Modelling the conversion between specific IgE test platforms for nut allergens in children and adolescents

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Abstract

Background: Multiplex tests allow for measurement of allergen-specific IgE responses to multiple allergen extracts and components and have several advantages for large cohort studies. Due to significant methodological differences, test systems are difficult to integrate in meta-analyses/systematic reviews since there is a lack of datasets with direct comparison. We aimed to create models for statistical integration of allergen-specific IgE to peanut/tree nut allergens from three IgE-test platforms.

Methods: Plasma from Canadian and Austrian children with peanut/tree nut sensitization and a cohort of sensitized, high-risk, pre-school asthmatics (total n=166) were measured with three R&D multiplex IgE test platforms: Allergy Explorer, ALEX (Macro Array Dx), MeDALL-chip (Mechanisms of Development of Allergy) (Thermo Fisher), and EUROLINE (EUROIMMUN). Skin prick test (n=51) and ImmunoCAP (n=62) results for extracts were available in a subset. Regression models (Multivariate Adaptive Regression Splines, local polynomial regression) were applied if >30% of samples were positive to the allergen. Intra-test correlations between PR-10 and nsLTP allergens were assessed.

Results: Using two regression methods, we demonstrated the ability to model allergen-specific relationships with acceptable measures of fit (r^2=94-56%) for peanut and tree nut sIgE testing at the extract and component-level, in order from highest to lowest: Ara h 2, Ara h 6, Jug r 1, Ana o 3, Ara h 1, Jug r 2, Cor a 9.

Conclusion: Our models support the notion that conversion is reasonably possible between sIgE multiplex platforms for allergen extracts and components and may provide options to aggregate data for future meta-analysis.

Keywords: allergen, component resolved diagnosis, conversion, food allergy, specific IgE

Introduction

Allergen-specific IgE sensitization is routinely assessed during the clinical workup of allergic diseases, including food allergy, allergic rhinitis, and allergic asthma[1]. To overcome the significant degree of cross-reactivity observed due to homology of allergens from the same protein family, component-resolved diagnostic (CRD) testing or molecular allergology was developed, which focuses on individual allergens[2]. Molecular allergology is useful for assessing and predicting food allergies, such as peanut allergy, with a high sensitivity and specificity[3]. To date, ~880 allergens have been acknowledged by the Allergen Nomenclature database of the World Health Organization/International Union of Immunological Societies[4]. As our knowledge of allergens expands, so does the number of platforms used to measure sensitization to these allergens. Consequently, there is a growing demand for strategies that can take advantage of molecular diagnostics by integrating data from different platforms used in cohort studies. However, previously undertaken systematic reviews examining the evidence on CRD testing have been largely limited from performing meta-analyses due to heterogeneity in methods and a shortage of studies comparing different platforms head-to-head[5].

While single-plex testing methods such as the ImmunoCAP® remain the most widely used for sIgE quantification[6], they limit the breadth of testing that can be performed because of high costs and serum demands. These are important considerations for birth cohort studies measuring early-life sensitization patterns, where it is important to assess both food and inhalant allergen-specific antibody responses but limited serum volumes available. Multiplex methods tend to have smaller sample requirements and are more cost-effective for large-scale studies relative to single-plex methods[6]. Thus, multiplex IgE assays are often advantageous for both clinical and research purposes, and different versions including some assessing viral responses have been developed[7,8].

Multiplex test systems that are currently available include the Immuno Solid-phase Allergen Chip (ISAC) (Thermo Fisher Scientific, Phadia AB, Uppsala, Sweden), also present in the R&D version as the Mechanisms of development of allergy (MeDALL)-chip[9]. The MeDALL-chip contains up to 176 allergen components analyzed using a fluorescence-based detection system[10]. Other tests using alternative approaches have also been launched, including blot-based systems used by EUROLINE (EUROIMMUN, Lubeck, Germany), which can be assembled into groups of allergens and extracts customized for the country of measurement, and colorimetric systems like

87 the Allergy Explorer (ALEX) (Macro Array Diagnostics, Vienna, Austria), measuring 156 extracts
88 and 126 components.

89 Despite the advantages of multiplex methods, they are reported to have higher coefficients of
90 variation and lower test sensitivity than single-plex methods[11]. Furthermore, multiplex systems
91 differ from one another in methodologies and units of measurement. For example, the amount of
92 allergen applied on the chip/surface can vary up to 10000-fold, making inhibition by IgG
93 antibodies more likely to influence IgE measurement[6]. Therefore, it has been argued that results
94 are not directly interchangeable between platforms such as the ImmunoCAP and ISAC[12].
95 Meanwhile, studies have observed strong correlations between different sIgE platforms for
96 inhalant allergens between ImmunoCAP and ISAC[12], ALEX and ISAC[13], and for peanut allergens
97 between ImmunoCAP and ISAC[14], and EUROLINE and ISAC[15], which supports the possibility
98 of developing conversion models between platforms despite differences in units of measurement.
99 Given the importance of longitudinal sensitization data, models or data that allow for conversion
100 between test platforms could facilitate data synthesis between studies utilizing different platforms
101 and directly impact knowledge generation.

102 Therefore, the objective of this study was to develop models that enable the conversion of sIgE
103 results between different platforms, with a focus on nut allergens. Clinical performance of the
104 platforms was not assessed, as this was beyond the scope of the study. Conversion was performed
105 using two statistical regression methods to harmonize sIgE results obtained by MeDALL-chip,
106 EUROLINE, and ALEX platforms from sensitized and/or nut allergic patients.

