Peanut allergy: Effect of environmental peanut exposure in children with filaggrin loss-of-function mutations

Helen A. Brough, MSc, FRCPCH,[a] Angela Simpson, MD, PhD,[b] Kerry Makinson, MSc,[a] Jenny Hankinson, PhD,[b] Sara Brown, MD,[d] Abdel Douiri, PhD,[e] Danielle C. M. Belgrave, MSc,[b,c] Martin Penagos, MD, MSc,[a] Alick C. Stephens, PhD,[a] W. H. Irwin McLean, PhD, DSc, FRSE, FMedSci,[d] Victor Turcanu, PhD,[a] Nicolaos Nicolaou, MD, PhD,[b] Adnan Custovic, MD, PhD,[b]* and Gideon Lack, MD, FRCPCH[a]* *London, Manchester, and Dundee, United Kingdom*

Background: Filaggrin *(FLG)* loss-of-function mutations lead to an impaired skin barrier associated with peanut allergy. Household peanut consumption is associated with peanut allergy, and peanut allergen in household dust correlates with household peanut consumption.
Objective: We sought to determine whether environmental peanut exposure increases the odds of peanut allergy and whether *FLG* mutations modulate these odds.
Methods: Exposure to peanut antigen in dust within the first year of life was measured in a population-based birth cohort. Peanut sensitization and peanut allergy (defined by using oral food challenges or component-resolved diagnostics [CRD]) were assessed at 8 and 11 years. Genotyping was performed for 6 *FLG* mutations.
Results: After adjustment for infantile atopic dermatitis and preceding egg skin prick test (SPT) sensitization, we found a strong and significant interaction between natural log *(ln [loge])* peanut dust levels and *FLG* mutations on peanut sensitization and peanut allergy. Among children with *FLG* mutations, for each *ln* unit increase in the house dust peanut protein level, there was a more than 6-fold increased odds of peanut SPT sensitization, CRD sensitization, or both in children at ages 8 years, 11 years, or both and a greater than 3-fold increased odds of peanut allergy compared with odds seen in children with wild-type *FLG*. There was no significant effect of exposure in children without *FLG* mutations. In children carrying an *FLG* mutation, the threshold level for peanut SPT sensitization was 0.92 μg of peanut protein per gram (95% CI, 0.70-1.22 μg/g), that for CRD sensitization was 1.03 μg/g (95% CI, 0.90-1.82 μg/g), and that for peanut allergy was 1.17 μg/g (95% CI, 0.01-163.83 μg/g).
Conclusion: Early-life environmental peanut exposure is associated with an increased risk of peanut sensitization and allergy in children who carry an *FLG* mutation. These data support the hypothesis that peanut allergy develops through transcutaneous sensitization in children with an impaired skin barrier. (J Allergy Clin Immunol 2014;134:867-75.)

Key words: FLG *loss-of-function mutations, filaggrin, skin barrier, peanut sensitization, peanut allergy, environmental peanut exposure, dust, threshold*

From [a]the Department of Pediatric Allergy, Division of Asthma, Allergy and Lung Biology, King's College London and Guy's and St. Thomas' NHS Foundation Trust, London; [b]the Centre for Respiratory Medicine and Allergy, Institute of Inflammation and Repair, Manchester Academic Health Sciences Centre, University of Manchester and University Hospital of South Manchester NHS Foundation Trust, Manchester; [c]the Centre for Health Informatics, Institute of Population Health, University of Manchester; [d]the Centre for Dermatology and Genetic Medicine, College of Life Sciences and College of Medicine, Dentistry and Nursing, University of Dundee; and [e]the Department of Public Health Science, School of Medicine, King's College London.

*These authors contributed equally to the manuscript and are joint senior authors.

The research was funded by Action Medical Research (S/P/4529) and supported by the National Institute for Health Research (NIHR) Clinical Research Facility at Guy's & St Thomas' NHS Foundation Trust and the NIHR Biomedical Research Centre based at Guy's and St Thomas' NHS Foundation Trust and Kings College London. The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health. The Manchester Asthma and Allergy Study is supported by Medical Research Council grants G0601361 and MR/K002449/1, the JP Moulton Charitable Foundation, North West Lung Centre Charity, and the National Institute for Health Research Clinical Research Facility at the University Hospital of South Manchester NHS Foundation Trust. The Centre for Dermatology and Genetic Medicine, University of Dundee, is funded by a Wellcome Trust Strategic Award (098439/Z/12/Z; to W.H.I.M.). S.B. holds a Wellcome Intermediate Clinical Fellowship (086398/Z/08/Z).

