The nuclear factor I/A (*NFIA*) gene is associated with the asthma plus rhinitis phenotype

Marie-Hélène Dizier, PhD,[a,b] Patricia Margaritte-Jeannin, PhD,[a,b] Anne-Marie Madore, PhD,[c] Miriam Moffatt, PhD,[d] Myriam Brossard, MSc,[a,b] Nolwenn Lavielle, MSc,[a,b] Chloé Sarnowski, MSc,[a,b] Jocelyne Just, MD,[e] William Cookson, MD,[d] Mark Lathrop, PhD,[f] Catherine Laprise, PhD,[c] Emmanuelle Bouzigon, MD, PhD,[a,b] and Florence Demenais, MD[a,b] *Paris, France, Chicoutimi and Montreal, Quebec, Canada, and London, United Kingdom*

Background: A previous genome-wide linkage scan in 295 families of the French Epidemiological Study on the Genetics and Environment of Asthma (EGEA) showed strong evidence of linkage of the 1p31 region to the combined asthma plus allergic rhinitis (AR) phenotype.

Objective: Our purpose was to conduct fine-scale mapping of the 1p31 linkage region to identify the genetic variants associated with asthma plus AR.

Methods: Association analyses with the asthma plus rhinitis phenotype were first conducted in the EGEA family sample using the family-based association method (FBAT) and logistic regression. The test of homogeneity of association between asthma plus AR versus asthma alone or AR alone was also applied. Replication of EGEA findings was sought in French-Canadian and United Kingdom family samples.

Results: We found a significant association between asthma plus rhinitis and a 1p31 genetic variant ($P = 2 \times 10^{-5}$ for rs12122228, which reached the multiple testing–corrected threshold) in EGEA using FBAT. There was evidence of heterogeneity of association between asthma plus AR versus asthma alone or AR alone ($P = .03$). A Meta-analysis of FBAT results from EGEA and French-Canadian families improved evidence for both association and heterogeneity ($P = 5 \times 10^{-6}$ and $P = .008$, respectively), whereas a meta-analysis of EGEA, French-Canadian, and United Kingdom samples based on logistic regression slightly increased the evidence for heterogeneity.

Conclusion: The single nucleotide polymorphism specifically associated to asthma plus rhinitis is located in the flanking 5′ untranslated region of the nuclear factor I/A *(NFIA)* gene, a strong candidate gene for asthma and AR. (J Allergy Clin Immunol 2014;■■■:■■■-■■■.)

Key words: *Positional cloning, association study, family-based association study, logistic regression, asthma, rhinitis, family sample*

Allergic rhinitis (AR) is an allergic manifestation of the upper airways induced by an IgE-mediated inflammation of the nasal membranes. Asthma, a common respiratory disease, is characterized by variable obstruction and inflammation of the lower airways and bronchial hyperresponsiveness. These 2 diseases are common comorbidities: approximately 60% of asthmatic patients have rhinitis, and 20% to 30% of patients with rhinitis have asthma.[1] The evidence for a genetic component in both asthma and AR has been well established by using family[2] and twin[3] studies. Moreover, familial association between AR and asthma suggests shared genetic determinants.[4] Asthma and AR are strongly associated with atopy: most subjects with rhinitis, asthma, or both are atopic, whereas only some atopic subjects have rhinitis, asthma, or both. In addition to atopy, asthma and AR might share other common pathophysiologic pathways involved in eosinophilic inflammation, airway remodeling, or both.

Numerous genetic studies based on candidate gene and positional cloning approaches and, more recently, genome-wide association studies (GWASs) have been conducted for asthma and have identified a large number of susceptibility genes.[5] Fewer genomic studies have been carried out for AR and have shown evidence for association with a few genes: *MRPL4* (19q13) and *BCAP* (10q24) were detected in a Chinese population,[6] and *C11orf30* and *LRRC32* (11q13.5) and *TMEM232* and *SLC25A46* (5q22) were identified by means of a meta-analysis of GWASs in 4 European populations.[7] To our knowledge, no genetic association study has yet been conducted for the comorbidity of asthma and AR.