Methods

Study Population and Clinical Assessment

A total of 166 subjects were included in this analysis who met the criteria for one of two studies on pediatric nut allergy or preschool asthma. Subjects of the Markers of Nut Allergy Study (MONAS) (n = 124) from age 6 months to 17 years were recruited at the allergy clinic at the Hospital for Sick Children (Toronto, Canada; n=98) or the Department of Pediatrics, Medical University of Vienna (Vienna, Austria; n=26) based on a peanut or tree nut allergy diagnosis or sensitization that was determined by clinical history, positive SPT, or sIgE tests. Sensitization by SPT (Omega Laboratories LTD, Montreal, Canada) or sIgE (ImmunoCAP, Phadia AB, Sweden) was assessed as part of routine clinical testing. This study was approved by the ethics board of the Hospital for Sick Children (REB #:1000053791) and the Medical University of Vienna (EK Nr. 2302/2016). Additionally, 42 children with sensitizations were selected from the Wheezy ER study on preschool asthma between 3-6 years of age (REB #:1000041089), based on inclusion criteria reported previously[16,17]. These children presented to the Emergency Department at the Hospital for Sick Children with an acute wheezing exacerbation, a history of at least two wheezing episodes during the last 12 months, and had displayed sensitization to food and/or inhalant allergens[18].

Test systems

The Allergy Explorer (ALEX, Macro Array Diagnostics) is a nitrocellulose chip-based immunoassay, containing 156 allergen extracts and 126 allergen molecules. Patient plasma (100µl) was incubated on the chip in a 1:5 dilution with a cross-reactive carbohydrate determinant (CCD) inhibitor-containing diluent. Detection was performed with alkaline phosphatase-labelled anti-human IgE, as previously described[18]. The sIgE was quantified with Raptor software (Macro Array Diagnostics) within a reportable range of 0.3-40 kUA/L. Positive sensitizations were defined as ≥ 0.30 kUA/L. Allergens in a different formulation on the new ALEX-2 were not considered for analysis. All kits were purchased by the authors without involvement of the companies.

The Mechanisms of development of allergy (MeDALL)-chip is a fluorochrome-based system measuring sIgE to 176 allergen components that are spotted in triplicates onto a glass slide[19–22]. Microarrays were incubated with 35 µl of patient plasma and detected using a fluorochrome-

conjugated IgE detection antibody according to previously published methods within the reportable range of 0.3-100 ISU[10,23]. Positive sensitizations were defined as ≥ 0.3 ISU.

The EUROLINE test (EUROIMMUN) is a blot-based test system. Two R&D test panels were performed per patient sample for a total of 12 extracts, 17 components and a CCD marker. Each strip was incubated with 175 µl of patient plasma. Detection was performed with an enzyme-conjugated anti-human IgE antibody, followed by addition of enzyme substrate according to manufacturer's instructions. EuroLineScan software (EUROIMMUN) was used to evaluate strips in the reportable range of 0.35-100 kUA/L. Positive sensitizations were defined as ≥ 0.35 kUA/L.

Statistical Analysis

For each platform (ALEX, MeDALL-chip, and EUROLINE) we determined a dynamic range based on our current data and previous observations. For ALEX measurements (n = 166), this was defined as between 0.3 kUA/L and 40 kUA/L, for MeDALL-chip (n = 166) between 0.3 ISU and 100 ISU, and for EUROLINE (n = 121) between 0.35 kUA/L and 100 kUA/L. Any values greater or less than these values were set to the maximum value or 0 respectively. Since our aim was to directly compare values presented by each platform, we did not perform any additional scaling or centering.

Each extract or component was assessed within each platform in a pairwise fashion if at least one other platform contained the corresponding allergen. Among these allergens, only those with at least 30% of the samples within the dynamic ranges in one of the tests stated above were used for modelling. All other extracts and components were excluded from regression analysis. A visual inspection of the pairwise values of some pairwise scatter plots revealed that while there are visible trends, these were not strictly linear and were specific to each comparison. To be able to accurately and consistently model this behavior, we opted to employ local polynomial regression (loess)[24] and multivariate adaptive regression splines (MARS)[25].

Local polynomial regression was conducted as follows: before beginning model training, each data matrix was first row-wise randomized to minimize any potential data collection effects. For local regression, the only parameter we needed to optimize per model was the span parameter to control the local environment of the polynomial regression. To find the best span parameter, after

randomization, the data matrix was split into 5 sections for 5-fold cross validation. Four of these 5 sections are then used to fit loess regressions for any given span value and the remaining section is used for calculation of the residual sum of squares (rss). This procedure is repeated five times using each section as a test set for each model and span value. The mean of the rss is calculated for these models and was used as the minimization target for numerical optimization using base R's optimize function, which utilizes a combination of golden section search and successive parabolic interpolation[26]. Whenever a span value failed to converge for the loess algorithm, an arbitrarily large number was used instead of rss as penalty. For each comparison, this winning span parameter is then used to fit the entire dataset for the final model. For each model trained we manually inspected model parameters, scatterplots overlaid with model fits and residual vs fitted plots for any aberrant behavior.

For MARS regression, we employed 5-fold cross validation (nfold) with 30 iterations (ncross) in order to avoid overfitting. Due to our small dataset, our variance method (varmod.method) was constructed using linear regression for standard deviation estimation. To avoid excessive model complexity, we finalized the resulting regression model using "exhaustive" pruning. All analysis was conducted using R statistical software[27] version 3.6.0. For MARS regression, earth package version 5.1.1[28], and for loess regression, stats package within the base R distribution was used.

Results

Patient characteristics

The mean age of the 166 patients was 8.2 years $\pm$ 4.6 years with a male predominance (62%) (Table S1). The majority of the patients had a clinical history of atopy, including atopic eczema (66%), food allergy (76%), asthma (68%), or allergic rhinitis (47%). Food allergy was more prevalent in the MONAS group (92%), enrolling children with peanut and/or tree nut allergy or sensitization, whereas asthma was more prevalent in the Wheezy ER group, focusing on moderate-to-severe pre-school asthmatics (100%; Table S1). Since only IgE test results were compared, these two groups were combined to allow for investigation of allergen extracts and molecules in children with significant sensitizations to food and inhalants.

Agreement of sensitization status across different test methods

Different test systems operate with different amounts of protein, extracts or allergens, and dynamic ranges. The alignment and sensitivity of detection was first evaluated across test systems measuring the same outcome (Fig 1). Among the twelve extracts common to the ALEX and EUROLINE, the blot-based approach (EUROLINE) reported more sensitizations at the extract level than the colorimetric, chip-based approach (ALEX), with over twice as many positive signals to macadamia, sesame and brazil nut, and substantial differences for milk, egg and almond. Almost perfect alignment in detection of peanut extract sIgE was achieved (Fig 1A).

