Mouse Chromosome 4 Is Associated with the Baseline and Allergic IgE Phenotypes

Cynthia Kanagaratham,* Pierre Camateros,† John Ren,‡‡ Robert Sladek,*‡†† Silvia M. Vidal,*‡‡ and Danuta Radzioch*‡†

*Department of Human Genetics, †Division of Experimental Medicine, Department of Medicine, Faculty of Medicine, §Department of Microbiology and Immunology, **Institute of Parasitology, and ††Genome Quebec Innovation Centre, McGill University, Montreal, Quebec, H3A 1B1, Canada

ABSTRACT Regulation of IgE concentration in the blood is a complex trait, with high concentrations associated with parasitic infections as well as allergic diseases. A/J strain mice have significantly higher plasma concentrations of IgE, both at baseline and after ovalbumin antigen exposure, when compared to C57BL/6J strain mice. Our objective was to determine the genomic regions associated with this difference in phenotype. To achieve this, we used a panel of recombinant congenic strains (RCS) derived from A/J and C57BL/6J strains. We measured IgE in the RCS panel at baseline and following allergen exposure. Using marker by marker analysis of the RCS genotype and phenotype data, we identified multiple regions associated with the IgE phenotype. A single region was identified to be associated with baseline IgE level, while multiple regions were associated with the phenotype after allergen exposure. The most significant region was found on Chromosome 4, from 81.46 to 86.17 Mbp. Chromosome 4 substitution strain mice had significantly higher concentration of IgE than their background parental strain mice, C57BL/6J. Our data presents multiple candidate regions associated with plasma IgE concentration at baseline and following allergen exposure, with the most significant one located on Chromosome 4.

Plasma IgE antibodies are commonly associated with allergic disorders, and are often elevated in patients with allergic asthma (Jackola et al. 2004). Epidemiological studies have shown that elevated plasma IgE level is a risk factor for asthma (Ishizaka and Ishizaka 1971), and results from family and twin studies indicate that regulation of circulating IgE level is a risk factor for asthma (Ishizaka and Ishizaka 1971), and results from family and twin studies indicate that regulation of circulating IgE level is largely genetically determined (Bazaral et al. 1971). Best fitting model studies have attributed several possible modes of inheritance for the phenotype including polygenic and recessive, dominant and co-dominant (Gerrard et al. 1978; Meyers et al. 1982, 1991; Hasstedt et al. 1983; Martinez et al. 1994; Dizier et al. 1995). Linkage and genome wide association studies in humans have found associations between serum concentrations of IgE and various loci, with the most replicated loci being STAT6 (on Chromosome 12) and IL-13 (on Chromosome 5) (Vercelli 2008; Potaczek and Kabesch 2012).

Few genetic studies for IgE levels have been done using animal models, and they were focused on the IgE concentration after exposure to parasites (Badalova et al. 2002; Lipoldova et al. 2002; Menge et al. 2003). Examples include association studies for the IgE phenotype in response to Leishmania major infection in a BALB/c × STS/A recombinant congenic panel (Lipoldova et al. 2000; Badalova et al. 2002), in response to Heligmosomoides polygyrus resistance in a CBA × SWR strain cross (Menge et al. 2003), and in response to ovalbumin allergen sensitization and challenge in an SM/J × A/J recombinant inbred strain panel (Ohno et al. 2013). These studies all investigated the IgE phenotype following antigen exposure. We believe that studying the genetic signature of baseline levels of IgE (prior to allergen exposure), and how the signature is changed following allergen exposure, can provide valuable information about the innate predisposition of expressing heightened levels of IgE, and the modulation of IgE levels following allergen exposure. This would help determine if an allergy susceptibility region can be identified even before allergen exposure. In humans, baseline levels of IgE have been shown to be a predictor of long-term disease outcome, such as in
patients with atopic dermatitis (Kiiski et al. 2015). Furthermore, it has been shown in certain populations that sensitization to environmental allergens, increasing baseline levels of plasma IgE, is a risk factor for the development of asthma, other allergic disorders, and lung infections (Leung et al. 1997; Skaaby et al. 2017).

