamed Lydia
Université Alger1, Faculté de Pharmacie
Service d'immunologie médicale, CHU Béni-Messous



INTRODUCTION



- ✓ Allergies are on the rise with significant burden both on affected individuals as well as on the societies: major public health issue.
- ✓ Relatively few data are available regarding the prevalence of allergy in Africa.
- ✓ Algeria: some regional studies have been published:
 - A.Benyounes et al (2017): multicenter, respiratory (HDM, olive pollen).
 - B.Bioud et al (2019): Setif, respiratory (HDM, grass pollen).
 - S.Lahiani et al (2020): Algiers, respiratory (HDM, cypress pollen).
 - S.Bounil et al (2020): Algiers, respiratory (HDM, olive pollen).

Exploration immunologique

Tests multi allergéniques
(uniplex) de screening
(anamnèse? Histoire clinique?)



Tests Unitaires d'identification



Tests moléculaires

Allergologie moléculaire (CRD =Component-resolved diagnostics)

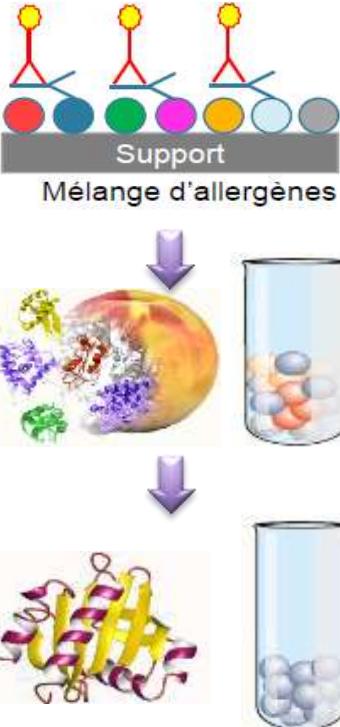
- ❖ Établir le **profil précis de sensibilisation**.
- ❖ Évaluer le **risque clinique** de la réaction (sévérité: famille biochimique).
- ❖ Elle explique les **réactions croisées** entre différentes sources allergéniques.
- ❖ **Adapter les mesures d'évitement et la sélection des patients pour ITA.**

3 niveau de recherche des IgE spécifiques

- La détection des IgE spécifiques contre plusieurs allergènes (**mélange**)
- permet d'évaluer la sensibilisation à un ensemble d'allergène
- Résultat + ou - Sans possibilité d'identifier l'allergène incriminé

- Utilisation des **extraits allergéniques** natifs produits à partir **d'une source allergénique** après extraction et purification (ex: cultures d'acariens , grains de pollens.....) ➔ 1 test =1 seule source
- Identification de source allergénique à laquelle le patient est sensibilisé

- Utilisation d'un allergène moléculaire unique recombiné ou natif
- Identification de l'allergène moléculaire précis de l'allergie
- Diagnostic à l'échelle moléculaire de l'allergie IgE médiée



INTRODUCTION



source allergénique
=système multi allergénique



Source allergénique =Mélange complexe de molécules allergéniques et non allergéniques

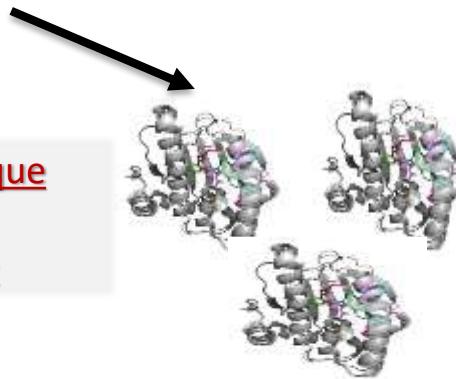
Source allergénique

Allergène = déterminant allergénique

Allergène ≠ source allergénique



Molécules allergisantes



Molécules non allergisantes



Allergène spécifique

Exprimée exclusivement par une source unique



« Pan allergène »

Commun à plusieurs sources allergéniques

Réactions croisées



un allergène moléculaire :

- une molécule individuelle reconnue par des anticorps spécifiques de l'allergène
- Structure moléculaire bien définie , appartenant à une famille biochimique précise
- soit **natif** purifié à partir de sources allergéniques naturelles
- soit « **recombinant** » synthétisé par génie génétique
- A ce jour (14/02/23), **1090 allergènes** (<http://www.allergen.org/treeview.php>) ont été identifiés

Nomenclature officielle des allergènes moléculaires

selon l'IUIS (International Union of Immunological Societies)

(exemple : un allergène de l'arachide: *Arachis hypogaea*)

Mode d'obtention:

- n=natif
- r=recombinant

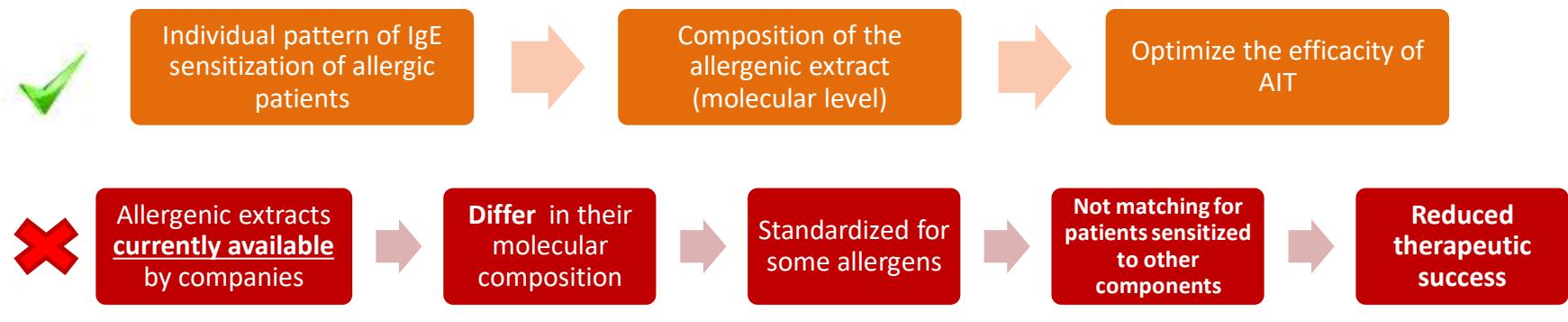


- ordre chronologique de découverte

INTRODUCTION



- ✓ Allergen immunotherapy (AIT): **curative approach** for allergic diseases → **repeat** administration of allergen extracts → **reeducate** the immune system in a **specific** manner → tolerance to sensitizer allergens.



- ✓ Selection of patients suitable for immunotherapy.
- ✓ **Molecular diagnostic algorithms.**

Chen KW, Zieglmayer P, Zieglmayer R, Lemell P, Horak F, Bunu CP, et al. Selection of house dust mite-allergic patients by molecular diagnosis may enhance success of specific immunotherapy. *J Allergy Clin Immunol.* mars 2019;143(3):1248-1252.e12.

Rodríguez-Domínguez A, Berings M, Rohrbach A, Huang HJ, Curin M, Gevaert P, et al. Molecular profiling of allergen-specific antibody responses may enhance success of specific immunotherapy. *J Allergy Clin Immunol.* 1 nov 2020;146(5):1097-108.



Algeria /north African countries

AIT → clinical + SPT and/or sIgE to **the whole extract!**

Allergenic extract (AIT) developed by other countries

- Sensitization de the same major allergens ?
 - IgE sensitization to allergens differ noticeably among distinct geographic areas

→ allergen extract-based testing: Cannot discriminate between genuine sensitization to the given allergen source and between co- and cross-sensitization

→ **This information can only be obtained by molecular testing**

El Fassy Fahy MT, El Gueddari Y, Jniene A, Douagui H, Beji M. Étude de la décision d'immunothérapie spécifique dans 3 pays du Maghreb pour des patients atteints de rhinite et/ou asthme avec sensibilisation allergique. Rev Fr Allergol. 1 déc 2014;54(8):570-4.

Daboussi S, Mhamdi S, Aichaouia C, Moetamri Z, Mejri I, Khadraoui M, et al. Immunothérapie allergénique sublinguale en Tunisie : profil de tolérance et d'efficacité. Rev Fr Allergol. 2018.

Weghofer M, Thomas WR, Kronqvist M, Mari A, Purohit A, Pauli G, et al. Variability of IgE reactivity profiles among European mite allergic patients. Eur J Clin Invest. déc 2008;38(12):959-65.

Muddaluru V, Valenta R, Vrtala S, Schleuderer T, Hindley J, Hickey P, et al. Comparison of house dust mite sensitization profiles in allergic adults from Canada, Europe, South Africa and USA. Allergy. 23 janv 2021.

OBJECTIFS



- ✓ The IgE sensitization profile with micro-arrayed allergen molecules in a cohort of Algerian allergic patients from Algiers.
 - ✓ Understand the local epidemiology
- ✓ Draw practical conclusions for the use of these components in daily diagnosis and for the selection of patients suitable for immunotherapy.
- ✓ Investigate whether the sensitization profile is related to age and clinical phenotype.

PATIENTS

June 2018 and June 2022

Anamnesis and clinical evaluation of patients

Clinical signs suggestive of allergic diseases

Allergy tests (measurement of specific IgE to allergenic extracts by IMMULITE 2000 Xpi)

Respiratory allergy

- Rhinitis
- Conjunctivitis
- Asthma
- Clinical and/or radiological features evocating ABPA

Food allergy

Local or systemic symptoms with precision of the suspected offending food(s)

Hymenoptera venom allergy

Local or systemic symptoms (Muller's classification) after an insect sting

Latex allergy

Clinical symptoms associated with latex gloves wearing, surgical procedures or exams which require latex gloves

HDM, Cat and dog dander, Pollens (olive, cypress, timothy grass, wall pellitory) and *Aspergillus fumigatus* (if suspicion of ABPA)

Suspected offending food (s)

Bee (*Apis mellifera*)

Wasp (*Vespa vulgaris*)

Latex

1038 patients were confirmed to be allergic and sensitized to at least one of the allergenic extracts tested

539 patients included

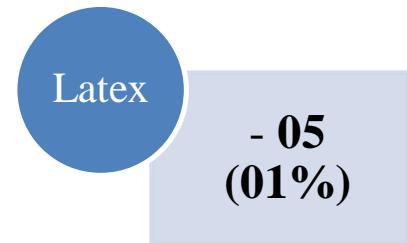
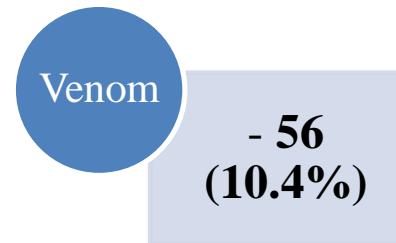
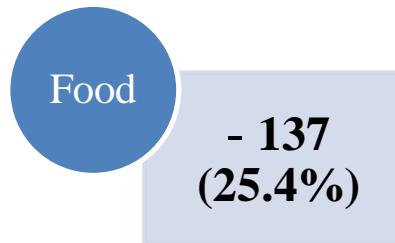
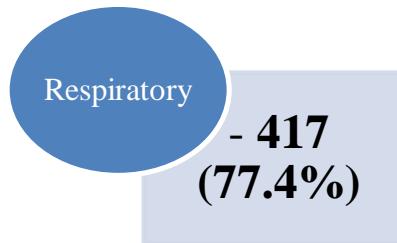
Patients excluded