Detection of twelve allergen components from peanut, milk, and egg was also compared between the three platforms (Fig 1B). The peanut components Ara h 1, 2, and 3 were more frequently positive on the ALEX and MeDALL-chip compared to EUROLINE, whereas sensitization to the cow's milk and egg components, Bos d 4, Gal d 2, and Gal d 3, were more frequently positive on EUROLINE (Fig S1, S2). In general, the ALEX and MeDALL-chip platforms reported comparable numbers of sensitization (Fig S3) and strong positive correlations for the majority of individual allergen components (Fig S4). Among the 85 components common to ALEX and MeDALL-chip, the food allergens Gal d 1, Gal d 5, Ara h 8, Ara h 9, Cor a 9, and Fag e 2, and the environmental allergens Art v 1, Can f 2, and Bla g 2 were more frequently positive on the ALEX platform than on the MeDALL-chip (Fig S3). Allergens more frequently recognized by MeDALL included Bet v 2, Fel d 2, Der f 2, Mus m 1, Gly m 5, Amb a 1, Can f 3, Art v 3, Der p 10, 11, Bla g 1, Hev b 1, 3, Pen m 1, Api m 1 and Ves v 5, among others (Fig S3).

Conversion models for nut extract allergens

To assess the conversion between different sIgE test platforms, the loess and MARS modelling approaches were applied to each pairwise comparison of platforms. In the MARS method, the fit of the model can be assessed by the coefficient of determination (r^2), with 1 indicating a perfect model fit where 100% of the variability in the data is explained by the model. Regions where the two modelling approaches aligned were considered as an indicator of the robustness of the models. After filtering for allergen comparisons with over 30% data points within the dynamic range (Table S2), the viable comparisons included peanut extract (Fig 2) and the components Ara h 1, 2, and 6 (Fig 3).

Peanut extract sIgE results were compared between platforms, alongside clinical ImmunoCAP (n = 59) and skin prick test (SPT) (n = 62) results in a subset of patients. As ImmunoCAP is the clinical standard, we assessed conversions between ALEX vs. ImmunoCAP and EUROLINE vs. ImmunoCAP (Fig 2A, 2B). The values predicted by both loess and MARS models exhibited good alignment within the range of 0.35-25 kUA/L of ImmunoCAP units. Overall, the MARS modelling was able to account for 89% of the variability in the conversion between peanut ALEX vs. ImmunoCAP, and 71% of the variability in the conversion between peanut EUROLINE vs. ImmunoCAP comparison (Table S3). Parameter estimates for all model equations can be found in the online supplement. Acceptable convertibility was observed between ALEX vs. EUROLINE platforms for peanut extract as well (r^2 = 84%) (Fig 2C).

Models were also created for tree nut extract sIgE to almond, walnut, and hazelnut (Fig S5). The MARS modeling for ALEX vs. ImmunoCAP values accounted for the majority of variability in walnut sIgE measurements (92%) and a moderate degree of variability in almond (70%) and hazelnut (66%) (Fig S5, Table S3). Similarly, EUROLINE vs. ImmunoCAP and ALEX vs. EUROLINE comparisons reported higher r^2 values for walnut extract at 82% and 92%, compared to almond extract at 69% and 70% respectively.

In contrast, interconversion of sIgE and SPT using this modelling approach was less useful. The MARS conversion models for peanut extract accounted for only 24% of the variability between ALEX vs. SPT and 32% of variability between ImmunoCAP vs. SPT (Fig 2D, 2E).

Conversion models for molecular nut allergens

Modelling was applied to molecular peanut allergens Ara h 1, 2, and 6 (Fig 3). Comparing ALEX vs. MeDALL-chip, ALEX exhibited a reduced dynamic range above the amount of 25-35 kUA/L (based on internal ImmunoCAP calibration standard on ALEX). The MARS model fit varied between allergens. Ara h 2 exhibited the highest overall r^2 values in the range of 84-94%, which was greatest for the conversion between ALEX vs. MeDALL-chip. This was followed by Ara h 6 (85-88%) and Ara h 1 (63-72%). Tree nut component sIgE was also compared between ALEX vs. MeDALL-chip, listed in order of model fit: Jug r 1 (78%), Ana o 3 (76%), Jug r 2 (62%), and Cor a 9 (56%) (Fig 4).

Correlations between PR-10 and nsLTP components

Cross-reactivity within protein families due to sequence and structural homology are of significant importance. The PR-10 and nsLTP families play a central role in this context. We sought to quantitatively compare sIgE levels detected by ALEX and MeDALL-chip using the Pearson correlation coefficient (r) as a surrogate for the degree of cross-reactivity. Among the PR-10 family proteins (Aln g 1, Ara h 8, Bet v 1, Cor a 1.0401, Gly m 4, Mal d 1), the observed intra-test correlations tended to be lower on ALEX (Fig 5A) than on MeDALL-chip (Fig 5B). MeDALL-chip also reported a larger proportion of positive PR-10 co-sensitizations compared to ALEX when sIgE was evaluated at the positive test cut-off (Fig S6).

In the context of protein sequence homology, the prototypical Bet v 1 allergen shares higher sequence similarity with other tree pollen allergens such as Aln g 1 (84%) and Cor a 1 (67%) as compared to homologous food allergens such as Ara h 8 (44%) (Fig 5C). Accordingly, there was a strong correlation between the pollen allergens Bet v 1-Cor a 1 on both the ALEX ($r = 0.88$) and MeDALL-chip ($r = 0.82$). In comparison, a weaker correlation was observed on the ALEX for the correlations between the pollen-food pairs Bet v 1-Ara h 8 (ALEX: $r = 0.28$, MeDALL-chip: $r= 0.68$) and Cor a 1-Ara h 8 (ALEX: $r = 0.37$, MeDALL-chip: $r = 0.80$). Among nsLTP allergens (Ara h 9, Art v 3, Cor a 8, Pru p 3), weaker correlations also tended to be reported by ALEX (Fig 5C) than MeDALL-chip (Fig 5D). However, a strong correlation between Ara h 9-Pru p 3 was present on both platforms (ALEX: $r = 0.83$, MeDALL-chip: $r = 0.92$), mirroring the high sequence similarity (Fig 5F).

