

ORIGINAL ARTICLE

IgE and IgG4 binding to lentil epitopes in children with red and green lentil allergy

Cansin Sackesen^{1,2,3}  | Baran Erman^{1,4}  | Gustavo Gimenez³ | Galina Grishina³ | Ozlem Yilmaz¹  | Suleyman T. Yavuz²  | Umit M. Sahiner²  | Betul Buyuktiryaki²  | Ebru A. Yilmaz²  | Ozlem Cavkaytar²  | Hugh A. Sampson³ 

¹Division of Pediatric Allergy, School of Medicine, Koc University, Istanbul, Turkey

²Division of Pediatric Allergy, Hacettepe University School of Medicine, Ankara, Turkey

³Division of Pediatric Allergy and Immunology, Icahn School of Medicine at Mount Sinai, New York, NY, USA

⁴Department of Molecular Biology and Genetics, Istinye University, Istanbul, Turkey

Correspondence

Hugh A. Sampson, Department of Pediatrics, Jaffe Food Allergy Institute, Icahn School of Medicine at Mount Sinai, 1425 Madison Ave, Box 1089, New York, NY 10029, USA.
Email: hugh.sampson@mssm.edu

Funding Information

This study was supported in part by the David H. and Julia Koch Research Program in Food Allergy Therapeutics.

Editor: Alexandra Santos

Abstract

Background: The consumption of lentil is common in the Mediterranean area and is one of the causes of IgE-mediated food allergy in many countries. Len c 1 is a well-defined allergen of lentil and approximately 80% of the patients with lentil allergy recognize the purified Len c 1 protein. We sought to identify IgE and IgG4 sequential epitopes of Len c 1 in patients with red and/or green lentil allergy. We also aimed to determine IgE and IgG4 binding differences between those patients who had outgrown or remained reactive to lentil.

Methods: Children with IgE-mediated lentil allergy were included in the study. We applied a microarray immunoassay to determine the characterization of positive IgE and IgG4 binding to Len c 1 epitopes in the patients' sera.

Results: The peptides specifically recognized by IgE and IgG4 antibodies were mainly detected between peptides 107 and 135 of Len c 1. The signal intensities of positive epitopes were significantly greater in reactive patients than tolerant ones ($P = .008$ for IgE and $P = .002$ for IgG4). Moreover, IgE and IgG4 antibodies bound largely the same sequential epitopes in patients who remained reactive or outgrew their allergy.

Conclusion: IgG4-binding epitopes in lentil allergy were identified and IgE and IgG4 binding to epitopes in both red and green lentils was compared. Our data regarding signal intensity differences between reactive and outgrown patients and overlap binding of IgE and IgG4 antibodies may be important for the development of more accurate diagnostic tests and understanding of natural tolerance development.

KEYWORDS

Len c 1, lentil allergy, microarray-based immunoassay, natural tolerance

1 | INTRODUCTION

Legumes comprise an important part of the human diet in many parts of the world because they have a high protein content and variable amounts of lipids and vitamins.¹ The lentil (*Lens culinaris*) is a member of legume family and a major food source in the nutrition of children.

Lentils are frequently consumed in the Middle East, Asia, and the Mediterranean area, such as Turkey.² However, IgE-mediated lentil allergy is common in many countries and affects both children and adults.³ In Turkey, lentils are reported as the sixth most common food allergen and in Spain, it is fourth in the pediatric population.^{4,5} Consumption of lentils has been increasing in the United States and Northern Europe, as well. The predominant types of lentils consumed in Turkey are red

Sackesen and Erman contributed equally to the work and are co-first authors.

and green lentils, and allergic reactions were described to either or both. In Spain the most frequently consumed lentils are the green and brown-red lentil, Pardina, whereas in the United States and in Europe more diverse lentils were eaten due to the greater heterogeneity in the populations.⁶ Moreover, allergy to other legumes such as chickpea, pea, peanut, and tree nuts is common in children with lentil allergy.⁵

To date, Len c 1, Len c 2, and Len c 3 have been identified as three major allergens in lentil.⁶ Approximately, 80% of the patients with lentil allergy recognize the purified Len c 1 which is a vicilin with a size of 47 kDa.^{7,8}

Vereda et al identified the location of IgE-binding sequential epitopes of the major lentil protein Len c 1.⁹ However, there has been no comprehensive study in the literature regarding IgE and IgG4 binding to lentil epitopes in separate groups of red and green lentil allergic patients. Here, we identified positive IgE- and IgG4-binding epitopes in patients with red, green, and red & green lentil allergy by using a peptide microarray-based immunoassay (MIA). We also showed the differences in IgE- and IgG4-binding patterns between patients who remained reactive or outgrew their lentil allergy.

2 | METHODS

2.1 | Study population

The study population included children with IgE-mediated lentil allergy who were followed in the Division of Pediatric Allergy, Hacettepe University School of Medicine. Demographic information and physical examination findings of all participants were recorded.² Children were diagnosed as having IgE-mediated lentil allergy according to the following criteria¹⁰:

1. A consistent and clear-cut history of lentil-related symptoms that developed within minutes to hours following the ingestion of lentils,
2. Positive serum levels of sIgE (>0.35 kU_A/L), or
3. Positive skin prick test (SPT) for lentil (a wheal diameter of 3 mm or greater than the negative control)

To determine the prognosis for lentil allergy, all children whose tolerance status was unknown underwent open food challenges (OFCs). Children with a history of anaphylaxis within the last year and patients who consumed lentils uneventfully did not undergo OFCs.

The children were grouped as red, green, or both red and green lentil allergic patients based on the previous history of reaction to different types of lentils, OFCs, and SPT results. Participants were then grouped as reactive or tolerant according to the outcome of OFCs.

