DNA methylation within melatonin receptor 1A *(MTNR1A)* mediates paternally transmitted genetic variant effect on asthma plus rhinitis

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Background: Asthma and allergic rhinitis (AR) are common allergic comorbidities with a strong genetic component in which epigenetic mechanisms might be involved.

Objective: We aimed to identify novel risk loci for asthma and AR while accounting for parent-of-origin effect.

Methods: We performed a series of genetic analyses, taking into account the parent-of-origin effect in families ascertained through asthma: (1) genome-wide linkage scan of asthma and AR in 615 European families, (2) association analysis with 1233 single nucleotide polymorphisms (SNPs) covering the significant linkage region in 162 French Epidemiological Study on the Genetics and Environment of Asthma families with replication in 154 Canadian Saguenay-Lac-Saint-Jean asthma study families, and (3) association analysis of disease and significant SNPs with DNA methylation (DNAm) at CpG sites in 40 Saguenay-Lac-Saint-Jean asthma study families.

Results: We detected a significant paternal linkage of the 4q35 region to asthma and allergic rhinitis comorbidity (AAR; $P = 7.2 \times 10^{-5}$). Association analysis in this region showed strong evidence for the effect of the paternally inherited G allele of rs10009104 on AAR ($P = 1.1 \times 10^{-5}$, reaching the multiple-testing corrected threshold). This paternally inherited allele was also significantly associated with DNAm levels at the cg02303933 site ($P = 1.7 \times 10^{-4}$). Differential DNAm at this site was found to mediate the identified SNP-AAR association.

Conclusion: By integrating genetic and epigenetic data, we identified that a differentially methylated CpG site within the melatonin receptor 1A *(MTNR1A)* gene mediates the effect of a paternally transmitted genetic variant on the comorbidity of asthma and AR. This study provides a novel insight into the role of epigenetic mechanisms in patients with allergic respiratory diseases. (J Allergy Clin Immunol 2016;■■■:■■■-■■■.)

Key words: *Linkage analysis, positional cloning, asthma, allergic rhinitis, parent-of-origin effect, DNA methylation, genetic association study*

The prevalence of allergy-related diseases, such as asthma and rhinitis, has reached epidemic proportions in high-income countries and represents a global health problem for all ages and all ethnic groups. Asthma and allergic rhinitis (AR) affect 5% to 16% and 10% to 25% of persons worldwide, respectively.[1,2] Asthma and AR frequently coexist in the same subjects.[3] Indeed, more than 60% of patients with asthma have concomitant AR, whereas 10% to 40% of patients with AR have asthma comorbidity,[4] suggesting that these diseases might share a common cause, overlapping pathogenic pathways, or both.

The risk of asthma and AR has a strong genetic component, with estimated heritability ranging from 35% to 75%.[2] Genome-wide association studies (GWASs) have been successful in identifying loci associated with asthma and AR, with a number of loci

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Abbreviations used
AAR: Asthma and allergic rhinitis comorbidity
AR: Allergic rhinitis
CIT: Causal inference test
CNG: Centre National de Génotypage
CTCF: CCCTC-binding factor
DNAm: DNA methylation
EGEA: Epidemiological Study on the Genetics and Environment of Asthma
GWAS: Genome-wide association study
MCPPAT: Monte Carlo Pedigree Parental Asymmetry Test
MTNR1A: Melatonin receptor 1A
PO-LRT: Parent-of-origin likelihood ratio test
POO: Parent of origin
QC: Quality control
SLSJ: Saguenay-Lac-Saint-Jean asthma study
SNP: Single nucleotide polymorphism

shared by both diseases.[5,6] A few genetic studies have examined asthma and AR comorbidities and led to the identification of new loci that were not detected when they were analyzed separately; these include *ZBTB10*, *CLEC16A*, and *NFIA* genes.[7,8]

Despite the success of GWASs for discovering common risk alleles associated with asthma, AR, or both, the genetic factors identified to date account only for a small part of the genetic component of these diseases.[1] Sources of this hidden heritability might include epigenetic mechanisms, such as DNA methylation (DNAm). Epigenetic modifications are heritable changes influencing gene expression without alteration of the underlying DNA sequence. Several lines of evidence support a role for epigenetics in asthma and allergy, which are immune-mediated diseases characterized by a T_H2-type immune response.[9] Epigenetic mechanisms are known to play a role in T-cell differentiation and regulation.[10] Moreover, environmental factors associated with asthma and allergy have been reported to induce epigenetic changes in experimental settings.[10] The importance of epigenetics in allergy-related diseases has been recently evidenced in human subjects by using an epigenome-wide association study of total IgE levels, where DNAm at 3 CpG sites was found to account for 13% of IgE variation, which is 10-fold higher than that accounted for by GWAS single nucleotide polymorphisms (SNPs).[11] DNAm can cause partial or complete silencing of 1 parental allele (imprinting) at a specific locus and can lead to an effect of that polymorphism on disease in offspring that differs according to the parental origin (paternal or maternal) of the allele (parent-of-origin [POO] effect).

