

THESIS

3

2004

56959847

This is to certify that the
dissertation entitled

STUDIES OF THE GATA-3 TRANSCRIPTION FACTOR AS A
POSITIONAL CANDIDATE GENE FOR A MURINE MODEL
OF ASTHMA

presented by

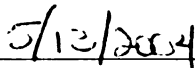
Xingnan Li

has been accepted towards fulfillment
of the requirements for the

Ph.D. degree in Genetics Graduate Program



Major Professor's Signature



Date



PLACE IN RETURN BOX to remove this checkout from your record.
TO AVOID FINES return on or before date due.
MAY BE RECALLED with earlier due date if requested.

DATE DUE	DATE DUE	DATE DUE
APR 09 2005		JUN 27 2005
04 11 05		04 11 05

**STUDIES OF THE GATA-3 TRANSCRIPTION FACTOR AS A POSITIONAL
CANDIDATE GENE FOR A MURINE MODEL OF ASTHMA**

By

Xingnan Li

A DISSERTATION

**Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of**

DOCTOR OF PHILOSOPHY

Genetics Graduate Program

2004

ABSTRACT

STUDIES OF THE GATA-3 TRANSCRIPTION FACTOR AS A POSITIONAL CANDIDATE GENE FOR A MURINE MODEL OF ASTHMA

By

Xingnan Li

Asthma is a respiratory inflammatory disorder due to inappropriate immune responses to common environmental allergens in genetically susceptible persons. Asthma is characterized by increased serum immunoglobulin E levels, airway infiltration of eosinophils, airway hyperresponsiveness (AHR), mucus hypersecretion, and overexpression of T helper 2 cell (Th2) type cytokines.

Quantitative trait loci for allergen-induced AHR, termed *Abhr1* and *Abhr2*, mapped to chromosome 2 in a murine model of asthma. The GATA-3 gene (*Gata3*) mapped within the *Abhr1* interval and was a strong positional candidate gene for this phenotype because it underlies the Th2 immune pathway. Thus, the working hypothesis for this study was that the allergen-induced AHR phenotype was due to over-expression of GATA-3.

To more closely determine the location of *Abhr1* and *Abhr2* relative to *Gata3*, *Abhr1* and *Abhr2* regions were fine-mapped (~2 cM) by genotyping 450 backcross mice derived from hyper- (A/J) and hypo-responsive (C3H/HeJ) parental strains for 37 densely clustered DNA markers on chromosome 2. *Gata3* was mapped within the *Abhr1* confidence interval (lod = 4.3).

Genomic DNA was sequenced to determine *Gata3* polymorphisms between A/J and C3H/HeJ mice. A polymorphic microsatellite was identified in intron 5. No

other inter-strain polymorphisms were found in exons, splicing sites, and part of the promoter region and 3' untranslated region (12,069 bp total).

A time course of the allergen-induced mRNA levels of GATA-3, interleukin (IL)-4, IL-5, IL-12, IL-13 and interferon gamma was determined in A/J and C3H/HeJ mice using real-time reverse transcriptase-polymerase chain reaction (RT-PCR). GATA-3 expression was induced by ovalbumin (OVA) treatment at the earliest time point (6 hr) in lymph nodes and spleens of A/J mice, but not C3H/HeJ mice. Unexpectedly, no GATA-3 expression difference was observed in lungs at any time point. Th2 cytokine expression was in general greater in A/J mice than in C3H/HeJ mice, while C3H/HeJ mice tended to surpass A/J mice in Th1 cytokine expression.

To investigate the role of GATA-3 in mediating allergen-induced AHR, Western blot was used to determine the levels of GATA-3 protein in splenocytes examined 72 hr following allergen exposure of these strains. A/J mice showed a strong GATA-3 signal subsequent to OVA challenge, but C3H/HeJ mice did not.

In summary, we found strain-specific and treatment-specific quantitative differences in GATA-3 mRNA and protein levels, but no qualitative differences between A/J and C3H/HeJ mice were detected in *Gata3* at the DNA level. These results reconfirmed the important role of GATA-3 in allergen-induced AHR. However, they do not provide direct evidence in support of *Gata3* as a positional candidate gene for *Abhr1* in the A/J and C3H/HeJ murine model of asthma.

Copyright by
XINGNAN LI
2004

DEDICATION

This dissertation is dedicated to: My lovely wife, Huashi Li, whose love is the most important stimulation for my career; my parents, Dawan Li and Zhongxi Li, who always encourage and support me by all hearts; my parents-in-law, Rongjie Li and Chunfu Quan, who support my family spiritually and commercially; and my sister, Changshu Li's family, for their love and support.

ACKNOWLEDGMENTS

This dissertation could not be completed without the excellent guidance, insight, and encouragement of Dr. Susan L. Ewart. Dr. Ewart is not only a great mentor but also a good friend.

I am grateful to Dr. Marsha Wills-Karp, who acts as my co-adviser. I am also grateful to my committee members, Dr. Sarah H. Elsea, Dr. Jack R. Harkema, and Dr. Patrick J. Venta, for their invaluable suggestions.

I would like to thank Dr. Norbert E. Kaminski's laboratory, especially Robert B. Crawford for their suggestions and technique support on TaqMan real-time RT-PCR and Western Blot.

I would like to thank the colleagues in my lab, especially Dennis Shubitowski for his technique support on DNA sequencing and genetic linkage analysis. I would also like to thank Annette S. Hamilton and Ravisankar A. Ramadas for their help with inbred mouse production and sacrifice.

Finally, I would like to thank my wife for her statistical inputs as well as her support and love.

TABLE OF CONTENTS

LIST OF TABLES.....	ix
LIST OF FIGURES.....	x
LIST OF ABBREVIATION.....	xii
Chapter One: Background and Significance.....	1
A. Asthma.....	1
B. Th1 and Th2 pathways.....	8
C. Transcription factors in the Th1/Th2 pathways.....	13
D. GATA-3.....	18
E. A/J and C3H/HeJ mouse model for asthma.....	27
F. Summary.....	31
Chapter Two: Fine-mapping of <i>Abhr1</i> and <i>Abhr2</i> loci.....	32
A. Introduction.....	32
B. Massachusetts Institute of Technology (MIT) microsatellite markers.....	34
C. Self-designed microsatellite markers.....	37
D. Hemolytic complement (<i>Hc</i>) RFLP marker.....	42
E. Polymerase chain reaction-based genotyping.....	45
F. Multipoint linkage analysis.....	47
G. Comparison of our linkage results to published linkage maps.....	50
H. Positional candidate genes.....	55
I. Summary.....	57
Chapter Three: Comparison of <i>Gata3</i> sequence in two murine strains.....	58
A. Introduction.....	58
B. <i>Gata3</i> gene structure.....	59
C. Sequencing of <i>Gata3</i>	61
D. Comparison of our sequence to public databases.....	83
E. Summary.....	86
Chapter Four: Comparison of GATA-3 and cytokine gene mRNA expression in two murine strains.....	87
A. Introduction.....	87
B. TaqMan real-time RT-PCR.....	89
C. Experimental time line.....	98
D. Expression profiles for cytokine genes : <i>Il4</i> , <i>Il5</i> , <i>Il12</i> , <i>Il13</i> , and <i>Ifng</i>	100
E. GATA-3 gene expression profile.....	107
F. Summary.....	111

Chapter Five: Comparison of GATA-3 protein in two murine strains.....	113
A. Introduction.....	113
B. GATA-3 Western blot assay development.....	115
C. Experimental time line.....	117
D. Western blot of GATA-3.....	119
E. Summary.....	122
Discussion.....	123
Materials and Methods.....	132
A. Genomic DNA isolation from mouse kidney.....	132
B. Polymerase chain reaction (PCR).....	133
C. <i>Bsg</i> I digestion for <i>Hc</i> genotyping.....	135
D. MAPMAKER program.....	135
E. JoinMap program.....	136
F. DNA primer design.....	137
G. DNA sequencing.....	137
H. Ovalbumin sensitization and challenge.....	139
I. TaqMan real-time RT-PCR.....	140
J. Statistic analysis of mRNA expression data.....	142
K. Nuclear protein isolation from EL-4 cells and splenocytes.....	144
L. GATA-3 protein quantitation by Western blot.....	146
Appendix.....	149
Table 8. Mouse ID reference and APTI value.....	149
Table 9. Genotyping data of 450 A/J backcross mice by 37 markers.....	155
Table 10. Genes within <i>Abhr1</i> and <i>Abhr2</i>	169
Table 11. GATA-3 expression in lungs.....	185
Table 12. GATA-3 expression in tracheobronchial lymph nodes.....	200
Table 13. GATA-3 expression in spleens.....	203
Bibliography.....	213

LIST OF TABLES

Table 1. MIT microsatellite markers on chromosome 2.....	35
Table 2. Self-designed microsatellite markers.....	41
Table 3. Comparison of our linkage results with MIT linkage map.....	53
Table 4. Comparison of our linkage results with Jackson lab RH map, Celera physical map, and UCSC physical map.....	54
Table 5. Positional candidate genes on chromosome 2.....	56
Table 6. Sequencing primers for <i>Gata3</i>	62
Table 7. Comparison of our sequencing data with public databases.....	85
Table 8. Mouse ID reference and APTI value.....	149
Table 9. Genotyping data of 450 A/J backcross mice by 37 markers.....	155
Table 10. Genes within <i>Abhr1</i> and <i>Abhr2</i>	169
Table 11. GATA-3 expression in lungs.....	185
Table 12. GATA-3 expression in tracheobronchial lymph nodes.....	200
Table 13. GATA-3 expression in spleens.....	203

LIST OF FIGURES

Figure 1. Th1 and Th2 pathways.....	12
Figure 2. Processes of identifying microsatellite markers in <i>Gad2</i>	38
Figure 3. <i>Hc</i> genotyping.....	43
Figure 4. Genotyping microsatellite marker <i>D2Mit416</i>	46
Figure 5. Mouse chromosome 2 genetic linkage map.....	49
Figure 6. <i>Gata3</i> gene structure.....	60
Figure 7. An example of a <i>Gata3</i> sequencing gel	66
Figure 8. An example of automated sequencing chromatograph of <i>Gata3</i>	67
Figure 9. <i>Gata3</i> gene sequence of A/J and C3H/HeJ mice.....	69
Figure 10. The process of TaqMan real-time RT-PCR.....	92
Figure 11. The threshold cycle of TaqMan real-time RT-PCR.....	93
Figure 12. GATA-3 and 18s rRNA amplification plots.....	96
Figure 13. Standard curves of GATA-3 and 18s rRNA.....	97
Figure 14. Experimental time line of in vivo allergen exposure.....	99
Figure 15. IL-4 expression in lungs and tracheobronchial lymph nodes.....	102
Figure 16. IL-5 expression in lungs and tracheobronchial lymph nodes.....	103
Figure 17. IL-13 expression in lungs and tracheobronchial lymph nodes.....	104
Figure 18. IL-12p40 expression in lungs and tracheobronchial lymph nodes..	105
Figure 19. IFN- γ expression in lungs and tracheobronchial lymph nodes.....	106
Figure 20. GATA-3 TaqMan primer pair and probe.....	108
Figure 21. GATA-3 expression in lungs, TaqMan and SYBR green assay....	109

Figure 22. GATA-3 expression in tracheobronchial lymph nodes and spleens.110

Figure 23. EL-4 cell Western blot gel.....116

Figure 24. Experimental time line for splenocyte nuclear protein isolation.....118

Figure 25. Western blot of GATA-3 8hr splenocytes samples..... 120

Figure 26. Western blot of GATA-3, 4, 12, 24 hr splenocytes samples.....121

LIST OF ABBREVIATIONS

AHR	airway hyperresponsiveness
APC	antigen-presenting cells
APTI	airway pressure time index
BAL	bronchoalveolar lavage
BHR	bronchial hyperresponsiveness
cAMP	cyclic adenosine monophosphate
CD	cluster of differentiation antigens
CDC	Centers for Disease Control and Prevention
CTLA-4	cytolytic T lymphocyte-associated antigen 4
DC	dendritic cells
ELISA	enzyme-linked immunosorbent assay
FEV	forced expiratory volume
FOG	friend of GATA
FVC	forced vital capacity
GATA-3	GATA-binding protein 3 (protein or mRNA)
<i>Gata3</i>	GATA-binding protein 3 (DNA)
HLA	human leucocyte associated antigens
ICAM-1	intercellular adhesion molecule-1
IFN- γ	interferon gamma
IgE	immunoglobulin E
I κ B	NF- κ B inhibitor
IL	interleukin
Jaks	Janus family protein tyrosine kinases
LFA-1	lymphocyte function-associated antigen 1
MHC	major histocompatibility complex
MIT	Massachusetts Institute of Technology
NFAT	nuclear factor of activated T cells
NF- κ B	nuclear factor of kappa light chain gene enhancer in B-cells
OVA	ovalbumin
PBS	phosphate-buffered saline
PMA	phorbol 12-myristate 13-acetate
QTL	quantitative trait locus
RFLP	restriction fragment length polymorphism
ROG	repressor of GATA-3
RT-PCR	reverse transcription-polymerase chain reaction
SNP	single nucleotide polymorphism
STAT	signal transducer and activator of transcription
T _c	cytotoxic T cells
TCR	T cell receptors
TGF- β	transforming growth factor- β
Th	T helper cells
TNF	tumor necrosis factor
T _s	suppressor T cells

Chapter One: Background and Significance

A. Asthma

Phenotype description

Asthma is a chronic respiratory disease characterized by airway hyperresponsiveness (AHR), infiltration of eosinophils, mast cells and lymphocytes, airway obstruction, increased immunoglobulin E (IgE) level, increased mucus secretion, and overexpression of T helper cell type 2 (Th2) cytokines, such as interleukin (IL)-4, IL-5, and IL-13 [1, 2]. Asthma is a chronic syndrome of the airway with recurrent wheezing, coughing, chest tightness, and shortness of breath. Persistent airway inflammation, which leads to AHR, is critical to the pathogenesis of asthma. Asthma is an inflammatory disorder due to inappropriate immune responses to common environmental antigens in genetically susceptible persons. Expression of asthma phenotype is affected by both genetic and environmental factors. Various environmental factors, such as viral infection, allergen exposure, and exercise, contribute to asthma. Various genes, such as IL-4, IL-13, T-cell antigen receptor, high-affinity IgE receptor, may be involved in asthma. Thus, asthma is a complex genetic syndrome [3]. However, the mechanism and the genetic reasons for asthma remain unclear.

Epidemiology

The prevalence, morbidity, and mortality of asthma have increased dramatically in recent years, especially in developed countries. According to National Health Interview Survey (NHIS), in 2001, 20.3 million (7.3 %) people of the United States had asthma at the time of the interview [4]. The rates of asthma decreased with age: 8.7% (6.3 million) of 0-17 years children had asthma compared with 6.9% (14 million) 18 years and over adults [4]. The rates of asthma varied among different racial groups: asthma prevalence of non-Hispanic blacks was approximately 10% and 40% higher than non-Hispanic whites and Hispanics, respectively [4]. The rates of asthma were also different between sexes: adult females had a 30% higher prevalence than adult male; however, boys had over 30% higher prevalence than girls [4].

Various forms of asthma

Asthma is a complex genetic disorder with numerous expressions, such as AHR, bronchoconstriction, airway edema, and atopy. Defining the phenotype of asthma is the first step to study it. According to its severity, asthma can be classified into intermittent, mild persistent, moderate persistent, and severe persistent [3]. Based on the level of IgE-mediated response, it can be classified into atopic and non-atopic. Additionally, etiologic classifications include occupational, exercise-induced, aspirin sensitive, and persistent respiratory infected [5]. Based on ages, four

asthma phenotypes are defined in children: transient infant wheezing, nonatopic wheezing of the toddler, IgE-mediated wheezing/asthma, and late-onset childhood asthma [5]. In adult, asthma can be either adult-onset or childhood-related, however, the phenotypes of adult-onset asthma are poorly defined [5]. Due to the heterogeneous genotypes and phenotypes, asthma is more likely to be a syndrome rather than a specific disease entity [1, 3].

Diagnosis

Various diagnostic methods exist based on different aspects of the asthma phenotypes. Airway obstruction can be determined by pulmonary function tests such as the decreasing ratio of forced expiratory volume to forced vital capacity (FEV1/FVC). Total serum IgE levels can be tested by enzyme-linked immunosorbent assay (ELISA). Airway eosinophil infiltration can be shown by cell counting from bronchoalveolar lavage (BAL). AHR refers to the ability of the airways to constrict when exposed to small concentrations of inhaled bronchoconstrictor mediators or naturally occurring bronchoconstrictor stimuli. AHR is a subset phenotype of asthma and considered a key feature [6], therefore AHR was used as an index of the asthma phenotype in the current study.

Therapy

Asthma has become a global disease; effective and specific therapy is urgently needed. Because asthma is driven by chronic airway inflammation, anti-inflammatory therapy forms the basis for asthma control. Glucocorticoids are the most effective and widely used anti-inflammatory agents in the treatment of asthma. Glucocorticoids can repress the translocation of nuclear factor of kappa light chain gene enhancer in B-cells (NF- κ B) through inducing the expression of NF- κ B inhibitor (I κ B). Since NF- κ B stimulates inflammatory cytokines, such as tumor necrosis factor alpha (TNF- α), IL-1 β , and IL-2, glucocorticoids have potential anti-inflammatory function by interfering with the NF- κ B pathway [7].

β 2-adrenergic agonists are also widely used to treat asthma due to their bronchodilatory function. β 2-adrenergic receptors are widely located in airway smooth muscle and activated when associated with trimeric Gs protein and guanosine triphosphate (Gs-GTP). Activated β 2-adrenergic receptors can increase cyclic adenosine monophosphate (cAMP) levels, which will induce airway smooth muscle relaxation. β 2-adrenergic agonists may stabilize activated β 2 receptors-Gs-GTP complex and thus act as a bronchodilator [8].

Other anti-inflammatory drugs or drugs that act on the nervous system, such as leukotriene receptor antagonists, anticholinergics, theophylline, cromolyn, and nedocromil, are also used [1]. However, all

the currently used drugs are non-specific and may have side effects. The only way to resolve this problem is to uncover the basic mechanism of asthma, so that targeted therapies can be developed. New ways to treat asthma such as antisense DNA, CpG oligodeoxynucleotides, and transcription factor agonists or antagonists may be available in the future [9, 10].

Genetics

Asthma as a heritable disease has been established by twin studies and family studies. Genetic linkage analysis and association studies further show that asthma is a complex trait instead of a simple Mendelian disease. Twin studies have contributed a lot to our understanding of the genetics of asthma and the interaction between genes and the environment [11]. In a study of 381 Australian twin pairs, strong genetic associations between asthma, atopy, and bronchial hyperresponsiveness (BHR) were indicated [12]. A nation-wide Finnish twin study showed that genetic factors could explain 87% of variations of asthma incidence in twins with affected parents, however susceptibility to asthma in twins with unaffected parents was largely explained by environmental effects [13]. Interestingly, a comparative study of monozygotic and dizygotic twins showed that pollen allergen had a greater genetic correlation, while indoor allergen was more environmentally correlated [14].

Genome-wide screens and genetic linkage analyses have been used to locate the quantitative trait loci (QTL) for asthma and positional candidate genes. A number of different linkages have been identified in different populations. One group of genes that have been repeatedly linked with asthma phenotypes are the genes involved in the Th2 cell pathway. One of the earliest studies was a sib-pair study of 11 Amish families that revealed the linkage of chromosome 5q31.1 with total serum IgE concentration and suggested *IL4* or nearby genes on 5q31.1 may be involved [15]. The linked region of chromosome 5q contains a cytokine gene cluster: *IL3*, *IL4*, *IL5*, *IL9*, *IL12B*, *IL13*, and granulocyte-macrophage colony-stimulating factor (*GMCSF*). Recent studies have shown a sequence variant on the *IL4* promoter region (C589T) associated with FEV1 and asthma [16]. A variant in the *IL13* coding region (Gln110Arg) has also been associated with asthma [17]. The receptors of IL-4 and IL-13 share the IL-4 receptor alpha chain and are both involved in IgE regulation. IL-5 is important for maturation and differentiation of eosinophils, and asthma. The linkage of chromosome 5q to circulating eosinophils have been shown [18]. Further studies on 5q regions revealed some new candidate genes including the hepatitis A virus receptor (*HAVCR1*), interleukin 12 p40 (*IL12B*) [19], *CD14* [20], and serine protease inhibitor, kazal type 5 (*SPINK5*) [21].

Besides the chromosome 5q region, other genes on other chromosomes have also been consistently identified with asthma-related

phenotypes: major histocompatibility complex (MHC) [22] and tumor necrosis factor alpha (TNF- α) on chromosome 6 [23], β -chain of the high-affinity receptor for IgE (Fc ϵ RI- β) on chromosome 11q13 [24]. According to an extensive review by Hoffjan et al, to date variants in 64 genes have been shown to be associated with asthma or related phenotypes among different populations and they are located on chromosome 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 16, 17, 19, 21, and X [25].

In summary, asthma is a complex genetic syndrome due to inappropriate immune responses to common environmental antigens in genetically susceptible persons. Lots of environmental factors and genes are involved in asthma, which leads to the heterogeneity of asthma phenotypes and genotypes. Genetic linkage analyses based on different human populations often reveal different QTL, which may not be applicable to other populations because of the genetic heterogeneity. To study this complex disease more effectively and easily, a murine model was utilized in the current study. Although the results of the murine model cannot be directly applied to humans, it is invaluable for revealing mechanisms of asthma due to molecular homology and common pathways between mice and humans.

B. Th1 and Th2 pathways

Antigen-presenting cells (APC)

Major histocompatibility complex (MHC) molecules, also known as histocompatibility antigens, are glycoproteins expressed on the cell surface of higher vertebrates. They were first discovered in mice and called H-2 antigens, which are encoded by genes on chromosome 17. The counterparts in human are called human leucocyte associated antigens (HLA), which are encoded by genes on chromosome 6 [26, 27]. High polymorphism is one of the distinctive features of MHC molecules, which is important for antigen processing and presenting functions. There are two principal classes of MHC molecules: MHC I and MHC II. MHC I molecules are expressed on all nucleated cells and deal with intracellular microbes such as viruses and antigens in the cytosol. By contrast, MHC II molecules are expressed on specialized cells, such as B cells, macrophages, and other antigen-presenting cells (APC) that take up foreign antigens from intracellular vesicles [27]. When foreign antigens enter the respiratory tract, antigen-presenting cells residing there can recognize and process them. The main APC in the lung are dendritic cells (DC), which have two subtypes: DC1 and DC2 [28]. DC2 or immature DCs, located on mucosal surface of the lung, express mainly IL-10 and low levels of MHC II. In contrast, DC1 or mature DCs, located in the lymphoid organs, express mainly IL-12 and higher level of MHC II on their surface [29]. CD28 is a glycoprotein molecule that is expressed on the

surface of T cells and serves as a receptor for the B7 costimulatory molecule, which is expressed on APC, activated T cells, or activated B cells. Antigens presented by MHC II and co-stimulatory signals presented by B7 molecules on the surface of DCs are recognized by T cell receptors (TCR) and CD28 molecules, respectively, on naïve T helper (Th0) cells located on peripheral lymphoid organs. Both TCR signals and costimulatory signals are required for Th0 cell activation [27].

T helper (Th) cells

Specific cluster of differentiation (CD) antigens also are required for DCs and T cells recognition besides MHC-TCR and B7-CD28 interactions. CD8 antigen is expressed on cytotoxic T (Tc) cells and suppressor T (Ts) cells, which recognizes MHC I molecules. CD4 antigen is expressed specially on T helper (Th) cells and recognizes MHC II molecules. Tc (CD8+) cells respond to viruses, tumors or transplanted organs. Th (CD4+) cells can stimulate nonspecific effector cells to produce specific immunity during cell-mediated immune response. With regard to the humoral immune response, Th (CD4+) cells are essential for inducing antibody production by B cells. Th cells make up the predominant lymphocyte population that infiltrates the airways in asthmatics [30]; Th cells multiply rapidly following certain stimuli; Th cells can also lead to cell-mediated or humoral immune responses. For these reasons, Th cells are believed to play a central role in asthmatic inflammation [2, 28].

T cell differentiation

Antigen-activated naïve Th0 (CD4+) cells can differentiate into two subsets, Th1 and Th2, based on their distinct functions and the profile of secreted cytokines [2, 28] (Figure 1).

The cytokine environment, type and dose of antigen, and costimulatory signals are all important for Th cell differentiation [31, 32]. Pfeiffer and colleagues have shown that strong binding of TCR and MHC II/antigen polarized Th1-like cells, but weak binding of TCR and MHC II/antigen favored Th2-like cells [33]. Weak TCR signaling favored IL-4 expression and Th2 differentiation by increasing nuclear factor of activated T cells (NFAT)c to NFATp ratio [34]. Dosage of antigen also affects Th0 cell differentiation. For example, naïve Th cells secreted more IL-4, a Th2 type cytokine, when treated with low dosage of ovalbumin (5 to 50 nM); but secreted more interferon gamma (IFN- γ), a Th1 type cytokine, when high dosage of antigen ($\geq$ 50 nM) was used [35]. Since the costimulatory signal through the interaction between B7 and CD28 is required for T cell stimulation, the two types of B7 molecule, B7-1 and B7-2, may be also important for Th0 cell differentiation. For example, the dominant presence of B7-2 molecules on Th0 cells may favor the Th2 pathway [31]. Based on a mitogenic anti-CD28 study, strong costimulation of CD28 versus TCR signal might favor the Th2 versus Th1 pathway [36]. CD28 costimulation signals might also control histone hyperacetylation and activation of IL-5 gene through the NF κ B and GATA-3 pathway [37]. Recently, cytolytic T

lymphocyte-associated antigen 4 (CTLA-4) was shown to be an important T cells differentiation regulator. CTLA-4 is also a receptor for the B7 costimulatory signal. CTLA-4 deficient T cells polarized to Th2 differentiation with increased activation of NF- κ B and expression of GATA-3 by a STAT-6 independent mechanism [38]. The ligation of intercellular adhesion molecule-1 (ICAM-1) on T cells and lymphocyte function-associated antigen 1 (LFA-1) on DC favored human Th1 development in a IL-12 independent manner [39]. However, the cytokine environment is the most important factor determining the Th cell differentiation. IL-12 can induce Th0 cells to differentiate into the Th1 subset through signal transducer and activator of transcription 4 (STAT4) and inhibit the Th2 pathway. IL-4 is critical for Th2 subset formation through STAT6 and inhibit Th1 pathway. Th1 cells produce IL-2, IFN- γ , and TNF- β , and promote strong cell-mediated immune responses. On the contrary, Th2 cells produce IL-4, IL-13, and IL-5 [28]. IL-4 and IL-13 are important for stimulating B cells to produce IgE [40]. IL-5 is essential for eosinophil maturation and infiltration [41]. Based on airway lavage and biopsy samples, Th2 cytokines (IL-4 and IL-5) were overexpressed in asthmatics [30]. Elevated IL-4 expression in asthmatics was correlated with elevated IgE level, and increased IL-5 expression was associated with eosinophilia [30]. Thus, it is believed that Th2 cells are critical in asthma.

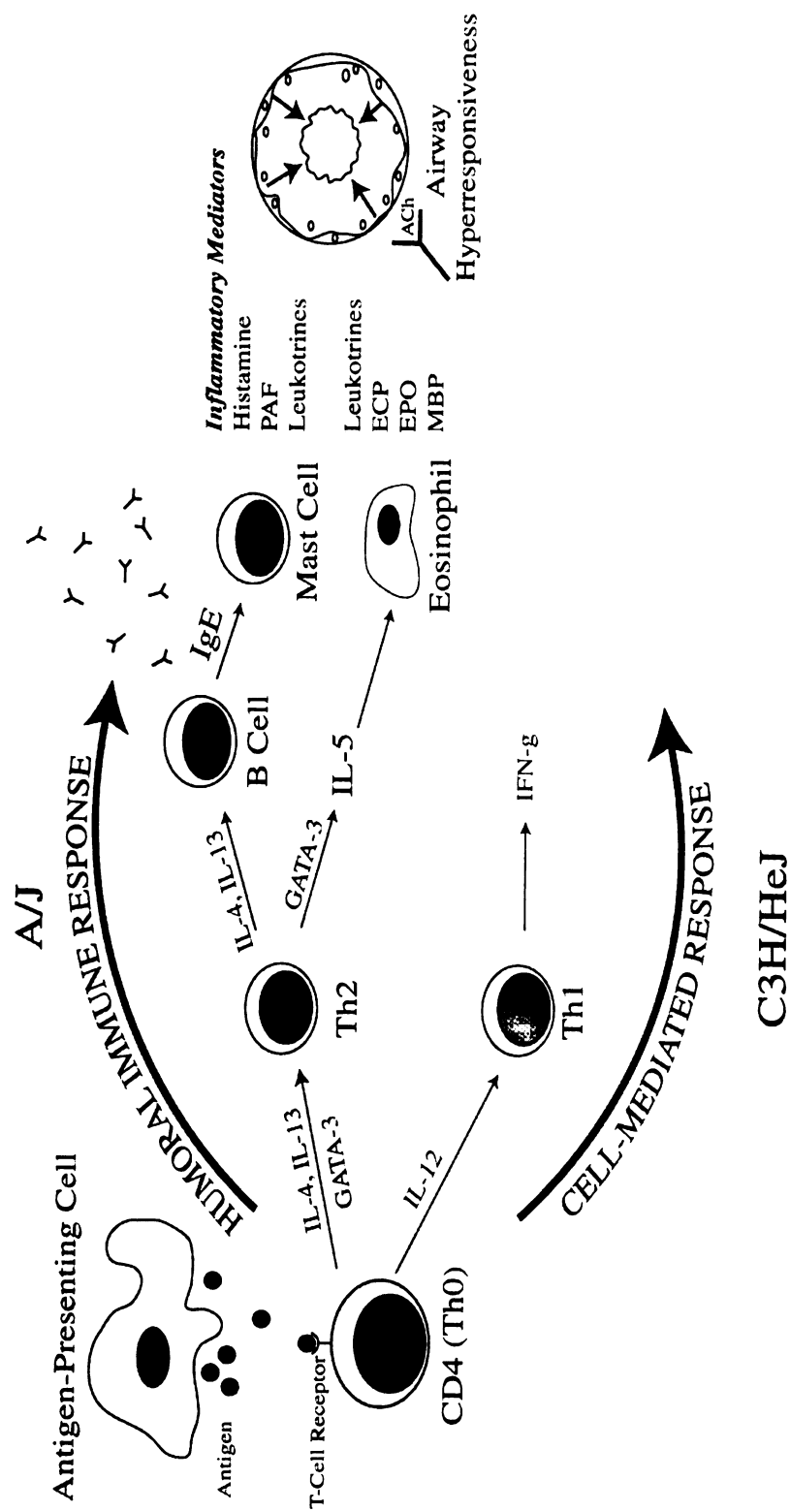


Figure 1. Th1 and Th2 pathways. Naïve T helper cells (Th0) can differentiate into type 1 T helper cells (Th1) or type 2 T helper cells (Th2) following antigen stimulation.

C. Transcription factors in the Th1/Th2 pathways

Introduction

IL-4 and IL-12 are believed to be the main cytokines polarizing naïve Th cells to Th2 and Th1 pathways, respectively. However, the regulation of Th1/Th2 differentiation is a complicated signal transduction network in which lots of transcription factors are involved, such as STAT, NFAT, c-Maf, NF- κ B, T-bet, and GATA-3 [28, 42, 43]. Among these transcription factors, GATA-3 and T-bet are believed to be the key players for the Th2 and Th1 pathways, respectively.

Signal transducer and activator of transcription (STAT)

The signal transducer and activator of transcription (Stat) family, with six family members (Stat1-6), is a group of transcription factors involved in cytokine-induced cell responses. The binding of a specific cytokine to its receptor can dimerize cytokine receptors and activate the Janus family protein tyrosine kinases (Jaks) associated with the cytokine receptors. Jaks are a group of tyrosine kinases with four family members (Jak1-3 and Tyk2). The activated Jaks can phosphorylate and activate specific Stats. The activated Stats will form dimers, translocate into nuclei, and act as transcription factors to regulate downstream gene expression. Association of IL-4 or IL-13 with its receptors will activate Jak1 and Jak3, which will in turn activate Stat6. Stat6 will up-regulate IgE production, B cell proliferation, and Th2 pathway [44]. In contrast, IL-12 and IL-12 receptor

recognition will activate Tyk2, and then Stat4. Stat4 will subsequently up-regulate IFN- γ expression and the Th1 pathway [44]. Thus, IL-4 and IL-12 can induce the Th2 and Th1 pathways through Stat6 and Stat4, respectively.

Nuclear factor of activated T cells (NFAT)

The NFAT gene family has five members: NFAT1 (NFATp), NFAT2 (NFATc), NFAT3, NFAT4, and NFAT5 [45]. NFAT is a transcription complex composed of subunits that exist in the cytoplasm of unstimulated T cells. Upon T cell stimulation, NFAT translocates to the nuclear where it acts as a transcriptional regulator [43]. NFATc deficient animals showed decreased serum IgE level. NFATc deficient Th (CD4+) cells lost the IL-6-induced IL-4 expression [46]. On the contrary, T cells in atopic patients had high levels of IL-4 but low levels of NFATp binding to the IL-4 promoter [47]. This evidence indicates NFATc and NFATp are positive and negative regulators for the Th2 pathway, respectively.

c-Maf

c-Maf is a basic-leucine zipper (b-Zip) transcription factor, which can bind to AP-1 and CRE sites of the target DNA promoters [48]. c-Maf is a Th2 specific transcription factor and favors Th2 differentiation mainly through IL-4 dependent mechanisms. c-Maf can also stimulate the Th2 pathway through an IL-4-independent and CD25-mediated mechanism

[49]. A c-Maf deficient mice study showed that IL-4 expression was dramatically decreased, but IL-5 and IL-13 expressions were still at normal levels [50]. Thus, c-Maf is not only Th2 specific, but also an IL-4 specific transcription factor.

NF- κ B

In mammals, the NF- κ B transcription factor family has five members: NF- κ B1 (p50/p105), NF- κ B2 (p52/p100), RelA (p65), RelB, and c-Rel [51]. NF- κ B functions as homo- or hetero-dimers dependent on the specific stimuli. In unstimulated cells, NF- κ B dimers reside in the cytoplasm as an inactive form associated with inhibitors of κ B (I κ B). I κ B family members include I κ B α , I κ B β , I κ B ϵ , I κ B ζ , I κ B δ (p100), I κ B γ (p105), and Bcl-3 [51, 52]. In stimulated cells, the NF- κ B dimer dissociates from I κ B and translocates into the nucleus to act as a transcription factor. NF- κ B family members are involved in numerous immune responses, such as innate immunity, T-cell activation, and B-cell activation, depending on the components of the homo- or hetero-dimer [51]. p50 deficient mice were unable to produce Th2 type cytokines such as IL-4, IL-5, and IL-13, and airway eosinophilic inflammation [53]. Thus, NF- κ B is a positive regulator of Th2 differentiation.

T-bet

T-bet is a newly identified transcription factor of the T-box family. T-bet is the only Th1 specific transcription factor found to date that induces IFN- γ expression and inhibits the Th2 pathway [54]. In human asthmatic subjects, the expression of T-bet was much lower than in healthy controls. Furthermore, mice with a targeted T-bet deletion showed spontaneous inflammatory phenotypes without allergen exposure [55]. The Th1 pathway may be induced by IL-12 through the STAT-4 pathway or by IFN- γ through the STAT-1 pathway. Recently, T-bet was found to be induced by IFN- γ through the STAT1 signaling pathway, instead of IL-12 through STAT-4, and upregulated IL-12R β 2 and IFN- γ expression [56]. Thus, T-bet is believed to be the key player of Th1/Th2 differentiation as a Th1 specific transcription factor. In contrast, GATA-3 is believed to be the other key player of Th1/Th2 differentiation, but as a Th2 specific transcription factor. Ectopic expression of GATA-3 or T-bet in human T cells without polarizing cytokines could lead T cells to Th2 or Th1 phenotypes, respectively [57]. The histone hyperacetylation of IL-4 or IFN- γ locus during Th2/Th1 differentiation was dependent on GATA-3 or T-bet, respectively [58]. It was believed that the chromatin remodeling of IL-4 and IFN- γ was initiated by TCR signaling, and was maintained and extended by GATA-3 and T-bet, respectively [59]. The mRNA expression ratio of GATA-3 to T-bet reflected the changes of IL-4 to IFN- γ expression, and could be quantitated by RT-PCR as an index of Th2/Th1 balance [60].

Other transcription factors

Many other transcription factors are also involved in the Th1/Th2 pathways. AP-1, C/EBP β , and JunB are positive regulators of the Th2 pathway, while BCL-6 is a negative regulator of the Th2 pathway [28, 42]. In fact, all transcription factors involved in Th1/Th2 pathways form a signal transduction network and may be important for asthma.