- incomplete medical information
 - insufficient serum
 - immunotherapy

PATIENTS



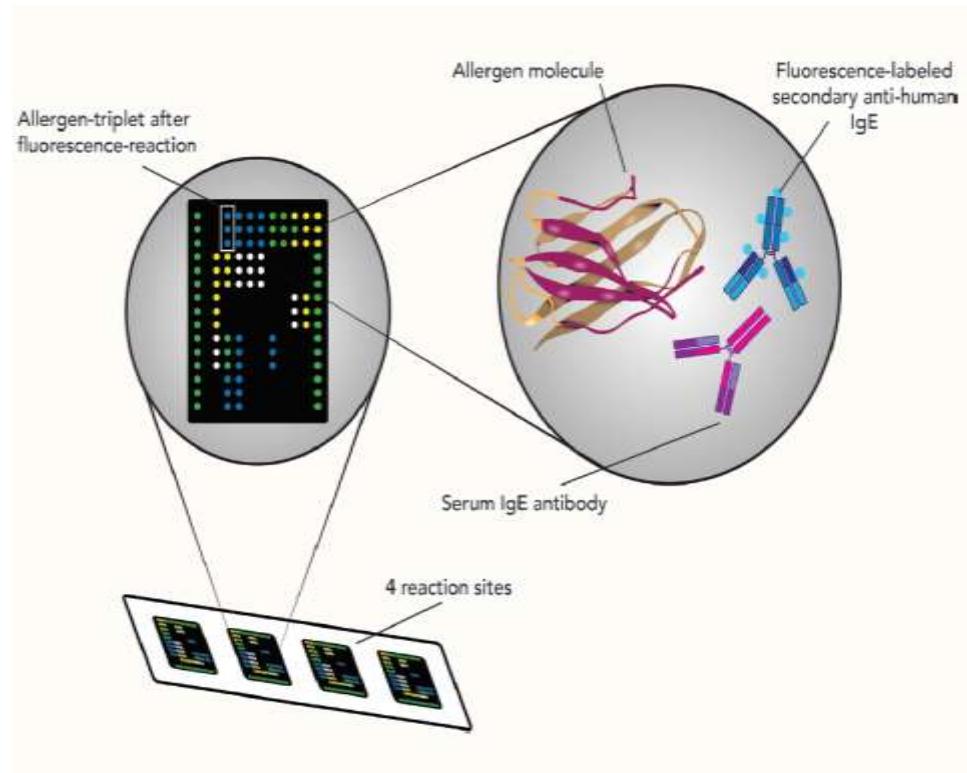
- 539 patients included.
- Age: 22 ± 16.4 [2 months-72 years]
 - 259 (48.1%) children.
 - 280 (51.9%) adults.
- Gender: 287 (53.2%) males and 252 (46.8%) females.



NB: some patients had more than one type of allergy.

METHODS

- Customized allergen-chip based on the ISAC technology (spIgE).
- 108 molecular allergens (13 native and 95 recombinants).
- Calibrator serum (calibration) and sample diluent (detection of background signals) were included in each analysis.
- ISAC standardized units (ISU-E, 0.3–150 ISU-E range) → low (0.3-1 ISU), medium (1-15 ISU), and high (>15 ISU) sensitization.



METHODS (Allergen-chip)



108 molecular allergens (13 native and 95 recombinants).

GD	Act d 2	Act d 5	Act d 8	Alt a 1	Amb a 1	Amb a 4	Amb a 8	Anis 3	Api m 1	Api m 2	Ara h 1	Ara h 2	Ara h 3	Ara h 6	Ara h 8	Ara h 9	Art v 1	Art v 3	Art v 6	Asp f 1	Asp f 3	GD
Asp f 6	Bet v 1	Bet v 2	Bet v 4	Blag 1	Blag 2	Blag 5	Blag 7	Blot 1	Blot 2	Blot 5	Blot 8	Blot 10	Blot 12	Blot 13	Blot 21	Bos d 4	Bos d 5	Bos d Lf	Bos d 8	Bos d 6	Can f 1	Can f 2
Can f 3	Can f 4	Can f 6	Cla h 8	Cor a 1.0401	Der p 1	Der p 2	Der p 4	Der p 5	Der p 7	Der p 10	Der p 18	Der p 21	Der p 23	Der p 37	Equ c 1	Equ c 3	Fel d 1	Fel d 2	Fel d 3	Fel d 4	Gad c 1	Gal d 1
Gal d 2	Gal d 3	Gal d 4	Gly m 4	Gly m 5	Gly m 6	Hel as 1	Hev b 3	Hev b 5	Hev b 8	Mal d 1	Mus m 1	Ole e 1	Ole e 2	Ole e 3	Ole e 5	Ole e 6	Ole e 7	Ole e 8	Ole e 9	Ole e 10	Par j 2	Pen m 1
GD	Pen m 2	Pla a 1	Pla l 1	Phl p 1	Phl p 2	Phl p 3	Phl p 5b	Phl p 6	Phl p 7	Phl p 12	Pru p 1	Pru p 3	Sal k 1	Tria 20	Tria 37	Ves v 1	Ves v 2	Ves v 5	CCD			GD

✓ Respiratory allergens (68 allergens)

METHODS (Allergen-chip)



108 molecular allergens (13 native and 95 recombinants).

GD	Act d 2	Act d 5	Act d 8	Alt a 1	Amb a 1	Amb a 4	Amb a 8	Anis 3	Api m 1	Api m 2	Ara h 1	Ara h 2	Ara h 3	Ara h 6	Ara h 8	Ara h 9	Art v 1	Art v 3	Art v 6	Asp f 1	Asp f 3	GD
Asp f 6	Bet v 1	Bet v 2	Bet v 4	Blag 1	Blag 2	Blag 5	Blag 7	Blot 1	Blot 2	Blot 5	Blot 8	Blot 10	Blot 12	Blot 13	Blot 21	Bos d 4	Bos d 5	Bos d Lf	Bos d 8	Bos d 6	Can f 1	Can f 2
Can f 3	Can f 4	Can f 6	Cla h 8	Cor a 1.0401	Der p 1	Der p 2	Der p 4	Der p 5	Der p 7	Der p 10	Der p 18	Der p 21	Der p 23	Der p 37	Equ c 1	Equ c 3	Fel d 1	Fel d 2	Fel d 3	Fel d 4	Gad c 1	Gal d 1
Gal d 2	Gal d 3	Gal d 4	Gly m 4	Gly m 5	Gly m 6	Hel as 1	Hev b 3	Hev b 5	Hev b 8	Mal d 1	Mus m 1	Ole e 1	Ole e 2	Ole e 3	Ole e 5	Ole e 6	Ole e 7	Ole e 8	Ole e 9	Ole e 10	Par j 2	Pen m 1
GD	Pen m 2	Pla a 1	Pla l 1	Phl p 1	Phl p 2	Phl p 3	Phl p 5b	Phl p 6	Phl p 7	Phl p 12	Pru p 1	Pru p 3	Sal k 1	Tri a 20	Tri a 37	Ves v 1	Ves v 2	Ves v 5	CCD			GD

✓ Food allergens (31 allergens)

METHODS (Allergen-chip)



108 molecular allergens (13 native and 95 recombinants).

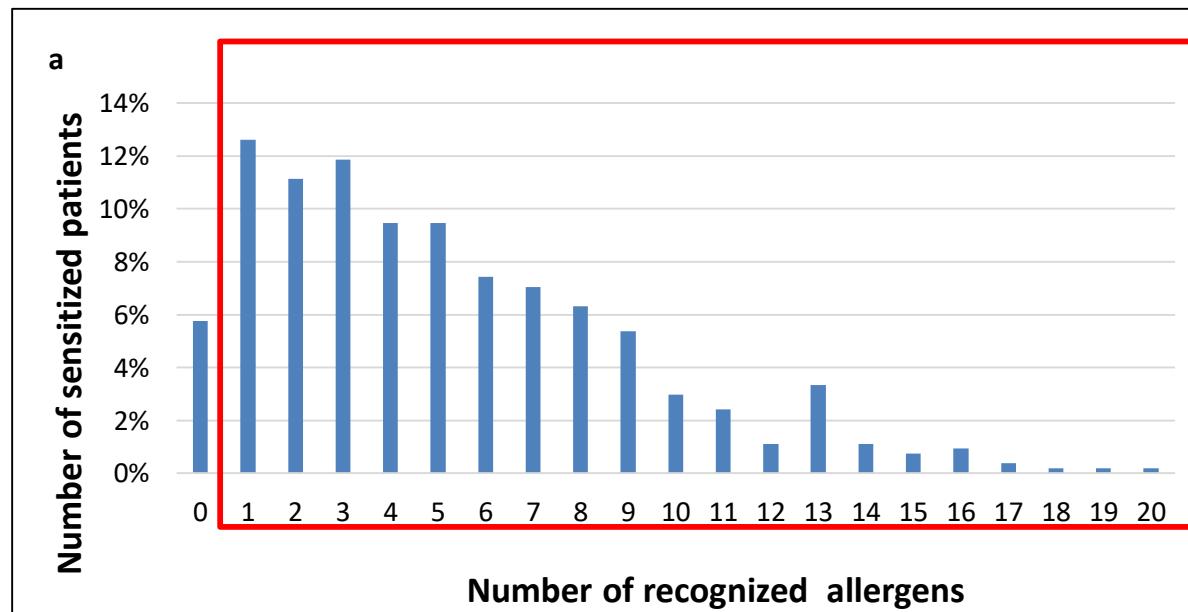
GD	Act d 2	Act d 5	Act d 8	Alt a 1	Amb a 1	Amb a 4	Amb a 8	Anis 3	Api m 1	Api m 2	Ara h 1	Ara h 2	Ara h 3	Ara h 6	Ara h 8	Ara h 9	Art v 1	Art v 3	Art v 6	Asp f 1	Asp f 3	GD
Asp f 6	Bet v 1	Bet v 2	Bet v 4	Blag 1	Blag 2	Blag 5	Blag 7	Blot 1	Blot 2	Blot 5	Blot 8	Blot 10	Blot 12	Blot 13	Blot 21	Bos d 4	Bos d 5	Bos d Lf	Bos d 8	Bos d 6	Can f 1	Can f 2
Can f 3	Can f 4	Can f 6	Cla h 8	Cor a 1.0401	Der p 1	Der p 2	Der p 4	Der p 5	Der p 7	Der p 10	Der p 18	Der p 21	Der p 23	Der p 37	Equ c 1	Equ c 3	Fel d 1	Fel d 2	Fel d 3	Fel d 4	Gad c 1	Gal d 1
Gal d 2	Gal d 3	Gal d 4	Gly m 4	Gly m 5	Gly m 6	Hel as 1	Hev b 3	Hev b 5	Hev b 8	Mal d 1	Mus m 1	Ole e 1	Ole e 2	Ole e 3	Ole e 5	Ole e 6	Ole e 7	Ole e 8	Ole e 9	Ole e 10	Par j 2	Pen m 1
GD	Pen m 2	Pla a 1	Pla l 1	Phl p 1	Phl p 2	Phl p 3	Phl p 5b	Phl p 6	Phl p 7	Phl p 12	Pru p 1	Pru p 3	Sal k 1	Tria 20	Tria 37	Ves v 1	Ves v 2	Ves v 5	CCD			GD

