

Mouse Chromosome 4 is associated with the baseline and allergic IgE phenotypes

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Short title: Baseline and allergic IgE phenotypes in RCS mice

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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 - 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.

Keywords

animal models, allergy, QTL mapping, complex traits, IgE

Introduction

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 levels 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; HASSTEDT *et al.* 1983; MEYERS *et al.* 1991; 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).

There are few genetic studies for IgE levels done using animal models, and they are mostly 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 x STS/A recombinant congenic panel (LIPOLDOVA *et al.* 2000; BADALOVA *et al.* 2002), in response to *Heligmosomoides polygyrus* resistance in a CBA x SWR strain cross (MENGE *et al.* 2003), and in response to ovalbumin allergen sensitization and challenge in an SM/J x 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 terms 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 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 approximately 12.5% of genetic material from one parental strain (minor genetic donor) and approximately 87.5% from the other (major genetic donor) (FORTIN *et al.* 2001). Here we use this panel to identify the genomic regions associated with the 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/NaJ (CSS4), and C57BL/6J-Chr 12A/J/NaJ (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-hour 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 weeks 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 100µg of ovalbumin (Sigma) adsorbed to 1.5mg of aluminum hydroxide (Imject Alum, Pierce) in a total volume of 0.2mL phosphate buffered saline (PBS). One week following the third sensitization, animals were aerosol challenged for three consecutive days with a 30-minute exposure to either 1% ovalbumin solution or PBS. Three hours after the final challenge, mice were euthanized by CO₂ exposure and blood was collected by cardiac puncture in EDTA coated tubes. Blood samples were spun at 3000 RPM for seven minutes at 4°C to isolate the plasma.

IgE measurements

Total IgE in the plasma or cell supernatants 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 qqman package for R (TURNER 2014).

Statistical analysis

Unless otherwise specified, data was 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

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).

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 concentration (Figure 1) (MOISAN *et al.* 2006). Our goal was to uncover the genetic determinants which 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, B), after sensitization to ovalbumin (Figure 2 C, D), and after sensitization and aerosol challenged with ovalbumin (Figure 2 E, 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 phenotype. The AcB strain with the lowest phenotype is always 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 regions involved in the regulation of the IgE trait following antigen exposure (Figure 3). Interestingly, only one region on Chromosome 4 (81.46 – 86.17 Mbp) is significantly associated with the IgE phenotype at baseline and is also associated with the phenotype following antigen sensitization with or without antigen challenge.

Mouse Chromosome 4 and IgE

Based on the marker association results we hypothesized that mouse Chromosome 4 likely harbours 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

In our ovalbumin allergen exposed mice we were able to confirm the polygenic nature of the plasma IgE phenotype 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 (81.46 – 86.17 Mbp). 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 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* detected a suggestive association between ovalbumin induced IgE and Chromosome 17 at 35Mbp, which falls within the Chromosome 17 region we identified using our ovalbumin sensitized and ovalbumin challenged model (28). Chromosome 4 (from 13.95 to 31.66Mbp) was associated with house dust mite antigen induced IgE in the 151 incipient lines of the Collaborative Cross (29). Differences in the mouse strains and antigens used in each study can largely 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 – 86.17 Mbp is found on Chromosome 9 from 13.31-18.60 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 *Bnc2* and *Psip1*, which are involved in immune system development and are associated to 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*, *Ttc39b*, *Ccdc171*, *Cntln*, *Adamts11*, *Haus6*, *Gm12551*, *Dennd4c* 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 have also been observed by other groups, that AHR may be IgE independent since it can develop in B-cell and mast cell deficient mice (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 post-allergen exposure (BADALOVA *et al.* 2002; LIPOLDOVA *et al.* 2002; MENGE *et al.* 2003; OHNO *et al.* 2013; KELADA *et al.* 2014). By studying both baseline and allergen exposure IgE phenotypes, 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.

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Conflict of Interest

The authors declare no conflict of interest.

