Mold allergen sensitization in adult asthma according to integrin β3 polymorphisms and Toll-like receptor 2/+596 genotype

Lidwien A. M. Smit, PhD,[a,b,c] Emmanuelle Bouzigon, MD, PhD,[d,e,f] Jean Bousquet, MD,[b,g] Nicole Le Moual, PhD,[b,c] Rachel Nadif, PhD,[b,c] Isabelle Pin, MD,[h,i,j] Mark Lathrop, PhD,[k] Florence Demenais, MD,[d,e,f] Francine Kauffmann, MD,[b,c] and Valérie Siroux, PhD,[h,i] on behalf of the Epidemiological Study on the Genetics and Environment of Asthma

Utrecht, The Netherlands, and Villejuif, Paris, Montpellier, Grenoble, and Evry, France

Background: Integrin β3 (*ITGB3*) and Toll-like receptor 2 (*TLR2*) are candidate genes for asthma and sensitization to mold allergens. Integrin β3 forms a complex with TLR2, and this biological interaction is required for the response of monocytes to TLR2 agonists such as fungal glucan.

Objective: To study whether genetic interaction between single nucleotide polymorphisms (SNPs) in genes encoding the TLR2-ITGB3 complex enhances susceptibility to mold sensitization.

Methods: Association analysis was conducted in 1243 adults (524 with asthma) who participated in the follow-up of the Epidemiological Study on the Genetics and Environment of Asthma. Allergic sensitization to mold allergens was determined by skin prick testing. Association of mold sensitization with 14 *ITGB3* SNPs was tested under an additive genetic model. Interaction between *ITGB3* SNPs and *TLR2*/+596, which was previously shown to be associated with asthma, was studied.

Results: A positive skin prick test to mold was found in 115 subjects with asthma (22.0%) and in 61 subjects without asthma (8.5%). The *ITGB3* rs2056131 A allele was associated with mold sensitization in subjects with asthma with an odds ratio (95% CI) of 0.60 (0.43-0.83; $P = .001$). Ten other *ITGB3* SNPs were significantly associated with mold sensitization in *TLR2*/+596TT subjects with asthma ($P = .03$-$.002$), whereas much weaker associations were found in carriers of the *TLR2*/+596 C allele ($P = .60$-$.04$). Interaction between *TLR2*/+596 and these *ITGB3* SNPs was statistically significant (*P* interaction = .05-.001).

Conclusion: *TLR2/+596* genotype may influence the association between *ITGB3* SNPs and mold sensitization in adults with asthma. (J Allergy Clin Immunol 2011;128: 185-91.)

Key words: Alternaria, Aspergillus, *allergy, asthma,* Cladosporium, *epidemiology, epistasis, genetics, innate immunity*

Allergic sensitization to molds such as *Alternaria* and *Cladosporium* is a risk factor for asthma, asthma severity, and allergic rhinitis.[1-3] The prevalence of mold sensitization depends strongly on geographic and climatic conditions,[3,4] and exposure to indoor mold allergens has been associated with mold allergy and asthma symptoms in children and adults.[5-8] Besides the influence of the environment, heritable factors were shown to contribute to mold sensitization as well. Concordance for skin prick testing to a mixture of *Alternaria* allergens was significantly greater among identical twins than nonidentical twins,[9] and maternal sensitization to *Alternaria alternata* significantly increased the risk of matched sensitization in their children.[10] Only a small number of candidate gene studies investigated the role of specific genetic variants in mold sensitization.[11-14] Weiss et al[12] found that single nucleotide polymorphisms (SNPs) in the integrin β3 gene (*ITGB3*), which is located on chromosome 17q21.32, were associated with asthma and sensitization to mold allergens in Hutterites, a founder population, and in 3 outbred replication populations, whereas other allergens showed no association. A candidate gene study and a study that used genome-wide genotyping to assess the reproducibility of previously published asthma genes also found associations between *ITGB3* SNPs and asthma, but these studies did not investigate mold sensitization as an outcome.[15,16]

ITGB3 encodes the β-chain of the receptor for a wide array of ligands, including vitronectin and fibrinogen. Integrin β3 and its ligands play a key role in cell adhesion, cell proliferation and differentiation, platelet activation, and various other biological processes.[17] Vitronectin may participate in the remodeling process during lung development or response to injury by downregulating the expression of α-smooth muscle actin and reducing the contractile ability of human lung fibroblasts.[18]