D. GATA-3

GATA family

The GATA family is a group of transcription factors with specific distributions and functions. Six members (GATA-1-6) of the GATA family were originally described in chicken erythroid cells [61]. Their homologues in other species, such as mice and human, have also been found [62]. According to sequence alignment, GATA family members share quite similar sequences. The most striking feature of the GATA family is that they all have a conserved C4 zinc finger structure, through which they can bind to WGATAR (W=A/T, R=A/G) conserved sequence on DNA [63].

The six GATA family members can be classified into two groups based on their expression sites and functions: GATA-1, GATA-2, and GATA-3 belong to the hematopoietic group because they are normally expressed in hematopoietic cells and are required for hematopoietic processes; GATA-4, GATA-5, and GATA-6 belong to the nonhematopoietic group because they are normally expressed in nonhematopoietic cells and involved in cardiogenesis and gut epithelium differentiation [64, 65]. GATA-1 is expressed in hematopoietic cells, spleen, lymph nodes, and thymus. GATA-1 mutations have been implicated in Down syndrome, transient myeloproliferative disorder, and acute megakaryoblastic leukemia [66]. GATA-2 is broadly expressed in almost all kinds of tissues. The functions of GATA-2 and GATA-1 were overlapped for normal hematopoiesis at the yolk sac stage [67]. GATA-4 is expressed in heart,

intestine, and testis. GATA-4 was found to regulate cardiac morphogenesis by inducing N-cadherin gene expression [68]. GATA-6 is expressed in lung, liver, colon, kidney, and spleen. GATA-6, together with GATA-4 was required for adrenal gene regulation and development [69]. GATA-5 as well as GATA-4 and GATA-6 is expressed in heart and intestine, and involved in intestinal gene regulation [70].

GATA-3 expression tissues

GATA-3 was first identified in chicken, where it was found to be expressed in adult erythrocytes and also abundantly expressed in T lymphocytes and brain tissue [61]. Murine and human homologs (mGATA-3 and hGATA-3) to chicken GATA-3 (cGATA-3) were expressed mainly in T cells but not B cells with a great amino acid sequence similarity ($\geq 95\%$) among these three species [71]. mGATA-3 was also expressed in embryonic central nervous system, peripheral nervous system, kidney, thymus, placenta, inner ear, epidermis, lens fibers, whisker follicles, and in the primary palate besides the whole T lymphocyte differentiation process [62, 72]. GATA-3 has been found to be important for embryonic development, for example, the mutation of one GATA-3 allele around the zinc finger domain in humans generated the hypoparathyroidism, sensorineural deafness, renal anomaly syndrome due to haplo-insufficiency [73, 74].

More important, GATA-3 was expressed in naïve spleen Th0 cells at a very low level, inhibited in Th1 cells, and induced and constitutively expressed in Th2 cells differentiated from Th0 cells [75]. According to a conditional knockout study, GATA-3 was required for development and maintenance of Th2 cells in vivo and in vitro [76]. Thus, GATA-3 is a transcription factor specific for Th2 cells.

GATA-3 structure

The mGATA-3 gene contains approximately 23 kb of coding region, 13 kb of 5' promoter region, and 8 kb of 3' flanking region [62]. It is composed of six exons with sizes ranging from 125 bp to 828 bp and five introns with sizes from 418 bp to 6.5 kb [62]. The functional domains of mGATA-3 protein are two zinc fingers (Cys-X₂-Cys-X₁₇-Cys-X₂-Cys): (1) an amino zinc finger (N-finger) (2) a carboxyl zinc finger (C-finger), located in exon 4 and exon 5, respectively [62, 71]. Although both N-finger and C-finger are necessary and important for GATA-3 DNA-binding activity, they may have different specificities. DNase I hypersensitivity assay revealed that the C-finger but not the N-finger was essential for IL-4/IL-13 chromatin remodeling; however, the N-finger was required for IL-5 promoter binding and induction [77, 78]. Normally, the N-finger and the C-finger function together to recognize the GATA motif on the DNA target; however, the N-finger with two basic regions on either side can independently bind to the GATC motif without the help of the C-finger [65].

The range of the GATA-3 promoter region has not been clearly identified. The enhancer region is located within 3 kb surrounding the transcriptional initiation site, including exon 1 and intron 1 [62]. A new promoter region (exon 1a), which is important for Th2 development, was recently identified approximately 10 kb upstream [79]. A silencer region, located around 8.3 to 5.9 kb upstream of the transcription initiation site was also found [80]. Astonishingly, a 625 kb yeast artificial chromosome (YAC), containing ~450 kb 5' and ~150 kb 3' flanking regions of GATA-3 contained insufficient sequence to support fully the tissue-specific expression of GATA-3 in thymus and specific neural crest-derived cells based on a LacZ reporter gene study [81]. Therefore, there are additional regulatory sequences that exist either further upstream or downstream of the 625 kb GATA-3 region.

GATA-3 in T cell differentiation

GATA-3 recognizes and activates the human T cell receptor δ gene (TCR δ) through the GATA motif located on the TCR δ gene enhancer [71]. Similarly, GATA-3 binds to the GATA motif on the enhancer of other TCR genes, such as TCR α and TCR β [82, 83]. Furthermore, the GATA-3(-/-) embryonic stem cells failed to produce CD4/CD8(-/-) T cells in a C57BL/6 complementation systems [84]. This evidence indicates that GATA-3 is important for T cell development.

Besides its function in T cell development, GATA-3 also determines the Th1/Th2 balance, and favors Th2 differentiation. GATA-3 was expressed at low levels in naïve Th cells, induced dramatically in Th2 cells, but inhibited in Th1 cells [75]. Zheng and colleagues have shown that the expression of Th2 type cytokines (IL-4, IL-5, and IL-13) in Th2 cells was inhibited following antisense GATA-3 treatment, while the expression of Th2 type cytokines (IL-4, IL-5, and IL-10) in Th1 cells of GATA-3 transgenic mice was induced. Thus, they concluded that GATA-3 was essential and sufficient for Th2 cytokine expression in CD4⁺ T cells [85]. Furthermore, Zhang and colleagues have shown that GATA-3 and cAMP were necessary for IL-5 expression in a murine thymoma EL-4 cell line through the two overlapping GATA-3 binding motifs on the promoter of IL-5 [75, 86]. Based on the studies of ectopic GATA-3 expression in B cells and antisense GATA-3 treatment in Th2 cells, GATA-3 was sufficient for IL-5 expression, but not for IL-4 expression [87]. The study of retroviral transduction of GATA-3 into developing T cells has shown that GATA-3 was helpful but not sufficient for inducing IL-4 expression to normal levels [88]. According to studies of ectopic expression of GATA-3 in committed Th1 cells and DNaseI hypersensitivity test, GATA-3 induced Th2 cytokine genes expression in Th1 cells and led to chromatin remodeling around IL-4/IL-13 locus [77, 89]. A conserved GATA-3 response element upstream of IL-13 was identified and might be important for GATA-3 binding, histone hyperacetylation, and activation of IL-13 expression [90]. Thus, GATA-3

may regulate IL-5 expression by directly binding to the IL-5 promoter, but regulate IL-4 and IL-13 expression by indirect chromatin remodeling. Besides inducing Th2 cytokine expression, GATA-3 downregulates Th1 cytokines (IL-12 and IFN- γ) expression in developing Th1 cells through an IL-4 independent mechanism [91, 92]. Retroviral expression of GATA-3 in developing Th1 cells could block Th1 development by repressing STAT-4 expression, but had no effect on IL-12 β 2 chain or T-bet expression [93]. Thus, GATA-3 is a key player for T cell development and Th1/Th2 differentiation.

GATA-3 in asthma

It is clear that the Th2 cells correlate with the asthmatic phenotype, such as AHR, eosinophil infiltration and elevated serum IgE. GATA-3 is critical for Th2 cell differentiation. Through regulating IL-5 expression, GATA-3 is associated with airway infiltration by eosinophils. GATA-3 is related to IgE synthesis via regulation of IL-4 and IL-13 expression. Finally, GATA-3 inhibits or even inverses the Th1 pathway by way of IL-12 and IFN- γ expression inhibition. Thus, GATA-3 is an important determinant of Th1/Th2 balance making it a molecule of interest in the study of asthma. GATA-3 expression in the airway of atopic asthmatic patients was significantly higher than in healthy controls and correlated with AHR and reduced airway caliber [94]. GATA-3 expression after allergen challenge in the nasal mucosa of patients was also much higher than controls and

strongly correlated with the number of cells expressing IL-5 [95]. Since GATA-3 is required for the early stages of T cell and nervous system development, GATA-3 gene knockout in mice have defects of embryonic liver hematopoiesis and nervous system, with embryonic lethality [96]. To bypass this barrier, dominant negative mutant and antisense GATA-3 approaches were used. A GATA-3 dominant negative mutant (KRR mutant) was generated by site directed mutagenesis of the KRR amino acid sequence located between the N-finger and the C-finger [97]. Through the overexpression of this dominant negative mutant of GATA-3 in a murine model of asthma, the function of endogenous wild-type GATA-3 was inhibited, which led to the inhibition of airway eosinophilia, mucus production, Th2 cytokines expression, and IgE synthesis in mice sensitized and challenged with ovalbumin [98]. Through a local antisense GATA-3 treatment in a different murine model of asthma, endogenous GATA-3 translation was blocked, which led to the inhibition of allergic airway inflammation and AHR [99]. Thus, GATA-3 is an important factor for asthma, as well as a potential point for asthma therapy.

GATA-3 expression regulation

Although the genes regulated by GATA-3 have been extensively studied, the regulation of GATA-3 itself remains largely unclear. The IL-4-induced STAT-6 pathway is the typical way to induce GATA-3 expression. The initial sources of IL-4 may come from cytotoxic (CD8+) T cells, natural

killer (NK) cells, or naïve Th cells [32, 100]. Ectopic expression of STAT-6 could induce GATA-3 and c-Maf expression, and inhibit IL-12 β 2 chain and IFN- γ expression in the absence of IL-4, which indicated that STAT-6 was upstream of GATA-3 [101]. Ouyang et al found a new STAT-6 independent GATA-3 autoactivation pathway that favored Th2 development in an IL-4-independent manner [102]. This GATA-3 autoactivation might function through the binding of GATA-3 to a GATA motif located within GATA-3 gene [103]. The expression of GATA-3 by STAT-6 dependent or independent pathways can induce Th2 type cytokine expression and determine the Th2 differentiation.

NF- κ B p50 subunit deficient (p50^{-/-}) mice could not generate eosinophilia due to their inability to produce IL-4, IL-5, and IL-13 [53]. Furthermore, CD4⁺ T cells from the p50^{-/-} mice could not produce GATA-3 under Th2 differentiating conditions, and the inhibition of NF- κ B activity blocked GATA-3 expression in developing, but not committed Th2 cells, which indicated that NF- κ B might be an upstream positive regulator of GATA-3 [53]. In a polycomb group gene (Mei18) disrupted mouse, the expression of GATA-3 and Th2 type cytokines were significantly reduced, which indicated that Mei18 might be a positive regulator of GATA-3 [104].

Repressor of GATA-3 (ROG) is a lymphoid specific and GATA-3 interacting protein. The overexpression of ROG inhibited the GATA-3 induced Th2 cytokine expression, and Th1 type cytokines as well [105]. Friend of GATA (FOG), a zinc finger transcription factor, might be another

repressor of GATA-3 because it blocked the GATA-3 induced Th2 cell development [106, 107]. Fetal liver zinc finger protein 1 (Fliz1) could repress GATA-3 expression in vivo and in vitro through binding to an intronic negative cis-acting element of GATA-3 [108]. Runx1, a transcription factor, might be another negative regulator of GATA-3 because expression of Runx1 in T cells inhibited the Th2 differentiation by repressing GATA-3 expression [109]. Transforming growth factor β (TGF- β) could suppress Th2 development through inhibition of GATA-3 expression at the transcription level and Th1 differentiation as well. Thus, TGF- β was a general immune response suppressor [110].

The post-translation regulation of GATA-3 is also important for GATA-3 activity. The increased phosphorylation of GATA-3 and Th2 cytokines expression is due to the activation of p38 mitogen-activated protein kinase by cAMP [111]. The acetylation of amino acids 305-307 (KRR) of GATA-3 was required for its activity because the mutation of these amino acids led to the dominant negative mutant [112]. Thus, while there are many potential mechanisms for GATA-3 regulation, the putative positive and negative regulators need to be further explored.

E. A/J and C3H/HeJ mouse model for asthma

Animal models

The results obtained from human studies can be applied to human disease directly, however, humans are not laboratory animals precluding the use of many experimental techniques. Studying complex genetic traits in humans is even more difficult due to genetic heterogeneity, incomplete penetrance, gene interaction, phenocopies, and rarity [113, 114]. For asthma studies in humans, the availability of human airway tissues, types of medications that can be used, and the difficulty of utilizing repeated invasive procedures in clinical studies are also limiting [115]. To avoid these problems, animal models are widely used in human disease research, as well as in asthma studies. Based on the phenotypic similarity to humans, animal models of asthma can be divided into three classes: animals that have naturally occurring recurrent airway obstruction, such as cats and ponies; animals that have naturally occurring AHR, such as dogs, rats, and mice; animals that develop reversible airway obstruction and/or increased AHR after allergen exposure, such as rabbits, sheep, guinea pigs, rats, dogs, monkeys, and mice [115].

Inbred Mouse models

Although naturally occurring reversible airway obstruction has not been observed in mice, they are still the most widely used animal model for asthma. There are many advantages to using inbred mice instead of

humans for genetic and physiologic studies. Inbred mice have genetic homozygosity (identical alleles) at all genetic loci and genetic identity among individuals. This largely reduces the problem of genetic complexity of humans and facilitates genetic studies. As laboratory animals, mice can be maintained in carefully controlled environments, eliminating the gene-environment interactions that confound the study of human complex genetic diseases. Inbred mice have a short life span (1.5-2.5 years), good litter size (~6-8), and early sexual maturity (~6 weeks), which means we can generate ample numbers of study subjects within a relatively short period. Furthermore, there is extensive information regarding mouse genetics and immunology, thus we can compare the mouse genome and immune pathways to those of humans. Finally, it is relatively easy to manipulate the mouse genome by means of creating congenic mice, transgenics, or mice with gene-targeted deletions (knock-outs) to further probe basic genetic mechanisms.

One thing to keep in mind is that animal models are not identical to humans, so there are limitations that mandate care when applying these data to humans. However, information obtained from inbred mouse models is invaluable to reveal the common pathways of human disease [115].

A/J and C3H/HeJ inbred mouse model

The characteristics of human asthma include airway inflammation, increased IgE level, eosinophil infiltration, and AHR. The A/J and C3H/HeJ mouse model utilized in the current study has all these asthma-related phenotypes. Airway hyperresponsiveness or hyporesponsiveness to acetylcholine (ACh) were observed in A/J or C3H/HeJ mice, respectively, without allergen challenge. Linkage analysis mapped this QTL for non-inflammatory AHR to murine chromosome 6, including IL-5 receptor as a positional candidate gene [116]. Following an allergen exposure protocol (sensitize with ovalbumin (OVA) or phosphate-buffered saline (PBS) on day 0, challenge with OVA or PBS on day 14, and collect data on day 17), A/J mice showed increased airway responsiveness, increased IgE level, and eosinophil infiltration. However, C3H/HeJ had no airway responsiveness increase, much lower IgE increase and limited eosinophil infiltration after allergen exposure [117]. Among these asthma-related phenotypes, AHR was pursued in the current studies because it is an outcome that most closely mimics the clinical manifestations of asthma. AHR was determined in anesthetized mice instrumented to measure airway pressure and flow. The time-integrated rise in peak inspiratory pressure subsequent to intravenous acetylcholine challenge was calculated and reported as the airway pressure time index (APTI). [118].

Without OVA treatment, A/J mice had higher APTI than that of C3H/HeJ mice. Following OVA treatment, the APTI of A/J mice increased

sharply relative to that of untreated A/J mice. In contrast, no significant changes occurred in C3H/HeJ mice following allergen challenge. The APTI of F1 mice (A/J x C3H/HeJ or C3H/HeJ x A/J) was intermediate to the parental strains and no changes occurred following OVA treatment [117]. To determine the inheritance pattern of allergen-induced airway responsiveness the frequency distributions of APTI were measured in C3H/HeJ, A/J, F1, F2 (F1 x F1), A/J backcross ((C3H/HeJ x A/J) F1 x A/J) and C3H/HeJ backcross ((C3H/HeJ x A/J) F1 x C3H/HeJ) mice following OVA sensitization and challenge [117]. A/J backcross mice had a broad APTI distribution, almost covering those of A/J and C3H/HeJ mice; therefore they were suitable for linkage analysis. Thus, to determine the chromosomal locations that contribute to allergen-induced AHR, a genome wide linkage analysis was performed in A/J backcross mice by using 164 microsatellite markers spaced at approximately 10 cM intervals [117]. Two QTL were found on chromosome 2: *Abhr1* (between *D2Mit359* and *D2Mit416*, with lod=4.2) and *Abhr2* (between *D2Mit238* and *D2Mit298*, with lod=3.7). The *Gata3* gene maps within *Abhr1* region. Since *Gata3* is critical in Th1/Th2 differentiation and is related to asthma, it was chosen as the strongest positional candidate gene for *Abhr1*. Complement factor 5 (C5) is a positional candidate gene for *Abhr2* and has been extensively pursued in related studies [119].

F. Summary

Asthma is a chronic airway inflammatory disease due to inappropriate immune responses to common environmental factors, which is characterized by AHR, infiltration of eosinophils, airway obstruction, increased IgE level, increase mucus secretion, and overexpression of Th2 type cytokines (IL-4, IL-5 and IL-13). The differentiation of naïve T helper cells into Th2 cells is believed to be critical in asthma. Among many transcription factors involved in Th1/Th2 pathways, GATA-3 is a key player in driving the Th2 pathway and thus, may be related to asthma. A genome-wide linkage analysis was performed in A/J backcross mice ((C3H/HeJ x A/J) F1 x A/J) revealed two QTL on chromosome 2: *Abhr1* and *Abhr2*. The *Gata3* gene maps within the *Abhr1* region. Because of its location and function, *Gata3* was considered the most important positional candidate gene for *Abhr1*. To investigate its role in allergen-induced AHR in our mouse model, *Gata3* was studied at DNA, mRNA, and protein levels in this project.

Chapter Two: Fine-mapping of *Abhr1* and *Abhr2* loci

A. Introduction

Prior to the initiation of this dissertation research, two quantitative trait loci (QTL) for allergen-induced AHR were identified on murine chromosome 2: *Abhr1* (between *D2Mit359* and *D2Mit416*, with lod = 4.2) and *Abhr2* (between *D2Mit 238* and *D2Mit298*, with lod = 3.7). This was accomplished by means of a genome-wide linkage analysis for airway hyperresponsiveness (AHR) in 176 A/J backcross mice ([A/J x (C3H/HeJ x A/J)F1] or [(C3H/HeJ x A/J)F1 x A/J]) using 164 microsatellite markers spaced at approximately 10 cM intervals [117]. Based on the current understanding of the immunologic basis of asthma, GATA-3 was the most attractive positional candidate gene in the *Abhr1* region. To more closely determine the locations of *Abhr1* and *Abhr2* relative to GATA-3, these QTL were fine mapped by genotyping more A/J backcross mice (n = 450) for additional densely clustered markers (n = 37) on chromosome 2.

Many types of DNA sequence-based markers are available: (1) restriction fragment length polymorphisms (RFLP), based on the different length of DNA fragments following endonuclease digestion, (2) single nucleotide polymorphisms (SNP), based on alternate nucleotides at a single position, (3) sequence-tagged sites (STS), which are also due to the DNA sequence polymorphisms and can be identified through polymerase chain reaction (PCR), which include expressed sequence tag (EST) and microsatellites [120]. EST are a subset of STS markers that

can be expressed into mRNA. Microsatellites (or simple sequence repeats, SSR) are a subset of STS markers, with variability in the copy number of a tandem repetition of a short DNA sequence, usually 2 to 4 nucleotides [121]. They are ubiquitously distributed throughout the genome with high polymorphism information content, and are relatively easy to be analyzed by PCR. Furthermore, a comprehensive database of murine microsatellite markers has been characterized in 12 of inbred strains by the Massachusetts Institute of Technology (MIT) Genome Center (www-genome.wi.mit.edu). Primers for these markers are commercially available (Research Genetics, Inc., Huntsville, AL). Based on these attractive features, microsatellite markers were most commonly used for genotyping in this project.

B. Massachusetts Institute of Technology (MIT) microsatellite markers

The Massachusetts Institute of Technology (MIT) Genome Center (www-genome.wi.mit.edu) identified 6,331 microsatellite markers, mapped on an Ob (C57/6J-Ob, an obese variant of C57 black mouse) x Cast (*Mus castaneus*) F2 intercross with an average resolution of 1.1 cM. The polymorphic repeats were characterized on a series of inbred mouse strains, including A/J and C3H/HeJ strains. Those MIT microsatellite markers located on chromosome 2 and with polymorphisms between A/J and C3H/HeJ strains were chosen for fine mapping the *Abhr1* and *Abhr2* loci. Thirty-one MIT microsatellite markers met these criteria (Table 1).

Table 1. MIT microsatellite markers on chromosome 2

Marker	Primers (5' - 3')	PCR annealing temp. (°C)	A/J size (bp)	C3H/HeJ size (bp)
<i>D2Mit355</i>	(Forward) GTCTTCCTTTGGGTAGTTCTCG	56	142	147
	(Reverse) AAATAAATGTCTGCCTCTTTCCC			
<i>D2Mit359</i>	(Forward) GGATCTAGAGATACGTTACATTCCTT	51	96	106
	(Reverse) TGAAATCTAACTCTGGTTGAAAAGC			
<i>D2Mit117</i>	(Forward) CCCAAAGAACATACATCAATGTG	55	170	176
	(Reverse) TGGAGATGCATGTTTAAACTCA			
<i>D2Mit31</i>	(Forward) ATTTGAAAAGACTGGACTCCTCC	56	140	136
	(Reverse) TGA CTGGGAATAACCTCTCTGC			
<i>D2Mit416</i>	(Forward) CCGACATTTGGTTGTGTATACG	55	116	124
	(Reverse) ATGTATGTGTTTCCCATGTGTACC			
<i>D2Mit6</i>	(Forward) AACAAACAAACCCCTTGCCC	59	124	132
	(Reverse) CTCTAACACAGCCCCAGGTG			
<i>D2Mit80</i>	(Forward) TAGCCTACAGAGTGGACAGCC	52	180	192
	(Reverse) CTAGGCTTTATGTAGCCTCTTTGC			
<i>D2Mit465</i>	(Forward) CCTTGGGGTTTTGATTACCA	55	122	128
	(Reverse) TCTAACTTCCCTGCCTCCC			
<i>D2Mit60</i>	(Forward) TGCCAGACAATGAACTCTGG	55 ^a	148	150
	(Reverse) AGAGGGTTCATCCTTGAGG			
<i>D2Mit81</i>	(Forward) ATGAGTTCTGCGGGCACTAT	55	156	150
	(Reverse) GCCCTACTAACTAATCAGAATGCA			
<i>D2Mit293</i>	(Forward) TACACACTTGGGATAAAGTAAGTGTG	55	194	180
	(Reverse) ATTCAGCACCTTTAGCCACA			
<i>D2Mit82</i>	(Forward) GGACAATGGCTCTGGGTTTA	61-53 ^b	214	198
	(Reverse) GCTTTCTAACCTCAAGCATTAAGC			
<i>D2Mit417</i>	(Forward) GTTCTTATTTTACTGGGGTCATGG	55	128	122
	(Reverse) TGGGTCACCTTAACAAGAATAATT			
<i>D2Mit152</i>	(Forward) CACAGATCTTGTAAGACCACGTG	53	107	113
	(Reverse) TGCCATGAGTGTGGGACTAA			
<i>D2Mit235</i>	(Forward) AGCACTGCCAACTGAGCC	52	126	120
	(Reverse) TCTTTCCCAATTTTGTTC			
<i>D2Mit367</i>	(Forward) GCCTGTGCTAAAAAAGAGGTG	55	162	149
	(Reverse) GCCCTGAGAACTACCCTCCT			
<i>D2Mit238</i>	(Forward) ACCATCATTTTTGAACTCCACC	57	153	135
	(Reverse) ACGCATATGCTCAGGACACA			
<i>D2Mit203</i>	(Forward) CACAAATTGCCCTCTAACTTCC	54	139	149
	(Reverse) GAATCTTTCCCCGGGATTAA			

Table 1 (Cont'd).

Marker	Primers (5' - 3')	PCR annealing temp. (°C)	A/J size (bp)	C3H/HeJ size (bp)
D2Mit298	(Forward) AGGGGACGAACTCTGGTTT	55 ^a	128	124
	(Reverse) AGCCAGGGCTACACAGAGAA			
D2Mit323	(Forward) AGAATCCTAAGTGGTGGTTAGAGG	55	126	110
	(Reverse) ACCCAAAGTTGTCTTTAAGTACACA			
D2Mit182	(Forward) CTGAGAGCAAATCCCTCCTG	55 ^a	147	151
	(Reverse) GAGAAACAGATGAGTAGACCCCC			
D2Mit380	(Forward) CCTCAGGTCTGAAATGAGGTG	51	132	120
	(Reverse) AATGATGTGCATGTGCGC			
D2Mit91	(Forward) CCATTCTTTGCTTCTGTCTCTG	55	180	174
	(Reverse) CCACCTTTGCAAAATACATGC			
D2Mit11	(Forward) CAAACCCCCAGCTCTCTCTT	57 ^a	232	226
	(Reverse) CCATACCCAGGCTCCATCTA			
D2Mit10	(Forward) CATCAGGAAACACAGGACCA	55 ^a	156	150
	(Reverse) ACCTAACCTAATGATGGGG			
D2Mit63	(Forward) GCAGTCTACCAGGAGCAACC	53 ^a	232	218
	(Reverse) TGGATGTAGGCATGTGCCT			
D2Mit307	(Forward) AGTTCCAGGGAATGAAACACC	57	150	172
	(Reverse) ACACCTCTGCCAACAGTGC			
D2Mit451	(Forward) CATTAGATAGACTGGGCAAGGG	57	116	142
	(Reverse) TCCTCCCTCCAAACCCTC			
D2Mit53	(Forward) GTGGACATTCCCTGAGAAACA	57 ^a	148	150
	(Reverse) GGGGTTTGATCAGCTCATGT			
D2Mit113	(Forward) CTCACGTGAGGGTCATGAGA	55	128	146
	(Reverse) CTTCTCTACCTTCCTCAGAAGCC			
D2Mit200	(Forward) ATGGCCTCTGCTAAATGGTG	57	136	114
	(Reverse) GCTAGCAGGAGCGTCATAGG			

a. Amplicons with small size differences (≤ 4 bp) between strains were amplified with incorporation of [α -³³P] dCTP and separated on acrylamide gels. All other primer pairs were amplified without radionuclides and separated on agarose gels.

b. Two-step PCRs were performed with two continuous annealing steps due to T_m differences within the primer pair.

C. Self-designed microsatellite markers

Since the number of informative MIT microsatellite markers on mouse chromosome 2 was limited, more markers were required for further fine-mapping of the *Abhr1* and *Abhr2* regions. The Celera Database (www.celeraDiscovery.com) was searched to obtain the genomic sequences of several genes (*Gata3*, *Prkcq*, *Il2ra*, *Mrc1* and *Gad2*) located in the *Abhr1* and *Abhr2* regions. We focused on the most common microsatellites positions, which are normally located in the intron region of the genes. After identifying simple nucleotide tandem repeats (≥ 15 repeats), we designed primer pairs covering the repeat region, amplified the regions in A/J, C3H/HeJ, and F1 (C3H/HeJ x A/J) mice, and compared the product sizes on 4.5% agarose gels. We were able to detect product size differences as small as 4 bp by this method. Clean products with different band sizes in A/J and C3H/HeJ strains were indicative of sequence polymorphisms in the target region. To confirm potential polymorphisms, we sequenced the PCR products of A/J and C3H/HeJ strains. For example, four pairs of primers covering four different simple tandem repeats regions of the *Gad2* gene were designed and tested by PCR, one of the primer pairs showed a clear product size difference between A/J and C3H/HeJ mice. The PCR products of this primer pair were sequenced: the A/J product was 119 bp, with 16 tetranucleotide (CTTT) repeats, while the C3H/HeJ product was 107 bp, with 13 CTTT repeats (Figure 2).

(A) A C F A C F A C F A C F

| 1 | 2 | 3 | 4 | Primer pair #



(B) Primer pair # 4:

CACATTGTAA AAGTATCCCT TGGGATTGGT TTTCTTTCTT

(Forward primer)

TCTTTCTTTC TTTCTTTCTT TCTTTCTTTC TTTCTTTCTT

(CTTT tetranucleotide repeats)

TCTTTCTTTC TTTCTTTAAG ATTTGCTTAT TTAATGTGA

(Reverse primer)

(C)

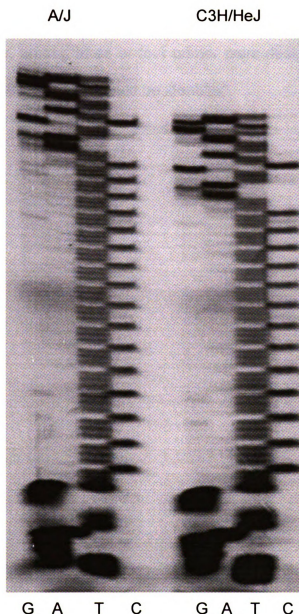


Figure 2. Processes of identifying microsatellite markers in *Gad2*. (A) Four primer pairs within the *Gad2* gene were tested in A/J (A), C3H/HeJ (C), and (C3H/HeJ x A/J)F1 (F) mice. PCR products were separated on a 4.5% agarose gel. A strain-specific size distinction was apparent in primer pair 4, while smaller differences may be present in primer pairs 1 and 3. (B) The location of the forward and reverse primer in relationship to the target sequence is shown. The target sequence was based on Celera database. (C) Sequencing gel of the primer pair #4 PCR products of A/J and C3H/HeJ. A/J has 16 CTTT repeats, and C3H/HeJ has 13 CTTT repeats.

Using the same methods, four other microsatellite markers were found in the intron regions of *Gata3*, *Prkcq*, *Il2ra* and *Mrc1* genes (Table 2). Typically, among three or four primer pairs designed for each gene, one microsatellite marker could be identified.

Table 2. Self-designed microsatellite markers

Marker	Primer pair (5' - 3')	PCR annealing temp. (°C)	A/J size (bp)	C3H/HeJ size (bp)
<i>Gata3</i>	(Forward) AGTGTAGACAGCGCAAAGT	56	192	196
	(Reverse) CCTAGAATTCTATGGCTCCGT			
<i>Prkcq</i>	(Forward) GGCCAAGGAACTCTAAAC	51	310	322
	(Reverse) TCCCACCCACAGAAATCATAC			
<i>Il2ra</i>	(Forward) GATGCGGCATGGTCAG	53	315	311
	(Reverse) CCATCCTAGCATAGGCGTTGT			
<i>Mrc1</i>	(Forward) AAAAATCCAATAGAAGTTTGT	51	318	313
	(Reverse) ATATGTTCTCCACATCTATC			
<i>Gad2</i>	(Forward) CACATTGTAAAAGTATCCC	44	119	107
	(Reverse) TCACATTAAATAAGCAAATCT			

D. Hemolytic complement (*Hc*) RFLP marker

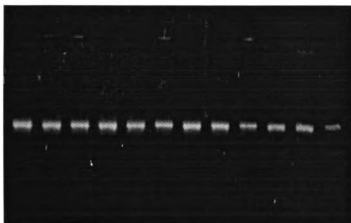
The murine *Hc* gene is an important positional candidate gene in the *Abhr2* region. Its human analog is the fifth complement component (*C5*) gene. It has been found that a two base pair (TA) deletion in a 5'-exon of *Hc* results in *C5* deficiency in *A/J* mice, however, *C3H/HeJ* *Hc* has no such deletion and thus is *C5* sufficient [122]. Subsequently, *A/J* mice have low *C5* gene expression, *C3H/HeJ* mice have relatively high *C5* gene expression, as determined by microarray analysis [119]. A primer pair, based on the primer pair designed by Wetsel [122], was designed to amplify *Hc* genomic DNA that covers the two base pair deletion region (forward primer: 5'-CAATTAAAGCTAACTATAAGAAGGATTTTACAA-3' and reverse primer: 5'-CAAGTTAGAACTAAGCACTAGCTACTCAAACAA-3'). The 2 bp deletion in *A/J* mice produces the endonuclease *BsgI* recognition site. *C3H/HeJ* mice have no such recognition site and thus still have a single 210 bp product; the *A/J* amplicon can be digested by *BsgI* to form two fragments of 180 bp and 28 bp sizes, respectively (Figure 3).

(A)

(*BsgI* cutting site) ↓
CAATTAAAGC TAACTATAAG AAGGATTTTA CAACAACTGG
(Forward primer) →
AACTGCATAC TTTGAAATTA AAGAATATGG TAATCTCTAA
(*BsgI* recognition site & TA deletion site)
TATTAATATA TTATTATAAC TAAGAGGGAT TTGCAGTAC
CTTTGGTATG ACTGGAAGTA CCAGAAGAGG GTGGGGGGAG
ACAGATGTAC GTAGTCATTG TTTGAGTAGC TAGTGCTTAG
(Reverse primer)
TTCTAACTTG

(B)

1 2 3 4 5 6 7 8 9 10 11 12



(C)

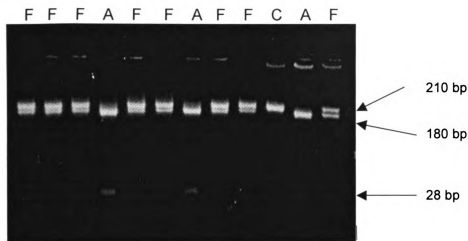


Figure 3. *Hc* genotyping (A) *Hc* primers, *BsgI* recognition and cutting sites, and target sequence. (B) PCR products from 12 A/J backcross mice were separated on a 1.5% agarose gel. (C) Fragments after *BsgI* digestion were separated on a 4.5% agarose gel. Strain specific product sizes were C3H/HeJ (C) = 210 bp, A/J (A) = 180 bp + 28 bp, and (C3H/HeJ x A/J)F1 (F) = 210 bp + 180 bp + 28 bp.

E. Polymerase chain reaction-based genotyping

Polymerase chain reactions were performed on 31 MIT microsatellite markers, 5 self-designed microsatellite markers, and the *Hc* RFLP marker. Since polymorphisms existed in these markers between A/J and C3H/HeJ strains, the sizes of the PCR products differed between these two strains. The smaller PCR product migrates faster and the larger product migrates slower during gel electrophoresis, thus A/J homozygotes (A), C3H/HeJ homozygotes (C), or A/J and C3H/HeJ heterozygotes (F) were distinguishable by this method (Figure 4).

Using the polymerase chain reaction-based genotyping method, 37 markers (36 microsatellite markers and 1 *Hc* RFLP marker) on chromosome 2 were genotyped on 450 A/J backcross mice with extreme APTI values ($APTI < 620$ or $APTI > 797$ cmH₂O.sec) (Table 8 and Table 9). On average, the 13 markers in the *Abhr1* region (0 – 11 cM) were genotyped in 408 mice, the 16 markers in the *Abhr2* region (11 – 40 cM) was genotyped in 343 mice, and each of the other 8 markers in more distant region (40 – 90 cM) was genotyped in 108 mice. 25 double crossover sites were found for all genotyping data (N = 11656) at rate of 0.2% (25/11656). Because double crossover sites may be the result of genotyping error, they were double checked by re-genotyping. Based on re-genotyping of these 25 double crossovers, 18 were reconfirmed and kept as original data, 4 were not sure and deleted from database, and 3 were genotyping errors and changed to the right data.



Figure 4. Genotyping microsatellite marker *D2Mit416*. PCR products were separated on a 4.5% agarose gel. Lanes identifiers are as follows: L = 100 bp DNA ladder, B = blank, A = A/J, C = C3H/HeJ, F = (C3H/HeJ x A/J)F1. The A/J amplicon is 116 bp and the C3H/HeJ amplicon is 124 bp.

F. Multipoint linkage analysis

The data from 37 markers genotyped in 450 A/J backcross mice were analyzed using MAPMAKER software [123, 124]. MAPMAKER/EXP 3.0 was used to perform multipoint linkage analysis on the raw genotyping data and to calculate the map order and map distance. MAPMAKER/QTL 1.1 was used to link phenotype with genotype and identify QTL by interval mapping and simultaneous search techniques (Table 9). Multipoint linkage analysis was also performed by the JoinMap 3.0 program [125]. The order, distance, and lod score of the markers were calculated by the JoinMap program (Figure 5).

Lod scores above 3.3 (P value = 1.0×10^{-4}) were accepted as significant evidence for linkage; lod scores above 1.9 (P value = 3.4×10^{-3}) were considered as suggestive evidence for linkage [126]. The first QTL, *Abhr1*, was maximized at *D2Mit355* (2.3 cM, lod = 4.42); the second QTL, *Abhr2*, was maximized at *D2Mit238* (19.9 cM, lod = 3.55). The interval of *Abhr1* region was narrowed to about 1 cM (13 markers/11 cM). GATA-3 is located within *Abhr1* region, with the lod score of 4.26.

