

Journal Pre-proof

Correlation of Skin Prick Testing to Environmental Allergens

Lillian Grobman, MD, Jessica Kitsen, BS, Diba Mortazavi, BS, Bob Geng, MD

PII: S1081-1206(20)31143-1

DOI: <https://doi.org/10.1016/j.anai.2020.10.010>

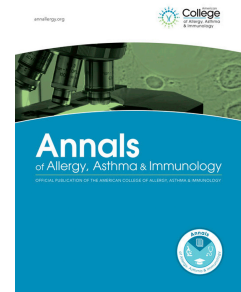
Reference: ANAI 3405

To appear in: *Annals of Allergy, Asthma and Immunology*

Received Date: 21 July 2020

Revised Date: 15 October 2020

Accepted Date: 22 October 2020



Please cite this article as: Grobman L, Kitsen J, Mortazavi D, Geng B, Correlation of Skin Prick Testing to Environmental Allergens, *Annals of Allergy, Asthma and Immunology* (2020), doi: <https://doi.org/10.1016/j.anai.2020.10.010>.

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2020 American College of Allergy, Asthma & Immunology. Published by Elsevier Inc. All rights reserved.

Title: Correlation of Skin Prick Testing to Environmental Allergens

Authors: Lillian Grobman, MD¹, Jessica Kitsen, BS^{1*}, Diba Mortazavi, BS^{1*}, Bob Geng, MD¹

¹ University of California, San Diego, Department of Allergy Immunology

*these authors contributed equally to this work

Corresponding Author: Lillian Grobman, 9500 Gilman Drive, MC 0912, La Jolla, CA 92023,
206-234-3142, lilyerika@gmail.com

Conflicts of Interest: None

Funding Source: None

Keywords: Environmental allergies, skin prick test, co-sensitization, cross-reactivity, allergy immunotherapy

Abbreviations/Acronyms: SPT: skin prick testing. AIT: allergen immunotherapy.

Word count: 2340 (manuscript)

Figures: 4

Tables: 4, plus 3 eTables

20-07-0469R2

Background To diagnose and adequately treat allergies, identification of sensitizing allergens is crucial. Skin prick or serum IgE testing determines sensitization to potential allergens. Consensus varies regarding degree of cross reactivity between aero-allergens.

Objective This retrospective analysis aims to quantify correlations between aero-allergen skin prick test results.

Methods 11832 patients' quantitative skin prick test results and basic characteristics were obtained from a tertiary academic center's electronic medical record and de-identified. Data was analyzed using Spearman's rank correlation coefficients to detect non-linear associations between wheal sizes of skin prick test results.

Results The highest correlation amongst grasses was 0.903 (Fescue and Red Top). Thirteen more grass pairs had correlation over 0.8 and nineteen had correlation between 0.7 and 0.8. The Northern grass comparative results are from a satellite clinic that tested more grasses than the main clinic, where only Kentucky Blue and Bermuda were tested (correlation 0.768, $n = 9348$). Highest correlation amongst weeds was 0.74 (Lambs Quarter and Pigweed). Six more weed pairs had correlation over 0.7. Highest correlation amongst trees was 0.724 (Palm and Alder). Six more tree pairs had correlation over 0.7. Highest correlation amongst molds was 0.711 (Helminthosporium and Epicoccum). Dust mite correlation between *D. farinae* and *D. pteronyssinus* was 0.848 ($n = 10022$).

Conclusion This study confirmed expected high degrees of correlation between skin prick test results of related environmental allergens, but also between less closely related Northern grasses and Bermuda grass. This has implications for simplification of testing and immunotherapy protocols in the future.

1 Introduction

2 Skin prick testing (SPT) is an important procedure used by allergists to confirm the presence of
3 IgE-mediated sensitization to allergens. It is used alone and sometimes in combination with in
4 vitro testing for evaluation and subsequent management of allergic rhinitis, asthma, eczema, food
5 allergy, insect sting allergy and drug allergy.

6 Understanding allergen co-sensitization and cross-reactivity is important for counseling and
7 managing patients with allergic disease. Co-sensitization occurs when there is parallel but
8 independent sensitization to multiple allergens. This is different from cross-reactivity, where an
9 antigen is recognized without prior sensitization due to either shared epitopes or
10 conformationally similar epitopes (1). Cross-reactivity allows for allergic reactions to allergens
11 without prior sensitization. Some cross-reactivities are well known and clinically relevant. Bet v
12 1 from birch trees confers the most well-known cross-reactivity, with cross-reactive IgE-epitopes
13 described in pollen, fruit, vegetables, nuts and seeds leading to oral allergy syndrome in Bet v 1-
14 sensitized patients (2,3). Extensive cross-reactivity has been described among different ragweed
15 species (4,5). Cross-reactivity often reflects taxonomy, with plants in the same genus being
16 expected to have the greatest number of shared allergens. Despite this theory there have been
17 reports of unexpected cross-reactivity between unrelated plants, sometimes remarkably distant
18 ones (1).

19 Understanding cross-reactivity can help reduce the number of allergens needed for SPT as well
20 as allergen immunotherapy (AIT). Current US allergen immunotherapy guidelines recommend
21 selecting a single pollen within a genus for immunotherapy when there is substantial cross-
22 reactivity (6).

23 In this study we analyzed the correlation of SPT results to environmental allergens.

Methods

Data Collection

11,832 patients' quantitative SPT results and basic characteristics (age, gender and whether the patient has a history of asthma) were retrospectively obtained from the electronic medical record and de-identified. SPT was performed between January 18, 2012 through January 18, 2017 at two locations, a tertiary care pediatric academic medical center (Rady Children's Hospital, UCSD, San Diego, CA) and a satellite clinic (Allergy & Asthma Medical Group, AAMG, San Diego, CA) where care is provided for both pediatric and adult patients.

