Loss-of-function variants in the filaggrin gene are a significant risk factor for peanut allergy

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Background: IgE-mediated peanut allergy is a complex trait with strong heritability, but its genetic basis is currently unknown. Loss-of-function mutations within the filaggrin gene are associated with atopic dermatitis and other atopic diseases; therefore, filaggrin is a candidate gene in the etiology of peanut allergy.

Objective: To investigate the association between filaggrin loss-of-function mutations and peanut allergy.

Methods: Case-control study of 71 English, Dutch, and Irish oral food challenge–positive patients with peanut allergy and 1000 non peanut-sensitized English population controls. Replication was tested in 390 white Canadian patients with peanut allergy (defined by food challenge, or clinical history and skin prick test wheal to peanut ≥8 mm and/or peanut-specific IgE ≥15 kUL^{-1}) and 891 white Canadian population controls. The most prevalent filaggrin loss-of-function mutations were assayed in each population: R501X and 2282del4 in the Europeans, and R501X, 2282del4, R2447X, and S3247X in the Canadians. The Fisher exact test and logistic regression were used to test for association; covariate analysis controlled for coexistent atopic dermatitis.

Results: Filaggrin loss-of-function mutations showed a strong and significant association with peanut allergy in the food challenge–positive patients ($P = 3.0 \times 10^{-6}$; odds ratio, 5.3; 95% CI, 2.8-10.2), and this association was replicated in the Canadian study ($P = 5.4 \times 10^{-5}$; odds ratio, 1.9; 95% CI, 1.4-2.6). The association of filaggrin mutations with peanut allergy remains significant ($P = .0008$) after controlling for coexistent atopic dermatitis.

Conclusion: Filaggrin mutations represent a significant risk factor for IgE-mediated peanut allergy, indicating a role for epithelial barrier dysfunction in the pathogenesis of this disease. (J Allergy Clin Immunol 2011;127:661-7.)

Key words: *Atopic dermatitis, filaggrin, IgE, peanut allergy, risk factor*

An adverse immune response to peanut ingestion may be severe and is potentially life-threatening.[1] The prevalence of IgE-mediated peanut allergy in the United Kingdom (UK) and the United States has increased significantly over the past decades[2,3] but may now have stabilized in the UK[4] and Canada.[5] The prevalence of peanut allergy in preschool and school-age children is approximately 1.2% to 1.6%,[3-5] whereas the prevalence in US adults is estimated to be 0.6%.[3]

Peanut allergy is strongly heritable, with a monozygotic twin concordance of 64% compared with 7% in both dizygotic twins[6]

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Abbreviations used
AD: Atopic dermatitis
ALSPAC: Avon Longitudinal Study of Parents and Children
FLG: Filaggrin
OR: Odds ratio
SPT: Skin prick test
UK: United Kingdom

and other siblings.[7] A previously reported association with HLA class II genes[8] has not been replicated,[9] and the genetic basis of this disorder remains poorly understood.

Loss-of-function mutations in the filaggrin gene (*FLG*) are a strong and significant risk factor for atopic dermatitis (AD)[10] as well as asthma in association with AD,[10] allergic rhinitis,[11] and elevated IgE, indicating sensitization to certain foods.[11] The *FLG* gene encodes profilaggrin, an insoluble polyprotein that is expressed in the granular layer of the epidermis and is broken down to release filaggrin monomers in the stratum corneum.[12] Filaggrin plays a key role in epithelial barrier function, but this protein is expressed in neither the bronchial airways nor the upper gastrointestinal tract beyond the oral mucosa[13] or possibly the esophagus.[14] It has been hypothesized that allergic sensitization in the atopic state occurs via either transcutaneous or transmucosal passage of allergens, a process that may be facilitated by filaggrin deficiency.[15,16] Experimental evidence supporting a prominent role of epithelial barrier deficiency as a facilitating early event in allergic priming comes from the recent analysis of a filaggrin-deficient mouse mutant.[17]

In view of the strong association of *FLG* null mutations with atopic disease and impaired skin barrier function, we aimed to investigate their role as a risk factor for IgE-mediated peanut allergy.

