Patterns of *GATA3* and *IL13* gene polymorphisms associated with childhood rhinitis and atopy in a birth cohort

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Background: *GATA3* activates transcription of the T_H2 cytokines, including *IL13*, an important step in the allergic inflammatory pathway.

Objective: We sought to identify associations of single nucleotide polymorphisms of the genes *GATA3* and *IL13* and their interactions with rhinitis and allergic sensitization during childhood.

Methods: We performed genetic association studies in a cohort of children (n = 923) who have been evaluated for the development of rhinitis and allergic sensitization by means of skin prick tests (SPTs) at age 10 years. Pyrosequencing was used to genotype 7 polymorphisms from *GATA3* and 5 from *IL13*. A novel model-selection procedure combining logistic regression models and classification was used to study the contributions of the polymorphisms and their interactions.

Results: Combinations of polymorphisms and their interactions increase the risk for rhinitis and allergic sensitization at age 10 years. A model with rs1058240, rs379568, and rs4143094 *(GATA3)* and rs1800925 *(IL13)* and their interactions was selected to predict rhinitis and positive SPT responses. rs1058240 was associated with rhinitis and allergic rhinitis ($P < .05$), and the gene-gene interaction rs1058240:rs1800925 was associated with rhinitis ($P = .043$). The odds ratios for 4 genotype combinations were significant for rhinitis or SPTs ($P < .044$).

Conclusion: Gene-gene interaction between *GATA3* and *IL13* polymorphisms can influence the risk of childhood rhinitis. Our study suggests that set associations of polymorphisms are important in studying genetic associations for complex phenotypes, such as rhinitis and atopy. (J Allergy Clin Immunol 2008;121:408-14.)

Key words: *Rhinitis, allergic rhinitis, atopy,* GATA3, IL13*, genetic association, gene-gene interactions*

Abbreviations used
AIC: Akaike Information Criterion
SNP: Single nucleotide polymorphism
SPT: Skin prick test
UTR: Untranslated region

Rhinitis is a disease of high prevalence, especially in industrialized countries.[1] This high prevalence translates into high cost to society in terms of overall health care use and quality of life of those with moderate-to-severe disease. Recent studies showed that the upward trend in prevalence seen during the last few decades might not yet have reached a peak.[2] The reasons for these trends remain unclear but probably reflect environmental influence on genetic predisposition. Noninfectious rhinitis is classified into allergic and nonallergic rhinitis, depending on the presence or absence of atopic sensitization. Familial predisposition for rhinitis, both allergic and nonallergic, is well established.[3,4] Atopy is characterized by production of specific IgE after exposure to allergens. In our birth cohort maternal and paternal rhinitis and atopy were shown to be independent risk factors for investigator-diagnosed rhinitis at age 10 years.[5] Both atopy and rhinitis have multifactorial inheritance, and it is likely that different combinations of genes increase the risk of phenotypic expression, resulting in allergic and nonallergic rhinitis.

GATA3 was selected as a candidate gene for rhinitis based on its action in driving the T_H2 cytokine response. It has been associated with human asthma[6] and is located within a quantitative trait locus for allergic asthma in a murine model.[7] *IL13* is an important cytokine involved in the IgE pathway, and *IL13* is one of the genes most consistently associated with asthma and IgE-related phenotypes in association studies.[8-14] An association of the *IL13* gene with serum IgE levels was shown in patients with allergic rhinitis but not with allergic rhinitis itself.[15] Because of the critical role of *GATA3* in T_H2 cell development and its role in regulating expression of *IL4*, *IL5*, and *IL13*, we believed it is important to examine *GATA3* along with *IL13*. We tested for the association of human *GATA3* and *IL13* gene polymorphisms with rhinitis and for positive skin prick test (SPT) responses at age 10 years. The combinations of single nucleotide polymorphisms (SNPs) and possible interactions that were statistically significant for rhinitis were then further tested for allergic rhinitis or nonallergic rhinitis and compared with results in the control group of children without rhinitis and with negative SPT responses. We used statistical variable selection procedures and cross-validation to investigate the influence of SNP patterns in the *IL13* and *GATA3* genes.