All SPT results shown are from the main allergy clinic, except for the results of grass allergens which are from the satellite clinic which tested for more grass allergens than the main Rady's allergy clinic which only tested Kentucky Blue and Bermuda grass.

The study was approved by the local institutional review board (UCSD Human Research Protections Program, Project #170209X).

Skin prick testing

All skin prick testing was done per standard clinic protocol, but this differed between the two different sites. The main allergy clinic used individual Greer testers and the satellite clinic used Lincoln Diagnostics multi testers. Correlations are only made between tests within each site, thus no comparisons were made between tests done with different methods or testers.

SPT results were only included in analysis when there was a negative saline control and a positive histamine control (≥ 3 mm). Skin test results were recorded according to millimeters of wheal and flare. Not all patients were tested to the same allergens. For patients with multiple SPT results, only the first available test result was used. All SPT were performed and read by

medical assistants who were trained by a team of allergists in the division with constant quality control.

Statistics

Correlation between allergens was based on wheal size. Spearman's rank correlation coefficient was used to detect non-linear associations between variables, and to account for possible outliers. Jennrich's test was used to compare correlation matrices by each subgroup. Sparse groups were excluded in the comparison. All statistical analyses were performed in R (V.3.6.1, R Core Team (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Results

Data from main allergy clinic

Data was collected and analyzed for 10,245 patients from the main allergy clinic. Patients were 42.3% female (n=4330) and 57.7% male (n=5915). Age in years was 10.6% less than 2 (n=1101), 31.6% 2 to 5.99 (n=3233), 38.1% 6 to 11.99 (n=3899), and 19.6% 12 and up (n=2011, range 12 – 44.5 years old, mean age 14.78). It should be noted that there were two patients in their 40's; the next oldest patient was 21 years old. In regard to asthma 40.4% of the patients had asthma (n=4142) and 59.6% of the patients did not have asthma (n=6105). In regard to SPT location 99.6% of patients had SPT performed on their back (n=10093) and 0.4% of patients had SPT performed on their arm (n=36). A summary of the patient characteristics is shown in eTable 1. SPT was performed to dust mites, trees, weeds, grasses and molds commonly found in the Southwest United States. Taxonomic distribution of the tested allergens is shown in eTable 2.

The allergens with the highest correlation coefficients are shown in eTable 3 with regard to their taxonomic relationship. Positive SPT results ranged from 9.3% to 33.4%, with no significant differences in SPT positivity rates based on diagnosis of asthma.

There was high correlation between the two dust mites, *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*, with a Spearman correlation coefficient of 0.848 (n=10022). Of tree allergens tested, the highest correlation was 0.724 between Palm and Alder (n=5465). Six more tree pairs had correlation over 0.7: Acacia and Palo Verde 0.722 (n=5693), Mulberry and Alder 0.722 (5467), Palm and Elm 0.72 (n=5480), Acacia and Eucalyptus 0.709 (5490), Privet and Olive 0.706 (5665), Bottlebrush and Alder 0.703 (n=3059). All Spearman correlation coefficients between tree allergens are shown in Table 1. A heat map to visualize Spearman correlation coefficients between tree allergens is shown in Figure 1A.

Of weed allergens tested, the highest correlation was 0.74 between Lambs Quarter and Pigweed (n=5448). Six more weed pairs had correlation over 0.7: Plantain and Lambs Quarter 0.725 (n=5631), Iodine Bush and Plantain 0.718 (n=5439), Iodine Bush and Lambs Quarter 0.71 (n=5438), Sage and Western Ragweed 0.705 (n=9099), Lambs Quarter and Russian Thistle 0.705 (n=5632), Pigweed and Russian Thistle 0.704 (n=9055). All Spearman correlation coefficients between weed allergens are shown in Table 2. A heat map to visualize Spearman correlation coefficients between weed allergens is shown in Figure 1B.

Of mold allergens tested, the highest correlation was 0.711 between *Helminthosporium* and *Epicoccum* (n=5459). No other mold pairs had correlation over 0.7. All Spearman correlation coefficients between mold allergens are shown in Table 3. A heat map to visualize Spearman correlation coefficients between mold allergens is shown in Figure 1C.

The only grasses tested at the main allergy clinic were Kentucky and Bermuda, which had a correlation of 0.768 (n=9348).

Subgroup analyses were performed on SPT results from the main allergy clinic. The subgroups were age (less than 2, 2 - 5.99, 6 – 11.99, 12 and up), gender (female, male), and presence of asthma (yes, no). Average Spearman correlation coefficients of wheal SPT results were highest for the age less than 2 groups for trees 0.899, weeds 0.761 and mold 0.743 as well as the 12 and up group for weeds 0.701. Average Spearman correlation coefficients of wheal SPT results for the other subgroups ranged from 0.495 to 0.686. Heat maps to visualize Spearman correlation coefficients within each age group are shown in Figure 2 for tree allergens, Figure 3 for weed allergens, and Figure 4 for mold allergens.

Jennrich's test compares two correlation matrices to see if they are equal or not. Getting a significant result in this test ($p < 0.05$) means that the two correlation matrices are different. Jennrich's test showed significant differences (p-value <0.001) between all of the subgroup analyses (ages, gender and presence of asthma) of tree, weed and mold results except for the age less than 2, where there were not enough samples to assess.

Data from Satellite Clinic

Data was collected and analyzed for 1,587 patients from the satellite clinic. Patients were 50.5% female (n=802) and 49.5% male (n=785). Age in years was 5.4% less than 2 (n=85), 25.5% 2 to 5.99 (n=405), 27.1% 6 to 11.99 (n=431), and 42% 12 and up (n=666, range 12 – 85 years old, mean age 31.49). There was no data regarding diagnosis of asthma or SPT location. A summary of the patient characteristics is shown in eTable 1. Taxonomic distribution of the tested allergens is shown in eTable 2.

