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Association of variants in innate immune genes with asthma and eczema

Sunita Sharma[1,2,3,*], **Audrey Poon**[1,3,*], **Blanca E. Himes**[1,3], **Jessica Lasky-Su**[1,3], **Joanne E. Sordillo**[1], **Kathleen Belanger**[4], **Donald K. Milton**[5], **Michael B. Bracken**[4], **Elizabeth W. Triche**[6], **Brian P. Leaderer**[4], **Diane R. Gold**[1,3], and **Augusto A. Litonjua**[1,2,3]

[1]Channing Laboratory, Department of Medicine, Brigham and Women's Hospital, Boston, MA, USA

[2]Division of Pulmonary and Critical Care Medicine, Department of Medicine, Brigham and Women's Hospital, Boston, MA, USA

[3]Harvard Medical School, Boston, MA, USA

[4]Center for Perinatal, Pediatric and Environmental Epidemiology, Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, CT, USA

[5]University of Maryland School of Public Health, College Park, MD, USA

[6]Department of Community Health and Epidemiology, Brown University School of Medicine, Providence, RI, USA

Abstract

Background—The innate immune pathway is important in the pathogenesis of asthma and eczema. However, only a few variants in these genes have been associated with either disease. We investigate the association between polymorphisms of genes in the innate immune pathway with childhood asthma and eczema. In addition, we compare individual associations with those discovered using a multivariate approach.

Methods—Using a novel method, case control based association testing (C2BAT), 569 single nucleotide polymorphisms (SNPs) in 44 innate immune genes were tested for association with asthma and eczema in children from the Boston Home Allergens and Asthma Study and the Connecticut Childhood Asthma Study. The screening algorithm was used to identify the top SNPs associated with asthma and eczema. We next investigated the interaction of innate immune variants with asthma and eczema risk using Bayesian networks.

Correspondence: Sunita Sharma, MD, Channing Laboratory, Department of Medicine, Brigham and Women's Hospital, 181 Longwood Avenue, Boston, MA 02115, USA. Tel.: (617) 525 2107, Fax: (617) 525 0958, sunita.sharma@channing.harvard.edu.
*These authors contributed equally to the manuscript.

Supporting Information
Additional Supporting Information may be found in the online version of this article:
Table S1. Number of SNPs tested for each innate immunity gene.
Table S2. SNPs tested.
Table S3. Comparison of white children with and without DNA in the Boston Home Allergens and Asthma Study.
Table S4. Comparison of white children with and without DNA in the Connecticut Childhood Asthma Study.
Table S5. Asthma associations (new definitions: cases = asthma + eczema [N = 82]; controls = eczema only [N = 123]).
Table S6. Eczema associations (new definitions: cases = eczema with no asthma [N = 123]; controls = no eczema, no asthma [N = 120]).
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Results—After correction for multiple comparisons, 7 SNPs in 6 genes *(CARD25, TGFB1, LY96, ACAA1, DEFB1, and IFNG)* were associated with asthma (adjusted p-value<0.02), while 5 SNPs in 3 different genes *(CD80, STAT4, and IRAKI)* were significantly associated with eczema (adjusted p-value < 0.02). None of these SNPs were associated with both asthma and eczema. Bayesian network analysis identified 4 SNPs that were predictive of asthma and 10 SNPs that predicted eczema. Of the genes identified using Bayesian networks, only CD80 was associated with eczema in the single-SNP study. Using novel methodology that allows for screening and replication in the same population, we have identified associations of innate immune genes with asthma and eczema. Bayesian network analysis suggests that additional SNPs influence disease susceptibility via SNP interactions.

Conclusion—Our findings suggest that innate immune genes contribute to the pathogenesis of asthma and eczema, and that these diseases likely have different genetic determinants.

Keywords

asthma; Bayesian network; genetic association; eczema; innate immunity

Atopic diseases including asthma and eczema are amongst the most common chronic diseases of childhood and are increasing in prevalence worldwide (1). The ‘atopic march’, reflects the clinical observation that eczema often predates the onset of allergic rhinitis, and that both these diseases can subsequently result in intermittent wheeze and asthma. Possible explanations for this association include common genetic determinants, shared environmental exposures, and gene-by-environment interactions. These explanations are supported by epidemiological evidence that suggests that environmental factors influence susceptibility to eczema and asthma (2), although familial aggregation and twin studies suggest a genetic contribution to these atopic diseases (3).