Discussion

This study outlines statistical models for interconversion between three multiplex sIgE tests: the ALEX, MeDALL-chip, and EUROLINE platforms. Using two regression methods, we demonstrated the ability to model allergen-specific relationships with acceptably high fit for peanut and tree nut sIgE testing at the extract and component-level, including Ara h 1, Ara h 2, Ara h 6, Ana o 3, Cor a 9, Jug r 1, and Jug r 2. Our findings support the notion that conversion is reasonably possible between sIgE multiplex platforms for allergen extracts and components.

For peanut allergens, the MARS regression models explained 63-94% of the variability in the data from different platforms. Importantly, these models reported high coefficients of determination for Ara h 2 (r^2 = 84-94%) and Ara h 6 (r^2 = 85-88%), which are the most discriminative marker proteins for peanut allergy[29]. For tree nut allergens, our models explained 56-92% of variability in the data of extracts and components from almond, walnut, hazelnut and cashew.

Previous studies comparing multiplex sIgE platforms have primarily reported only the level of semi-quantitative/quantitative agreement or correlation. Buzzulini et al.[30] compared food allergens on the ALEX and ImmunoCAP and observed an overestimation of ALEX at lower sIgE levels and an underestimation at higher sIgE levels compared to ImmunoCAP. Our models reflected the tendency for the linear range of the ALEX to end at approximately 30 kUA/L in ImmunoCAP units. Although the linear ranges of the ImmunoCAP and MeDALL-chip exceed that of the ALEX, their ranges encompass the frequently used 95% cut-off of 15 kUA/L for peanut sIgE, which could allow for interconversion in the range that is most important for extrapolation of clinical relevance[31]. Correlations beyond 30 kUA/L between ALEX and ImmunoCAP require individual assessment of each allergen. For inhalant allergens, additional comparison studies have found significant correlations between ALEX and ISAC within three cohorts[13,32,33], supporting the potential utility of these conversion methods for other allergens.

Cross-reactivity patterns on the multiplex test platforms were also compared. We observed that the MeDALL-chip tended to report higher intra-family correlations between PR-10 and nsLTP allergens, suggesting that cross-reactive antibodies may have a broader impact on test results in this context. Similarly, Heffler et al. observed that PR-10 and nsLTP allergens were more frequently positive and had greater mean values on the ISAC chip compared to ALEX[13]. Discrepancies may result from different concentrations of allergen spotted on the respective platforms or variations in allergen production. The CCD blocking protocols also differed between test systems. The use of a CCD-inhibitor in the ALEX but not the EUROLINE and MeDALL-chip may account for the lower number of positive sensitizations identified, in particular for glycosylated allergens.

Strengths of this study include the large cohort of individuals with food allergen sensitizations, allowing for the generation of models for several major food allergens. Our analysis utilized two

independent modelling methods that were consistently applied to each comparison, but which were capable of capturing trends specific to different allergens and platforms. Moreover, two independent cohorts and two sites (Toronto and Vienna) were included. Although cross-validation of these models was performed, limitations of this study include the lack of an independent validation in a third cohort large enough to assess the generalizability of these proposed models. With additional cohorts using these different test systems, further validation of these models is possible. Lastly, all measurements were performed using research chips in a research laboratory and without provocation tests, therefore conclusions for clinical decision-making are limited. Applications of this methodology are targeted towards addressing the challenges of performing global collaborations, such as the pooling of data and harmonization of phenotypic definitions between studies that is required for genetic research on food allergy[31]. This methodology could also be useful in combination with alternative IgE quantification strategies to expand allergy research in countries where established test platforms are cost-prohibitive[34,35].

In summary, this study illustrates the ability to integrate sIgE results from different test systems using statistical modelling. Further validation of these conversion models for peanut and tree nut allergens could establish a method for the harmonization of sIgE data produced by different test systems.

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Figure Legends:

Figure 1: Number of positive sensitizations reported by sIgE test platforms illustrates test agreement between A) allergen extracts on ALEX and EUROLINE and B) allergen components on ALEX, MeDALL-chip, and EUROLINE. Only the subjects with corresponding measurements in all 3 platforms are shown (n=121).

Figure 2: Modelling of peanut extract sIgE pairwise comparisons demonstrates convertibility between pairwise comparisons of ImmunoCAP, ALEX, and EUROLINE (A, B, C), but poor model fit for conversions with skin prick test (D, E). Blue = loess modelling, red = MARS modelling, ribbon = 95% confidence interval.

Figure 3: Modelling of Ara h 1, Ara h 2, and Ara h 6 sIgE demonstrates convertibility between pairwise comparisons of ALEX, MeDALL-chip, and EUROLINE sIgE test platforms. Blue = loess modelling, red = MARS modelling, ribbon = 95% confidence interval.

Figure 4: Modelling of Ana o 3, Cor a 9, Jug r 1, and Jug r 2 sIgE demonstrates convertibility between pairwise comparisons of ALEX and MeDALL-chip sIgE test platforms. Blue = loess modelling, red = MARS modelling, ribbon = 95% confidence interval.

Figure 5: Correlations of sIgE levels to PR-10 and nsLTP allergens are reflective of cross-reactivity resulting from protein sequence similarities and differ between multiplex platforms. A) Correlation of PR-10 components on ALEX, B) correlation of PR-10 components on MeDALL-chip, C) percentage of PR-10 protein sequence similarity, D) correlation of nsLTP components on ALEX, E) correlation of nsLTP components on MeDALL-chip F) percentage of nsLTP protein sequence similarity. Pearson correlation coefficient (r) is depicted by both color intensity and circle size.

