Associations and interactions of genetic polymorphisms in innate immunity genes with early viral infections and susceptibility to asthma and asthma-related phenotypes

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Background: The innate immune system is essential for host survival because of its ability to recognize invading pathogens and mount defensive responses.

Objectives: We sought to identify genetic associations of innate immunity genes with atopy and asthma and interactions with early viral infections (first 12 months of life) in a high-risk birth cohort.
Methods: Three Canadian family-based studies and 1 Australian population-based case-control study (n = 5565) were used to investigate associations of 321 single nucleotide polymorphisms (SNPs) in 26 innate immunity genes with atopy, asthma, atopic asthma, and airway hyperresponsiveness. Interactions between innate immunity genes and early viral exposure to 3 common viruses (parainfluenza, respiratory syncytial virus, and picornavirus) were examined in the Canadian Asthma Primary Prevention Study by using both an affected-only family-based transmission disequilibrium test and case-control methods.
Results: In a joint analysis of all 4 cohorts, IL-1 receptor 2 *(IL1R2)* and Toll-like receptor 1 *(TLR1)* SNPs were associated with atopy after correction for multiple comparisons. In addition, an *NFKBIA* SNP was associated with atopic asthma. Six SNPs (rs1519309 *[TLR3]*, rs740044 *[ILIR2]*, rs4543123 *[TLR1]*, rs5741812 *[LBP]*, rs917998 *[IL18RAP]*, and rs3136641 *[NFKBIB]*) were significant ($P < .05$, confirmed with 30,000 permutations) in both the combined analysis of main genetic effects and SNP-virus interaction analyses in both case-control and family-based methods. The *TLR1* variant (rs4543123) was associated with both multiple viruses (respiratory syncytial virus and parainfluenza virus) and multiple phenotypes.
Conclusion: We have identified novel susceptibility genes for asthma and related traits and interactions between these genes and early-life viral infections. (J Allergy Clin Immunol 2012;130:1284-93.)

Key words: *Asthma, atopy, atopic asthma, airway hyperresponsiveness, genetics, association, gene-environment interactions, viral exposures*

The worldwide prevalence of asthma and other allergic diseases has increased during the past 2 decades, particularly in developed countries.[1-3] Currently, asthma affects 8% to 10% of the population in the United States and is the most common chronic disease in children. The innate immune system plays an important role in the development of allergic disorders by sensing various microbial compounds and mounting defensive responses. The hygiene hypothesis states that early childhood exposure to infection or endotoxins alters the T_H1/T_H2 balance, attenuating the T_H2 immune response and thus inhibiting the development of

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Abbreviations used
AHR: Airway hyperresponsiveness
CAPPS: Canadian Asthma Primary Prevention Study
GWIS: Genome-wide interaction study
IL-1R: IL-1 receptor
LD: Linkage disequilibrium
OR: Odds ratio
PIV: Parainfluenza virus
QQ: Quantile-quantile
RSV: Respiratory syncytial virus
SAGE: Study of Asthma Genes and the Environment Study
SLSJ: Saguenay–Lac-Saint-Jean and Quebec City Familial Asthma Collection
SNP: Single nucleotide polymorphism
TLR: Toll-like receptor

asthma and other allergic diseases.[4] In contrast, a microbe-free environment can polarize T_H cells to a T_H2 immune response and therefore predispose to the development of atopy.

A vital part of the innate immune response is the IL-1 receptor (IL-1R)/Toll-like receptor (TLR) superfamily.[5] The genes of the IL-1R/TLR pathways are widely expressed in different cell types in the human lung.[6] The IL-1R subgroup has been implicated in the development of atopy and asthma,[7-9] and genetic associations with atopic diseases have been reported.[8] Single nucleotide polymorphisms (SNPs) of *TLR2*,[10,11] *TLR4*,[11] *TLR6*,[12] *TLR9*,[11,13] and *TLR10*[14] have also been reported to be associated with asthma or allergic diseases. There has been no report yet on genetic variants of Toll/IL-1 receptor adaptors in allergic diseases.

Viral infections are well recognized as an environmental risk factor for both the development of asthma and asthma exacerbations,[15-21] although it is not clear whether viruses are causal, affecting and modifying the growth and development of the immune system, or whether the relationship is indicative of a susceptible subject with impaired lung function and innate immune responses or a combination of the two.[20,22,23] The "2-hit" hypothesis has been postulated, whereby viral infections promote asthma mainly in predisposed children.[20] It is entirely plausible that viral infections initiate asthma in children genetically predisposed to the disease. If we are going to understand the cause of asthma, we need to examine the larger complex picture of genetic susceptibility, environmental exposures, and their interactions.