The Northern grass comparative results are from the satellite clinic where more grasses were used in SPT than the main allergy clinic. The highest correlation was 0.893 between Fescue and Red Top (n=404). Thirteen more grass pairs had correlation over 0.8: Red Top and Brome 0.881 (n=404), Brome and Rye 0.86 (n=407), Fescue and Brome 0.857 (n=405), Timothy and Johnson 0.843 (n=732), Rye and Orchard 0.836 (n=409), Fescue and Orchard 0.836 (n=407), Red Top and Rye 0.833 (n=405), Fescue and Rye 0.831 (n=411), Red Top and Orchard 0.831 (n=403), Brome and Orchard 0.823 (n=403), Perennial Rye and Timothy 0.822 (n=294), Johnson and Bermuda 0.817 (n=745), Kentucky Blue and Bermuda 0.803 (n=76). The remainder of the grass pairs had correlation over 0.7. All Spearman correlation coefficients between grass allergens are shown in Table 4. A heat map to visualize Spearman correlation coefficients between grass allergens is shown in Figure 1D.

The sample size was too low for grass allergens from the satellite clinic to perform subgroup analysis.

Discussion

This data confirms high correlation of SPT results among allergens in the same genus, suggestive of cross-reactivity. Cross-reactivity is the ability of an antigen to bind an antibody that was created in response to a different antigen. It can arise due to either shared epitopes on multivalent antigens or conformational similarity of epitopes. The general patterns of botanical cross-allergenicity are that there is rarely significant cross-allergenicity between families, there is generally a degree of cross-allergenicity between tribes or genera of a family, and there is generally a high degree of cross-allergenicity between species of the same genus. (7)

Where there is high correlation across genera, this could represent cross-reactivity, ~~or~~ co-sensitization, or likely a combination of the two and future serologic studies of potential cross-reactivity are warranted. Low correlation between allergens is suggestive against cross-reactivity. Dust mite cross-reactivity and co-sensitization is common, and the high correlation between the two most allergenically relevant dust mites testing in this population, *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*, confirm this.

This study confirms high correlation between trees in the same families, such as Oleaceae and Fabaceae. There is also high correlation between several trees in different families, which is more likely to represent co-sensitization.

The weed results showed high correlation within the Amaranthaceae and Asteraceae families ~~family~~, but also between several families. In general amongst weeds there is great diversity and minimal cross-reactivity, with some notable exceptions. There is significant cross-reactivity seen within the Amaranthaceae family, Asteraceae members show variable cross-reactivity, and the four major ragweeds (short, giant, western, and false) strongly cross-react (1).

Amongst molds this study only showed high correlation between *helminthosporium* and *epicoccum*. Cross-reactivity between fungal allergens is poorly delineated, although certain patterns exist. There are some associations between Deuteromycetes, with *Aspergillus* and *Penicillium* species showing some cross-reactivity and *Alternaria* and *Cladosporium* have shared allergens (1).

Grass allergic patients often have multiple grass pollen sensitivities. Because many grass species coexist in the same geographical region, this likely represents co-sensitization.

Radioallergosorbent test and enzyme-linked immunosorbent assay inhibition assays have revealed extensive allergenic cross-reactivity among taxonomically related grasses (1). Among

grasses, cross-reactivity is thought to be present between the Panicoids with both Pooids and Chloridoids, but not between the Pooid and Chloridoid subfamilies. Given this belief, many use one representative grass from each family for testing and therapy, such as Timothy (Pooid), Bermuda (Chloridoid) and Johnson (Panicoid) grasses. (2). This study confirms high correlation among grasses in the Pooid family, Pooids with Johnson grass (the one Panicoid tested), as well as between Bermuda grass, Johnson grass and the Pooids. There is also high correlation between Bermuda grass, the one Chloridoid tested, and many of the Pooids. It is possible this correlation is due to co-sensitization, as patients in Southern California are exposed to both families of grass. In analyzing subgroups and their effect on skin test result correlations, co-morbid asthma, race and gender did not have any effect. Age was associated with differences in correlation coefficients, but with no clear pattern. The youngest patients (less than 2 years old) had the highest degree of correlation. This could be due to higher co-sensitization at that age, however the opposite would typically be expected, as people become sensitized to more allergens as they age. In a study of 404 children tested longitudinally from 0 to 6 years of age, sensitization assessed by serum IgE levels to inhalant allergens (birch, grass, mugwort, *D. Pteronyssinus*, *D. farinae*, dog, cat, horse, *Alternaria*, and *Cladosporium*) increased with age (8). It should be noted that the number of patients in the less than 2 years old group was lower than the other groups, which may have impacted the correlation seen in this age group.

This study has several limitations. As it was done retrospectively, there are limitations in the data available to collect from patients. We were not able to correlate SPT results with true symptoms of allergy to assess sensitization versus allergy. The SPT was done per clinical standards at these two sites, which did not include any dilutions of SPT or intradermal testing to further quantify the degree of sensitization to the allergens. Correlation coefficients between different categories

184 of environmental allergens were not compared due to the difficulty in interpreting co-
185 sensitization versus cross-reactivity. The only species tested are those present in this
186 geographical area (Southern California), so sensitization patterns for the patients in this study
187 may differ from patients who live in other locations with different pollen exposures, limiting the
188 generalizability of these results. However, given the temperate climate in Southern California,
189 seasonal variability in SPT results is less likely to be a confounder. Importantly, as this was
190 retrospective, we did not analyze any patient serum for cross-reactivity, so we are only able to
191 report correlation coefficients and are unable to assess whether these correlations represent cross-
192 reactivity or just co-sensitization. It is likely both are playing a role in the correlations seen in
193 this study.

Acknowledgements

Statistical analysis was performed by Euyhyun Lee and James Proudfoot.