From [a]the Institute for Risk Assessment Sciences, Division of Environmental Epidemiology, Utrecht University; [b]INSERM, CESP Center for Research in Epidemiology and Population Health, U1018, Respiratory and Environmental Epidemiology Team, Villejuif; [c]Université Paris Sud 11, UMRS 1018, Villejuif; [d]INSERM, U946, Paris; [e]Université Paris Diderot, Paris 7, Institut Universitaire d'Hématologie; [f]Fondation Jean Dausset-Centre d'Etude du Polymorphisme Humain, Paris; [g]Hôpital Arnaud de Villeneuve, Montpellier; [h]INSERM, U823, Grenoble; [i]Université Joseph Fourier-Grenoble 1; [j]Centre Hospitalier Universitaire de Grenoble; and [k]Commissariat à l'Energie Atomique, Institut de Génomique, Centre National de Génotypage, Evry.

Supported by the French Ministry of Higher Education and Research, University Paris Diderot-Paris 7, grants from the French Agency for Environmental and Occupational Health Safety (grant no. AFSSET-APR-SE-2004), the French National Agency for Research (grant nos. ANR 05-SEST-020-02/05-9-97 and ANR 06-CEBS), Merck Sharp & Dohme, the Hospital Program of Clinical Research—Paris, and The Netherlands Organization for Scientific Research Van Gogh Programme for French-Dutch cooperation. L.A.M.S. was supported by a European Academy of Allergology and Clinical Immunology–Global Allergy and Asthma European Network exchange fellowship award.

Disclosure of potential conflict of interest: The authors have declared that they have no conflict of interest.

Received for publication May 21, 2010; revised March 25, 2011; accepted for publication April 1, 2011.

Available online May 13, 2011.

Reprint requests: Lidwien A. M. Smit, PhD, Institute for Risk Assessment Sciences, Division of Environmental Epidemiology, Utrecht University, PO Box 80178, Utrecht, The Netherlands. E-mail: L.A.Smit@uu.nl.

0091-6749/$36.00

doi:10.1016/j.jaci.2011.04.007

Abbreviations used
EGEA: Epidemiological Study on the Genetics and Environment of Asthma
FDR: False discovery rate
ICS: Inhaled corticosteroid
ITGB3: Integrin β3
LD: Linkage disequilibrium
OR: Odds ratio
RR: Relative risk
SNP: Single nucleotide polymorphism
SPT: Skin prick test
TLR2: Toll-like receptor 2
UTR: Untranslated region

Integrin β3 forms a complex with Toll-like receptor 2 (TLR2), and vitronectin and integrin β3 are required for the response of monocytes to bacterial lipopeptide and other TLR2 agonists such as fungal glucan.[19] We therefore hypothesized that variants in genes encoding the TLR2-ITGB3 complex may play a role in susceptibility to asthma and mold sensitization.

The current study is the first epidemiologic study to address a gene-gene interaction between *ITGB3* and *TLR2*. In adults from the French Epidemiological study on the Genetics and Environment of Asthma (EGEA), the *TLR2*/+596 (rs3804099) C allele was associated with asthma in both case-control and family-based analyses.[20] We aimed to study whether *TLR2*/+596 genotype modified associations between *ITGB3* SNPs and asthma and sensitization to mold allergens. In addition, we aimed to confirm associations between *ITGB3* SNPs and asthma and mold sensitization.

METHODS

Population

The current analysis uses data from the 12-year follow-up of the EGEA survey. The design and protocol of EGEA, a family study and a case-control study of asthma, have been reported in detail elsewhere.[21,22] Briefly, 2047 subjects were enrolled at baseline (1991-1995): 388 patients with asthma (age, 7-70 years) from 6 chest clinics in 5 French cities, their 1244 first-degree relatives, and 415 population-based controls. At follow-up (2003-2007), 92.2% of the alive cohort returned a self-completed questionnaire, and 77.1% completed a detailed questionnaire.[23] At the follow-up survey, all subjects were adults. For the current cross-sectional analysis, we used 1243 subjects (62.8% of the alive cohort) with complete data on asthma, mold sensitization, and genotyping (see flowchart in this article's Fig E1 in the Online Repository at www.jacionline.org). The 1243 subjects with complete data were slightly older, had more often studied at university level, and reported rhinitis more often than the 300 subjects who were excluded because of missing genotype or phenotype (mold sensitization) data (see this article's Table E1 in the Online Repository at www.jacionline.org). All participants gave written informed consent.