MAPMAKER is a MS-DOS based program that can be downloaded without cost from the MIT Genome Center website. Chromosome cross-over positions are most clearly depicted in the MAPMAKER program (Table 9). JoinMap is a Windows-based program. JoinMap advantages over MAPMAKER include a user-friendly interface and superior graphics data display capabilities (Figure 5). Both linkage analysis programs are

based on multipoint interval linkage analysis. Comparing the results of these two programs, the relative position of markers and lod scores match quite well.

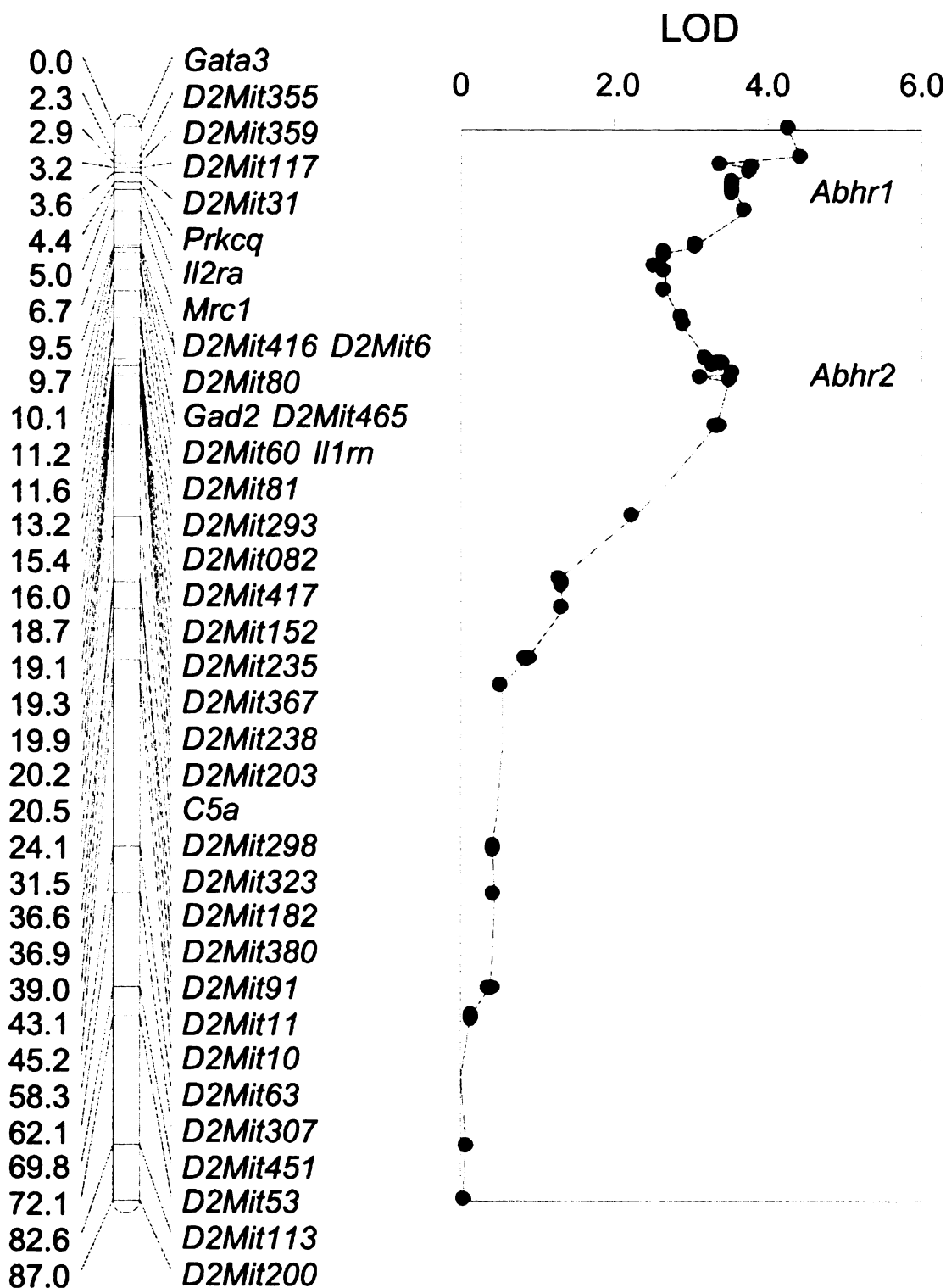


Figure 5. Mouse chromosome 2 genetic linkage map. The map was drawn using JoinMap program. Markers and their genetic locations are listed on the left figure, the lod scores of these markers are shown on the right figure.

G. Comparison of our linkage results to published linkage maps

We compared our map constructed of 31 MIT microsatellite markers genotyped on A/J backcross mice [A/J X (C3H/HeJ X A/J) F1 and (C3H/HeJ X A/J) F1 X A/J] with the MIT Genome Center map based on the same microsatellite markers (except *D2Mit60*, whose information comes from the Jackson lab) in an Ob (C57/6J-Ob, an obese variant of C57 black mouse) x Cast (*Mus castaneus*) F2 intercross mice. Overall, the order and relative spacing of our markers matched quite well with the MIT genetic linkage map (Table 3). Comparison between these two maps indicated that the markers within the *Abhr1* and *Abhr2* regions were particularly well aligned, with the exception of the distance between the most centromeric two markers, which was smaller in our dataset than in the MIT map. Indeed, our map was more detailed due to the large number of mice ($n = 450$) genotyped in the *Abhr1* and *Abhr2* regions, compared with that of the MIT map. For example, *D2Mit359*, *D2Mit117*, and *D2Mit31* were distinguished at 2.9, 3.2, and 3.6 cM, respectively in our linkage map, while these three markers were indistinguishable at 7.7 cM in the MIT map. The inter-map alignment was not as highly conserved for markers in the more distal portion of chromosome 2, which may be due to the smaller number of mice genotyped ($n = 108$) through this region. The orders of two pairs of neighboring markers (*D2Mit380* and *D2Mit91*, and *D2Mit11* and *D2Mit10*) were inversed on our map as compared to the MIT map.

DNA markers can be classified into Type I and Type II markers. Type I markers are located within genes, while Type II markers are located outside genes. We genotyped 7 Type I markers in A/J backcross mice: 5 self-designed microsatellite markers in *Gata3*, *Prkcq*, *Il2ra*, *Mrc1*, and *Gad2* respectively, 1 RFLP marker in *Hc*, and the *D2Mit60* marker that located in *Il1m* gene. We compared our Type I marker genetic map with The Jackson Laboratory (JAX) T31 radiation hybrid (RH) mouse mapping panel (www.jax.org). The JAX RH database integrates its RH mapping data with many other databases, such as the Whitehead Institute (www.wi.mit.edu), the MRC Mouse Genome Center (www.mrc.ac.uk), and Genoscope CNS (www.genoscope.cns.fr). Thus, the JAX map is a composite linkage map, with the data derived from many different experiments in different mouse strains. This likely contributed to the disparity we observed in marker order between our map and JAX, as two pairs of neighboring genes had reversed orders (Table 4). However, the relative map distances were surprisingly consistent for 5 of the 7 genes. Furthermore, our linkage map order is identical to those of the Celera and UCSC (genome.ucsc.edu) physical maps (Table 4). Physical maps are based on the absolute DNA sequences, therefore, they are the standards for the relative orders of markers. In contrast, genetic linkage maps are based on the frequency of chromosome crossovers during meiosis. Although the genetic linkage map distances between markers are relative and may vary due to the number of meioses genotyped, the relative

orders should align with the physical maps. Based on the conservation of marker order between our map and the Celera and UCSC physical maps, we believe that our genetic linkage map is accurate and is a valuable tool for genetic studies.

Table 3. Comparison of our linkage results with MIT linkage map

Marker	Current study		MIT linkage map (cM)
	Lod score	Linkage map (cM)	
<i>D2Mit355</i>	4.42	2.3	2.2
<i>D2Mit359</i>	3.39	2.9	7.7
<i>D2Mit117</i>	3.79	3.2	7.7
<i>D2Mit31</i>	3.76	3.6	7.7
<i>D2Mit416</i>	3.06	9.5	12
<i>D2Mit6</i>	3.06	9.5	12
<i>D2Mit80</i>	3.06	9.7	12
<i>D2Mit465</i>	2.64	10.1	12
<i>D2Mit60</i>	2.52	11.2	10 ^a
<i>D2Mit81</i>	2.66	11.6	14.2
<i>D2Mit293</i>	2.66	13.2	15.3
<i>D2Mit82</i>	2.87	15.4	17.5
<i>D2Mit417</i>	2.9	16	20.8
<i>D2Mit152</i>	3.2	18.7	23
<i>D2Mit235</i>	3.37	19.1	24
<i>D2Mit367</i>	3.29	19.3	26.2
<i>D2Mit238</i>	3.55	19.9	27.3
<i>D2Mit203</i>	3.13	20.2	27.3
<i>D2Mit298</i>	3.34	24.1	29.5
<i>D2Mit323</i>	2.25	31.5	31.7
<i>D2Mit182</i>	1.28	36.6	38.3
<i>D2Mit380</i>	1.32	36.9	40.4
<i>D2Mit91</i>	1.33	39	38.3
<i>D2Mit11</i>	0.86	43.1	42.6
<i>D2Mit10</i>	0.54	45.2	41.5
<i>D2Mit63</i>	0.43	58.3	54.6
<i>D2Mit307</i>	0.44	62.1	61.2
<i>D2Mit451</i>	0.38	69.8	73.2
<i>D2Mit53</i>	0.14	72.1	84.2
<i>D2Mit113</i>	0.07	82.6	87.4
<i>D2Mit200</i>	0.04	87	97.3

a. *D2Mit60* is located within the *Il1m* gene. Subsequent to our data collection, *D2Mit60* was removed from the Whitehead Institute website (www.wi.mit.edu). Therefore, its map location comes from the JAX database (www.jax.org).

Table 4. Comparison of our linkage results with Jackson lab RH map, Celera physical map, and UCSC physical map

Marker	Lod score	Linkage maps (cM)		Physical maps (bp)	
		Current study	JAX RH	Celera	UCSC
<i>Gata3</i>	4.26	0	7	6,795,337 - 6,815,972	9,795,868 - 9,815,813
<i>Prkcq</i>	3.54	4.4	2	8,093,753 - 8,222,740	11,110,400 - 11,239,245
<i>Il2ra</i>	3.55	5	6.4	8,560,395 - 8,608,396	11,646,316 - 11,696,658
<i>Mrc1</i>	3.71	6.7	5	11,132,506 - 11,233,984	14,231,672 - 14,334,048
<i>Gad2</i>	2.64	10.1	9	19,352,183 - 19,421,655	22,825,513 - 22,894,186
<i>Il1m</i> (D2Mit60)	2.52	11.2	10	21,056,292 - 21,070,434	24,603,317 - 24,617,939
<i>Hc (C5a)</i>	3.5	20.5	23.5	31,677,653 - 31,755,923	35,256,298 - 35,334,364

H. Positional candidate genes

Our genetic linkage map indicated that the *Abhr1* region spans 0 – 11 cM and the *Abhr2* region is from 11 – 40 cM on murine chromosome 2. Based on the Celera mouse database (www.celeradiscoverysystems.com) and the Jackson lab mouse database (www.jax.org), there are approximately 200 genes located within the *Abhr1* and the *Abhr2* regions (Table 10).

Based on their locations relative to the *Abhr* QTL and functions, several positional candidate genes were identified (Table 5). The *Gata3* gene was prioritized and selected for further study based on its located within the *Abhr1* region (lod = 4.26) and its involvement in the Th2 pathway.

Table 5. Positional candidate genes on chromosome 2

Positional candidate gene	Position (cM)	Human homologue	Functions
Protein Kinase C, theta (<i>Prkcq</i>)	2.0	PRKCQ 10p15	TCR-mediated T cell activation
Macrophage Mannose Receptor, C type 1 (<i>Mrc1</i>)	5.0	MRC1 10p13	Recognize glycoprotein antigens
Interleukin 15 Receptor, alpha chain (<i>Il15ra</i>)	6.4	IL15RA 10p15-p14	NK cells, CD8+ T lymphocytes development
Interleukin 2 Receptor, alpha chain (<i>Il2ra</i>)	6.4	IL2RA 10p15-p14	T cells proliferation
GATA-binding Protein 3 (<i>Gata3</i>)	7.0	GATA3 10p15	Induce Th2 pathway
Interleukin 1 Receptor Antagonist (<i>Il1rn</i>)	10.0	IL1RN 2q14.2	Inhibit IL-1 signal transduction
Complement Component 8, gamma subunit (<i>C8g</i>)	12.3	C8G 9q34.3	Component of complement attack complex
Hemolytic Complement (<i>Hc</i>)	23.5	C5 9q34.1	Component of complement system
Lipocalin 2 (<i>Lcn2</i>)	27.0	LCN2 9q34	Transport LPS and formylpeptides
Prostaglandin-endoperoxide Synthase 1 (<i>Ptgs1</i>)	29.0	PTGS1 9q32-q33.3	Synthesize prostaglandin

I. Summary

To fine map the *Abhr1* and *Abhr2* loci, we identified 31 informative MIT microsatellite markers and 1 RFLP marker, and designed 5 microsatellite markers within 5 genes on murine chromosome 2, concentrating on the regions around *Abhr1* and *Abhr2*. We genotyped these 37 markers in up to 450 A/J backcross mice and created a refined linkage map of murine chromosome 2. GATA-3 was located within *Abhr1* region (lod = 4.26), which is a strong positional candidate gene in our mouse model. Thus, further testing of DNA, mRNA, and protein of GATA-3 in A/J and C3H/HeJ were required.

Chapter Three: Comparison of *Gata3* sequence in two murine strains

A. Introduction

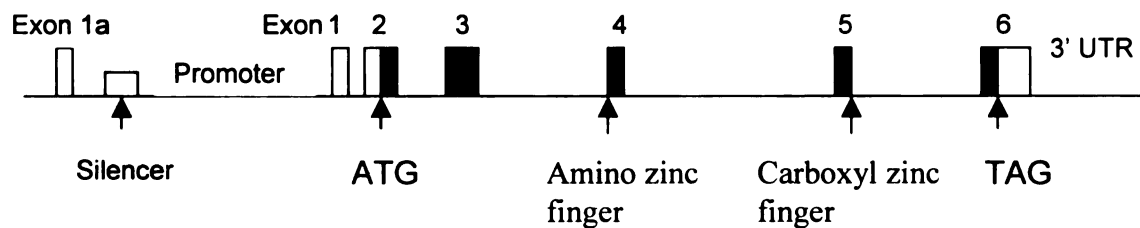
According to the refined linkage map described in chapter 2, *Gata3* is located within the *Abhr1* region (lod = 4.26) on murine chromosome 2. GATA-3 also has a tight relationship with the Th2 pathway and asthma. Thus, it was selected as the strongest positional candidate gene for allergen-induced AHR in our mouse model of asthma.

To investigate the role of GATA-3 in allergen-induced airway hyperresponsiveness (AHR), the first step was to compare its gene sequence between A/J and C3H/HeJ mice to determine whether DNA polymorphisms were present.

Gata3 was sequenced at the MSU Genomics Technology Support Facility (genomics.msu.edu) using the fluorescence-labeled dideoxy method. Coding regions are translated to protein and variations in these regions are most likely to have functional consequences; therefore, we first sequenced the coding region. Furthermore, to ensure the sequence accuracy, we repeated the sequencing reactions of the coding region using the radioactivity-labeled dideoxy method. Because errors of splicing sites may lead to altered protein products, we also sequenced all 5' and 3' splicing sites. Since the protein expression is regulated by the promoter region, we sequenced the identified promoter region. We also sequenced the 3' untranslated region (3' UTR), as it is involved in mRNA transportation and stability.

B. *Gata3* gene structure

The *Gata3* gene contains approximately 23 kb of coding region, 13 kb of 5' promoter region, and 8 kb of 3' flanking region [62]. It is composed of six exons with size ranging from 125 bp to 828 bp and five introns with sizes from 418 bp to 6.5 kb [62]. The functional domains of GATA-3 protein are two zinc fingers: an amino zinc finger and a carboxyl zinc finger, located in exon 4 and exon 5, respectively (Figure 6) [62, 71]. The range of the *Gata3* promoter region has not been clearly identified. The enhancer region is located within 3 kb surrounding the transcriptional initiation site, including exon 1 and intron 1 [62]. A new promoter region (exon 1a), which is important for Th2 development, was recently identified approximately 10 kb upstream [79]. A silencer region, located around 5.9 to 8.3 kb upstream of the transcription initiation site was also found [80]. Astonishingly, even the 625 kb YAC, containing ~450 kb 5' and ~150 kb 3' flanking regions of *Gata3* is still not enough to support fully the tissue-specific expression of *Gata3* [81].



Component	Size and characteristics	Component	Size and splicing sites
Exon 1	189 bp, 5' noncoding end	Intron 1	420 bp, with GT...AG
Exon 2	632 bp, with start codon (ATG)	Intron 2	2.5 kb, with GT...AG
Exon 3	537 bp	Intron 3	5.5 kb, with GT...AG
Exon 4	146 bp, with amino zinc finger	Intron 4	6.5 kb, with GT...AG
Exon 5	126 bp, with carboxyl zinc finger	Intron 5	4.5 kb, with GT...AG
Exon 6	802 bp, with stop codon (TAG)		

Figure 6. *Gata3* gene structure. The size and characteristics of *Gata3* exons come from published sequence [62]. Intron sizes and characteristics are based on the Celera database (www.celeradiscoverysystems.com).

C. Sequencing of *Gata3*

Primer pairs were designed for the coding region based on the sequence published at www.ncbi.nlm.gov (entry number: NM_008091). Primers for the promoter region come from BALB/c mice *Gata3* sequence published by George KM [62]. The primers for the introns, 3' UTR, and the remaining regulatory regions were based on the sequence of 129X1/SvJ, 129S1/SvImJ, DBA/2J, and A/J strains from the Celera database (www.celeradiscoverysystems.com). In total, 24 pairs of primers were designed to cover *Gata3* exons, splicing sites, 3' UTR region and parts of the promoter regions (Table 6).

Table 6. Sequencing primers for *Gata3*

Primer pair (5'-3')	Position	Product size (bp)
(Forward) CCCACCCGGTCCAAC (Reverse) ACCCCAAGTTCTGCTAGCTC	Exon 1a, -9371 to -8753	619
(Forward) CCTCCAGTTCCAATATACCA (Reverse) CCCTTCGGTCCTACTCAC	Silencer, -8307 to -7212	1096
(Forward) GGAACCCAACCCGTACCTT (Reverse) ATTTGGAAGCCCTGATTGAA	Promoter, -4566 to -3559	1008
(Forward) CGGAGTCAGGTTCCGGTTGTT (Reverse) TCCGGGCCTCGTTAAG	Promoter, -3674 to -2943	732
(Forward) AGGCAGCAGCGGCAACA (Reverse) TCCAGCCTGTAGGGGGTATTT	Promoter, -3130 to -2462	669
(Forward) GCGGACCGGCTGGGAATTAC (Reverse) CTAAAAGGCCCGGGATTAAGA	Promoter, -2507 to -1929	579
(Forward) GAAGCTGTGAAGTAGGGA (Reverse) CCCCTTTTACATCTGATCTT	Promoter, -2043 to -1600	444
(Forward) CTGGCAGCTTGATTGG (Reverse) TTGGCTTTAGAGGGTTACTG	Promoter, -1816 to -1308	509
(Forward) GAAAGGGCTTGTCGGGAATC (Reverse) TGGACCCAGCCGCAACTTC	Promoter, -1413 to -938	476
(Forward) CCTCTCTACTGGGCGTCTTC (Reverse) CCGCGTTATTGTTATGGTCT	Promoter, -1045 to -625	421
(Forward) CATCGCGTTGTCTACTAAGGTC (Reverse) CATTCCGGGCTCAGTAGATT	Promoter, -757 to -344	414
(Forward) GGCAGCCGGGTTTCACTCGTA (Reverse) CCCGGTCAGATTGCGTAGCTC	Promoter, -389 to 143	532
(Forward) GGCGCGGATGACACTAGAAC (Reverse) CTGCAGGGCCGACTCAC	Exon 1 & Intron 1 (5' splicing site), -50 to 521	571
(Forward) CTTTTGGAGGATTTGTAGTG (Reverse) TCTTTGCGGGATAGTTTAG	Intron 1 (3' splicing site), 326 to 815	490
(Forward) CCCTTGCAGGTGATCGGAAGA (Reverse) GGACCCAGGAAAACAAACGC	Exon 2 & Intron 2 (5' splicing Site), 601 to 1296	696
(Forward) ACCACGTCCCGTCCTACT (Reverse) AACTCTCCCAACCAGCGACAC	Intron 2 (5' splicing site), 1173 to 1424	252
(Forward) CTGGGCCTTTCGTCTTAGAGT (Reverse) AGCCAGGGCAGAGATCCGT	Intron 2 (3' splicing site), 3391 to 3716	326

Table 6 (cont'd).

Primer pair (5'-3')	Position	Product size (bp)
(Forward) GCTCTCACTGCGGTATTCT (Reverse) AGCCCTCACAGAGACCCTTAG	Intron 2 (3' splicing site), Exon 3 & Intron 3 (5' splicing site), 3580 to 4319	740
(Forward) ACGCTTTCCTTCCCTAAGTGA (Reverse) AATTCAGAAGCGATTTAACC	Intron 3 (5' splicing site), Exon4 & Intron 4 (5' splicing site), 9643 to 10076	434
(Forward) AGAAAGAAAGTTCGGTAT (Reverse) ATATAATAAACTCAAGCCTAC	Intron 4 (3' splicing site), Exon 5 & Intron 5 (5' splicing site), 15121 to 15658	538
(Forward) AGGAAGAAGAGGCAATCAACA (Reverse) GGAGGAACTCTTCGCACAC	Intron 5 (3' splicing site), Exon 6, 19833 to 20300	468
(Forward) ATGCTGACCACACCGA (Reverse) CAATTACAGGGACTGATTAC	Exon 6 & 3' UTR (poly A tail), 20152 to 20996	845
(Forward) GGGCAATGGGTGTGTGATCTC (Reverse) GCCGTCACTTGATACAATG	3' UTR, 20910 to 21561	652
(Forward) GCCCCTGTGTGTTCTGTATTA (Reverse) AGCTCTCCACCGCATGTC	3' UTR, 21438 to 22295	858

Sequencing was performed either by radioactivity-labeled dideoxy method, using the Thermo Sequenase Radiolabeled Terminator Cycle Sequencing kit (Figure 7) or by fluorescence-labeled dideoxy method, using the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction kit (Figure 8). A total of 12,069 bp were sequenced in both A/J and C3H/HeJ mice (Figure 9). Some important sites related to the *Gata3* gene are indicated in Figure 9 and described here: (1) Position -9371 to -8753 is exon 1a [79]. (2) Position -8307 to -7212 is the silencer position [80]. (3) Position 1 is the starting point of exon 1 [62]. (4) Position 1004 to 1006 “ATG” is the start codon. (5) Positions 1 to 189, 610 to 1241, 3668 to 4204, 9687 to 9832, 15372 to 15497, and 19957 to 20758 are exon 1, 2, 3, 4, 5, and 6 respectively. (6) N-terminal zinc finger is in exon 4; C-terminal zinc finger is in exon 5 (shown as CXXC.....CXXC). (7) Positions 190 to 609, 1242 to 3667, 4205 to 9686, 9833 to 15371, and 15498 to 19956 are intron 1, 2, 3, 4, and 5 respectively. (8) Positions 190 to 191, 1242 to 1243, 4205 to 4206, 9833 to 9834, and 15498 to 15499 “GT” are the 5’ splicing site for intron 1, 2, 3, 4, and 5 respectively. (9) Positions 608 to 609, 3666 to 3667, 9685 to 9686, 15370 to 15371, and 19955 to 19956 “AG” are the 3’ splicing site for intron 1, 2, 3, 4, and 5 respectively. (10) Position 17346 to 17537 is the microsatellite marker for *Gata3* in intron 5. A/J = (GT)₂₆, C3H/HeJ = (GT)₂₈. (11) Position 20239 to 20241 “TAG” is the stop codon.

A microsatellite marker was found on intron 5 of *Gata3* (Figure 8). No other sequence polymorphisms were found between A/J and C3H/HeJ mice. This microsatellite sequence contained (GT)₂₆ in A/J and (GT)₂₈ in C3H/HeJ mice. We developed a PCR-based marker for this microsatellite, which was used in genotyping experiments to determine the *Gata3* position on the refined linkage map. However, this microsatellite is not likely to be functionally important for regulation of *Gata3* gene expression.

200

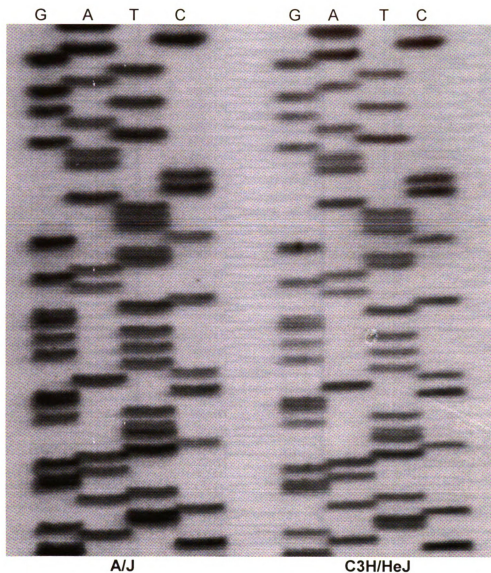
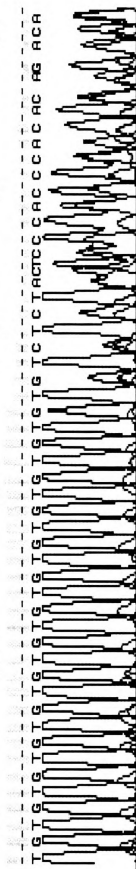
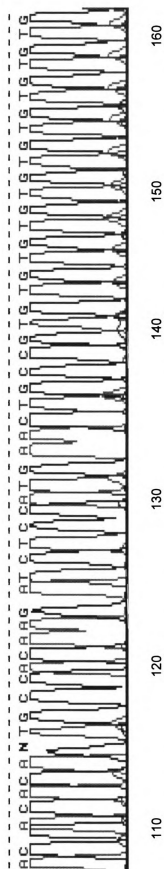
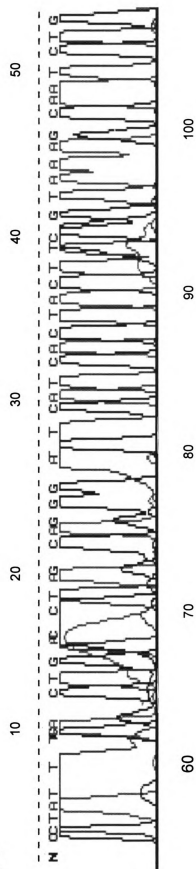


Figure 7. An example of a *Gata3* sequencing gel. [α - 32 P] dideoxynucleotide (ddNTP) chain termination sequencing was performed. PCR products were separated on polyacrylamide gel. Both A/J and C3H/HeJ mice have the same sequence and read as 5'-GCAGTTCATGGAGATCTTGTGGCACTGTGTG GTCAGATTGCTTTACCAAGTAGTGATGACA-3'

(A)



(B)

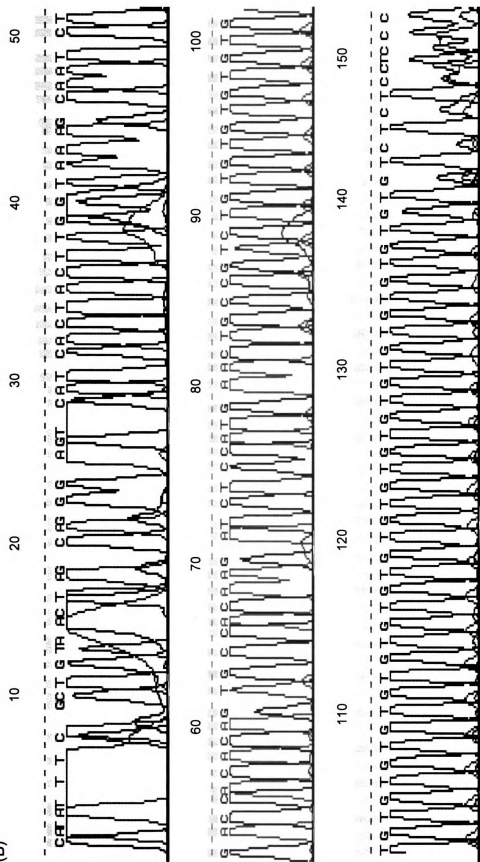


Figure 8. An example of automated sequencing chromatograph of *Gata3*. *Gata3* intron 5 was sequenced using the fluorescence-labeled dideoxy method (A) A/J microsatellite marker in intron 5 with (GT)₂₆. (B) C3H/HeJ microsatellite marker in intron 5 with (GT)₂₈.

Figure 9. *Gata3* gene sequence of A/J and C3H/HeJ mice

-9400	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXXC	CCACCCGGTC	} (Exon 1a)
-9360	CAACTCAGTT	TCACACACCT	CTGATGTGCG	GTTTGTGTGT	
-9320	TTGCAATTCC	CTCCCTCCCC	CTCCTTCTTT	CCTCCTCCCC	
-9280	CCTCCTCCTA	CCCCTTTGCA	ACCCGGAAGA	GCTCTGCTGT	
-9240	CTACACTGAG	CCAAGGGAGG	CAGAGGGGAG	GGGGCTCTTA	
-9200	GCAAGGATTC	ACATCAGCCA	GGTTTTACCT	CTGGCTGGAA	
-9160	GCCTGGCTGA	GATGCAGTGA	AGCAGCCGCC	GCTGCTGTGG	
-9120	GAGCGTCAGC	AACAGTGAAG	TCTGCCTGTC	ATTTCTACCT	
-9080	TCAGATCTCC	AGCAAGGCGG	GTGGAAAAAA	AACAAAAAAA	
-9040	CAAAAACCAA	ACCAGCTGGC	CTTCAGGAGA	GAGCTGCCCT	
-9000	GGTCAGTGAG	TACATAGCTA	TCTCCTTTTC	CTCTGTTATC	
-8960	CTTCTCCTTG	ATTCCCATGG	CGAGAATTAA	GAGGAGGTCT	
-8920	CCGCCATGGG	GATAGCTGCC	TCTGAAAGAA	AGTGAACCAG	
-8880	ACCCCAGCCC	TTTATTGTTC	TTTCTGGGCA	CTGGAAGTCT	
-8840	TTGGCTTTCT	GCAGTGACCT	TGGGTGATCA	TGGTGTGGAA	
-8800	GTGTGTGGAT	GCCCTTGTTT	GGAAGAAGGA	GCTAGCAGAA	
-8760	CTTGGGGT				
.....					
-8320	XXXXXXXXXX	XXXCCTCCAG	TTCCAATATA	CCATTCAGAA	} (Silencer)
-8280	ATAGAACACA	CACACACACA	CACACACACA	CACACACACA	
-8240	CACACACACA	CACACACAGG	ATAATGTTAA	AATGCAAGGA	
-8200	AAGGAGAGTC	TGGTGAATCT	GAGATATGAG	CAGAGCCGTT	
-8160	GCTTTATTAT	TTTTTTTAAA	GGCGATTCTT	TTAATAGAGC	

-8120 AGGGTAACAA ACTGACAGGT GTGATTCCAT CCAGAATAGG
 -8080 AATGAAGTAG CTGTGCCCCG TGGACCCCTT GCTGTAGGTG
 -8040 TAGTTCCATA TTTTTTGACA TCTCCTCCCC TTATAGACCA
 -8000 CACCAGGACC CTGAAATCAC AGTTTGAGAG ACCCAGAATT
 -7960 CTGAAGGGGG ACAAATAATG AAGGTACCCT TTGTGCAGCC
 -7920 CCTCACTCTT CCGCAGTGGT GGTAGTGGGC TGTCCCCCA
 -7880 GCGGCAGGTT ATTTCTAAAC AAGAAGCTTG GCTGCCCAA
 -7840 TCGCTATATG TGGGGAAGAG GGCTTCCTAT AAAATGAGAA
 -7800 CAAAATGTT CTCTATCAGT TTGCACTAGG TCTAGATGTA
 -7760 ATTTCTGTCC TGCTAGAACA AGACTCATGC AGGCATCAAT
 -7720 GAACTCTGTT TACTGATTGA TTTAGCATTG GGATTTAGCA
 -7680 GAAGGCCCCAG CTTCAAGGTT CAAGCCAGTG GCTCAATGCG
 -7640 GCCTAAGGTT TCTGGCTCTG GGACACAACA GGGCATAGAG
 -7600 AATAGCATGC ATGGCAGTGG TTGACATCAC CTTCTACATA
 -7560 ATTGGTCTTT GCTAGCAGCC CCAGATTCTC TAGTCCTCTT
 -7520 CTGTTACAAT CCAAAGCCCA GGGCAGGCGA TCTGCATTTT
 -7480 CCTTTATTTT CTATGTTGAC TCTGCCTTCT TGCAGAAAAT
 -7440 GTGTAGTGGG GTTCAGGCAT AGGTGGAGTG AAAAGATGGG
 -7400 TGCAAGCCTT AGGACAGGTA GTGTCCTTCT GTGTGTTTGC
 -7360 CAATTGCCCCA AAGCCACACA GAAAGGCCAA GGAAGTTGCA
 -7320 GGTGTATGGA AGTCCTAAGG CTTCCATCTT TTTTGACAAT
 -7280 TTGTGTGCCC TGCAGTGAAG ACTTTGATAG AGAAATAAGA
 -7240 ATTCTGACCC CGTGAGTAGG ACCGAAGGG

(Silencer)
 (cont'd)

-4600	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXGGAACC
-4560	CAACCCGTAC	CTTCACAACC	AGACCCTCCG	GGTTATCCCA
-4520	GGCCTGCATA	TCGCAGAGCC	CGGGCGGGGA	TTGCAGCGTG
-4880	CACGGAATCG	AATTAAACGC	ATCTCGGTGG	TTATTGTCTC
-4840	TCGCTAATTG	CTTCAACAAT	GGCCACTGTT	AAAACGGATA
-4800	CACCCAATTA	AAGGTCCCGC	GCGGTCTCCC	TGGGTCTCTG
-4760	CCACCGCCCC	GCTTCCCTAG	TCCGAGGCGA	TGTAGACCTC
-4720	CGGGTTGTGA	ACCGGTGGTA	AGGGGGTACC	GGCGCCGGCC
-4680	TGGATACCTC	TGCCCTTCGC	TTCCTTCCCA	GACGCACAGC
-4640	GACCACGGTC	TTTTCACTTC	GTTTTCTATT	AATCAATCGC
-4600	ATGGAAGGAA	GATTGATCGA	GCTGGAATGG	GAAGTGAATT
-4560	TTTCCCTAGT	CCTTGGGTCG	TCGGGGTCCC	GGGCTGTGAA
-4520	TTTAATTTCT	GGCCTTCGGT	GCTGCTCTGC	TTTCTCCCCG
-4480	ACCTCCCAGC	CCTTCAGGTT	CCGGGCTTCA	CTTCAGTCGG
-4440	TAACCGCGCC	ATCAGATCCG	CCCAGGCCGG	CGCCTTGGCT
-4400	CCGGGAGGAG	GGACAAGGCA	GCACGCCCCG	CTCCGCTTCG
-4360	TTTTCCAGCC	TGCAAACATT	TGCTGCCTCT	AGGAGAATCC
-4320	TACTATTCCG	CTTGCGCTCG	GCGTGCAAAG	TCCCTAAGTA
-4280	AACACGCTCA	AATGACAGCC	CTAGGCATTC	CGAGGCACGC
-4240	TTTCTACCCC	TGCCCAGGAT	TAGTAACTAT	AGTACTTCGA
-3800	CCCCCGGGGT	TTGGCTTTGC	AGGTCACTCT	CTGGAGGGCT
-3760	GCGCTCAACA	AACCCCCCCC	ACCCCCGCGA	CCCCCAAAGT
-3720	AGCATCTTTT	CGCATCTCGG	CCCCGCGGAG	CTCGCCTGGT
-3680	CTCTCTCGGA	GTCAGGTTTCG	GGTTGTTGAC	TCTGGGTGGA

(Promoter)

