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Mass spectrometry confirmation that clinically important peanut protein allergens are present in household dust

To the editor

The route of exposure of peanut in the first year appears to be crucial in determining whether peanut allergy or tolerance develops. Epicutaneous peanut exposure via an inflamed disrupted skin barrier increases the risk of peanut allergy.¹ Furthermore, there is evidence that perinatal exposure to peanut in dust is associated with the development of peanut sensitization and allergy in children with a disrupted skin barrier.² Environmental exposure to peanut in early life is therefore an important factor to quantify and consider in the development of peanut allergy. Previous studies have quantified peanut protein (using a polyclonal whole peanut protein ELISA) and specific peanut allergens (using a monoclonal Ara h 1

ELISA) in household dust and surfaces.^{2,3} Studies linking peanut protein in dust to peanut sensitization used an ELISA employing a polyclonal antibody which has been validated for sensitivity, specificity, and reproducibility with regards analysis of dust samples⁴; however, given the specificity of the ELISA,^{5,6} it does not confirm the presence of peanut allergens (eg, Ara h 2) and isoforms thereof. We therefore used mass spectrometry (MS) to confirm the presence of peanut proteins present in dust samples previously indicated by ELISA and characterize the repertoire of peanut allergens present.

Dust samples were collected from UK households using a method previously described⁴; they had high (n = 2, infant's play-area

TABLE 1 Detection of example allergen sequence isoforms by MS and quantitation of peanut content by ELISA

Allergen	UniProt accession	Dust sample					
		1		2		3	
		Protein coverage (%)	-10logP (peptides)	Protein coverage (%)	-10logP (peptides)	Protein coverage (%)	-10logP (peptides)
Cupin barrel family							
Ara h 1	P43237	62	50.74-132.26	19	57.8-109.99	Not identified	
Ara h 3	Q0GM57	51	50.08-127.56	24	50.46-122.36	4	
Prolamin family							
Ara h 2	Q6PSU2*	79	53.23-136.9	39	51.84-92.56	15	
Ara h 6	Q647G9	64	50.35-133.47	29	72.88-101.96	Not identified	
Ara h 7	B4XID4	45	53.34-121.31	15	60.01-95.76	Not identified	
Ara h 9	B6CG41	21	73.4	Not identified		Not identified	
Bet v 1 family							
Ara h 8	B0YIU5	9 [one peptide]	50.35	Not identified		Not identified	
Oleosin family							
Arah10,11	Q9AXI1	14 [one peptide]	62.95	Not identified		Not identified	
Profilin family							
Ara h 5	Q9SQI9	Not identified		Not identified		Not identified	
ELISA peanut quantitation							
Peanut protein detected (mg/g)		85		80		0	

Note: Quality criteria for identifications of example isoforms of allergen families are given and are described hereafter. Protein coverage (%) indicates the amount of sequence of each protein accession for which peptide identities were assigned (higher is better). The range of -10logP scores for peptides used in these protein identifications are given as ranges encompassing detected peptides. These -10logP scores (higher is better) are comparable to P-values with a -10logP score of 20 being equivalent to a P-value of 1%. An FDR cutoff of <0.1% was used to ensure confident identifications (usual standard is < 1%).