2.2 | Open food challenges and diagnosis of anaphylaxis

OFC tests were performed according to standard methods as previously described.² Diagnosis of anaphylaxis was made according to recently published guidelines.¹¹

Key Message

Although identification of IgE and IgG4 binding epitopes have been shown in different food allergens, the data regarding Len c 1 (lentil) binding is very limited. We identified IgE and IgG4 binding profiles to Len c 1 peptides in patients with lentil allergy. IgG4 binding to lentil epitopes and both IgE and IgG4-binding patterns in isolated red and isolated green lentil allergic patient groups have been demonstrated for the first time. We also determined the signal intensity differences between lentil reactive and outgrown patients. These data may be informative for a better understanding of natural tolerance development to lentils.

2.3 | Study procedures and measurements

SPTs with lentil allergen extracts and other implicated foods were performed in children (Alyostal ST-IR, Stallergenes SA). Histamine and saline solution were used as positive and negative controls, respectively

Total serum IgE and sIgE levels were measured with the ImmunoCAP system (Phadia AB).

The study was approved by the Ethics Committee of Hacettepe University. All patient materials were obtained in accordance with the Declaration of Helsinki. Prior to the initiation of the study, informed consent forms were obtained from parents.

2.4 | Peptide microarray immunoassay

Peptide libraries, slides, and printing were prepared and performed as described previously.^{12,13} Immunolabeling was performed as previously described, with some modifications.^{9,12,14-16} In brief, 50 μ L of patient serum diluted 1:5 in PBS-T/HSA was applied to slides and incubated for 24 hours at 4°C. Slides were washed and incubated for 24 hours at 4°C with a cocktail of two monoclonal biotinylated anti-human IgE and IgG4 antibodies (Invitrogen and BD Biosciences Pharmingen) diluted 1:250 each. To test for non-specific binding of secondary antibody or dendrimers to peptides, two immunolabeling steps were performed without patient's serum. The immunoassay was performed as explained above except for substitution of serum by blocking solution. The slides were then washed and scanned using a ScanArray®Gx (PerkinElmer). Serum samples obtained from two healthy subjects were used as negative controls.

2.5 | Data analysis

Statistical analysis was performed with SPSS 22.0 for Windows (IBM SPSS Statistics). Lentil sIgE, age, and age at onset of lentil allergy were summarized as median with the interquartile range (IQR).

Fluorescence signals from one channel (for IgE and IgG4 binding) were digitized with the ScanArray Express Microarray Analysis

TABLE 1 Patient characteristics

Patient No.	Age	Gender	Age of onset	Symptoms after lentil intake	SPT	Lentil allergy	Lentil extract slgE	Lentil challenge test	Symptoms from history	Reactive or Outgrown
1	13	M	9	R ^a	ND	Red	1.29	ND	Tolerated at follow-up	O
2	6	M	1	An ^a	Pos.	R & G	43.6	ND	Anaphylaxis	R
3	4	M	1	C	Pos.	R & G	0.3	Neg.		O
4	4	F	1	C	ND	Green	3.4	Neg.		O
5	5	M	2	C	Neg.	R & G	2.26	Pos.		R
6	5	F	1	C	Pos.	Green	5.75	Pos.		R
7	12	M	7	C	Pos.	Green	4.66	ND	Tolerated at follow-up	O
8	4	F	1	C, G	Pos.	R & G	61.9	Pos.		R
9	11	M	1	An ^a	Pos.	Red	2.56	ND	Tolerated at follow-up	O
10	7	M	3	C	Pos.	Green	0.3	Neg.		O
11	13	F	2	An	Pos.	Red	106	Pos.		R
12	2	M	1	C	Pos.	R & G	23.5	Pos.		R
13	4	F	1	C	Pos.	R & G	96.5	Pos.		R
14	3	M	1	C	Pos.	R & G	1.71	Neg.		O
15	9	M	1	C	Pos.	Red	2.6	Neg.		O
16	3	M	1	An	Pos.	Red	0.65	Pos.		R
17	3	M	1	C	Pos.	R & G	10.6	Neg.		O
18	2	M	1	C	Neg.	Green	6.6	Neg.		O
19	3	M	1	An ^a	ND	Green	1.35	Pos.		R
20	5	F	1	C	Pos.	Green	0.3	Neg.		O
21	2	M	1	C	ND	Red	1.38	Neg.		O
22	2	F	1	C	Neg.	R & G	2.18	Pos.		R
23	3	M	2	C	ND	Red	0.88	Neg.		O
24	11	F	10	An	Pos.	Red	1.12	Neg.		O
25	2	M	1	C	Pos.	Green	3.7	Neg.		O
26	1	M	1	C	Pos.	R & G	52.6	ND	Anaphylaxis	R
27	2	M	1	C	Neg.	R & G	1.01	Pos.		R
28	1	M	1	C	Neg.	R & G	5.07	Pos.		R

Abbreviations: An, Anaphylaxis; C, Cutaneous; F, Female; G, Gastrointestinal; M, Male; ND, Not done or not determined; O, Outgrown; R & G, Red and green; R, Reactive; R, Respiratory; SPT, Skin prick test.

^aSymptoms after exposure to steam from cooked lentils.

System (PerkinElmer), exported as comma-delimited (CSV) files and transformed to z score using R analysis (R development Core Team. R: A language and environment for statistical computing. Version 2.5.0: R foundation for Statistical Computing, 2007. <http://r-project.org>). The differences of binding intensity between reactive and outgrown patients were determined using *T*-test and one-way ANOVA. Pearson's chi-square test was used for the identification of predictivity values. Intensity signals were calculated as mean values.