METHODS

Subjects

Seventy-one patients with peanut allergy confirmed by oral food challenge, 1000 nonsensitized controls, and the respective population controls were collected from the white European populations of England, The Netherlands, and Ireland, as follows:

English patients with peanut allergy. Records of thirty-five children with peanut allergy were obtained from the Avon Longitudinal Study of Parents and Children (ALSPAC).[18] In this longitudinal birth cohort study, peanut allergy was defined by a suggestive clinical history plus a positive double-blind placebo-controlled food challenge. Subjects reported by parents as having flexural dermatitis at 2 or more time points (up to a maximum of 4) between the ages of 6 and 42 months were designated as having AD.

Dutch patients with peanut allergy. Twenty patients with peanut allergy were recruited from pediatric allergy clinics. Peanut allergy was defined by a positive double-blind placebo-controlled oral food challenge test to peanut. AD was defined on the basis of a dermatologist's examination.

Irish patients with peanut allergy. Sixteen patients with peanut allergy were recruited from pediatric allergy clinics on the basis of a suggestive clinical history and a positive open oral food challenge test to peanut. AD was defined on the basis of a pediatrician's examination or parental report.

Non–peanut-sensitized control group. ALSPAC is a longitudinal, population-based birth cohort study that originally included over 14,000 English children, as previously described.[18] One thousand consecutive non–peanut-sensitized individuals (ie, having had a negative skin prick test [SPT] result for peanut) for whom AD status was recorded were drawn from the ALSPAC cohort as normal controls for the primary analysis. In this control group, AD was defined in the same way as for the ALSPAC patients—that is, as having flexural dermatitis at 2 or more time points between the ages of 6 and 42 months.

Demographic data for the food challenge–positive patients with peanut allergy and non–peanut-sensitized controls are shown in Table I.

Population control groups. Within the ALSPAC population birth cohort, relevant phenotype data were available for 6895 individuals successfully typed for the 2 most common *FLG* null mutations (R501X and 2282del4). The 6851 individuals without peanut allergy from this collection were used as an English population control group. One hundred Dutch population control samples were obtained from healthy adult blood donors attending the University Medical Centre, Groningen. Irish population controls were 100 healthy adults from the population-based Trinity Biobank control samples.

Replication study

The replication of findings from the food challenge–proven patients was tested in a larger Canadian peanut allergy case collection, as follows:

Canadian patients with peanut allergy. From an established Canadian peanut allergy cohort,[19-22] 390 white patients with peanut allergy were recruited between July 2008 and April 2009. They were defined as having peanut allergy on the basis of a positive oral food challenge (n = 25) or, in the non–food-challenged subjects, a peanut-specific IgE ≥15 kUL^{-1} (n = 65) or a SPT wheal to peanut ≥8 mm (n = 214) or both sIgE ≥15 kUL^{-1} and SPT wheal ≥8 mm (n = 86).[23-25] A total of 249 (68%) of the patients defined by immunologic parameters had a clinical history of anaphylaxis to peanut on the basis of the consensus definition from the National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network,[26] whereas 116 (32%) had a history strongly suggestive of type I hypersensitivity to peanut.[27] These thresholds have gained general acceptance, and in the Canadian context, it would be ethically difficult to justify the use of a food challenge in children with values exceeding these thresholds.

The clinical and immunologic data relating to the Canadian patients with peanut allergy are summarized in Table II.

Canadian population controls. A total of 891 Canadian controls of white ethnicity were derived from a population collection of adult volunteers from the Toronto, Ontario, area, for whom peanut allergy status and the presence or absence of coexistent AD were unknown.

Demographic data and clinical characteristics for the replication collection are shown in Table III.

Ethical considerations

The peanut allergy case collections were approved by the relevant local research ethics committees, and all subjects or the subjects' guardians gave written informed consent. DNA was collected and analyzed from individuals within each of the control groups with ethical approval and written informed consent.

Genotyping for *FLG* loss-of-function mutations

DNA was extracted from blood or saliva samples by using standard protocols. The *FLG* loss-of-function mutations that are most prevalent in the European population were genotyped by using a previously published methodology.[28] R501X and 2282del4 had previously been typed in the ALSPAC cohort; R501X, 2282del4, R2447X, and S3247X were typed in the Dutch, Canadian, and Irish case collections in this study.

Statistical analysis

Case-control analysis to test the association between filaggrin genotype and peanut allergy. The Fisher exact test and logistic regression were used to test for association