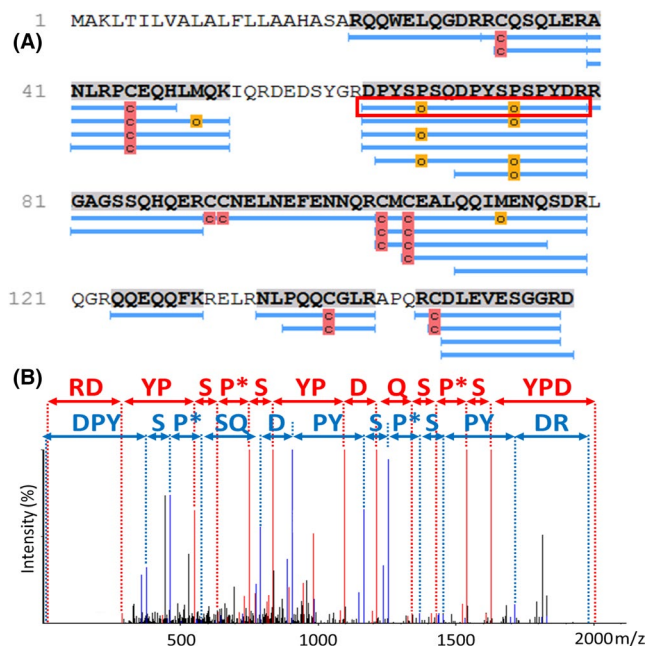


FIGURE 1 Confirmation of the presence of the Ara h 2 isoform Q6PSU2-3 in dust sample 1. Protein coverage map (A) shows all observed peptides (indicated by blue line) at FDR < 0.1% confidence, with modifications indicated (c = carbamidomethyl, o = oxidation). The peptide boxed in red is described further in inset (B), which shows the MS2 spectrum of the peptide DPYS(P*)SQDPYS(P*)SQDPDR (P* denoting hydroxyproline). This peptide is unique to the peanut allergen Ara h 2 (Q6PSU2 isoforms). Major y-ions (red) and b-ions (blue) used to confirm peptide presence are shown

(1) and bed-sheet (2)) or low ($n = 1$, maternal bed-sheet (3)) peanut concentration (determined by the Veratox polyclonal peanut ELISA (detecting total peanut protein), Neogen Europe) were used for the MS analysis.

1 | SAMPLE PREPARATION

Chemicals were obtained from Sigma-Aldrich unless otherwise indicated. Dust (5 mg) was extracted 1:20 (weight: volume) in 50 mmol/L Tris Cl⁻ (pH 8.8), 50 mmol/L DTT, 0.04% (w/v) Rapigest™ (kind gift of Waters Corporation). Samples were reduced, alkylated, and digested using trypsin (Promega) as described previously,⁷ prior to sample cleanup using Pierce C18 spin columns (Thermo Scientific) according to manufacturer's instruction. Samples were stored frozen at -20°C prior to analysis.

2 | MASS SPECTROMETRY

Samples were analyzed by the Biological Mass Spectrometry Facility of the University of Manchester using reverse phase HPLC electrospray ionization MS (RP-HPLC-ESI-MS) using a Nanoacquity nano-LC (Waters) coupled to an Orbitrap Elite mass spectrometer

(Thermo Scientific).^{7,8} In order to maximize the likelihood of peanut detection, the total digests derived from 5 mg of dust were analyzed using a single injection. Data Analysis was performed as described in the Methods S1. The FASTA database used was downloaded from Uniprot (TAXID 3818: *A. hypogaea*) (<https://unl.box.com/s/gg8xoql7k8aj6fjoomr0mnsfk47od8o0>).

The polyclonal ELISA quantified the concentration of peanut protein in dust samples 1 and 2 as 85 and 80 µg/g, respectively, whereas that in dust sample 3 was below the lower limit of quantitation (<0.625 µg/g). Abbreviated analysis of the MS/MS data obtained from the same extracted dust samples is shown in Table 1 (more extensive reporting available: <https://unl.box.com/s/gg8xoql7k8aj6fjoomr0mnsfk47od8o0>). In dust samples 1 and 2, many peptides derived from peanut proteins were evident even at FDR < 0.1%, the highest stringency allowable with the PEAKS data package. In the case of dust sample 3, (suggested by ELISA analysis to contain little or no peanut protein) there was an indication of far fewer peanut derived peptides. Results followed a similar trend for both ELISA and number of peanut peptides identified, with dust sample 3 having the smallest amount of peanut, followed by 2 and 1 having the most.