Epidemiological studies have consistently shown a protective effect of endotoxin exposure on allergic sensitization (4). However, its effect on asthma has been less consistent with some studies showing a protective effect (5), whereas others have found adverse effects (4). It is hypothesized that genetic polymorphisms may contribute to the variability in an individual’s innate immune response to endotoxin resulting in differing susceptibility to atopic disease. However, candidate gene studies of innate immune genes and atopic disorders have been inconsistent. For example, genetic association studies for the toll-like receptor 4 (*TLR4*) gene and asthma and allergy have produced variable results (6, 7). In addition, *CD14*, one of the most studied genes in asthma, has also shown conflicting genetic association results (8, 9). Since asthma and eczema are complex disorders that are determined by multiple genes and environmental exposures, it is hypothesized that other genes in the innate immune pathway may be involved in the development of these diseases. In addition, variants in these genes may interact to produce particular disease phenotypes.

Our aim was to use a novel method developed for population-based genetic association studies that incorporates initial screening and replication testing within the same population, to demonstrate associations between variants in genes in innate immune pathways with childhood asthma and eczema. In addition to performing single-SNP association tests, we used Bayesian networks to create multivariate predictive models of asthma and eczema using genetic variants in innate immune genes.

Methods

Study populations

Boston Home Allergens and Asthma Study—Subjects were recruited as part of a prospective birth cohort in the Boston metropolitan area to investigate the relationship between indoor allergen exposure and the development of asthma and other allergic diseases. Details of the study design have been previously described in detail (10). Between September 1994 and June 1996, 505 infants from 499 families were recruited after delivery at the Brigham and Women's Hospital in Boston, Massachusetts. Inclusion criteria were, maternal age ≥18 yr, residence considered safe without intention to move in the next 12 months, maternal ability to speak English or Spanish, residence within the metropolitan area, and parental report of a doctor's diagnosis of asthma, hay fever, or allergy to inhaled allergens. Children who were born prematurely (<36 wk), had a major congenital anomaly, or were hospitalized in the neonatal intensive care unit after delivery were excluded from the study. For subjects recruited into the Boston Home Allergens and Asthma Study, blood samples were collected at age 2–3 yr, and again at 4–5 yr of age. Questionnaires about home environmental characteristics (i.e. home dampness, smoking, and rugs/carpets) and other relevant exposures (i.e. daycare attendance, other siblings in the home, and presence of pets) were obtained at the first home visit at age 2–3 months and then annually thereafter. Non-Hispanic white children with a parental report, a nurse's report, or physician diagnosed asthma or eczema were considered as either asthma or eczema cases. Approval for this analysis was obtained from the Institutional Review Board (IRB) of the Brigham and Women's Hospital. Informed consent was obtained from the mother at the time of the first home visit.

Connecticut Childhood Asthma Study—Between September 1996 and December 1998, 1002 families were recruited after having had a child at one of five Connecticut hospitals or a hospital in south central Massachusetts. To be eligible, a family had to have a newborn infant (index child) with a sibling less than 11 yr old with physician diagnosed asthma. Study design and primary outcomes for this study have also been previously published (11). Questionnaires were administered at the first home visit, quarterly for the first 3 years, and then annually thereafter. Non-Hispanic white children with physician diagnosed asthma or eczema during the first 6 yr of life were defined as cases. Approval was obtained from the IRB of each participating institution and informed consent was obtained from the mother of the study participant prior to entry.

SNP selection and genotyping

Candidate genes were selected based on their role in the innate immune pathway. The SNPs in 46 innate immune genes were selected if they were (1) tagging SNPs located within 10 kb of the gene of interest with an $r^2 < 0.80$ and a minor allele frequency >5% (2) non-synonymous SNPs resulting in an amino acid change with a minor allele frequency> 1%, or (3) variants that had been previously associated with either asthma or asthma-related phenotypes (Tables S1 and S2). The SNP genotyping was performed using the Illumina BeadStation 500G (San Diego, CA, USA). Of the SNPs genotyped using the Illumina platform, 609 markers passed stringent quality control measures with overall genotype completion rates >99.5% for these markers, 100% concordance on repeat samples, and markers without mendelian inconsistencies. All SNPs that were included in the analysis were in Hardy–Weinberg equilibrium.

To assess for potential population stratification, a total of 100 unlinked SNPs were genotyped in all subjects and were analyzed according to the method proposed by Pritchard and Rosenberg (12). Population stratification SNPs were chosen from the Celera dataset if

they had a minor allele frequency ≥0.25 in Caucasians, were >100 kb apart, and did not map to a gene in SNPper (http://snpper.chip.org/).

