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Search for quantitative trait loci of atopy-associated immune responses using allergen-specific IgG1 as an “endophenotype”

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Summary Inherited atopic diseases of humans arise from adverse adaptive humoral responses to noninfectious environmental allergens. We previously reported that allergen-specific IgG1 provides more reliable heritability estimates for responses to allergens than total IgE. Genome scans were done for 91 Caucasian nuclear families with history of atopy for total IgE and IgG1 produced against a common major allergen from house dust mite, Der p 1. Suggestive associations for Der p 1-IgG1 production were found at 7 quantitative trait loci (QTL) (logarithm of the odds, $\text{LOD} \geq 1.23$; $p \leq 0.009$) with QTL-specific heritabilities of 73%–80%. Scans using total IgE found suggestive associations for 12 QTLs ($\text{LOD} \geq 1.44$; $p \leq 0.004$), but QTL-specific heritabilities only in the range of 30%–35%. Allergen-specific IgG1 is a suitable “endophenotype” to be used in searches for genes associated with atopy-associated humoral immune responses to common aeroallergens.

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Introduction

Atopic disorders, such as allergic rhinitis or atopic bronchial asthma, are common inherited diseases that result from adverse immune system responses to noninfectious environmental allergens. They are generally characterized by elevated serum total IgE levels or evidence for the production of allergen-specific IgE manifested by positive skin prick test

(SPT) results. However, genome scans using these phenotypes demonstrated numerous chromosomal regions putatively associated with these traits, but there was no clear consensus [1].

Numerous metabolic pathways contribute to the production of IgE [2] that can be influenced by numerous environmental factors, such as history of infectious disease, environmental pollution levels, and population health practices. The incidence of atopic disease varies widely in different geographic locales due to the impact of these factors [3]. Quantitative IgE traits associated with atopy show a great

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ABBREVIATIONS

Der p	house dust mite allergen (<i>Dermataphagoides pteronyssinus</i>)
LOD	logarithm of the odds
QTL	quantitative trait locus
SPT	(percutaneous) skin prick test

amount of phenotypic variability, which probably accounts for much of the observed variation observed in genome scan results [1].

Production of allergen-specific IgE is the result of an adaptive, memory-forming humoral immune response to noninfectious environmental antigens (allergens). With the exception of specific IgE production, it is equally likely for anyone to mount a T-cell-dependent humoral response to allergens involving varying amounts of the IgA and IgG isotype subclasses [4,5]. Of special note, specific IgG1 is elevated among individuals with atopic sensitization to a particular allergen [6].

In this journal, we previously demonstrated, among nuclear families with medical histories of atopic diseases, that the total narrow sense heritability of serum total IgE was about 48% [7]. However, this estimate was significantly influenced by age, gender, and atopic clinical status. In contrast, using the major Der p 1 allergen from house dust mite the total narrow sense heritability of atopy-associated Der p 1-IgG1 responsiveness was about 62%. Importantly, this estimate was not significantly influenced by age, gender, or overall atopic status [7]. Thus, for gene searches for atopy-associated immune response, allergen-specific IgG1 may be a suitable “endophenotype” [8].

We conducted genome scans in these families using Der p 1-IgG1 as an endophenotype and compared them with results for total IgE. As with the heritability estimates [7], the results suggest that allergen-specific IgG1 is a more robust phenotype for conducting gene searches for these complex human immune system responses to common aeroallergens.

Methods

Study population

Caucasian families residing in the Minneapolis/St. Paul, Minnesota, metropolitan area that included two children with a physician's diagnosis of asthma were recruited through media advertisements as part of studies for the genetics of asthma. We evaluated 367 people from 91 nuclear families that included both parents, the asthmatic children, and their siblings. A total of 169 parents (mean age = 43.7 years; range = 22-75 years) and 198 children (mean age = 14.9 years; range = 6-42 years) were screened. There were 176 females and 191 males. Each person, or legal guardian, provided written, informed consent according to the guidelines of the Institutional Review Board of the University of Minnesota.