- ✓ Venom allergens (5 allergens)
- ✓ Latex allergens (3 allergens)
- ✓ CCD



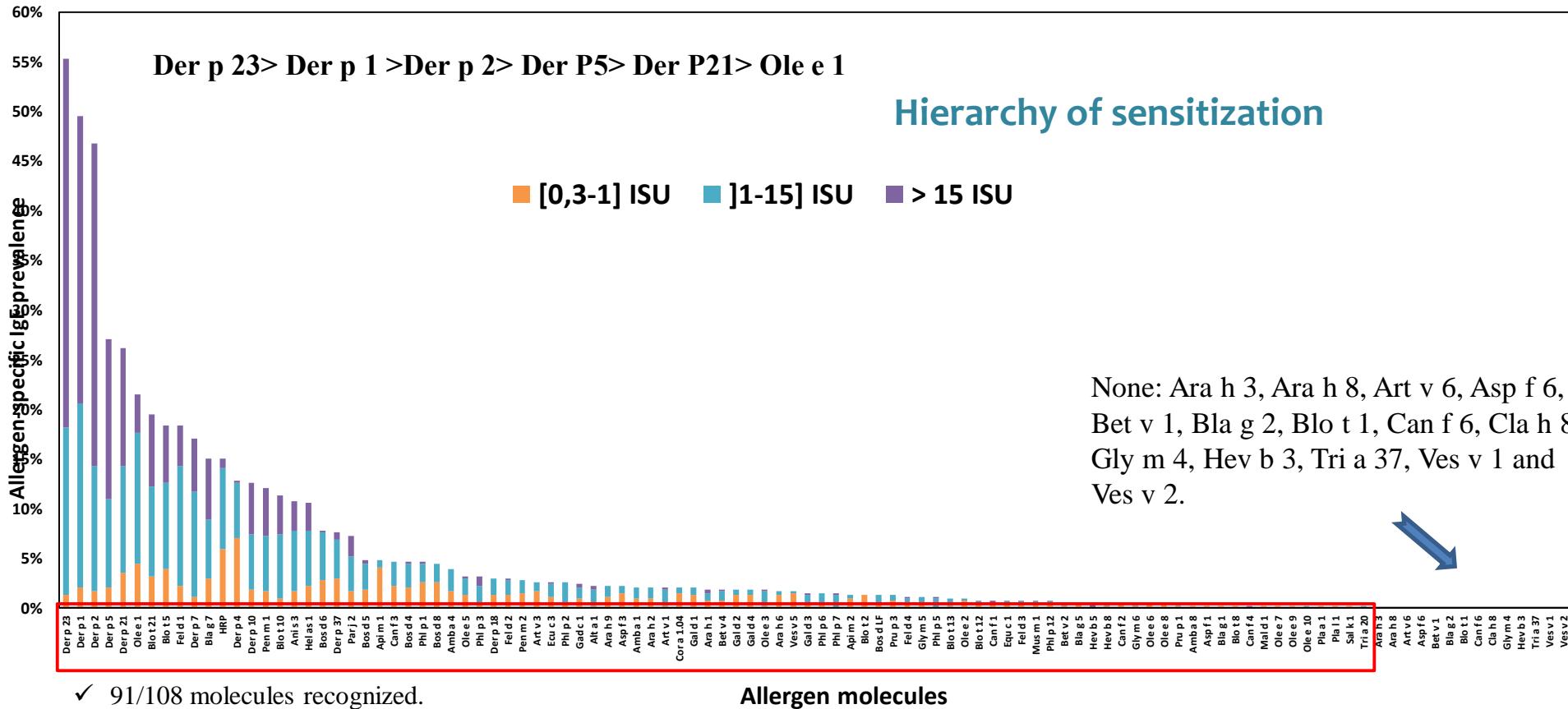
Hierarchy of sensitization

- ✓ 94.2% (508/539) were sensitized to at least one molecular allergen.



- ✓ On average, individuals reacted to **5 single allergens**.

RESULTS AND DISCUSSION



RESULTS AND DISCUSSION

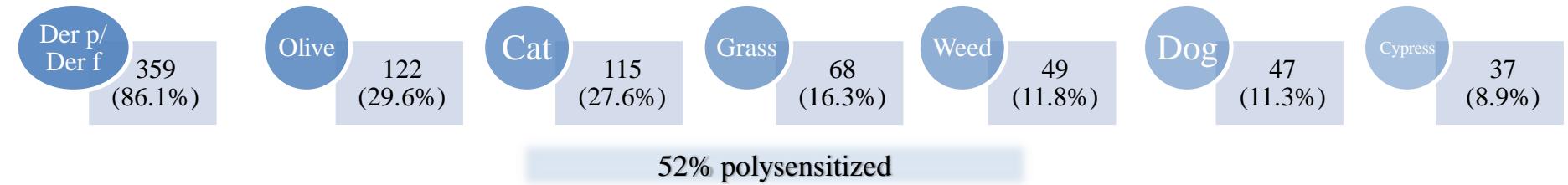
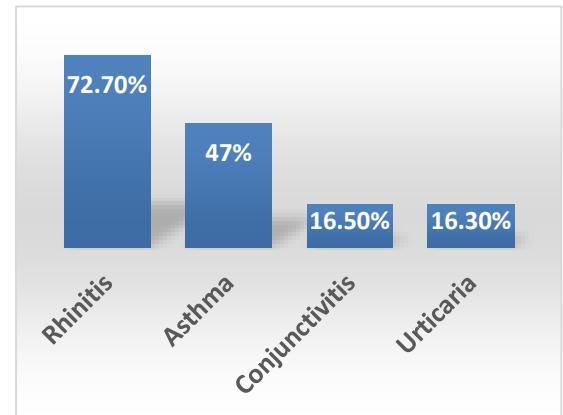


Patients with respiratory allergy

- 417 patients (Symptoms related to inhalant allergens (asthma, rhinitis and/or conjunctivitis) + IgE test (≥ 0.35 kU/l) to at least one: house dust mite, cat and/or dog dander's, pollens (grass, weed, olive, cypress)).
- Age: 23.9 ± 15.7 [2-72] years.
 - 173 (41.5%) children.
 - 244 (58.5%) adults.
- Gender: 202 (48.4%) males and 215 (51.6%) females.
 - Sensitization profile (sp IgE to the whole extract IMMULITE)



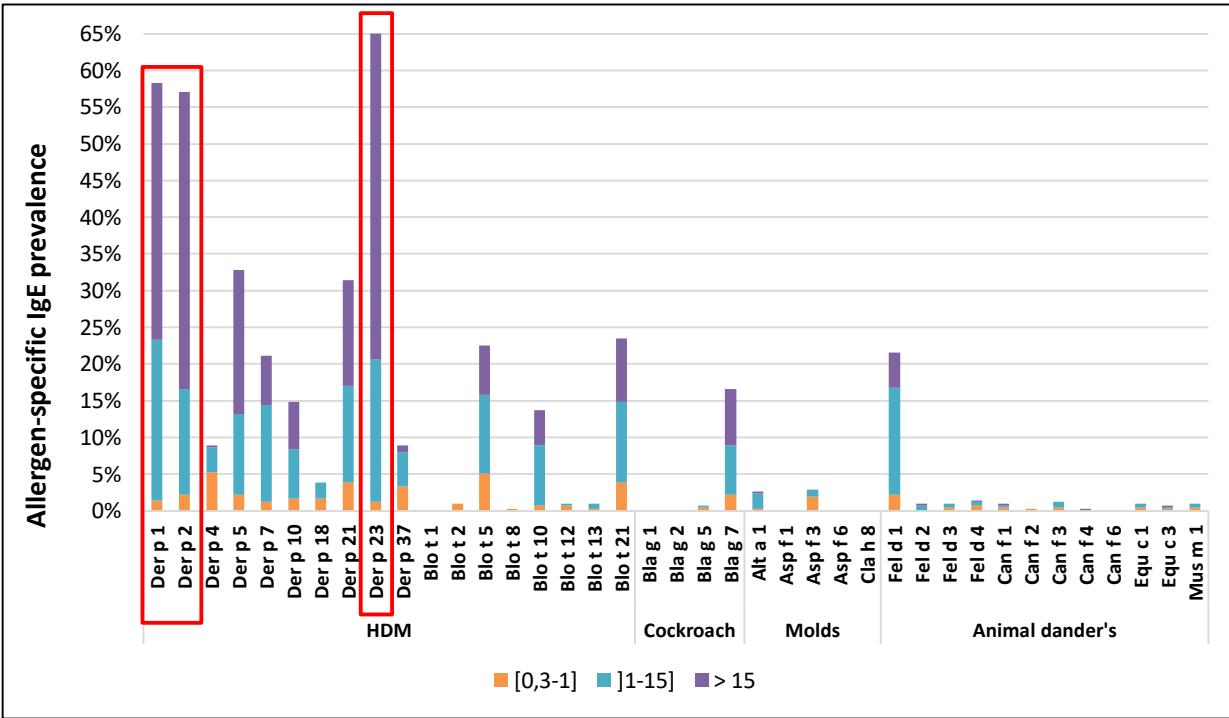
29 (9.4%) vs 279 (90.6%)



RESULTS AND DISCUSSION



Patients with respiratory allergy (indoor molecular allergens)



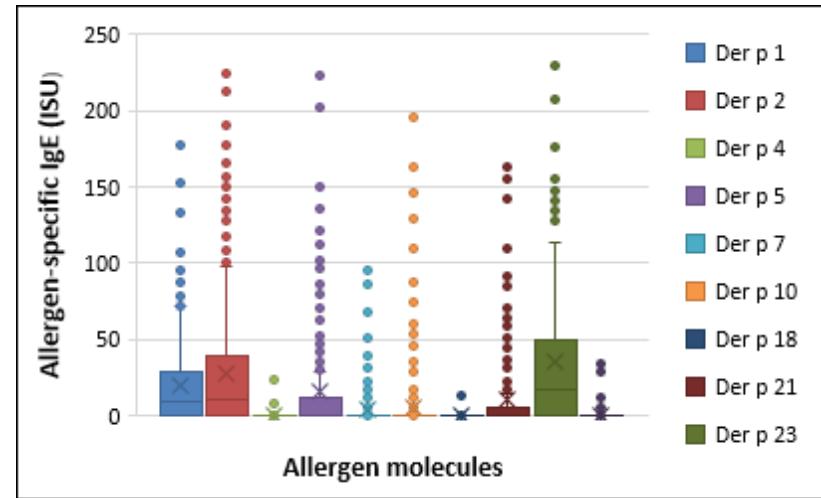
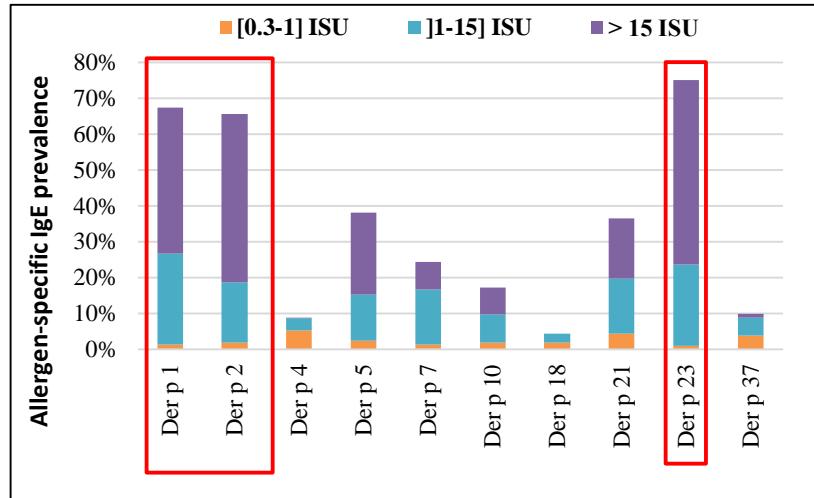
Sensitization to aeroallergens was **dominated** by the three major allergens from *Dermatophagoides pteronyssinus*: **Der p 23 (65%)**, **Der p 1 (58.3%)** and **Der p 2 (57.1%)**.