The present study is aimed at identifying regions associated with IgE concentration at baseline, following allergen sensitization, and following allergen sensitization and challenge. To achieve this, we used an AcB/BcA panel of recombinant congenic strains (RCS), and an ovalbumin induced model of allergic asthma. The AcB/BcA panel of RCS was derived from the parental strains A/J and C57BL/6J, two strains with significantly differing IgE phenotypes. To date, these strains have been used in studies on topics such as resistance to infectious diseases like influenza and Salmonella, psychiatric disorders and addiction, and lung responsiveness (Gill and Boyle 2005; Roy et al. 2006; Camateros et al. 2010; Boivin et al. 2012). Each RCS is fully inbred, and composed of ~12.5% of genetic material from one parental strain (minor genetic donor) and ~87.5% from the other (major genetic donor) (Fortin et al. 2001). Here, we use this panel to identify the genomic regions associated with levels of circulating IgE at baseline, and after antigen exposure.

**MATERIALS AND METHODS**

**Mice**

Breeding pairs for A/J, C57BL/6J, C57BL/6J-Chr 4A/J/Nal (CSS4), and C57BL/6J-Chr 12A/J/Nal (CSS12) mice were purchased from Jackson Laboratories (Boston, MA). The AcB/BcA panel of RCS were generated at the Montreal General Hospital Research Institute from A/J and C57BL/6J mice (Fortin et al. 2001). All mice were bred and housed at the Montreal General Hospital Research Institute animal facility in specific pathogen-free conditions with a 12-hr light/dark cycle. Animals were housed at a maximum of five animals per cage, and had ad libitum access to food and water. Only male mice aged 9–12 wk were used in all experiments. Animal protocols were approved by the Montreal General Hospital Facility Animal Care Committee, and were in compliance with the regulations set by the Canadian Council for Animal Care.

**Allergic model and sample collection**

To generate the allergic model, mice were sensitized by three weekly intraperitoneal injections of 100 μg of ovalbumin (Sigma) adsorbed to 1.5 mg of aluminum hydroxide (Imject Alum, Pierce), in a total volume of 0.2 ml phosphate buffered saline (PBS). At 1 wk following the third sensitization, animals were aerosol challenged for 3 d consecutively with a 30-min exposure to either 1% ovalbumin solution or PBS. At 3 hr after the final challenge, mice were killed by CO2 exposure, and blood was collected by cardiac puncture in EDTA-coated tubes. Blood samples were spun at 3000 rpm for 7 min at 4°C to isolate the plasma.

**IgE measurements**

Total IgE in the plasma was measured by enzyme-linked immunosorbent assay (ELISA) using the mouse BD OptEIA kit (BD Biosciences) following the manufacturer’s instructions.

**Identification of significantly associated regions**

A marker-by-marker analysis was conducted to identify associations between genotype and phenotype, as previously described (Camateros et al. 2010). Each phenotype was analyzed individually using log2-transformed phenotype data, and an established list of 1215 single nucleotide polymorphism (SNP) and microsatellite markers (Boivin et al. 2012). The analysis was done using the statistical software R, version 3.3.2. Manhattan plots were produced using the qman package for R (Turner 2014).

**Statistical analysis**

Unless otherwise specified, data were analyzed by GraphPad version 5.03 (GraphPad Software Inc.). One-way ANOVA followed by a Dunnet’s post-test procedure was used to compare each RCS to its respective major parental strains. All other comparisons between three groups was done by one-way ANOVA followed by Bonferroni post-test. P values ≤0.05 were considered to be significant.

**Data availability**

Supplemental Material, File S1, contains the phenotyping data of the RCS and parental strains. Genotyping marker ID and location, along with genotype for each strain, has been previously published (Boivin et al. 2012, File S1).