An overview of the specific allergen detection by mass spectrometry which underlies the detection of peanut allergen families (eg, Ara h 1 or Ara h 2) and example isoforms thereof in dust are shown in Table 1. We indicate only one isoform for each allergen, but unique peptides indicating the presence of different isoforms were detected (see link: <https://unl.box.com/s/gg8xoql7k8aj6fjoomr0mnsfk47od8o0>). It should be noted that MS analysis is limited by availability of sequences and that this study relied upon the UniProt sequences available for peanut which are not complete. Protein sequence coverage was poorer compared with previous profiling of allergens from peanut flour using data-independent acquisition but was similar between allergens, with better coverage being observed for Ara h 2 and 6 compared with Ara h 1 and 3 in the dust samples. The allergens Ara h 3, 1, 2, and 6 dominate the peanut seed proteome, and this is reflected in high-quality assignment of peptide spectra. However, the less abundant allergens Ara h 7, 8, 9, 10, and 11 are also detectable in dust sample 1. We did not detect the profilin allergen Ara h 5, consistent with previous work.⁷ Dust sample 3 contained poor coverage of the most abundant allergens (Ara h 1, 2, and 3). The detection of the allergen Ara h 2 in dust sample 1 is shown in detail in Figure 1. Multiple peptides and MS2 fragmentation thereof allow for the confident demonstration of allergen presence. When signal peptides and those peptides too small for detection using our methodology are discounted, we observed all expected peptides at a FDR < 0.1%. Furthermore, we were able to confidently detect hydroxyproline modifications, which have been found to be important for IgE binding.⁸ Although number of identified peptides is correlated with protein quantity, variable factors such as ionisation efficiency and MS2 spectral quality make derivation of protein quantity from this data imprecise. For this reason, we have not attempted quantitation of allergens using this data.

We have confirmed the presence of the clinically important peanut allergens Ara h 1, 2, 3, and 6 in peanut containing dust samples using MS analysis which offers more specific, sequence-specific, detection than ELISA method. The findings from this MS analysis confirm the utility of mass spectrometry in detection of food allergens in complex environmental samples such as house dust and furthermore the detection of specific proteins known to cause allergic reactions. We suggest that more rapid, targeted, quantitative MS methodology based on that presented here could form the basis of broader environmental analysis to further investigate the role of food allergens in dust may play in sensitizing individuals through environmental exposure.

CONFLICT OF INTEREST

Dr Brough reports grants from Food Allergy Research & Education (FARE), other from UK Dept of Health through NIHR, during the conduct of the study; being on scientific advisory board for DBV Technologies, and research support from Thermo Scientific outside the submitted work. Dr Mills reports grants from The UK Food Standards Agency, during the conduct of the study; grants from Reacta Biotech Ltd, outside the submitted work; in addition, Dr Mills has a patent PCT/GB2016/051637 pending to Reacta Biotech Ltd, and a patent PCT/GB2016/053829 pending to Reacta Biotech Ltd and is Chief Scientific Officer and shareholder of Reacta Biotech Ltd, a start-up developed to commercialize foods for use in oral food challenges. Dr Lack reports other from UK Dept of Health through NIHR during the conduct of the study; personal fees and other from DBV Technologies, other from Mighty Mission Me, personal fees from Aravax, outside the submitted work. Mrs Richards and Dr Johnson have nothing to disclose.

AUTHOR CONTRIBUTIONS

Dr Brough and Dr Johnson designed the study, analyzed and prepared the results for publication, drafted the letter, and critically revised it. Mrs Richards acquired the data for the dust using the polyclonal ELISA and contributed to the revising of the manuscript. Professor Lack and Professor Mills made substantial contributions to the conception and design of the study as well as interpretation of data and were involved in drafting and revising the letter. All authors have given final approval for the version to be published and agree to be accountable for all aspects of the work.

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SUPPORTING INFORMATION

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

MS data (.raw files) were analysed using PEAKS Client 8.5 build 20180507 (Bioinformatic Solutions Inc, Waterloo, Canada). Bioworks v. 3.3.1 (ThermoScientific, Waltham, USA) was installed to allow PEAKS to process raw files directly. Analytical variables were set as follows: parent ion mass tolerance 5ppm, MS2 ion tolerance 0.5 Da, 2+ and 3+ charge states only. Up to 1 missed cleavage (trypsin) were allowed at either end of the peptide. Carbamidomethyl (cysteine) was set as a required modification and oxidation (methionine) and hydroxylation (proline) set as variable modifications. The false discovery rate (FDR) for protein identities was set at 0.1%.