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2S protein Ara h 7.0201 has unique epitopes compared to other Ara h 7 isoforms and is comparable to 2S proteins Ara h 2 and 6 in basophil degranulation capacity

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Summary

Background: Screening for specific IgE against 2S albumin proteins Ara h 2 and 6 has good positive predictive value in diagnosing peanut allergy. From the third 2S member Ara h 7, 3 isoforms have been identified. Their allergenicity has not been elucidated.

Objective: This study investigated the allergenicity of Ara h 7 isoforms compared to Ara h 2 and 6.

Methods: Sensitization of 15 DBPCFC-confirmed peanut-allergic patients to recombinant Ara h 2.0201, Ara h 6.01 and isoforms of recombinant Ara h 7 was determined by IgE immunoblotting strips. A basophil activation test (BAT) was performed in 9 patients to determine IgE-cross-linking capacities of the allergens. Sensitivity to the allergens was tested in 5 patients who were sensitized to at least 1 Ara h 7 isoform, by a concentration range in the BAT. 3D prediction models and sequence alignments were used to visualize differences between isoforms and to predict allergenic epitope regions.

Results: Sensitization to Ara h 7.0201 was most frequent (80%) and showed to be equally potent as Ara h 2.0201 and 6.01 in inducing basophil degranulation. Sensitization to Ara h 7.0201 together with Ara h 2.0201 and/or 6.01 was observed, indicating the presence of unique epitopes compared to the other 2 isoforms. Differences between the 3 Ara h 7 isoforms were observed in C-terminal cysteine residues, pepsin and trypsin cleavage sites and 3 single amino acid substitutions.

Conclusion & clinical relevance: The majority of peanut-allergic patients are sensitized to isoform Ara h 7.0201, which is functionally as active as Ara h 2.0201 and 6.01. Unique epitopes are most likely located in the C-terminus or an allergenic loop region which is a known allergenic epitope region for Ara h 2.0201 and 6.01. Due to its unique epitopes and allergenicity, it is an interesting candidate to improve the diagnostic accuracy for peanut allergy.

KEYWORDS
allergens and epitopes, basophil, food allergy, IgE

1 | INTRODUCTION

It is estimated that approximately 11.4%-13.1% of children (0-17 years) and 3.2%-5.1% of adults (>18 years) in European countries are sensitized against at least 1 food allergen, based on the detection of specific IgE in serum.[1] Most food allergies are IgE-mediated, and symptoms develop within minutes to a few hours after ingestion of the specific allergen. Among food allergies, peanut allergens are most frequent in eliciting a fatal food reaction, and it is estimated that 0.6% of the total population is affected by peanut allergy.[2] By determining specific IgE to recombinant or purified peanut proteins rather than crude peanut extract, component-resolved diagnostics (CRD) has proven to be a useful tool to improve diagnostic accuracy for peanut allergy.[3] However, the functionality of several potentially relevant allergens remains unknown, as their capacity to induce effector cell degranulation has never been tested in patient samples before.

To date, 17 peanut allergens (Ara h 1-17) are known and most of them have been sequenced and cloned.[3-6] Previous research indicated that screening for specific IgE against 2S albumins Ara h 2 and 6 to date is most effective in diagnosing peanut allergy, as the majority of peanut-allergic patients have specific IgE against these allergens.[6-9] Ara h 2 and Ara h 6 are both proteins belonging to the conglutin family, and Ara h 6 has approximately 53% sequence identity with Ara h 2.[5,6]

Next to determining specific IgE against Ara h 2 and Ara h 6 for diagnosing peanut allergy, a third 2S protein Ara h 7 is currently gaining attention as predictor for peanut allergy.[10] Although Ara h 7 makes up only 0.5% of total peanut protein,[3] it is a storage protein and therefore considered an allergen with a potential strong diagnostic value.[11] In comparison, the abundance of Ara h 2 ranges between 5.9% and 9.3% of total peanut protein, and for Ara h 6, this is 4%-14%.[3,12] By phage display technology, isoforms Ara h 7.0101 and Ara h 7.0201 were previously identified as allergens, but only Ara h 7.0201 was identified in peanut extract.[5] Ara h 7.0101 shares 42% sequence identity with Ara h 2 and 45% with Ara h 6, whereas Ara h 7.0201 shares 44% sequence identity with Ara h 2 and 52% with Ara h 6.[5,13] Next to these 2 isoforms of Ara h 7, a third isoform labelled Ara h 7.0301 shares 70% sequence identity with Ara h 7, and was also identified in peanut extract.[5,14] Previous research indicated the presence of unique epitopes in these Ara h 7 isoforms, and therefore, they may be relevant in peanut allergy diagnosis.[10]

As the functionality of Ara h 7 has not yet been studied extensively, the aim of this study was to identify whether the capacity of Ara h 7 to induce basophil degranulation using whole blood from peanut-allergic patients is similar to Ara h 2 and 6. In addition, the allergen recognition pattern of the 3 isoforms of Ara h 7 by peanut-allergic patients was studied. Furthermore, in relation to their functionality, the amino acid sequence and a 3D prediction protein model were used to predict epitopes or regions of Ara h 7 that are important in inducing basophil degranulation and allergenicity.