References

1. Weber RW. Cross-reactivity of plant and animal allergens. *Clinic Rev Allerg Immunol*. 2001 Oct;21(2-3):153-202.
2. Lockey RF, Ledford DK, editors. Allergens and allergen immunotherapy: subcutaneous, sublingual and oral. 5th edition. Baton Rouge, FL: CRC Press, Taylor & Francis Group. 2014.
3. Aalberse RC. Assessment of allergen cross-reactivity. *Clin Mol Allergy*. 2007 Feb 9;5:2.
4. Bernstein IL, Perera M, Gallagher J, Michael JG, Johansson SG. In vitro cross- allergenicity of major aeroallergenic pollens by the radioallergosorbent technique. *J Allergy Clin Immunol*. 1976 Feb;57(2):141-52.
5. Leiferman KM, Gleich GJ, Jones RT. The cross-reactivity of IgE antibodies with pollen allergens. II. Analyses of various species of ragweed and other fall weed pollens. *J Allergy Clin Immunol*. 1976 Jul;58(1 PT. 2):140-8.
6. Cox L, Nelson H, Lockey R, Calabria C, Chacko T, Finegold I, et al. Allergen immunotherapy: A practice parameter third update. *J Allergy Clin Immunol*. 2011 Jan;127(1 Suppl):S1-55.
7. Burks AW, Holgate ST, O'Hehir RE, et al. 2020. Middleton's Allergy: Principles and Practice: Ninth Edition. Elsevier.
8. Kjaer HF, Eller E, Andersen KE, Høst A, Bindslev-Jensen C. The association between early sensitization patterns and subsequent allergic disease. The DARC birth cohort study. *Pediatr Allergy Immunol*. 2009 Dec;20(8):726-34.

Table 1: Spearman correlation coefficients between wheal size of tree allergen SPT results. Number of patients (n) in parenthesis.

	Olive	Mesquite	Privet	Palo Verde	Acacia	Eucalyptus	Oak	Walnut	Pepper	Willow	Elm	Palm	Alder	Mulberry	Cedar
Mesquite	0.544 (9318)														
Privet	0.706 (5665)	0.560 (5666)													
Palo Verde	0.630 (5691)	0.582 (5680)	0.665 (5672)												
Acacia	0.572 (5695)	0.539 (5683)	0.624 (5671)	0.722 (5693)											
Eucalyptus	0.534 (5490)	0.526 (5474)	0.591 (5470)	0.658 (5484)	0.709 (5490)										
Oak	0.606 (9149)	0.519 (9111)	0.573 (5469)	0.668 (5486)	0.663 (5494)	0.650 (5489)									
Walnut	0.572 (5471)	0.541 (5467)	0.588 (5468)	0.681 (5474)	0.673 (5476)	0.686 (5477)	0.693 (5474)								
Pepper	0.572 (5466)	0.541 (5466)	0.577 (5468)	0.670 (5472)	0.669 (5472)	0.647 (5471)	0.666 (5469)	0.689 (5469)							
Willow	0.527 (5480)	0.531 (5467)	0.567 (5468)	0.623 (5482)	0.638 (5484)	0.641 (5484)	0.631 (5478)	0.665 (5474)	0.687 (5469)						
Elm	0.549 (5692)	0.536 (5677)	0.557 (5668)	0.674 (5689)	0.647 (5693)	0.624 (5483)	0.645 (5493)	0.658 (5473)	0.663 (5467)	0.674 (5477)					
Palm	0.534 (5483)	0.531 (5467)	0.573 (5466)	0.645 (5477)	0.669 (5486)	0.659 (5483)	0.630 (5483)	0.669 (5468)	0.660 (5465)	0.653 (5474)	0.720 (5480)				
Alder	0.555 (5467)	0.540 (5464)	0.589 (5465)	0.662 (5469)	0.672 (5469)	0.665 (5470)	0.659 (5471)	0.676 (5467)	0.649 (5466)	0.652 (5466)	0.693 (5469)	0.724 (5465)			
Mulberry	0.567 (5479)	0.543 (5465)	0.574 (5465)	0.663 (5478)	0.650 (5480)	0.642 (5481)	0.645 (5479)	0.667 (5471)	0.668 (5466)	0.643 (5476)	0.664 (5477)	0.694 (5475)	0.722 (5467)		
Cedar	0.483 (5479)	0.479 (5466)	0.533 (5467)	0.595 (5480)	0.620 (5480)	0.617 (5481)	0.567 (5480)	0.619 (5470)	0.601 (5467)	0.602 (5474)	0.608 (5482)	0.634 (5478)	0.656 (5467)	0.664 (5474)	
Bottlebrush	0.596 (3056)	0.546 (3059)	0.600 (3059)	0.640 (3061)	0.656 (3061)	0.650 (3061)	0.646 (3059)	0.689 (3060)	0.648 (3059)	0.657 (3059)	0.653 (3059)	0.693 (3060)	0.703 (3059)	0.692 (3059)	0.675 (3060)

Table 2: Spearman correlation coefficients between wheal size of weed allergen SPT results. Number of patients (n) in parenthesis.

	West Ragweed	Sage	Russian Thistle	Pigweed	Lambs Quarter	Plantain
Sage	0.705 (9099)					
Russian Thistle	0.682 (9296)	0.674 (9070)				
Pigweed	0.640 (9049)	0.652 (9022)	0.704 (9055)			
Lambs Quarter	0.642 (5629)	0.641 (5430)	0.705 (5632)	0.740 (5448)		
Plantain	0.628 (5671)	0.652 (5473)	0.651 (5664)	0.691 (5446)	0.725 (5631)	
Iodine Bush	0.643 (5436)	0.641 (5425)	0.699 (5436)	0.697 (5438)	0.710 (5438)	0.718 (5439)

Table 3: Spearman correlation coefficients between wheal size of mold allergen SPT results. Number of patients (n) in parenthesis.