The accuracy of selected IgE and IgG4 epitopes for predicting clinical reactivity was determined by receiver operating characteristics (ROC) curve analysis. For each selected cut-off point that significantly differentiated between tolerant and reactive patients,

sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were accordingly calculated. Two-by-two tables were used to calculate sensitivity, specificity, PPV, and NPV. Youden index (sensitivity + specificity-1) was preferred to denote cut-off level of binding intensity of selected IgE and IgG4 epitopes.¹⁷

3 | RESULTS

3.1 | Study population

Among the 28 patients in the study population 8 had red lentil allergy, 8 had green lentil allergy, and 12 had both red & green lentil

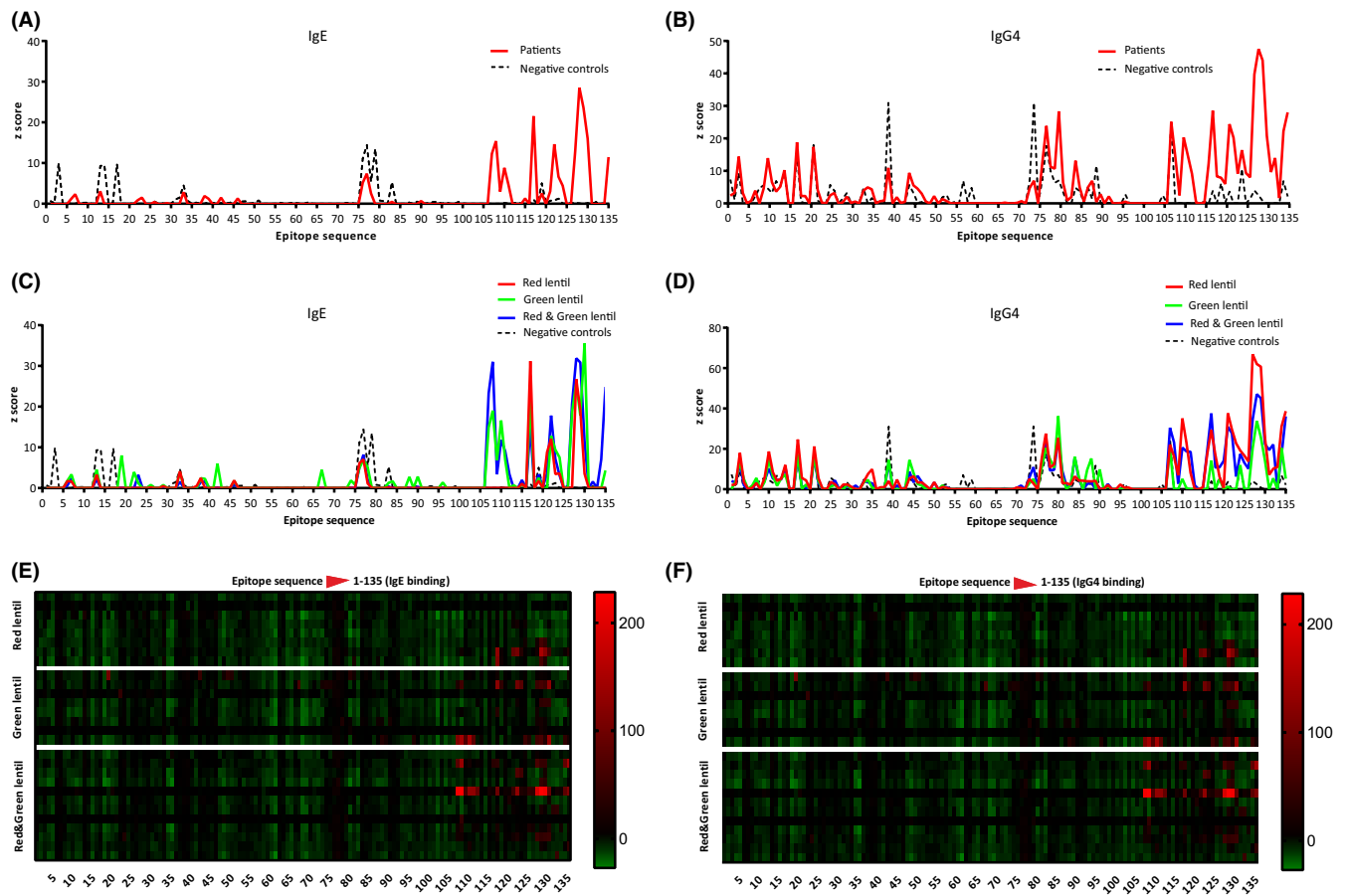


FIGURE 1 Mean signal intensity of IgE and IgG4 binding in the patients. A-B, IgE and IgG4 binding in patients and negative controls. C-D, IgE and IgG4 binding in patient groups and negative controls. E, Heatmap of IgE signal intensity for each epitope in patient groups. F, Heatmap of IgG4 signal intensity for each epitope in patient groups

allergy. The median age of onset of symptoms was 1.2 years (0.9-1.9). Lentil sIgE levels ranged between 0.3 and 106 kU_A/L with a median of 32.4 kU_A/L (IQR: 1.16-9.6). Four patients also reported symptoms after exposure to steam from cooked lentils, 70% had atopic dermatitis, and 47% had bronchial asthma. The most frequent symptoms were immediate cutaneous (97%) and respiratory (30%) symptoms. A significant number of patients reported symptoms following ingestion of other legumes or nuts (Table 1). The other concomitant food allergies were egg and milk allergies. Fifteen patients outgrew lentil allergy during the study.

3.2 | Epitope recognition by serum IgE and IgG4 antibodies

The mean signal intensity present in peaks in distinct regions of the protein showed that the peptides at the C-terminal side, between 107 and 135 of Len c 1, specifically recognized IgE and IgG4 antibodies (Figure 1A,B). This positive binding pattern was seen in all patient groups (red, green, red & green lentil allergy; Figure 1C-F). The signal intensity of IgG4 binding was significantly greater ($P < .01$) than IgE in isolated patient groups (Figure 1D). The number of positive IgG4 epitopes was greater than IgE epitopes in overall patients and also in red and green lentil allergy groups separately. However, overlap

binding between IgE and IgG4 epitopes was observed, especially at the C-terminal side of the protein. The number of all positive epitopes is listed in Table S1.

3.3 | The IgE- and IgG4-binding epitope profile in red and green lentil allergic patients

While IgE-binding epitopes in red lentil allergic patients were between peptides 115 and 130, in green lentil allergic patients were between peptides 107 and 135 (Figure 1C,E); the patients who were allergic to both red and green lentil simultaneously showed the binding profile of red and green lentil at the same time. However, IgG4-binding epitope profiles were almost the same in both red and green lentil allergic patients (Figure 1D,F).