2 | MATERIALS & METHODS

2.1 | Study design and study population

Assessment of sensitization to 2S peanut allergens was performed with residual plasma of 15 peanut-allergic patients who visited the outpatient clinic of Dermatology/Allergology at the University Medical Center Utrecht in 2015-2017 for clinical research. Table 1 shows data on gender, age and historical data on SPT, subjective and objective doses determined by DBPCFC, and Müller score. Of these 15 patients, 9 random patients who were scheduled for visiting the UMC for clinical research were able to donate blood for the functional basophil activation test. Five random patients from the complete cohort who were sensitized to at least 1 Ara h 7 isoform were recruited for the concentration range BAT. Inclusion criteria consisted of a type I allergic reaction to peanut, confirmed by a positive double-blind placebo-controlled food challenge (DBPCFC). Use of prednisone, other immunosuppressants and pregnancy were exclusion criteria. Informed consent was obtained of all patients prior to the study. The study was reviewed and approved by the Ethics Committee of the University Medical Center Utrecht (NL51606.041.15).

TABLE 1 Patient characteristics. Sex, age, skin prick test (SPT), DBPCFC results and Müller score per peanut-allergic subject

Patient	Sex (M/F)	Age (y)	SPT peanut (mm)	Subjective ED (mg)	Objective ED (mg)	Müller score[a]
N01	F	41	3+	10	–	2
N02	M	37	3+	0.1	300	4
N03	M	45	4+	100		2
N04	F	50	4+	10	10	3
N05	F	35	4+	0.1	–	4
N06	F	27	4+	4	40	2
N07	M	42	5+	Not known	300	3
N08	M	24	4+	100	>3000	1
N09	F	24	3+	Not known	>3000	3
N10	F	18	4+	300	1000	3
N11	F	32	4+	10	3000	2
N12	M	27	5+	0.1	1000	3
N13	M	25	3+	10	–	2
N14	F	26	4+	0.1	100	3
N15	F	34	4+	40	12 000	2

Skin prick test (mm)—a diameter of 3 mm (3+) was considered positive. Subjective and objective effective dose (ED) during DBPCFC indicated in mg.

[a]Müller score 0: symptoms of oral cavity; 1: symptoms of the skin and mucous membranes; 2: gastrointestinal symptoms; 3: respiratory symptoms; and 4: cardiovascular symptoms.

2.2 | Expression and purification of crude peanut extract and recombinant allergens

Crude peanut extract (CPE) was obtained by blending raw peanuts, followed by extraction at room temperature with Tris/NaCl buffer (20 m mol L^{-1} Tris, 150 m mol L^{-1} NaCl, pH 7.2). Supernatant was filtered twice and diluted in 1× PBS to the appropriate concentration. Recombinant peanut allergens Ara h 2.0201 (Acc. no. Q6PSU2), Ara h 6.01 (Acc. no. Q647G9), Ara h 7.0101 (Acc. no. Q9SQH1), Ara h 7.0201 (Acc. no. B4XID4) and Ara h 7.0301 (Acc. no. Q647G8) were provided by EUROIMMUN and produced as described previously.[10,15]

2.3 | Immunoblot

Immunoblots (Euroline; EUROIMMUN, Lübeck, Germany) were used to determine sensitization of 15 patients to isoforms of recombinant peanut proteins of Ara h 2.0201, Ara h 6.01, Ara h 7.0101, Ara h 7.0201 and Ara h 7.0301. Immunoblots and reagents were kindly provided by EUROIMMUN, and experiments were performed according to the manufacturer's instructions. In short, the Euroline strips were incubated on a rocking shaker overnight at RT with 100 μL of 1:11 diluted patient plasma in universal buffer. Binding of antibodies was visualized with an enzyme-labelled anti-human IgE antibody in combination with substrate nitro blue tetrazolium/5-bromo-4-chloro-3′-indolyphosphate. The results were evaluated with EuroLineScan software. The intensity of the bands was measured, and an intensity level of 3 or higher was considered positive (arbitrary units). Line blot intensity values of 2 and lower are considered negative.