Statistical analysis: genetic association analysis

Both cohorts were similar with respect to their geographic ascertainment area, recruitment scheme, data collection time frames, and data collection methods. In addition, the linkage disequilibrium patterns in genotyped SNPs were similar between the Caucasian children in the two cohorts, and were thus combined for this analysis as has been done previously (9). Association testing was performed using the case control based association testing (C2BAT) methodology, as proposed by McQueen MB, et al. (http://rss.acs.unt.edu/Rdoc/library/pbatR/html/c2bat.html). This method was used to select the top 15 SNPs with the highest power for subsequent association testing in the cohort.

The C2BAT allows for both the screening and testing of SNPs for association using the same dataset by randomly splitting the data into observed estimation and testing sets using the calculated selection probabilities for each genotype. An imputed set is created by using the margins of the 2 × 3 table of the testing set; cell counts from the observed estimation set are used to impute cell counts for a new 2 × 3 table (called the imputed set). Finally, the observed estimation set and the imputed set are combined to generate the initial screening set. C2BAT then estimates the effect size of each of the candidate SNPs in the screening set. For example, the higher the effect size (i.e. noncentrality parameter [NCP]), the more likely the SNP would be detected if associated with the outcome of interest. The top n (determined *a priori*) SNPs with the highest NCP can then be tested for association in the original data set. Since the statistics generated in the screening set is independent of the original data set, C2BAT allows for screening and testing of SNPs in the same data set. A Cochrane-Armitage trend test is computed from the screening set for all SNPs. The highest powered SNPs are then selected using the NCP and tested for association with the disease of interest in the original dataset. This strategy of screening eliminates the need to adjust the significance level for the analysis done in the screening step since it is constructed to be statistically independent of the subsequent association tests.

Multivariate analyses using conditional logistic regression was then performed to estimate the odds ratios (ORs) for asthma and eczema attributable to each SNP while adjusting for relevant covariates under an additive genetic model using SAS software (SAS Institute, Inc., Cary, NC, USA). A false discovery rate (FDR) of $\alpha = 0.05$ was used to control for multiple testing by adjusting the p-values of the top 15 most powered SNPs.

Statistical analysis: Bayesian network creation

Bayesian networks were constructed using a ‘phenocentric’ search algorithm in which SNPs that directly modulate asthma or eczema were found. This set consisted of nodes with edges originating from the phenotype (i.e. children with asthma/eczema) and those with edges directed to children of the phenotype (i.e. parents of children of asthma/eczema). The search algorithm proceeded by greedily selecting SNPs associated with the phenotype based on Bayes factors comparing the likelihood that asthma/eczema was dependent vs. independent on each SNP, and, for each asthma/eczema child, greedily selected its parental SNPs based on Bayes factors comparing the likelihood that the new SNP should be connected vs. not connected to the child with the phenotype of interest. The final network returned is that with the highest likelihood of predicting asthma/eczema, given a set of SNPs, among the networks explored.

Statistical analysis: Bayesian network predictive validation

The predictive accuracy of the network was determined via a fivefold cross-validation in which each of five non-overlapping data subsets, obtained by randomly splitting the original dataset, was used as an independent dataset while the remaining four are used to learn the network dependencies. The probability of asthma/eczema given the genotype of an individual subject was calculated using the Clique algorithm implemented in Bayesware Discoverer (13). The predictive performance of the network was evaluated with receiver operating characteristic (ROC) curves calculated with both fitted values and cross-validation results. Predictive accuracy was measured as the area under the ROC curve (AUROC), and significance for this accuracy was obtained by comparing the classification ability of models obtained to random classification. The standard error (SE) for AUROCs and for the difference between AUROCs of two curves were estimated using the nonparametric asymptotic method (14).