	Alternaria	Epicoccum	Helminthosporium	Penicillium	Cladosporium
Epicoccum	0.685 (5455)				
Helminthosporium	0.621 (5457)	0.711 (5459)			
Penicillium	0.495 (5489)	0.579 (5458)	0.617 (5459)		
Cladosporium	0.508 (5496)	0.583 (5452)	0.581 (5454)	0.688 (5486)	
Aspergillus	0.498 (5480)	0.569 (5430)	0.562 (5432)	0.647 (5475)	0.698 (5459)

Table 4: Spearman correlation coefficients between wheal size of grass allergen SPT results. Number of patients (n) in parenthesis. AAMG data only.

	Bermuda	Johnson	Timothy	Orchard	Rye	Brome	Red Top
Johnson	0.817 (745)						
Timothy	0.764 (733)	0.845 (732)					
Orchard	0.706 (410)	0.725 (411)	0.766 (410)				
Rye	0.730 (431)	0.701 (432)	0.770 (431)	0.836 (409)			
Brome	0.760 (406)	0.754 (407)	0.793 (406)	0.823 (402)	0.860 (407)		
Red Top	0.746 (404)	0.751 (405)	0.786 (404)	0.831 (403)	0.833 (405)	0.881 (404)	
Fescue	0.715 (410)	0.682 (411)	0.737 (410)	0.836 (407)	0.831 (411)	0.857 (405)	0.893 (404)
Perennial Rye	0.720 (295)	0.756 (294)	0.822 (294)				

Figure Legends

Figure 1: Visualization of correlations between wheal results of SPT to environmental aero-allergens. Clockwise from top left: trees (A), weeds (B), grasses (C) and molds (D).

Figure 2: Visualization of correlations between wheal results of skin prick testing to trees. Clockwise from top left: ages less than 2, 2 to 5.99, 6 to 11.99, and 12 and up.

Figure 3: Visualization of correlations between wheal results of skin prick testing to weeds. Clockwise from top left: ages less than 2, 2 to 5.99, 6 to 11.99, and 12 and up.

Figure 4: Visualization of correlations between wheal results of skin prick testing to molds. Clockwise from top left: ages less than 2, 2 to 5.99, 6 to 11.99, and 12 and up.

Figure 1: Spearman correlation coefficient heat maps.

Journal Pre-proof

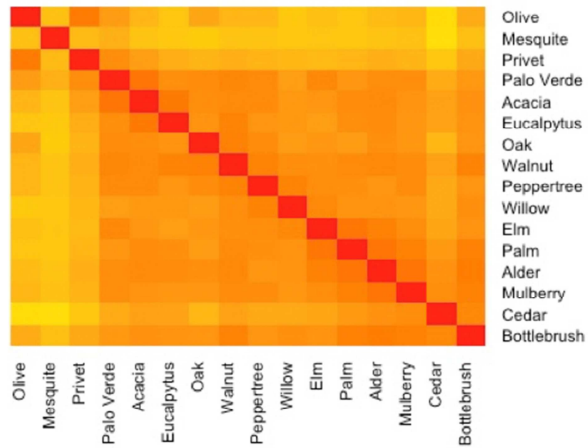
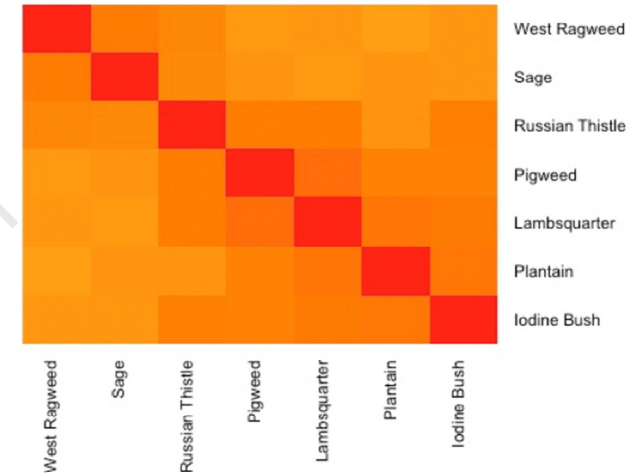
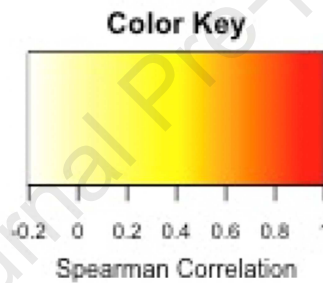
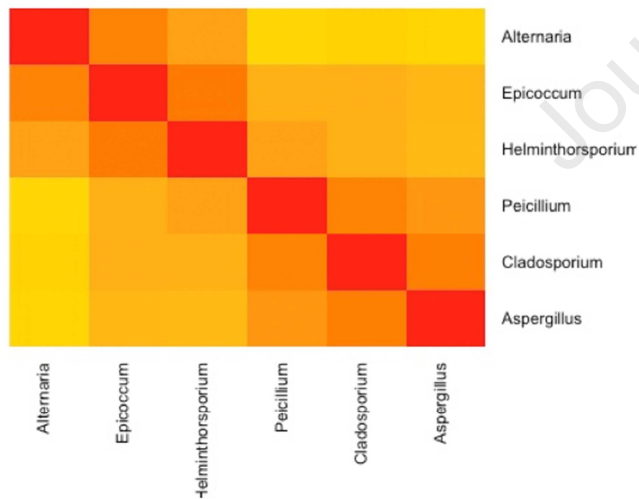
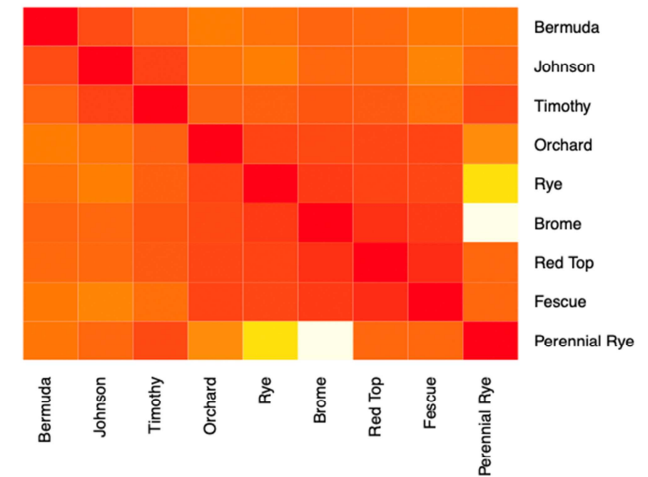
A) Trees ($n = 3056 - 9318$)**B) Weeds ($n = 5425 - 9296$)****C) Molds ($n = 5430 - 5496$)****D) Grasses ($n = 294-745$)**