Statement of Contribution: JAH performed sample measurements, contributed to data analysis, and wrote the manuscript. AC performed the statistical modelling and wrote the manuscript. CL contributed to sample measurement. RV critically reviewed the manuscript. LD, RD, MGB, AD, CL, SW, MAF, AM, KS collected data. PS, JEMU, ZS, TE designed the studies. AR contributed to statistical analysis. TE designed the study, contributed to generation of the manuscript and supervised the project. All authors discussed the results and commented on the manuscript.

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Conflicts of Interest: Dr. Eiwegger is a site PI of a DBV sponsored global study, reports grants from Innovation Fund Denmark, and is the Co-I or scientific lead in three investigator-initiated oral immunotherapy trials supported by the Allergy and Anaphylaxis Program at SickKids, outside of the submitted work. He reports personal fees from ALK and is on a local and international advisory board of ALK. Outside of this project Dr. Eiwegger collaborates with MADX in a government funded project. Dr. Eiwegger serves as associate editor for Allergy and is in the editorial board of the IAA and the review board of JACI. Dr. Upton reports personal fees from Kaleo, personal fees from ALK, is a member of the advisory board of Emerade, and is a sub-investigator on clinical trials of DBV Therapeutics, Aimmune, Regeneron, and CIHR, outside the submitted work. Dr. Lupinek reports personal fees from Thermo Fisher, outside the submitted work. Dr. Valenta has received research grants from Viravaxx, Vienna, Austria and serves as a consultant for this company, outside the submitted work.

Abbreviations Used:

ALEX: Allergy Explorer

Ana o: cashew allergen

Ara h: peanut allergen

Cor a: hazelnut allergen

CRD: component-resolved-diagnosis

ISU: ISAC standardized units

Jug r: walnut allergen

loess: locally estimated scatterplot smoothing

MARS: multivariate adaptive regression spline

MeDALL-chip: Mechanisms of development of allergy chip

nsLTP: non-specific lipid transfer protein

sIgE: allergen specific IgE

SPT: skin prick test

Pru du: almond allergen

PR-10: pathogenesis related subfamily 10

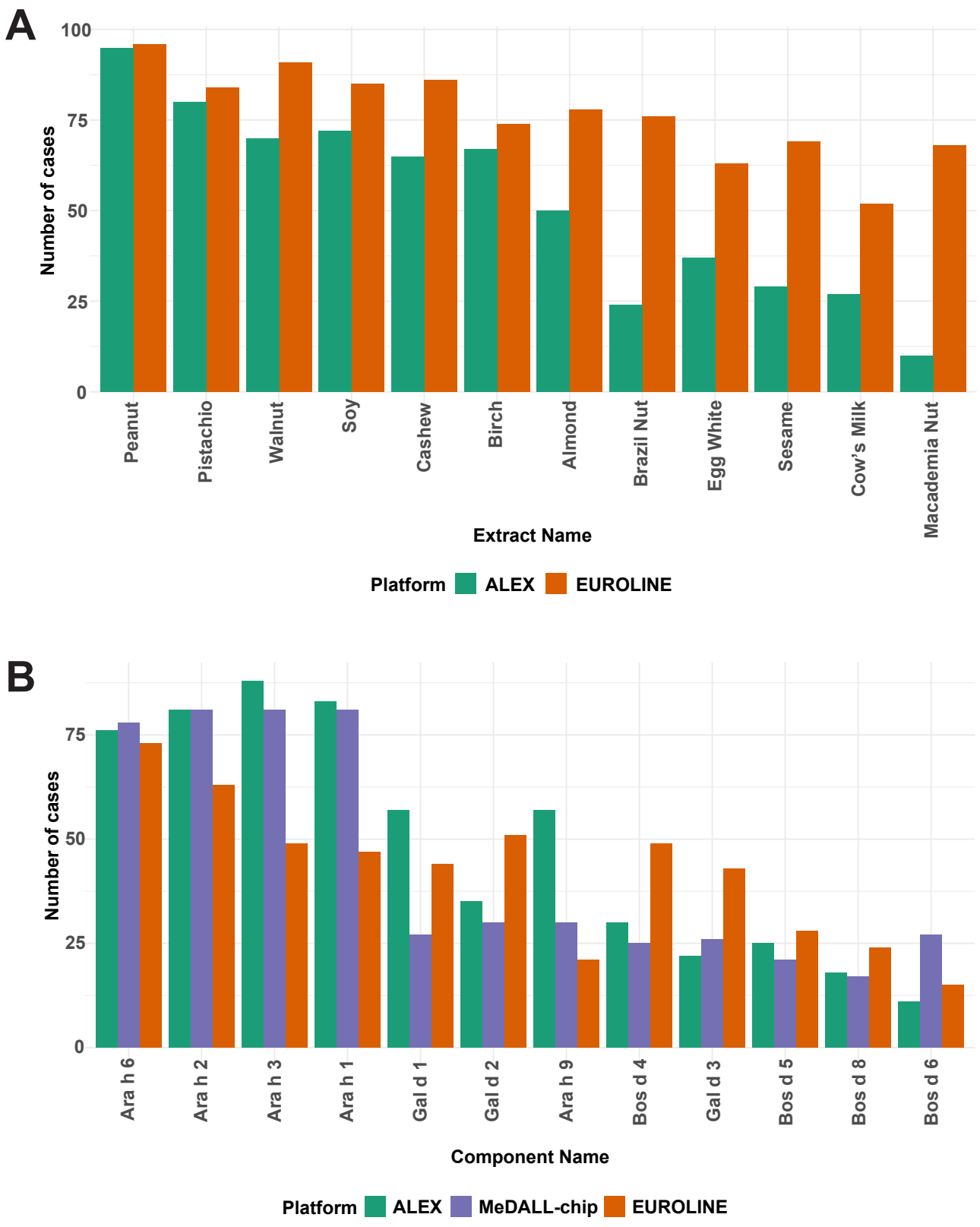
A
100
75
50
25
0
Number of cases
Peanut
Pistachio
Walnut
Soy
Cashew
Birch
Almond
Brazil Nut
Egg White
Sesame
Cow's Milk
Macademia Nut
Extract Name
Platform ALEX EUROLINE
B
75
50
25
0
Number of cases
Ara h 6
Ara h 2
Ara h 3
Ara h 1
Gal d 1
Gal d 2
Ara h 9
Bos d 4
Gal d 3
Bos d 5
Bos d 8
Bos d 6
Component Name
Platform ALEX MeDALL-chip EUROLINE