Figure: Percentage and level of specific IgE among patients with **respiratory allergy (N=417)** for indoor molecular allergens .

RESULTS AND DISCUSSION



Patients with respiratory allergy (indoor molecular allergens)



Patients with HDM allergy (N=359)

- Der p 23 > Der p 1 > Der p 2 > 50% → major allergens.
- Der p 5 > Der p 21 > Der p 7 [20-50%] → mid-tier allergens.
- Der p 18 Der p 4 Der p 37 Der p 10 20% → minor allergens.

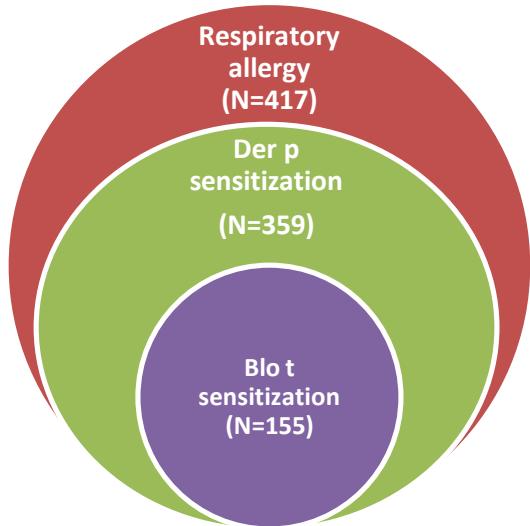
12% (43/359): Der p 23 (+) Der p 1 (-) Der p 2 (-)

AIT standardized only for Der p 1 and Der p 2 will be relevant for these patients?

RESULTS AND DISCUSSION

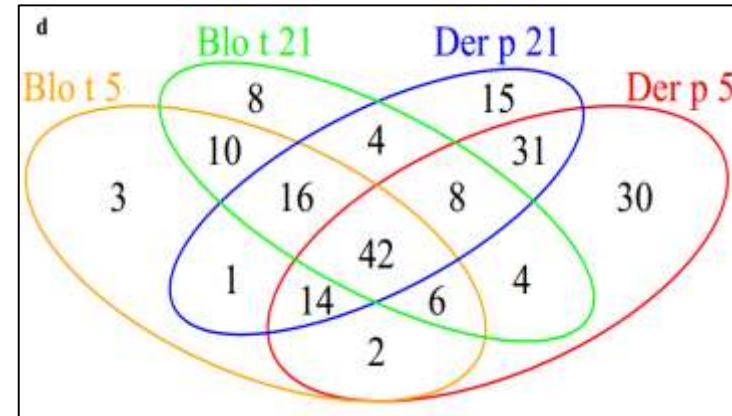
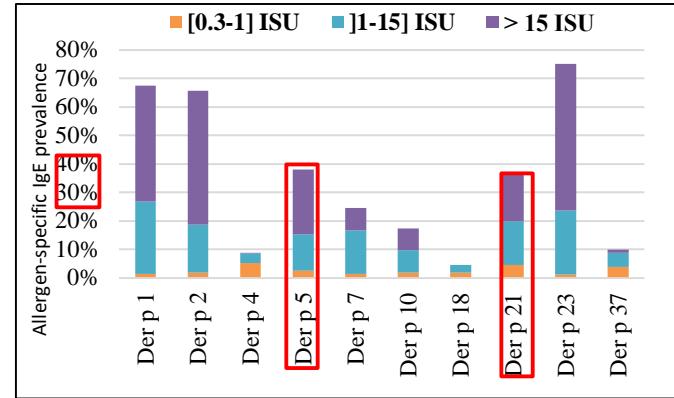
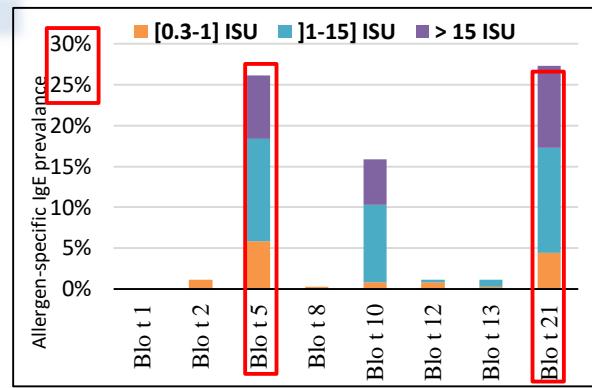
Patients with respiratory allergy (indoor molecular allergens)

Patients with HDM allergy (N=359)



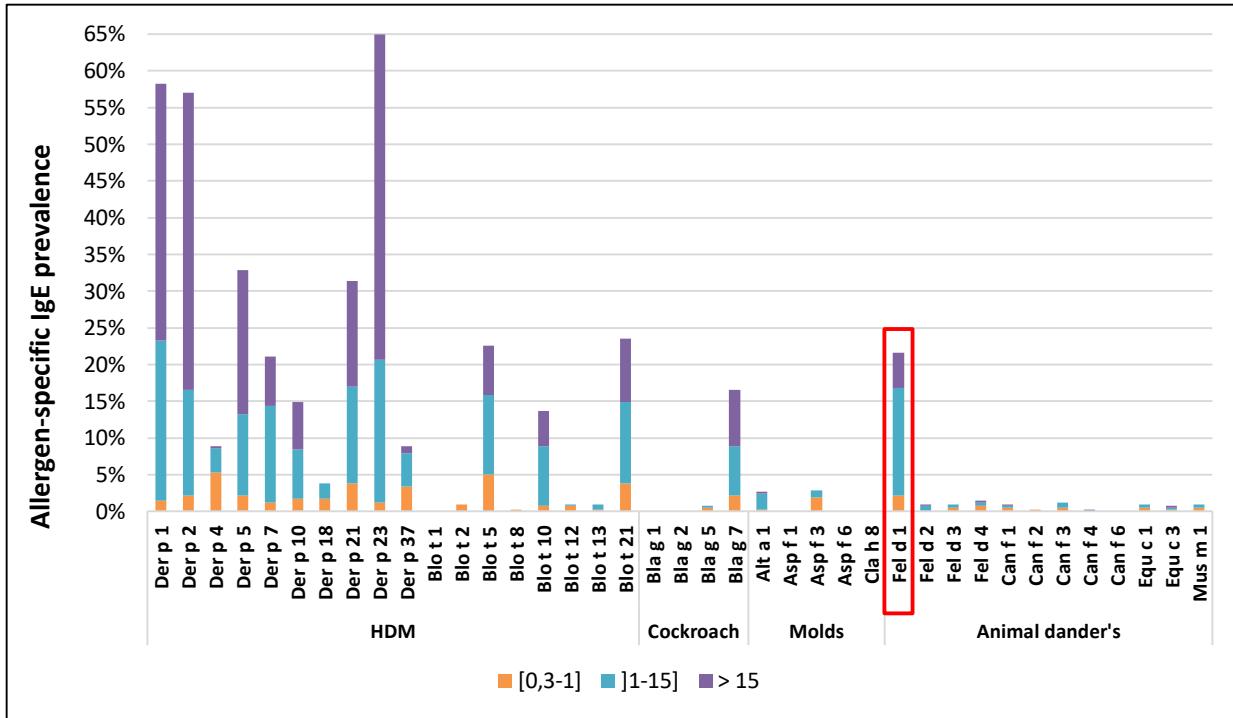
Cross-sensitization??

For these patients a deeper anamnesis is necessary to look if they already leaved in a tropical region.





Patients with respiratory allergy (indoor molecular allergens)



- ✓ **Fel d 1:** dominate the IgE reactivity in the group of animal danders'.
- ✓ Sensitizations to other animal-derived molecules (dog, horse, mouse) <1.5% and were mostly associated with cat allergens reactivity (in 97.9%).

Figure: Percentage and level of specific IgE among patients with **respiratory allergy (N=417)** for indoor molecular allergens .



Patients with respiratory allergy (indoor molecular allergens)

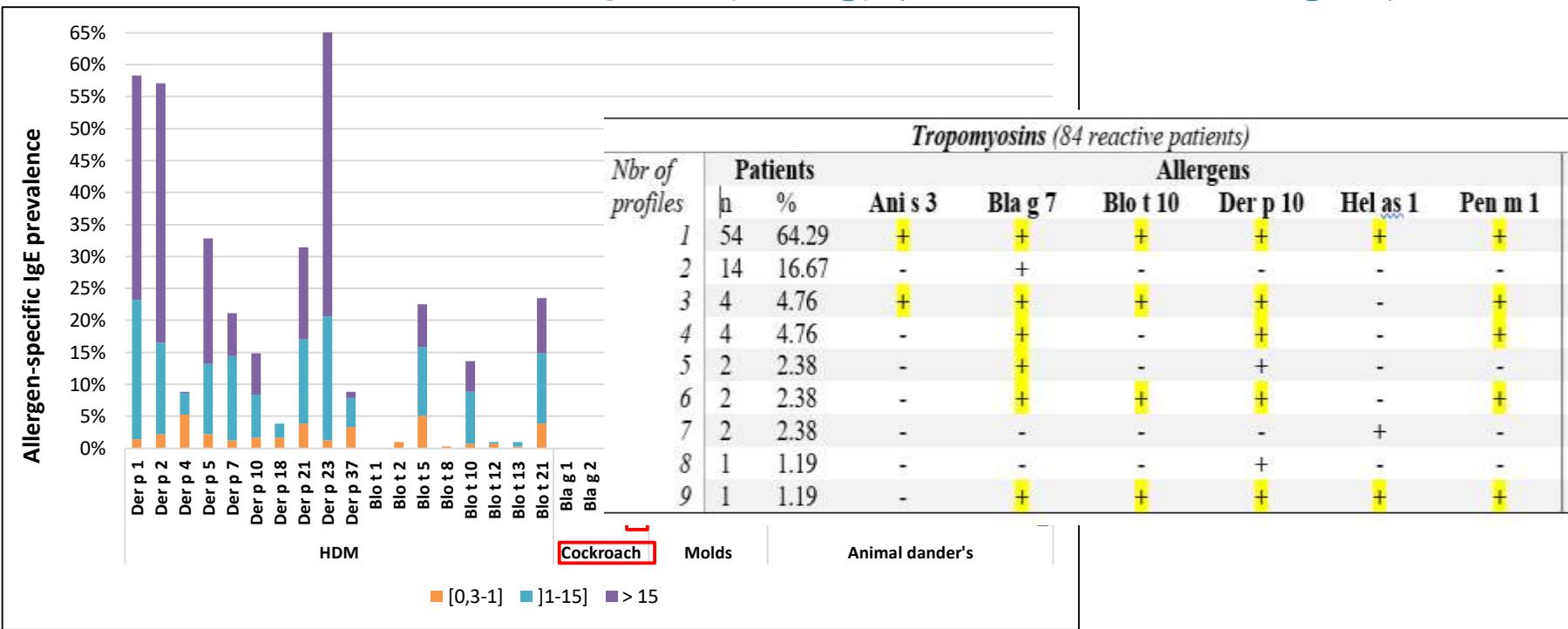


Figure: Percentage and level of specific IgE among patients with **respiratory allergy (N=417)** for indoor molecular allergens .