Disclosure of potential conflict of interest: H. A. Brough has received research support from the Department of Health through the National Institute for Health Research (NIHR) comprehensive Biomedical Research Centre award to Guy's & St. Thomas' NHS Foundation Trust in partnership with King's College London and King's College Hospital NHS Foundation Trust and Action Medical Research, UK. A. Simpson has received research support from the Medical Research Council, JP Moulton Charitable Foundation, and the National Institute of Health Research. K. Makinson has received research support from the Department of Health through the National Institute of Health Research comprehensive Biomedical Research Centre award to Guy's & St. Thomas' NHS Foundation Trust in partnership with King's College London and King's College Hospital NHS Foundation Trust and the Immune Tolerance Network, National Institutes of Health. S. Brown has received research support from the Wellcome Trust Intermediate Clinical Fellowship and has received payment for lectures from the American Academy of Allergy, Asthma & Immunology. A. Douiri has received research support from the National Institute of Health Research. A. C. Stephens has received research support from the Department of Health through the NIHR comprehensive Biomedical Research Centre award to Guy's & St. Thomas' NHS Foundation Trust in partnership with King's College London and King's College Hospital NHS Foundation Trust. W. H. I. McLean has received research support from the Wellcome Trust. A. Custovic has consultant arrangements with Circassia; has received research support from the Medical Research Council and the Moulton Charitable Foundation; and has received payment for lectures from GlaxoSmithKline, Thermo Fisher Scientific, Novartis, and ALK-Abelló. G. Lack has received research support from the Department of Health through the NIHR comprehensive Biomedical Research Centre award to Guy's & St. Thomas' NHS Foundation Trust in partnership with King's College London and King's College Hospital NHS Foundation Trust and Action Medical Research, UK; is a board member of DBV Technologies; has consultant arrangements with the Anaphylaxis Campaign and the National Peanut Board; has received payment for lectures from Sodilac, Novartis, Nestle Nutrition, GlaxoSmithKline, and the Serono Symposia International Foundation; and has stock/stock options with DBV Technologies. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication April 2, 2014; revised August 20, 2014; accepted for publication August 20, 2014.

Corresponding author: Gideon Lack, MD, Children's Allergy Unit, 2nd Floor, Stairwell B, South Wing, Guy's and St Thomas' NHS Foundation Trust, Westminster Bridge Road, London SE1 7EH, United Kingdom. E-mail: Gideon.lack@kcl.ac.uk.

0091-6749/$36.00

http://dx.doi.org/10.1016/j.jaci.2014.08.011

Abbreviations used
AD: Atopic dermatitis
CRD: Component-resolved diagnostics
FLG: Filaggrin
GEE: Penalized generalized estimating equations methodology
ISU: ISAC standardized unit
LLQ: Lower limit of quantitation
MAAS: Manchester Asthma and Allergy Study
OFC: Oral food challenge
OR: Odds ratio
sIgE: Allergen-specific IgE
SPT: Skin prick test

There is a clear association between early-onset atopic dermatitis (AD) and food allergy.[1,2] Children with AD have an impaired skin barrier, which might allow antigen to penetrate the skin and sensitize the subject.[3,4] In children with a history of AD, 90% of those who went on to have peanut allergy had been exposed topically to creams containing *Arachis* species (peanut) oil in the first 6 months of life.[2] In mice epicutaneous exposure to food allergens after skin stripping induces a potent allergic T_H2-type response associated with high IL-4, IL-5, and allergen-specific IgE (sIgE) levels and systemic anaphylaxis after oral challenge.[5,6]

Filaggrin is responsible for the strength and integrity of the stratum corneum[7] and regulates the permeability of the skin to water and antigens.[8] Loss-of-function mutations in the gene encoding filaggrin *(FLG)* are present in up to 50% of patients with moderate-to-severe AD[9,10] and have been shown to increase the risk of inhalant allergic sensitization, allergic rhinitis, asthma,[11,12] and peanut allergy.[13] In the flaky tail mouse, which has a 1-bp deletion mutation (5303delA) within the murine *flg* gene (analogous to common human *FLG* loss-of-function mutations), topical allergen application leads to cellular infiltration and allergen-specific antibody response, even without skin stripping.[14] This suggests that filaggrin deficiency, even in the absence of dermatitis, might be sufficient for transcutaneous sensitization.