2.4 | Basophil activation test

Whole heparinized blood was obtained from 9 of 15 peanut-allergic patients, and a BAT was performed. Blood samples were stimulated for 30 minutes with increasing concentrations or 1000 ng/mL of crude peanut extract (CPE), or separate recombinant peanut allergens in RPMI-1640 medium (Gibco, Life Technologies) supplemented with 1 ng/mL IL-3 (R&D Systems). Control samples for the basophil activation test were rVP40 (recombinant VP40, control protein) and buffer. Leucocytes were stained with an antibody cocktail of CD45-PO (Life Technologies), CD123-FITC (Biolegend), HLA-DR-PB (Biolegend), CD63-PE (Monosan), CD41-PE-Cy7 (Beckman Coulter) and CD203c (Biolegend). Basophils were defined as $CD45^{+}$ $CD203c^{+}$ $CD123^{+}$ and $HLA\text{-}DR^{-}$ $CD41^{-}$, and degranulation was quantified by determining the surface expression of CD63. Results are expressed as percentage of CD63-bright basophils. A threshold above 5% degranulation was considered positive.

2.5 | 3D protein models and distance mapping

3D protein models were created with Protein Homology/analogY Recognition Engine (PHYRE).[16] Further graphics and analyses such as distance mapping were performed with UCSF Chimera, developed by the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco.[17]

2.6 | Data analysis and statistics

Correlation between percentage degranulation of basophils and intensity of the Euroline strips was determined with Spearman's

(A)

		Line blot intensity (arbitrary units)				
Patient	CAP peanut (kU/L)	Ara h 2.0201	Ara h 6.01	Ara h 7.0101	Ara h 7.0201	Ara h 7.0301
N01	1.7	4	2	2	2	2
N02	44	67	71	5	3	0
N03	1.8	3	11	0	7	0
N04	12	4	0	2	5	1
N05	85	18	31	10	34	5
N06	12.8	3	62	8	3	1
N07	42.7	57	58	26	74	25
N08	1.9	4	5	1	2	0
N09	1	3	2	2	2	0
N10	>100	167	81	56	115	106
N11	n.d.	12	26	1	5	1
N12	66	74	96	6	106	12
N13	11.2	29	4	3	40	4
N14	9.7	50	61	8	54	4
N15	1.55	2	17	8	12	3
		14/15 (93%)	12/15 (80%)	9/15 (60%)	12/15 (80%)	7/15 (47%)

(B)

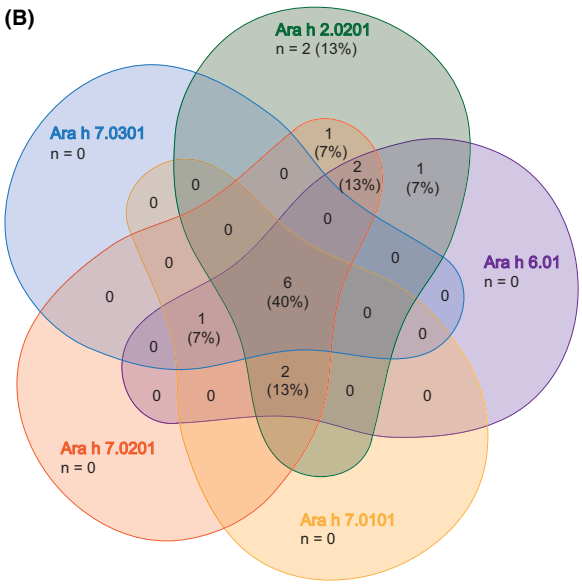

FIGURE 1 Cosensitization to peanut allergens of 15 peanut-allergic patients. A, Specific IgE (kU/L) against peanut determined by ImmunoCAP are indicated per patient in the left column. Sensitization to allergens Ara h 2.0201, 6.0101, 7.0101, 7.0201 and 7.0301 by line blot are shown as intensity values (arbitrary units) with intensity values > 3 considered positive. B, Venn diagram illustrating cosensitization to peanut allergens Ara h 2.0201, 6.0101, 7.0101, 7.0201 and 7.0301 based on the intensity level of the Euroline strips (n = 15)

