Genome-wide association studies of asthma indicate opposite immunopathogenesis direction from autoimmune diseases

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Background: Genome-wide association studies (GWASs) of asthma have consistently implicated the ORM1-like 3 and gasdermin B *(ORMDL3-GSDMB)*, *IL33*, IL-1 receptor–like 1 and IL-18 receptor 1 *(IL1RL1-IL18R1)*, *RAD50-IL13*, thymic stromal lymphopoietin and WD repeat domain 36 region *(TSLP-WDR36)*, and *HLA-DR/DQ* regions.

Objective: A GWAS of asthma was performed in a non-Hispanic white population.

Methods: A GWAS was performed in 813 Severe Asthma Research Program/Collaborative Studies on the Genetics of Asthma/Chicago Asthma Genetics Study cases and 1564 control subjects. The GWAS results were compared with those of the published GWASs of autoimmune diseases.

Results: Multiple single nucleotide polymorphisms in the *TNFAIP3* interacting protein 1 *(TNIP1)* gene, which interacts with *TNFAIP3* and inhibits the TNF-α–induced nuclear factor κB inflammation pathway, were associated with asthma: rs1422673 ($P = 3.44 \times 10^{-7}$) and rs10036748 ($P = 1.41 \times 10^{-6}$, $r^2 = 0.67$). rs1422673 was also associated with asthma in the published GABRIEL ($P = .018$) and EVE ($P = 1.31 \times 10^{-5}$) studies. The minor allele T of rs20541 in *IL13* is the risk allele for asthma but the protective allele for psoriasis. The minor allele T of rs2395185 in *HLA-DRA* is the risk allele for asthma but the protective allele for ulcerative colitis. The minor allele A of rs2872507 in *GSDMB* is the protective allele for asthma but the risk allele for rheumatoid arthritis, Crohn disease, and ulcerative colitis. The T allele of rs10036748 in the *TNIP1* gene is the minor protective allele for asthma but the minor or major risk allele for systemic lupus erythematosus and systemic sclerosis in non-Hispanic white or Chinese subjects, respectively.

Conclusions: Our study suggests that single nucleotide polymorphisms associated with both asthma and autoimmune diseases might have opposite effects on immunopathogenesis.

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Key words: *Asthma, genetics, genome-wide association study,* TNFAIP3 *interacting protein 1*

Asthma is a common inflammatory airway disease that can be triggered in genetically susceptible subjects by various environmental exposures. Asthma is characterized by bronchial hyperresponsiveness, bronchodilator reversibility, and often by increased expression of T_H2 cytokines, increased serum IgE levels, and atopy. In allergic asthmatic patients eosinophilic inflammation and T_H2 cytokines dominate; in patients with severe/refractory asthma, neutrophilic inflammation and TNF-α/T_H17 cytokines are involved.[1] Genome-wide association studies (GWASs) of asthma and asthma-related traits have consistently identified 6 major regions: the ORM1-like 3 and gasdermin B (*ORMDL3-GSDMB*) region,[2-4] interleukin 33 (*IL33*),[3-5] the IL-1 receptor–like 1 and IL-18 receptor 1 (*IL1RL1-IL18R1*) region,[3-5] the RAD50 homolog and IL-13 (*RAD50-IL13*) region,[3,6] the thymic

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Abbreviations used
CAG: Chicago Asthma Genetics Study
CSGA: Collaborative Studies on the Genetics of Asthma
GSDMB: Gasdermin B
GWAS: Genome-wide association study
IL1RL1: IL-1 receptor–like 1
IL18R: IL-18 receptor 1
LD: Linkage disequilibrium
OR: Odds ratio
ORMDL3: ORM1-like 3
SARP: Severe Asthma Research Program
SLE: Systemic lupus erythematosus
SNP: Single nucleotide polymorphism
TENOR: The Epidemiology and Natural History of Asthma: Outcomes and Treatment Regimens
TNIP1: *TNFAIP3* interacting protein 1
TSLP: Thymic stromal lymphopoietin
WDR36: WD repeat domain 36

stromal lymphopoietin and WD repeat domain 36 region *(TSLP-WDR36)* region,[3-5,7] and the major histocompatibility complex class II DR/DQ (*HLA-DR/DQ*) region.[3,6,7]

Autoimmune diseases arise through abnormal immune responses to self-antigens and are generally characterized by T_H1-mediated inflammation. Autoimmune diseases cause extensive comorbidity among psoriasis, systemic lupus erythematosus (SLE), rheumatoid arthritis, Crohn disease, type I diabetes, multiple sclerosis, ulcerative colitis, and celiac disease, for example.[8] Although both Crohn disease and ulcerative colitis are inflammatory bowel diseases, the T_H1 process is dominant in patients with Crohn disease, whereas ulcerative colitis is likely to be a T_H2 disease.[9] Comparison of published GWAS results among patients with a variety of autoimmune diseases reveals that significant association at the gene or single nucleotide polymorphism (SNP) level is common among autoimmune diseases.[8]

Systematic comparison of genes or SNPs found to be significant in GWASs of both asthma and autoimmune diseases is very limited.[8] Asthma and autoimmune diseases share extensive immunologic pathways but generally are believed to have different or opposite pathogenic T-cell mechanisms (oversimplified as the T_H2 vs T_H1 model). A counterregulatory model emphasizes the importance of regulatory T cells in patients with immune diseases, which inhibit both T_H2-mediated allergic diseases and T_H1-mediated autoimmune diseases.[10] Here we report a GWAS of asthma in a non-Hispanic white population (813 cases and 1564 control subjects) from the Severe Asthma Research Program (SARP)/Collaborative Studies on the Genetics of Asthma (CSGA)/Chicago Asthma Genetics Study (CAG) to identify novel genes and to confirm previously identified genes involved in asthma. Several asthma candidate genes (*HLA*, *IL13*, and *TNFAIP3* interacting protein 1 *[TNIP1]*) identified by us and others were associated with autoimmune diseases as well. Hence we compared SNPs in genes identified by using GWASs of asthma with those in autoimmune diseases to explore common genetic factors and disease causes.