Figure 2: Spearman correlation coefficient heat maps of tree allergens separated by patient age.

Journal Pre-proof

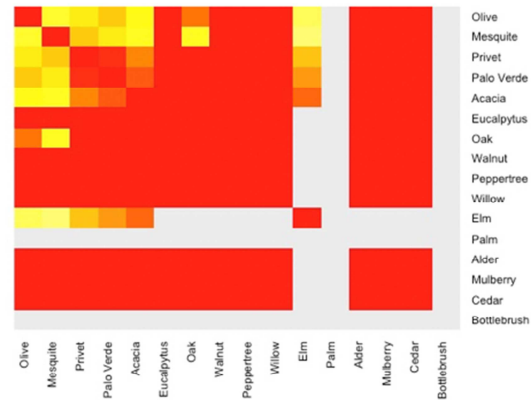
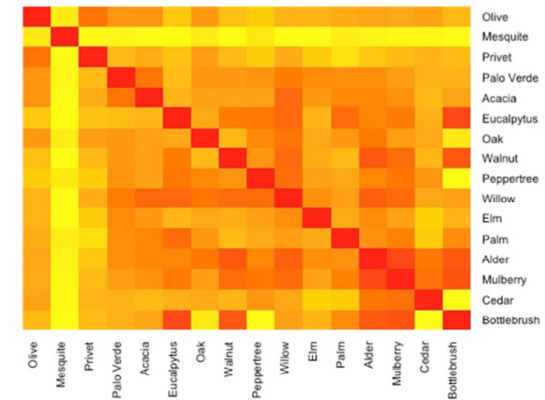
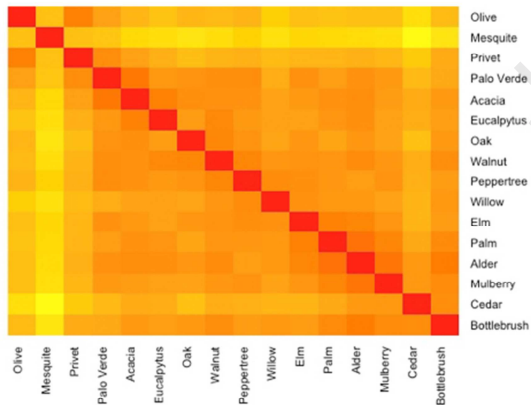
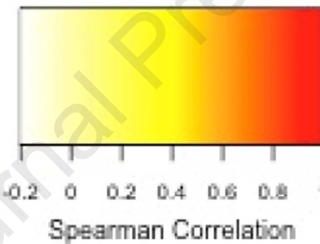
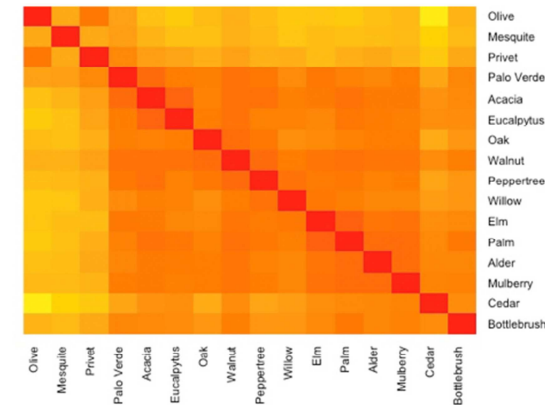
A) Age ≤ 2 ($n = 1 - 598$)**B) Age 2 – 5.99 ($n = 46 - 2979$)****C) Age 6 – 11.99 ($n = 1934 - 3785$)****Color Key****D) Age ≥ 12 ($n = 1073 - 1963$)**

Figure 3: Spearman correlation coefficient heat maps of weed allergens separated by patient age.