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METHODS

Study population

A whole population birth cohort (n = 1456) was established on the Isle of Wight, United Kingdom, in 1989 to study the natural history of asthma and allergic disorders and identify genetic and environmental risk factors important in their development. Approval was given by the local research ethics committee, and parental informed consent was obtained. The population is largely white (99%) and living in a semirural environment with no heavy industry. At birth, information was collected on the family history of atopy and potential environmental risk factors. These children have been followed at the ages of 1, 2, 4, and 10 years.[16] At every follow-up, detailed questionnaires were completed with the parents for each child regarding the prevalence of asthma, rhinitis, and atopic dermatitis. Information was also collected and updated on environmental risk factors. At 4 and 10 years, SPTs were performed to 14 common food allergens and aeroallergens (ALK-Abelló, Hørsholm, Denmark) on 981 and 1036 children, respectively.[17] Anticoagulated blood samples were collected at age 10 years and stored frozen for subsequent DNA analysis (n = 923).

Genotyping

Genomic DNA was isolated from blood samples by using QIAamp DNA Blood Kits (Qiagen, Valencia, Calif) or the ABI PRISM 6100 Nucleic Acid PrepStation (Applied Biosystems, Foster City, Calif). Polymorphisms in the *GATA3* and *IL13* genes were examined with the SNPper (http://snpper.chip.org/) and Applied Biosystems (http://www.appliedbiosystems.com/) databases. Genotyping was conducted by using biotin-streptavidin–based pyrosequencing performed on PSQ-96 instrumentation (Biotage AB, Uppsala, Sweden) or by using fluorogenic 5′ nuclease chemistry PCR with Assays on Demands kits cycled on a 7900HT Sequence Detection System (Applied Biosystems). Haploview 3.32 software (Broad Institute, Cambridge, Mass) was used to conduct marker quality checks and generate linkage disequilibrium plots for each gene (Fig 1).

The SNPs were selected to provide data across the *GATA3* and *IL13* genes. *IL13* is a small gene, approximately 3 kb in size, that has been genetically evaluated in a number of studies.[8-14,18] The *IL13* SNPs genotyped in the present study span from the promoter to the 3′ untranslated region (UTR) and identify 1 linkage disequilibrium block. The *GATA3* gene is approximately 20 kb in length, including introns. Initially, 10 validated SNPs identified by using the SNPper database were screened in a subset of our population. Based on minor allele frequencies and the linkage disequilibrium structure of the gene obtained in these preliminary studies,[19] 7 SNPs located in the promoter, introns, exon, and 3′ UTR were genotyped in the entire population. These SNPs provide information on the entire *GATA3* gene. Seven SNPs from the *GATA3* gene and 5 from *IL13* were used in this study (Table I).

Outcomes

Outcomes of rhinitis and SPTs were evaluated at age 10 years. The relevant question for the period prevalence of rhinitis was as follows: Has your child, in the last 12 months, had a problem with sneezing or a runny or blocked nose when he or she did not have a cold or the flu? A positive response was taken as indicating rhinitis. Children with rhinitis were further classified into allergic and nonallergic rhinitis. A child with rhinitis and a positive reaction on an SPT to 1 or more allergens was said to have allergic rhinitis. Nonallergic rhinitis was defined as the presence of rhinitis in the absence of a positive SPT response. We focused on rhinitis at age 10 years because rhinitis is a clinical diagnosis, and in early childhood responses to standardized questions are not specific to make a firm diagnosis. Furthermore, upper respiratory tract infections are frequent in early childhood, blurring the distinction between infectious and noninfectious rhinitis. The allergic rhinitis and nonallergic rhinitis subgroups were compared with the control group of children without rhinitis and with a negative SPT response. Furthermore, SPT responses at age 10 years were examined as a separate outcome for genetic association analysis because the mechanisms of allergic sensitization, although related, might be different from those specific for nasal symptoms.