The goal of the present study was to identify novel risk loci for allergic diseases defined by asthma, AR, and asthma and allergic rhinitis comorbidity (AAR) through a positional cloning approach taking into account the POO effect and to determine whether the effect of associated SNPs is mediated by DNAm. For that purpose, we developed a 3-step analysis strategy in family samples ascertained through asthma: (1) genome-wide linkage scan of asthma, AR, and AAR in 3 European family data sets; (2) association analysis of SNPs covering the significant linkage region in the French Epidemiological Study on the Genetics and Environment of Asthma (EGEA) discovery data set with validation in a French Canadian data set; and (3) association analyses of DNAm levels with both genetic variants and disease, followed by the causal inference test (CIT).

METHODS

Analysis strategy and family samples

The study design is described in Fig E1 in this article's Online Repository at www.jacionline.org. The first step consisted of a genome-wide linkage analysis of allergic diseases conducted in 615 families from 3 independent European family samples: EGEA,[12] the Medical Research Council Asthma UK National family collection,[13] and Italian families.[14] All families were ascertained through at least 1 asthmatic proband recruited in chest clinics, as previously described.[12-14] Asthma was based on a physician's diagnosis and international standardized questionnaires. AR was defined by the report of at least 1 of the following symptoms: sneezing or runny nose to hay/flowers, animals, or dust. AAR was defined as the coexistence of the 2 diseases (asthma and AR) in the same subject. Details on study characteristics and phenotype definitions are provided in the Methods section and Table E1 in this article's Online Repository at www.jacionline.org.

In the second step the region of linkage found significantly linked to AAR in the presence of the POO effect was explored for association in the EGEA study. This study was the only one of the 3 data sets used for linkage analysis that had genotyped SNPs in both parents and offspring, thus allowing testing for the POO effect. After applying stringent quality control (QC) criteria, there were 531 family members belonging to 162 families with at least 1 offspring with AAR and 2 genotyped parents (see the Methods section in this article's Online Repository for details). The EGEA top associated SNPs were then tested for validation in an independent family study, the Saguenay-Lac-Saint-Jean asthma study (SLSJ), which included 253 French Canadian multigenerational families ascertained through 2 probands with asthma.[15] Asthma definition in probands was based on at least 2 of the following criteria: (1) a minimum of 3 clinic visits for acute asthma within 1 year, (2) 2 or more asthma-related hospital admissions within 1 year, or (3) steroid dependency. In relatives asthma was defined by (1) a history of asthma (validated by a physician) or (2) asthma-related symptoms and bronchial hyperresponsiveness (see Table E1). The definition of AR was similar to the one used in the EGEA study (see Table E1). After QC of the genotypic data, the SLSJ analysis sample included 486 family members from 154 families with at least 1 genotyped offspring with AAR and 2 genotyped parents (see the Methods section in this article's Online Repository for details).

In the third step we investigated whether the significant POO effects of SNPs on AAR were driven by differential DNAm levels measured in 40 SLSJ families by using CIT.[16]

For all independent studies, ethical approval was obtained from the appropriate institutional ethic committees, and all subjects provided written informed consent.

Genotyping

The 3 data sets used for linkage analysis had been genotyped by using the same panel of 396 microsatellites at the Centre National de Génotypage (CNG, Evry, France) as part of our linkage analysis program.[17] After QC of the genotypic data, the analysis sample included a total of 2702 subjects belonging to 615 families with at least 2 genotyped sibs.

The discovery and replication association analyses were based on SNP data generated in the EGEA and SLSJ data sets by using the Illumina 610K array (Illumina, San Diego, Calif) at CNG as part of the European GABRIEL asthma consortium.[18] The same QC criteria were applied to both data sets, as described elsewhere.[19] The region analyzed for association included a total of 1233 genotyped SNPs covering the 6-Mb linkage region (95% CI defined by a 1.5-LOD decrease from the linkage peak).

Methylation levels at CpG sites were measured by using DNA from peripheral blood leukocytes in a subset of SLSJ subjects (n = 167) by using the Infinium HumanMethylation450 BeadChip at Genome Quebec Innovation Centre (Montreal, Quebec, Canada).[11] After QC, a total of 159 subjects belonging to 40 nuclear SLSJ families were available for DNAm association analysis (see the Methods section in this article's Online Repository for details). Differential white cell counts (eosinophils, neutrophils, basophils, monocytes, and lymphocytes) were measured by using an automated counter in SLSJ data.