Figures and tables (presented in the order mentioned in manuscript)

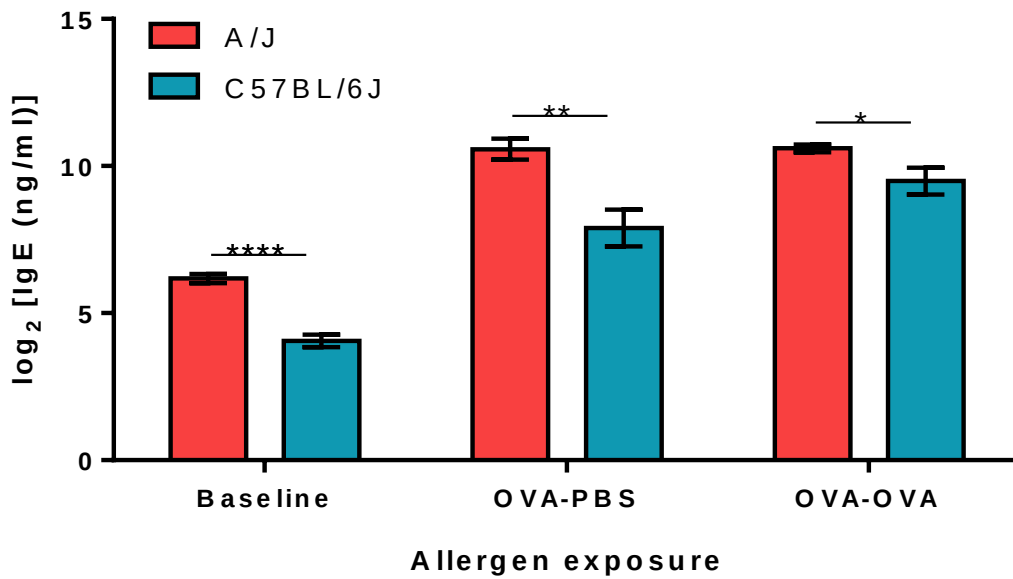


Figure 1: Comparison of plasma IgE levels in A/J and C57BL/6J mice at baseline and following allergen exposure. The A/J strain has a higher IgE concentration in all three conditions compared to the C57BL/6J strain. Results are presented for unsensitized (Baseline), ovalbumin (OVA) sensitized and PBS challenged (OVA-PBS), and OVA sensitized and OVA challenged animals (OVA-OVA). Data are presented as mean of log₂ transformed values $\pm$ SEM. Significant differences between strains was determined by a two tailed t-test. $n > 6$ per strain, *, **, and *** represent $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