A previous genome-wide linkage scan carried out in 295 families of the French Epidemiological Study on the Genetics and Environment of Asthma (EGEA) showed strong evidence of linkage of 1p31 to asthma and AR and even more to the combined asthma plus AR phenotype.[8] Further analysis demonstrated heterogeneity of linkage within the 1p31 region, which was found linked to the asthma plus rhinitis phenotype, whereas there was no linkage to the asthma alone or AR alone phenotype.[9] Our objective was to conduct fine-scale mapping of this linkage region to identify the genetic variant or variants specifically associated to asthma plus AR comorbidity. This study, which is the first applied to this comorbidity, was based on a discovery phase performed in the EGEA families. Two statistical methods were used for internal validation:

From [a]INSERM U946, Genetic Variation and Human Diseases Unit, Paris; [b]Université Paris Diderot, Sorbonne Paris Cité, Institut Universitaire d'Hématologie, Paris; [c]Université du Québec, Chicoutimi; [d]the National Heart and Lung Institute, Imperial College, London; [e]Centre de l'Asthme et des Allergies, APHP, Hôpital Trousseau, UMPC Paris; and [f]McGill University, Montreal.

Supported in part by the GWIS-AM grant (ANR-11-BSV1-027). Genotyping of the asthma-ascertained samples was supported by grants from the European Commission (018996), the French Ministry of Higher Education and Research, the Wellcome Trust (WT084703MA), and Asthma UK. C.L. is the chairholder of the Canada Research Chair on genetic determinants in asthma and the director of the Inflammation and Remodeling Strategic Group of the Respiratory Health Network of the Fonds de la recherche en santé du Québec (FRSQ) and member of Allergen network, which partially support the French-Canadian study.

Disclosure of potential conflict of interest: The authors declare that they have no relevant conflicts of interest.

Received for publication July 5, 2013; revised November 20, 2013; accepted for publication December 19, 2013.

Corresponding author: Marie-Hélène Dizier, PhD, UMR946–Université Paris Diderot, 27 rue Juliette Dodu, F-75010 Paris, France. E-mail: Marie-Helene.Dizier@inserm.fr.

0091-6749/$36.00

http://dx.doi.org/10.1016/j.jaci.2013.12.1074

Abbreviations used
AR: Allergic rhinitis
EGEA: Epidemiological Study on the Genetics and Environment of Asthma
FBAT: Family-based association study
GWAS: Genome-wide association study
LD: Linkage disequilibrium
MRC: Medical Research Council
MRCA: Medical Research Council Asthma UK Center in Allergic Mechanisms of Asthma
NFIA: Nuclear factor I/A
QC: Quality control
SLSJ: Saguenay-Lac-Saint-Jean
SNP: Single nucleotide polymorphism
UK: United Kingdom
UTR: Untranslated region

the family-based association method (FBAT) and logistic regression. To assess whether the association was specific to the combined asthma plus AR phenotype, we tested for heterogeneity of association according to disease status defined by the presence of the 2 diseases (ie, asthma plus AR) versus the presence of only 1 disease (ie, asthma only or AR only). The replication phase used 2 family samples of European ancestry, French-Canadian families and United Kingdom (UK) families, which, similarly to EGEA families, were ascertained through asthmatic probands and were part of the GABRIEL consortium asthma GWAS.[10]

METHODS

Discovery sample

The EGEA study has been described in detail previously.[11] The EGEA family sample consisted of 388 French nuclear families that included 253 families ascertained through offspring with asthma (1 offspring proband in 90% of families and 2 offspring probands in the remainder) and 135 families ascertained through 1 parent with asthma. Inclusion criteria for probands have been described previously.[11] The definition of AR was the same as that used for the previous linkage analysis[8] and was defined by the presence of at least 1 of the following symptoms: sneezing or runny nose to hay/flower or animals or dust. The definition of asthma used for analysis was also the same as that used for the previous linkage analysis[8] and was defined by a positive answer to at least 1 of the following 2 questions: (1) Have you ever had attacks of breathlessness at rest with wheezing associated with either the presence of bronchial hyperresponsiveness, hospitalization for asthma in life, or asthma therapy? (2) Have you ever had an attack of asthma associated with either the presence of bronchial hyperresponsiveness, hospitalization for asthma in life, or asthma therapy? After applying stringent quality control (QC) criteria to the genome-wide genotype data (see the genotyping paragraph), 1492 EGEA family members were kept for analysis.

Replication samples

French-Canadian family collection. The Saguenay-Lac-Saint-Jean (SLSJ) asthma study included 253 French-Canadian multigenerational families ascertained through 2 probands with asthma.[12] Inclusion criteria for probands have been described elsewhere.[12] Definitions of AR and asthma were similar to those used in the EGEA sample. After QC of the genotypic data, the analysis sample included 1172 family members.