SPT

Skin prick testing was done as previously described [9] using 14 standardized allergen extracts for dust mites (2), including Der p allergen extract, ragweed, molds (3), trees (4), grasses (2), and animal danders (2). A positive SPT result was recorded for a wheal area ≥ 9 mm² above negative saline control.

Der p 1 allergen

The major allergen Der p 1 from house dust mite was purified by methods previously described [10]. We chose Der p 1 allergen as a model, because we previously demonstrated that the atopic response to any particular allergen was not obligately inherited. Rather, it is a random variable independent of any family history of atopic allergen sensitivity [11,12]. Der p 1 is a common nonseasonal indoor allergen that induced the highest incidence of atopic sensitization among all of the common indoor allergens tested in the SPT screens.

Serum antibody tests

Serum total IgE tests were done using the ACCESS Immunoassay System (Beckman Coulter, Chaska, MN, USA), according to the manufacturer's specifications, with results reported in international units per milliliter (1 IU = 2.4 ng). Measures of Der p 1-specific IgG1 were performed using modifications of “reverse sandwich” ELISA assays previously described [7]. This assay gave a linear response on a log-log scale over the range of 0.05 to 100 ng/ml.

Genotyping

DNA was isolated from peripheral blood white cells by standard methods [9]. DNA samples were genotyped using a Weber Screening Set 6 composed of 360 markers whose positions spanned all chromosomes ($\approx$ 10-cM spacing) by the Marshfield Center for Medical Genetics, Marshfield, Wisconsin, USA (<http://research.marshfieldclinic.org/genetics>).

Genome scans

Variance components linkage methods were employed to account for pedigrees of arbitrary size and complexity [13]. This approach is based upon specifying the expected genetic covariances between relatives as a function of their identity-by-descent (IBD) relationships at a quantitative trait locus (QTL). The QTL mapping procedure was implemented in two phases: (1) estimation of IBD probabilities for all pairs of relatives in all pedigrees at all chromosomal positions conditional on their marker genotypes, followed by (2) estimation of effects of loci by correlating genetic IBD similarity at those loci for pairs of relatives with the phenotypic similarity of those relatives. IBD and variance component estimations were generated with the software program MERLIN [14] using the supercomputer facilities of the Minnesota Supercomputing Institute.

Table 1 Genome scan results

CHR	Position (cM)	Der p 1-IgG1			Total IgE		
		LOD	QTL-H2 ^a	<i>p</i> value	LOD	QTL-H2	<i>p</i> value
1	13.4	—			2.46	0.35	0.0004
1	156.7	2.06	0.80	0.001	1.54	0.33	0.004
2	84.3	1.23	0.73	0.009	—		
2	252.1				2.05	0.34	0.0011
3	115.9	—			2.52	0.37	0.0003
4	51.2	1.31	0.78	0.007	—		
4	100.4	—			1.91	0.38	0.002
7	119.8	—			1.44	0.33	0.005
7	193.6	1.61	0.77	0.003	—		
8	72.5	1.43	0.73	0.005	1.56	0.35	0.004
10	113.6	—			2.65	0.37	0.0002
12	150.5	—			2.45	0.35	0.0004
13	97.9	—			2.37	0.35	0.0005
16	36.0	—			2.75	0.36	0.0002
17	112.1	1.31	0.79	0.007	—		
20	10.6	—			3.04	0.40	0.00009
20	90.4	1.39	0.74	0.006	—		

^a QTL-H2, quantitative trait locus-specific heritability estimate.

Results

Clinical characteristics

A total of 367 individuals (176 female, 191 male) from 91 Caucasian nuclear families with a history of atopic asthma were screened. Using a battery of 14 common aeroallergens, there was a high proportion of skin prick test positive (SPT [+]) response: 262/367 (71%). Among those with any SPT [+] result, 127/262 (48.5%) were SPT [+] for the house dust mite Der p allergen. The study population showed significant atopic sensitivity to common aeroallergens and to dust mite (Der p) allergen in particular. (Additional clinical characteristics were previously reported [7].)