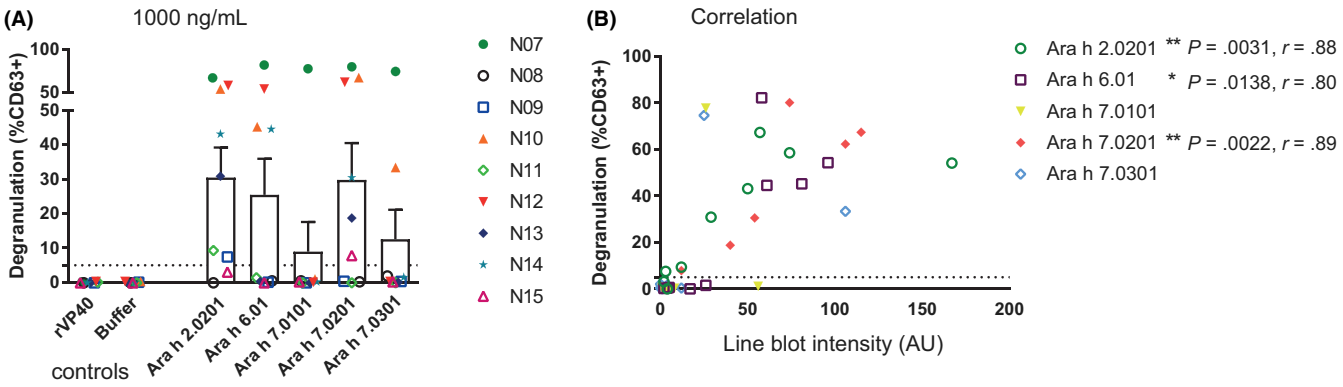

FIGURE 2 Functionality Ara h 7 isoforms in basophil activation test. A, BAT assay in peanut-allergic patients at an allergen concentration of 1000 ng/mL. Degranulation is indicated as percentage of $CD63^+$ cells (n = 9). B, Spearman correlation between degranulation in the BAT assay vs the intensity levels of the line blot strips (n = 9), * $P < .05$, ** $P < .01$

correlation coefficient, as the data were not normally distributed. GraphPad Prism 7 (GraphPad Software, USA) was used for statistical testing and visualizing data.

3 | RESULTS

3.1 | Peanut-allergic patients can be sensitized to Ara h 2.0201, Ara h 6.01 and all isoforms of Ara h 7

In 15 peanut-allergic patients, sensitization to Ara h 2.0201, Ara h 6.01 and the 3 isoforms of Ara h 7 was established by means of the immunoblot strips (Figure 1A). Sensitization to Ara h 2.0201 was most abundant; 14 of 15 patients were sensitized to this allergen, followed by sensitization to Ara h 6.01 and 7.0201, which were both recognized by 12 patients (80%). Two patients were monosensitized to Ara h 2.0201 (N01, N09), while also cosensitization for multiple allergens occurred. 40% of the patients were sensitized to all allergens, while 1 patient (N15) recognized all allergens except Ara h 2.0201 (Figure 1B). Ara h 7.0201 sensitization in combination with Ara h 2.0201 and/or Ara h 6.01 was found for 3 patients (N03, N04 and N11), while sensitization to Ara h 7.0101 or Ara h 7.0301 was never observed in the absence of Ara h 7.0201.

