

DR. HELEN ANNARUTH BROUGH (Orcid ID : 0000-0001-7203-0813)

PROF. GIDEON LACK (Orcid ID : 0000-0001-7350-4021)

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

Short running title: Mass spectrometry of peanut allergens in dust

Helen A. Brough FRCPCH PhD,^{1,2,3} E.N. Clare Mills PhD,⁴ Kerry Richards MSc,^{1,2} Gideon Lack FRCPCH MB BCh, ^{1,2,3} Philip E. Johnson PhD,^{4,5}

¹ Paediatric Allergy Group, Department of Women and Children's Health, School of Life Course Sciences, King's College London, St. Thomas' Hospital, London SE1 7EH, UK

² Paediatric Allergy Group, Peter Gorer Dept of Immunobiology, School of Immunology & Microbial Sciences, King's College London, Guys' Hospital, London, UK

³ Children's Allergy Service, Evelina Children's Hospital, Guy's and St. Thomas' Hospital NHS Foundation Trust, London SE1 7EH, UK

⁴ Division of Infection, Immunity & Respiratory Medicine, School of Biological Sciences, Manchester Academic Health Science Centre, Manchester Institute of Biotechnology, The University of Manchester, 131 Princess Street, Manchester, M1 7DN, UK

⁵ Department of Food Science and Technology, University of Nebraska, Lincoln, Nebraska, USA.

Corresponding author:

Dr Helen A Brough
Children's Allergy Service
Evelina London
2nd Floor, Stairwell B
South Wing, St Thomas' Hospital
Email: helen.brough@gstt.nhs.uk
Telephone: +44 2071889525

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Abbreviations

Mass spectrometry: MS

False Discovery Rate: FDR

Enzyme-linked immunosorbent assays: ELISAs

High Performance Liquid Chromatography: HPLC

Key words: Mass spectrometry, environment, peanut allergy, household dust, Ara h 2

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 samples,⁴ however, given the specificity of the ELISA,⁵ it does not confirm the presence of peanut allergens (e.g. 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 described;⁴ they had high (n=2, infant's play-area (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) and were used for the MS analysis. *Sample preparation:* Chemicals were obtained from Sigma-Aldrich (Poole, Dorset, UK) unless otherwise indicated. Dust (5mg) was extracted 1:20 (weight:volume) in 50 mM Tris Cl⁻ (pH 8.8), 50 mM DTT, 0.04% (w/v) RapigestTM (kind gift of Waters Corporation Milford, MA, USA). Samples were reduced, alkylated and digested using trypsin (Promega, Southampton, UK) as described previously,⁶ prior to

sample cleanup using Pierce C18 spin columns (Thermo Scientific, Loughborough, UK) according to manufacturer's instruction. Samples were stored frozen at -20°C prior to analysis.

Mass Spectrometry: Samples were analysed by the Biological Mass Spectrometry Facility of the University of Manchester using reverse phase HPLC electrospray ionisation MS (RP-HPLC-ESI-MS) using a Nanoacquity nano-LC (Waters, Manchester, UK) coupled to an Orbitrap Elite mass spectrometer (Thermo Scientific, Waltham, USA).^{6,7} In order to maximize the likelihood of peanut detection the total digests derived from 5mg of dust were analysed using a single injection. Data Analysis was performed as described in the supplementary methods. 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 85µg/g and 80µg/g respectively whereas that in dust sample 3 was below the lower limit of quantitation (<0.625µg/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 (e.g. 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 to 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 to 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

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detect the profilin allergen Ara h 5, consistent with previous work⁶. 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.⁷ 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.

References

1. Leung DY, Guttman-Yassky E. Deciphering the complexities of atopic dermatitis: shifting paradigms in treatment approaches. [Review]. *J Allergy Clin Immunol* 2014; 134:769-79.
2. Brough HA, Liu AH, Sicherer S, Makinson K, Douiri A, Brown SJ, et al. Atopic dermatitis increases the effect of exposure to peanut antigen in dust on peanut sensitization and likely peanut allergy. *J Allergy Clin Immunol* 2015; 135:164-70.
3. Perry TT, Conover-Walker MK, Pomes A, Chapman MD, Wood RA. Distribution of peanut allergen in the environment. *J Allergy Clin Immunol* 2004:973-6.
4. Brough HA, Makinson K, Penagos M, Maleki SJ, Cheng H, Stephens AC, et al. Distribution of peanut protein in the home environment. *J Allergy Clin Immunol* 2013; 132:623-9.
5. Jayasena S, Smits M, Fiechter D, de Jong A, Nordlee J, Baumert J, Taylor SL, Pieters RH, Koppelman SJ. Comparison of six commercial ELISA kits for their specificity and sensitivity in detecting different major peanut allergens. *J Agric Food Chem* 2015, 63, (6), 1849-55.
5. Johnson PE, Sayers RL, Gethings LA, Balasundaram A, Marsh JT, Langridge JI, et al. Quantitative Proteomic Profiling of Peanut Allergens in Food Ingredients Used for Oral Food Challenges. *Anal Chem* 2016; 88:5689-95.
6. Sayers RL, Johnson PE, Marsh JT, Barran P, Brown H, Mills EN. The effect of thermal processing on the behaviour of peanut allergen peptide targets used in multiple reaction monitoring mass spectrometry experiments. *Analyst* 2016 141(13):4130-41 .
7. Bernard H, Guillon B, Drumare MF, Paty E, Dreskin SC, Wal JM, et al. Allergenicity of peanut component Ara h 2: Contribution of conformational versus linear hydroxyproline-containing epitopes. *J Allergy Clin Immunol* 2015; 135:1267-74 e1-8.

Brough HA FRCPCH PhD^{1,2,3}

Mills ENC PhD⁴

Richards K MSc^{1,2}

Lack G FRCPCH MB BCh^{1,2,3}

Johnson PE PhD^{4,5}

¹ Paediatric Allergy Group, Department of Women and Children's Health, School of Life Course Sciences, King's College London, St. Thomas' Hospital, London SE1 7EH, UK

² Paediatric Allergy Group, Peter Gorer Dept of Immunobiology, School of Immunology & Microbial Sciences, King's College London, Guys' Hospital, London, UK

³ Children's Allergy Service, Evelina Children's Hospital, Guy's and St. Thomas' Hospital NHS Foundation Trust, London SE1 7EH, UK

⁴ Division of Infection, Immunity & Respiratory Medicine, School of Biological Sciences, Manchester Academic Health Science Centre, Manchester Institute of Biotechnology, The University of Manchester, 131 Princess Street, Manchester, M1 7DN, UK

⁵ Department of Food Science and Technology, University of Nebraska, Lincoln, Nebraska, USA.

Conflict of interest statement: 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 commercialise 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, analysed 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

Legends:

Table 1: Detection of example allergen sequence isoforms by MS and quantitation of peanut content by ELISA. 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 $-10\log P$ scores for peptides used in these protein identifications are given as ranges encompassing detected peptides. These $-10\log P$ scores (higher is better) are comparable to P-values with a $-10\log P$ score of 20 being equivalent to a P-value of 1%. An FDR cut-off 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.

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	76.62-117.08
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	