Figure 1

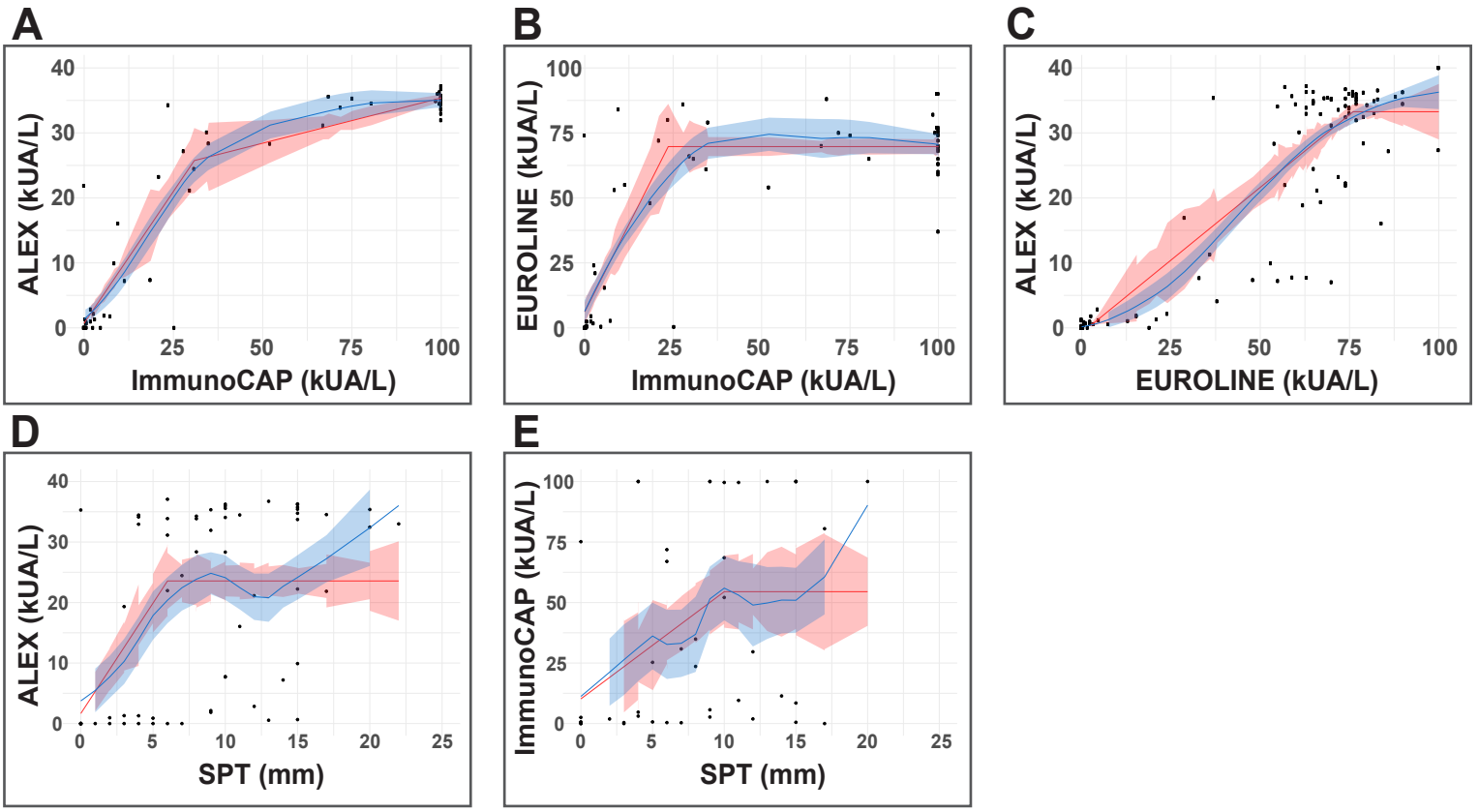
Peanut Extract
A
ALEX (kUA/L)
ImmunoCAP (kUA/L)
B
EUROLINE (kUA/L)
ImmunoCAP (kUA/L)
C
ALEX (kUA/L)
EUROLINE (kUA/L)
D
ALEX (kUA/L)
SPT (mm)
E
ImmunoCAP (kUA/L)
SPT (mm)