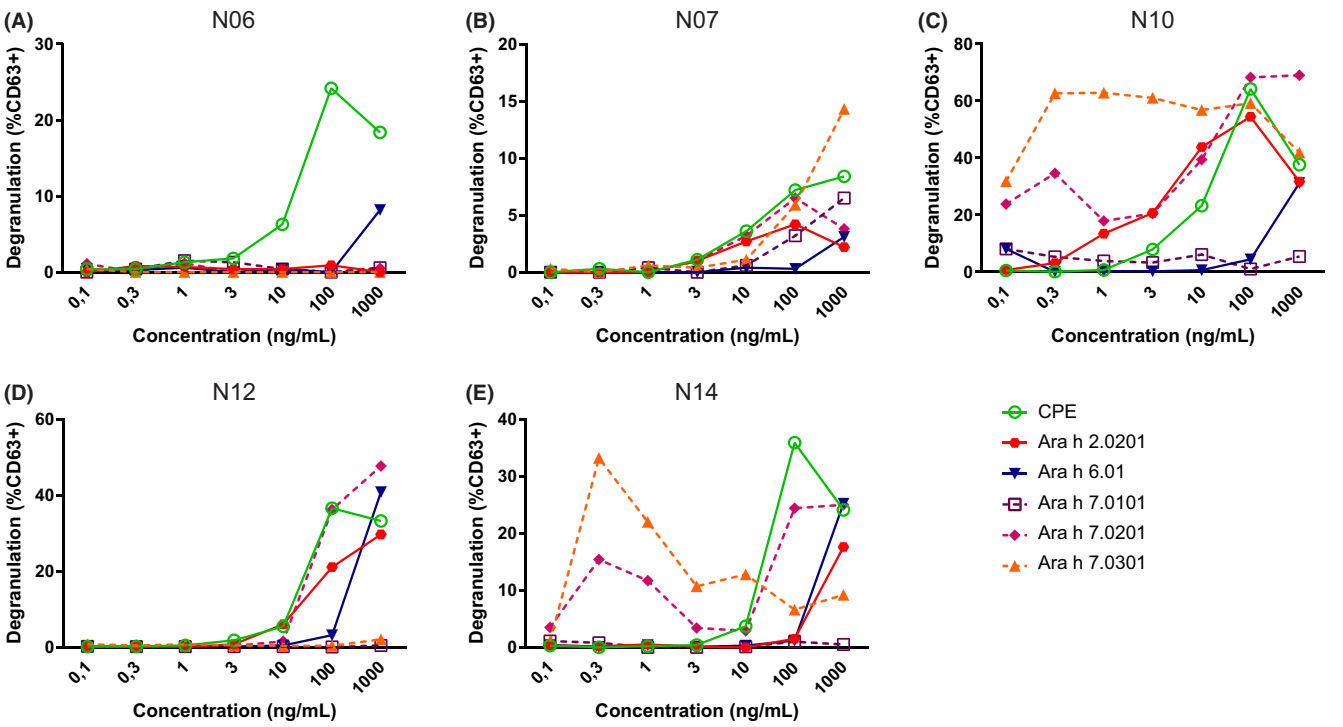

FIGURE 3 Degranulation of basophils after exposure to different allergen concentrations. A-E, Basophil activation of 5 peanut-allergic patients in a BAT assay with an allergen concentration range (CPE, Ara h 2.0201, 6.0101, 7.0101, 7.0201 and 7.0301)

3.2 | All Ara h 7 isoforms can induce basophil degranulation, and their line blot intensity levels correlate with basophil degranulation

Basophil degranulation experiments were performed for 9 peanut-allergic patients, with the optimal allergen concentration of 1000 ng/mL (Figure 2A). All allergens were able to induce basophil degranulation. Of the 3 Ara h 7 isoforms, Ara h 7.0201 was most effective, as it induced basophil degranulation in 6 patients, followed by Ara h 7.0301 which induced basophil degranulation in 2 patients. Ara h 7.0101 was able to induce degranulation in only 1 patient. Next to basophil degranulation, the correlation between the intensity levels of the Euroline strips, which are directly related to levels of specific IgE, and the percentage of basophil degranulation was determined (Figure 2B). A significant correlation ($P < .05$), r = .8-.9, was observed between the line blot intensity and basophil degranulation upon Ara h 2.0201, 6.01 or 7.0201 exposure (Figure 2B).

3.3 | Isoforms Ara h 7.0201 and Ara h 7.0301 can induce basophil degranulation at least as well as Ara h 2.0201 and 6.01

To compare the ability to induce basophil degranulation at low allergen concentrations, a concentration range of allergens was used in the BAT in whole blood of 5 patients who were sensitized against at least 1 isoform of Ara h 7. Patient N06 was sensitized to Ara h 2.0201, 6.01, 7.0101 and 7.0201. However, only basophil degranulation was detected upon exposure to CPE and Ara h 6.01 (Figure 3A), which is probably related to relatively low intensity levels of sensitization for Ara h 2.0201 and 7. N10 and N14 showed already high basophil degranulation upon low concentrations (0.3 ng/mL) of isoforms Ara h 7.0201 and Ara h 7.0301 (Figure 3C, E), while basophils of other patients degranulated around 10 ng/mL of Ara h 2, 6 and 7. In the other 2 patients (N07 and N12), the maximal degranulation upon exposure to the Ara h 7 isoforms was comparable to Ara h 2.0201 and 6.01. This indicates that isoform Ara h 7.0201 is at least as effective as recombinant Ara h 2.0201 and Ara h 6.01 in terms of inducing basophil degranulation.

3.4 | Sequential differences in sequence alignment of Ara h 7 isoforms compared to Ara h 2.0201 and 6.01

A sequence alignment between Ara h 2.0201, 6.01 and the 3 Ara h 7 isoforms was performed, to explain the differences in the efficacy of Ara h 7.0201 to induce basophil degranulation in more patients than the other 2 Ara h 7 isoforms (Figure 4A). Known linear epitopes of Ara h 2 and 6 recognized by allergic patients are highlighted in colour.[18] Of the 3 isoforms, Ara h 7.0201 showed most sequence similarity with Ara h 2.0201 and 6.01 in the C-terminal regions that are known to be allergenic linear epitopes in Ara h 2 and 6 (orange underlined sequence).[18] Similar to the conserved cysteine pattern of at least 8 conglutins of Ara h 2.0201 and Ara h 6.01, Ara h 7.0201 is the only isoform containing 8 cysteine residues (underlined C-residues), whereas Ara h 7.0101 and Ara h 7.0301 only contain 6 cysteine residues. Cysteine residues play an important role in the folding and stability of proteins.[11] Furthermore, Ara h 7.0201 differed in 3 amino acid positions from both other isoforms (Figure 4B, arrows). These differences influence polarity, hydrophobicity, charge