Allergen-specific IgG1 as an endophenotype

As expected, there was a significant positive correlation between serum total IgE values and the number of SPT [+] results: $r = 0.56$; $t(366) = 12.8$; $p \ll 0.001$. However, as previously reported, total IgE values varied significantly due to age and gender [7]. Also, total IgE values were elevated among those with any SPT [+] result and were highest among those with any SPT [+] who also had asthma (Table 1 in [7]). IgE levels are influenced both by allergen sensitivity and by asthma-associated inflammation.

In contrast, allergen-specific IgG1 was not significantly influenced by these variables. Using the major dust mite allergen Der p 1 as a model, we also reported for this study population that Der p 1-IgG1 values are not influenced by age, gender, or asthmatic respiratory involvement [7]. Der p 1-IgG1 values are, however, elevated among those who are also sensitized to the Der p allergen extract (Figure 1 in [7]). As reported, for those who were SPT [+] for the Der p allergen extract, the average log-10 value for Der p 1-IgG1 was

−0.64 (SD = 0.51; $N = 127$). For those who were SPT [−] for the Der p extract, average log-10 Der p 1-IgG1 = −0.80 (SD = 0.25; $N = 240$). By two-sided t test, these values were significantly different: $t(365) = 3.17$; $p = 0.0008$.

There was no significant correlation between Log [Total IgE] and Log [Der p 1-IgG1] values: $r = 0.09$; $t(366) = 1.26$; $p = 0.21$. There was, however, a slight although statistically significant correlation between Log [Der p 1-IgG1] values and the number of SPT [+] results: $r = 0.15$; $t(366) = 2.91$; $p = 0.004$. This result may have been largely due to the high proportion of people who were SPT [+] for the Der p allergen in this study population.

Der p 1-IgG1 is a suitable endophenotype for gene searches for atopy-associated immune responses to aeroallergens [8]; it is not influenced by age or gender effects, and it is not elevated due to asthmatic respiratory involvement, but it is elevated among those with established atopic sensitivity to allergen(s).

Comparisons for total IgE and Der p 1-IgG1 of gene scan results for QTL

Whole genome scans were done using 360 polymorphic markers comprising Weber Screening Set 6, with average inter-marker distance of ≈ 10 cM. Searches were done for QTL on all chromosomes for serum total IgE values and allergen-specific IgG1 using house dust mite Der p 1-IgG1 as a model. Screen results were conducted using the computer software MERLIN [14], which provided best estimates for the logarithm of the odds for association (LOD Score), its associated QTL-specific heritability, (QTL-H2) and the p value for these estimates. Table 1 shows the results of these scans.

Overall, seven QTLs were identified with suggestive association to Der p 1-IgG1 production (LOD ≥ 1.23 ; $p \leq 0.009$). Of interest, the QTL-specific heritability estimates for these

loci were in the range of 73% to 80%. We previously reported for this study population that the total narrow sense heritability for Der p 1-IgG1 was 61.5% [7].

Using total IgE values provided suggestive evidence at 12 QTLs ($\text{LOD} \geq 1.44$; $p \leq 0.004$). For comparison, the QTL LOD scores for total IgE were generally higher than that of Der p 1-IgG1. However, the QTL-specific heritability estimates for the IgE scans (30%-35%) suggest that these results were less robust. This was previously suggested by the total narrow sense heritability estimate for total IgE of 47.8% that was significantly influenced by age, gender, and clinical status effects [7].

Although the results are not shown, we also did gene scans using the number of SPT [+] results as a quantitative trait. No LOD score higher than 0.74 ($p = 0.03$; $\text{QTL-H}^2 = 31\%$) was found. These results are consistent with previous findings that the number of SPT [+] results is a random variable whose quantitative expressions are not an inherited trait [11,12]. In contrast, Der p 1-IgG1 production is evident in all the subjects studied and is a marker associated with an immune response to a well-defined allergen known to be associated with atopy.