Figure 2

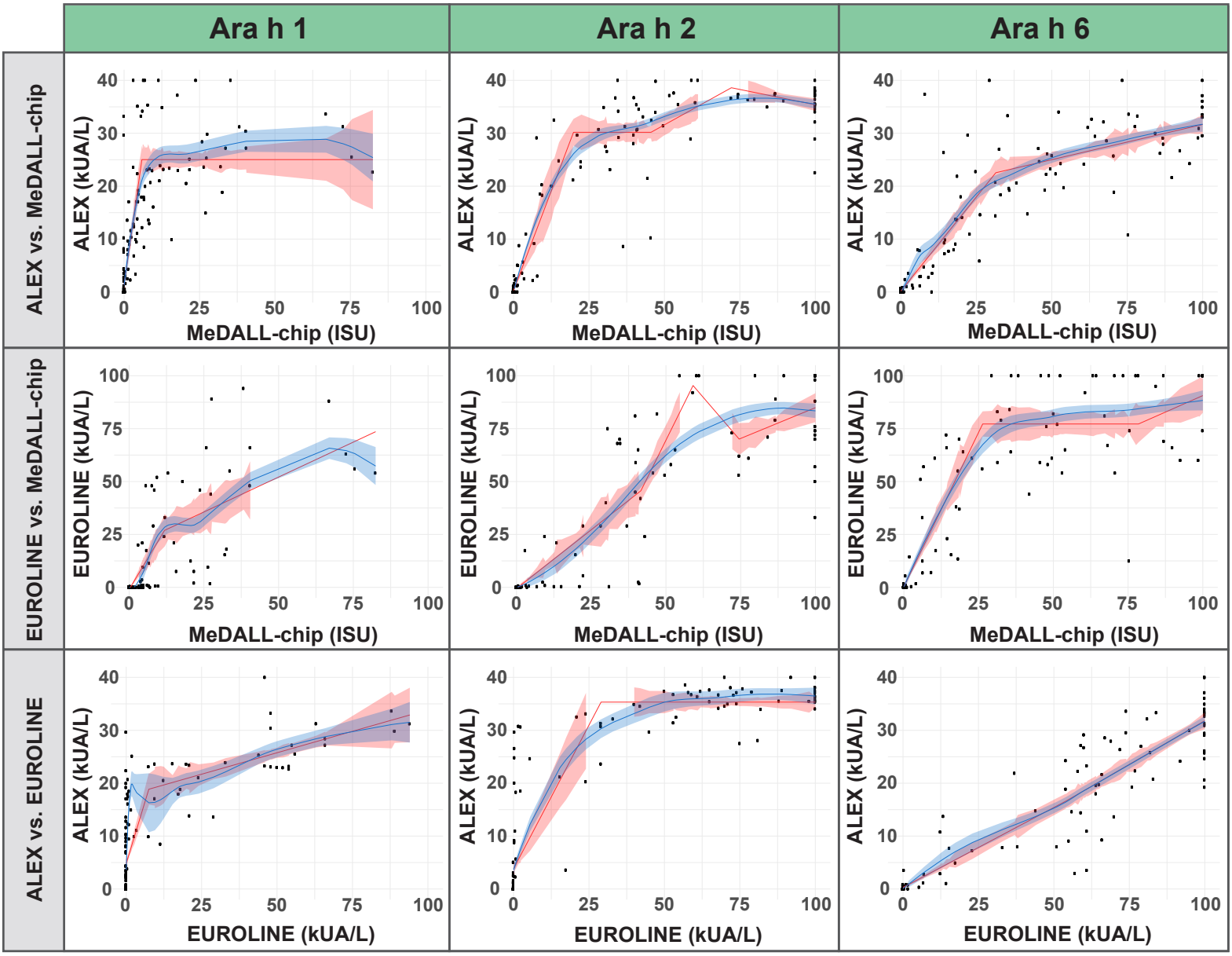
Ara h 1
Ara h 2
Ara h 6
ALEX vs. MeDALL-chip
EUROLINE vs. MeDALL-chip
ALEX vs. EUROLINE
ALEX (kUA/L)
EUROLINE (kUA/L)
MeDALL-chip (ISU)
EUROLINE (kUA/L)
0
25
50
75
100
10
20
30
40

Figure 3

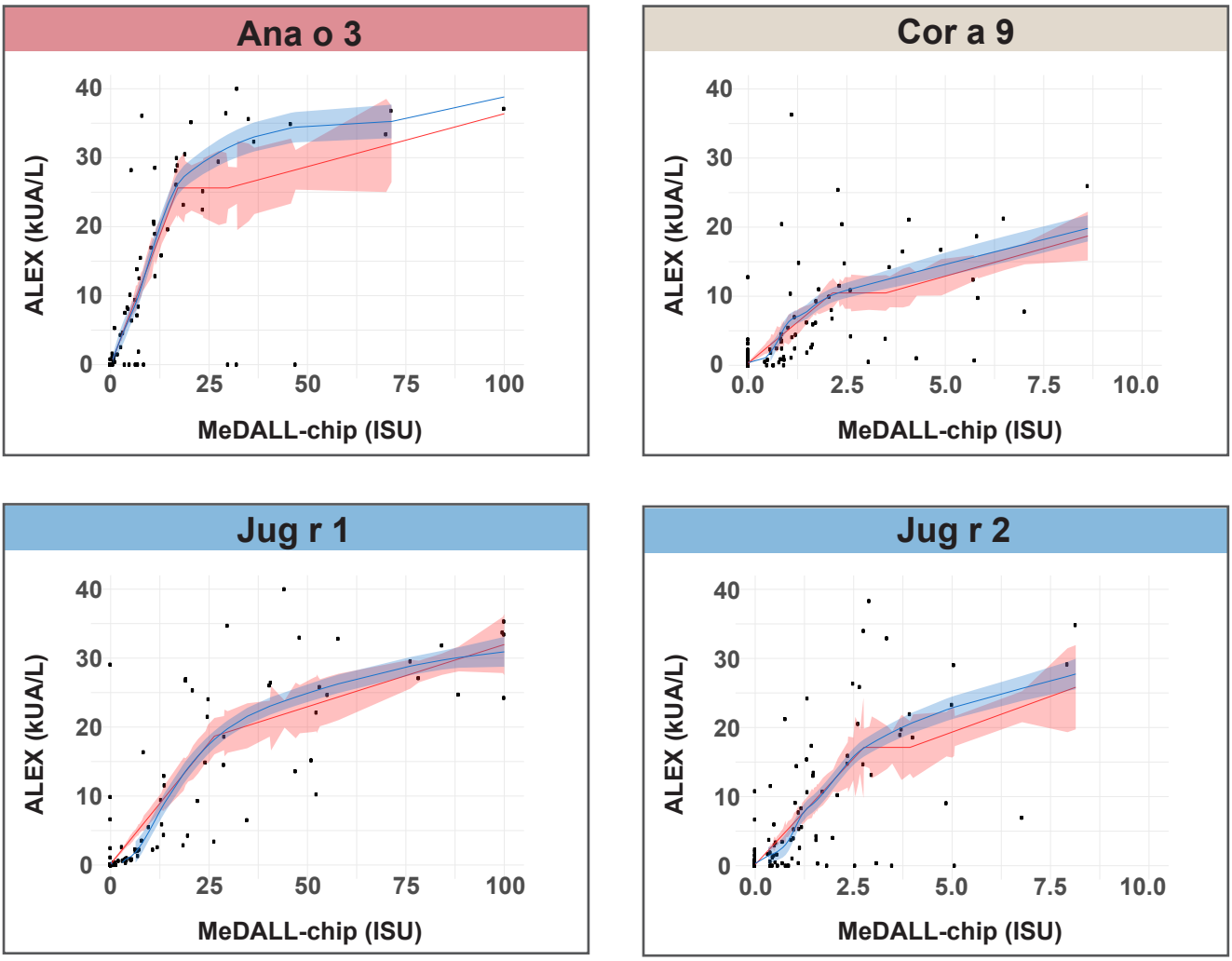
Ana o 3
Cor a 9
Jug r 1
Jug r 2
ALEX (kUA/L)
MeDALL-chip (ISU)
0
10
20
30
40
0
25
50
75
100
0.0
2.5
5.0
7.5
10.0