3.4 | The IgE- and IgG4-binding profile in outgrown and reactive patients

First, we observed that IgE and IgG4 antibodies bound largely to the same sequential epitopes in reactive and in patients who had outgrown their allergy (Figure 2A,B). The signal intensities of positive IgE and IgG4 epitopes were significantly higher in reactive patients than tolerant ones (IgE $P < .01$; IgG4 $P < .01$, Figure 2A,B,G and H).

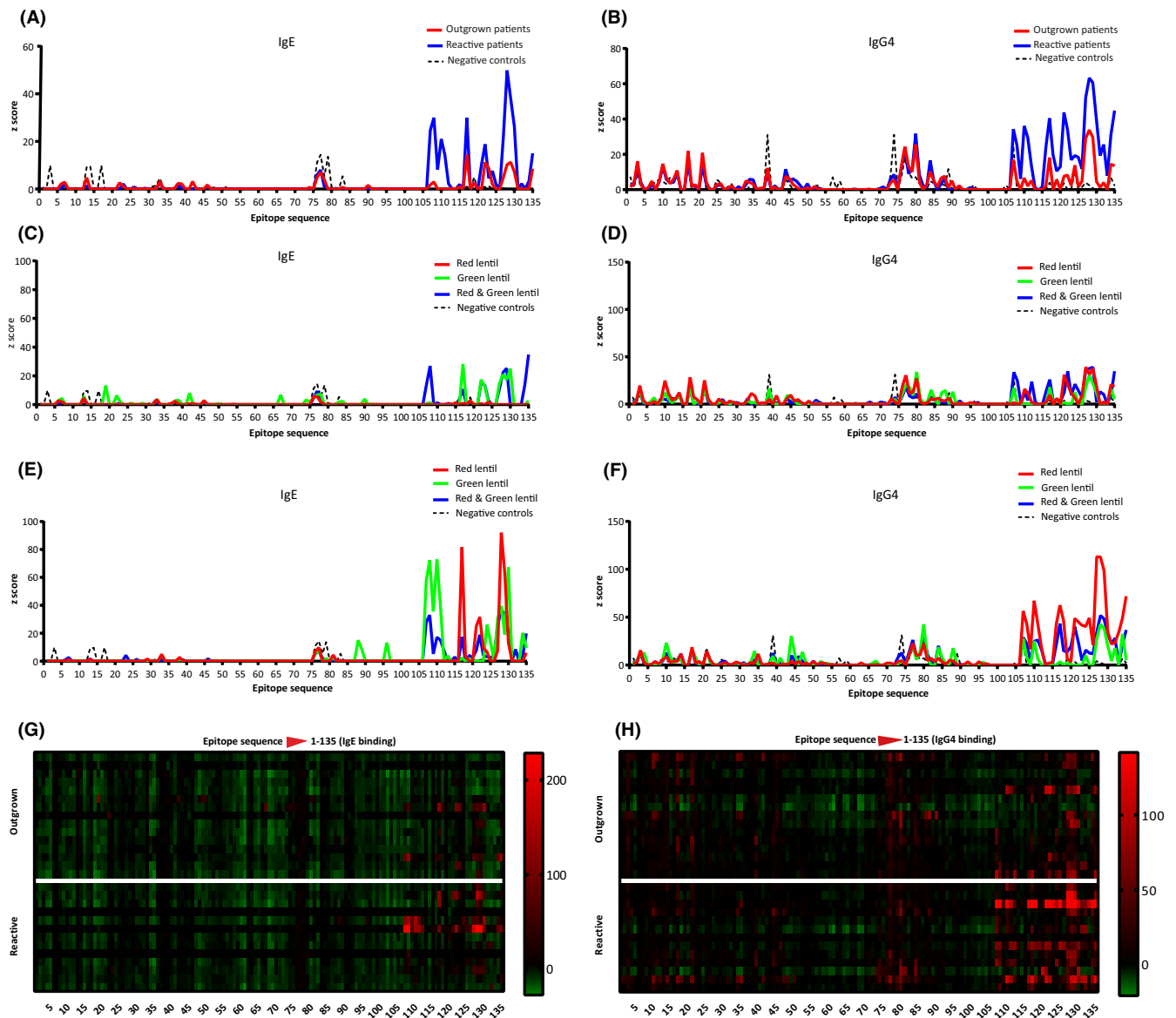


FIGURE 2 Mean signal intensity of IgE and IgG4 binding in outgrown and reactive patients. A-B, IgE and IgG4 binding in patients. C-D, IgE and IgG4 binding in outgrown patient groups. E-F, IgE and IgG4 binding in reactive patient groups. G-H, Heatmap of IgE and IgG4 signal intensity for each epitope in outgrown and reactive patients

This significant difference was found at the C-terminal region (peptides 107-135) in red, green, and red & green lentil allergic patient groups ($P = .01$, $.01$, and $.04$ for red, green, red & green lentil allergic groups, respectively; Figure 2C-F).

There were several significant informative IgG4 epitopes in reactive patients. The intensities of IgG4 binding to peptides 111 (mean: 27.1 ± 9.6), 128 (mean: 64.4 ± 11.5), and 132 (mean: 25.4 ± 9.3) in reactive patients were statistically higher compared to tolerant group [(mean: 4.2 ± 4.1 , $P = .03$), (mean: 32.9 ± 7.8 , $P = .03$), and (mean: 4 ± 3.8 , $P = .04$) for 111, 128, and 132 IgG4 peptides, respectively]. We found five individual informative epitopes in red lentil allergic and one epitope in green lentil allergic ones with significantly higher binding intensity in the reactive group than in the outgrown group (Table S2).

We determined the differences in signal intensity between reactive and outgrown patients with each positive z score on all Lenc 1 peptides recognized by IgE and IgG4 antibodies. The mean intensities of IgE and IgG4 binding were significantly higher in reactive patients (Figure 3A,B). When we performed the same analysis for patient groups, we realized that a similar pattern for IgE binding was seen in only red allergic groups (Figure 3C). The similarity of IgG4 binding was observed in red and red & green lentil allergic groups (Figure 3D). The mean intensity of IgE and IgG4 signals are given in Table 2.