Journal Pre-proof

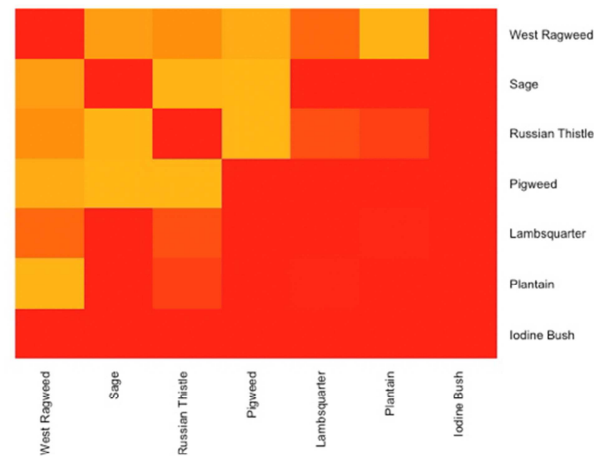
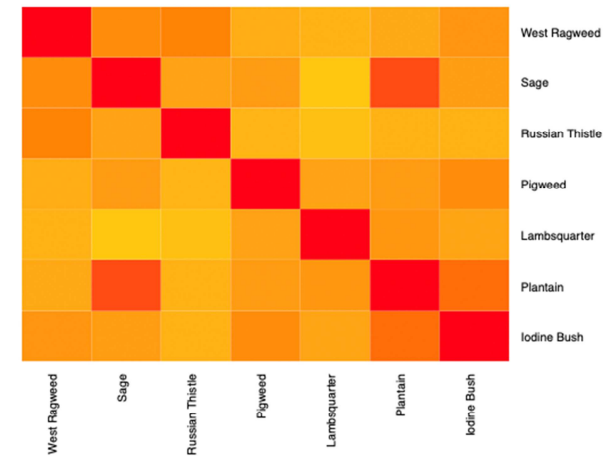
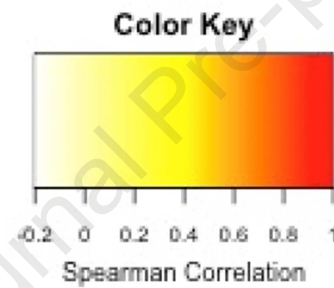
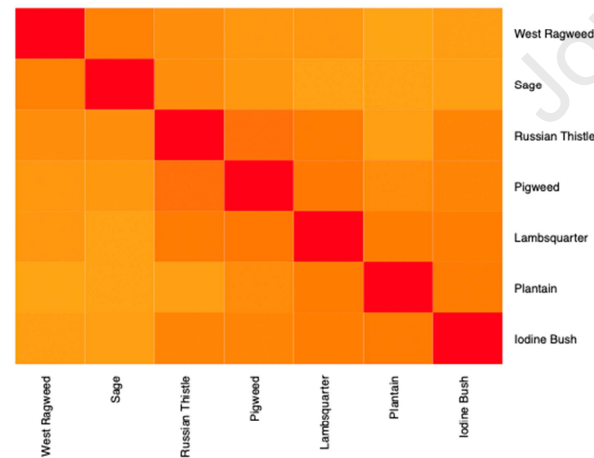
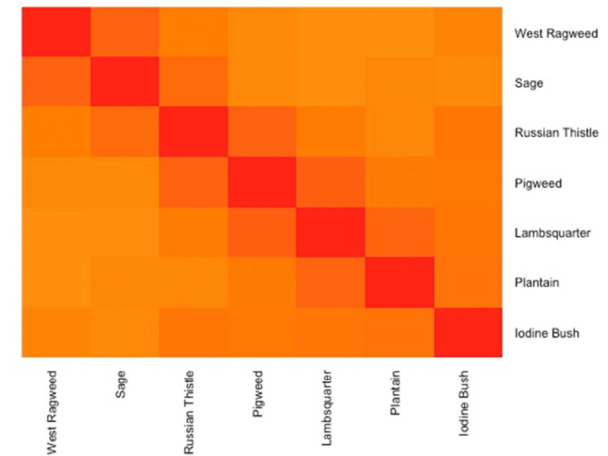
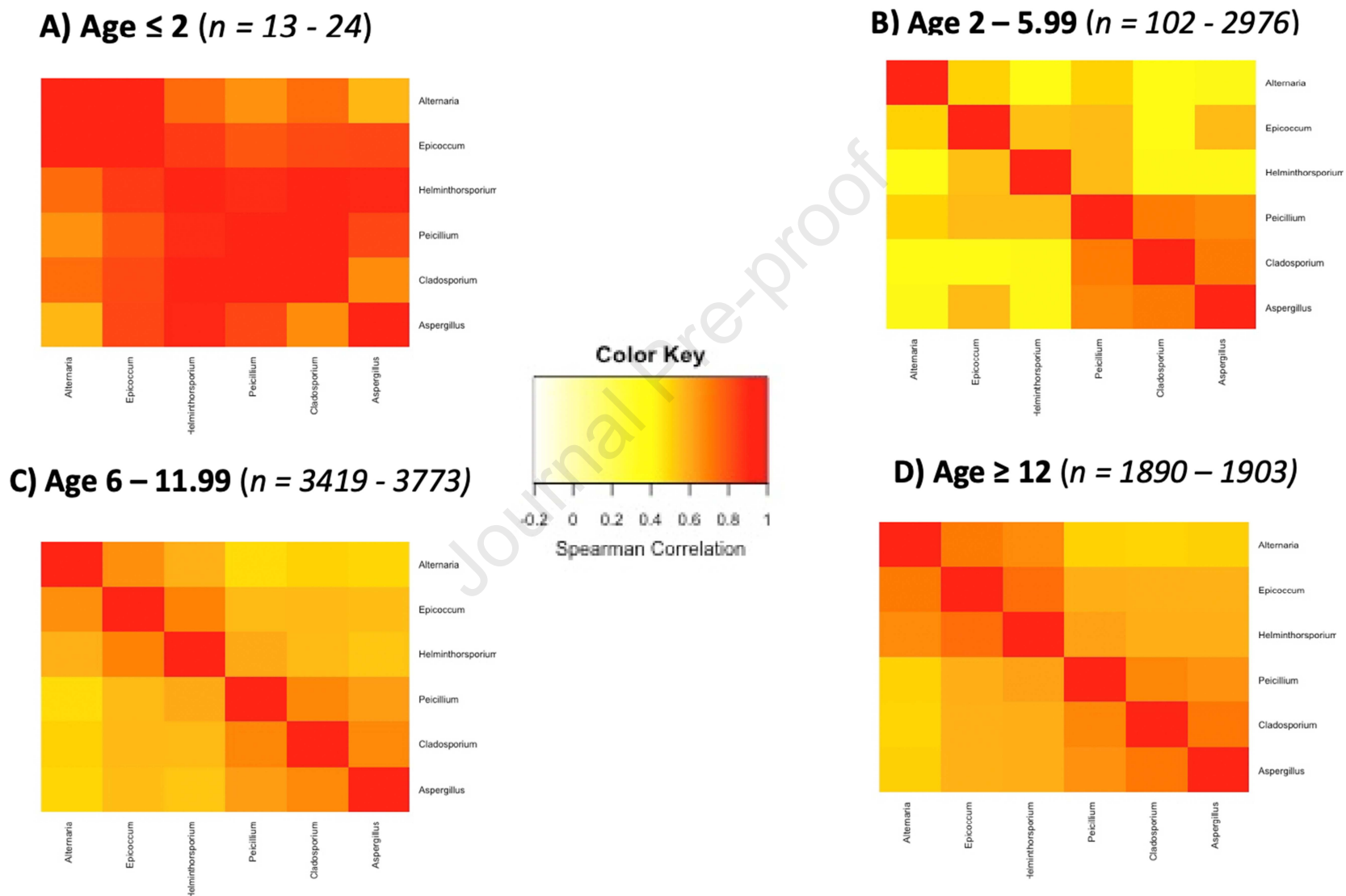
A) Age ≤ 2 ($n = 3 - 594$)**B) Age 2 – 5.99 ($n = 102 - 2976$)****C) Age 6 – 11.99 ($n = 3419 - 3773$)****D) Age ≥ 12 ($n = 1899 - 1953$)**