UK family sample. The Medical Research Council (MRC) UK National family collection included 207 nuclear families recruited through at least 1 proband with asthma (Medical Research Council Asthma UK Center in Allergic Mechanisms of Asthma [MRCA] sample).[13] Rhinitis definition was based on a positive answer to the following question: Has your child ever had a problem with sneezing or a runny or blocked nose, when he/she did not have a cold or the "flu?" Asthma was defined either by attacks of asthma or by a doctor's diagnosis. To increase the number of unaffected subjects (control subjects), we included subjects from another MRC UK collection of families that were recruited through proband having eczema (we called this sample MRCE). Only subjects without asthma and those without eczema were used as control subjects in logistic regression analyses. We checked that the age and sex distributions were similar in the 2 MRCA and MRCE samples.

After QC of the genotypic data, the analysis sample included 254 siblings from MRCA plus 98 additional unaffected subjects from MRCE. The whole UK sample will be subsequently designated as the MRC sample.

Genotyping and imputations

The EGEA sample was genotyped with the Illumina 610 Quad array (Illumina, San Diego, Calif) at the Centre National de Génotypage (CNG, Evry, France), as part of the European GABRIEL consortium asthma GWAS (http://www.gabriel-fp6.org/).[10] Stringent quality criteria, as detailed by Imboden et al,[10,14] were used to select both subjects and single nucleotide polymorphisms (SNPs) for analysis. For this study, we used 2042 SNPs belonging to the 1p31 region and that spanned 5 Mb on each side of the linkage peak located 58 Mb from pter. The SLSJ study replication sample was also genotyped at CNG with the Illumina 610 Quad array. The offspring in MRC families were genotyped with the 317k Illumina array, as part of a previous GWAS (parents were not genotyped either in the MRCA or the MRCE).[10,13] The same QC criteria for subjects and markers as used for EGEA were applied to these data sets. To have the same number of SNPs across the EGEA, SLSJ, and MRCA samples and to increase SNP density, imputations of nongenotyped SNPs were performed with MACH v1.00 software (http://www.sph.umich.edu/csg/abecasis/MACH/) and HapMap2 release 21 CEU haplotypes as a reference panel. These imputations were carried out in each of the 3 data sets (EGEA, SLSJ, and MRC sets) separately. Imputed SNPs were kept for analysis if their imputation quality score (rsq)[15] was greater than or equal to 0.9 and if their minor allele frequency was 5% or greater.

Statistical methods

Because the 1p31 region was detected through linkage analysis, association of asthma plus rhinitis with the 2042 genotyped SNPs spanning the linkage region was first investigated in the EGEA families by using the FBAT approach,[16] which tests for association in the presence of linkage. We assumed an additive genetic model and took into account the presence of linkage with the –e option.[17] The sibship distribution of EGEA families contributing to FBAT analysis is shown in Table E1 in this article's Online Repository at www.jacionline.org. To correct for multiple testing, a threshold corresponding to a type 1 error of 5% was calculated by using the method of Gao et al[18] and was estimated to be equal to 5×10^{-5}. For SNPs reaching the significance level of association by using FBAT analysis, we investigated whether the association was specific to the combined asthma plus AR phenotype by testing for heterogeneity of association according to disease status defined by the presence of the 2 diseases (ie, asthma plus AR) versus the presence of only 1 disease (ie, asthma only or AR only) by using FBAT. Replication of these results was then sought in the SLSJ sample for both tests of association and heterogeneity with FBAT. The sibship distribution of SLSJ families contributing to FBAT analysis is shown in Table E1. Note that FBAT analyses could not be conducted in MRC families because only the offspring had been genotyped. Meta-analyses of FBAT results from the EGEA and SLSJ samples were conducted for both tests of association and heterogeneity by using the Stouffer z score method. The combined z score is a weighted average of the study-specific z scores in which the weight is a function of the number of informative families.

At a second step, logistic regression analysis was carried out in EGEA siblings, allowing an internal validation of FBAT results. Affected status was defined by the presence of both asthma and AR, and unaffected status was defined by the absence of both. This analysis was conducted with SNPs reaching the critical threshold in FBAT analysis in EGEA plus all genotyped and imputed SNPs spanning 10 kb on each side of each significant SNP. We used allele dosage to take into account the uncertainty of imputed genotypes. The first 2