Although there was a significant difference in signal intensity, we did not detect any discrepancy in the number of IgE- and IgG4-binding peptides between reactive and outgrown patients.

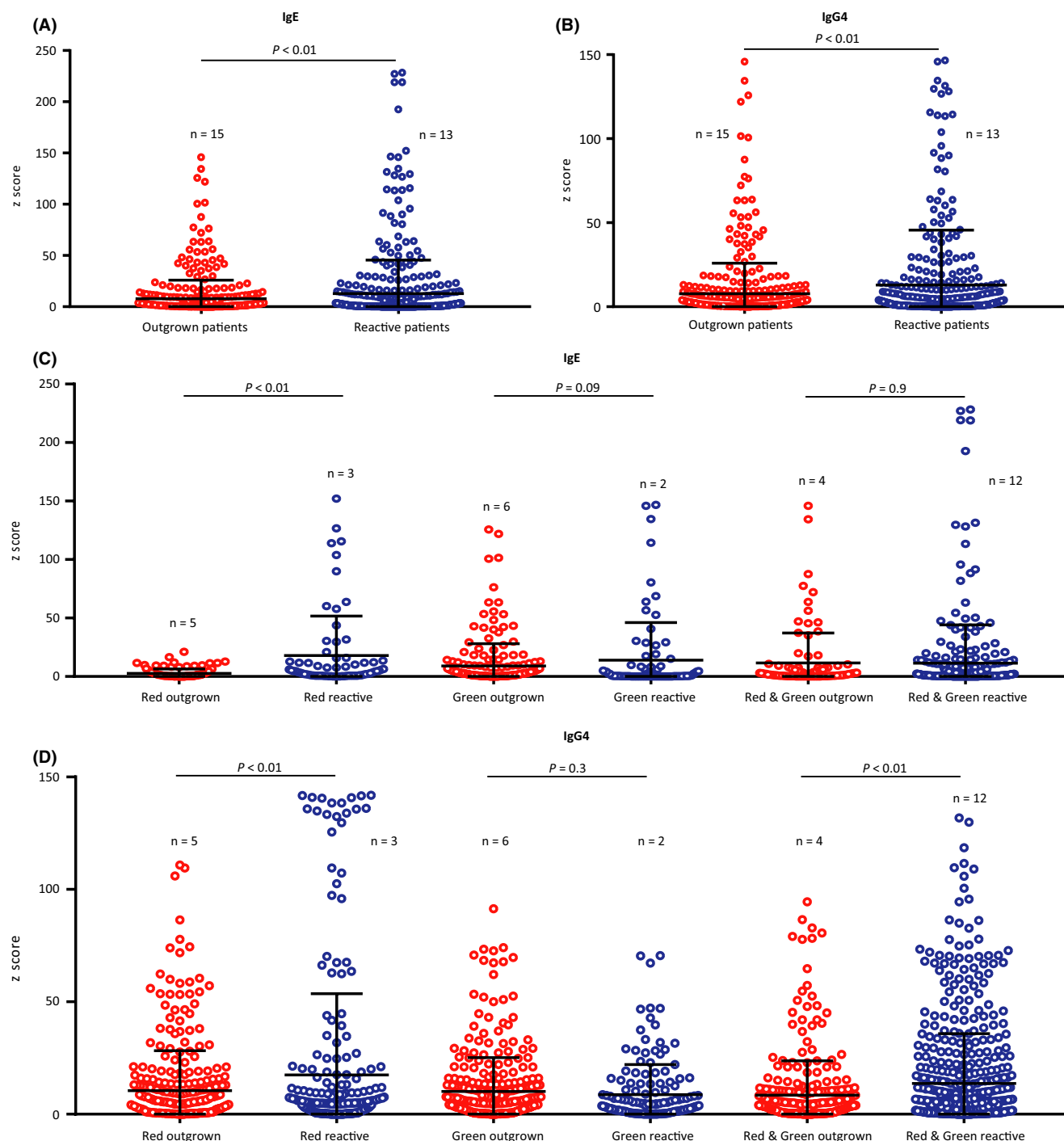


FIGURE 3 Dot-plot graphics of each positive epitopes in the patients. A-B, Positive IgE and IgG4 epitopes in outgrown and reactive patients. C, Positive IgE epitopes in outgrown and reactive patient groups. D, Positive IgG4 epitopes in outgrown and reactive patient groups

3.5 | Cut-off levels of lentil extract sIgE, and IgE- and IgG4-binding epitopes for predicting clinical reactivity

Receiver operating characteristics (ROC) curve analysis against reactivity was used for predicting clinical reactivity. We determined area under curve (AUC) values of lentil sIgE and IgE- and IgG4-binding intensity to Len c 1 epitopes. While the AUC value of lentil extract sIgE was 0.644 ($P = .1$), it was statistically significant for the signal

intensity of 1 IgE and 8 IgG4 epitopes (Table S3 and Figure S1). These data suggest that single epitope values are more useful than sIgE for predicting clinical reactivity. Then we calculated significant binding intensity cut-off levels (z scores) of those nine epitopes with sensitivity and specificity (Youden index). The cut-off levels of seven epitopes were found to be statistically significant. Sensitivity, specificity, PPV, and NPV of these seven epitopes are listed in Table 3. The cut-off z score levels of epitope 111 and epitope 131 binding to IgG4 were 1.56 (sensitivity: 76.9, specificity: 80) and 1.55 (sensitivity:

TABLE 2 Mean signal intensity of each positive epitope in outgrown and reactive patient groups

	All cases			Red lentil			Green lentil			Red & Green lentil		
	O	R	P	O	R	P	O	R	P	O	R	P
IgE	7.7	12.9	<.01	2.7	17.9	<.01	9.5	14.1	.09	11.5	11.5	.9
IgG4	9.73	13.87	<.01	10.5	17.5	.01	10.1	8.7	.3	8.5	13.8	.01

Abbreviations: O, Outgrown; P, P value; R, Reactive.