Patients with respiratory allergy (indoor molecular allergens)

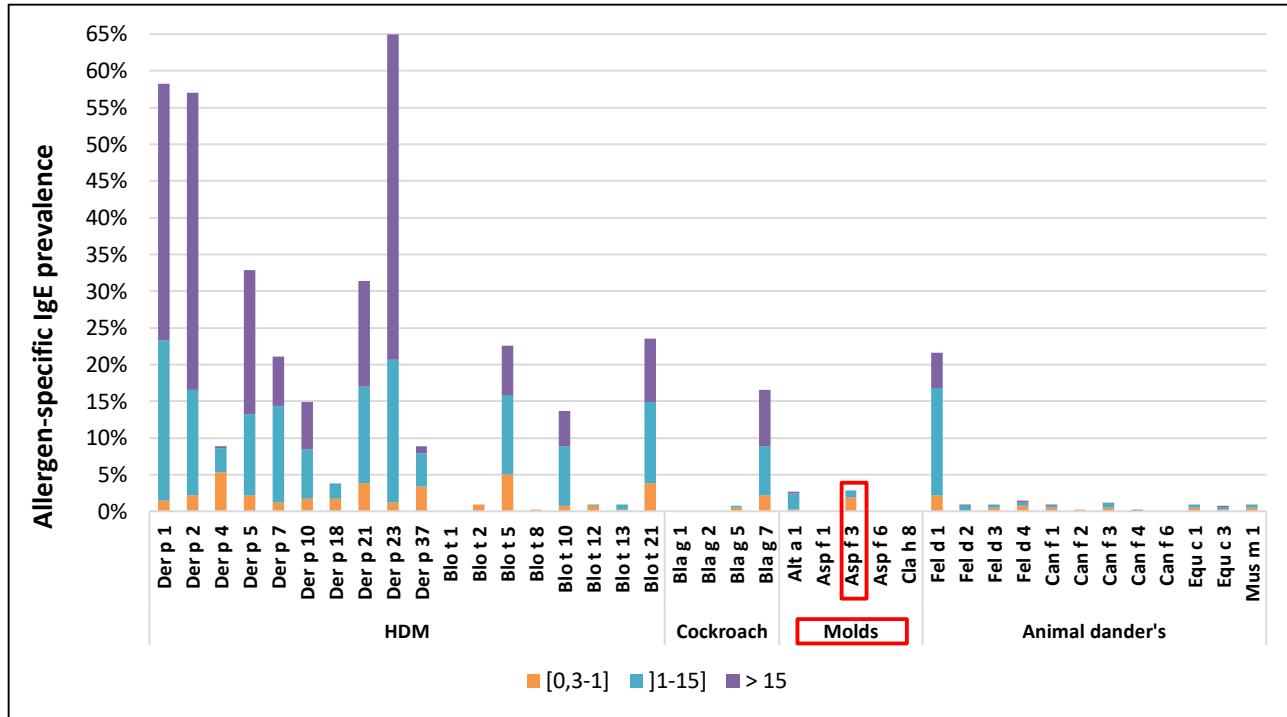


Figure: Percentage and level of specific IgE among patients with **respiratory allergy (N=417)** for indoor molecular allergens .

- ✓ *Aspergillus fumigatus*: Asp f 3 (12patients: 7 ABPA).
- ✓ 11 cases: Alt a 1.

RESULTS AND DISCUSSION



Patients with respiratory allergy (outdoor molecular allergens)

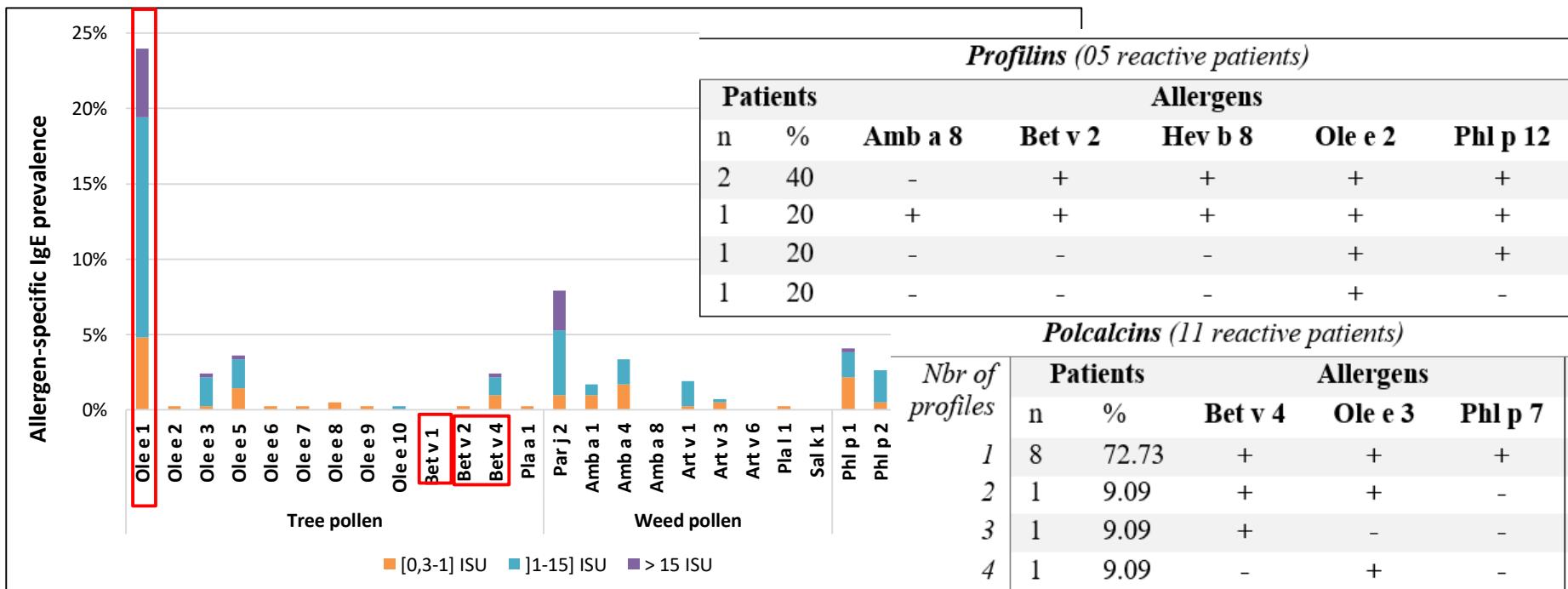


Figure: Percentage and level of specific IgE among patients with **respiratory allergy** (N=417) for outdoor molecular allergens .

Patients with respiratory allergy (outdoor molecular allergens)

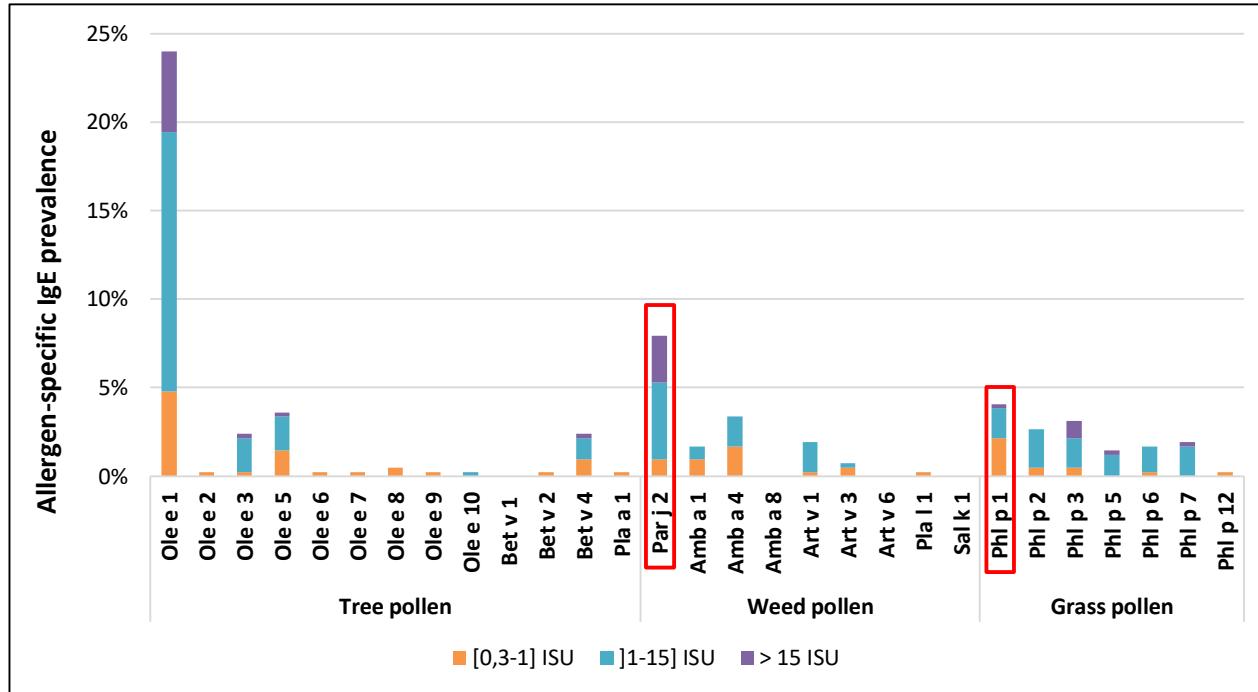


Figure: Percentage and level of specific IgE among patients with **respiratory allergy (N=417)** for outdoor molecular allergens .

- ✓ Parietaria was the next important pollen allergen source as indicated by the major allergen Par j 2.
- ✓ Whereas the other weed pollen allergens were less frequently recognized < 5%.
- ✓ Grass pollen reactivity was dominated by Phl p 1.