Results

Population characteristics

The combined cohort consists of 117 non-Hispanic white asthma cases and 255 non-asthmatic controls with both DNA and phenotypic data available for analyses. The subset of subjects with DNA available from both the Boston Home Allergens and Asthma Study and the Connecticut Childhood Asthma Study used for the genetic analyses were similar to the overall population and to those individuals without DNA thereby limiting the concern for selection bias (See Tables S3 and S4). The frequency of eczema was relatively similar between the asthma cases (48%) and controls in the combined population (70%) ($p = 0.04$). No evidence of population stratification was detected in the combined cohort (p-value = 0.90). Baseline characteristics of the asthma cases and controls were similar except that asthmatic children were more likely to be boys, were more likely to have eczema, and were more likely to have a maternal history of asthma and eczema (Table 1). For example, 41% of asthmatic cases had asthmatic mothers whereas only 24% of controls had mothers with a history of asthma ($p < 0.05$). Furthermore, in concordance with previous epidemiological data, asthmatic children were more likely to have mothers with a history of eczema (32% cases vs. 22% controls, $p < 0.05$). Of note, 71% of asthmatics were diagnosed with eczema before the age of 6, which is significantly higher than in the controls ($p < 0.05$).

There were 205 children who had a diagnosis of eczema by the age of 6, and 167 non-Hispanic white children without eczema who were classified as controls. In these individuals the frequency of asthma was similar in cases (22%) and controls (40%) ($p = 0.30$). Children with and without eczema had similar baseline characteristics with respect to the proportion of boys, a maternal or paternal asthma history, history of day care attendance 7–12 months after birth, and pet ownership in the first year of life (Table 1). However, children with eczema were more likely to have a maternal and/or paternal history of eczema, and were less likely to have attended daycare during the first 6 months of life (26% vs. 35%) when compared to controls.

Associations with asthma

A total of 609 SNPs in 44 innate immune genes were genotyped in this combined cohort. After removal of SNPs with minor allele frequency <5%, and those out of Hardy–Weinberg equilibrium, 569 SNPs in 44 innate immune genes were analyzed. Using C2BAT, the top 15 SNPs with the highest NCP, a proxy for measuring effect size, were analyzed for association with asthma under an additive genetic model. A total of seven SNPs in six genes (*TGFB1*, *DEFB1*, *LY96*, *ACAA1*, *CARD15*, and *IFNG*) were associated with asthma after adjustment for multiple testing by FDR (Table 2). Of the seven SNPs, the minor alleles of

rs6957 (allele G in *TGFB1*), rs12980942 (allele C in *TGFB1*), rs5743404 (allele C in *DEFB1*), and rs5743291 (allele A in *CARD15*) were associated with increased asthma susceptibility, with ORs ranging from 1.57 (95% confidence interval [CI] [1.12–2.19], adjusted p = 0.01) for rs5743404 of *DEFB1* to 1.85 (CI [1.12–3.06], adjusted p-value = 0.02) for rs5743291 of *CARD15*. The minor alleles of rs156265 (*ACAA1*, allele G), rs16938758 (*LY96*, allele T) and rs2069718 (*IFNG*, allele T) were found to be protective against the development of asthma with ORs of 0.52 (CI [0.31, 0.87]), 0.55 (CI [0.34, 0.89]), and 0.69 (CI [0.50, 0.95]), respectively.

Associations with eczema

The C2BAT screening algorithm was used to select the 15 SNPs with the largest effect estimates for genetic association testing with eczema susceptibility. After adjustment for multiple comparisons, a total of five SNPs from three genes (*CD80*, *STAT4* and *IRAK2*) were significantly associated with eczema (Table 3). The minor alleles of variants rs7630595 (allele A in *CD80*), rs6808536 (allele T in *CD80*), rs13071247 (allele C in *CD80*), and rs263408 (allele C in *IRAK2*) were associated with an increased risk of eczema. The ORs ranged from 1.75 (CI [1.73–2.73]) for rs6808536 (*CD80)* to 2.44 (CI [1.46–4.07], adjusted $p = 7 \times 10^{-3}$) for rs7630595 (*CD80*). Of note, the minor allele of variant rs925847 (allele T in STAT4) conferred protection against the development of eczema (OR = 0.63 (CI [0.44, 0.91]), adjusted p-value = 0.01). Of the SNPs demonstrating an association with eczema, none were associated with asthma susceptibility.

Bayes networks of asthma and eczema

Bayesian network analysis for asthma susceptibility identified four SNPs in three genes NGFIA-binding protein 2 (*NAB2*), *KIAA0286*, and signal transducer, and activator of transcription (*STAT6*) that were predictive of asthma (Fig. 1). The AUROC for the asthma network, which was 0.55, was not significantly better than that of a random classifier (p = 0.065). Interestingly, none of the SNPs identified using this method was identified in the individual SNP association analysis with asthma in this population.