TABLE 3 Different cut-off levels and predictive values of informative epitopes

Epitope	Youden index	Cut-off z score	Sensitivity	Specificity	PPV	NPV	P value
IgG4-110	1.55	11.8	69.2	86.7	81.8	76.5	<.01
IgG4-111	1.56	1.16	76.9	80	76.9	80	<.01
IgG4-120	1.50	0.03	92.3	60	66.7	90	<.01
IgG4-125	1.41	0.006	61.5	86.7	80	72.2	<.01
IgG4-131	1.55	1.04	69.2	86.7	81.8	76.5	<.01
IgG4-132	1.43	0.38	76.9	66.7	66.7	76.9	.02
IgG4-133	1.54	0.03	61.5	93.3	88.9	73.7	<.01

Abbreviations: NPV, Negative predictive value; PPV, Positive predictive value.

69.2, specificity: 86.7), respectively. PPV and NPV for IgG4 binding to epitope 111 were 76.9% and 80%, while those for IgG4 binding to epitope 131 were 81.8% and 76.5%, respectively.

4 | DISCUSSION

This first detailed research about IgE and IgG4 epitope mapping of a major lentil allergen identified IgE and IgG4 sequential epitopes in lentil allergic patients. IgE and IgG4 antibodies of the patients recognized mainly the C-terminal region of the Len c 1 (peptides 107-135). Red, green, and red & green lentil allergic patients showed the same binding patterns. The binding intensity of IgE and IgG4 antibodies was significantly higher in reactive patients than in tolerant ones. Moreover, we determined several individual epitopes for predicting clinical reactivity in the patients.

Sequential epitope-mapping studies of food allergens have been of increasing interest to allergologists in the last 10 years because identification of epitope diversity can facilitate the diagnosis and predict the prognosis of the disease. There are many studies concerning epitope sequencing of food allergens such as hazelnut, walnut, peanut, egg, milk, and lentil.^{9,15,18-23} However, there has been no comprehensive study regarding the identification of IgE- and IgG4-binding epitopes of Len c 1 in red and green lentil allergy separately.

The major IgE- and IgG4-binding epitopes specifically recognized by patients were mainly detected between peptides 107 and 135 of the Len c 1 molecule, which was compatible to the results of the study by Vereda et al.⁹ Analysis of the patient groups led to the discovery of a similar binding pattern for both IgE and IgG4 in all red, green, and red & green lentil allergy groups. This result was compatible with the previous studies that showed the similar binding pattern for both IgE and IgG4 in milk, peanut, and egg allergy.^{16,22,24} Our

result showed that patients with lentil allergy recognize largely the same IgE and IgG4 epitopes.

The analysis of patients as reactive vs outgrown noted higher intensity of binding by both IgE and IgG4 antibodies in reactive than outgrown patients for each positive epitope, particularly at the C-terminal region of the protein. However, subgroup analysis showed that the intensity of binding for each positive IgE epitope was higher in red and red & green lentil-reactive patients compared to those of tolerant patients. The same intensity difference was observed in reactive patients of red and green lentil allergic groups for IgG4 epitope binding. There are only a few studies investigating IgE and IgG4 epitope binding profiles in patients with persistent food allergy and those who naturally became tolerant.^{14,18,22,23,25} All of those publications were carried out in patients allergic to cow milk. Our findings are in line with the results from previous studies, as our data showed that IgE- and IgG4-binding intensity decreased during natural tolerance acquisition. Although the mechanism of natural tolerance development in food allergy remains poorly understood, our findings suggest that there is an important association between IgE and IgG4 binding in natural tolerance acquisition. Moreover, overlapping binding patterns between IgE and IgG4 support the hypothesis²⁶ of blocking IgG4 in natural tolerance development.

In our study, even though the patterns of IgE and IgG4 binding to epitopes were similar between isolated red and isolated green reactive patients, we tried to identify individual epitopes to distinguish the different profiles of binding by IgE and IgG4 antibodies in red and green lentil allergy. We identified several informative epitopes in reactive patients. We suggest that these specific epitopes may be useful for the diagnosis or prognosis of the disease. In the report of Shreffler and colleagues,²⁷ the number of IgE-binding epitopes to peanut allergen was lower in patients with mild symptoms compared

to patients suffering from severe symptoms. These data also support the notion that specific epitopes may be more informative than the quantity of recognized epitopes.

There are some limitations in our study, such as the small number of patients. Another probable limitation is the lack of time points during natural tolerance development. Thus, further studies including more patients are needed for a better understanding of tolerance mechanism in different lentil allergic patients.

In conclusion, IgG4 binding to lentil epitopes and both IgE- and IgG4-binding patterns in isolated red and isolated green lentil allergic patient groups have been demonstrated for the first time. Our findings revealed significantly stronger binding in reactive children than in those who are naturally tolerant to lentil allergies. These data are informative for natural tolerance acquisition. Moreover, we determined several informative epitopes for the prediction of clinical reactivity in red and green lentil allergic patients.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

CS, G Gimenez, and G Grishina performed experiments. CS, STY, UMS, BB, EAY, and OC provided clinical care of the patients and clinical data. CS, BE, and OY participated in the analytic framework of the study, interpretation of data, and manuscript preparation. CS and BE wrote the manuscript. HAS conceived this study, provided laboratory resources, and together with CS and BE was involved in critical scientific discussions. All authors critically reviewed the manuscript and agreed to its publication.