RESULTS AND DISCUSSION

Patients with respiratory allergy

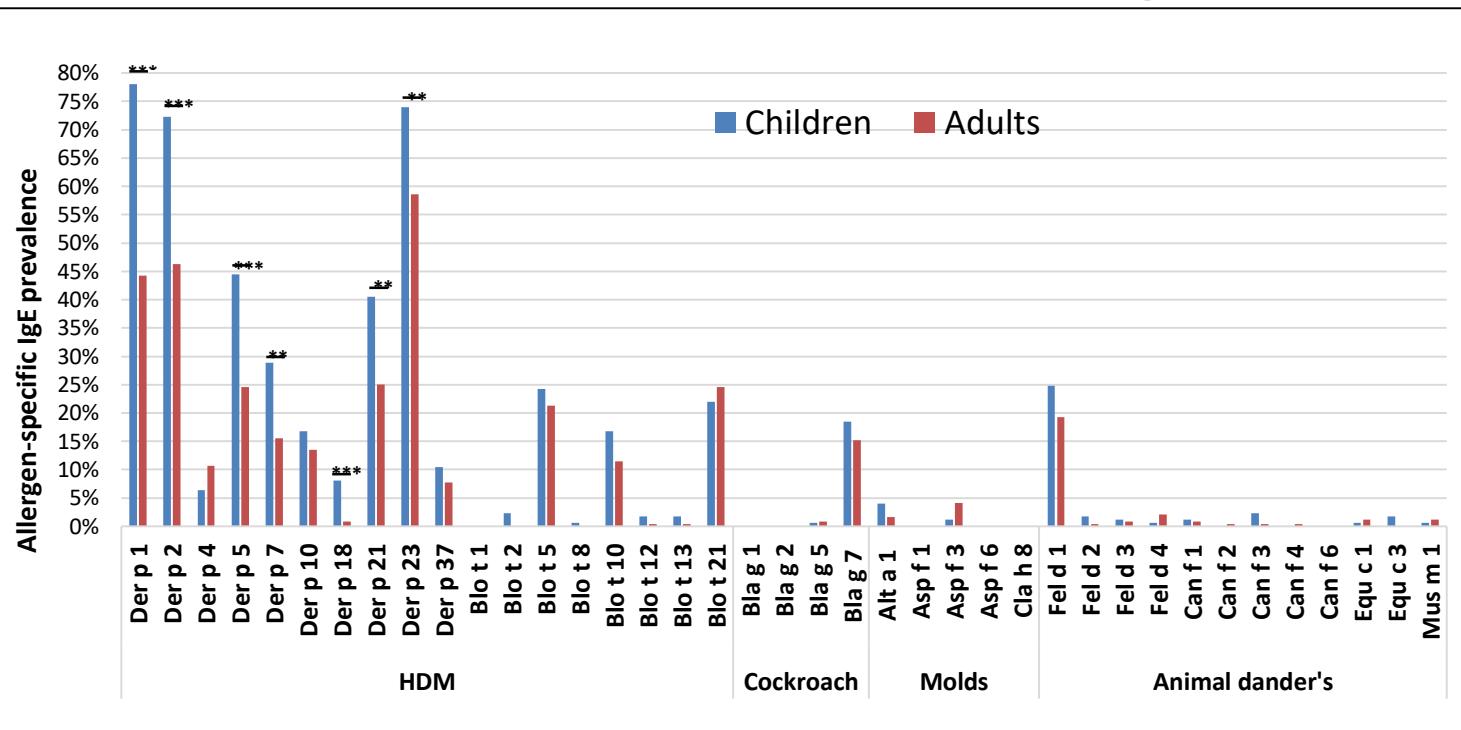


Figure: Comparison of allergen-specific IgE sensitization frequencies between children and adults for **indoor molecular allergens**.

✓ Older subjects: better control HDM exposure and practice better avoidance, assuming that allergen avoidance eventually reduces HDM allergen-specific IgE levels.

RESULTS AND DISCUSSION



Patients with respiratory allergy

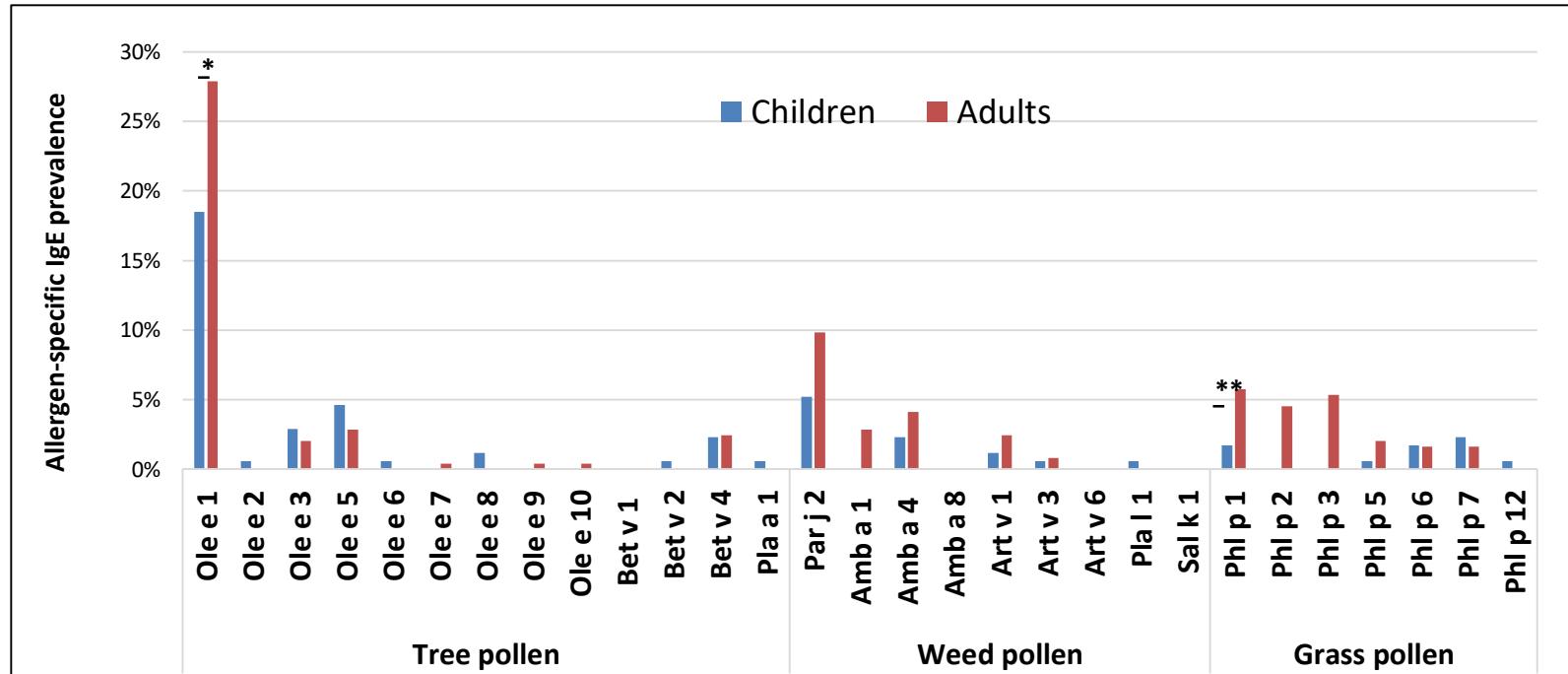
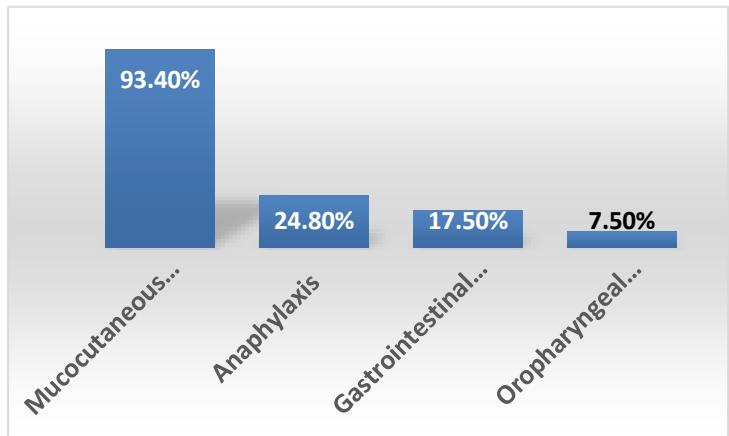
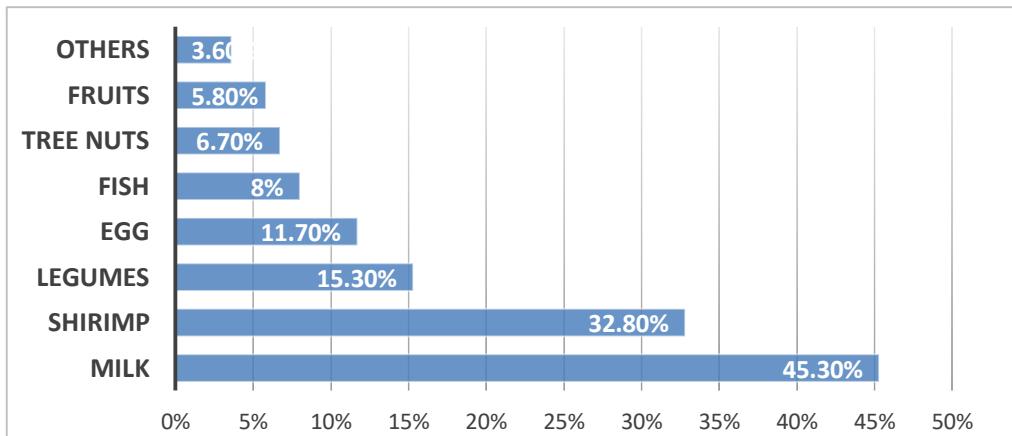


Figure: Comparison of allergen-specific IgE sensitization frequencies between children and adults for **outdoor molecular allergens**.



Patients with food allergy

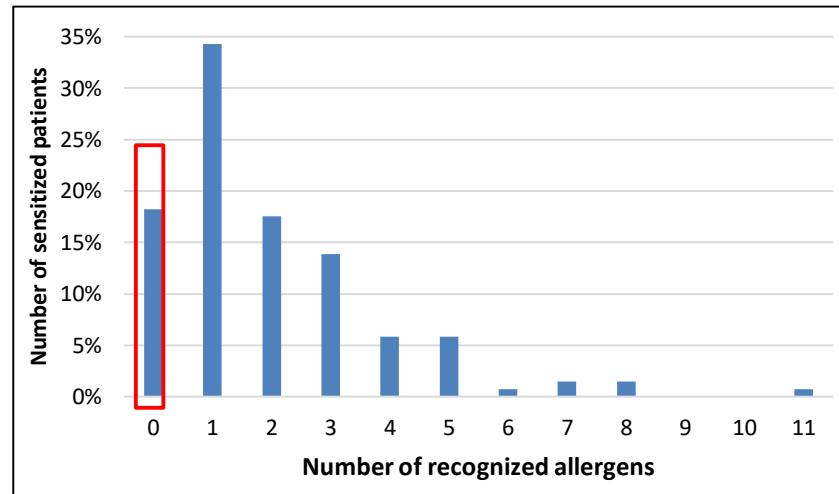
- 137 patients.
- Age: 14.8 ± 16.2 [2 months- 72 years].
 - 97 (70.8%) children.
 - 40 (29.2%) adults.
- Gender: 74 (54%) males and 63 (46%) females.





Patients with food allergy

- ✓ The number of allergens recognized per person range between 1 and 11



- ✓ **18.8%** were negative despite a clinical history of food allergy and a positive IgE result to at least one food crude extract.