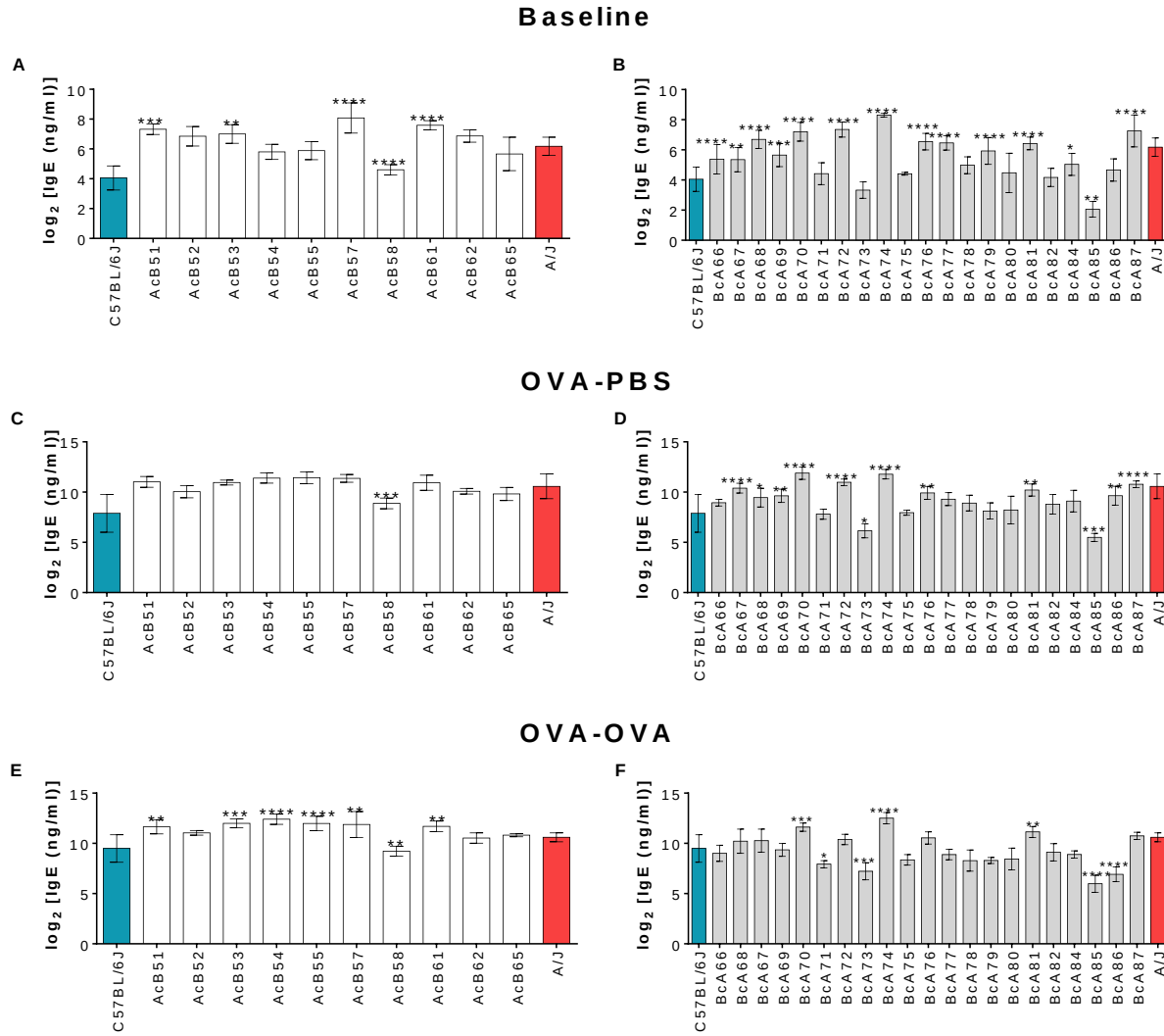


Figure 2: Distribution of the IgE phenotype in the RCS compared to parental strains. AcB strains were compared to A/J strain, while BcA strains were compared to C57BL/6J strain. Phenotype and genotype data allowed to identify genomic regions associated with the phenotype. AcB and BcA phenotype and marker analysis results are presented for unsensitized (baseline, A, B), ovalbumin (OVA) sensitized (OVA-PBS, C, D), and OVA sensitized and OVA challenged animals (OVA-OVA, E, F). Data are presented as mean of $\log_2$ transformed values $\pm$ SEM. Comparisons between RCS and respective major genetic donor parental strains were done by one-way ANOVA followed by Dunnett's correction. $n = 5 - 20$ per strain, 724 mice in total. *, **, and *** represent $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