**RESULTS**

**IgE phenotype in RCS panel with increased antigen exposure**

Previous studies have demonstrated that A/J and C57BL/6J strains of mice have significantly different levels of plasma IgE concentrations (Figure 1) (Moisan et al. 2006). Our goal was to uncover the genetic determinants that may cause this difference between these two strains, both at baseline and after antigen exposure. To achieve this, plasma IgE concentration was measured from each of the 10 AcB and 21 BcA strains of the RCS panel derived from the A/J and C57BL/6J parental strains. IgE was measured in all strains at baseline (Figure 2, A and B), after sensitization to ovalbumin (Figure 2, C and D), and after sensitization and aerosol challenged with ovalbumin (Figure 2, E and F). Informative strains, whose phenotypes are significantly different from their major parental strain, are mostly from the BcA family, and they most likely contain the segregating alleles influencing the IgE trait. Important informative strains include BcA85, which has the lowest IgE phenotype in all three models,
and BcA74, which has one of the highest phenotypes. The AcB strain, with the lowest phenotype, is AcB58.

**Marker association**

To identify the chromosomal regions associated with plasma IgE levels, we performed a marker by marker linear regression analysis using the phenotype data, and genotype data of each strain at 1215 markers distributed across the genome (Boivin et al. 2012). Separate analyses were done for IgE levels at baseline, following ovalbumin sensitization and mock PBS challenge, and following ovalbumin sensitization and ovalbumin challenge. Markers that surpassed the significance threshold were considered associated with the phenotype (Figure 3). The regions delimited by the markers that were significant are summarized in Table 1. We observed an increase in the number of regions involved in the regulation of the IgE trait following allergen exposure (Figure 3). Interestingly, only one region on Chromosome 4 (81.46–86.17 Mb) is significantly associated with the IgE phenotype at baseline, and is also associated with the phenotype following allergen sensitization with or without allergen challenge.

**Mouse Chromosome 4 and IgE**

Based on the marker association results, we hypothesized that mouse Chromosome 4 likely harbors one or more genes important in the control of plasma IgE levels. To test this hypothesis, we used CSS4 mice, which have a C57BL/6J background with Chromosome 4 from the A/J strain. Plasma IgE levels were measured at baseline, after ovalbumin sensitization, and after ovalbumin sensitization and aerosol challenge in the CSS4 mice. The results illustrated in Figure 4 demonstrate that substitution of A/J Chromosome 4 on a C57BL/6J background is sufficient to significantly increase the IgE phenotype within a range comparable to the A/J strain under all three tested conditions.

**DISCUSSION**

We were able to confirm the polygenic nature of the plasma IgE phenotype in our ovalbumin allergen-exposed mice through the identification of multiple genomic regions associated with the trait. On the other hand, the analysis of baseline IgE levels in the RCS mice identified only a single associated chromosomal region on Chromosome 4.
This region was associated with IgE levels under all three conditions. From our experiments involving CSS4 mice, we understand that the Chromosome 4 locus alone does not recapture the entire complex trait. At baseline, A/J and CSS4 mice have similar differences from C57BL/6J mice in their IgE levels; however, in the allergen-exposed models (ovalbumin sensitization followed by PBS challenge, and ovalbumin sensitization followed by ovalbumin challenge) A/J mice have a larger difference from C57BL/6J mice than CSS4 mice. These findings support the supposition that the genetic factors influencing baseline IgE levels, the increase in levels due to antigen

![Figure 3](image)

**Figure 3** Identification of markers associated with the IgE phenotype at (A) baseline, (B) following ovalbumin sensitization and PBS challenge (OVA-PBS), and (C) ovalbumin sensitization and ovalbumin challenge (OVA-OVA) was done using a linear regression; significance thresholds were calculated after 10,000 permutations. Lines representing significance thresholds at $\alpha = 0.05$ are presented for each graph (naïve = $3.498$, OVA-PBS = $3.447$, OVA-OVA = $3.598$). Markers with $P$-values greater than the threshold were considered significant. Regions associated with the phenotypes are listed in Table 1.