Patients with food allergy

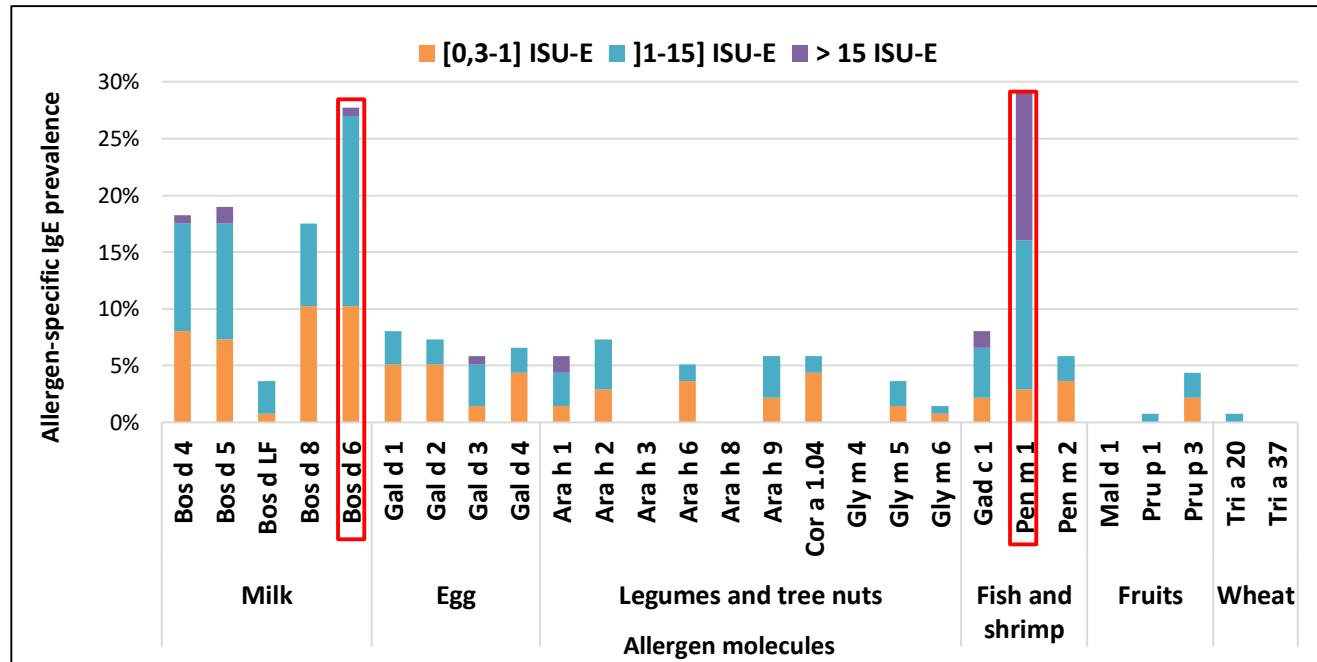


Figure: Percentage and level of specific IgE for food molecular allergens .



Patients with food allergy

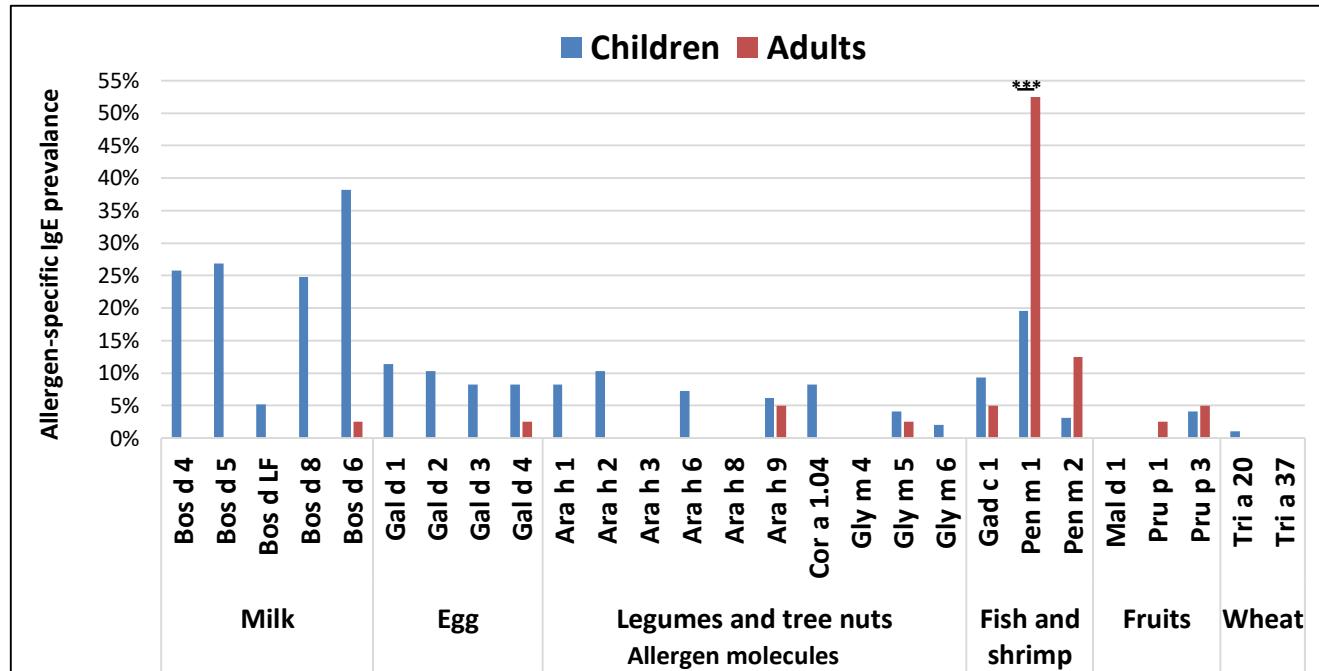


Figure: Comparison of allergen-specific IgE sensitization frequencies between children and adults for food molecular allergens.

IgEs d'allergènes moléculaires LV disponibles / pertinents



IgEs Source

Lait de vache (LV)

Composants

α lactalbumine
n Bos d 4

B lactoglobuline
n Bos d 5

Caséine
n Bos d 8

SAB
n Bos d 6

Thermolabiles

Stable à la chaleur
Réactivité croisée entre mammifères
Tx élevés = allergie persistante

Réactivité
croisée
LV-viande



Bos domesticus



Patients with food allergy (milk allergy)

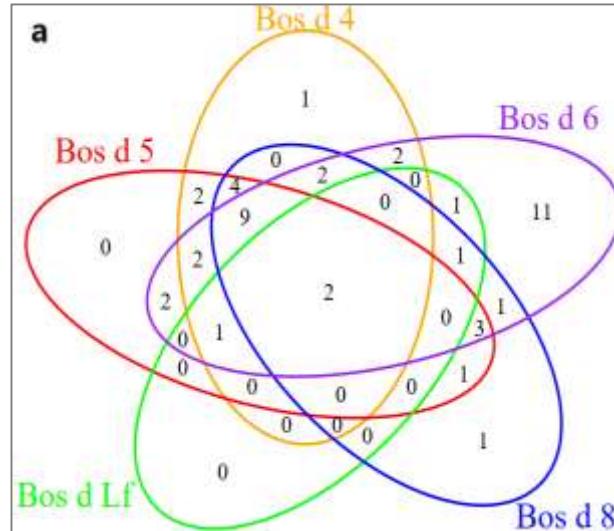
40.3% (25/62)

41.9% (26/62)

58.1% (36/62)

8.1% (05/62)

38.7% (24/62)



	Bos d 8 (-)	Bos d 8 (+)	p
Anaphylaxis	4 (10.5%)	9 (37.5%)	0.022



- Gal d 1, ovomucoïde
- Stable à la chaleur, hautement allergique
- Risque de réaction à l'œuf cuit/cru
- Taux élevés peuvent signer allergie persistante

- Gal d 2, ovalbumine
- Sensible à la chaleur
- Protéine la plus abondante du blanc œuf
- Risque de réaction à l'œuf cru ou peu cuit

- ✓ Affiner le Dg et prédire la tolérance aux œufs cuis et crus (profil de sévérité)
- ✓ Intérêt dans le suivi pour prédire l'évolution (résolution / persistance)
- ✓ Permet de guider la PEC (Régime d'évitement adapté / réintroduction / ITO)

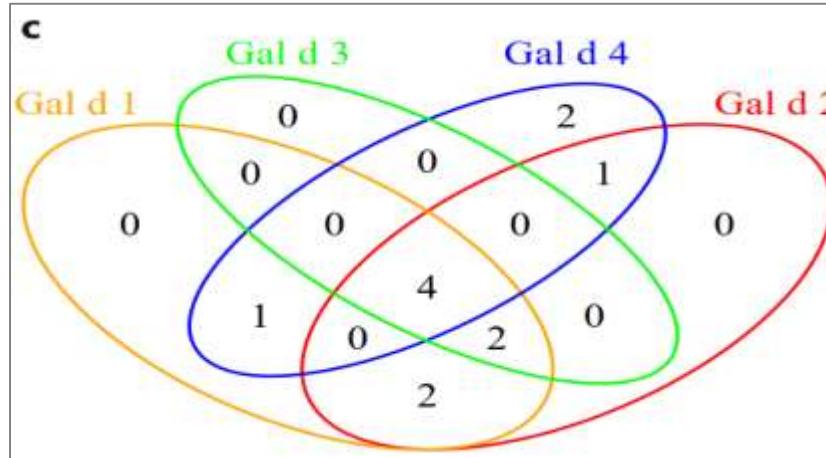


Patients with food allergy (egg allergy)

37.5% (6/16) 50% (8/16)