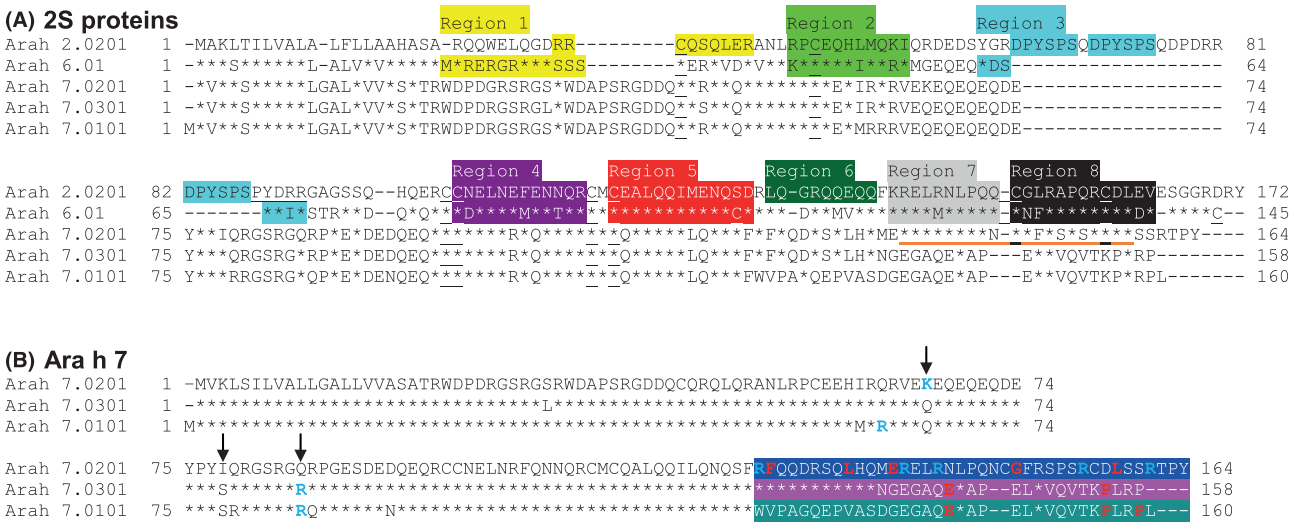

FIGURE 4 Sequence alignment of Ara h 2.0201, 6.0101, 7.0201, 7.0101 and 7.0301. A, Known allergenic epitopes of Ara h 2.0201 and 6 are colour-highlighted and explained below. Cysteine residues are underlined. Stars indicate similarities compared to Ara h 2.0201. C-terminal similarity of Ara h 7.0201 compared to Ara h 2.0201 and 6 is underlined in orange. B, Sequence alignment of Ara h 7.0201, 7.0101 and 7.0301. Similarities compared to Ara h 7.0201 are indicated with stars. Arrows indicate differences in amino acid sequence of Ara h 7.0201 to both other isoforms. Blue residues indicate trypsin cleavage sites and red residues pepsin cleavage sites. The C-terminus is highlighted at the end

and trypsin cleavage sites (blue residues). In addition, more differences in trypsin (blue) and pepsin (red) cleavage sites were observed in the C-terminus (highlighted end) between the 3 different isoforms, which plays an important role in the enzymatic digestion and thus can influence stability and allergenicity of proteins (Figure 4B).

3.5 | 3D structural differences between Ara h 7 isoforms related to known allergenic epitope sites of Ara h 2.0201 and 6.01

In contrast to Ara h 2 and 6, of which crystal structures have been described,[2,19] no crystal structure is available for the isoforms of Ara h 7 and isoforms Ara h 2.0201 and Ara h 6.01. In addition to the sequence alignment, predictive protein 3D models of all isoforms were therefore created with PHYRE and UCSF Chimera (Figure 5). Known allergenic linear epitopes of Ara h 2.0201 and 6.01 are highlighted in the same colours as displayed in Figure 4 (Figure 5A, B).[18] Figure 5C-E shows the predicted 3D models of the Ara h 7 isoforms. The sequence alignment indicated that most differences were located in the C-terminus. In the 3D models, a main structural difference with Ara h 7.0101 is observed in this C-terminus (turquoise), and some smaller differences are observed between Ara h 7.0201 and Ara h 7.0301 in this region (pink vs blue). The 3 amino acids that differ between these 3 isoforms (light blue) are all located in a loop which is a known epitope region for Ara h 2.0201 and 6.01 (region 3). As these changes in amino acids can influence hydrophobicity, polarity and charge, an amino acid distance analysis was performed (UCSF Chimera; Figure S1). Indeed, mainly in the loop region (green circle), differences in distance between amino acid residues were observed.

4 | DISCUSSION

Ara h 2 and Ara h 6 have proven to be 2 of the most informative peanut allergens in the diagnosis of peanut allergy, as most patients have specific IgE against 1 or both allergens.[7] In addition, the current study shows that 80% of the 15 peanut-allergic patients studied were sensitized to 1 or multiple isoforms of a third recombinant 2S albumin member Ara h 7, mostly in combination with recombinant