ORCID

Cansin Sackesen  <https://orcid.org/0000-0002-1115-9805>

Baran Erman  <https://orcid.org/0000-0001-9398-8465>

Ozlem Yilmaz  <https://orcid.org/0000-0003-2971-283X>

Suleyman T. Yavuz  <https://orcid.org/0000-0002-9315-0037>

Umit M. Sahiner  <https://orcid.org/0000-0003-0088-913X>

Betul Buyuktiryaki  <https://orcid.org/0000-0003-1206-969X>

Ebru A. Yilmaz  <https://orcid.org/0000-0002-6204-2754>

Ozlem Cavkaytar  <https://orcid.org/0000-0002-5747-7032>

Hugh A. Sampson  <https://orcid.org/0000-0003-1613-8875>

REFERENCES

- Duranti M. Grain legume proteins and nutraceutical properties. *Fitoterapia*. 2006;77(2):67-82.
- Yavuz ST, Sahiner UM, Buyuktiryaki B, et al. Role of specific IgE in predicting the clinical course of lentil allergy in children. *Pediatr Allergy Immunol*. 2013;24(4):382-388.
- Ibanez MD, Martinez M, Sanchez JJ, Fernandez-Caldas E. Legume cross-reactivity. *Allergol Immunopathol (Madr)*. 2003;31(3):151-161.
- Yavuz ST, Sahiner UM, Buyuktiryaki B, et al. Phenotypes of IgE-mediated food allergy in Turkish children. *Allergy Asthma Proc*. 2011;32(6):47-55.
- Martinez San Ireneo M, Ibanez MD, Sanchez JJ, Carnes J, Fernandez-Caldas E. Clinical features of legume allergy in children from a Mediterranean area. *Ann Allergy Asthma Immunol*. 2008;101(2):179-184.
- Andreae DA, Grishina G, Sackesen C, Ibanez MD, Sampson HA. High similarity between lentil and other lentil-like-proteins (dal) complicates recommendations on avoidance in lentil allergic patients. *J Allergy Clin Immunol Pract*. 2015;3(5):808-810.e2.
- Sanchez-Monge R, Pascual CY, Diaz-Perales A, Fernandez-Crespo J, Martin-Esteban M, Salcedo G. Isolation and characterization of relevant allergens from boiled lentils. *J Allergy Clin Immunol*. 2000;106(5):955-961.
- Lopez-Torres G, Salcedo G, Martin-Esteban M, Diaz-Perales A, Pascual CY, Sanchez-Monge R. Len c 1, a major allergen and vicilin from lentil seeds: protein isolation and cDNA cloning. *J Allergy Clin Immunol*. 2003;112(6):1208-1215.
- Vereda A, Andreae DA, Lin J, et al. Identification of IgE sequential epitopes of lentil (Len c 1) by means of peptide microarray immunoassay. *J Allergy Clin Immunol*. 2010;126(3):596-601.e1.
- Sicherer S, Sampson H. Food allergy. *J Allergy Clin Immunol*. 2006;117(2):S470-S475.
- Simons F, Arduo L, Bilò MB, et al. World Allergy Organization anaphylaxis guidelines: summary. *J Allergy Clin Immunol*. 2011;127(3):587-593.e22.
- Lin J, Bardina L, Shreffler WG. Microarrayed allergen molecules for diagnostics of allergy. *Methods Mol Biol*. 2009;524:259-272.
- Lin J, Bardina L, Shreffler WG, et al. Development of a novel peptide microarray for large-scale epitope mapping of food allergens. *J Allergy Clin Immunol*. 2009;124(2):315-322, 322 e311-313.
- Cerecedo I, Zamora J, Shreffler WG, et al. Mapping of the IgE and IgG4 sequential epitopes of milk allergens with a peptide microarray-based immunoassay. *J Allergy Clin Immunol*. 2008;122(3):589-594.
- Flinterman AE, Knol EF, Lencer DA, et al. Peanut epitopes for IgE and IgG4 in peanut-sensitized children in relation to severity of peanut allergy. *J Allergy Clin Immunol*. 2008;121(3): 737-743.e10.
- Shreffler WG, Lencer DA, Bardina L, Sampson HA. IgE and IgG4 epitope mapping by microarray immunoassay reveals the diversity of immune response to the peanut allergen, Ara h 2. *J Allergy Clin Immunol*. 2005;116(4):893-899.
- Youden WJ. Index for rating diagnostic tests. *Cancer*. 1950;3(1):32-35.
- Caubet JC, Lin J, Ahrens B, et al. Natural tolerance development in cow's milk allergic children: IgE and IgG4 epitope binding. *Allergy*. 2017;72(11):1677-1685.
- Jarvinen KM, Beyer K, Vila L, Bardina L, Mishoe M, Sampson HA. Specificity of IgE antibodies to sequential epitopes of hen's egg ovomucoid as a marker for persistence of egg allergy. *Allergy*. 2007;62(7):758-765.
- Offermann LR, Bublin M, Perdue ML, et al. Structural and functional characterization of the hazelnut allergen Cor a 8. *J Agric Food Chem*. 2015;63(41):9150-9158.
- Robotham JM, Hoffman GG, Teuber SS, et al. Linear IgE-epitope mapping and comparative structural homology modeling of hazelnut and English walnut 11S globulins. *Mol Immunol*. 2009;46(15):2975-2984.
- Sackesen C, Suarez-Farinas M, Sillva R, et al. A new Luminex-based peptide assay to identify reactivity to baked, fermented, and whole milk. *Allergy*. 2019;74(2):327-336.
- Savilahti EM, Kuitunen M, Valori M, et al. Use of IgE and IgG4 epitope binding to predict the outcome of oral immunotherapy in cow's milk allergy. *Pediatr Allergy Immunol*. 2014;25(3):227-235.