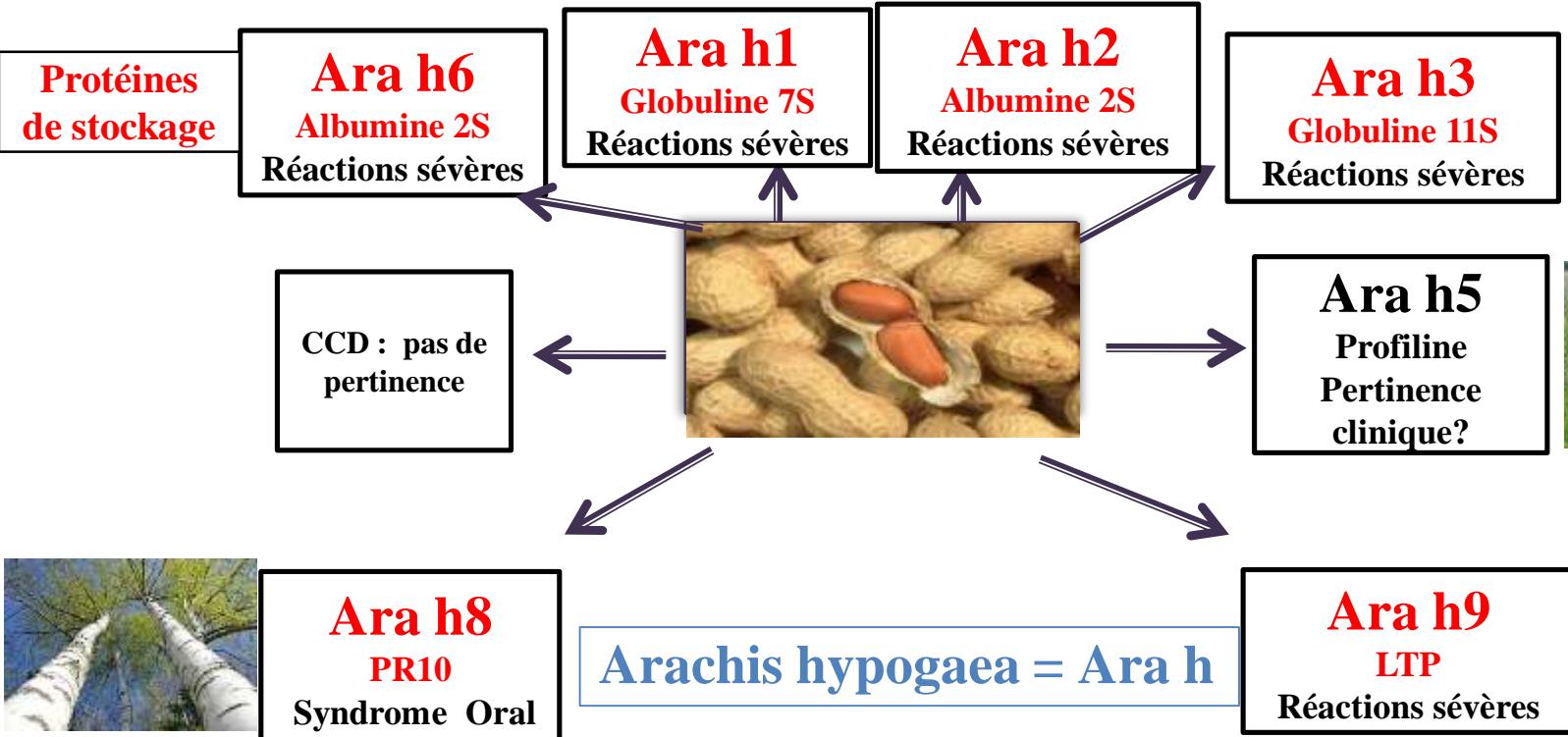
56.3% (9/16)

56.3% (9/16)



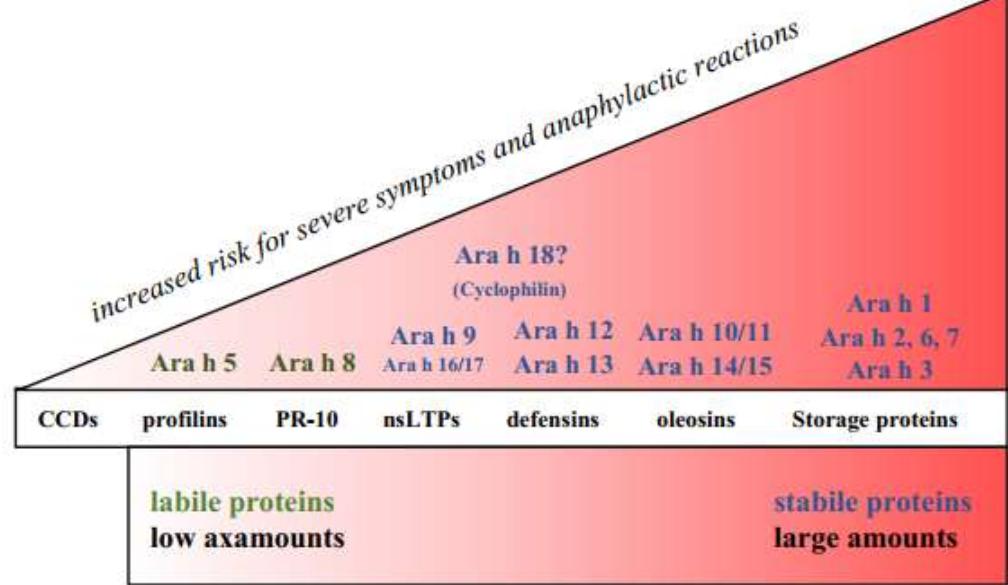
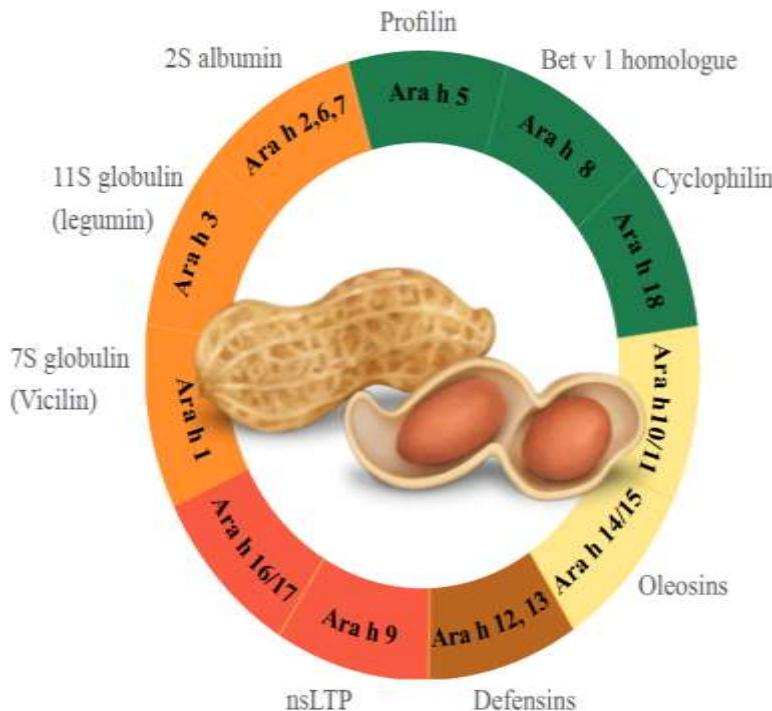
	Gal d 1 (-)	Gal d 1 (+)
Mucocutaneous	100%	55.6%
Gastrointestinal	0%	11.1%
Anaphylaxis	0%	33.3%

Arachide : apport des recombinants



17 allergènes décrits mais 06 dosables en pratique clinique !

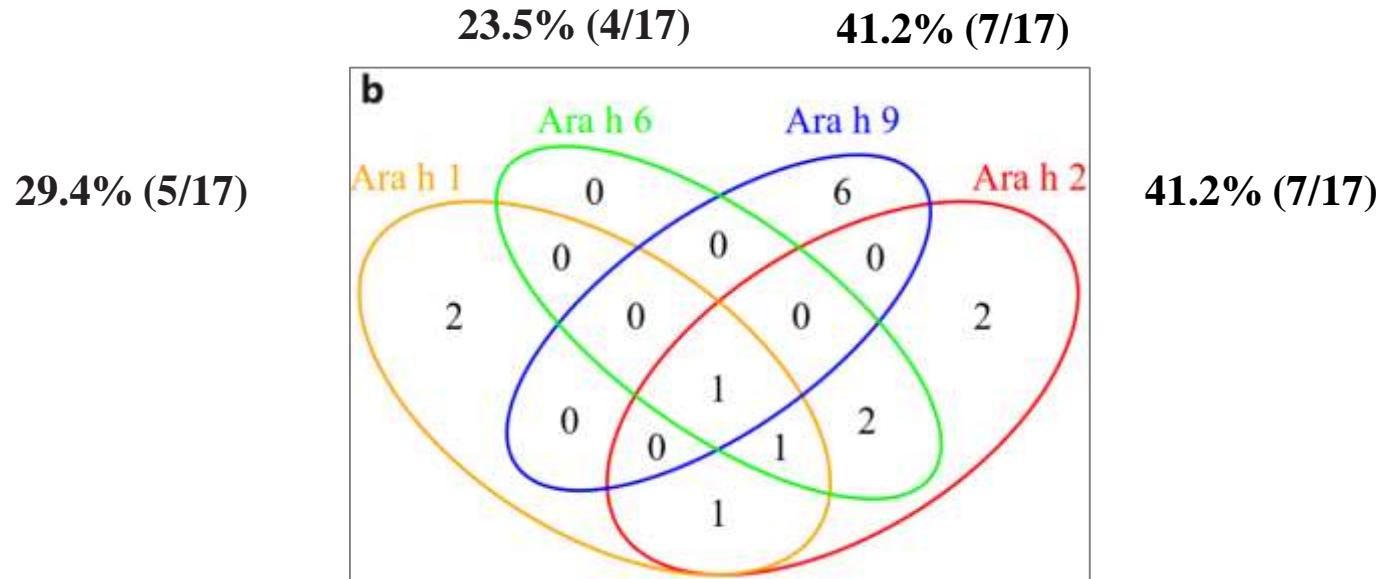
Arachide : apport des recombinants





Patients with food allergy (peanut molecular allergens)

Peanut allergy





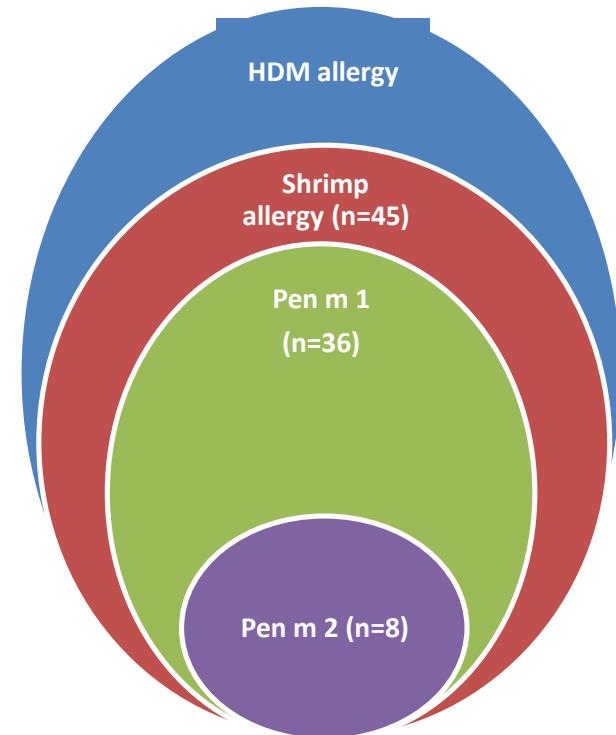
Patients with food allergy (seafood allergy)

Shellfish allergy (shrimp)

- ✓ Shrimp allergic patients (n=45), the majority (36/45, 80%) showed positivity to the tropomyosin Pen m 1 and 17.8% had an additional IgE reactivity to the arginine kinase Pen m 2.
- ✓ All the patients with shrimp allergy were allergic to the HDM. Among them 82.2% had a positive IgE result to at least one of the major HDM allergens (Der p 1, Der p 2 and/or Der p 23).

Fish allergy

- ✓ All fish allergic patients recognized the parvalbumin allergen Gad c 1.



CONCLUSION



- ✓ This study provided a deep insight into IgE sensitization profile of our cohort, which led to a better understanding of local epidemiology.
- ✓ It allowed the determination of allergen panels needed for diagnosis purpose.
- ✓ Children and adults seems to have different sensitization profile.
- ✓ Larger studies involving different regions of Algeria will be necessary to establish a complete molecular allergen map for the complete country.

Thank you for your
attention!