### Table 1 Chromosomal regions significantly associated with circulating levels of plasma IgE in AcB/BcA panel of congenic mice

| Chr. | Region (Mbp) | Peak P Value | Region (Mbp) | Peak P Value | Region (Mbp) | Peak P Value |
|------|--------------|--------------|--------------|--------------|--------------|--------------|
| 3    | 81.46–86.17  | $1.17 \times 10^{-4}$ | 81.46–86.17  | $2.53 \times 10^{-4}$ |              |              |
| 5    |              |              |              |              |              |              |
| 7    |              |              |              |              |              |              |
| 11   | 67.30–76.98  | $1.49 \times 10^{-4}$ |              |              |              |              |
| 12   |              |              |              |              |              |              |
| 13   |              |              |              |              |              |              |
| 15   |              |              |              |              |              |              |
| 17   |              |              |              |              |              |              |
| 19   |              |              |              |              |              |              |

Regions are based on reference "Genome Reference Consortium GRCh38," UCSC version mm10. 5% significance thresholds are: Baseline: $3.17 \times 10^{-4}$, OVA-PBS: $3.57 \times 10^{-4}$, OVA-OVA: $2.52 \times 10^{-4}$.

sensitization, and the further increase in levels due to antigen exposure, affect the phenotype in a stepwise manner at each level of allergen exposure in the process of developing an IgE-mediated allergic response.

In only two cases did we observe overlap between the IgE-associated regions identified in studies by other groups and our own. Ohno et al. 2013 detected a suggestive association between ovalbumin induced IgE and Chromosome 17 at 35. Mbp, which falls within the Chromosome 17 region we identified using our ovalbumin-sensitized and ovalbumin-challenged model (Ohno et al. 2013). Chromosome 4 (from 13.95 to 31.66 Mbp) was associated with house dust mite antigen induced IgE in the 151 incipient lines of the Collaborative Cross (Kelada et al. 2014). Differences in the mouse strains and allergens used in each study can account for the differences in linkage results. This lack of reproducibility in linkage results is also observed in human studies of complex traits, where different regions are identified in different populations.

The human region homologous to mouse Chromosome 4 from 81.46 to 86.17 Mbp is found on Chromosome 9 from 13.31 to 18.60 Mbp. While this region has not been previously associated with atopy, human Chromosome 9 has been associated with IgE levels in a prior study (Wjst et al. 1999). Our IgE-associated region contains 12 protein coding genes that could be explored as candidates for the phenotype, such as Bnc2 and Psip1, which are involved in immune system development, and are associated with other allergic immune disorders (Ochs et al. 2000; Moy et al. 2015). Mutation assays performed using the Mouse Genome Informatics database did not identify any mutations in the protein coding regions of these two genes. However, nonsynonymous mutations were identified in the coding sequences of Cer1, Tc39b, Cdc171, Cnth, Adamt61, Haus6, Gm12551, and Dnmuk4e, but these genes do not have any prior documented associations with allergy, IgE, or asthma (Eppig et al. 2012). Further studies exploring the functions of these genes and SNPs in the context of allergy need to be performed to validate their importance.

Plasma IgE concentration and airway responsiveness have often been shown to go hand-in-hand in allergic asthma (Burrows et al. 1989). However, no overlapping regions were identified in our current study on baseline IgE, and our previous study on baseline airway responsiveness (Camateros et al. 2010). Similar findings, i.e., that AHR may be IgE independent since it can develop in B-cell and mast cell deficient mice, have also been observed by other groups (Korsgren et al. 1997; Takeda et al. 1997).

To our knowledge, no other studies have reported exploring the genetics of baseline IgE concentration, but instead have focused on IgE levels postallergen exposure (Badalova et al. 2002; Lipoldova et al. 2002; Menge et al. 2003; Ohno et al. 2013; Kelada et al. 2014). By studying the IgE phenotypes at baseline and following allergen exposure, we identified a highly significant region on Chromosome 4 common to both. The methods we used can be applied to study other asthma phenotypes, such as the recruitment of eosinophils to the lungs and the production of allergy-associated cytokines.

**ACKNOWLEDGMENTS**

This work was supported by grants from American Asthma Foundation and the Canadian Institutes of Health Research (CIHR) awarded to D.R. (MOP-106544) and S.M.V. and R.S. (MOP-89821). C.K. is a recipient of doctoral awards from CIHR, the Fonds de recherche Santé Québec (FRQ-S) and the AllerGen Network of Centers of Excellence. P.C. is a recipient of Postgraduate Scholarship from the Natural Sciences and Engineering Research Council of Canada (NSERC) and a CIHR Doctoral Award. J.R. is a recipient of a summer studentship from AllerGen. R.S. is a recipient of a New Investigator Award from the CIHR and a Chercheur-boursier Junior 2 award from the FRQ-S. S.M.V. is a recipient of a Canada Research Chair. The authors declare no conflicts of interest.