The aim of this study was to identify novel genetic associations and potential gene-environment interactions between innate immunity genes and early viral infections (first 12 months of life) with 3 common respiratory viruses (parainfluenza virus [PIV], respiratory syncytial virus [RSV], and picornavirus) that have been implicated in the onset or exacerbation of asthma in children. Main genetic effects and interactions were tested for 4 allergic phenotypes: atopy, asthma, atopic asthma, and airway hyperresponsiveness (AHR). Innate immunity genes that were associated with asthma and atopic diseases in other populations were also investigated in our study cohorts, and these data have been previously reported.[24] We considered a variety of analytic approaches, including a genome-wide interaction study (GWIS), but given that we expected interaction effects to be modest (odds ratio [OR] < 2) and our small sample size (approximately 280 children), we believed that the multiple testing burden of a GWIS would overwhelm these modest effects. A more hypothesis-based pathway approach could have been undertaken, and this approach would have increased our power. However, our prior knowledge of biological pathways and gene-virus interactions is limited, and we believed our analyses could benefit from a more general approach. As such, we chose to look at a class of genes (innate immunity) with strong biological plausibility of involvement in gene interactions with the 3 common respiratory tract viruses. This nested hypothesis-based approach represents a compromise, it reduces the number of statistical tests performed compared with a GWIS, but it is not as focused as a genetic pathway approach.

METHODS

Study populations

Four well-characterized groups were used for this study of asthma and asthma-related phenotypes (Table I). Informed consent was obtained from each patient or his or her guardians, and the study was approved by the research ethics board of each recruiting center.

The Canadian Asthma Primary Prevention Study (CAPPS). This prospective birth cohort assessed the effectiveness of a multifaceted intervention program designed to prevent the development of asthma and other atopic disorders in 549 high-risk children who had a family history of allergies.[25] The children were recruited from Vancouver and Winnipeg, Canada, and have been assessed by a pediatric allergist at 3 time points for the presence of allergic phenotypes. A total of 545 families were initially recruited into the study, and 380 families were available for analysis by the end of 7 years of follow-up.[26] The presence of PIV, RSV, or picornavirus was tested at 2 weeks and 3, 8, and 12 months of age, as previously described.[19,27,28] Details of the laboratory protocols have been previously published.[19,27,28] Nasal swabs were tested for each virus, and the sensitivity for detection by means of RT-PCR was 10^2 to 10^3 copies of virus-specific nucleic acid within a specimen.[19]

The Study of Asthma Genes and the Environment (SAGE). This study included 723 children and their parents who were recruited from a population-based sample of 16,320 children born in Manitoba, Canada, in 1995.[29-31] A total of 3,586 children participated in a survey in 2002, of whom 392 had asthma, 192 had hay fever or food allergy, and 3,002 had none of these conditions.

All children with asthma or allergy were invited to participate in a nested case-control study in addition to a sample of control subjects representative of both urban and rural environments.

The Saguenay−Lac-Saint-Jean and Québec City Familial Asthma Collection (SLSJ). This collection is comprised of 306 families from the Saguenay−Lac-Saint-Jean (n = 227) and Quebec City (n = 79) regions of Quebec, Canada.[32-34] A family was deemed acceptable for the study on identification of an asthmatic proband whose 4 grandparents were of French-Canadian ancestry. Each subject completed a modified American Thoracic Society respiratory questionnaire.

The Busselton Health Study Population. Residents of the town of Busselton in the southwest of Western Australia have been involved in a series of health surveys since 1966. Subjects attended one of 6 cross-sectional surveys from 1966 to 1981 and the follow-up survey in 1994. A nested case-control study was designed from this population that included subjects who participated in 1 or more surveys and had a methacholine challenge. Cases and control subjects were defined by the presence (n = 644) or absence (n = 749) of asthma.[35,36]

Phenotype definitions

Asthma was defined as doctor-diagnosed asthma at 7 years of age in the CAPPS and SAGE cohorts based on history and physical examination without knowledge of the results of methacholine and allergy skin tests. In the SLSJ sample asthma was diagnosed by asthma specialists, and in the Busselton Study asthma was defined as self-reported doctor-diagnosed asthma. Atopy was defined as at least 1 positive response (ie, wheal diameter ≥3 mm or more than that elicited by the negative control at 10 minutes). AHR was defined as a PC_{20} FEV_1 of less than 3.2 mg/mL for CAPPS and SAGE subjects or less than