Health outcomes and exposure variables

Inclusion criteria used to define asthma in probands were based on self-reported answers to 4 questions—"Have you ever had attacks of breathlessness at rest with wheezing?" "Have you ever had asthma attacks?" "Was this diagnosis confirmed by a physician?" and "Have you had an asthma attack in the last 12 months?"—or a positive response to at least 2 questions and a positive review of the subject's medical record.[21] Asthma in relatives of probands was defined as a positive answer to at least 1 of the first 2 questions.[21]

Atopy was defined by the presence of a positive skin prick test (SPT; mean wheal diameter ≥3 mm) to at least 1 of 11 aeroallergens (*Aspergillus*, *Cladosporium herbarum*, *Alternaria alternata*, cat, *Dermatophagoides pteronyssinus*, *Blattella germanica*, olive, birch, *Parietaria judaica*, timothy grass, and ragweed pollen) using extracts made by Stallergènes (Antony, France). Mold sensitization was defined as a positive SPT to at least 1 of the 3 mold allergens. Mold species tested in EGEA were the same as in the study by Weiss et al.[12]

Environmental exposure to molds was assessed by questionnaire using items from the European Community Respiratory Health Survey.[6] A small lake near Montpellier was assumed to be a source of molds.[24] We therefore investigated recruitment in Montpellier as an environmental determinant of mold sensitization.

Genotyping

Fourteen SNPs in *ITGB3* (located at chromosome 17q21.32) with a minor allele frequency >5% were selected by using a tagging approach. All SNPs were in Hardy-Weinberg equilibrium ($P > .01$). This article's Fig E2 in the Online Repository at www.jacionline.org shows linkage disequilibrium (LD) between SNPs. Genotyping was performed by using Taqman Probes (Applied Biosystems, Foster City, Calif) on an ABI7900HT Sequence Detection System at the Centre National de Génotypage (Evry, France).

Data analysis

Analyses using mold sensitization as an outcome were conducted in subjects with and without asthma separately, because mold sensitization is strongly associated with asthma, and subjects were recruited through patients with asthma. Determinants of mold sensitization were first explored by using univariate analyses (χ^2 test or t test). All further analyses were performed by generalized estimating equation to account for dependence among subjects sharing the same household. Odds ratios (ORs) and 95% CIs were adjusted for age and sex unless stated otherwise. In the lack of evidence for a recessive or dominant genetic model, the effect of *ITGB3* SNPs on health outcomes was tested under an additive genetic model with the minor allele as a risk allele.[12,15,16] To test whether association between *ITGB3* SNPs and health outcomes were modified by *TLR2/+596* (rs3804099) genotype (TT or CC+CT), we introduced a multiplicative gene-gene interaction term in the generalized estimating equation model and used a generalized score test that follows a χ^2 distribution with 1 degree of freedom. False discovery rate (FDR)–adjusted P values were calculated to take multiple comparisons (n = 14 SNPs) into account.[25]

RESULTS

Association of mold sensitization and asthma

The current study included 524 subjects with asthma and 719 subjects without asthma. Mold sensitization was significantly more prevalent among subjects with asthma (n = 115; 22.0%) than in subjects without asthma (n = 61; 8.5%), with an adjusted OR (95% CI) of 2.80 (2.03-3.87). Exclusive mold sensitization was rare: only 13 (2.5%) subjects with asthma and 16 (2.2%) subjects without asthma were sensitized to mold without being sensitized to any of the other common allergens. *Alternaria, Cladosporium*, and *Aspergillus* sensitization was present in 71 (13.5%), 39 (7.4%), and 29 (5.5%) subjects with asthma and 27 (3.8%), 25 (3.5%), and 22 (3.1%) subjects without asthma, respectively. Atopy (sensitization to any of the 11 allergens tested) was found in 414 (79.0%) subjects with asthma and in 277 (38.5%) subjects without asthma. Subjects with asthma who were sensitized to mold had more often used corticosteroids in the past year and tended to have a lower age of onset of asthma