Discussion

Searches for chromosomal loci associated with the complex human atopic diseases produced widely conflicting results, with numerous loci putatively associated with disease-related traits such as serum total IgE levels or SPT results [1]. These studies assumed that the total phenotype expression was a simple summation of the contributions of multiple underlying causative elements (multiple gene effects). Yet, this strategy may overlook the behavior of the whole (complex) system that is influenced both by multiple genes as well as significant nongenetic, random environmental effects [15].

The choice of a particular phenotypic trait to be used in genome-wide scanning should be reflective of underlying physiological processes that are common to all, but are only abnormal in the advent of a disease [8]. In the case of the atopic diseases associated with immune system sensitivity to common, noninfectious environmental aeroallergens, it has been implicitly assumed that serum total IgE levels is such a trait. However, we previously argued that serum total IgE may actually comprise at least two physiologically distinct components [16].

One is a basal component unrelated to atopic disorders, whereas a second arises from the production of allergen-specific IgE. A simple manifestation of increased levels of this fraction is reflected by an increased number of SPT [+] results [16]. Each SPT [+] result represents a unique "clone" of lymphoid cells that respond specifically to and produce IgE against a particular allergen. But, the number and specific allergens to which people respond are random variables independent of any "inherited propensity" to adversely respond to aeroallergens [11,12].

Further, not only is serum total IgE elevated among those who have any SPT [+] result [16], but also it is higher still among those who are SPT [+] and have asthma [12]. This resulted from the observation that among those with any SPT [+] result, the average number of SPT [+] results was significantly higher among individuals with asthma compared to

those without asthma. The allergen-specific IgE fraction is influenced both by sensitivity to allergens and by asthmatic inflammatory processes. Total IgE is not a robust phenotype to be used for gene scans related to atopic immune responses to allergens.

This is borne out in the results shown in Table 1. As with many previous studies [1], a number of QTL show suggestive associations to IgE production. Yet, on average, the estimates for QTL-specific heritability are, at best, modest (30%-35%). This may have arisen from the inability to fully account for the several distributions of IgE that could arise.

There are numerous metabolic networks that can contribute to IgE production, although their complex interactions are not well understood [2]. From *in vivo* [17] and *in vitro* [18,19] studies, isotype class switching to IgE is far less common than a switch to IgG production. These results were derived from IgG or IgE production under identical experimental conditions. The principle difference was the total time until the appearance of these isotypes, with IgG appearing far sooner than IgE. Both IgG and IgE production share common metabolic pathways leading to their production, but in probabilistic terms it is far more likely for an IgG response to be manifest in the presence of a defined antigen than is IgE.

Allergen-specific IgG production is probably more common than is specific IgE, as independently observed by our group [6] and others [4, 5]. In particular, allergen-specific IgG1 is elevated among those who are also atopically sensitized to a particular allergen, like ragweed pollen [6] or house dust mite [7]. The production of allergen-specific IgG1 may not be atopic disease associated, but it is associated with immune system humoral response development to antigens known to be atopy associated. Further, as a quantitative trait for use in genome scans, specific IgG1 is more robust than total or specific IgE because it is not significantly influenced by age, gender, or overall atopic clinical status, and it is a reasonably well-defined immunological response to a well-defined source of antigenic stimulation [7].

Thus, allergen-specific IgG1 is an endophenotype [8] that appears to provide more reliable information from gene scans than do the IgE traits. This is borne out by the results in Table 1. Although we cannot say with certainty that the QTLs identified are linked to specific IgG1 production, it does appear that the results from the scan for this trait are more reliable than those for IgE, because the QTL-specific heritability estimates for specific IgG1 are significantly higher (73%-80%) compared with those for total IgE (30%-35%).

The complex atopic diseases of humans, like allergic rhinitis or bronchial asthma, are largely due to enhanced immune system responsiveness to otherwise biologically benign, noninfectious environmental allergens. Aside from disease manifestation, the atopic and nonatopic immune responses may have more in common than are disparate, including vigorous humoral responses to these antigens [6,20]. Before the genes associated with atopic disease can be found, it may be best to first unravel the genetic components involved with immune responses to these unique types of antigens.

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