-3640 TTTCTACCTT CTGGGTCCAT GGCCCCAAGT GATGAGGCCC
 -3600 AATCACTCGC TAAACCTTTT TTTTCAATCA GGGCTTCCAA
 -3560 ATTCTGAAGG GGACCGGTTG TGTCTACTTC TAGAGGGATG
 -3520 GGGTCGCCCT AGTGAAGGCG AGTGGGAGGG ACCTGAGGAT
 -3480 GGGCAAGTGT GTGTGTGTGT GTGTGTGTGT GTGTGTGTGT
 -3440 GTGTGTAGGG GGGTGAGGGG TGTCTCAGCT TACTCCACGC
 -3400 ATAGAGTAAG TCAGATCCAT TTCTTACCCT TTCCCCAAGA
 -3360 GGGCACACAT ACCCTGCTCA ACTTGACGCC TCCAGCAGCA
 -3320 GCCTCAAGGC CTGGGTTTTT AGATAGGCCA AATTGACTCC
 -3280 GGCTATCCAA GTACAACAGC CCCAGATCCC GGTTCTAGCT
 -3240 GAGCCTTTGC CTGCTCCGCT CCAGATGCTG AGTCCCCAGG
 -3200 GAACCGTAGC GTTCGTCAGC CCCTTACCCT ATAGAGAGAG
 -3160 GCCTCATGAA CTGCAGGAGC CAAAGACTCC AGGCAGCAGC
 -3120 GGCAACAGCA TTCTCCTTGA TGGCTTTCTC TTGCCTTTCC
 -3080 CCCAGCTCCG CCAGATGCTC CAGGGGAAGA AGGGGAATGC
 -3040 GGTACACCGA GCCAGACCCG GTGTGCCTTG GGGTTGTGGT
 -3000 GGTGGTGGCA CCCCAGGTCT TTAAGGGGGC ACCCAGACGT
 -2960 CCCTTAACGA GGCCCGGATC AGCTTTGTAG CTCTCTGGAA
 -2920 CTGAAGGATT GGGACCACCT TTCCCTCCGG CCTCAAGCCC
 -2880 CCTCCCCTCC CCCACCACCC GCCAAGGGAA GAGGAAGCCC
 -2840 GGCCTGCATT TGCGAGCAAG GCGGGTCAGA CCGGCGCCTG
 -2800 TCCTGGCCCA GACCCCCGGA GTCCAGCTCT CCGCCTCAGG
 -2760 CCACGCGCCG CAGCCTCTCC GGA CTGATC CCTTCCCCCT

(Promoter)
 (cont'd)

-2720 TCTTCCCGGT GGGTCTCGGA GCCGACCTCG GCCCTCCCTC
 -2680 TCCGGTATCC CCCTCCTCTC TTTTCCCTC CCCCTCCCCT
 -2640 GCTCTGTGTT TCTGACTCTG GGGCCGGCCC GTGACGTCAA
 -2600 ACCCAGTGTG GCGTCGGCGA GACTGGCCGG CGCGGGCCAT
 -2560 CAAAAGAGCA ACGTCCTCTT TCCCAATTAC CCACTGTCAG
 -2520 TCCGGGAACA GGGGCGGACC GGCTGGGAAT TACATGTTAA
 -2480 ATACCCCTTA CAGGCTGGAT CCAGTGCCCG GGGCGCCTAG
 -2440 CCTCAGGCCC CCTACCCCTC TCTGCCAACC CTAAACTACC
 -2400 TCAGACCTGG AGGGGCCTAT TAGATATTTT GTTTTCCAA
 -2360 GGGAGGGGAG TGTCTTTGCA TCCAGTCTAG CTCCTCTGGT
 -2320 GATTGAATTA AGACGAATAA TCCTCTCCAT CCCCCTACT
 -2280 ACATCTATTA GAGAGGCGCC CCTACTAGGG AAGCCATCCT
 -2240 TTTCAGAAGT ATATTCTTTT TCAGTCTTTA AAAATACGTT
 -2200 CTAAGCCGTA TAGACTAACA TGTTTCAGTTC TTGGCGTCAG
 -2160 CAGCTTTCTT TCTTGTCTCC AGAAGGATTT CTTTTGCGCA
 -2120 TTAAAAAAT TATTATTATT ATCATCTACA TCTCGGTGCC
 -2080 CGGGACCAAA GAGATATGCT TACTCGAGAC TTTTCTCGAA
 -2040 GCTGTGAACT AGGGACTGGC TGACTGATGC CTTATACAAC
 -2000 TCATGAGTGA TACAGCAATA GTATTTTCC CTTTAAACT
 -1960 CTCATTCTAG ATCTTAATCC CGGGCCTTTT AGCAAAAATG
 -1920 TGTGTGCGGT CTTCTCTGCT TGTTTATGGA ATGGTTTCCG
 -1880 GTGAATGTTA GACAGCAACT AAATAATTAA AAGGTTTTGA
 -1840 GAGCGCCGTG AGCTGGAAGA GATGCTGGCA GCTTGATTGG
 -1800 CCCAGGAGAG ATGGGTGGT CAAAGAGTGA AAGTAAAAA

(Promoter)
 (cont'd)

-1760 ATCGATATTC TAAGACTGGG CCGCCCTCAT AAACGTGGGA
 -1720 CCAAGGGGTG CAGGGTGAAC TCAGAAGGGA GGAAGACTC
 -1680 GTTTTAAAA AAAAGGTATC TTCGTAATTT GTATTAGAA
 -1640 ATTCAGCCTT GATTTCAGTC AAAGATCAGA TGTAAGGGG
 -1600 GGGGGGGTGT CAGGAGAAGC TCCAAACCCA AGCCCGCAGG
 -1560 GCACATTTCT AAATAAATTT TGTCTTCTTC AAGGGTTGTA
 -1520 TTGTAAAAAG AACGCACCTT CCCTCTCCCC GCACTCCACT
 -1480 GGCTTCAAGG TAGATATTGA TGGCTTTTCT CCACAGTAGA
 -1440 ATAGAAGAAG TTAAGTTGGA CTTTAAAGAA AGGGCTTGTC
 -1400 GGAATCCAA GTTTTGAATA AGTGGGAAAT ATTATGGGGT
 -1360 AAAGAAATGA AGAGGGGATG ACGCCTTCAG TTCCAGTAAC
 -1320 CCTCTAAAGC CAAGCTCTCT GCCCTGGAGG GTGGAATGGG
 -1280 AACACCTCTT AACTGCCCT GGGGTGCACC CGAAGTCTCT
 -1240 TTCGGACCTT GTCATCCGG GCAGTGTTTT CTGGACCCAG
 -1200 GCTGCAGAAA CCTCAACGCC ACTAGGGGAT GCAGTACTC
 -1160 AAAGGACGGA ACAGGGCCGC AGAGGTTGGA GCGCGTGGGG
 -1120 CGGGGCGACT GCGGCCCGGT CGTGGTATCT CCCGATGATT
 -1080 GGTCTTACA AATGGCCCCG CCCTCGTCCG CCCAGCCTCT
 -1040 CTAAGGGCG TCTTCCAGCC TCTCTCCTGT GTCAGCTTGC
 -1000 GGCCCTCCA CCAGACCTG TCCAGAGAC CAAAATGTC
 -960 CAGGAAGTT GCGGCTGGGT CCACCCCGGA GGTGAGGGT
 -920 GGATATTATA GAGGGGACCG TTTTCCTCGG GTGAAAGCAG
 -880 AATAAGTAG CACCCCGAGC TAGGCGAAGA GAAAAGTTAG

(Promoter)
 (cont'd)

-840	GCTATCGCAG CAAAGATCAA GTTAGTTGTA CACGGTACTT	} (Promoter) (cont'd)
-800	CGGGGACCAG TGAGCCTAGG TCTCGCTAAG CCAGCTTTTT	
-760	ATGCATCGCG TTGTCACTAA GGTCAAAAGC ACACATTGAT	
-720	GATATCATTG GATCCGAAAA AGACAGCTGC AACAGCTGAC	
-680	GCTGTTCGTT CTGGAGAGGT TTGGGAAAGC AAGCAGAGAC	
-640	CATAACAATA ACGCGGGCGT CCGAATCAAA GCCCAGGTCC	
-600	TCCGCGCGAT TCAGCAGCCT CCCGGAAGC TGGTTCGGAG	
-560	GCAAATCTTC AGTTACTTCG CCATGAAACT TTTCTAATCT	
-520	GTTTTACATG CACATAGCCC CTAAGCTGAT ATAAACTGAA	
-480	AAACGTTCTG GCTTGAATCC TAATTTAAAA GTTTGCTCAT	
-440	TTTTAAAAAA GTATCGAAAA GTCTGCTTAT GGAAATTAGC	
-400	TTTATGAATG GGGCAGCCGG GTTTCACCTCG TACGGAATAA	
-360	TCTACTGAGC CCGAATGAAT CGGCTTTGCT ACATTTAAAG	
-320	GGCCAGAGAG CGAATTCCCT CCTGCCTGTC CCCTCTGCCC	
-280	CAAGCCC3GG CGGGCCTCGC CCACCCTGCC CGCGGCCCT	
-240	CTGCCCTAGG CGGCCCTTGG CGGCGCCCCT TTCCGGTCAG	
-200	TAGAGGGGCG GGAGGAGGGG CGGCGGTGTG CCGGGGTGGG	
-160	TGGGGGGGAT GGGATGGGGG TGGAGGTGAA GTCCTGGAGC	
-120	GGGTTTGGGT TGCAGTTTCC TTGTGCTGAG GATCGTGTCC	
-80	CCTCCTGCCA GCGCCAGGCT CCTCCCCCTC GGCGCGGATG	
-40	ACACTAGAAC CTCCTTAAGT TGCGTCGCGC CACAGCTGTC	
+1	TGCGAACACT GAGCTGCCTG GCGCCGTCTT GATAGTTTCA	} (Exon 1)
+41	▲ exon 1) GAAAGAATGC ATTCCCTGTA AAAAAAAAT ACTGAGAGAG	
+81	GGAGAGGAGA AAGAGAGAGA GACTGAGAGA GCGAGACATA	

+121	GAGAGCTACG CAATCTGACC GGGCAGGTCA CACGCCTCCT	(Exon 1) (cont'd)
+161	CCTCCTCCTC TACGCTCCTT GCTACTCAGG TTGGTATTGT (exon 1 ▲ intron 1)	
+201	GACTTGTGAC TTTTTTTTTT TTTTAAAGTT TGATATCACT	(Intron 1)
+241	CCCTCCTCTT GGAGGGACTG TTTGTGTGTG TGTGTTTGGG	
+281	GGTTTGTTTG TTTGGTTTTT TGTAAACTAA AAGCTCAGCT	
+321	TTCTCCTTTT GGAGGATTTG TAGTGGTTGG AAAGATCCTG	
+361	GGGCCGGTGT CCTCCAGGGA GGA CTGTGCTC CCAAGTTTTA	
+401	ATTGCTTAGT CAGTCCTGGG CTCCTGCGAG CCTGGCTGTC	
+441	GGAGGTGTGC TGC GGAGCTT GGCTTCTTTC CCTCCGGGTC	
+481	TTCGCGTGCG TGTGCACGTC CCGGGTGAGT CGGCCCTGCA	
+521	GGAAGCTTGC GAAGACCTAG TGCCCTCCAG AATTTCAGGT	
+561	GTTAAAAGTT CTTACACCTC ATTCAGGTCT CTCTTTCTCT	
+601	CCCTTGCAGG TGATCGGAAG AGCAACCGTC TCTGAGCGCC (intron 1 ▲ exon 2)	(Exon 2)
+641	AAGGAATCAG TGTGCAGTGT GGTCACTC GGATTCCTCT	
+681	CTCCCTCCTT TTTTTTTTTT TTTTGTGACC CCTTTATTCC	
+721	TCCGTGTCTG CTTTTTTTTT GGGGGGGGGG ATCGCCCTCA	
+761	TTCTTTTCTT TTTCTTCTTT CCCTTCCTTT CTTTGTCTAA	
+801	ACTATCCCGC AAAGATTTTT CTTCCTCCC TAAACCCTCC	
+841	TTTTTGCTCT CTTTTTCTAT ACCCTTAACT GCAAACAAAC	
+881	CATTAAACGA CCCCTCTCCT GGGCCTCCGA CGGCAGGAGT	
+921	CCGCGGACCT CCCAGGCCGA CAGCCCTCCC TCTACCCGCG	
+961	AGGGTTCCGG GCCGGGCGAG AGGGCGCGAG CACAGCCGAG	
+1001	GACATGGAGG TGACTGCGGA CCAGCCGCGC TGGGTGAGCC M E V T A D Q P R W V S	(Start codon)

+1041	ACCATCACCC	CGCGGTCCTC	AACGGTCAGC	ACCCAGACAC	(Exon 2) (cont'd)
	H H H P A V L N G Q H P D T				
+1081	GCACCACCCG	GGCCTCGGCC	ATTCGTACAT	GGAAGCTCAG	
	H H P G L G H S Y M E A Q				
+1121	TATCCGCTGA	CGGAAGAGGT	GGACGTA	TTTAACATCG	
	Y P L T E E V D V L F N I				
+1161	ATGGTCAAGG	CAACCACGTC	CCGTCCTACT	ACGGAAACTC	
	D G Q G N H V P S Y Y G N S				
+1201	CGTCAGGGCT	ACGGTGCAGA	GGTATCCTCC	GACCCACCAC	
	V R A T V Q R Y P P T H H				
+1241	GGTGAGTCCA	CCTTGCGCCT	GGAACCTTTT	GGATCCGCGT	(Intron 2)
(exon 2 intron 2)					
+1281	TTGTTTTTCT	GGGTCCACGA	GACCGCCAGA	GACCCTGGCT	
+1321	GGGCAGAGCA	AGCCAAAGCT	CCTAAGTGTG	TTACTAGTTC	
+1361	ATTTAAAAAA	AGTATCGGGG	GCCCGGAAAT	GGGCGAGGAA	
+1401	GCAGTGTCGC	TGGTTGGGAG	AGTT		
+3391	CTGGGCCTTT	CGTCTTAGAG	TTGGCGAGTG	TGGACAGGGC	(Exon 3)
+3431	CTGCTTCTAT	CTTGAGGCAT	GTGGGCCTGG	TACTCCTGGG	
+3471	ACCACGGAGA	TTAGCGTATG	TTTGAGCTCA	ATTGCGGTTG	
+3511	AGCCTGGAGG	TTTGGCTGAC	TGGGAGGTTG	GAAGCGCCAG	
+3551	GGGGAAATCT	TCAGGTGTGT	CTCAGGGCAG	CTCTCACTGC	
+3591	GGTATTCTTC	TTTTTTTTTT	CTCCTCCTCA	TCTTCTCTCC	
+3631	CCCCTCGCC	CCGCCGCCGC	CATCGCTGGC	ATCACAGGGA	(Exon 3)
				(intron 2 AG	
+3671	GCCAGGTATG	CCGCCCGCCT	CTGCTGCACG	GATCTCTGCC	
	S Q V C R P P L L H G S L P				
+3711	CTGGCTGGAT	GGCGGCAAAG	CCCTGAGCAG	CCACCACACC	
	W L D G G K A L S S H H T				
+3751	GCCTCGCCCT	GGAACCTCAG	CCCCTTCTCC	AAGACGTCCA	
	A S P W N L S P F S K T S				
+3791	TCCACCACGG	CTCTCCGGGG	CCTCTGTCCG	TTTACCCTCC	
	I H H G S P G P L S V Y P P				
+3831	GGCTTCATCC	TCTTCTCTGG	CGGCCGGCCA	CTCCAGTCCT	
	A S S S S L A A G H S S P				

+3871	CATCTCTTCA	CCTTCCCGCC	CACCCCGCCG	AAAGACGTCT	(Exon 3) (cont'd)
	H L F T F P P T P P K D V				
+3911	CCCCAGACCC	GTCGCTGTCC	ACCCCGGGAT	CCGCCGGGTC	
	S P D P S L S T P G S A G S				
+3951	GGCCAGGCAA	GATGAGAAAG	AGTGCCTCAA	GTATCAGGTG	
	A R Q D E K E C L K Y Q V				
+3991	CAGCTGCCAG	ATAGCATGAA	GCTGGAGACG	TCTCACTCTC	
	Q L P D S M K L E T S H S				
+4031	GAGGCAGCAT	GACCACCCTG	GGTGGGGCCT	CATCCTCAGC	
	R G S M T T L G G A S S S A				
+4071	CCACCACCCC	ATTACCACCT	ATCCGCCCTA	TGTGCCCGAG	
	H H P I T T Y P P Y V P E				
+4111	TACAGCTCTG	GA CTCTTCCC	ACCCAGCAGC	CTGCTGGGAG	(Intron 3)
	Y S S G L F P P S S L L G				
+4151	GATCCCCCTAC	CGGGTTCGGA	TGTAAGTCGA	GGCCCAAGGC	
	G S P T G F G C K S R P K A				
+4191	ACGATCCAGC	ACAG G TAAGC	GCCACCTCTA	CCCCTGGGAA	
	R S S T ▲ intron 3)				
+4231	TCTTTTGCTC	CCTCCTATTT	GCGCTATATC	CAGGTCAGGG	
+4271	CAAAAAGGG	GGTTTTCTTT	ACTCCAAACT	AAGGGTCTCT	
+4311	GTGAGGGCT				
.....					
+9641	XXACGCTTTC	CTTCCCTAAG	TGACTTATCT	GTGACCTTGT	(Exon 4)
+9681	TTCC A GAAGG	CAGGGAGTGT	GTGAACTGCG	GGGCAACCTC	
	(intron 3) ▲ E G R E C V N C G A T S				
+9721	TACCCCACTG	TGGCGGCGAG	ATGGTACCGG	GCACTACCTT	
	T P L W R R D G T G H Y L				
+9761	TGCAATGCCT	GCGGACTCTA	CCATAAAATG	AATGGGCAGA	
	C N A C G L Y H K M N G Q				
+9801	ACCGGCCCT	TATCAAGCCC	AAGCGAAGGC	TG G TAAGTTC	
	N R P L I K P K R R L ▲ intron 4)				
+9841	TTGGAAGTAC	TGAGCTCATT	TATCTGTTTT	ACGTTTTCTG	
+9881	TGTCGTCTCT	GAGAAGAATG	GCTTTCTGCA	GGAAACTGGC	(Intron 4)
+9921	CTGGTAGTTT	TCTCTCCTTT	ATTTTCTTTC	CCTTCTTTTT	
+9961	TACTGTTTCT	CCTCCACCTT	TTATTTCTTT	TATCTCTTTT	
+10001	CCTTTTATAC	TTTTTCAAAT	TAAATAAAAA	AAAAC TTTGT	
+10041	ATTTTTATTT	TTCATGGTTA	AATCGCTTCT	GAAATT	
.....					

+15121 AGAAAGAAAG TTCGGTATTT GCCTGTTGAG GCCCAAAAAA
 +15161 GTCCAAGACT CTAGATTGGC TACATCTTTC TTCTCAAGGA
 +15201 TGATGTGGGA CCTTGATGAC TTTCATGTTG CCCACTTGCT
 +15241 GGTTTTGATT TCAATGTCTT TTTTTTTTTT TTTTTCGATG
 +15281 TCTTTGGAAG ACTTCAAGCC AGTCTTCATA GCTCTGCCAA
 +15321 CATATTTACT GTGTGTTCCA ATTTTATCTT CTCCTCTCTT
 +15361 CTCATCCCCA **G**TCGGCAGCA AGGAGAGCAG GGACATCCTG
 (intron 4 **▲S A A R R A G T S C**)
 +15401 CGCGAACTGT CAGACCACCA CCACCACCCT CTGGAGGAGG
A N C Q T T T T L W R R
 +15441 AACGCTAATG GGGACCCGGT CTGCAATGCC TGTGGGCTGT
N A N G D P V C N A C G L
 +15481 ACTACAAGCT TCATAAT**GTA** AGCGGGACTG GAACCAGCCG
Y Y K L H N▲ intron 5)
 +15521 GGATGGGGGT GGGGAGCCAT CCTGACCATG ACCTTGAAAC
 +15561 TTCACCAGCA TCCAGATTTC TGATGGGTTT CAGTCACCTT
 +15601 CCTTGGAGCT AAACAATTTG TTCAGATAAA TAATGCAGTA
 +15641 GGCTTGAGTT TATTATAT

 +17341 XXXXXAGTGT AGACAGCGCA AAGTCTATTT CAGCTGTCCT
 +17381 AGCAGGGTGT CATCACTACT TGGTAAAGCA ATCTGACCAC
 +17421 ACAGTGCCAC AAGATCTCCA TGAAGTCCG TGTGTGTGTG
 Microsatellite marker for *Gata3*
 +17461 TGTGTGTGTG TGTGTGTGTG TGTGTGTGTG TGTGTGTGTG
 A/J = (GT)₂₆, C3H/HeJ = (GT)₂₈
 +17501 TTTCTCTACC ACCTCCACGG AGCCATAGAA TTCTAGG

 +19831 XXAGGAAGAA GAGGCAATCA ACACAGGAAC ACATCCCTGG
 +19871 CCTGGGGCCT GTGCTACTGT GCAAAAGCTT TTATCATTTT
 +19911 AAAGGCAGCA AAAAAGTAAA AAAAAAAAAA ATTGATCTTT

(Intron 4)
(cont'd)

(Exon 5)

(Intron 5)

+19951	GTTT AG ATTA	ACAGACCCCT	GACTATGAAG	AAAGAAGGCA	
	(intron 5	▲I	N R P L	T M K	K E G
+19991	TCCAGACCCG	AAACCGGAAG	ATGTCTAGCA	AATCGAAAAA	
	I Q T R	N R K	M S S	K S K K	
+20031	GTGCAAAAAG	GTGCATGACG	CGCTGGAGGA	CTTCCCCAAG	
	C K K	V H D	A L E D	F P K	
+20071	AGCAGCTCCT	TCAACCCGGC	CGCTCTCTCC	AGACACATGT	(Exon 6)
	S S S	F N P A	A L S	R H M	
+20111	CATCCCTGAG	CCACATCTCT	CCCTTCAGCC	ACTCCAGCCA	
	S S L S	H I S	P F S	H S S H	
+20151	CATGCTGACC	ACACCGACGC	CCATGCATCC	GCCCTCCGGC	
	M L T	T P T	P M H P	P S G	
+20191	CTCTCCTTCG	GACCTCACCA	CCCTTCCAGC	ATGGTCACCG	
	L S F	G P H H	P S S	M V T	
+20231	CCATGGGTT TA	GAGAGGCAGA	GCCCTGCTCC	ACATGCGTGA	(Stop codon)
	A M G *				
+20271	GGAGTCTCCA	AGTGTGCGAA	GAGTTCCTCC	GACCCCTTCT	
+20311	ACTTGC GTTT	TTCGCAGGAG	CAGTATCATG	AAGCCCGAAA	
+20351	GCGACAGATC	TGTGTTTTTG	AAGGCAGAAA	GCAAAATGTT	
+20391	TGCTTCTTTT	TTCAAAGGAG	CTCGAGGTGG	TGTCTGCATT	
+20431	CCAACCACTG	AATCCGGATC	CCATTTGTGA	ATAAGCCATT	
+20471	CAGACTCATA	TTCCCTATTT	AACAGGGTCT	CTAGTGCTGT	
+20511	GAAAAAATA	TTGCTGAACA	TTGCATATAA	CTTATATTGT	
+20551	AAGAAATACT	GTACATTTGA	GGAAGACTTT	ATTGTACCTG	
+20591	GATAGCTGTA	AGAAAGGCAT	GAAGGACGCC	AAGAGTTTTA	(3' UTR)
+20631	AGGAATATAG	GGGGATTAAA	GTATGGAGAT	ACAGAAGAAA	
+20671	CCACTAAGTC	TGATGTCCAA	ATGGGCACAC	TGTCAGTTTT	
+20711	GTTTCCCTTC	AGTTGTTTGA	TGCATTTAAA	AAAAAAAAAA	
+20751	AGAAAGAAAA	AGAAAAAAG	GGGGGGGGGG	GAGAAAAAAA	
+20791	TAAATTAAAA	AAAAAAAAAA	AAAAAGAAAA	GAAAGAAAAA	
+20831	TCTAAGAAAA	AAAAAAAAAA	GGTTGTAGGC	AAATCATTTG	

+20871 TTCCAGGCTG TGAGCCTGTG CAAAAGAGAT TTCAGATCTG
 +20911 GGCAATGGGT GTGTGATCTC ACCCACTGAA GATCTGAGAA
 +20951 TGTCATGGCT AGGCCTACAT GCTCTGTGAA TCAGTCCCTG
 +20991 TAATTGTTGT TTGTATGTAT AATTCAGAAG CACCAAAATA
 +21031 AGAAAAGATG TAGATTTATT TCATCATATT ATACAGACTG
 +21071 AATTGTTGTA TAAATTTATT TACTGCTAGT GTTAGGAACT
 +21111 GCTTTTTTTT TTTTTTTTGG TTTTAATGTT TTTTTTTTTT
 +21151 TTGTTTTTTG TTTTTTTTTT TCTTTCTCTC TGGATTTTTG
 +21191 GTTGAATAAA CTAGATTGCT TTCAGTTGAC TTAAGGTGGA
 +21231 TGTACTCTGG AGGGTTTATT TTTCCTTTTA TTATTATTTT
 +21271 TGATGGTATT TATTAAATAG CTTCTATGGG CCCGGCGGTA
 +21311 CCTGTCTTTT TCGTCACTTT TCTTGCAGCC TAAACTATGA
 +21351 AGGTAGCAGC GTACCAGCTA CCAACATGCA TGTCAGAGAC
 +21391 CCGGCCACTC ACAGGCCTGG TCCTGAGAGC CACCTGGCTG
 +21431 ACTGTTAGCC CCTGTGTGTT CTGTATTAGT GATCACTGCC
 +21471 TTTAAACAGT CTGTTGGAAT AATACTATAA AAATAATAAT
 +21511 AAAGTTAAAA TATTTTAAAA CAACAAAGTA ACATTGTATC
 +21551 AACGTGACGG CCAAGCTCTT ACAGAAATTG GTAGCACCAG
 +21591 CTAATTTTCTAG AGTTGTTTTT TTTTAAAGCA TCCCAAAGCA
 +21631 AATATGGTAG TTATTCACCA GGAAATCATA TCCCTTGAAT
 +21671 GAGAGAATAG TGAGTTCTAC TATTGTATAG AGAAATCTAT
 +21711 CCCTGGGCAG GCACAGCTTC TGGGTCCTCC AGTTAGATGC
 +21751 TGAGATACGT GCTGTTCTTG AGTATCTCCT GAGACAAGCT

(3' UTR)
 (cont'd)

+21791 TAAAGGGAGA GTGGCAAAC TCGATGGGCGG TGGTGGCGCA
 +21831 CGCCTTTAAT CCCAGCACTT GGGAGGCAGA GGCAGGCGGA
 +21871 TTTCTGAGTT CGAGACAAGC CTGGTCTACA AAGTGAGGTC
 +21911 CAGGACAGGC AGGGCTACAC AGAGAAACCC TGTCTCGAAA
 +21951 AAAACAAAAC AAACAAAGAG TGGCAAAC TCCTCAGCTGAG
 +21991 GTTTCAGGT CTATCTACTG TCGGAGGGTG GATTCCTCTT
 +22031 TGCTACAGCA AGCCAGCCAG GTGCTCCTCA GTTCATCCCC
 +22071 ATCTTGCTTT GCCACTTCCT TTGCTACTCG CAATGGAGGG
 +22111 GTGACTGGGA TGTCTGCAAG ACACAAAGTT ACCCTTTTGA
 +22151 AGCCGGCTGG ATATTCATTC CCTTCGTGAC TCTGGCACAC
 +22191 ACTTATGAAT CTGTTACCTG CCTCTGCTTT CTTAGCAAAT
 +22231 AGTTTAAGTC CAAAGCTGAA TGTGAAATA GAAAGAGTAA
 +22271 GAGAAGGGAC ATGCGGTGGA GAGCT

(3' UTR)
 (cont'd)

D. Comparison of our sequence to public databases

Although no functional polymorphisms were found between A/J and C3H/HeJ mice, many sequence differences were identified between our sequence results and published sequences. The differences may be due to the different strains used for sequencing, or may be due to sequencing errors. We compared our sequencing results with the Celera database (129X1/SvJ, 129S1/SvImJ, DBA/2J, and A/J strains) as well as the results of George et al (BALB/c mice) [62]. Overall, Celera data match our results closely, while George and colleagues report many differences as compared to both our results and the Celera database (Table 7). Based on the facts that we obtained duplicate sequence in two strains, repeated sequencing of all exons, and identified high sequence similarity with the Celera database, we have high confidence in the accuracy of our sequence results.

It is not a surprise that no polymorphism within *Gata3* exons were found because *Gata3* is important for both T cell differentiation and neural development. *Gata3* knockout mice will die at embryonic stage, thus the mutations that totally destroy the function of *Gata3* are lethal.

However, it is a little surprise that only one microsatellite marker was found among 12,069 bp *Gata3* sequence. Wade and colleagues studied the rate of single nucleotide polymorphisms (SNPs) of inbred mice strains and found that the average rate was 14 SNPs per 10 kb [127]. However, the distribution of SNPs was highly non-uniform on long segments of

genome, either extremely high (40 SNPs/10 kb) or extremely low (0.5 SNPs/10 kb) [127]. *Gata3* may be located in the extremely low SNPs rate region, therefore, no SNP was found on approximately 12 kb sequence.

Table 7. Comparison of our sequencing data with public databases

Nucleotide position	Our sequence	Celera database	George et al.
-1562 to -1558	GGGCA	GGGCA	CAGG
-1000 to -999	GG	GG	G
-991	A	A	T
-979 to -977	CCC	CCC	CC
-961 to -960	CC	CC	C
-940 to -939	CC	CC	C
-931 to -930	GA	GTA	GA
-907 to -904	GGGG	GGGGG	GGGG
-693 to -962	TG	TG	TCG
54 to 56	CCC	CCCC	CCC
364	C	C	Y ^a
389 to 392	CCCC	CCCC	CCC
470 to 475	CCCTCC	CCCTCC	N ^b CN ^b CC
479	T	T	A
485	C	C	N ^b
547 to 548	CC	CC	CCC
689 to 706	(T) ₁₈	(T) ₁₈	(T) ₁₉
962 to 964	GGG	GGG	GG
20673	A	C	N/A ^c

- a. Y represents C or T.
- b. N represents A, G, C, or T.
- c. N/A means no sequence information available.

E. Summary

I sequenced 12,069 bp of *Gata3* gene in both A/J and C3H/HeJ strains, including all exons, splicing sites, part of the promoter and 3' UTR regions. A microsatellite marker in intron 5 was found; however, no other functional polymorphisms were found. The sequencing data do not support *Gata3* as a positional candidate gene. GATA-3 protein has no amino acid sequence difference between these two strains. However, the *Gata3* expression regulatory region has not been determined totally; thus, it may be located in further upstream, downstream, or even in intron regions. Since we cannot sequence the whole *Gata3* gene, it is still too early to exclude the possibility that *Gata3* expression levels are differently regulated in A/J and C3H/HeJ mice. That is the reason we continued to compare the expression level differences of these two strains.

Chapter Four: Comparison of GATA-3 and cytokine gene mRNA expression in two murine strains

A. Introduction

The comparative sequence analysis described in chapter 2 revealed no polymorphisms between A/J and C3H/HeJ mice in *Gata3* exons and splicing sites. Therefore, GATA-3 mRNA of these two strains should also have identical sequences. However, polymorphisms of *Gata3* regulatory regions may still exist in the unsequenced locations. Any such polymorphism may lead to expression level differences of GATA-3 mRNA of these two strains. Thus, we quantitated the GATA-3 mRNA expression in A/J and C3H/HeJ mice.

Cytokine environment is the most important factor that determines the Th1/Th2 differentiation, thus, the expression profiles of some important cytokine genes (IL-4, IL-5, IL-12, IL-13, and IFN- γ) involved in Th1/Th2 regulation were also studied.

IL-4 is the key cytokine determining the Th2 pathway through STAT-6 signaling. IL-4 and IL-13 are important for stimulating B cells to produce IgE [40]. IL-5 is essential for eosinophil maturation and infiltration [41]. Therefore, IL-4, IL-5, and IL-13 were chosen as the marks for Th2 responses.

The Th1 pathway may be induced by IL-12 through the STAT-4 pathway or by IFN- γ through the STAT-1 pathway. T-bet, the key player for the Th1 pathway, was found to be induced by IFN- γ through the STAT1

signaling pathway [56]. IL-12 is a heterodimer composed of IL-12p40 and IL-12p35 subunits. IL-12p40 expression is induced earlier than IL-12p35 and is an important interface between innate and adaptive immune responses [128]. Normally, the expression of IL-12p40 is believed to be interchangeable with that of IL-12. Therefore, IFN- γ and IL-12p40 were chosen as marks for Th1 responses.

GATA-3 is a transcription factor that induces Th2 cytokine and inhibits Th1 cytokine expressions; thus, the expression of GATA-3 is the upstream event of the cytokine gene regulations. GATA-3 expression in Th2 cells is constitutive due to autoactivation [102]. Therefore, GATA-3 mRNA expression were studied at both early (6 and 12 hr) and late (24, 48, and 72 hr) time points following allergen exposure.

B. TaqMan real-time RT-PCR

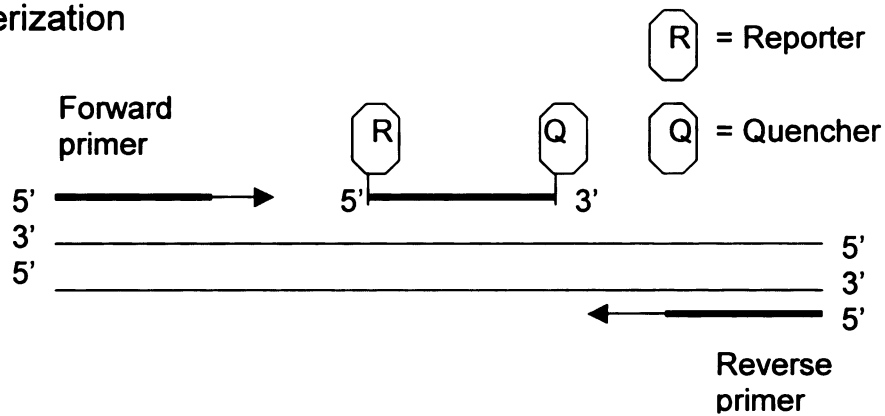
A variety of techniques are available to detect mRNA, such as Northern blot hybridization, competitive quantitative reverse transcription-polymerase chain reaction (RT-PCR), SYBR green real-time RT-PCR, and fluorogenic 5' nuclease assay (referred to by the commercial product name "TaqMan" real-time RT-PCR). Northern blot hybridization requires radioactive labeled DNA or RNA probes, and is not very sensitive: approximately 10 ug of total RNA or 5×10^5 copies of an RNA transcript are needed for each sample. Competitive quantitative RT-PCR is much more sensitive than Northern blot hybridization, but designing optimal PCR internal standards is laborious. SYBR green and TaqMan real-time RT-PCR are currently the most widely utilized assays, because these methods are very sensitive, detecting as few as 500 copies of RNA transcripts. Additionally, they are carried out as closed-tube reactions preventing contamination and they are quantitative. SYBR green real-time RT-PCR uses SYBR green I dye molecule to detect all double stranded DNA, which may also detect primer dimers and other non-specific products along with target products. SYBR green methods are flexible since no probes required and no fixed annealing temperatures are needed. Therefore, SYBR green methods have utility as preliminary screening tests and are valuable for those genes with sequence that is not amenable to TaqMan primer/probe design. Compared with SYBR Green methods, TaqMan real-time RT-PCR assays are more specific due to the

sequence-specific fluorescent probe, although they are more costly and difficult to design. Based on the desire for maximum sensitivity and specificity, we utilized TaqMan real-time RT-PCR assays as the primary method to quantitate mRNA.

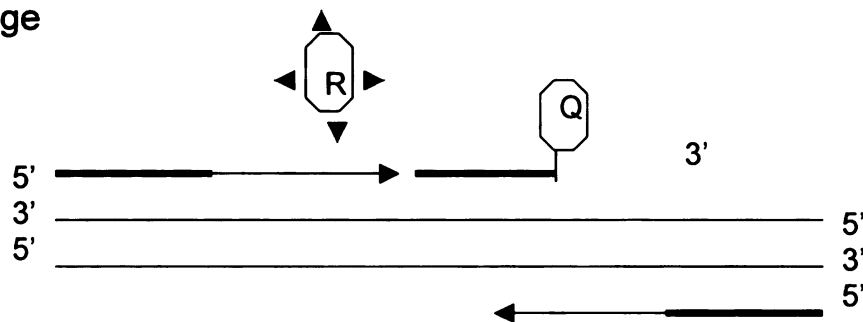
TaqMan real-time RT-PCR assays are commercially available (Applied Biosystems (www.appliedbiosystems.com) or target sequence-specific primer pairs and probes can be designed using Primer Express 6.0 software. The probes are labeled with 5' reporter (FAM or VIC) and 3' quencher (TAMRA or non-fluorescent) dyes [129] (Figure 10). When the probe is intact, the quencher dye suppresses the reporter dye, thus no fluorescent signal is detected. During the PCR process, the 5'-3' nucleolytic activity of the AmpliTaq Gold DNA polymerase cleaves the probe when it is bound to the target. The reporter dye linked to the cut fragment shows the fluorescent signal. The strength of the fluorescent signal is proportional to the number of copies of amplified target, and is detected by the ABI Prism 7700 sequence detection system (PE Biosystems, Foster City, CA). During the PCR process, the PCR product is exponentially amplified and the fluorescent signal increases proportional to the exponential amplification of the PCR product. The threshold cycle (CT) value is the cycle number at which a statistically significant increase in fluorescent signal over background is first detected [129] (Figure 11). The CT value is used as the index of the original copy numbers of target mRNA. The higher the original copy number of target mRNA, the lower the

CT value. One CT unit difference is equal to a two-fold difference in target mRNA difference.