**LITERATURE CITED**

Badalova, J., M. Svobodova, H. Havélkova, V. Vladimirov, J. Vojtkovskova et al., 2002 Separation and mapping of multiple genes that control IgE level in Leishmania major infected mice. Genes Immun. 3: 187–195.

Bazaral, M., H. A. Orgel, and R. N. Hamburger, 1971 IgE levels in normal infants and mothers and an inheritance hypothesis. J. Immunol. 107: 794–801.

Bovin, G. A., J. J. Bothellet, E. Skamene, E. G. Brown, J. C. Lerrodo-Ostí et al., 2012 Mapping of clinical and expression quantitative trait loci in a sex-dependent host susceptibility to mouse-adapted influenza H3N2/HK/1/68. J. Immunol. 188: 3949–3960.

Burrows, B., F. D. Martinez, M. Halonen, R. A. Barber, and M. G. Cline, 1989 Association of asthma with serum IgE levels and skin-test reactivity to allergens. N. Engl. J. Med. 320: 271–277.

Camateros, P., R. Marino, A. Fortin, J. G. Martin, E. Skamene et al., 2010 Identification of novel chromosomal regions associated with airway hyperresponsiveness in recombinant congenic strains of mice. Mamm. Genome 21: 28–38.

Dziżer, M. H., M. Hill, A. James, J. Faux, G. Ryan et al., 1995 Detection of a recessive major gene for high IgE levels acting independently of specific response to allergens. Genet. Epidemiol. 12: 93–105.

Eppig, J. T., J. A. Blake, C. J. Bult, J. A. Kadın, and J. E. Richardson, 2012 The mouse genome database (MGD): comprehensive resource for genetics and genomics of the laboratory mouse. Nucleic Acids Res. 40: D881–D886.

Fortin, A. E. Diez, D. Rochefort, L. Laroche, D. Malo et al., 2001 Recombinant congenic strains derived from A/J and C57BL/6J: a tool for genetic dissection of complex traits. Genomics 74: 21–35.

Gerrard, J. W., D. C. Rao, and N. E. Morton, 1978 A genetic study of immunoglobulin E. Am. J. Hum. Genet. 30: 46–58.

Gill, K., and A. E. Boyle, 2005 Genetic analysis of alcohol intake in recombinant inbred and congenic strains derived from A/J and C57BL/6J progenitors. Mamm. Genome 16: 319–331.

Hasstedt, S. J., D. A. Meyers, and D. G. Marsh, 1983 Inheritance of immunoglobulin E: genetic model fitting. Am. J. Med. Genet. 14: 61–66.

Hisizaka, K., and T. Ishizaka, 1971 IgE and reaginic hypersensitivity. Ann. N. Y. Acad. Sci. 190: 443–456.

Jackola, D. R., C. L. Liebeler, M. N. Blumenthal, and A. Rosenberg, 2004 Random outcomes of allergen-specific responses in atopic families. Clin. Exp. Allergy 34: 540–547.
Kelada, S. N., D. E. Carpenter, D. L. Aylor, P. Chines, H. Rutledge et al., 2014 Integrative genetic analysis of allergic inflammation in the murine lung. Am. J. Respir. Cell Mol. Biol. 51: 436–445.

Kiiski, V., O. Karlsson, A. Remitz, and S. Reitamo, 2015 High serum total IgE predicts poor long-term outcome in atopic dermatitis. Acta Derm. Venereol. 95: 943–947.

Korsgren, M., J. S. Erjefalt, O. Korsgren, F. Sundler, and C. G. Persson, 1997 Allergic eosinophil-rich inflammation develops in lungs and airways of B cell-deficient mice. J. Exp. Med. 185: 885–892.

Leung, R., P. Ho, C. W. Lam, and C. K. Lai, 1997 Sensitization to inhaled allergens as a risk factor for asthma and allergic diseases in Chinese population. J. Allergy Clin. Immunol. 99: 594–599.