Bayesian network analysis for susceptibility to eczema identified 10 SNPs in three genes that were predictive of eczema (Fig. 2). Of the genes identified (*CD80*, *TLR10*, and *IL4R*), only *CD80* was associated with eczema susceptibility in the single-SNP association study. The AUROC for the eczema network, which was 0.58, was significantly better than that of a random classifier (p = 0.0085).

Discussion

The innate immune pathway regulates an individual's response to endotoxin exposure resulting in atopic sensitization and the development of diseases like asthma and eczema. Genetic polymorphisms in innate immune genes have been postulated to result in the variability in endotoxin response, leading to differences in susceptibility to atopic disease. Using a novel statistical method, we have not only shown that variants in innate immune genes are associated with asthma and eczema, but that the genetic underpinnings for these two diseases may be different with different genes showing an association with asthma (*TGFB1*, *DEFB1*, *LY96*, *ACAA1*, *CARD15*, and *IFNG*) than with eczema (*CD80*, *STAT4* and *IRAK2*). Moreover, we have shown that additional SNPs may influence disease susceptibility via SNP interactions.

Epidemiological data suggests that children with eczema are more likely to develop other atopic diseases like asthma, suggesting the possibility of a common etiology for these diseases. Using a prospectively studied cohort of 94 children with eczema, Gustafsson et al.

demonstrated that 43% of children with eczema develop asthma within the first 7 yr of life, and that children with a family history of atopy are also more likely to develop asthma during that time period (15). Although several advances in our understanding of the pathobiology of atopic diseases have been made, investigation of the genetic determinants of these diseases may allow us to elucidate novel molecular mechanisms involved in disease pathogenesis.

Many candidate gene studies investigating the genetic determinants of atopic diseases have focused on genes involved in epidermal and epithelial barrier function. Morar, et al. demonstrated that a barrier defect in both the skin and lung epithelium leads to overstimulation of immune cells by invading allergens suggesting a potential mechanism whereby patients with atopic dermatitis are more susceptible to the development of asthma (16). Given the preponderance of data implicating the innate immune system in the development of atopic disorders, we set out to test variants in genes in the innate immune pathway as candidates for the common genetic origins of asthma and eczema.

We demonstrate significant associations for genetic variants in *TGFB1*, *CARD15*, and *DEFB1* with increased asthma susceptibility. The *TGFB1* is a pleiotropic cytokine that has been implicated in the pathogenesis of asthma. Murine models have demonstrated that *TGFB1* regulates differentiation of naive T cells into proinflammatory T helper (Th) – 17 cells (18). Human studies have shown that there is increased *TGFB1* expression in bronchoalveolar lavage fluid of asthmatic subjects (19). However, candidate gene studies of *TGFB1* and asthma have been inconsistent. Although we demonstrate significant associations of three SNPs in the *TGFB1* gene with asthma in this cohort, previous work from our group did not show associations of variants in *TGFB1* with asthma susceptibility in two other childhood asthma cohorts (20). These discrepancies may be explained by differences in either the ascertainment of subjects, study design (i.e. family-based vs. population-based), or environmental exposures of the cohorts.

We also demonstrate significant associations of SNP rs5743404 in *DEFB1* and rs5743291 in *CARD15* (also called *NOD2*) with asthma. Both *DEFB1* and *CARD15* are expressed on the epithelial surface of the respiratory tract, and have been found to play a role in an individual's resistance to infectious pathogens (21). Both genes have been found to mediate innate and adaptive immune response. Variants of *DEFB1* and CARD15 have been previously associated with asthma in several other populations (22). We also show a significant association between rs156265 in the *ACAA1* gene, located upstream of *MYD88* in a potential regulatory region, and decreased asthma risk. The *MYD88* is an adaptor molecule involved in innate immune signaling pathways that promotes Th1 response. Of note, although these genes have been implicated in asthma susceptibility in our study, they are not associated with increased susceptibility to eczema in our population.

We identified variants in three genes that were significantly associated with eczema. Three SNPs in the *CD80* gene (rs7630595, rs6808536, rs13071247) were associated with increased eczema susceptibility. A meta-analysis of previous linkage studies of eczema demonstrates evidence of linkage on chromosome 3 in the region of the *CD80* gene (23). We also identified rs263408 in the gene for Inerleukin-1 receptor associated kinase 2 (*IRAK-2*) with eczema susceptibility. The *IRAK2* is critical to TLR/IL1R signaling, as is has been shown to associate with the IL-1R complex leading to activation of NF-kB (24). In addition, we have identified a variant in *STAT4* that was also associated with eczema in this population. The STAT proteins are a family of transcription factors that are activated in response to a variety of cytokines. The *STAT4* is expressed in peripheral blood monocytes, dendritic cells, and macrophages at sites of inflammation, and mediates IL-12 signaling that is critical for host protection against infection (25).