Polymerization



Cleavage



Polymerization

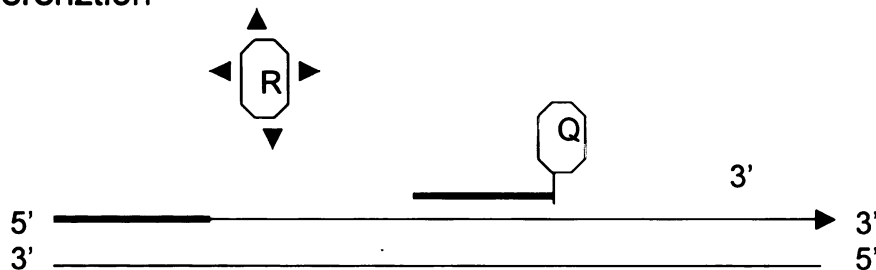


Figure 10. The process of TaqMan real-time RT-PCR. The TaqMan probe is linked with 5' reporter and 3' quencher dyes. The quencher dye is near to reporter dye and suppresses the reporter signal in intact probe. During PCR process, AmpliTaq Gold DNA polymerase not only amplify the target DNA through its polymerase activity, but also cleaves the probe by its 5' nuclease activity. The separated reporter dye shows the fluorescent signal whose strength is proportional to its concentration.

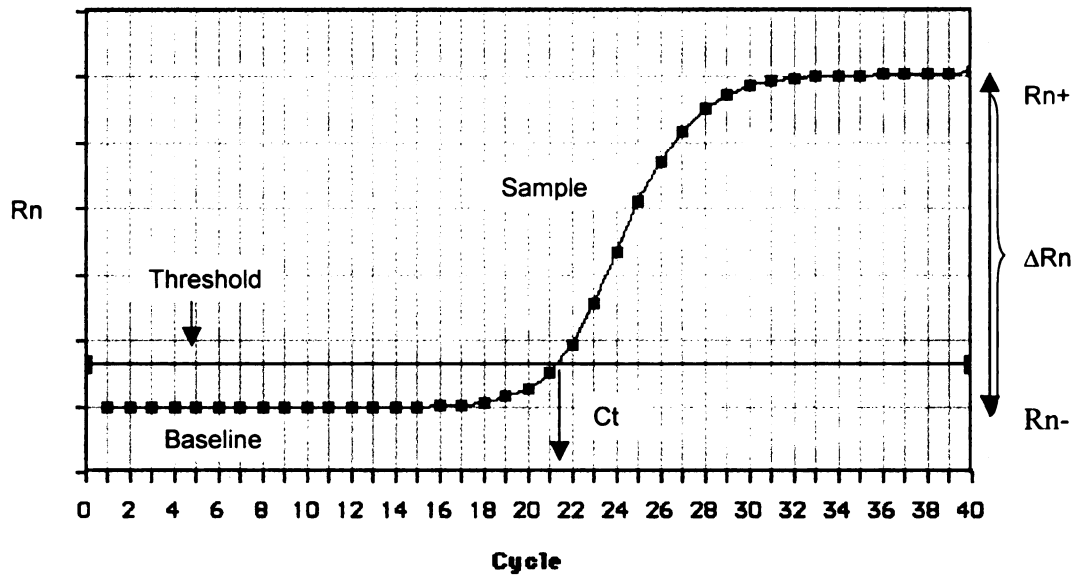


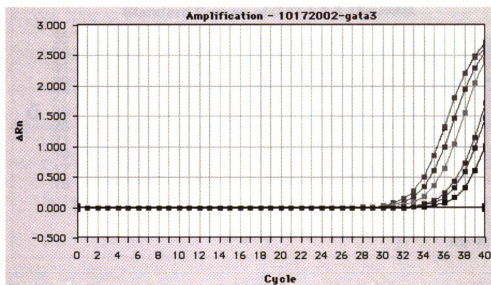
Figure 11. The threshold cycle of TaqMan real-time RT-PCR. Rn is the ratio of the emission intensity of the reporter dye to the passive reference (ROX). Rn- is the Rn value of the unreacted sample or early cycles without signal increases (baseline). Rn+ is the Rn value of a reaction sample. ΔRn is the difference between Rn+ and Rn-, which shows the signal increases due to PCR amplification. The threshold cycle (Ct) is the cycle at which a statistically significant signal increase in ΔRn is first detected.

Since we were more interested in the relative gene expression between A/J and C3H/HeJ mice and between PBS and OVA-treated mice, rather than the absolute gene copy numbers, a relative quantitation of gene expression method was used. Because 18s rRNA is stably expressed in all tissues, it was used as an internal control. The ratio of target product relative to 18s rRNA was used as the index of relative amount of target gene. An example of GATA-3 and 18s rRNA amplification plots is shown in Figure 12. The probes of GATA-3 and 18s rRNA were labeled with FAM dye and VIC dye, respectively. The concentrations of 18s rRNA and GATA-3 were quite different as CT values for 18s rRNA ranged from 12 to 20 and CT values for GATA-3 ranged from 30 to 36 (Figure 12). Along with concentration differences, there were also differences of amplification efficiency between the PCR amplicons as indicated by the values of the slopes in Figure 13. Multiplexing of the internal standard and the target amplicon in the same reaction vial results in data error when the difference in amplification efficiencies between the two amplicons is greater than 10%, therefore, separate tubes were used to amplify 18s rRNA and GATA-3. Two analysis methods are available CT data; the standard curve and the comparative CT methods [130]. The comparative CT method has the advantage of using a single sample as a calibrator, but requires similar amplification efficiencies of the target gene and internal control gene. Since the amplification efficiency of 18s rRNA was near 100% (standard curve slope = -3.2) and that of GATA-3 was near 90% (standard curve

slope = -4.1), they were too different for analysis by the comparative CT method (Figure 13). So, we used standard curve method to analyze our data. The standard curve method compares the results of each sample with a series of dilutions analyzed simultaneously with the test samples. A series dilution of 20, 10, 5, 2, 1, and 0.5 ng of total RNA of a specific sample were used as our standard curve. Samples were amplified in the same plate with standard curve, and the sample CT values were compared to the standard curve to calculate the relative amounts of target RNA.

In brief, every sample was duplicated and the CT values of duplicates were averaged to calculate the relative amounts of target RNA by referring to the standard curve. The amounts of target RNA were divided by the amounts of 18s rRNA to get the relative ratio. The samples from the same strain/treatment/exposure-time group were averaged to get group average. The strain/treatment/exposure-time group averages were compared to show the effects of strain, treatment, and exposure-time. The data were analyzed by two-factor analysis of variance and Student-Newman-Keul test was used for pairwise comparisons.

(A)



(B)

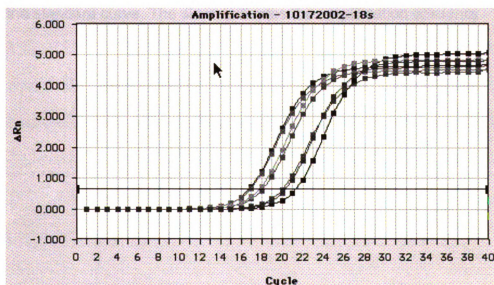


Figure 12. GATA-3 and 18s rRNA amplification plots. Taqman assays were performed on cDNA derived from total RNA isolated from murine lung tissues. (A) The GATA-3 probe was labeled with FAM dye (B) 18s rRNA probe was labeled with VIC dye. Note the earlier rise in signal in the more abundant 18s rRNA as compared to GATA-3.

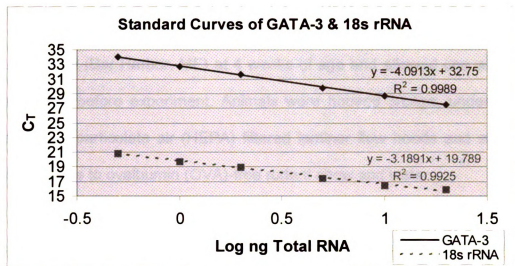


Figure 13. Standard curves of GATA-3 and 18s rRNA. A serial dilution of cDNA reverse transcribed from 20, 10, 5, 2, 1 and 0.5 ng total RNA from a lung sample was used as the standard curve. R^2 indicates the linearity of the standard curve. The slope indicates the amplification efficiency.

C. Experimental time line

A/J and C3H/HeJ male mice were obtained from the Jackson Laboratory (Bar Harbor, ME) at 4 weeks of age and allowed one week to acclimate before experiment. Animals were housed 3/cage, under high-efficiency particulate air (HEPA) filtered laminar flow hoods and allowed free access to ovalbumin (OVA)-free rodent chow and water.

On day 0, mice were sensitized (n = 6/group) intraperitoneally with 10 ug chicken egg OVA (crude grade IV; Sigma, St. Louis, MO) in 200 ul phosphate-buffered saline (PBS) or PBS alone. On day 14, mice were anesthetized (ketamine, 45 mg/kg and xylazine, 8 mg/kg, intraperitoneally), and challenged with a tracheopharyngeal instillation of 1.5% OVA (675 ug) in 45 ul PBS or PBS alone, then recovered from anesthesia. Mice were euthanized by overdosed pentobarbital. Lungs, tracheobronchial lymph nodes, and spleens were harvested at 6, 12, 24, 48, and 72 hr after challenge (Figure 14). The tissues were homogenized in Trizol reagent and stored at – 80 °C.

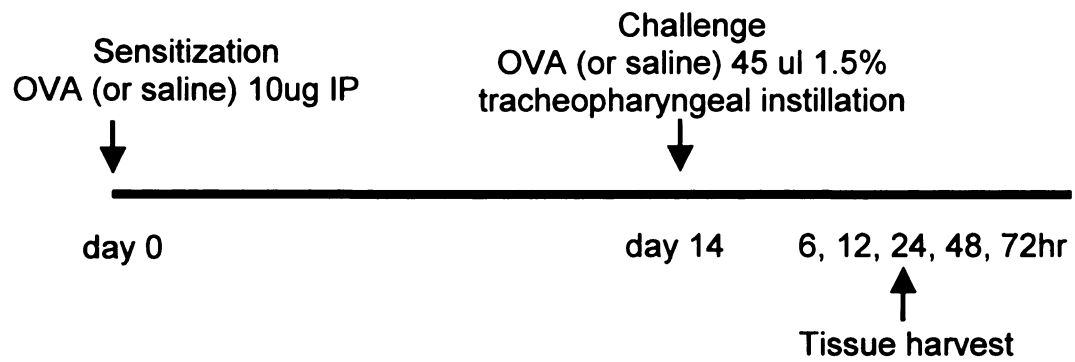


Figure 14. Experimental time line of in vivo allergen exposure

D. Expression profiles for cytokine genes: *Il4*, *Il5*, *Il12*, *Il13*, and *Ifng*

To support the hypotheses that A/J mice respond to allergen via the Th2 pathway and C3H/HeJ mice deviate to the Th1 pathway following OVA exposure, Th2 type cytokine genes (*Il4*, *Il5*, and *Il13*) and Th1 type cytokine genes (*Il12* and *Ifng*) were quantitated by TaqMan real-time RT-PCR.

IL-4 expression was induced early in lungs of A/J and C3H/HeJ mice by OVA exposure, reached maximal expression at 6 hr, and subsequently declined (Figure 15). IL-4 expression was also induced in tracheobronchial lymph nodes of A/J and C3H/HeJ mice by OVA exposure from 6 hr through 72 hr and did not vary in a time-dependent manner (Figure 15). Overall, A/J mice have higher expression of IL-4 following OVA exposure than C3H/HeJ mice.

IL-5 expression was induced by OVA exposure in lungs and tracheobronchial lymph nodes of A/J mice at all time points and in C3H/HeJ mice at the earlier time points (Figure 16). IL-5 expression was comparable between A/J and C3H/HeJ mice at the early time points, while expression was greater in A/J mice after 24 hr. IL-5 expression in tracheobronchial lymph nodes of A/J mice was biphasic with expression maximal at 6 hr, subsiding by 24 hr, and increasing again by 72 hr (Figure 16).

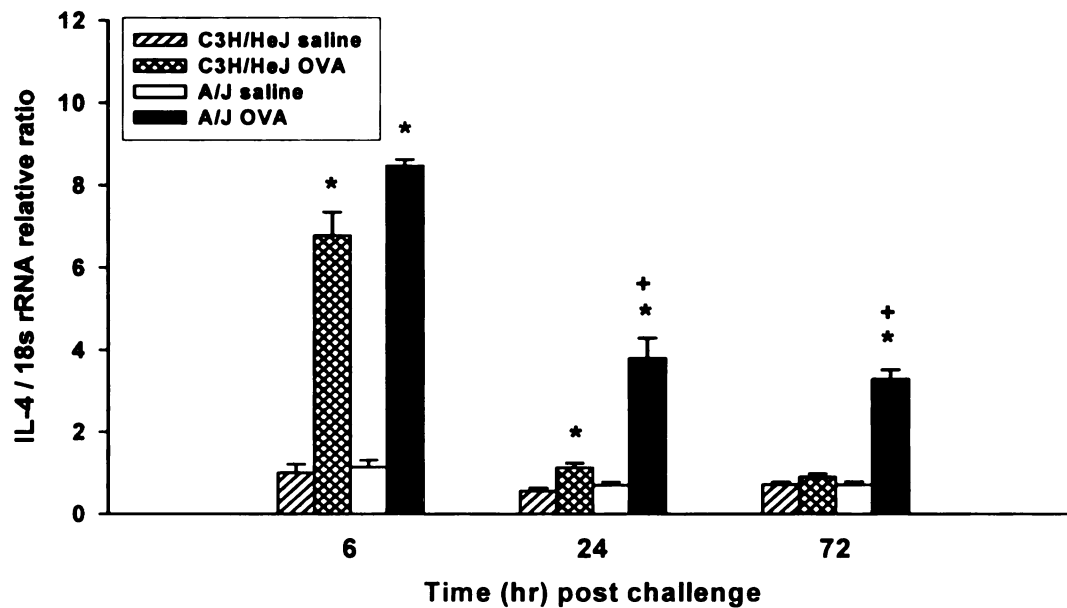
IL-13 expression was induced in lungs and tracheobronchial lymph nodes of both A/J and C3H/HeJ mice at all time points. IL-13 expression

decreased over time in lungs of C3H/HeJ mice, while expression increased in a time-dependent manner in A/J mice. It was greater in A/J mice than C3H/HeJ mice at 24 hr and beyond (Figure 17). IL-13 expression in tracheobronchial lymph nodes was comparable between A/J and C3H/HeJ mice at from 6 hr to 48 hr, while greater in A/J mice at 72 hr (Figure 17).

OVA treatment of A/J mice resulted in suppression of IL-12p40 expression in lungs at all time points and in tracheobronchial lymph nodes at 24 hr through 72 hr samples (Figure 18). IL-12p40 expression in C3H/HeJ mice was largely unaffected by OVA exposure. Overall, A/J mice tended to have lower expression of IL-12p40 following OVA exposure, as compared to C3H/HeJ mice (Figure 18).

IFN- γ expression was induced in lungs at 6 hr through 24 hr time points in C3H/HeJ mice. IFN- γ expression was induced in lungs of A/J mice at 24 hr and 72 hr time points, but the levels were significantly lower than those C3H/HeJ mice at 6 hr and 24 hr (Figure 19). IFN- γ expression in tracheobronchial lymph nodes was largely unchanged by OVA exposure in both A/J and C3H/HeJ mice, but the trend was for higher expression in C3H/HeJ mice as compared to A/J mice (Figure 19).

(A)



(B)

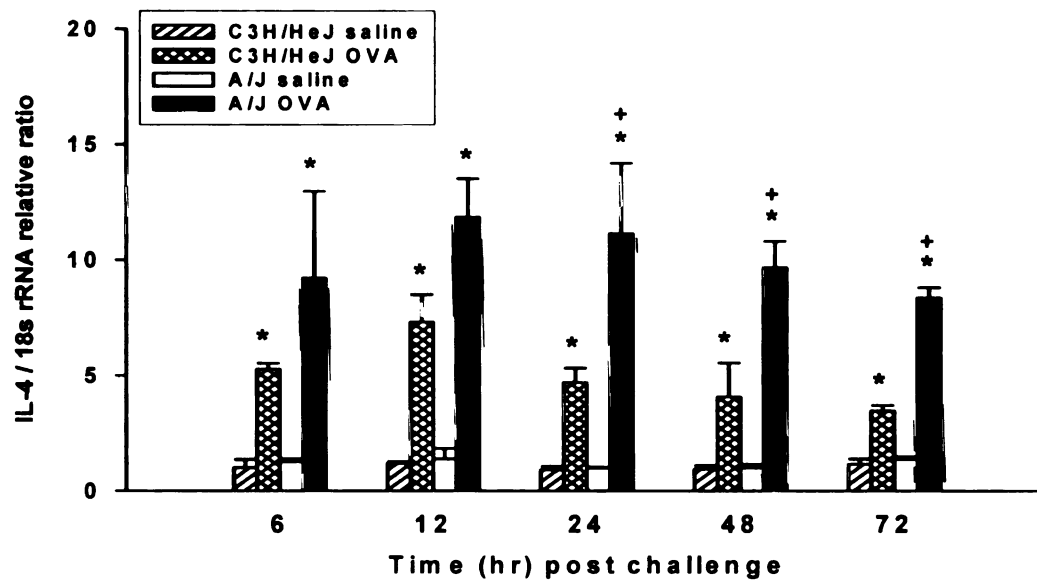


Figure 15. IL-4 expression in lungs and tracheobronchial lymph nodes. (A) IL-4 expression in lungs. (B) IL-4 expression in tracheobronchial lymph nodes. A/J or C3H/HeJ mice were exposed with PBS or OVA ($n = 6$ mice/group). Tissues were collected at 6, 12, 24, 48, and 72 hr following challenge. *Significant difference due to treatment ($p \leq 0.05$). +Significant difference due to strain ($p \leq 0.05$).

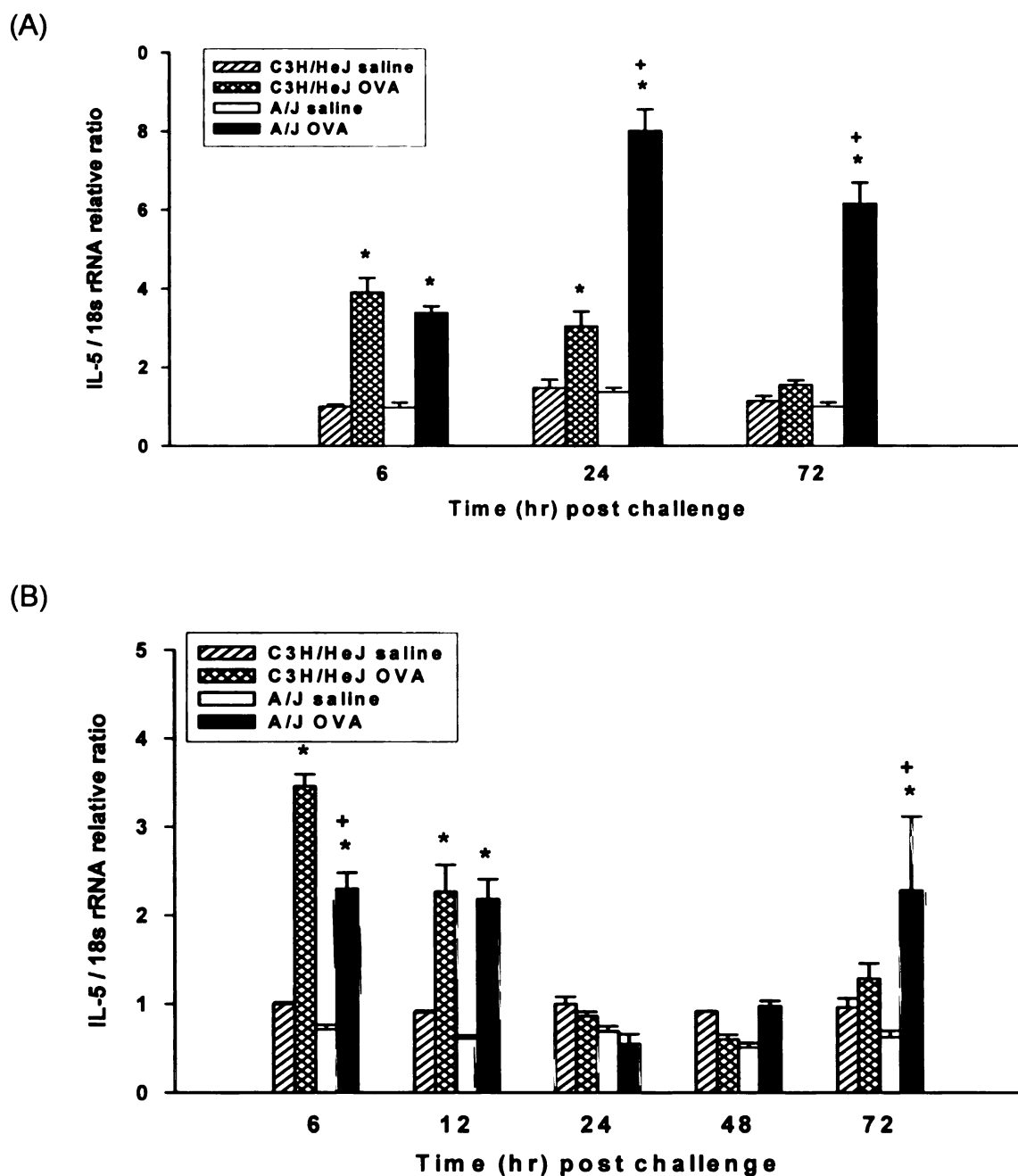
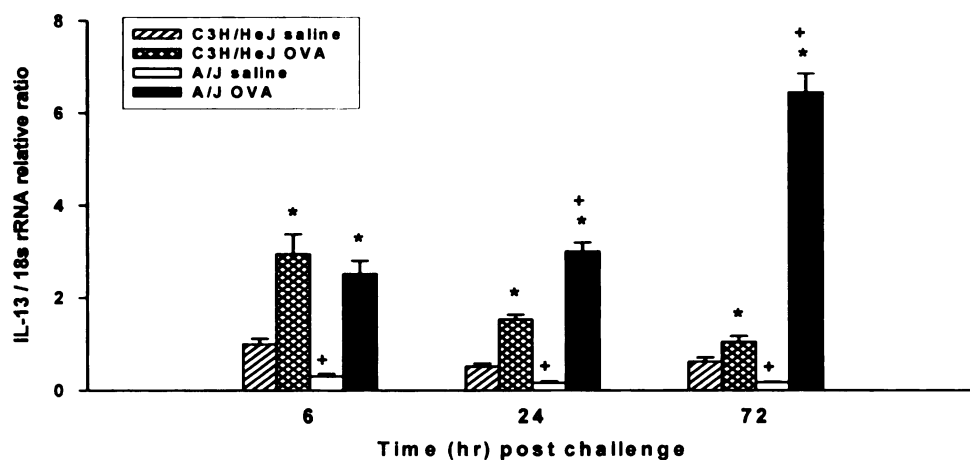


Figure 16. IL-5 expression in lungs and tracheobronchial lymph nodes. (A) IL-5 expression in lungs. (B) IL-5 expression in tracheobronchial lymph nodes. A/J or C3H/HeJ mice were exposed with PBS or OVA ($n = 6$ mice/group). Tissues were collected at 6, 12, 24, 48, and 72 hr following challenge. *Significant difference due to treatment ($p \leq 0.05$). +Significant difference due to strain ($p \leq 0.05$).

(A)



(B)

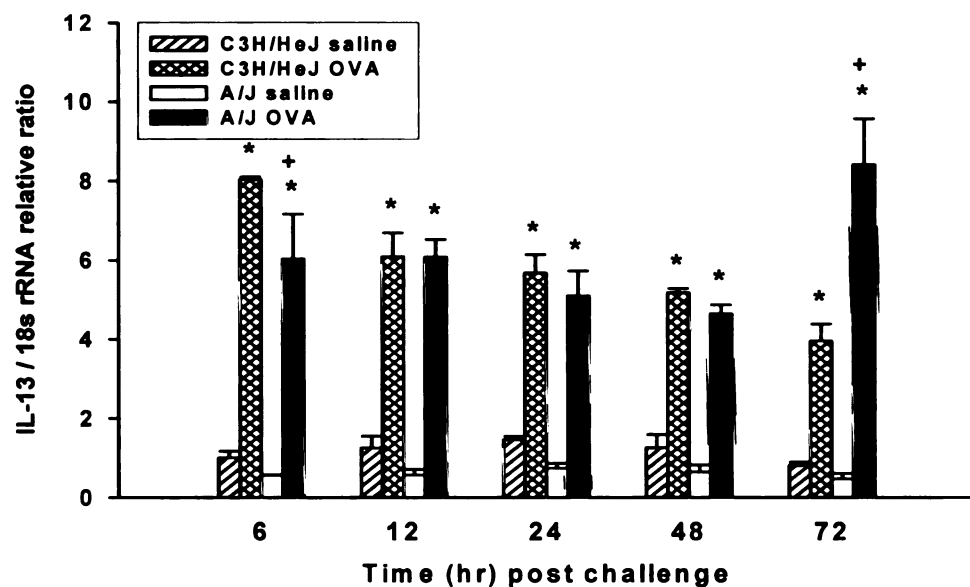


Figure 17. IL-13 expression in lungs and tracheobronchial lymph nodes. (A) IL-13 expression in lungs. (B) IL-13 expression in tracheobronchial lymph nodes. A/J or C3H/HeJ mice were exposed with PBS or OVA ($n = 6$ mice/group). Tissues were collected at 6, 12, 24, 48, and 72 hr following challenge. *Significant difference due to treatment ($p \leq 0.05$). +Significant difference due to strain ($p \leq 0.05$).

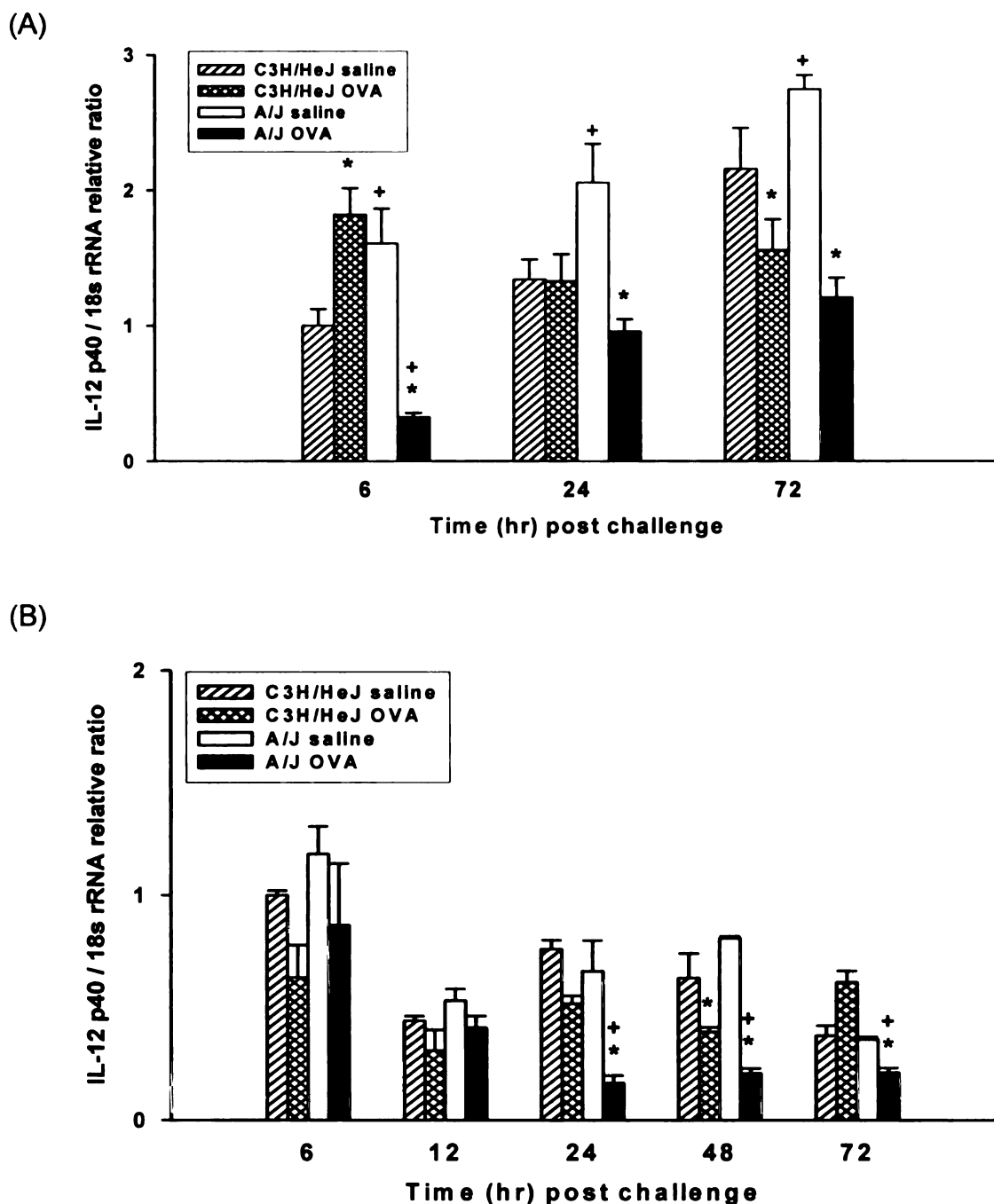


Figure 18. IL-12p40 expression in lungs and tracheobronchial lymph nodes. (A) IL-12p40 expression in lungs. (B) IL-12p40 expression in tracheobronchial lymph nodes. A/J or C3H/HeJ mice were exposed with PBS or OVA ($n = 6$ mice/group). Tissues were collected at 6, 12, 24, 48, and 72 hr following challenge. *Significant difference due to treatment ($p \leq 0.05$). *Significant difference due to strain ($p \leq 0.05$).

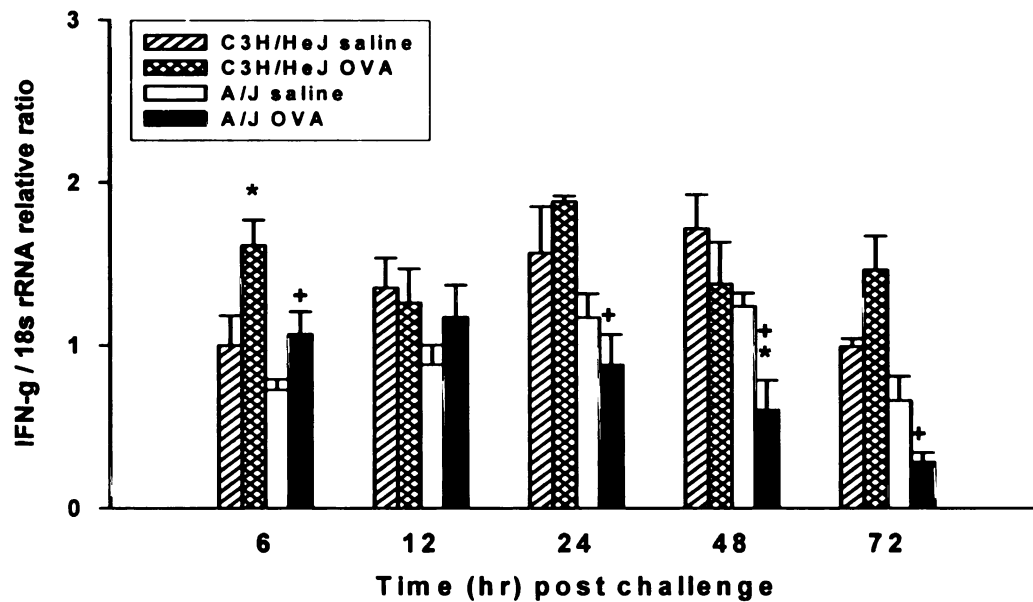
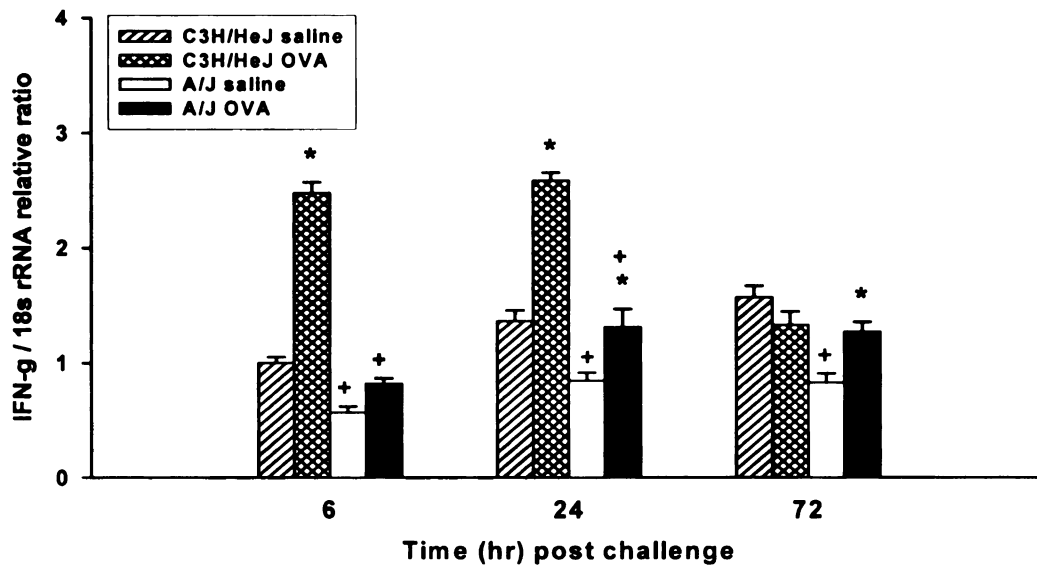


Figure 19. IFN- γ expression in lungs and tracheobronchial lymph nodes. (A) IFN- γ expression in lungs. (B) IFN- γ expression in tracheobronchial lymph nodes. A/J or C3H/HeJ mice were exposed with PBS or OVA ($n = 6$ mice/group). Tissues were collected at 6, 12, 24, 48, and 72 hr following challenge. *Significant difference due to treatment ($p \leq 0.05$). +Significant difference due to strain ($p \leq 0.05$).

E. GATA-3 gene expression profile

The GATA-3 TaqMan primer pair and probe were designed using Primer Express software. The forward primer was located in exon 3, and reverse primer was located at the border of exon 3 and exon 4, making it specific for RNA amplification by avoiding genomic DNA amplification (Figure 20). This primer pair was also used with SYBR green reagents.

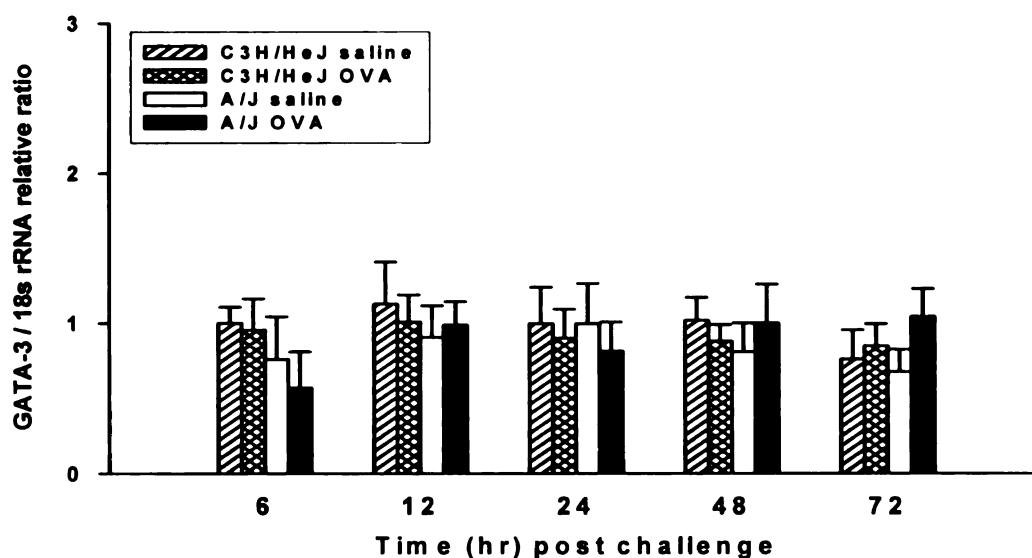
GATA-3 expression in lungs was unaffected by OVA exposure in both *A/J* and *C3H/HeJ* mice as assessed by both TaqMan and SYBR green assays (Figure 21 and Table 11). In contrast, GATA-3 expression was induced in *A/J* mice at 6 hr in both tracheobronchial lymph nodes and spleens (Figure 22, Table 12, and Table 13). A decrease in GATA-3 expression in tracheobronchial lymph nodes was observed in *C3H/HeJ* mice following OVA exposure at the 24 hr through 72 hr time points (Figure 22 and Table 12).