METHODS

Study subjects

Non-Hispanic white subjects were participants in the National Heart, Lung, and Blood Institute–funded SARP, the National Heart, Lung, and Blood Institute's CSGA, and the CAG (or CSGA enrolled in Chicago). Subjects with mild-to-severe asthma and nonasthmatic control subjects were recruited from SARP[11,12] and CSGA[13] centers with a similar protocol. CAG subjects with asthma and nonasthmatic control subjects were collected at the University of Chicago by using a similar protocol.[4] Results from the GWAS in SARP/CSGA/CAG were included in the EVE consortium meta-analysis.[4]

Subjects with difficult-to-treat or severe asthma were recruited from the Epidemiology and Natural History of Asthma: Outcomes and Treatment Regimens (TENOR) multicenter study.[6,14] SARP, CSGA, CAG, and TENOR studies were approved by the appropriate institutional review boards at the participating sites, including informed consent.

General population control subjects were obtained by using the Illumina iControlDB client (Illumina, Inc, San Diego, Calif) to download genotypes for 3294 white subjects with genotype data available from HumanHap 550 k products. General population control subjects (n = 1892) were matched with 473 TENOR cases, as described previously.[6] The remaining general population control subjects (n = 1011) and control subjects genotyped in SARP/CSGA/CAG (n = 553) were merged and used as control subjects for SARP/CSGA/CAG cases (n = 813).

DNA was isolated by using standard protocols, and SNP genotyping was performed with the Illumina HumanHap1M BeadChip or the Illumina HumanCNV370 BeadChip for SARP/CSGA/CAG[4,15] and TENOR,[6] respectively.

SARP/CSGA/CAG (813 cases vs 1564 control subjects) was used as the discovery dataset. The published TENOR,[6] EVE,[4] and GABRIEL[3] studies were used to replicate top findings from SARP/CSGA/CAG. Meta-analysis *P* values from EVE and GABRIEL were reported by using a fixed-effects model.

Statistical analysis

Quality control was applied to SARP/CSGA/CAG cases and control subjects, TENOR cases, and Illumina control subjects separately because they were genotyped by using slightly different Illumina products, as described previously.[6] In brief, subjects were removed if they (1) had genotyping call rates of less than 95%, (2) were discrepant or ambiguous for genetic sex (heterozygous haploid genotype percentage ≥0.01 for male subjects or X-chromosome homozygosity F ≥0.9 for female subjects), (3) failed the check for family relatedness (PI_HAT > 0.125), or (4) were detected as an outlier (>6 SDs for the first or second principal component). After subjects meeting these criteria were excluded, SNPs were removed if (1) call rates were 95% or less, (2) they were inconsistent with Hardy-Weinberg equilibrium ($P < 10^{-4}$), or (3) they had a minor allele frequency of 0.05 or less. After quality control, SNPs shared between cases and control subjects were merged for analysis.

Logistic regression, assuming an additive disease model, was used for genome-wide association analysis of asthma susceptibility by using PLINK[16] adjusted for age, sex, and significant principal components (n = 4) from EIGENSTRAT.[17] Linkage disequilibrium (LD) was estimated with Haploview.[18]

Logistic regression of SNPs with well-replicated associations (rs2872507 in *GSDMB*, rs3939286 in *IL33*, rs13431828 in *IL1RL1*, rs20541 in *IL13*, rs1837253 in *TSLP*, and rs2395185 in *HLA-DRA*) were performed in SARP/CSGA/CAG and TENOR populations by using either 6 SNPs together or genetic scores with age and sex adjusted. Genetic scores were defined as follows: genotypes with 1 or 2 minor alleles were merged together and recoded to 0 as a protective category (if the minor allele was a protective allele) or to 1 as a risk category (if the minor allele was a risk allele). The percentage of deviance explained by an SNP was defined as deviance explained by an SNP/deviance of the null model with age and sex adjusted. The area under the receiver operating characteristic curve was calculated by using SAS software (SAS Institute, Inc, Cary, NC).

GWAS results from the published GABRIEL study[3] were extracted from the European Genome-Phenome Archive (http://www.cng.fr/gabriel; accession no. EGAS00000000077 for the GABRIEL study). SNPs ($P < 1.0 \times 10^{-5}$) from GWASs of asthma or asthma-related traits[2-7] were extracted from the NIH GWAS database[19] (http://www.genome.gov/gwastudies/). SNPs ($P < 1.0 \times 10^{-5}$) from GWASs of autoimmune diseases were extracted from the NIH GWAS database[19] (http://www.genome.gov/gwastudies/) if the same SNPs were significant ($P < 1.0 \times 10^{-5}$) in a GWAS of asthma or asthma-related traits.[2-7]