Using single-SNP association analysis, we were unable to confirm previous associations of innate immunity genes (*CD14*, *TLR2*, and *TLR4*) with asthma or eczema. Although we have previously reported that a SNP in the promoter region of CD14 was associated with eczema before the age of 2 yr in our population, this association did not reach statistical significance at 6 yr of age in our current analysis. Whereas it is possible that the lack of association with these genes was due differences in the populations tested, it is also possible that other genes in the innate immune pathway are more important than the previously studied genes. To date, our study is the most comprehensive interrogation of this pathway, as most previous studies have investigated variants in only one or two genes at a time. Recently, Riejmerink et al. investigated 169 SNPs in 29 TLR pathway-related genes in a large sample of Dutch children (26). Although they did not find significant single-SNP associations with asthma, they did find significant gene-by-gene interactions amongst the variants tested. Although their findings underscore the fact that different genes may be identified in different populations due to varying environmental influences, it also demonstrates the importance of gene-by-gene interactions in disease pathogenesis. As with the Riejmerink study, our Bayes network analysis identified SNPs that did not have an effect on either phenotype on their own, but were identified only in the context of other SNPs. In addition, the Riejmerink study only investigated 2- and 3-SNP interaction models, whereas our analysis took into account all the genotyped SNPs.

Although single-SNP associations with disease susceptibility account for a small proportion of the heritability of complex diseases like asthma and eczema, elucidation of gene-by-gene interactions can also enhance our understanding of the underlying biologic mechanisms of disease. To investigate the effects of multiple innate immune pathway genetic variants on disease susceptibility simultaneously, we developed Bayesian networks to predict asthma and eczema status. The Bayesian network model for asthma prediction suggests interesting interactions among genes in the innate immune pathway. For example, two SNPs in the *STAT-6* gene appear to interact with a transmembrane gene *KIAA0286* whose function is not yet known. In a murine model of airway disease *STAT6* regulation was associated with airway goblet cell hyperplasia and increased airway hyperresponsiveness (27), two of the pathological hallmarks of asthma. The *NAB2*, which also interacts with the transmembrane protein *KIAA0286* in our asthma model, has been shown to modulate the vascular endothelial growth factor (*VEGF*) pathway. We and others have previously shown that the *VEGF* pathway is implicated in asthma pathogenesis and airway remodeling that occurs in persistent asthmatics (28). These gene relationships, which could not be identified by our single-SNP analysis, are interesting biologic candidates for follow-up investigation. Of note, our asthma network does not meet strict criteria for statistical significance ($p = 0.06$), which may be explained by our small sample size, interactions between genes outside of the innate immune pathway, and possible gene-by-environment interactions that were not modeled in our current analysis. In spite of these limitations, the gene-by-gene interactions suggested herein warrant furthermore investigation for their involvement in asthma pathogenesis.

Several genes were found to predict eczema susceptibility in our population. In addition, the eczema Bayesian network predicted eczema better than a random classifier ($p = 0.0085$). In addition to the individual SNP associations found in the *CD80* gene with eczema, this gene was also identified as an integral part of the eczema network. The *CD80* positive cells with the characteristics features of Langerhans cells (LC) were identified in skin lesions of individuals with atopic dermatitis (29), suggesting a role for these cells in chronic inflammation and the development of eczema. In addition, investigations in several pediatric asthma populations have found that *IL4R* and *TLR10* variants are associated with asthma susceptibility (30), but to date we are not aware of any reported associations of *TLR10* or *IL4R* with susceptibility to eczema. Interestingly, our single-SNP associations did not

identify either of these genes as being associated with eczema, again demonstrating that multivariate models can offer novel insights in the analysis of complex diseases.