CCTACCGGGTTCGGATGTAAGTCGAGGCCCAAGGCACGATCCAGCACAG**AAGGC**
Forward primer → probe ←

AGGGAG TGTGTG
Reverse primer

Figure 20. GATA-3 TaqMan primer pair and probe. Exon 3 is shown in regular font and exon 4 is shown in bold.

(A)



(B)

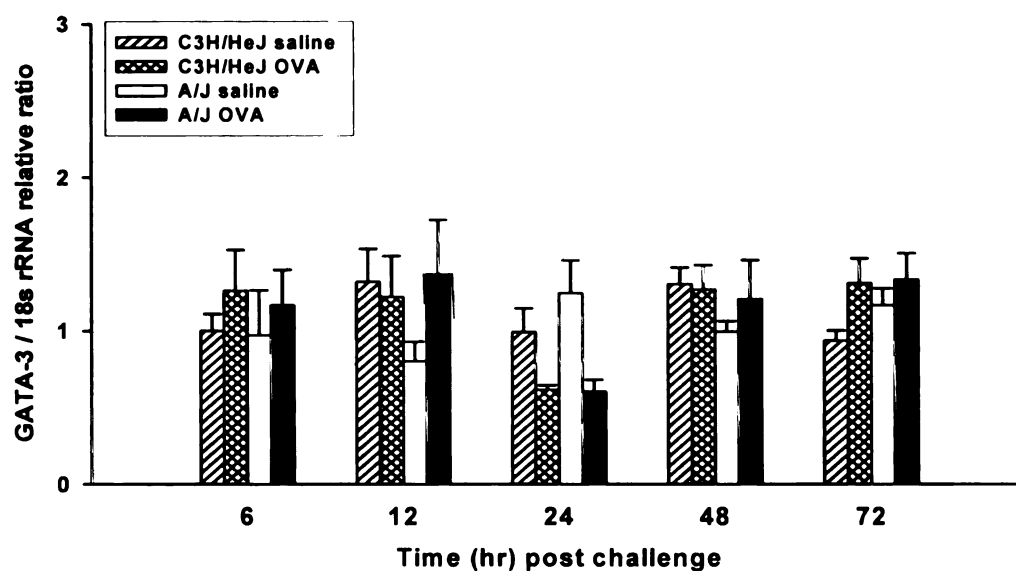
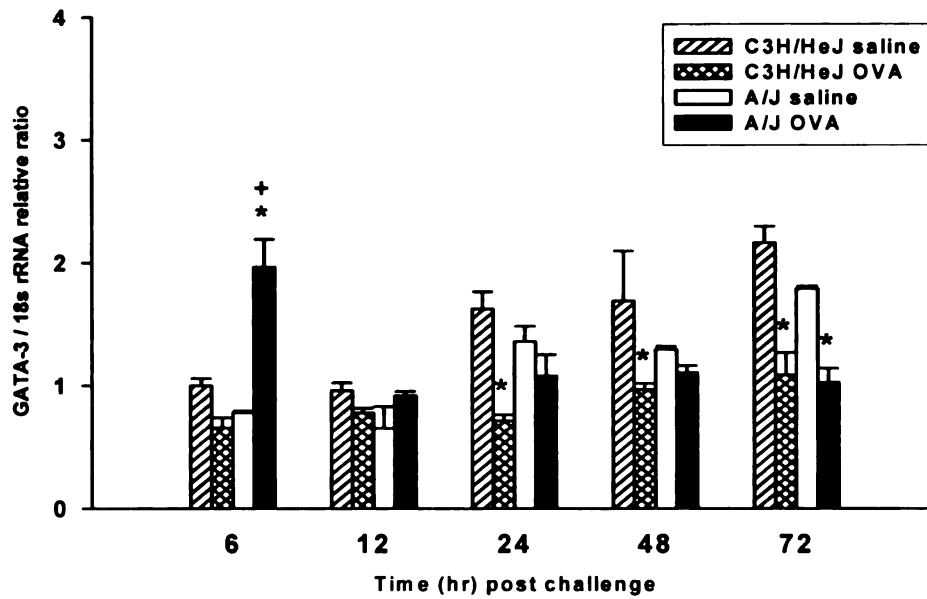


Figure 21. GATA-3 expression in lungs, TaqMan and SYBR green assay. (A) GATA-3 expression in lungs, TaqMan assay. (B) GATA-3 expression in lungs, SYBR green assay. A/J or C3H/HeJ mice were exposed with PBS or OVA (n = 6 mice/group). Tissues were collected at 6, 12, 24, 48, and 72 hr following challenge. *Significant difference due to treatment ($p \leq 0.05$). *Significant difference due to strain ($p \leq 0.05$).

(A)



(B)

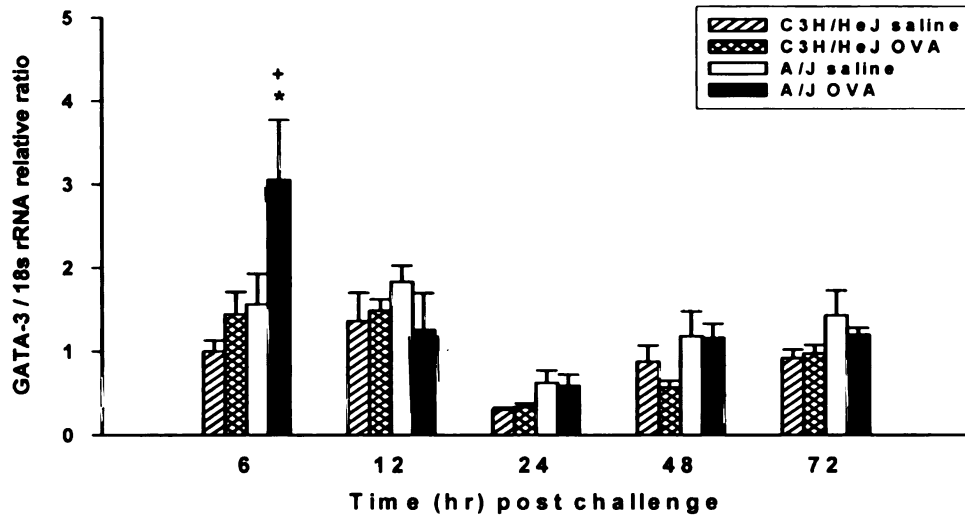


Figure 22. GATA-3 expression in tracheobronchial lymph nodes and spleens. (A) GATA-3 expression in tracheobronchial lymph nodes. (B) GATA-3 expression in spleens. A/J or C3H/HeJ mice were exposed with PBS or OVA (n = 6 mice/group). Tissues were collected at 6, 12, 24, 48, and 72 hr following challenge. *Significant difference due to treatment ($p \leq 0.05$). +Significant difference due to strain ($p \leq 0.05$).

F. Summary

According to TaqMan real-time RT-PCR data, Th2 type cytokines (IL-4, IL-5, and IL-13) were induced in A/J mice following OVA exposure at much higher levels than in C3H/HeJ mice. However, Th1 type cytokines (IL-12 and IFN-g) in lungs and tracheobronchial lymph nodes were generally higher in C3H/HeJ than A/J mice following OVA treatment, but this was as much a function of cytokine suppression in A/J mice as induction in C3H/HeJ mice. The cytokines expression profiles reconfirmed our hypothesis that A/J mice have stronger Th2 responses and C3H/HeJ have stronger Th1 responses following OVA treatment. GATA-3 expression was induced in A/J mice at 6 hr following OVA exposure, but not in C3H/HeJ mice in both lymph nodes and spleens. These results also reconfirmed our expectation since GATA-3 is a transcription factor and should be induced in A/J mice at earlier time points than the cytokine genes whose expression is regulated by GATA-3. Unexpectedly, GATA-3 expression was not induced in lungs of A/J mice at 6 hr or any other time points. The different results between lungs and lymphoid organs (spleens and tracheobronchial lymph nodes) might be due to the composition of cell types of each tissue. Spleen and tracheobronchial lymph nodes are mainly composed of immune response related cells, thus their gene expression profiles may reflect immune response more closely. Lungs contain a high proportion of epithelial cells, which may contribute to higher background GATA-3 expression. Perhaps, that is the reason why we did not observe

the GATA-3 induction by OVA treatment in A/J mice at 6 hr in lungs. Since the proteins are the final factor of biological pathways, GATA-3 protein level differences were compared between A/J and C3H/HeJ mice.

Chapter Five: Comparison of GATA-3 protein in two murine strains

A. Introduction

Since proteins are the real executants of biological functions, the phenotype difference between A/J and C3H/HeJ mice may be determined by either the GATA-3 protein amino acid sequence difference or by the concentration difference of GATA-3 protein. Since no coding polymorphisms were detected between these two strains, the GATA-3 protein is predicted to have the same amino acid sequences. Based on the results of GATA-3 expression studies described in chapter 4, concentration differences in GATA-3 protein may exist between these two strains.

Two popular methods are available for protein semi-quantitation; electrophoretic mobility shift assay (EMSA) and Western blot. EMSA uses a specific DNA probe to assay the binding between protein and DNA, while the Western blot is based on specific antigen-antibody recognition. Since GATA-3 is a transcription factor, it functions through binding to the target DNA promoter. Therefore, we initially attempted to use the EMSA method to study GATA-3. However, EMSA require delicate protein-DNA binding conditions which we were unable to achieve. After repeated unsuccessful attempts to optimize probe, salt and DNA concentrations, we abandoned the EMSA method and pursued the Western blot.

GATA-3 is a transcription factor; thus, it has to be transported into the nucleus where it binds to the target DNA promoter. For this reason we

isolated nuclear protein instead of whole cell protein. We demonstrated in the expression studies described in chapter 4 that cytokines were induced differently in A/J and C3H/HeJ mice even after 72 hr OVA exposure; therefore, we chose to examine protein levels at the 72 hr time point after challenge in spleen cells.

B. GATA-3 Western blot assay development

Miaw and colleagues [105] have previously demonstrated that GATA-3 mRNA is expressed in both stimulated (phorbol 12-myristate 13-acetate (PMA) + ionomycin) and unstimulated EL-4 (T lymphoma cell) cells. Dibutyryl cyclic AMP (Bt2cAMP) is a cAMP analogue, which can stimulate cytokine production in Th cells through the p38 mitogen-activated protein (MAP) kinase pathway. PMA activates T cells through the protein kinase C pathway (PKC). Based on this information, we anticipated stable expression of GATA-3 protein in EL-4 cells; thus, we tested them for use as a positive control. We treated EL-4 cells with 25 ng/ml PMA + 1 mM Bt2cAMP in ethanol or ethanol alone. EL-4 cells were harvested and nuclear proteins were isolated 2 hr and 8 hr after treatment. The nuclear proteins were examined by Western blot (Figure 23). GATA-3 proteins were expressed in both treated and untreated EL-4 cells. Comparing lanes 1 and 2 or lanes 3 and 4, GATA-3 protein was increased subsequent to PMA and Bt2cAMP treatment. Protein levels were higher in the 8 hr than the 2 hr samples.

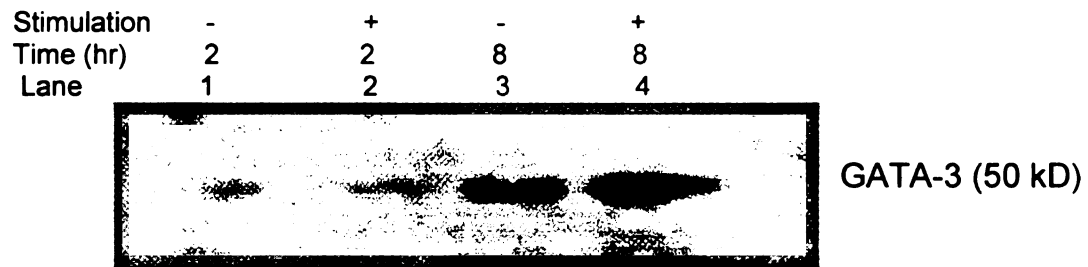


Figure 23. EL-4 cell Western blot gel. Lane 1, EL-4 untreated 2 hr (30 ug). Lane 2, EL-4 treated 2 hr (30 ug). Lane 3, EL-4 untreated 8 hr (60 ug). Lane 4, EL-4 treated 8 hr (60 ug).

C. Experimental time line

A/J and C3H/HeJ male mice were obtained from the Jackson Laboratory (Bar Harbor, ME) at 4 weeks of age and allowed one week to acclimate before experimentation. Animals were housed 4/cage, under high-efficiency particulate air (HEPA) laminar flow hoods and allowed free access to ovalbumin (OVA)-free rodent chow and water.

On day 0, mice were sensitized (n = 8/group) intraperitoneally with 10 ug chicken egg OVA (crude grade IV; Sigma, St. Louis, MO) in 200 ul phosphate-buffered saline (PBS) or PBS alone. On day 14, mice were anesthetized (ketamine, 45 mg/kg and xylazine, 8 mg/kg, intraperitoneally), and challenged with a tracheopharyngeal aspiration of 1.5% OVA in 45 ul PBS or PBS alone, then recovered from anesthesia. Spleens were harvested 72 hr after in vivo challenge (Figure 24). The 8 spleens within each group were combined. The spleens were disaggregated, and the cells spread on to 8 petri dishes under treated or control conditions. After 4, 8, 12, or 24 hr in vitro stimulation (25 ng/ml PMA + 1 mM Bt2cAMP), splenocytes were collected and nuclear proteins were isolated to perform Western blot.

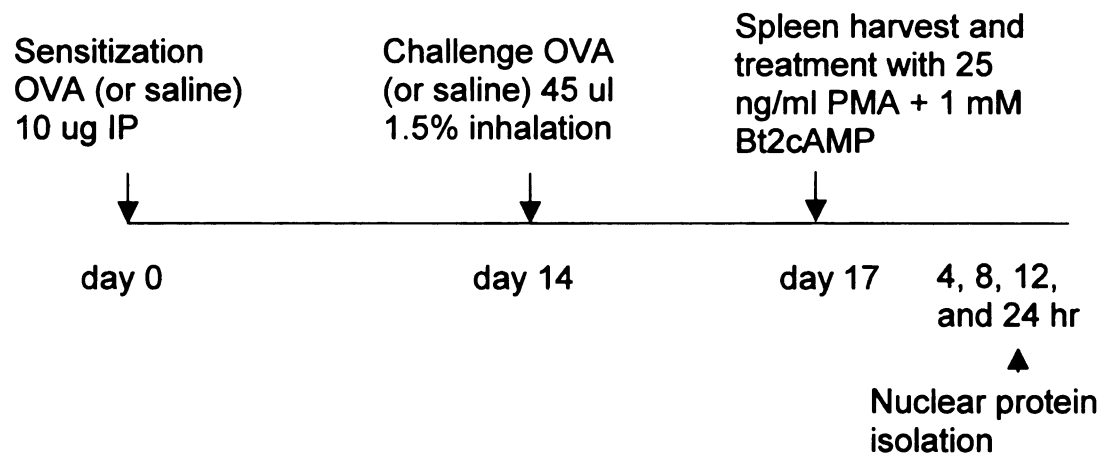


Figure 24. Experimental time line for splenocyte nuclear protein isolation

D. Western blot of GATA-3

Western blot of GATA-3 was first performed on the samples of splenocytes nuclear protein following 8 hr of in vitro stimulation. Actin was stained as an internal control (Figure 25). Only A/J OVA-treated mice showed a strong GATA-3 signal, which was independent of cAMP/PMA stimulation (lane 8 and 9). GATA-3 was not readily detected in OVA-exposed or control C3H/HeJ mice, nor in A/J mice not exposed to OVA. However, weak bands were visible for these other groups when the gel was developed longer, indicating far lower levels of GATA-3 production (data not shown). As we expected, GATA-3 protein expression was induced by OVA in A/J mice, but not in C3H/HeJ mice.

Western blot of GATA-3 was then performed in 4, 12, and 24 hr samples (Figure 26). Our results demonstrate that GATA-3 production was not induced by OVA treatment in C3H/HeJ mice even after 24 hr of in vitro stimulation (lane 2 and 3). On the contrary, in A/J mice GATA-3 protein production was induced by OVA at all time points (from lane 4 to lane 9) and increased in a time-dependent manner. However, in vitro cAMP/PMA stimulation had little effect over in vivo OVA exposure (compare lane 4 with lane 5, lane 6 with lane 7, and lane 8 with lane 9).

Strain	C	C	C	C	A	A	A	A
Rx	P	P	O	O	P	P	O	O
Stimulation	-	+	-	+	-	+	-	+
Lane	1	2	3	4	5	6	7	8

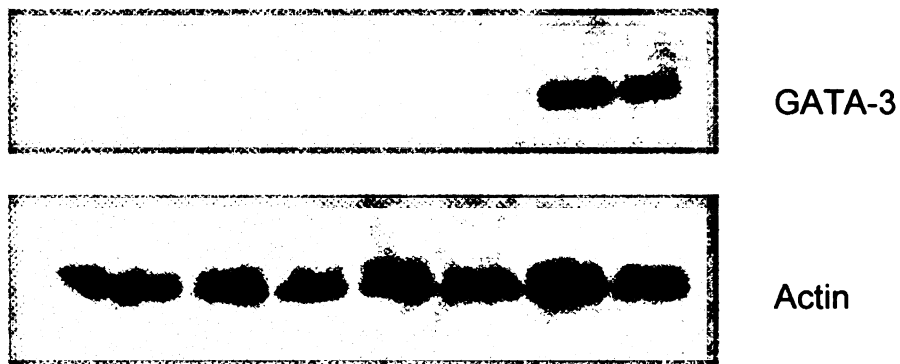


Figure 25. Western blot of GATA-3 8 hr splenocytes samples. Lane 1, C3H/HeJ PBS, unstimulated (10 ug). Lane 2, C3H/HeJ PBS, stimulated (10 ug). Lane 3, C3H/HeJ OVA, unstimulated (10 ug). Lane 4, C3H/HeJ OVA, stimulated (10 ug). Lane 5, A/J PBS, unstimulated (10 ug). Lane 6, A/J PBS, stimulated (10 ug). Lane 7, A/J OVA, unstimulated (10 ug). Lane 8, A/J OVA, stimulated (10 ug). Actin was used as internal control. C3H/HeJ (C), A/J (A), PBS (P), OVA (O).

Strain	E	C	C	A	A	A	A	A	A
Rx	O	O	O	O	O	O	O	O	O
Stimulation	-	-	+	-	+	-	+	-	+
Time (hr)	-	24	24	4	4	12	12	24	24
Lane	1	2	3	4	5	6	7	8	9

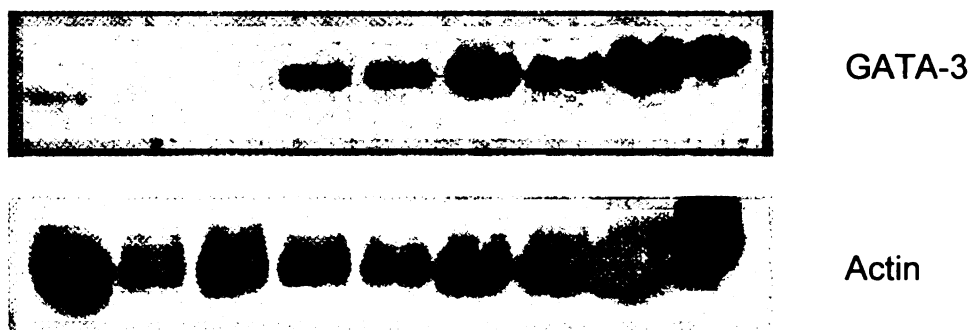


Figure 26. Western blot of GATA-3, 4, 12, and 24 hr splenocytes samples. Lane 1, EL-4 (30 ug). Lane 2, C3H/HeJ OVA, unstimulated, 24 hr (20 ug), Lane 3, C3H/HeJ OVA, stimulated, 24 hr (20 ug). Lane 4, A/J OVA, unstimulated, 4 hr (20 ug). Lane 5, A/J OVA, stimulated, 4 hr (20 ug). Lane 6, A/J OVA, unstimulated, 12 hr (20 ug). Lane 7, A/J OVA, stimulated, 12 hr (20 ug). Lane 8, A/J OVA, unstimulated, 24 hr (20 ug). Lane 9, A/J OVA, stimulated, 24 hr (20 ug). C3H/HeJ (C), A/J (A), PBS (P), OVA (O).

E. Summary

Western blot analysis of spleen cell nuclear proteins indicated that GATA-3 protein was induced in A/J mice, but not in C3H/HeJ mice, at 72 hr following OVA challenge. GATA-3 mRNA was induced only in A/J mice spleen at 6 hr following OVA challenge, but not in C3H/HeJ mice or other time points (shown in chapter 4). This discrepancy may be due to the relative stability of protein compared with mRNA. These results reconfirmed that GATA-3 was differently expressed in A/J and C3H/HeJ mice in response to OVA exposure. The induction of GATA-3 in A/J mice following OVA exposure indicated its important role in cytokine gene expression regulation, Th1/Th2 pathway determination, and airway hyperresponsiveness.

DISCUSSION

The goal of this project was to further define the loci and underlying genes controlling allergen-induced AHR in a murine model of asthma. The studies conducted for this dissertation research reflect our approach to refine the map positions of the *Abhr* loci and then test the highest priority positional candidate gene, which was *Gata3*.

To more closely determine the location of *Abhr1* and *Abhr2* relative to *Gata3*, these QTL were fine-mapped (~2 cM) by genotyping 450 A/J backcross mice for 37 densely clustered DNA markers on chromosome 2.

A/J backcross mice ($n = 450$) with extreme airway responsiveness phenotypes ($APTI \leq 620$ or $APTI \geq 797$ cmH₂O.sec) were selected for genotyping from approximately 1,000 A/J backcross mice. Because asthma is a complex disease, several genes may be involved and interact to lead to the asthmatic phenotype [1]. The mice with extreme phenotypes were chosen because it was likely that their genomes included more genes carrying asthma-supportive alleles.

Thirty one MIT microsatellite markers with polymorphisms between A/J and C3H/HeJ mice on chromosome 2 were available at the initiation of this project. To find more markers, especially the type 1 markers within positional candidate genes, the Celera mouse genome database was searched to obtain the sequences of identified positional candidate genes. Primer pairs were designed to amplify simple nucleotide tandem repeats regions, which were normally

located within introns. The primer pairs were used as microsatellite markers if the PCR products had clear size differences between A/J and C3H/HeJ strains. Five new type I markers within genes *Gata3*, *Mrc1*, *Prkcq*, *Gad2*, and *Il2ra* on chromosome 2 were designed. These five newly identified type I markers may be useful in other mouse strains that are polymorphic at these alleles.

The map constructed with the markers genotyped in this study was compared to published maps. The relative positions of our markers were similar to the MIT linkage map and the Celera physical map, thus ours appears to be a consistent and accurate genetic linkage map. Furthermore, the map constructed in this study was a detailed map, for example, the *Abhr1* interval contains 13 markers in 11 cM). In mice, 1 cM genetic (or recombination) unit is approximately equal to 2 Mb of genomic DNA, thus our linkage map is detailed to approximately 2 Mb. Due to the large number of mice ($n = 450$) used for genotyping, our map discerned the location differences of a number of markers that were not distinguishable by the MIT linkage map. More information regarding additional markers on chromosome 2 can in the future be easily incorporated into our current map to make an even more detailed map. This refined map will also be useful for accurate dissection the DNA fragments in a related congenic mouse project ongoing in our laboratory.

Within *Abhr1* and *Abhr2* regions, approximately 200 identified genes exist. *Hc* (*C5a*) was an important positional candidate gene for the *Abhr2* region (lod = 3.5) [119]. *Gata3* mapped within the *Abhr1* confidence interval (lod = 4.3) and was reconfirmed to be a strong positional candidate gene for this locus. Other

positional candidate genes, such as *Mrc1*, *Il1m*, *Lcn2*, and *Ptgs1*, also merit future attention.

To determine *Gata3* polymorphisms between A/J and C3H/HeJ mice, *Gata3* exons, splicing sites, and part of the promoter region and 3' untranslated region (12,069 bp total) were sequenced in both A/J and C3H/HeJ strains.

One microsatellite marker (type I) in intron 5 of *Gata3* was identified and used to genotype *Gata3* on our linkage map. However, this marker may not be functionally important because it is a simple tandem repeats marker. No additional polymorphisms were found on approximately 12 kb of *Gata3* sequence. Based on the sequence comparison between mouse strains B6 and 129, the average SNPs rate was 14 SNPs/10 kb [127]. However, the distribution of SNPs were mosaic rather than uniform throughout the genome. That is, the long segments of DNA had either extremely high (40 SNPs/10 kb) or extremely low (0.5 SNPs/ 10 kb) SNPs rate [127]. Our results indicate that *Gata3* gene is located in an extremely low SNPs rate DNA segment, therefore, no SNP was found in 12 kb sequences of A/J and C3H/HeJ strains. Similarly, based on the National Center for Biotechnology Information (NCBI) SNP database, only two SNPs were found in murine *Gata3*; in intron 4 (SNP ID: rs6312556) and intron 3 (SNP ID: rs6314548), but these SNPs were not polymorphic between A/J and C3H/HeJ strains. According to Roche mouse SNPs database (mousesnp.roche.com), there were two SNPs far upstream of *Gata3* (950,950 bp and 247,098 bp upstream) polymorphic between A/J and C3H/HeJ mice. The

functions of these two SNPs 5' to *Gata3* merit further study because they may belong to the *Gata3* promoter region.

Since no polymorphisms were found in exons and splicing sites of *Gata3*, there should be no qualitative difference in GATA-3 mRNA or amino acid sequences between A/J and C3H/HeJ mice. Although no functional polymorphisms were identified, polymorphisms may exist in unidentified regulatory regions or unsequenced introns. Polymorphisms in introns may lead to alternate splicing or expression level difference of GATA-3 mRNA. Only one alternative splicing form of *Gata3* promoter has been reported: exon 1a, which is selectively expressed in the brain, substitutes the previously identified exon 1 in the alternative form [79]. This alternative form of GATA-3 mRNA may lead to tissue-specific expression, but will not produce a new form of GATA-3 protein since it is located at *Gata3* promoter region. Polymorphisms in regulatory regions may lead to quantitative differences in GATA-3 mRNA and proteins. In future studies, larger ranges of the genome around the *Gata3* location could be sequenced to pursue regulatory region polymorphisms. Alternatively, the whole *Abhr1* and *Abhr2* regions may be sequenced and compared between A/J and C3H/HeJ mice to identify all the polymorphisms. This complete sequencing information would be useful to narrow and identify the positional candidate gene(s).

To compare GATA-3 expression between A/J and C3H/HeJ, the levels of GATA-3 mRNA at different time points (6, 12, 24, 48, 72 hr) following allergen exposure and in control mice of these strains were determined by real-time RT-

PCR. Both early and late time points were studied to show the relative complete immune response profile in the mice. Expression of cytokine genes IL-4, IL-5, IL-12, IL-13, and IFN- γ were also tested by TaqMan real-time RT-PCR. Overall, Th1 type cytokines (IL-12 and IFN- γ) have relatively higher gene expression levels following allergen treatment in the C3H/HeJ strain; however, Th2 type cytokines (IL-4, IL-5, and IL-13) were induced higher in the A/J strain. The results support our expectation that the hyperresponsive A/J strain has stronger Th2 responses due to allergen exposure. Interestingly, the expressions of Th2 type cytokines (IL-4, IL-5, and IL-13) were induced at similar levels at the earliest time point (6 hr) in both A/J and C3H/HeJ strains, but declined quickly in C3H/HeJ mice. IFN- γ was also induced in both strains in almost all time points, although it tended to be higher in C3H/HeJ than A/J mice. IL-12 was largely not changed in C3H/HeJ at all time points, although it was inhibited in A/J mice. In vitro CD4 T cell differentiation studies also showed that low levels of IL-4 and IFN- γ could be detected after 24 hr of Th1 and Th2 priming, however after 48 hr, Th1 and Th2 cells expressed only IFN- γ and IL-4, respectively [85]. Overall, it seems like C3H/HeJ mice or A/J mice did not develop purely Th1 or Th2 responses, especially at the earliest time point. It is quite possible that the early time point (6 hr) is still the determination stage with combined Th1/Th2 responses in both strains. However, with effects of transcription factors, such as GATA-3, A/J and C3H/HeJ mice favor Th2 and Th1 pathways, respectively, at later time points.

Because IL-5 is essential for eosinophil differentiation and maturation, the numbers of eosinophils lining the main axial airway of the left lung lobe following

OVA exposure in A/J and C3H/HeJ mice were counted in accordance with the IL-5 expression assay of the right lung lobe. At 6 hr following OVA exposure, the numbers of eosinophils were increased in both A/J and C3H/HeJ strains. However, at 72 hr after OVA exposure, only A/J mice had significantly increased number of eosinophils (unpublished data from Dr. Harkema). Eosinophil numbers at both early and late time points matched well with the IL-5 expression data. Since IL-4 can induce mucin gene expression and mucus secretion by goblet cells in the airways [131], the intraepithelial mucosubstances in the surface epithelium lining the main axial airway at generation 5 of the left lung lobe following OVA exposure in A/J and C3H/HeJ mice were stained and counted in accordance with the IL-4 expression assay of the right lung lobe. Mucosubstances increased only in A/J mice following OVA exposure at 24, 48, and 72 hr, and peaked at 72 hr (unpublished data from Dr. Harkema). IL-4 expression in lungs increased early (6hr) following OVA exposure in both A/J and C3H/HeJ mice, but decreased quickly in C3H/HeJ mice. At 72 hr after OVA challenge, IL-4 expression increased significantly only in A/J mice but not C3H/HeJ mice. Mucosubstances quantitation also matched well with IL-4 expression data. Overall, the histological data were consistent with the cytokine gene expression results.

GATA-3 expression was induced by OVA treatment at the earliest time point (6 hr) in lymph nodes of A/J mice, and reduced at late time points in C3H/HeJ mice. GATA-3 expression profile in spleen was similar to lymph nodes, specifically, GATA-3 was induced only in A/J mice at 6 hr by OVA. These results

reconfirmed the early induction of GATA-3 expression and may indicate its important role in cytokine expression regulation. Since both tracheobronchial lymph nodes and spleens are lymphoid organ, the cell types are similar and largely composed of immune response related cells, therefore the expression profiles of lymph nodes and spleens should be similar.

Unexpectedly, no difference of GATA-3 expression was observed in lungs at all time points, which may due to the high background expression in this tissue by epithelial cells (UniGene Cluster Hs.413375). Laser capture technique may be used to select specific immune response related cell types from lungs to prevent background expression of other cells.

In vitro T cell differentiation studies have shown GATA-3 mRNA was constitutively expressed and induced during Th2 cell differentiation even after 120 hr following Th2 priming [75, 92]. In contrast, our in vivo assay only showed that GATA-3 mRNA expression increased at 6 hr following OVA exposure in spleens and tracheobronchial lymph nodes. This discrepancy may come from the differences between in vivo and in vitro stimulation or single cell line and whole tissue message source. Since CD4 T cells are the most important cell type involved in asthma, the in vitro studies of T cell differentiation can show the profile of this asthma-related cell accurately. However, the in vivo whole animal study has its own advantages, that is it reflects the real events happening in the animals more closely.

Since GATA-3 is a transcription factor upstream of cytokine genes, the induction of GATA-3 expression should be earlier than that of Th2 type cytokines.

GATA-3 induction by OVA happened quite early at 6 hr, its expression at earlier time points such as 0.5, 1, 2, and 4 hr could be tested in future studies to show its complete expression profile. DNA is transcribed into mRNA before translation into protein, thus, the protein response should follow the mRNA regulation.

To investigate the role of GATA-3 in mediating allergen-induced AHR, the levels of GATA-3 protein in splenocytes at 72 hr following allergen exposure and in control mice of these strains were measured by Western-blot. A/J mice showed a strong GATA-3 signal following OVA challenge, but not C3H/HeJ mice. Cyclic AMP in vitro stimulation had no further effects on GATA-3 protein expression in this study. In contrast, the electrophoretic mobility shift assays (EMSA) have shown cAMP could induce the target DNA binding activity of GATA-3 [75, 86]. Because cAMP activates GATA-3 phosphorylation through the p30 MAP kinase pathway and phosphorylation may be important for the DNA binding activity of GATA-3 [111], cAMP may have little effects on the expression of GATA-3. It seems like GATA-3 proteins were constitutively induced in A/J mice following OVA exposure even after 72 hr. In contrast GATA-3 mRNA induction was only observed at 6 hr in spleens. It is possible that the GATA-3 protein is more stable than mRNA.

When antigens, such as OVA in this study, enter the respiratory airways of mice, dendritic cells will process antigens and circulate to peripheral lymphoid organs through blood and lymphoid fluids. Tracheobronchial lymph nodes and spleens are peripheral lymphoid organs where the naïve T cells are activated by antigen-carrying dendritic cells. The early (6 hr) induction of GATA-3 mRNA in

the tracheobronchial lymph nodes and spleens of A/J mice following OVA exposure can lead naïve T cells into Th2 cells. Our protein analysis showed that GATA-3 proteins were induced in splenocytes of A/J mice following OVA exposure even after 72 hr, which further indicated that A/J mice favored Th2 pathway following OVA exposure. The activated lymphocytes will move to the target organs, such as the lung in this study, to deal with the antigens. Although the induction of GATA-3 mRNA in the lungs of A/J mice following OVA exposure could not be detected in this study, the relatively higher induction of Th2 type cytokines in A/J mice lungs indicated the infiltration of Th2 cells and eosinophils. GATA-3 mRNA and protein expression data confirmed our hypothesis that GATA-3 is differently expressed in A/J and C3H/HeJ mice following allergen challenge. It indicates that the expression level difference of GATA-3 may be important for cytokine gene expression, Th1/Th2 pathway determination, and airway hyperresponsiveness.

In summary, we found strain-specific and treatment-specific quantitative differences in GATA-3 mRNA and protein levels, but no qualitative differences between A/J and C3H/HeJ mice were detected in *Gata3* at the DNA level. These results reconfirmed the important role of GATA-3 in allergen-induced AHR. However, they do not provide direct evidence in support of *Gata3* as a positional candidate gene for *Abhr1* in the A/J and C3H/HeJ murine model of asthma.

Materials and Methods

A. Genomic DNA isolation from mouse kidney

In preparation for this procedure the 5810R centrifuge (Hamburg, Germany) was cooled to 4°C and a waterbath (Fisher Scientific, Pittsburgh, PA) was warmed to 42-47°C. 15 ml polypropylene tubes were put on ice. One kidney was placed in each dounce with 4.5 ml cold Iso-Hi-pH [0.14 M NaCl (J.T. Baker, Phillipsburg, NJ), 0.01 M Tris (pH 8.4) (Invitrogen, Carlsbad, CA), 0.15 mM MgCl₂ (J.T. Baker, Phillipsburg, NJ), 0.05% NP-40 (Sigma, St. Louis, MO), and H₂O to suitable volume. Sterilize final solution by autoclave or filter, and store at 4°C.]. The tissues were homogenized using 7-10 strokes, the pestle rinsed with 1 ml cold Iso-Hi-pH and the homogenate transferred to a cold 15 ml tube using a transfer pipet. Homogenates were spun at 3,000 rpm, for 25 min, at 4°C, then supernatants were discarded. 400 ul Iso-Hi-pH buffer was added to 15 ml tubes and the pellets were resuspended using a transfer pipet, then the suspensions were transferred to new 2 ml eppendorf tubes. Iso-Hi-pH + SDS + PK (400 ul) [75% Iso-Hi-pH, 2% SDS (Sigma, St. Louis, MO) and 1 mg/ml proteinase K (Sigma, St. Louis, MO) were added. This solution must be made freshly each day.] and inverted to mix. Eppendorfs were incubated in a waterbath at 42-47°C for 2-3 hr. The eppendorfs were filled with phenol/chloroform/isoamyl alcohol mixture [phenol (Boehringer, Indianapolis, IN) / chloroform (Sigma, St. Louis, MO) / isoamyl alcohol

(Sigma, St. Louis, MO): at 11:11:0.48 volume ratios. It must be made freshly each week.]. Tubes were inverted to mix, and centrifuged at 12,000 rpm for 3 min. The top layer was transferred to a new 2 ml eppendorf and refilled with phenol/chloroform/isoamyl alcohol mixture. Tubes were inverted to mix, and centrifuged at 12,000 rpm for 3 min again. The top layer was transferred to a new 15 ml polypropylene tube. 70 μ l 3 M KCl were added, then 2-3X volumes (~2.5 ml) cold 95% alcohol. DNA was spooled onto flamed glass rods, rinsed with 70% alcohol. DNA was allowed to dry without contamination. DNA was dissolved in 300 μ l 10 mM TE buffer [0.5 ml 2 M Tris-Cl (pH 8.0), 0.2 ml 0.5 M EDTA (pH 8.0) (Invitrogen, Carlsbad, CA), and 99.3 ml ddH₂O. Sterilize by autoclave or 0.2 μ m filter.], and stored at 4°C.