High consumption of peanut by household members during the child's first year of life is associated with an increased risk of peanut allergy, possibly because of environmental peanut exposure in the child's home[15]; however, in this study questionnaire-based assessment of household peanut consumption was not validated against an objective measure of peanut in the environment and was potentially subject to retrospective bias. We recently showed that peanut protein in household dust is positively correlated with household peanut consumption.[16] In addition, we showed that peanut protein in dust activates basophils from children with peanut allergy in a dose-dependent manner and is thus biologically active.[16]

We hypothesized that peanut sensitization can occur through presentation of environmental peanut antigen through an impaired skin barrier to underlying antigen-presenting cells. To address this hypothesis, we investigated whether early-life environmental peanut exposure measured directly by quantifying peanut antigen in household dust was a risk factor for the development of peanut allergy and whether this relationship was modified by *FLG* genotype. Specifically, we predicted that an increase in the peanut protein concentration in household dust during infancy would be associated with an increase in school-age peanut sensitization and allergy and that this effect would be augmented in children with 1 or more *FLG* loss-of-function mutations.

METHODS

Study population

The Manchester Asthma and Allergy Study (MAAS) is an unselected birth cohort described in detail elsewhere (registration: ICRCTN72673620).[17] In brief, 1184 subjects were recruited prenatally from 1995 to 1997 and followed up at ages 1, 3, 5, 8, and 11 years. The study was approved by the local ethics committee; parents provided written informed consent.

Data sources

Validated questionnaires were interviewer administered to collect information on parentally reported symptoms and physicians' diagnoses. Parental report of a history of AD during infancy was assessed by using a modified International Study of Asthma and Allergies in Childhood questionnaire to apply the UK Working Party's diagnostic criteria for AD.[18] Peanut sensitization was assessed at ages 8 and 11 years by using skin prick tests (SPTs) to whole peanut extract (Hollister-Stier, Spokane, Wash)[19] and by measuring sIgE to whole peanut extract and peanut components Ara h 1, 2, and 3 with ImmunoCAP (age 8 years) or the ISAC Multiplex Immuno Solid-phase Allergen Chip (age 11 years; Thermo Fisher Scientific, Uppsala, Sweden).[20] Maternal peanut consumption during pregnancy and breast-feeding were collected retrospectively (aged 8 years) in a subset of patients assessed for peanut allergy by means of diagnostic oral food challenge (OFC).

Definition of outcomes

Peanut SPT sensitization. Peanut SPT sensitization was defined as a mean wheal diameter of 3 mm or greater than that elicited by the negative control.

Peanut component-resolved diagnostics sensitization. Peanut component-resolved diagnostics (CRD) sensitization was defined as sIgE to the peanut components Ara h 1, 2, or 3 of 0.35 kU_A/L or (8 years) or 0.35 ISAC standardized units (ISU) or greater (11 years).[20] Patients with Ara h 1, 2, or 3 levels of less than 0.35 kU_A/L (8 years) or 0.35 ISU (11 years) were deemed non-CRD sensitized. If no CRD analysis was available, then patients with peanut sIgE levels of less than 0.2 kU_A/L ImmunoCAP were considered not CRD sensitized.

Peanut allergy. All children with evidence of peanut sensitization at age 8 years (peanut SPT response ≥3 mm or sIgE level ≥0.2 kU_A/L) were offered an OFC to peanut to determine allergy versus tolerance.[19] Open OFCs were applied among children who had a history of tolerating peanut on consumption; all other children underwent a double-blind, placebo-controlled OFC.[19] OFC results were considered positive after development of 2 or more objective signs indicating an allergic reaction.[19] Children with a convincing history of an immediate hypersensitivity reaction on exposure to peanut combined with a peanut sIgE level of 15 kU_A/L or greater,[21] an SPT response of 8 mm or greater,[22] or both (age 8 years) were considered to have peanut allergy and did not undergo an OFC. Two children with a convincing history of an immediate hypersensitivity reaction on exposure to peanut and an SPT response of 3 mm or greater who refused consent for OFCs were considered to have peanut allergy based on an Ara h 2 level of 0.35 ISU or greater[19] at subsequent follow-up at age 11 years.

Quantitation of environmental peanut exposure in household dust

Dust samples were collected predominantly at 36 weeks' gestation from the lounge-sofa, as previously described.[23] If no antenatal dust sample was available from the lounge-sofa, then dust samples from 6 or 12 months were analyzed for peanut protein (where available). Dust samples were extracted