An application to help users identify IgE cross reactivity based on sequence similarity and identity

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Introduction

The main objective of this project is to provide guidance to a user to determine what foods or airborne allergens to avoid based on their verified allergens. Interactions between antibodies called Immunoglobulin E (IgE) and allergenic proteins trigger allergic reactions. When an individual has produced antibodies against a particular allergenic protein, sometimes the antibodies can fail to distinguish between the original protein and other proteins usually from the same protein family. This phenomenon is called cross-reactivity. There is a lot of prior research surrounding IgE and cross reactivity but that research is not easily available to the layman in a manner to be used practically. We use results from cross reactivity research and analyze which data can be reliably used for allergen prediction. This prediction can be further refined for an individual based on their allergen profile. Though clinical skin prick tests(SPTs) can determine the susceptibility of individuals to specific allergens, the proteins that an individual is tested for are limited in scope. So it is hard to determine if a protein for which an individual has not been tested for is safe or whether it can trigger an allergic reaction. Testing individuals for a plethora of proteins has proven to be not very effective. This solution aims to provide an answer to this question for proteins belonging to the same family by combining cross-reactivity risk score based on protein sequence similarity and identities between proteins of the same protein family and an individual's SPT to calculate risk associated with other proteins. The final classification maps allergens to their most common source of food, animal and plant based origin that are then marked as high risk, medium high risk, medium low risk and low risk. This analysis can be used to create avoidance guidelines and plans for immunotherapy.

Methods - Data acquisition and processing

We selected 3 protein families with the largest set of registered allergens: Profilins, nsLTP and PR-10. For each of these protein families, we generated identity and similarity score using the following steps

- 1. Acquire globally registered IUIS Genbank accession number from AllFam database, for all proteins in the family
- 2. Use CLUSTAL Omega to generate multiple sequence alignment of proteins
- 3. Use SIAS with CLUSTAL data to generate sequence similarity(S) and identities(I) using default parameters
- 4. Generate pairwise risk score for each protein using the formula Risk score = (I+S)/2
- 5. Store Risk score index map in mysql DB schema

"CREATE TABLE IF NOT EXISTS crossreactivity (name VARCHAR(255), origin VARCHAR(255), %s)" % (header)"

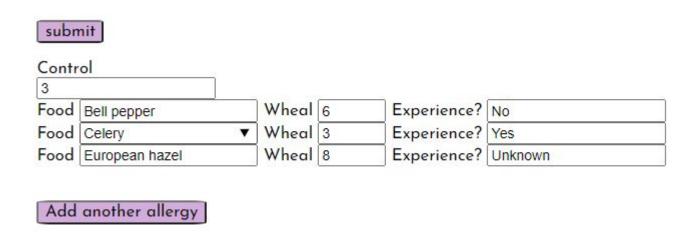
where header is composed of all protein names in the family

Methods - Sample data with pairwise Risk score

origin	P85524	BAA74451	CAA88831	AAB01092	AAQ91847	CAA42646	AAX19889	AHC08073	CAM31908	CAM31909	CAA50327	CAA47366	ADK39021	CAA33887	AAB24432	ABZ81045	ACJ23861	ABG54495	ACJ23864
Kiwi fruit 1	100	25	23.33	24.995	24	24.33	24.665	26.995	26.66	26.33	28.33	26.33	26.33	25.66	26.995	29.665	27.995	24.445	25.33
Mung bean	25	100	24.995	23.22	26.77	27.74	29.03	29.03	27.415	28.06	29.995	28.705	28.385	31.93	30.315	27.415	27.415	27	29.35
Celery	23.33	24.995	100	84.415	44.8	44.8	44.15	47.075	52.59	54.215	47.075	45.125	46.425	49.995	49.67	48.37	51.62	49.995	51.94
Carrot	24.995	23.22	84.415	100	41.395	42.085	41.61	42.185	50.31	51.585	44.06	41.815	43.435	43.75	44.06	45.595	48.425	46.71	47.81
Peanut	24	26.77	44.8	41.395	100	74.84	72.895	49.04	51.905	47.13	50.635	49.995	51.265	55.09	52.86	51.905	54.455	54.01	57.64
Soybean	24.33	27.74	44.8	42.085	74.84	100	79.67	49.675	53.475	50.63	53.475	51.575	52.845	54.425	55.695	55.375	55.695	53.28	58.54
Mung bean 1	24.665	29.03	44.15	41.61	72.895	79.67	100	48.06	49.03	48.38	48.06	46.12	47.415	51.285	49.995	52.575	53.865	51.455	55.8
Tomato	26.995	29.03	47.075	42.185	49.04	49.675	48.06	100	54.71	53.18	49.06	47.795	49.37	50.62	48.75	52.825	54.71	54.01	57.81
Gold kiwi fruit	26.66	27.415	52.59	50.31	51.905	53.475	49.03	54.71	100	74.2	57.86	52.825	55.97	55.025	57.545	58.485	60,685	59.12	61.63
Kiwi fruit	26.33	28.06	54.215	51.585	47.13	50.63	48.38	53.18	74.2	100	55.09	49.995	53.18	55.09	57	56.36	58.595	60.215	60.825
European hazel	28.33	29.995	47.075	44.06	50.635	53.475	48.06	49.06	57.86	55.09	100	90.25	92.495	76.875	80.31	59.745	62.26	60.215	67.81
European hornbeam	26.33	28.705	45.125	41.815	49.995	51.575	46.12	47.795	52.825	49.995	90.25	100	96.535	77.04	77.98	55.025	56.915	56.93	63.835
European hop hornbeam	26.33	28.385	46.425	43.435	51.265	52.845	47.415	49.37	55.97	53.18	92.495	96.535	100	78.12	79.685	56.915	58.8	57.295	65.625
European white birch	25.66	31.93	49.995	43.75	55.09	54.425	51.285	50.62	55.025	55.09	76.875	77.04	78.12	100	84.685	62.575	63.83	62.405	70.935
European alder	26.995	30.315	49.67	44.06	52.86	55.695	49.995	48.75	57.545	57	80.31	77.98	79.685	84.685	100	63.2	63.2	62.77	69.37
White oak	29.665	27.415	48.37	45.595	51.905	55.375	52.575	52.825	58.485	56.36	59.745	55.025	56.915	62.575	63.2	100	89.93	61.675	71.065
Sweet chestnut	27.995	27.415	51.62	48.425	54.455	55.695	53.865	54.71	60.685	58.595	62.26	56.915	58.8	63.83	63.2	89.93	100	64.595	73.265
Red raspberry	24.445	27	49.995	46.71	54.01	53.28	51.455	54.01	59.12	60.215	60.215	56.93	57.295	62.405	62.77	61.675	64.595	100	71.165
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Methods - Cross reactivity predictions based on skin prick test(SPT)