B. Polymerase chain reaction (PCR)

PCR reactions were generally performed in 15 μ l volumes containing: 1.5 μ l 10X PCR buffer (Invitrogen, Carlsbad, CA), 0.45 μ l 50 mM Mg⁺⁺ (Invitrogen, Carlsbad, CA), 1.2 μ l 1 mM dNTPs (Invitrogen, Carlsbad, CA), 1 μ l 10 μ M forward primer (IDT, Coralville, IA), 1 μ l 10 μ M reverse primer (IDT, Coralville, IA), 0.075 μ l 5 unit/ μ l Taq polymerase (Invitrogen, Carlsbad, CA), 6.775 μ l ddH₂O, and 3 μ l 10 ng/ μ l DNA template. A 100X master mixture of all components (except DNA template) was made for each 96 well plate, 96 well plates were loaded with master mix, and then 3 μ l DNA template was added separately to each well. PCR reactions were performed in a GeneAmp PCR System 9600 (Perkin Elmer, Norwalk, CT).

PCR products were checked on 1% agarose gels (Invitrogen, Carlsbad, CA) for common use, or on 4.5% agarose gels (3% of agarose + 1.5% of NuSieve GTG agarose (Cambrex Bio Science, Rockland, ME) for genotyping experiments. Gels were run in 1X TAE buffer (Diluted from 50X TAE: 242 g of Tris, 57.1 ml glacial acetic acid, and 100 ml 0.5 M EDTA (pH 8.0)) for common use, or in 0.5X TBE buffer (Diluted from 5X TBE: 54 g Tris, 27.5 g boric acid, and 20 ml 0.5 M EDTA (pH 8.0)) for genotyping experiments.

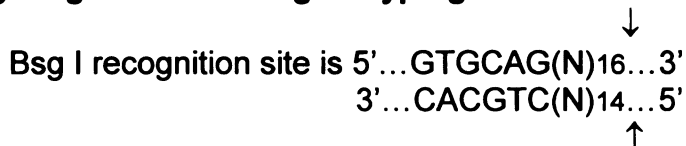
PCR cycling conditions were routinely performed as following: 94°C (denaturing, 4 min), followed by 35 cycles of 94°C (denaturing, 30 sec), *57°C (annealing, 60 sec), and 72°C (extension, **90 sec). After cycling, a final extension was performed (72°C, 10 min) followed by an indefinite hold at 4°C. *Annealing temperatures were dependent on the base pair content of the primer pairs, normally ranging from 50°C to 60°C. **Extension times were dependent on the length of the products (approximately, 1 min/1000 bp).

For two-step PCR (used for *D2Mit82*), all the components and reactions were the same as regular PCR, except there was a two-step annealing: 61°C (45 sec) and 53°C (45 sec), since the T_m of forward and reverse primers were dramatically different.

Radiolabeled reactions were required to distinguish small differences in product sizes. For these reactions all the components were the same as the regular PCR, except that 0.5 ul of 1000-3000 Ci/mmol [α -³³P] labeled

dCTP (ICN, Costa Mesa, CA) was added into the 100X master mixtures, and PCR products were separated on sequencing gels.

C. *Bsg* I digestion for *Hc* genotyping



PCR products (15 ul) were mixed with 2 ul 10X NE buffer 4, 0.05 ul 400X SAM, 3 ul of 50 mM MgCl₂, and 0.5 ul of 3 units/ul *Bsg* I (New England Biolabs Inc., Beverly, MA) and incubate at 37°C, overnight.

D. MAPMAKER program

MAPMAKER is a computer package for constructing genetic linkage maps and finding QTL of complex traits [123], [123]. MAPMAKER 3.0 contains two programs: MAPMAKER/EXP 3.0 and MAPMAKER/QTL 1.1.

MAPMAKER/EXP 3.0 performs multipoint linkage analysis based on raw genotyping data simultaneously to calculate map order and distance.

MAPMAKER/QTL 1.1 uses interval mapping and simultaneous search techniques to find QTL based on the data generated from MAPMAKER/EXP 3.0. Interval mapping calculates the map distance between markers and QTL. Simultaneous search can fit multiple QTLs to the genome at the same time.

Genotype data were prepared as text only files. The first line was "data type f2 backcross", which means A/J backcross. The second line was "450 37 1", which means 450 mice, 37 markers, and 1 quantitative

trait. The default codes for F2 backcross were homozygote (A), heterozygote (H), and missing data (-). Genotype data were listed as “*locus# genotypes”. Quantitative trait data came after genotype data and were listed as “*weight quantitative data”. Raw genotype data were analyzed by MAPMAKER/EXP to determine map order and distance; transformed data were analyzed by MAPMAKER/QTL to determine QTL locations. The computer codes were provided in the MAPMAKER protocols [123].

E. JoinMap program

JoinMap 3.0 is a computer program for the calculation of genetic linkage maps, designed by Plant Research International, Wageningen, Netherlands [125]. Overall, the principle of JoinMap program is similar to the MAPMAKER program, that is multipoint interval mapping methods. However, it is more user-friendly, since its interface is based on MS-Windows. The user only needs to provide genotype data, and JoinMap will select and group the subsets of loci and individuals.

Data were loaded into the project file, for example, genotype data were stored in locus genotype file. The genotype data were grouped and ordered by JoinMap 3.0. Finally, *LOD score and QTL was calculated by MapQTL 4.0 [132].

*LOD = $\log_{10}(\text{Probability (Data| Linkage)} / \text{Probability (Data| Unlinkage)})$

F. DNA primer design

DNA primers were designed by Oligo primer design software (Molecular Biology Insights, Inc., Cascade, CO). The program setting included: (1) mouse databases, (2) duplex-free oligonucleotides, (3) highly specific oligos (3'-end stability), (4) eliminate false priming oligonucleotides, (5) eliminate homo-oligomers/sequence repeats, (6) the length of primer is between 15 and 25 nt, and (7) GC content is approximately 50-60%.

Based on the exported results, the primers were further selected to eliminate potential primer dimer formation (especially 3' primer dimer), prevent hairpin structure, and avoid large melting temperature differences within pairs of primers.

G. DNA sequencing

Primer pairs were designed by the Oligo program (Method F. DNA primer design). PCR were performed as described above (Method B. Polymerase chain reaction). PCR products were separated on 1.8% agarose gels.

Target DNA bands were extracted using QIAquick gel extraction kits (QIAGEN Inc. Valencia, CA). The DNA fragments were excised from the agarose gels to which was added 3 volumes of Buffer QG to 1 volume of gel (100 mg ~ 100 ul). Typically, 500 ul was enough for one band. The mixture was incubate at 50 °C for 10 min and mixed by vortexing the tube

every 2-3 min until the gel had completely melted. The color of the mixture should be yellow. QIAquick spin columns were put in the provided 2 ml collection tubes. Samples were applied to QIAquick spin columns, and centrifuged for 1 min at 14,000 rpm to bind DNA to the membrane of the column. The flow-through was discarded, 0.5 ml of Buffer QG was added to column, and column was centrifuged 1 min at 14,000 rpm to remove any agarose remaining. 0.75 ml of Buffer PE was added to the columns and centrifuged for 1 min at 14,000 rpm to remove the salt. The flow-through was discarded and centrifuged again to remove trivial Buffer PE left. QIAquick columns were placed into new 1.5 ml eppendorf tubes. 30 μ l ddH₂O was added to the center of the QIAquick membrane, the column was allowed to set for 1 min, and then centrifuged for 1 min at 14,000 rpm. Purified DNA product was stored at 4 °C.

Sequencing reaction was based on [α -³³P]dideoxynucleotide (ddNTP) chain termination reactions. Protocols and reagents were from Thermo Sequenase Radiolabeled Terminator Cycle Sequencing kits (USB corporation, Cleveland, OH). The 4 termination (labeled as “G”, “A”, “T”, and “C”) mixes were prepared as follows:

<u>1 X Sequence (μl)</u>	<u>G</u>	<u>A</u>	<u>T</u>	<u>C</u>
dGTP or dTTP master mix	2.0	2.0	2.0	2.0
[α - ³³ P]ddGTP	0.5	-	-	-
[α - ³³ P]ddATP	-	0.5	-	-
[α - ³³ P]ddTTP	-	-	0.5	-
[α - ³³ P]ddCTP	-	-	-	0.5

The reaction mixtures were prepared as follows: 10 ul ddH₂O + 2 ul reaction buffer + 5ul DNA + 1ul 10 ng / ul primer + 2 ul Thermo sequenase, mix well. Transfer 4.5 ul mixture to each of four termination tubes (labeled as "G", "A", "T", and "C"), mix well. Cycle 30-60 times as follows:

<u>dGTP</u>	<u>dITP</u>
95 °C, 30 sec	95 °C, 30 sec
55 °C, 30 sec	50 °C, 30 sec
72 °C, 1 min	60 °C, 5-10 min

Reactions were stopped by adding 4 ul stop solution, store at 4 °C.

Long Ranger Singel (BioWhittaker Molecular Applications, Rockland, ME) gels were used for gel electrophoresis. The sequencing gels were pre-run at 75 watt, for about 2 hr. Samples were heated at 70 °C, for 5 min. Samples were loaded and run at 55 watt. The gels were dried at 80 °C, for about 45 min (Heto Dry GD-1 gel dryer, Heto Lab Equipment, Denmark). Dried gels were exposed to Kodak BioMax film (Eastman Kodak Company, Rochester, NY). The films were developed using a Canon gel development machine.

H. Ovalbumin sensitization and challenge

A/J and C3H/HeJ male mice were obtained from the Jackson Laboratory (Bar Harbor, ME) at 4 weeks of age and allowed one week to

acclimate before experiment. Animals were housed 3/cage, under high-efficiency particulate absolute (HEPA) laminar flow hoods and allowed free access to ovalbumin (OVA)-free rodent chow and water.

On day 0, mice were sensitized (n = 6/group) intraperitoneally with 10 ug chicken egg OVA (crude grade IV; Sigma, St. Louis, MO) in 200 ul phosphate-buffered saline (PBS) or PBS alone. On day 14, mice were anesthetized (ketamine, 45 mg/kg and xylazine, 8 mg/kg, intraperitoneally), and challenged with an aspiration of 1.5% OVA in 45 ul PBS or PBS alone, then recovered from anesthesia. Lungs, tracheobronchial lymph nodes, and spleens were harvested at 6, 12, 24, 48, and 72 hr after challenge.

I. TaqMan real-time RT-PCR

Total RNA was extracted from lung, lymph node or spleen tissues using TRIzol reagent (Invitrogen life technologies, Carlsbad, CA). Tissues were homogenized in 10 ml tissue dounces at room temperature in 2 ml TRIzol reagent using about 10 strokes. The pestle was rinsed with 1 ml TRIzol reagent. This 1.5 ml mixture was stored at -70°C for future use. The 1.5 ml mixture was poured into a sterile microfuge tube. The sample was incubated at room temperature for 5 min to allow complete dissociation of cells. Subsequently, 200 ul chloroform was added, tubes were capped securely and shaken vigorously by hand for 15 sec, then incubated at room temperature for an additional 3 min. Sample were

centrifuged at 12,000 rpm at 4°C for 15 min. The upper, aqueous phase containing the RNA was transferred to a new, sterile 1.5 ml microfuge tube and RNA precipitated by adding 500 ul isopropyl alcohol. This mixture was incubated at room temperature for 10 min. Samples were centrifuged at 12,000 rpm at 4°C for 10 min. The RNA precipitate formed a gel-like paste on the bottom of the tube. The supernatants were carefully removed and the pellets washed with 1 ml 75% ethanol. The samples were vortexed and centrifuged at 9,500 rpm for 5 min at 4°C. The supernatants were carefully removed and air-dried for 15-30 min being careful not to contaminate samples by aerosol or over dry. RNA pellets were dissolved in 300 ul 0.2% DEPC-treated water and stored at -70°C.

Genomic DNA was removed by DNase I treatment. Total RNA (300 ul) was combined with 20 ul 1 M Tris (pH = 7.5), 4 ul 1 M MgCl₂, 2 ul 10 mg/ml BSA, 4 ul DNase I (RNase free), and 70 ul DEPC-H₂O to make 400 ul total volume. This was incubated at 37°C for 30 min and then precipitated with 40 ul 3 M sodium acetate (pH = 5.2). The tubes were filled with ice-cold 95-100% ethanol and incubated at -20°C for 30 min, then centrifuged at 12,000 rpm for 5 min at room temperature. The supernatants were carefully removed and 1 ml 75% ethanol was added, the samples were vortexed, and re-spun under the same conditions. The pellets were air dried for 5-10 min and resuspended in 300 ul DEPC-H₂O.

cDNA was reversed transcribed from total RNA using Reverse transcription kit (Applied Biosystems, Foster City, CA). According to the

manufacturer's instructions, 10 ul 10X RT buffer was combined with 22ul 25 mM MgCl₂, 20 ul deoxy NTPs mixture, 5 ul random hexamers, 2 ul RNase inhibitor, 6.25 ul 50 unit/ul Multiscribe reverse transcriptase, 2 ug total RNA, and a suitable amount of DEPC-H₂O to make 100 ul total volume. At other times, a 10 ul total volume was prepared by combining these reagents in proportional amounts. The reagents were capped, gently tapped, and centrifuged to mix well in 0.2 ml Micro-Amp tubes. Thermal cycling was conducted as follows: 25°C (10 min) - 37°C (60 min) - 95°C (5 min). Samples were diluted to 5 ng cDNA/ul in DEPC-H₂O.

TaqMan Pre-Developed Assay Reagents (PDAR) and TaqMan universal PCR master mix were obtained from Applied Biosystems. For these assays, 10.25 ul DEPC-H₂O was combined with 12.5 ul 2 x TaqMan universal PCR master mix, 1.25 ul 20 x PDAR of target gene, and 1 ul cDNA template to make a 25 ul reaction system. TaqMan Real-time RT-PCR was performed as: 50°C (2 min) - 95°C (10 min) – 95°C (15 sec)/ 60°C (1 min) (40 cycles).

J. Statistic analysis of mRNA expression data

cDNA samples from a single time point (n = 24) as well as a standard curve were loaded into 96 well plates in duplicate. The standard curve was made from a single A/J OVA lung sample as a series dilution of 10, 2, 1, 0.2, 0.1 ng of total RNA based on spectrometer. The CT values of duplicates were averaged to calculate the relative nanogram of the target

RNA by referring to the nanogram of the standard curve. The amounts of target RNA were divided by the amounts of 18s rRNA to get the relative ratio. After a ratio was calculated for each individual sample, the ratios from each strain/treatment/exposure-time group were averaged to get the group average. The standard error (SEM) of a group was calculated using the formula: $SEM = ((\sum (X_i - \bar{X})^2) / (n - 1))^{0.5}$. The group averages were divided by the group average of 6 hr C3H/HeJ PBS and termed as Normalizer1. The SEM of each group was divided by its Normalizer1 to obtain SEM1. The Normalizer1 and SEM1 of strain/treatment/exposure-time groups were compared to show the effects of strain, treatment, and exposure-time.

The data of the relative ratio of target gene/18s rRNA were analyzed using the SigmaStat 2.03 program (SPSS Inc., Chicago, IL). Due to the unbalanced data, the three-way ANOVA did not work for SigmaStat program. The data were analyzed by two-way analysis of variance (ANOVA). The normality of the data was scanned by SigmaStat program. If the normality test was failed, the data were transformed by logarithm to match the normality requirement. Student-Newman-Keul test was used for pairwise comparisons of the mean responses among different treatment groups. P value ≤ 0.05 was used as the standard for significant difference.

K. Nuclear protein isolation from EL-4 cells and splenocytes

A/J and C3H/HeJ mice (8 mice/group) were sensitized and challenged as described above. Mice were sacrificed and spleens were harvested at 72 hr after challenge. 8 spleens of the same group were disaggregated by squeezing in 20 ml of RPMI medium. Cells were centrifuged at 1,200 rpm for 8 min, supernatants were discarded, and pellets suspended with 16 ml of RPMI (1 spleen/2 ml RPMI, 1 spleen = 10^8 cells). RPMI medium was made as follows: RPMI medium powder (for IL, with L-glutamine, without Na_2CO_3 , GIBCO) was dissolved into 800 ml ddH₂O, 2 g of Na_2CO_3 and 15 μl of 1M HEPES were added. The mixture was spun and mixed well, and brought up to 1 L using ddH₂O. 500 ml medium were filtered through a 0.25 μm filter and kept for future use at 4°C. 50 ml of 10X serum stock and 5 ml of 100X penicillin/streptomycin was added to the other 500 ml medium, which was now ready for cell culture. 32 petri dishes were labeled as the combination of "A/J or C3H/HeJ", "PBS or OVA", "4 hr, 8 hr, 12 hr, or 24 hr", "unstimulated or stimulated". 18 ml RPMI was added to each of them, then 2 ml of spleenocytes were added to each dish and mixed well (5×10^6 cells/ml). For stimulated dishes, 20 μl 1000X PMA (Sigma, St. Louis, MO) and 200 μl 100 x Bt2cAMP (Sigma, St. Louis, MO) were added. For unstimulated dishes, 20 μl ethanol and 200 ddH₂O were added. Spleenocytes were cultured at 37°C, in a 5% CO₂ incubator. Cells were harvested after 4, 8, 12, or 24 hr stimulation. Spleenocytes were centrifuged at 3,000 rpm, 5 min, 4°C. Supernatants were discarded, 0.5 ml

Gey's buffer was added, and the samples were mixed well. Then 3 ml Gey's lysis solution was added and samples were incubated on ice for 5 min, mixing well every 1 min. An additional 10 ml Gey's buffer was added, centrifuged at 3,000 rpm, 5 min, 4°C. 4 ml HB buffer (0.5 M HEPES 1 ml, 1M MgCl₂ 75 ul, use ddH₂O to fill up to 50 ml) was added and incubated on ice for 15 min. Samples were centrifuged at 3,000 rpm, 5 min, 4°C and supernatants discarded. Nuclear pellets were washed twice with 1 ml MDHK (0.5 M HEPES 5 ml, 2 M NaCl 5 ml, 1 M MgCl₂ 0.3 ml, using ddH₂O to fill up to 100 ml), transferred to 1.5 ml eppendorf tubes, centrifuged and supernatants again discarded. 100 ul of Buffer C was added (0.5 M HEPES 1.5 ml, 1M MgCl₂ 37.5 ul, 0.5 M EDTA 15 ul, 100% glycerol 2.5 ml, 2 M NaCl 5.625 ml, Igepal CA-630 25 ul, use ddH₂O to fill up to 25 ml. Freshly add protease inhibitor before use). Nuclei in Buffer C can be stored at - 80°C stably. Samples were rocked on ice for 1 hr, then centrifuged at 14,000 rpm, 15 min, 4°C. Supernatants (about 90 ul) were removed and combined with equal volume of Buffer D (0.5 M HEPES 3.0 ml, 1M MgCl₂ 75 ul, 0.5 M EDTA 30 ul, 100% glycerol 5 ml, use ddH₂O to fill up to 50 ml. Freshly add protease inhibitor before use). Protein concentration was determined by Bicinchoninic Acid Protein Assay kit (Sigma).

EL-4 cells (2×10^7 cells/vial) were removed from storage in a liquid nitrogen tank. EL-4 cells were thawed at 37°C, 1 ml was pipetted into 50 ml RPMI medium, the cells were grown in cell grow flasks at 37°C, in 5%

CO₂, overnight. Cells were transferred to 50 ml centrifuge tubes, centrifuged at 200 g, for 5 min, in 4°C. Supernatants were discarded, 10 ml fresh RPMI medium was added, and mixed well. Cell numbers were counted on a Bright-line hemacytometer after staining with Trypan Blue solution. Petri dishes were labeled as “EL-4, unstimulated, 2hr”, “EL-4, stimulated, 2 hr”, “EL-4, unstimulated, 8 hr”, or “EL-4, stimulated, 8 hr”. EL-4 cells were diluted to make 10⁶ cells/ml, 20 ml/dish. For stimulated dishes, 20 ul 1000 x PMA and 200 ul 100 x Bt2cAMP were added. For unstimulated dishes, 20 ul ethanol and 200 ddH₂O were added. EL-4 cells were cultured at 37°C, in a 5% CO₂ incubator. Cells were harvested after 2 or 8 hr stimulation. Cells were spun down at 200 g, for 5 min, in 4°C. The cells were washed with 1 ml ice cold PBS and spun down at 3,000 rpm, for 5 min, in 4°C. The following nuclear protein isolation steps were the same as spleenocytes nuclear protein isolation. Nuclear proteins were stored in a -80°C freezer.

L. GATA-3 protein quantitation by Western blot

Separating gels (10%) were made as follows: ddH₂O 4 ml, 4 x separating buffer 2.5 ml (Tris 36.3 g dissolve in ddH₂O, use HCl to adjust pH to 8.8, use ddH₂O to fill up to 200 ml), Ultrapure ProtoGel (30% Acrylamide:0.8% Bisacrylamide, National Diagnostics, Atlanta, GA) 3.3 ml, 10% SDS 0.1 ml, 10% APS (freshly made) 0.1 ml, and 10 ul TEMED were combined to make 10 ml separating gel. The separating gels were poured

and sealed with water-saturated butanol for 30 min until polymerization. The water-saturated butanol was then removed by suction. Stacking gels (4%) were made as follows: ddH₂O 3 ml, 4 x stacking buffer 1.25 ml (Tris 6 g dissolve in ddH₂O, use HCl to adjust pH to 6.8, use ddH₂O to fill up to 100 ml), Ultrapure ProtoGel (0.66 ml, 10% SDS 0.05 ml, 10% APS (freshly made) 0.05 ml, and 5 ul TEMED were combined to make 5 ml separating gels. The stacking gels were poured on the top of the polymerized separating gel. The gels were allowed to polymerize overnight at 4°C. Then 4 x protein loading buffer was added to nuclear protein samples, which were then boiled at 95-100°C for 5-10 min. The protein ladder and samples were loaded, and run in 1 X running buffer (5 x stock: Tris 15.1 g (Sigma, St. Louis, MO), Glycine 72 g, SDS 5 g (Sigma, St. Louis, MO), use ddH₂O to fill up to 1L) at 100 v for about 2 hr. The gels were soaked in Transfer buffer (Tris 3.03 g, Glycine 14.4 g, Methanol 200 ml, use ddH₂O to fill up to 1L) for 2 x 15 min. Six pieces of filter papers and two pieces of sponges were soaked in Transfer buffer. Nitrocellulose membranes were soaked in ddH₂O to activate, then soaked in Transfer buffer just before use. The gel transfer equipment was set up in the order of cathode side of folder, sponge, 3 filter papers, gel, membrane, 3 filter papers, sponge, and anode side of folder. The gels were transferred at 15 v in 4°C, overnight, with a stir bar to circulate the buffer. The membranes were removed and rinsed with TBST (Tris 3.636 g, NaCl 26.31 g, Tween20 3 ml, use ddH₂O to fill up to 3L) 2 x 15 min. The membranes were blocked in 5% non-fat

milk for 2 hr. Then GATA-3 antibody (Santa Cruz Biotechnology, Santa Cruz, CA) 1 ug/ml was added and shaken for 1 hr. Membranes were washed with TBST 5 x 5 min. Sheep-Anti-mouse IgG-HRP conjugated antibody (Amersham) was added at 1:5000 dilution, and incubated at room temperature for 20 min. Membranes were washed with TBST 10 x 5 min. A mixture of 1 ml of Super Signal West Femto Stable Peroxide buffer was combined with 1 ml of Lumino/Enhancer Solution (Pierce) and applied to the membrane for 4 min. Membranes were then exposed and developed on film.

To quantitate Actin, the internal control protein, the membrane was submerged in stripping buffer (100 mM 2-mercaptoethanol, 2% sodium dodecyl sulphate, 62.5 mM Tris-HCl pH6.7), and incubated at 37°C for 30 min with occasional agitation. The membrane was washed 2 X 10 min in TBST at room temperature. The membrane was blocked in 5% blocking reagent for 1 hr at room temperature. Immunodetection was performed as described above except using for first antibody to Actin (1:25,000 dilution, Sigma).

Table 8. Mouse ID reference and APTI value

Mouse ID*	Mouse #	APTI	Mouse ID*	Mouse #	APTI
1	Ag-ABX 0002	514	44	Ag-ABX 0132	1881
2	Ag-ABX 0005	407	45	Ag-ABX 0135	1335
3	Ag-ABX 0006	2251	46	Ag-ABX 0136	1465
4	Ag-ABX 0007	606	47	Ag-ABX 0137	580
5	Ag-ABX 0008	1685	48	Ag-ABX 0138	480
6	Ag-ABX 0016	350	49	Ag-ABX 0140	1679
7	Ag-ABX 0018	355	50	Ag-ABX 0142	610
8	Ag-ABX 0019	508	51	Ag-ABX 0144	534
9	Ag-ABX 0021	447	52	Ag-ABX 0150	469
10	Ag-ABX 0023	524	53	Ag-ABX 0151	1352
11	Ag-ABX 0026	1418	54	Ag-ABX 0159	436
12	Ag-ABX 0028	1240	55	Ag-ABX 0160	389
13	Ag-ABX 0029	1123	56	Ag-ABX 0162	606
14	Ag-ABX 0044	1280	57	Ag-ABX 0163	1429
15	Ag-ABX 0052	1586	58	Ag-ABX 0165	518
16	Ag-ABX 0057	2168	59	Ag-ABX 0166	471
17	Ag-ABX 0082	264	60	Ag-ABX 0168	1166
18	Ag-ABX 0083	585	61	Ag-ABX 0169	1149
19	Ag-ABX 0084	2349	62	Ag-ABX 0179	415
20	Ag-ABX 0085	403	63	Ag-ABX 0181	424
21	Ag-ABX 0086	461	64	Ag-ABX 0185	440
22	Ag-ABX 0088	544	65	Ag-ABX 0188	1500
23	Ag-ABX 0089	444	66	Ag-ABX 0190	404
24	Ag-ABX 0091	620	67	Ag-ABX 0191	2138
25	Ag-ABX 0092	265	68	Ag-ABX 0192	1420
26	Ag-ABX 0094	384	69	Ag-ABX 0195	508
27	Ag-ABX 0095	1057	70	Ag-ABX 0197	545
28	Ag-ABX 0096	1171	71	Ag-ABX 0200	1127
29	Ag-ABX 0097	542	72	Ag-ABX 0201	1339
30	Ag-ABX 0098	467	73	Ag-ABX 0202	1795
31	Ag-ABX 0099	409	74	Ag-ABX 0203	1530
32	Ag-ABX 0101	442	75	Ag-ABX 0204	605
33	Ag-ABX 0102	387	76	Ag-ABX 0206	1283
34	Ag-ABX 0103	1357	77	Ag-ABX 0207	1589
35	Ag-ABX 0105	476	78	Ag-ABX 0212	526
36	Ag-ABX 0107	466	79	Ag-ABX 0213	2323
37	Ag-ABX 0109	436	80	Ag-ABX 0214	490
38	Ag-ABX 0116	2217	81	Ag-ABX 0217	455
39	Ag-ABX 0117	1041	82	Ag-ABX 0218	483
40	Ag-ABX 0124	1309	83	Ag-ABX 0222	441
41	Ag-ABX 0126	1367	84	Ag-ABX 0224	1358
42	Ag-ABX 0130	1406	85	Ag-ABX 0225	256
43	Ag-ABX 0131	1520	86	Ag-ABX 0232	1469

Table 8 (cont'd).

Mouse ID*	Mouse #	APTI	Mouse ID*	Mouse #	APTI
87	Ag-ABX 0233	384	130	Ag-ABX 0320	1122
88	Ag-ABX 0236	385	131	Ag-ABX 0321	1018
89	Ag-ABX 0242	379	132	Ag-ABX 0322	1818
90	Ag-ABX 0246	408	133	Ag-ABX 0323	1062
91	Ag-ABX 0248	284	134	Ag-ABX 0324	1436
92	Ag-ABX 0249	320	135	Ag-ABX 0325	1293
93	Ag-ABX 0250	1261	136	Ag-ABX 0326	1204
94	Ag-ABX 0251	1691	137	Ag-ABX 0327	1650
95	Ag-ABX 0253	460	138	Ag-ABX 0328	1436
96	Ag-ABX 0258	532	139	Ag-ABX 0334	1652
97	Ag-ABX 0261	457	140	Ag-ABX 0336	491
98	Ag-ABX 0262	1400	141	Ag-ABX 0337	447
99	Ag-ABX 0264	613	142	Ag-ABX 0341	1497
100	Ag-ABX 0265	592	143	Ag-ABX 0345	1417
101	Ag-ABX 0267	546	144	Ag-ABX 0346	601
102	Ag-ABX 0268	576	145	Ag-ABX 0351	1125
103	Ag-ABX 0270	387	146	Ag-ABX 0357	329
104	Ag-ABX 0271	1239	147	Ag-ABX 0358	241
105	Ag-ABX 0272	354	148	Ag-ABX 0360	271
106	Ag-ABX 0273	501	149	Ag-ABX 0362	267
107	Ag-ABX 0276	1009	150	Ag-ABX 0364	255
108	Ag-ABX 0277	1208	151	Ag-ABX 0365	406
109	Ag-ABX 0278	353	152	Ag-ABX 0366	254
110	Ag-ABX 0279	286	153	Ag-ABX 0368	366
111	Ag-ABX 0280	1571	154	Ag-ABX 0369	313
112	Ag-ABX 0281	573	155	Ag-ABX 0372	482
113	Ag-ABX 0282	371	156	Ag-ABX 0373	302
114	Ag-ABX 0283	608	157	Ag-ABX 0376	1790
115	Ag-ABX 0284	573	158	Ag-ABX 0377	1940
116	Ag-ABX 0285	511	159	Ag-ABX 0379	488
117	Ag-ABX 0290	991	160	Ag-ABX 0384	335
118	Ag-ABX 0291	377	161	Ag-ABX 0388	1329
119	Ag-ABX 0292	379	162	Ag-ABX 0390	1532
120	Ag-ABX 0294	385	163	Ag-ABX 0393	510
121	Ag-ABX 0295	604	164	Ag-ABX 0394	532
122	Ag-ABX 0297	401	165	Ag-ABX 0395	589
123	Ag-ABX 0300	1395	166	Ag-ABX 0396	1296
124	Ag-ABX 0310	1439	167	Ag-ABX 0398	410
125	Ag-ABX 0315	1480	168	Ag-ABX 0399	413
126	Ag-ABX 0316	1038	169	Ag-ABX 0401	1166
127	Ag-ABX 0317	1873	170	Ag-ABX 0403	2117
128	Ag-ABX 0318	1407	171	Ag-ABX 0404	1331
129	Ag-ABX 0319	994	172	Ag-ABX 0405	489

Table 8 (cont'd).

Mouse ID*	Mouse #	APTI	Mouse ID*	Mouse #	APTI
173	Ag-ABX 0407	1589	216	Ag-ABX 0522	2075
174	Ag-ABX 0409	510	217	Ag-ABX 0523	562
175	Ag-ABX 0411	1995	218	Ag-ABX 0524	1175
176	Ag-ABX 0412	493	219	Ag-ABX 0526	1133
177	Ag-ABX 0420	1196	220	Ag-ABX 0527	1000
178	Ag-ABX 0425	1025	221	Ag-ABX 0528	1302
179	Ag-ABX 0427	1163	222	Ag-ABX 0533	1787
180	Ag-ABX 0428	1000	223	Ag-ABX 0534	408
181	Ag-ABX 0429	617	224	Ag-ABX 0537	1053
182	Ag-ABX 0430	1157	225	Ag-ABX 0538	570
183	Ag-ABX 0431	1512	226	Ag-ABX 0540	340
184	Ag-ABX 0432	602	227	Ag-ABX 0541	315
185	Ag-ABX 0433	1804	228	Ag-ABX 0542	1065
186	Ag-ABX 0434	1114	229	Ag-ABX 0543	291
187	Ag-ABX 0436	2124	230	Ag-ABX 0544	2565
188	Ag-ABX 0453	267	231	Ag-ABX 0547	565
189	Ag-ABX 0455	421	232	Ag-ABX 0549	614
190	Ag-ABX 0456	1003	233	Ag-ABX 0550	1065
191	Ag-ABX 0457	1228	234	Ag-ABX 0551	497
192	Ag-ABX 0458	577	235	Ag-ABX 0553	468
193	Ag-ABX 0459	566	236	Ag-ABX 0554	452
194	Ag-ABX 0460	588	237	Ag-ABX 0555	334
195	Ag-ABX 0463	499	238	Ag-ABX 0556	1322
196	Ag-ABX 0465	1190	239	Ag-ABX 0557	1527
197	Ag-ABX 0466	573	240	Ag-ABX 0558	530
198	Ag-ABX 0467	307	241	Ag-ABX 0561	1500
199	Ag-ABX 0468	1536	242	Ag-ABX 0562	611
200	Ag-ABX 0489	561	243	Ag-ABX 0563	620
201	Ag-ABX 0490	1077	244	Ag-ABX 0564	1192
202	Ag-ABX 0491	1354	245	Ag-ABX 0565	1201
203	Ag-ABX 0492	1501	246	Ag-ABX 0566	1484
204	Ag-ABX 0493	1743	247	Ag-ABX 0568	478
205	Ag-ABX 0496	1535	248	Ag-ABX 0569	1686
206	Ag-ABX 0498	1499	249	Ag-ABX 0570	1891
207	Ag-ABX 0501	1324	250	Ag-ABX 0571	568
208	Ag-ABX 0509	476	251	Ag-ABX 0572	2162
209	Ag-ABX 0512	1965	252	Ag-ABX 0575	1027
210	Ag-ABX 0514	1047	253	Ag-ABX 0576	1545
211	Ag-ABX 0516	1599	254	Ag-ABX 0586	1627
212	Ag-ABX 0517	1191	255	Ag-ABX 0587	482
213	Ag-ABX 0518	1642	256	Ag-ABX 0590	1737
214	Ag-ABX 0519	573	257	Ag-ABX 0593	1339
215	Ag-ABX 0521	1248	258	Ag-ABX 0617	1086

Table 8 (cont'd).

Mouse ID*	Mouse #	APTI	Mouse ID*	Mouse #	APTI
259	Ag-ABX 0618	1335	302	Ag-ABX 0741	587
260	Ag-ABX 0620	1208	303	Ag-ABX 0742	1381
261	Ag-ABX 0621	1242	304	Ag-ABX 0743	1516
262	Ag-ABX 0623	2433	305	Ag-ABX 0744	1760
263	Ag-ABX 0624	997	306	Ag-ABX 0745	1151
264	Ag-ABX 0628	453	307	Ag-ABX 0746	1592
265	Ag-ABX 0630	515	308	Ag-ABX 0747	1248
266	Ag-ABX 0631	375	309	Ag-ABX 0748	610
267	Ag-ABX 0633	549	310	Ag-ABX 0749	467
268	Ag-ABX 0662	1031	311	Ag-ABX 0750	571
269	Ag-ABX 0663	1200	312	Ag-ABX 0751	2072
270	Ag-ABX 0664	1101	313	Ag-ABX 0753	553
271	Ag-ABX 0665	1432	314	Ag-ABX 0754	561
272	Ag-ABX 0668	1361	315	Ag-ABX 0755	1417
273	Ag-ABX 0669	1310	316	Ag-ABX 0758	594
274	Ag-ABX 0670	1713	317	Ag-ABX 0759	1145
275	Ag-ABX 0672	1319	318	Ag-ABX 0760	1166
276	Ag-ABX 0673	1075	319	Ag-ABX 0762	1343
277	Ag-ABX 0674	1224	320	Ag-ABX 0764	434
278	Ag-ABX 0676	1985	321	Ag-ABX 0765	1025
279	Ag-ABX 0678	2192	322	Ag-ABX 0766	407
280	Ag-ABX 0679	1684	323	Ag-ABX 0767	1410
281	Ag-ABX 0680	2512	324	Ag-ABX 0774	1207
282	Ag-ABX 0681	1045	325	Ag-ABX 0776	1269
283	Ag-ABX 0684	1521	326	Ag-ABX 0777	1102
284	Ag-ABX 0685	1739	327	Ag-ABX 0778	1066
285	Ag-ABX 0686	1282	328	Ag-ABX 0779	1810
286	Ag-ABX 0687	1546	329	Ag-ABX 0780	388
287	Ag-ABX 0688	619	330	Ag-ABX 0793	1399
288	Ag-ABX 0689	984	331	Ag-ABX 0796	1841
289	Ag-ABX 0691	1295	332	Ag-ABX 0817	508
290	Ag-ABX 0693	1563	333	Ag-ABX 0819	396
291	Ag-ABX 0719	1009	334	Ag-ABX 0820	345
292	Ag-ABX 0720	507	335	Ag-ABX 0821	472
293	Ag-ABX 0721	1037	336	Ag-ABX 0825	369
294	Ag-ABX 0729	1446	337	Ag-ABX 0838	471
295	Ag-ABX 0730	335	338	Ag-ABX 0839	525
296	Ag-ABX 0731	1164	339	Ag-ABX 0844	1283
297	Ag-ABX 0734	1179	340	Ag-ABX 0845	1031
298	Ag-ABX 0737	616	341	Ag-ABX 0847	984
299	Ag-ABX 0738	1480	342	Ag-ABX 0849	1111
300	Ag-ABX 0739	1422	343	Ag-ABX 0853	203
301	Ag-ABX 0740	1501	344	Ag-ABX 0854	1054

Table 8 (cont'd).