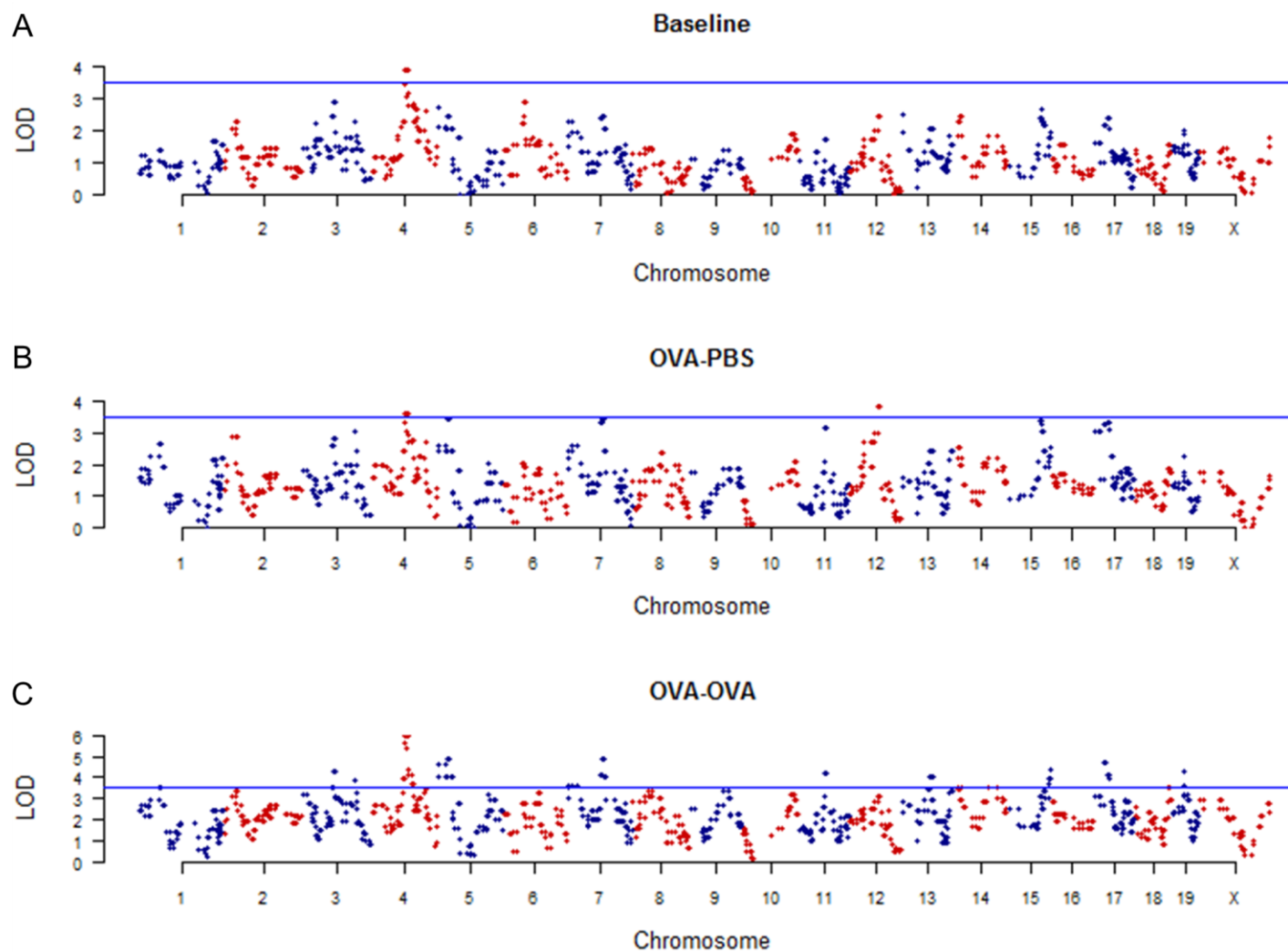


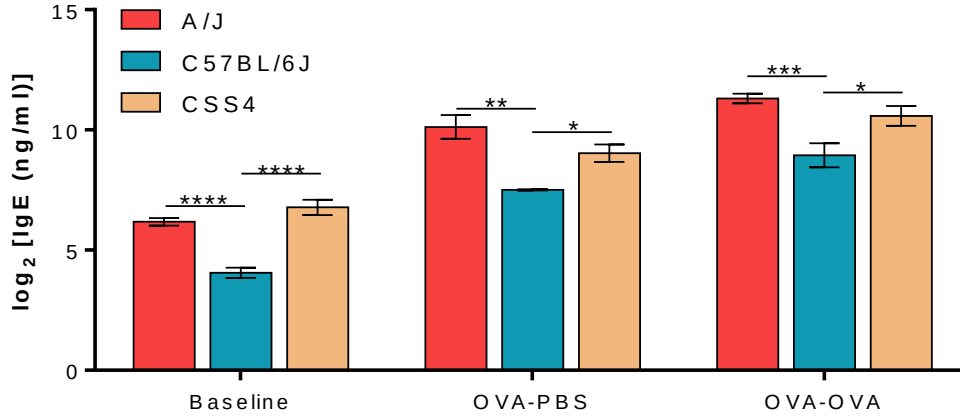
Figure 3: Analysis of markers associate with the IgE phenotype at baseline (A), following ovalbumin sensitization and PBS challenge (OVA-PBS, B) and ovalbumin sensitization and ovalbumin challenge (OVA-OVA, C) was done using a linear regression and the significance thresholds were calculated after 10,000 permutations. A 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 phenotype are listed in Table 1.