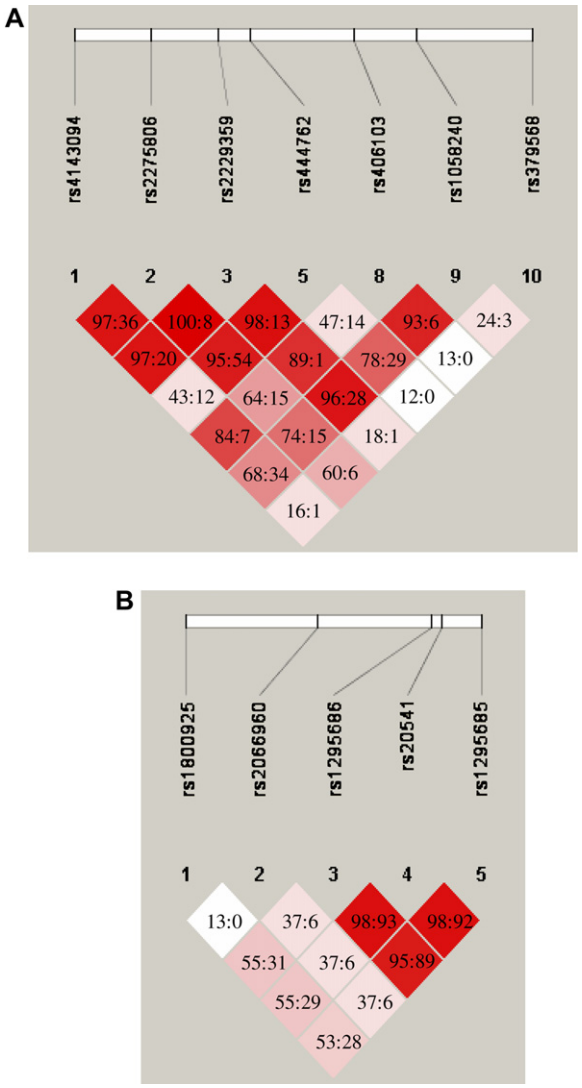

FIG 1. Linkage disequilibrium plot using the Haploview program for the genes *IL13* and *GATA3*. D′ and r^2 are pairwise linkage disequilibrium determinants. The standard D′/LOD color scheme for Haploview is used as follows: D′ = 1 and LOD ≥ 2 is bright red, D′ < 1 and LOD ≥ 2 is shades of red/pink, and D′ < 1 and LOD < 2 is white. **A**, *GATA3* linkage disequilibrium plot. D′:r^2 values are displayed. **B**, *IL13* linkage disequilibrium plot. D′:r^2 values are displayed.

Statistical analysis

In this article a 2-step procedure with logistic regression and classification of selecting SNP patterns among the 12 SNPs was used. We performed an exhaustive search of logistic regression models for 12 SNPs in 2 genes involved in the IgE pathway. The model search for the best subset of variables was based on the Akaike Information Criterion (AIC).[20] The AIC is a measure of goodness of fit with a trade-off for complexity versus how well the model fits the data. Among the models with up to 4 main effects and all possible 2-way SNP-SNP interactions, we selected those with the smallest AIC, such that the SNPs included in the model have highest rank, as measured in the classification procedure random forests.[21] This novel approach allowed the search for unique SNP patterns in combination with selected SNP-SNP interactions. All statistical analyses were performed with R v2.4.1 (http://cran.r-project.org) to identify genetic associations and SNP-SNP interactions with rhinitis, allergic rhinitis, and nonallergic rhinitis at age 10 years. For the model comparisons with the AIC criterion to be valid, only observations with complete