Lipoldova, M., M. Svobodova, M. Krulova, H. Havelkova, J. Badalova et al., 2000 Susceptibility to Leishmania major infection in mice: multiple loci and heterogeneity of immunopathological phenotypes. Genes Immun. 1: 200–206.

Lipoldova, M., M. Svobodova, H. Havelkova, M. Krulova, J. Badalova et al., 2002 Mouse genetic model for clinical and immunological heterogeneity of leishmaniasis. Immunogenetics 54: 174–183.

Martinez, F. D., C. J. Holberg, M. Halonen, W. J. Morgan, A. L. Wright et al., 1994 Evidence for Mendelian inheritance of serum IgE levels in Hispanic and non-Hispanic white families. Am. J. Hum. Genet. 55: 555–565.

Menge, D. M., J. M. Behnke, A. Lowe, J. P. Gibson, F. A. Iraqi et al., 2003 Mapping of chromosomal regions influencing immunological responses to gastrointestinal nematode infections in mice. Parasite Immunol. 25: 341–349.

Meyers, D. A., W. B. Bias, and D. G. Marsh, 1982 A genetic study of total IgE levels in the Amish. Hum. Hered. 32: 13–23.

Meyers, D. A., T. H. Beatty, C. R. Colyer, and D. G. Marsh, 1991 Genetics of total serum IgE levels: a regressive model approach to segregation analysis. Genet. Epidemiol. 8: 351–359.

Moisan, J., P. Camateros, T. Thuraisingam, D. Marion, H. Koohsari et al., 2006 TLR7 ligand prevents allergen-induced airway hyperresponsiveness and eosinophilia in allergic asthma by a MYD88-dependent and MK2-independent pathway. Am. J. Physiol. Lung Cell. Mol. Physiol. 290: L1987–L1995.

Moy, A. P., M. Murali, D. Kroshinsky, L. M. Duncan, and R. M. Nazarian, 2015 Immunologic overlap of helper T-cell subtypes 17 and 22 in erythrodermic psoriasis and atopic dermatitis. JAMA Dermatol. 151: 753–760.

Ochs, R. L., Y. Murow, Y. Si, H. Ge, E. K. Chan et al., 2000 Autoantibodies to DFS 70 kd/transcription coactivator p75 in atopic dermatitis and other conditions. J. Allergy Clin. Immunol. 105: 1211–1220.

Ohno, T., M. Okamoto, T. Hara, N. Hashimoto, K. Imaizumi et al., 2013 Detection of loci for allergic asthma using SMXA recombinant inbred strains of mice. Immunogenetics 65: 17–24.

Potaczek, D. P., and M. Kubes, 2012 Current concepts of IgE regulation and impact of genetic determinants. Clin. Exp. Allergy 42: 852–871.

Roy, M. F., N. Riendeau, J. C. Loredo-Osti, and D. Malo, 2006 Complexity in the host response to Salmonella Typhimurium infection in AcB and BcA recombinant congenic strains. Genes Immun. 7: 655–666.

Skaaby, T., L. L. Husemoen, B. H. Thuesen, R. V. Fenger, and A. Linneberg, 2017 IgE sensitization to inhalant allergens and the risk of airway infection and disease: a population-based study. PLoS One 12: e0171525.

Takeda, K., E. Hamelmann, A. Joetham, L. D. Shultz, G. L. Larsen et al., 1997 Development of eosinophilic airway inflammation and airway hyperresponsiveness in mast cell-deficient mice. J. Exp. Med. 186: 449–454.

Turner, S. D., 2014 qman: an R package for visualizing GWAS results using Q-Q and manhattan plots. bioRxiv. doi: https://doi.org/10.1101/005165.

Vercelli, D., 2008 Discovering susceptibility genes for asthma and allergy. Nat. Rev. Immunol. 8: 169–182.

Wjst, M., G. Fischer, T. Immervoll, M. Jung, K. Saar et al., 1999 A genomewide search for linkage to asthma. German asthma genetics group. Genomics 58: 1–8.

Communicating editor: D. W. Threadgill