In the current study, we demonstrate genetic association of variants in *TGFB1*, *CARD15*, and *DEFB1* with increased asthma susceptibility. In addition, we demonstrate that variants in *CD80*, *STAT4*, and *IRAK2* are associated with susceptibility to eczema. Moreover, we demonstrate how genetic variants in others gene interact to increase susceptibility to these diseases. The strengths of our study include a comprehensive genetic analysis of the innate immune pathway in a phenotypically well-characterized cohort. Furthermore, we utilize C2BAT a novel statistical methodology that allows for population-based single-SNP association testing and replication within the same cohort. The C2BAT is a powerful statistical methodology that can be used for genetic association studies in the future. Previous studies investigating the role of the innate immune pathway in asthma and eczema have resulted in conflicting results in part due to possible gene-gene interactions that may result in disease susceptibility. To our knowledge, we are the first to use a Bayesian network analysis to investigate possible epistatic interactions in genetic variants in the innate immune pathway that result in increased asthma and eczema susceptibility.

Our current study suffers from some limitations. First, in spite of combining two well-characterized asthma and allergy cohorts, our sample size remains relatively small. However, in this study we used C2BAT, which allows us to screen and test SNPs in the same population. Since C2BAT does not bias the nominal statistics, allowing for screening and testing in the same cohort, it allows for an overall reduction in the number of statistical tests performed. Therefore, C2BAT is a powerful method that can be implemented in population-based genetic association studies to reduce the chance of false positive results. Replication of our current results in independent populations would increase the likelihood that the statistical associations represent true biological differences. A recent study suggests that environmental exposures may modify the effect of genetic variants in the innate immune pathway on disease susceptibility (31). We were unable to investigate the effect of common environmental exposures on genetic associations with asthma and eczema in innate immune pathway genes, but analysis of gene-by-environment interactions could further our understanding of the role of the innate immune genes in the pathogenesis of atopic disorders. In addition, we recognize that some of the genetic determinants of asthma and eczema may not have been identified in the current analysis due to the population prevalence of eczema in the asthma cases and controls and the prevalence of asthma in the eczema cases and controls. Given the small sample size of our population, we were unable to perform an analysis of asthma or eczema alone. Although the prevalence of atopic disease was similar in cases and controls, we performed a sensitivity analysis for the asthma and eczema single-SNP associations where asthmatics without eczema were defined as cases and subjects with eczema as controls which demonstrated similar results to our initial model (Table S5). We conducted an additional sensitivity analysis for the eczema association that included eczema cases without asthma vs. controls without eczema or asthma (none of the subjects in this subset had asthma). These results of the sensitivity analysis for eczema (Table S6) also showed ORs with corresponding p values that were very similar to the original analysis of genetic polymorphisms and eczema.

Conclusion

In conclusion, we have found that different innate immunity genes are implicated in the pathogenesis of asthma and eczema. In addition, there are differences among the potential interactions between variants in innate immune genes. Furthermore investigation is necessary to fully understand the role of the innate immune genes in atopic disease susceptibility.

Using novel methodology that allows for screening and replication in the same population, we have identified associations of innate immune genes with asthma and eczema. Bayesian network analysis suggested that additional SNPs influence disease susceptibility via SNP interactions. Our findings suggest that innate immune genes contribute to the pathogenesis of asthma and eczema, and that these diseases likely have different genetic determinants.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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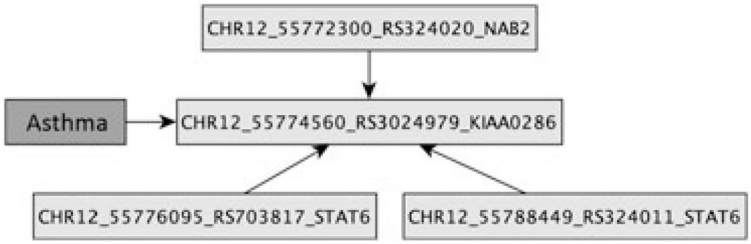

Fig. 1.

Bayesian network for asthma susceptibility. Network of genes identified from the innate immune pathway for asthma susceptibility. The area under the receiver operating characteristic curve for the asthma network, which was 0.55, was not significantly better than that of a random classifier ($p = 0.065$).

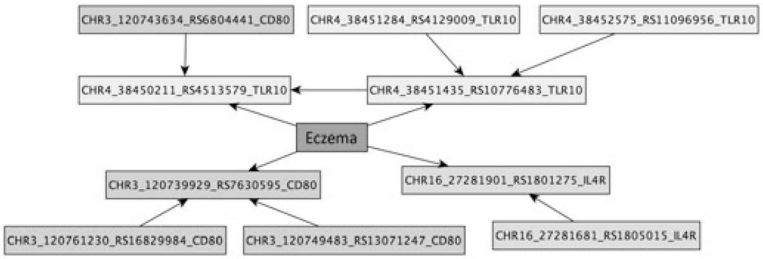

Fig. 2.