- In clinical skin prick tests, a substance that may potentially cause allergies to an individual manifests as a wheal, the size of the wheal relative to a negative control wheal size is used as an indicator of allergic potential. With this tool Users are able to input data about multiple proteins from a skin prick test.
 - Input Wheal size for Control
 - Select individual substances (s) from a list of common name proteins in the protein family nsLTP as it contains one of the largest set of allergenic proteins. For substances that have multiple variants of proteins, we select one variant with a representative risk score
 - Input wheel size and any allergic reactions experienced on exposure to substances that have been tested



Methods - Cross reactivity predictions based on skin prick test(SPT)

We use the following heuristics to combine cross-reactivity score with SPT input to determine a risk category for allergens. Food allergens eliciting wheal diameters of at least 3 mm or larger than the negative control are considered positive test results. Negative skin tests have a high negative predictive accuracy, thus usually excluding food allergy to common foods. If there is a history of a no allergic reaction to a food and the skin test is positive, the test may be viewed as non diagnostic

- Filter out substances (s) that an individual is not allergic to.
 - o If wheel size of s control wheel size < 3mm, then that is considered a negative result. Exclude the substance s
 - o If exposure by ingestion has not resulted in allergic reaction, then exclude the substance s
- Generate one risk score for each protein by picking max cross-reactivity risk score
 - o crossReactivityRiskScore_p = max(crossReactivity_s_p) for each substance s in input list that has not been excluded
- Display predictions about potential allergies to substances as a sorted list based on risk score
 - o Show cross-reactivity reaction potential in high, medium-high, medium-low and low risk categories

Methods – IgE cross reactivity visualization

- Present a list of common substance names for proteins in the family. User can get more details about computed score and Genbank accession number
- Use php to allow user to select any protein substance in the family

Medium Low Risk

• Use sql queries to retrieve cross reactivity data and display common names from proteins in order of highest potential cross reactivity with the selected protein, based on risk index



card for strawberry is flipped and is showing the computed score and the genbank accession number

Results

Risk category based on SPT and cross-reactivity score from nsLTP

Clinical SPT data from an individual was used to validate the risk category generated by the application. Data was input for a subset of proteins that showed high wheal size compared to negative control. The risk category generated by the app for proteins corresponding to the ones in test panel show similar results. In the result set below, input data from 3 proteins marked by the red arrow with large wheal size generated correctly that the proteins that showed non allergenic in SPT test also show as non-cross reactive in the output generated by the app. So using nsLTP protein family with SPT can be a good way of deciding what proteins to avoid.