Figure 4

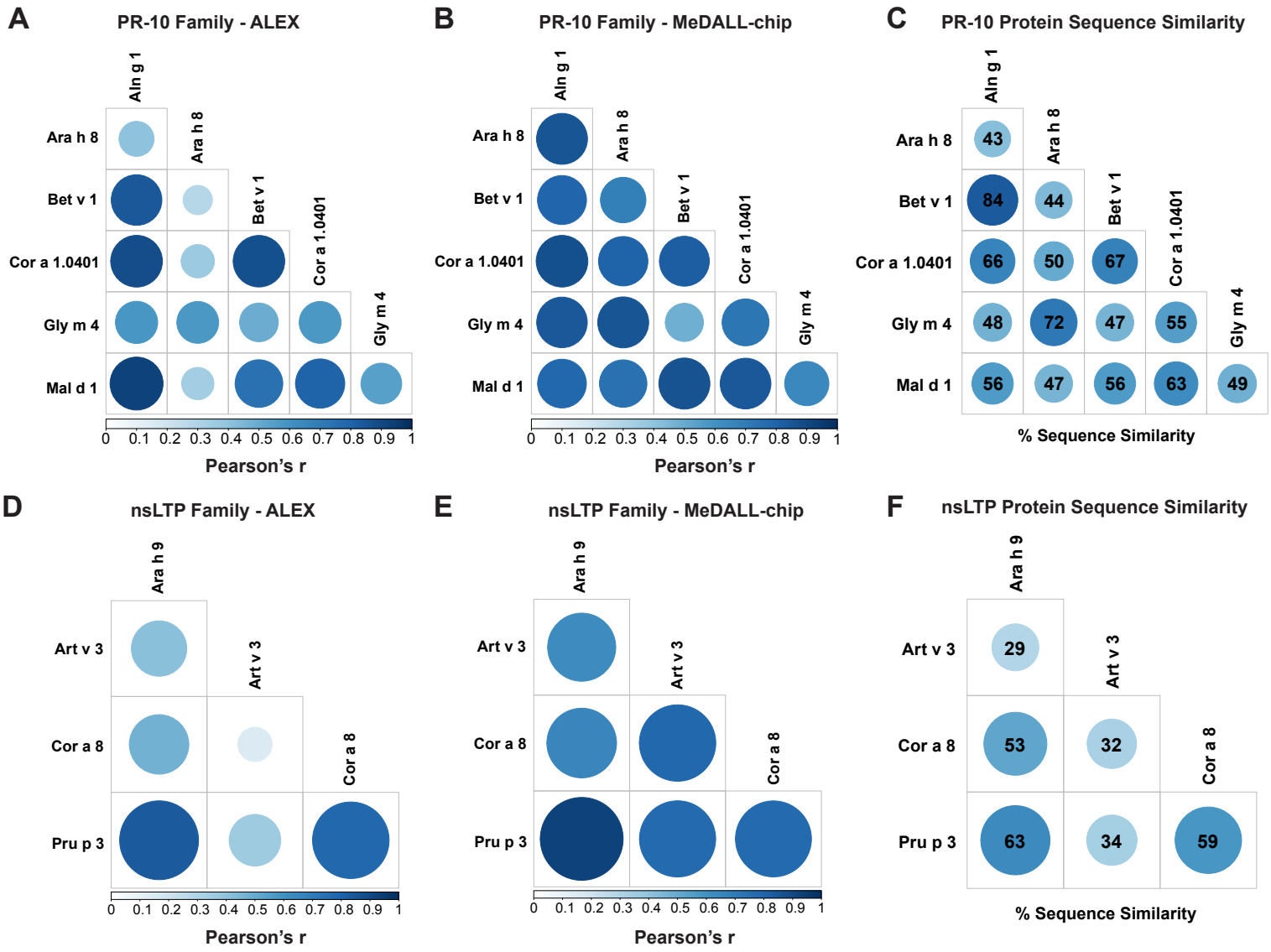
A
PR-10 Family - ALEX
Aln g 1
Ara h 8
Bet v 1
Cor a 1.0401
Gly m 4
Mal d 1
0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1
Pearson's r
B
PR-10 Family - MeDALL-chip
Aln g 1
Ara h 8
Bet v 1
Cor a 1.0401
Gly m 4
Mal d 1
0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1
Pearson's r
C
PR-10 Protein Sequence Similarity
Aln g 1
Ara h 8
Bet v 1
Cor a 1.0401
Gly m 4
Mal d 1
43
84 44
66 50 67
48 72 47 55
56 47 56 63 49
% Sequence Similarity
D
nsLTP Family - ALEX
Ara h 9
Art v 3
Cor a 8
Pru p 3
0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1
Pearson's r
E
nsLTP Family - MeDALL-chip
Ara h 9
Art v 3
Cor a 8
Pru p 3
0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1
Pearson's r
F
nsLTP Protein Sequence Similarity
Ara h 9
Art v 3
Cor a 8
Pru p 3
29
53 32
63 34 59
% Sequence Similarity

Figure 5