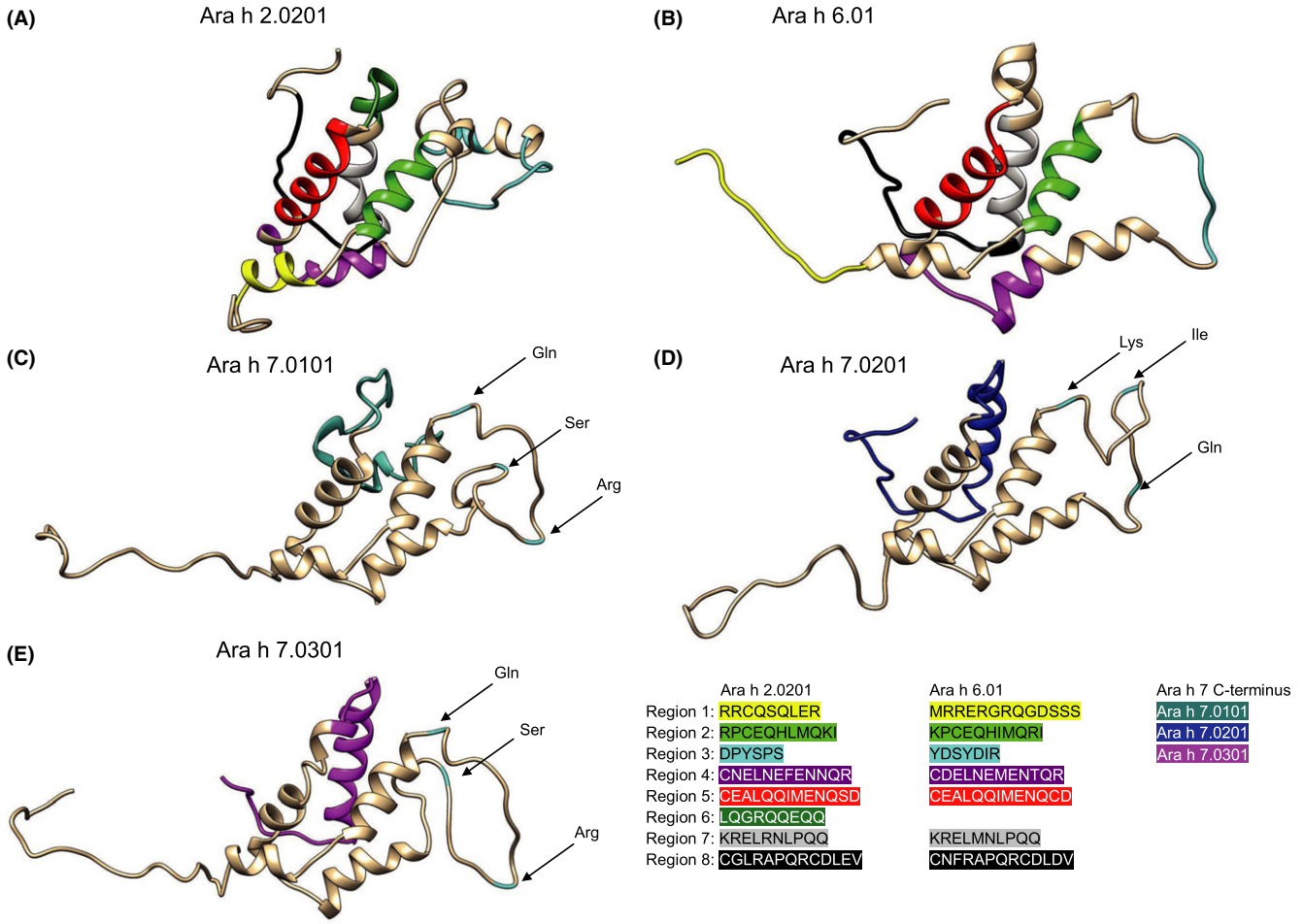

FIGURE 5 3D protein prediction models of Ara h 2.0201, 6.0101 and 7. A-B, 3D model of Ara h 2.0201 and 6; known allergenic epitopes are indicated in colour according to the colour scheme of Figure 4. C-E, 3D prediction models of the 3 Ara h 7 isoforms. Turquoise (Ara h 7.0101), blue (Ara h 7.0201), or pink (Ara h 7.0301) indicates the C-terminus, and light blue residues indicated with arrows indicate the main differences between Ara h 7.0201 and the other 2 isoforms in loop region 3

Ara h 2.0201 or Ara h 6.01. This is probably explained by the sequence identity between these 3 isoforms and Ara h 2.0201 and 6.01.[2,5,6,8,9] Ara h 7.0201 showed the highest sensitization frequency among peanut-allergic patients (80%), which was comparable to sensitization to Ara h 2.0201 and 6.01 (93 and 80%, respectively).