24. Martinez-Botas J, Cerecedo I, Zamora J, et al. Mapping of the IgE and IgG4 sequential epitopes of ovomucoid with a peptide microarray immunoassay. *Int Arch Allergy Immunol*. 2013;161(1):11-20.
25. Savilahti EM, Rantanen V, Lin JS, et al. Early recovery from cow's milk allergy is associated with decreasing IgE and increasing IgG4 binding to cow's milk epitopes. *J Allergy Clin Immunol*. 2010;125(6):1315-1321.e9.
26. Till SJ, Francis JN, Nouri-Aria K, Durham SR. Mechanisms of immunotherapy. *J Allergy Clin Immunol*. 2004;113(6):1025-1034.
27. Shreffler WG, Beyer K, Chu TH, Burks AW, Sampson HA. Microarray immunoassay: association of clinical history, in vitro IgE function, and heterogeneity of allergenic peanut epitopes. *J Allergy Clin Immunol*. 2004;113(4):776-782.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Sackesen C, Erman B, Gimenez G, et al. IgE and IgG4 binding to lentil epitopes in children with red and green lentil allergy. *Pediatr Allergy Immunol*. 2020;31:158–166. <https://doi.org/10.1111/pai.13136>

IgE and IgG4 binding to lentil epitopes in children with red and green lentil allergy

Table S1. Positive IgE and IgG4 epitopes

Epitope number	IgE					IgG4					
	7	22	23	38	46	19	22	34	38	41	46
	107	108	109	110	111	47	50	82	83	90	108
	115	117	121	122	123	109	110	111	112	116	117
	124	127	128	129	130	118	120	121	122	123	125
	135					126	127	128	129	130	131
						132	133	134	135		

Bold numbers indicate overlap epitope binding of IgE and IgG4 antibodies

Table S2. Significantly higher binding epitopes in reactive patient groups

Mean signal intensity (standard error mean)					
Epitope	Red outgrown	Red reactive	Green outgrown	Green reactive	p value
IgE-109	-	-	0	35.6 (1.4-33)	0.05
IgE-117	0.9	81 (1.2-38.8)	-	-	0.03
IgE-128	0.9	92 (23.9-30)	-	-	<0.01
IgE-129	0	63 (2.4-26.6)	-	-	0.01
IgG4-127	39.1	113.1 (13.7-24.8)	-	-	0.03
IgG4-128	31.5	112.8 (9.1-21.5)	-	-	0.01

Table S3. AUC values of statistically significant epitopes and lentil sIgE against reactivity

Epitope	AUC	p value
Lentil sIgE	0,644	0,1
IgE-128	0.718	0.04
IgG4-110	0.774	0.01
IgG4-111	0.800	<0.01
IgG4-120	0.759	0.02
IgG4-125	0.779	0.01
IgG4-128	0.723	0.04
IgG4-131	0.795	<0.01
IgG4-132	0.728	0.04
IgG4-133	0.785	0.01

AUC: Area under curve

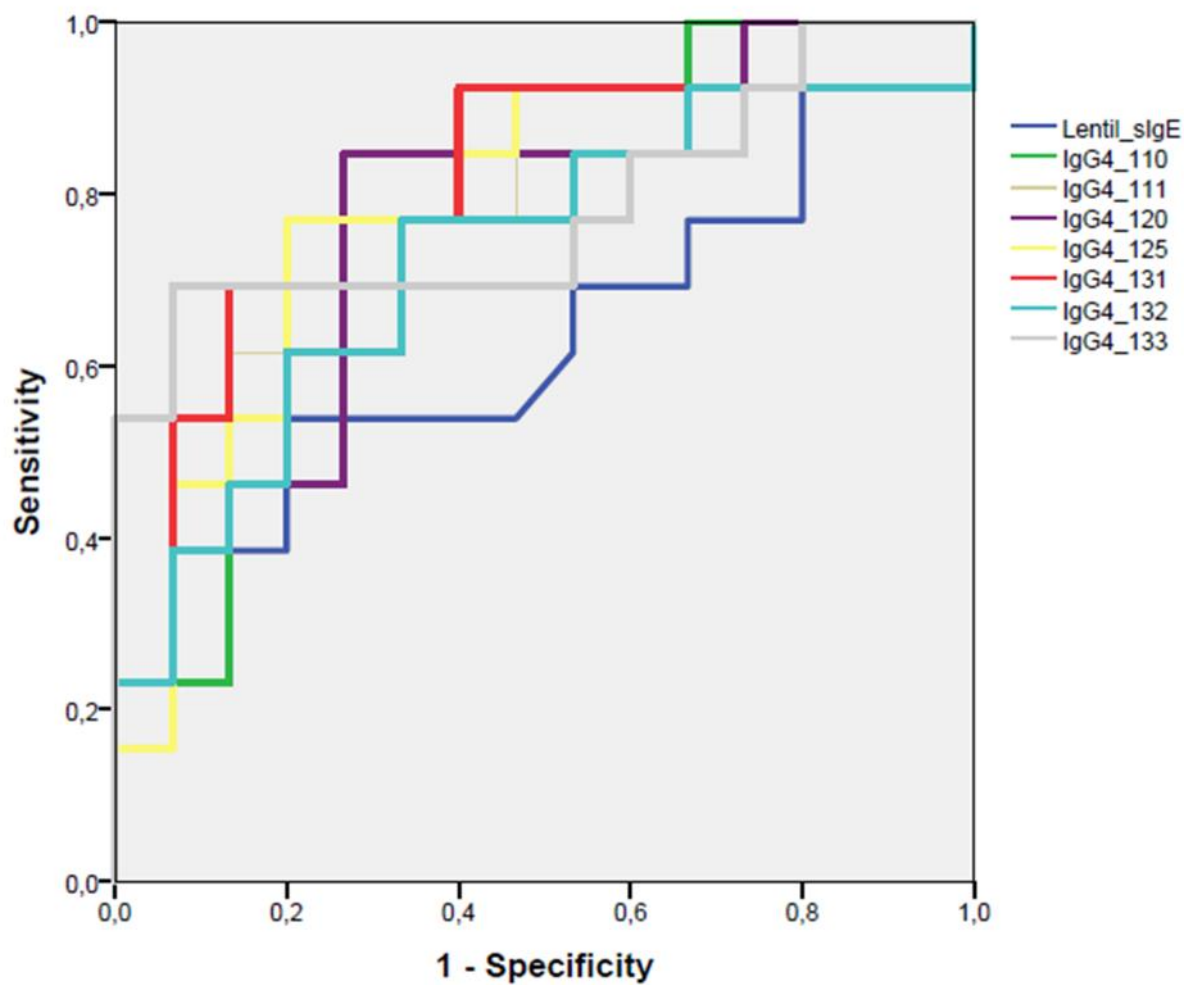


Figure S1. Receiver operating characteristic (ROC) curve predicting clinical reactivity for statistically significant IgE and IgG4 epitopes