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

3

Chr.	Baseline		OVA-PBS		OVA-OVA	
	Region (Mbp)	Peak <i>p</i> value	Region (Mbp)	Peak <i>p</i> value	Region (Mbp)	Peak <i>p</i> value
3					73.51-76.06 121.46-126.41	5.15x10 ⁻⁵ 1.25x10 ⁻⁴
4	81.46-86.17	1.17x10 ⁻⁴	81.46-86.17	2.53x10 ⁻⁴	75.06-101.43	1.08x10 ⁻⁶
5					3.06-34.77	1.24x10 ⁻⁵
7					3.07-29.93 70.81 – 84.21	2.46x10 ⁻⁴ 1.24x10 ⁻⁵
11					63.66 – 76.96	6.68x10 ⁻⁵
12			67.30-76.98	1.49x10 ⁻⁴		
13					67.76 – 73.80	8.67x10 ⁻⁵
15					96.69 – 103.45	3.96x10 ⁻⁵
17					26.61 – 41.02	2.0x10 ⁻⁵
19					26.75 – 28.74	5.34x10 ⁻⁵

Regions are based on reference “Genome Reference Consortium GRCm38”, UCSC version mm10. 5% significance thresholds - Baseline: 3.17x10⁻⁴, OVA-PBS: 3.57x10⁻⁴, OVA-OVA: 2.52x10⁻⁴



6

7 **Figure 4: IgE phenotype in chromosome 4 consomic strain mice.** Plasma IgE concentration of
8 Chromosome 4 substitution strain (CSS4) relative to parental strains A/J and C57BL/6J following different
9 degrees of antigen exposure. Results are presented for unsensitized (Baseline), ovalbumin (OVA) sensitized
10 and PBS challenged (OVA-PBS), and OVA sensitized and OVA challenged animals (OVA-OVA).
11 Comparisons between strains for each phenotype was done by one-way ANOVA followed by Bonferonni
12 correction. Data are presented as mean of log₂ transformed values $\pm$ SEM. $n \geq 5$ per strain; *, **, and ***
13 represent $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

References

- Badalova, J., M. Svobodova, H. Havelkova, V. Vladimirov, J. Vojtiskova *et al.*, 2002 Separation and mapping of multiple genes that control IgE level in *Leishmania major* infected mice. *Genes Immun* 3: 187-195.
- Bazaraal, 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.
- Boivin, G. A., J. Pothlichet, E. Skamene, E. G. Brown, J. C. Loredon-Osti *et al.*, 2012 Mapping of clinical and expression quantitative trait loci in a sex-dependent effect of host susceptibility to mouse-adapted influenza H3N2/HK/1/68. *J Immunol* 188: 3949-3960.
- Burrows, B., F. D. Martinez, M. Halonen, R. A. Barbee 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.
- Dizier, 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. Kadin 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.
- Ishizaka, K., and T. Ishizaka, 1971 IgE and reaginic hypersensitivity. *Ann NY Acad Sci* 190: 443-456.
- Jackola, D. R., C. L. Liebler, 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, H. Havelkova, M. Krulova, J. Badalova *et al.*, 2002 Mouse genetic model for clinical and immunological heterogeneity of leishmaniasis. *Immunogenetics* 54: 174-183.

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.

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., T. H. Beaty, 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.

Meyers, D. A., W. B. Bias and D. G. Marsh, 1982 A genetic study of total IgE levels in the Amish. *Hum Hered* 32: 15-23.

Moisan, J., P. Camateros, T. Thuraingam, 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: L987-L995.

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. Muro, 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. Kabesch, 2012 Current concepts of IgE regulation and impact of genetic determinants. *Clin Exp Allergy* 42: 852-871.

Roy, M. F., N. Riendeau, J. C. Loredó-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 qqman: an R package for visualizing GWAS results using Q-Q and manhattan plots. *bioRxiv*.

103 Vercelli, D., 2008 Discovering susceptibility genes for asthma and allergy. Nat Rev Immunol 8:
104 169-182.
105 Wjst, M., G. Fischer, T. Immervoll, M. Jung, K. Saar *et al.*, 1999 A genome-wide search for
106 linkage to asthma. German Asthma Genetics Group. Genomics 58: 1-8.
107