To the best of our knowledge, this is the first time that the functionality of recombinant Ara h 7 isoforms was tested, rather than only determining specific IgE binding in patient samples. Although the BAT assay can be a variable assay, grouped results indicate that overall, Ara h 7.0201 was able to induce basophil degranulation comparable to Ara h 2.0201 and 6.01. In 2 independent patients, Ara h 7.0201 and Ara h 7.0301 were able to induce basophil degranulation at relatively low concentrations of allergen compared to CPE and Ara h 2.0201 and 6.01, suggesting that these specific Ara h 7 epitopes can be recognized by sensitized individuals and increase efficacy in stimulating basophil degranulation. While this could not be directly related to sensitization levels of the line blot strips, it indicates that some patients can react to low concentrations of Ara h 7. Although Ara h 7 represents only 0.5% of peanut protein content, in contrast to 4%-14% for Ara h 2 and 6,[3,12] this allergen has the potency to induce responses at low concentrations. Sensitization to isoform Ara h 7.0101 was observed in 60% of peanut-allergic patients, although biologic activity was observed in only 1 patient. Ara h 7.0101 was identified with phage display technology, but could not be retrieved in peanut extract,[5] which is most likely the explanation for this reduced biologic activity. Cross-reactivity between the 3 isoforms may explain the observed sensitization for this isoform.

A limitation of this study is that currently there is no native Ara h 7 available. All experiments were performed with recombinant proteins. Native Ara h 2 and 6 have been shown to induce basophil degranulation at lower concentrations of allergen than those of the recombinant proteins used in this study.[9] Nevertheless, the recombinant Ara h 7 proved to be able to induce basophil degranulation in some patients at already low concentration, indicating that it might even be more reactive in crude peanut extract.

A limitation of the BAT assay is that the response of patients to specific allergens can be significantly variable and not always follows the typical bell-shaped dose-response curve,[20] as, for instance, is observed in patients N10 and N14. The BATs performed in this study were only performed once for each patient. Combining the obtained results, Ara h 7.0201 appears the most promising Ara h 7 isoform in optimizing peanut allergy diagnosis, as it possesses a similar sensitization profile and efficacy in basophil degranulation as recombinant Ara h 2.0201 and 6.01. Ara h 7.0201 contains more unique epitopes than the other 2 Ara h 7 isoforms, as patients are sensitized more often to this isoform, and they can be sensitized to this particular isoform combined with only Ara h 2.0201 and Ara h 6.01 (N03, N04 and N11). Sensitization to either of the other 2 isoforms in combination with Ara h 2.0201 and/or 6.01 was not observed.

Although Ara h 7.0201 contains cross-reactive epitopes with Ara h 2.0201 and 6.01, a previous study indicated that monosensitization against Ara h 7.0201 was observed in 2 of 15 patients.[10] This suggests that Ara h 7.0201 indeed contains 1 or more epitopes not present on the other Ara h 7 isoforms or Ara h 2.0201 and 6.01. The similarity of Ara h 7.0201 in sensitization and basophil degranulation with Ara h 2.0201 and 6.01 is most likely related to the C-terminus of Ara h 7.0201, as it fits into the conserved cysteine conglutin family pattern of at least 8 cysteine residues, in contrast to the other 2 isoforms.[5] These C-terminal cysteine residues are important for protein stability and determine the IgE binding of allergens.[21] By 3D protein modelling, the 3 main differences in amino acid sequence of the Ara h 7 isoforms were visualized in a loop region that is a known allergenic epitope for Ara h 2.0201 and 6.01. Due to these amino acid substitutions, small changes in distance between amino acids occur, which could contribute to enhanced exposure to an epitope. Combining these 2 findings, it is expected that the unique epitopes of Ara h 7.0201 are located either in the C-terminus or in this loop region 3. Therefore, differences in enzymatic digestion by pepsin and trypsin may influence the allergenicity of Ara h 7 isoforms.

Taking together previous data[10] and the data presented in this study, we hypothesize that determining specific IgE for Ara h 7.0201 can be of additional value in peanut allergy diagnosis. Ara h 7.0201 contains unique epitopes and is functionally as active as Ara h 2.0201 and Ara h 6.01 in inducing basophil degranulation. In addition, in some patients Ara h 7 can already provoke basophil degranulation at low concentrations. Due to cross-reactivity between Ara h 2.0201, 6.01 and 7, the latter one could have a potential strong diagnostic value.

CONFLICT OF INTEREST

J. Garssen is employed by Nutricia Research. W. Suer is an employee of EUROIMMUN AG. E.F. Knol has received a fee for speaking and reimbursement for attending a symposium from Thermo Fisher Diagnostics. A.M. Ehlers was an employee of EUROIMMUN AG until 2016. Her position at UMC Utrecht is funded by EUROIMMUN AG.

AUTHOR CONTRIBUTIONS

SH, LW and HO designed the experiments; WS provided the EUROIMMUN reagents; AK assisted in recruitment of patients; and SH and CHJ performed the experimental procedures. SH performed data collection and analyses and drafted the manuscript. EK, AE, JG, WS, CHJ, AK, LW and HO contributed to data interpretation and critically revised the manuscript.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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