Bayesian network for eczema susceptibility. Network of genes in the innate immune pathway identified for susceptibility to eczema. The area under the receiver operating characteristic curve for the eczema network, which was 0.58, was significantly better than that of a random classifier ($p = 0.0085$).

Table 1

Baseline characteristics of study subjects

Variable	Asthma cases	Asthma controls	Eczema cases	Eczema controls
Subjects (with DNA)	117	255	205	153
Gender (male) *	70/117 (0.60)	130/254 (0.51)	117/205	77/153
Asthma diagnosis	117/117	0/255	82/205	33/153
Eczema diagnosis	82/117	123/255	205/205	0/153
Asthmatic mother *	48/117 (0.41)	60/254 (0.24)	67/205	36/153
Asthmatic father	24/114 (0.21)	39/253 (0.16)	45/202	18/152
Mother with eczema *†	37/114 (0.32)	56/251 (0.22)	61/201 (0.30)	31/152 (0.20)
Father with eczema †	15/113 (0.13)	31/246 (0.09)	33/199 (0.17)	11/149 (0.07)
Attended day care for the first 6 months of life †	34/116 (0.29)	73/253 (0.29)	53/205 (0.26)	54/153 (0.35)
Attended day care between months 7–12 after birth	45/116 (0.39)	92/253 (0.36)	71/205	64/153
Dog in home – year 1	33/117 (0.28)	74/255 (0.29)	53/176	50/134
Cat in the home – year 1	65/117 (0.56)	77/255 (0.30)	68/180	42/129
Eczema diagnosis before age 6 *	82/115 (0.71)	123/234 (0.53)		

* Covariate varies significantly between asthma cases and asthma controls ($p < 0.05$).

† Covariate varies significantly between eczema cases and eczema controls ($p < 0.05$).

Table 2

Asthma associations

Gene	SNP	Location of SNP	Base change	Minor allele frequency	Case genotype frequencies (AA, Aa, aa)	Control genotype frequencies (AA, Aa, aa)	OR	95% CI	Adjusted p-value
TGFB1	rs6957	5′ UTR	A>G	0.18	69 (0.61), 37 (0.32), 8 (0.07)	175 (0.73), 55 (0.23), 9 (0.04)	1.8	1.21–2.67	0.0037
DEFB1	rs5743404	Promoter	T>C	0.38	35 (0.30), 57 (0.50), 23 (0.20)	104 (0.42), 115 (0.47), 28 (0.11)	1.57	1.12–2.19	0.0083
TGFB1	rs12980942	5′ UTR	G>A	0.15	74 (0.66), 32 (0.29), 6 (0.05)	184 (0.76), 54 (0.22), 5 (0.02)	1.78	1.16–2.74	0.0086
ACAA1	rs156265	Intron	C>G	0.15	91 (0.82), 19 (0.17), 1 (0.01)	164 (0.70), 64 (0.27), 2 (0.03)	0.52	0.31–0.87	0.0123
LY96	rs16938758	Intron	A>T	0.17	88 (0.77), 27 (0.23), 0 (0.00)	159 (0.65), 79 (0.32), 8 (0.03)	0.55	0.34–0.89	0.0141
CARD15	rs5743291	Exon	G>A	0.10	86 (0.76), 23 (0.20), 4 (0.04)	205 (0.84), 37 (0.15), 2 (0.01)	1.85	1.12–3.06	0.0158
IFNG	rs2069718	Intron	C>T	0.42	51 (0.45), 40 (0.36), 21 (0.19)	75 (0.31), 120 (0.49), 49 (0.20)	0.69	0.50–0.95	0.0216

CI, confidence interval; OR, odds ratio; SNP, single nucleotide polymorphism.

Table 3

Eczema associations

Gene	SNP	Location of SNP	Base change	Minor allele frequency	OR	95% CI	Adjusted p-value
CD80	rs7630595	Intron	G>A	0.13	2.436	1.46–4.07	0.0007
CD80	rs6808536	Intron	G>T	0.17	1.749	1.13–2.70	0.0118
CD80	rs13071247	Intron	A>C	0.16	1.753	1.13–2.72	0.0129
STAT4	rs925847	Intron	C>T	0.28	0.633	0.44–0.91	0.0137
IRAK2	rs263408	Intron	T>C	0.1	1.966	1.13–3.41	0.0166

CI, confidence interval; OR, odds ratio; SNP, single nucleotide polymorphism.