Figure 4: Spearman correlation coefficient heat maps of mold allergens separated by patient age.



eTable 1: Summary of patient characteristics.

	Main Clinic	Satellite Clinic
Gender		
Female	4330 (42.3%)	802 (50.5%)
Male	5915 (57.7%)	785 (49.5%)
Age		
Less than 2	1101 (10.7%)	85 (5.4%)
2 to 5.99	3233 (31.6%)	405 (25.5%)
6 to 11.99	3899 (38.1%)	431 (27.1%)
12 and up	2011 (19.6%)	666 (42.0%)
Asthma		
No	6105 (59.6%)	
Yes	4142 (40.4%)	
SPT Location		
Arm	36 (0.4%)	
Back	10093 (99.6%)	

Table 2: Taxonomic distribution of environmental allergens tested based on family (trees, weeds, grasses and insects) or phylum (molds).

Trees	Weeds	Grasses	Molds	Insects
• Anacardiaceae - o Pepper • Arecaceae - o Palm • Betulaceae - o Alder • Cupressaceae - o Cedar • Fabaceae - o Acacia - o Mesquite - o Palo Verde • Fagaceae - o Oak • Juglandaceae - o Walnut • Moraceae - o Mulberry • Myrtaceae - o Eucalyptus - o Bottlebrush • Oleaceae - o Olive - o Privet • Salicaceae - o Willow • Ulmaceae - o Elm	• Amaranthaceae - o Iodine Bush - o Lamb's Quarter - o Pigweed - o Russian Thistle • Asteraceae - o Sage - o Western Ragweed • Plantaginaceae - o Plantain	• Chloridoideae - o Bermuda • Panicoideae* - o Johnson • Pooideae - o Brome - o Fescue - o Kentucky blue - o Orchard - o Perennial Rye - o Red Top - o Rye - o Timothy	• Ascomycota - o Alternaria - o Aspergillus - o Cladosporium - o Epicoccum - o Helminthosporium - o Penicillium	• Pyroglyphidae - o D. pteronyssinus - o D. farniae

* Allergically cross-reactive with both pooids and chloridoids

eTable 3: High correlation coefficients and taxonomic relationships

Same Family				Different Families		
Correlation coefficient	Family	Allergen	Allergen	Correlation coefficient	Allergen (Family)	Allergen (Family)
0.893	Pooideae	Fescue grass	Red Top grass	0.845	Johnson grass (Panicoideae)	Timothy grass (Pooideae)
0.881	Pooideae	Brome grass	Red Top grass	0.817	Bermuda grass (Chloridoideae)	Johnson grass (Panicoideae)
0.860	Pooideae	Brome grass	Rye grass	0.725	Lamb's Quarter (Amaranthaceae)	Plantain (Plantaginaceae)
0.857	Pooideae	Brome grass	Fescue grass	0.724	Alder (Betulaceae)	Palm (Arecaceae)
0.848	Pyroglyphidae	D. farinae	D. pteronyssinus	0.722	Alder (Betulaceae)	Mulberry (Moraceae)
0.836	Pooideae	Orchard grass	Rye grass	0.720	Elm (Ulmaceae)	Palm (Arecaceae)
0.836	Pooideae	Fescue grass	Orchard grass	0.718	Iodine Bush (Amaranthaceae)	Plantain (Plantaginaceae)
0.833	Pooideae	Red Top grass	Rye grass	0.709	Acacia (Fabaceae)	Eucalyptus (Myrtaceae)
0.831	Pooideae	Fescue grass	Rye grass	0.703	Alder (Betulaceae)	Bottlebrush (Myrtaceae)
0.831	Pooideae	Orchard grass	Red Top grass			
0.823	Pooideae	Brome grass	Orchard grass			
0.822	Pooideae	Perennial Rye grass	Timothy grass			
0.740	Amaranthaceae	Lamb's Quarter	Pigweed			
0.722	Fabaceae	Acacia	Palo Verde			
0.711	Ascomycota	Helminthosporium	Epicoccum			
0.710	Amaranthaceae	Iodine Bush	Lamb's Quarter			
0.706	Oleaceae	Olive Tree	Privet Tree			
0.705	Asteraceae	Sage	Western Ragweed			
0.705	Amaranthaceae	Lamb's Quarter	Russian Thistle			
0.704	Amaranthaceae	Pigweed	Russian Thistle			