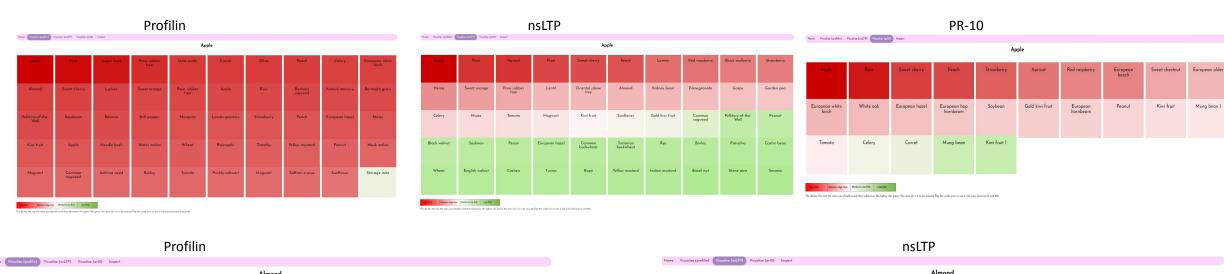
Clinical SPT results PRICK Order Score Wheal Flare Loc Allergen Order Panel: Inhalant Skin Tests *D. Farinae 10,000 (Environment) Yes 3 30 LA No Panel: FOOD 32 Diluent control (Control) Yes 4 LA No Cows Milk 1:20 (Food) Yes 4 LA No *Egg Whole 1:20 (Food) Yes 3 10 30 LA No Wheat 1:20 (Food) Yes 4 LA 3 No *Peanut 1:20 (Food) Yes 15 LA No *Histamine (Histamine) Yes 20 LA No Soybean 1:20 (Food) Yes 4 LA No Hazel Nut 1:20 (Food) Yes 4 LA No *Cashew 1:20 (Food) Yes 18 40 LA No Walnut 1:20 (Food) Yes 0 4 LA No *Almond (Food) Yes 35 LA No



Results - Contd.

Comparison of cross reactivity across different protein family

This result compares the cross reactivity risk score for the same substance based on the presence of proteins from different protein families. As shown below, the proteins in the profilin family have strong cross-reactivity between almost all protein pairs. So this protein cross reactivity score is not very useful for determining potential for allergic reactions. However both PR10 and nsLTP cross reactivity scores show similar results for the same substance. They also show results inline with the expected relationships of allergies between substances like Almond and sweet cherry or apple and pear. So nsLTP protein family with it's larger size, is a good candidate for predicting potential allergens.



				Alm	ond				
Almond	Peach	Sweet cherry	Sweet orange	Apple	Sugar beet	Pellitory-of-the- Wall	Bell pepper	Para rubber tree	Pear
Kiwi fruit	Apple	Mesquite	Lychee AAL07320 86.255	Para rubber tree	Redroot pigweed	Strawberry	Bermuda grass	Banana	Olive
Needle bush	Pineapple	Rice	Tomato	Peach	Carrot	Date palm	Celery	Soyabean	Yellow mustard
Musk melon	Water melon	Wheat	European white birch	Lambs-quarters	Barley	European hazel	Maize	Apple	Annual mercury
Peanut	Mugwort	Timothy	Prickly saltwort	Sunflower	Mugwort	Asthma weed	Saffron crocus	Common ragweed	Storage mite

				Alm	ond				
Almond	Black mulberry	Plum	Apple	Sweet cherry	Apricot	Peach	Red raspberry	Pear	Hemp
Strawberry	Oriental plane tree	Grape	Pomegranate	Lentil	Kidney bean	Kiwi fruit	Tomato	Para rubber tree	Garden p
Lemon	Mugwort	Maize	Celery	Gold kiwi fruit	Sunflower	Sweet orange	Common ragweed	Rye	Black wali
Castor bean	Pellitory-of-the- Wall	European hazel	Soybean	Peanut	Pecan	Pistachio	Turnip	Cashew	English wa
Tartarian buckwheat	Rape	Common buckwheat	Brazil nut	Wheat	Sesame	Yellow mustard	Indian mustard	Barley	Stone pir

Discussion

A lot of the already established research is lacking in accessibility and can be very misleading. The profilin family—for example—has a lot of crossreactivity between proteins, making it seem as if every single item on the list is crossreactive with every other item. Through the course of the project, it eventually became apparent that some of these results were not indicative of reality, so we began to look into different families which were verifiable with my real life results. Looking at the families nsltp and pr10 we began to see much more diverse correlations, allowing us to make more representative predictions. Besides from simply choosing the best family, the project also simplifies the results to make it much more accessible to the general public. Rather than using complex allergen nomenclature, the display is simplified to real-world origins and organisms.

Conclusion

We show that using SPT and cross reactivity score to determine risk for allergenic proteins can be a useful tool for determine what foods to avoid and for creating immunotherapy plans. We also show that some protein families are inherently unsuited for food avoidance type usage because the proteins in the family are highly similar leading to a superset of potential allergens. Selection of numerous foods to be eliminated from the diet based on large numbers of poorly selected potential allergens may lead to diets that are difficult for families to follow and may eliminate foods that are clearly tolerated, making adherence to the diet poor. In addition SPT results need to be judiciously used as positive results are not always diagnostic and negative results are more reliable. Our application gives greater weight to negative results and to real experiences with allergic reaction based on exposure, to increase reliability of risk prediction. Combining SPT data for multiple allergens further increases the reliability of the result. This tool is a step in the right direction for allergies and accessibility. It can warn users about possible allergens and can be especially useful in avoiding serious allergic reactions by avoiding high risk substances until a formal diagnosis can occur. In situations where users experience allergies especially with food and cannot determine the cause, they can use this tool to identify potential allergens. Though our analysis was constrained by the availability of only one dataset for verification, it confirms our basic hypothesis.

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