Mouse ID*	Mouse #	APTI	Mouse ID*	Mouse #	APTI
345	Ag-ABX 0855	502	388	Ag-ABX 0947	270
346	Ag-ABX 0856	1693	389	Ag-ABX 0948	526
347	Ag-ABX 0857	1333	390	Ag-ABX 0949	396
348	Ag-ABX 0860	1088	391	Ag-ABX 0950	539
349	Ag-ABX 0861	1557	392	Ag-ABX 0951	393
350	Ag-ABX 0862	1570	393	Ag-ABX 0952	401
351	Ag-ABX 0864	618	394	Ag-ABX 0953	578
352	Ag-ABX 0865	1015	395	Ag-ABX 0954	515
353	Ag-ABX 0867	1091	396	Ag-ABX 0955	1056
354	Ag-ABX 0869	1287	397	Ag-ABX 0956	381
355	Ag-ABX 0870	1571	398	Ag-ABX 0957	401
356	Ag-ABX 0872	1389	399	Ag-ABX 0958	439
357	Ag-ABX 0873	1240	400	Ag-ABX 0959	588
358	Ag-ABX 0875	1147	401	Ag-ABX 0960	565
359	Ag-ABX 0876	997	402	Ag-ABX 0961	364
360	Ag-ABX 0877	571	403	Ag-ABX 0962	504
361	Ag-ABX 0878	1251	404	Ag-ABX 0963	400
362	Ag-ABX 0879	593	405	Ag-ABX 0964	581
363	Ag-ABX 0880	1044	406	Ag-ABX 0966	390
364	Ag-ABX 0882	600	407	Ag-ABX 0984	346
365	Ag-ABX 0883	594	408	Ag-ABX 0985	382
366	Ag-ABX 0891	1039	409	Ag-ABX 0986	373
367	Ag-ABX 0892	570	410	Ag-ABX 0987	308
368	Ag-ABX 0898	511	411	Ag-ABX 0989	346
369	Ag-ABX 0899	1090	412	Ag-ABX 0990	383
370	Ag-ABX 0902	398	413	Ag-ABX 0991	434
371	Ag-ABX 0905	605	414	Ag-ABX 0992	438
372	Ag-ABX 0906	603	415	Ag-ABX 0993	388
373	Ag-ABX 0908	521	416	Ag-ABX 0994	491
374	Ag-ABX 0909	403	417	Ag-ABX 0995	494
375	Ag-ABX 0910	480	418	Ag-ABX 0997	195
376	Ag-ABX 0911	292	419	Ag-ABX 0998	797
377	Ag-ABX 0912	238	420	Ag-ABX 1000	507
378	Ag-ABX 0933	578	421	Ag-ABX 1019	1082
379	Ag-ABX 0935	619	422	Ag-ABX 1020	529
380	Ag-ABX 0938	450	423	Ag-ABX 1022	518
381	Ag-ABX 0939	104	424	Ag-ABX 1026	1650
382	Ag-ABX 0940	369	425	Ag-ABX 1028	1567
383	Ag-ABX 0942	475	426	Ag-ABX 1029	1161
384	Ag-ABX 0943	323	427	Ag-ABX 1031	1033
385	Ag-ABX 0944	474	428	Ag-ABX 1033	394
386	Ag-ABX 0945	323	429	Ag-ABX 1034	1123
387	Ag-ABX 0946	474	430	Ag-ABX 1035	1015

Table 8 (cont'd).

Mouse ID*	Mouse #	APTI	Mouse ID*	Mouse #	APTI
431	Ag-ABX 1036	1174	441	Ag-ABX 1048	241
432	Ag-ABX 1037	1131	442	Ag-ABX 1049	1116
433	Ag-ABX 1038	471	443	Ag-ABX 1050	400
434	Ag-ABX 1040	607	444	Ag-ABX 1051	537
435	Ag-ABX 1041	543	445	Ag-ABX 1053	1011
436	Ag-ABX 1042	998	446	Ag-ABX 1055	2778
437	Ag-ABX 1043	4361	447	Ag-ABX 1056	1025
438	Ag-ABX 1044	565	448	Ag-ABX 1057	513
439	Ag-ABX 1046	1138	449	Ag-ABX 1058	602
440	Ag-ABX 1047	463	450	Ag-ABX 1059	1881

*450 A/J backcross mice with extreme APTI values ($104 \leq \text{APTI} \leq 620$ and $797 \leq \text{APTI} \leq 4361$) were given ID numbers (1-450).

Table 9. Genotyping data of 450 A/J backcross mice by 37 markers

[illegible]

[illegible]

D2M465		-----HHNHNHAAHHNNHHNAAAHHNNHNHAAAAHNHNHAAA--HANHNAAAAHNAN
	0.7 cM	
D2M060		-----HHNHNHAAH--HNA-AHHNNHAN-----H-A-AAH-----A--HA-
	0.7 cM	
D2M081		-----HHNHNHAAHHNN--HAAAHHNNHNHAAAAHNHNHAAAAHNANHNAAAAHNAN
	1.4 cM	
D2M293		-----HHNHNHAA--HHNNHAA--HH---HAAAAHNHNHAAAAHNANHNAA--AAHNAN
	1.6 cM	X
D2M082		-----HHNHNHAAHHNNHHNAAAHHNNHNHAAAAHNHNHAAAAHNANHNHAAAHNA-
	0.5 cM	
D2M417		-----HHNHNHAAHHNNHHNAAAHHNNHNHAA--AHANHNAAAAHNANHNHAAAHNA-
	2.4 cM	
D2M152		-----HHNHNHAAHHNNHHNAAAHHNNHNHAAAAHNHNHAAAAHNANHNHAAAHNAN
	0.2 cM	
D2M235		-----HHNHNHAAHHNNHHNAAAHHNNHNHAAAAHNHNHAAAAHNANHNHAAAHNAN
	0.2 cM	
D2M367		-----HHNHNH--AHNNHHNAAAHHNN--AHAAAAHNHNHAAAAHNANHNHAAAHNAN
	0.5 cM	
D2M238		-----HHNHNHAAHHNNHHNAAAHHNNHNHAAAAHNHNHAAAAHNANHNHAAAHNAN
	0.2 cM	
D2M203		-----HHNHNHAA--HHNNHAAAHHNNHNHAAAAHNHNHAAAAHNANHNHAAAHNAN
	0.7 cM	
Hc		-----HHNHNHAAHHNNHHNAAAHHNNHNHAAAAHNHNHAAAAHNANHNHAAAHNAN
	5.4 cM	
D2M298		-----HNNH-HAAH--HNA-AHH-NAN-----H-A-AAH-----A--HA-
	5.4 cM	X X X
D2M323		-----HHNHNHAAAHH--AAA-HNANHNAAAAHHNNHAAAAHNANHNHNNHNAN
	5.9 cM	
D2M182		-----H---H-A-----HH-H-----H-A-AAH-----A-
	1.2 cM	
D2M380		-----HHNHNHNANA--HAA-AHNNANAN-----H-A-AAH-----H--HA-
	1.5 cM	
D2M091		-----HHNHNHNANHHNAAAAHNANHNAAAAHHNNHAAAAHHNAAHNNHNAN
	7.6 cM	X
D2M011		AHNAHHNNHHNAN---H-A-AHNNANA-----H-A-AAH-----A-
	1.4 cM	X
D2M010		-----HHNNHHNANA--HAA-AHNNHHNN-----H-A-AAH-----H--AA-
	13.5 cM	
D2M063		-----HHH-H-HA---AA-A---HH-----H--A--
	3.9 cM	
D2M307		-----HHH-H-HA---AA-A--A-HH-----H--A--
	8.7 cM	X
D2M451		-----HHH-A-HA---AA-A--A-HH-----H--A--
	3.0 cM	X
D2M053		-----HHH-A-HA---AA-A--A-HH-----A--A--
	11.6 cM	X
D2M113		AHNA-HHH-A-H---AA-H--A-H-----
	4.4 cM	
D2M200		-----HHH-A-HA---AA-H--A-HH-----A--A--

	#Recs:	00000001010010001001000011000000000000000001200000

=====

[illegible]

D2M380	1.2 cM	A-HHH-A--A-H--A---H-H-----A-HAAH---A-----
D2M091	1.5 cM	AHHHHHAAHAAHHA-AAAHHHH--AAHHHAHHA-HHHAHAAAAHAAHAAH
D2M011	7.6 cM	X
D2M010	1.4 cM	A--AH-A--A-H--A---H-H-----A--AAH-----
D2M063	13.5 cM	-----H-----A-----
D2M307	3.9 cM	--A-----H-----A-----
D2M451	8.7 cM	-----X-----X-----
D2M053	3.0 cM	--A-----A-----H-----
D2M113	11.6 cM	-----
D2M200	4.4 cM	--A-----A-----H-----

	#Recs:	10011010211010001001100100000020000000200001000000

[illegible]

D2M465		HHHHAAAHNNHHHAAAAAHANNHAAAHNNHHNNHHNNHANNHANNHAAHNNHHNNHAA
D2M060	0.7 cM	-----H-----
D2M081	0.7 cM	HHHHAAAHNNHHHAAAAAHANNHAAAHNNHHNNHHNNHANNHANNHAAHNNHHNNHAA
D2M293	1.4 cM	HHHHAAAHNNHHHAAAAAHANNHAAAHNNHHNNH-NAHHHANNHAAHNNHHNNHAA
D2M082	1.6 cM	HHHHAAAHNNHHHAAAAAHANNHAAAHNNHHNNHHNNHANNHANNHAAHNA-HAAA
	0.5 cM	
D2M417		HHHHAAAHNNHHHAAAAAHANNHAAAHNNHHNNHHNNHANNH-NAAHNNHNAHAA
D2M152	2.4 cM	X X
D2M152	0.2 cM	HHHHAAAHNNHANAHA-ANAHNNHAAAHNNHHNNHHNNHANNHAAHNNHNAHAA
D2M235	0.2 cM	HHHHAAAHNNHANAHA
D2M367	0.5 cM	HHHHAAAHNNHANAHA
D2M238	0.2 cM	HHHHAAAHNNHANAHA
D2M203	0.7 cM	HHHHAAAHNNHANAHA
Hc	5.4 cM	HHHHAAAHNNHANAHA
D2M298	5.4 cM	-----H-----
D2M323	5.9 cM	HANNHAAAHNNHANAHA
D2M182	1.2 cM	-----H-----
D2M380	1.5 cM	-----H-----
D2M091	7.6 cM	HANNHAAAHNNHANAHA
D2M011	1.4 cM	-----H-----
D2M010	13.5 cM	-----H-----
D2M063	3.9 cM	-----H-----
D2M307	8.7 cM	-----H-----
D2M451	3.0 cM	-----H-----
D2M053	11.6 cM	-----H-----
D2M113	4.4 cM	-----H-----
D2M200		-----H-----
		#Recs: 000000100010000000000000000000200000010000000000000110

=====

[illegible]

D2M380	1.2 cM	-----A-----A
D2M091	1.5 cM	HHHAAHAAAAAAAAAAHAAH-HAHHHHHHA-AAHHHHHAA
D2M011	7.6 cM	-----A-----A-----A
D2M010	1.4 cM	-----A-----A-----A
D2M063	13.5 cM	-----A-----A-----A
D2M307	3.9 cM	-----A-----A-----A
D2M451	8.7 cM	-----X-----H-----A
D2M053	3.0 cM	-----
D2M113	11.6 cM	-----A-----H-----H
D2M200	4.4 cM	-----A-----H-----H
#Recs:		1000120000010000000000000000100000000001000000000

Table 10. Genes within *Abhr1* and *Abhr2*

Symbol	Gene name	Celera physical map position (bp)	Jackson lab RH map position (cM)	Human homolog name & position	Function & Phenotype
<i>Nmt2</i>	N-myristoyltransferase 2	236,028 - 278,108	N/A	N/A	Glycylpeptide N-tetradecanoyltransferase-related
<i>E230009</i>	RIKEN cDNA	293,170 - 318,824	N/A	N/A	Similar to medium-chain S-acyl fatty acid synthetase thio ester hydrolase (MCH)
<i>B14Rik</i>	E230009B14 gene	409,610 - 425,951	N/A	SUV39H2, 10p13	Chromatin binding activity, methyltransferase activity
<i>Suv39h2</i>	Suppressor of variegation 3-9 homolog 2 (Drosophila)	439,506 - 463,399	2.5	N/A	Heat shock protein 70 related; protein APG-2 related
<i>Hsp70-4</i>	Heat shock protein 4	1,836,888 - 1,864,613	N/A	N/A	Selenophosphate synthase
<i>11T0046</i>	RIKEN cDNA	1,874,118 - 1,893,818	N/A	N/A	Phytanoyl-CoA hydroxylase gene; lupus nephritis-associated peptide 1
<i>B24Rik</i>	1110046B24 gene	2,253,186 - 2,664,623	N/A	N/A	CamKII-like protein kinase
<i>Phyh</i>	Phytanoyl-CoA hydroxylase	2,796,081 - 2,819,597	N/A	N/A	ADP-ribose pyrophosphatase related
<i>E030025</i>	RIKEN cDNA	2,822,472 - 2,848,546	N/A	N/A	Protein translocase subunit
<i>C11Rik</i>	E030025C11 gene	3,479,513 - 3,822,511	N/A	N/A	CUG triplet repeat RNA binding protein 2; elav-type RNA-binding protein 3; CUG triplet repeat RNA-binding protein 2
<i>Nudt5</i>	Nudix (nucleoside diphosphate linked moiety X)-type motif 5	6,795,337 - 6,815,972	7	GATA3, 10p15	Regulation of transcription, transcription factor activity
<i>Sec61a2</i>	SEC61, alpha subunit 2 (S. cerevisiae)	6,998,538 - 7,019,585	N/A	N/A	ATP synthase gamma chain
<i>Cugbp2</i>	CUG triplet repeat RNA binding protein 2				
<i>Gata3</i>	GATA binding protein 3				
<i>Atp5c1</i>	ATP synthase, H+ transporting, mitochondrial F1 complex, gamma polypeptide 1				

Table 10 (cont'd).

Symbol	Gene name	Celera physical map position (bp)	Jackson lab RH map position (cM)	Human homolog name & position	Function & Phenotype
<i>Itih2</i>	Inter-alpha trypsin inhibitor, heavy chain 2	7,033,645 - 7,069,816	1	ITIH2, 10p15	Serine protease inhibitor activity
<i>Itih5-pending</i>	Inter-alpha trypsin inhibitor heavy chain precursor 5	7,092,688 - 7,192,530	N/A	N/A	Inter-alpha-trypsin inhibitor heavy chain related
<i>Prkq</i>	Protein kinase C, theta	8,093,753 - 8,222,740	N/A	PRKQ, 10p15	Intracellular signaling cascade, protein phosphorylation
<i>Pfkfb3</i>	6-phosphofructo-2-kinase/fructose-2, 6-bisphosphatase 3	8,390,163 - 8,420,416	N/A	N/A	Similar to Inducible 6-phosphofructo-2-kinase
<i>2700027</i>	RIKEN cDNA	8,504,038 - 8,522,409	N/A	N/A	45kDa splicing factor, SPF 45
<i>J02Rik</i>	Interleukin 2 receptor, alpha chain	8,560,395 - 8,608,396	N/A	IL2RA, 10p15-p14	Cell surface receptor linked signal transduction
<i>Il15ra</i>	Interleukin 15 receptor, alpha chain	8,622,938 - 8,651,692	6.4	IL15RA, 10p15-p14	Cell surface receptor linked signal transduction
<i>Fbxo18</i>	F-box only protein 18	8,660,216 - 8,695,047	6.4	N/A	DNA helicase related
<i>D430029</i>	RIKEN cDNA	8,696,068 - 8,707,873	N/A	N/A	Similar to ankyrin3, isoform 1; ankyrin-3, node of Ranvier, ankyrin-G
<i>B21Rik</i>	D430029B21 gene	8,707,873	N/A	N/A	
<i>Pter</i>	Phosphotriesterase related	9,835,576 - 9,913,267	N/A	N/A	Resiniferatoxin-binding, phosphotriesterase-related protein
<i>C1ql-pending</i>	C1q-like	9,913,253 - 9,923,463	N/A	N/A	Complement C1q factor related
<i>Rsu1</i>	Ras suppressor protein 1	9,987,301 - 10,181,196	N/A	N/A	Similar to Ras suppressor protein 1 (Rsu-1) (RSP-1)
<i>Dnmt2</i>	DNA methyltransferase 2	10,421,222 - 10,454,776	N/A	N/A	DNA (cytosine-5) methyltransferase 2

Table 10 (cont'd).

Symbol	Gene name	Celera physical map position (bp)	Jackson lab RH map position (cM)	Human homolog name & position	Function & Phenotype
<i>Itga8</i>	Integrin alpha 8	N/A	7	ITGA8, 10p13	Matrix adhesion; Cell adhesion receptor activity
<i>Vim</i>	Vimentin	10,484,839 - 10,493,270	7	VIM, 10p13	Intermediate filament, kinesin complex
<i>Siat8f-pending</i>	Sialyltransferase 8 (alpha-2, 8-sialyltransferase) F	10,564,430 - 10,699,691	N/A	N/A	Similar to sialyltransferase 8 (alpha-2, 8-sialyltransferase) F
<i>Ptpia</i>	Protein tyrosine phosphatase-like (proline instead of catalytic arginine), member a	10,931,647 - 10,960,710	N/A	N/A	Protein tyrosine phosphatase-like (proline instead of catalytic arginine), member a
<i>Stam</i>	Signal transducing adaptor molecule (SH3 domain and ITAM motif) 1	10,978,515 - 11,050,719	N/A	N/A	Signal transducing adaptor molecule (SH3 domain and ITAM motif)
<i>Mrc1</i>	Mannose receptor, C type 1	11,132,506 - 11,233,984	5	MRC1, 10p13	ATP binding activity, mannose binding activity
<i>Cacnb2</i>	Calcium channel, voltage-dependent, beta 2 subunit	11,505,143 - 11,890,184	14.5	CACNB2, 10p12	Calcium channel activity
<i>4930587 A11Rik</i>	RIKEN cDNA 4930587A11 gene	11,956,966 - 11,978,239	N/A	N/A	ADP-ribosylation factor-like 5
<i>Tem7r-pending</i>	Tumor endothelial marker 7 related precursor	13,260,851 - 13,656,298	N/A	N/A	Tumor endothelial marker 7-related precursor
<i>Milt10</i>	Myeloid/lymphoid or mixed lineage-leukemia translocation to 10 homolog	14,961,511 - 15,108,395	N/A	N/A	Type I AF10 protein

Table 10 (cont'd).

Symbol	Gene name	Celera physical map position (bp)	Jackson lab RH map position (cM)	Human homolog name & position	Function & Phenotype
<i>DnaJc1</i>	DnaJ (Hsp40) homolog, subfamily C, member 1	15,114,567 - 15,289,780	N/A	N/A	Similar to DnaJ (Hsp40) homolog, subfamily C, member 1; DnaJ-like protein 1
<i>Bup</i>	Bmi1 upstream gene	15,564,346 - 15,568,101	9	BUP, 10pter-q22.1	BUP
<i>Bmi1</i>	B lymphoma Mo-MLV insertion region 1	15,569,672 - 15,577,489	9	BMI1, 10p11.23	Cell growth and/or maintenance; protein binding activity
<i>Pip5k2a</i>	Phosphatidylinositol-4-phosphate 5-kinase, type II, alpha	15,734,114 - 15,892,638	N/A	N/A	Phosphatidylinositol-4-phosphate 5-kinase type II alpha
<i>Pilb-pending</i>	Pilin-like transcription factor	16,270,463 - 16,293,742	N/A	N/A	Peptide methionine sulfoxide reductase
<i>Prtf1a</i>	Pancreas specific transcription factor, 1a	16,344,730 - 16,346,560	N/A	N/A	bHLH transcription factor
<i>5330427</i>	RIKEN cDNA	18,246,725 - 18,708,101	N/A	N/A	Metabotropic glutamate receptor
<i>MR13Rik</i>	5330427M13 gene	19,084,520 - 19,348,334	N/A	N/A	Similar to myosinIIIA
<i>Myo3a</i>	Myosin IIIA	19,352,183 - 19,421,655	9	GAD2, 10p11.23	Amino acid metabolism, neurotransmitter biosynthesis
<i>Gad2</i>	Glutamic acid decarboxylase 2	N/A	9	CUBN, 10p12.31	Amino acid activation, receptor mediated endocytosis
<i>Cubn</i>	Cubilin (Intrinsic factor cobalamin recep)	N/A	9	N/A	Trans-prenyltransferase
<i>Tpif-pending</i>	Trans-prenyltransferase	19,626,847 - 19,671,616	N/A	N/A	Abelson interactor 1, cell growth and/or maintenance
<i>Ssh3bp1</i>	Spectrin SH3 domain binding protein 1	19,671,727 - 19,771,825	15	SSH3BP1, 10p11.2	ATP-dependent metalloprotease FtsH1
<i>Yme111</i>	YME1-like 1	19,888,188 - 19,930,138	N/A	N/A	

Table 10 (cont'd).

Symbol	Gene name	Celera physical map position (bp)	Jackson lab RH map position (cM)	Human homolog name & position	Function & Phenotype
<i>Hnmt</i>	Histamine N-methyltransferase	20,732,677 - 20,779,270	N/A	N/A	Histamine N-methyltransferase
<i>Il1f9</i>	Interleukin 1 family, member 9	20,912,455 - 20,918,123	10.25	IL1F9, 2q12-q21	Immune response; cytokine activity
<i>Il1f6</i>	Interleukin 1 family, member 6	20,934,861 - 20,945,148	10.2	IL1F6, 2q12-q14.1	Immune response; inflammatory response
<i>Il1f5</i>	Interleukin 1 family, member 5 (delta)	20,996,389 - 21,002,847	10.1	IL1F5, 2q14	Biological process unknown, immune responsiveness activity
<i>Il1f8</i>	IL1 family, member 8	N/A	10.3	IL1F8, 2q12-q14.1	Immune response; cytokine activity
<i>Il1f10</i>	Interleukin 1 family, member 7	21,010,633 - 21,013,187	10.3	IL1F10, 2q14.1	Immune response; cytokine activity
<i>Il1f11</i>	Interleukin 1 receptor antagonist	21,056,292 - 21,070,434	10	IL1RN, 2q14.2	Cell surface receptor linked signal transduction
<i>Pax8</i>	Paired box gene 8	21,140,049 - 21,195,181	13.5	PAX8, 2q12-q14	Cell differentiation, transcription factor activity
<i>Cacna1b</i>	Calcium channel, voltage-dependent, N type, alpha 1B subunit	21,325,633 - 21,485,478	18	CACNA1B, 9q34	Calcium channel, voltage-dependent, N type, alpha 1B subunit; calcium channel, voltage-dependent, L type, alpha 1B subunit
<i>2700064</i>	RIKEN cDNA	21,671,752 - 21,683,087	N/A	N/A	Similar to melanin-concentrating hormone receptor interacting zinc finger protein
<i>H14Rik</i>	2700064H14 gene	21,781,486 - 21,789,966	N/A	N/A	Nasal embryonic LHRH factor
<i>Nelf</i>	Nasal embryonic LHRH factor	21,812,675 - 21,822,357	N/A	N/A	NADPH oxidase activator 1
<i>Noxa1</i>	NADPH oxidase activator 1	21,908,544 - 21,911,117	N/A	N/A	Ankyrin-repeat protein Nrarp

Table 10 (cont'd).

Symbol	Gene name	Celera physical map position (bp)	Jackson lab RH map position (cM)	Human homolog name & position	Function & Phenotype
<i>Npttic-pending</i>	Sodium-phosphate cotransporter IIC	21,956,682 - 21,962,073	N/A	N/A	Na ⁺ /Pi-cotransporter type IIC
<i>Anapc2-pending</i>	Anaphase-promoting complex subunit 2	22,000,196 - 22,013,424	N/A	N/A	Similar to anaphase-promoting complex 2
<i>Grim1</i>	Glutamate receptor, ionotropic, NMDA1 (zeta 1)	22,019,756 - 22,046,480	12	GRIN1, 9q34.3	Potassium ion transport
<i>C8g</i>	Complement component 8, gamma subunit	N/A	12.3	C8G, 9q34.3	Complement activation; complement activity
<i>Dpp7</i>	Dipeptidylpeptidase 7	22,079,795 - 22,083,865	N/A	N/A	Dipeptidyl peptidase7
<i>Entpd2</i>	Ectonucleoside triphosphate diphosphohydrolase 2	22,123,355 - 22,128,781	N/A	N/A	Ectonucleoside triphosphate diphosphohydrolase 2
<i>Npdc1</i>	Neural proliferation, differentiation and control gene 1	22,130,623 - 22,136,991	N/A	N/A	Neural proliferation, differentiation and control gene 1
<i>Fut7</i>	Fucosyltransferase 7	22,150,995 - 22,153,882	N/A	N/A	Alpha - (1,3)-fucosyltransferase
<i>Abca2</i>	ATP-binding cassette, sub-family A (ABC1), member 2	22,156,280 - 22,175,762	12.6	ABCA2, 9q34	ATP binding cassette, (ABC) transporter activity
<i>Plgds</i>	Prostaglandin D2 synthase (brain)	N/A	12.9	PTGDS, 9q34.2	PG biosynthesis, PG-D synthase activity
<i>Clc3</i>	Chloride intracellular channel 3	22,184,328 - 22,186,268	N/A	N/A	Chloride intracellular channel 3
<i>Fbw5</i>	F-box and WD-40 domain protein 5	22,228,336 - 22,232,992	N/A	N/A	Similar to f-box and WD-40 domain protein 5; F-box protein Fbw5

Table 10 (cont'd).

Symbol	Gene name	Celera physical map position (bp)	Jackson lab RH map position (cM)	Human homolog name & position	Function & Phenotype
<i>Edf1</i>	Endothelial differentiation-related factor 1	22,285,666 - 22,289,849	N/A	N/A	Endothelial differentiation-related factor 1 isoform beta, multiprotein bridging factor 1
<i>Fcna</i>	Ficolin A	22,353,028 - 22,356,377	N/A	N/A	Ficolin A
<i>Lcn8-pending</i>	Lipocalin 8	22,381,430 - 22,384,530	N/A	N/A	Epididymal 17 kDa protein precursor
<i>Lcn5</i>	Lipocalin 5	22,386,314 - 22,389,958	N/A	N/A	Epididymal retinoic acid binding protein
<i>Lcn3</i>	Lipocalin 3	22,491,662 - 22,494,194	N/A	N/A	Lipocalin 3
<i>Lhx3</i>	LIM homeobox protein 3	22,922,740 - 22,930,792	16	LHX3, 9q34.3	Homeodomain protein, electron transport, regulation of transcription,
<i>Ags3-pending</i>	Activator of G-protein signaling 3	23,038,194 - 23,066,478	N/A	N/A	Activator of G-protein signaling 3
<i>Inpp5e</i>	Inositol polyphosphate-5-phosphatase	23,113,926 - 23,126,918	N/A	N/A	Mitochondrial matrix processing protease, alpha subunit
<i>Notch1</i>	Notch gene homolog 1	23,176,171 - 23,221,734	15	NOTCH1, 9q34.3	ATP binding activity, calcium ion binding activity
<i>Egfr7</i>	EGF-like domain 7	23,298,062 - 23,309,769	N/A	N/A	NOTCH4-like protein
<i>Lcn4</i>	Lipocalin 4	23,394,975 - 23,398,584	N/A	N/A	Lipocalin 4
<i>Abo</i>	ABO blood group	23,552,918 - 23,574,718	N/A	N/A	Cis-AB transferase
<i>Surf6</i>	Surfeit gene 6	23,598,731 - 23,612,623	15.5	SURF6, 9q34.2	Surfeit gene 6

Table 10 (cont'd).

Symbol	Gene name	Celera physical map position (bp)	Jackson lab RH map position (cM)	Human homolog name & position	Function & Phenotype
<i>Surf5</i>	Surfeit gene 5	23,615,011 - 23,620,387	15.5	SURF5, 9q34.2	Similar to surf5t5 isoform b; surf5t locus protein 5
<i>Rpl7a</i>	Ribosomal protein L7a	23,620,542 - 23,623,048	18	RPL7A, 9q34	Surfeit 3 protein, protein biosynthesis, structural constituent of ribosome
<i>Surf1</i>	Surfeit gene 1	23,623,132 - 23,626,275	15.5	SURF1, 9q34.2	Cytochrome c oxidase activity
<i>Surf2</i>	Surfeit gene 2	23,626,170 - 23,629,638	N/A	N/A	Surfeit gene 2
<i>Surf4</i>	Surfeit gene 4	23,629,782 - 23,643,172	N/A	N/A	Similar to surf5t gene 4
<i>Dbh</i>	Dopamine beta hydroxylase	23,868,135 - 23,886,111	15.5	DBH, 9q34	Dopamine beta monoxygenase activity
<i>Sardh</i>	Sarcosine	23,891,307 - 23,950,326	N/A	N/A	Sarcosine dehydrogenase
<i>Vav2</i>	Vav2 oncogene	23,968,691 - 24,129,634	15.3	VAV2, 9q34.1	Intracellular signaling cascade, DAG binding activity
<i>Brd3</i>	Bromodomain containing 3	24,150,203 - 24,178,266	N/A	N/A	Similar to bromodomain-containing FSH-like protein
<i>Wdr5</i>	WD repeat domain 5	24,217,886 - 24,239,265	N/A	N/A	WD repeat domain 5 protein; WD-repeat protein 5; likely ortholog of mouse WD repeat protein BIG-3(BMP-2-induced gene 3 kb)
<i>Rxra</i>	Retinoid X receptor alpha	24,379,677 - 24,463,625	17	RXRA, 9q34.3	Regulation of transcription, DNA binding activity
<i>Col5a1</i>	Procollagen, type V, alpha 1	24,589,456 - 24,740,321	18	COL5A1, 9q34.2- q34.3	Cell adhesion; extracellular matrix structural constituent
<i>Olfm1</i>	Olfactomedin 1	24,895,739 - 24,933,255	N/A	N/A	Olfactomedin 1

Table 10 (cont'd).

Symbol	Gene name	Celera physical map position (bp)	Jackson lab RH map position (cM)	Human homolog name & position	Function & Phenotype
<i>Mips2</i>	Mitochondrial ribosomal protein S2	25,170,229 - 25,175,446	N/A	N/A	Mitochondrial ribosomal protein S2
<i>Fs-</i> <i>pendg</i>	Forsman glycolipid synthetase	25,201,940 - 25,209,576	N/A	N/A	Forsman glycolipid synthetase
<i>Ralgds</i>	Ral guanine nucleotide dissociation stimulator	25,217,504 - 25,257,246	N/A	N/A	Ral guanine nucleotide dissociation stimulator
<i>Cel</i>	Carboxyl ester lipase	25,259,997 - 25,267,338	16	CEL, 9q34.3	Lipid catabolism, carboxylic ester lyase activity
<i>2700084</i> <i>A09Rik</i>	RIKEN cDNA 2700084A09 gene	25,270,433 - 25,287,929	N/A	N/A	Similar to general transcription factor IIIC, polypeptide 5 (63kD)
<i>Grlf1b</i>	Growth factor independent 1B	25,313,962 - 25,326,447	N/A	N/A	Growth factor independence-1B
<i>Tsc1</i>	Tuberous sclerosis 1	25,345,746 - 25,391,859	N/A	N/A	Tuberous sclerosis1
<i>Dnm</i>	Dynamin	N/A	17	DNM1, 9q34	Endocytosis, ATP binding activity, GTP binding activity
<i>Barhl1</i>	BarH-like 1 (Drosophila)	25,613,808 - 25,622,536	17	BARHL1, 9q34	Hearing, regulation of transcription
<i>Ttf1</i>	Transcription termination factor 1	25,766,284 - 25,808,075	N/A	N/A	RNA polymerase I transcription termination factor 1
<i>Nlrg2</i>	Netrin G2	25,900,631 - 25,954,383	N/A	N/A	Similar to Netrin-G2b
<i>Odf2</i>	Outer dense fiber of sperm tails 2	26,596,700 - 26,626,474	N/A	N/A	Outer dense fiber protein
<i>Set</i>	SET translocation	26,765,384 - 26,775,453	N/A	N/A	Similar to set gene [Rattus norvegicus]

Table 10 (cont'd).

Symbol	Gene name	Celera physical map position (bp)	Jackson lab RH map position (cM)	Human homolog name & position	Function & Phenotype
<i>Zdhhc12</i>	Zinc finger, DHHC domain containing 12	26,794,461 - 26,797,175	N/A	N/A	Ankyrin-repeat containing protein
<i>Endog</i>	Endonuclease G	26,875,395 - 26,877,941	N/A	N/A	Endonuclease G
<i>Sh3glb2</i>	SH3-domain GRB2-like endophilin B2	27,049,814 - 27,064,372	N/A	N/A	SH3-containing protein SH3GLB2
<i>Crat</i>	Carnitine acetyltransferase	27,106,890 - 27,120,260	18	CRAT, 9q34.1	Fatty acid metabolism, acetyltransferase activity
<i>Spna2</i>	Spectin alpha 2	N/A	18	SPTAN1, 9q33-q34	Actin binding activity, calcium ion binding activity
<i>Ppp2r4</i>	Protein phosphatase 2A, regulatory subunit B (PR 53)	27,120,838 - 27,152,667	N/A	N/A	Phosphotyrosyl phosphatase activator
<i>Prrx2</i>	Paired related homeobox 2	27,550,150 - 27,586,619	N/A	N/A	Paired-like homeodomain protein PRX2 [Homo sapiens]
<i>Ptges</i>	Prostaglandin E synthase	27,597,630 - 27,608,659	24	PTGES, 9q34.3	Prostaglandin E synthase
<i>Usp20</i>	Ubiquitin specific protease 20	27,688,127 - 27,728,455	N/A	N/A	pVHL-interacting deubiquitinating enzyme
<i>Freq</i>	Frequenin homolog (Drosophila)	27,984,569 - 27,997,283	N/A	N/A	Frequenin homolog (Drosophila) [Homo sapiens]
<i>Ass1</i>	Argininosuccinate synthetase 1	28,171,205 - 28,221,605	20	ASS, 9q34.1	Argininosuccinate synthetase, ATP binding activity
<i>2810003</i>	RIKEN cDNA	28,653,185 - 28,674,909	N/A	N/A	Ionized calcium binding adapter molecule 2 [Mus musculus]
<i>C17Rik</i>	Protein-O-mannosyltransferase 1	28,940,996 - 28,959,961	N/A	N/A	Pulative protein O-mannosyltransferase POMT1

Table 10 (cont'd).

Symbol	Gene name	Celera physical map position (bp)	Jackson lab RH map position (cM)	Human homolog name & position	Function & Phenotype
<i>Ciz1-pending</i>	Cip1-interacting zinc finger protein	29,068,436 - 29,085,635	N/A	N/A	Cip1-interacting zinc finger protein
<i>Pges2</i>	Prostaglandin E synthase 2	29,101,412 - 29,108,380	N/A	N/A	Prostaglandin E synthase 2
<i>Dpm2</i>	Dolichol-phosphate (beta-D) mannosyltransferase 2	29,276,681 - 29,279,356	N/A	N/A	DPM2 protein
<i>Siat7d</i>	Sialyltransferase 7D	29,292,936 - 29,306,373	N/A	N/A	Sialyltransferase 7D isoform b; NeuAc-alpha-2,3-Gal-beta-1,3-GalNAc-alpha-2,6-sialyltransferase alpha2,6-sialyltransferase
<i>Siat7f</i>	Sialyltransferase 7F	29,313,702 - 29,327,466	N/A	N/A	Sialyltransferase 7 ((alpha-N-acetylneuramyl) 2,3-betagalactosyl-1,3)-N-acetyl galactosaminide alpha-2,6-sialyltransferase) F
<i>Ak1</i>	Adenylylate kinase 1	29,336,145 - 29,341,694	21.6	AK1, 9q34.1	Adenylylate kinase 1 cytosolic adenylylate kinase
<i>Eng</i>	Endoglin	29,353,176 - 29,388,722	21.4	ENG, 9q33-q34.1	Angiogenesis, cell adhesion; Binding activity
<i>Cdk9</i>	Cyclin-dependent kinase 9 (CDC2-related kinase)	29,414,046 - 29,419,362	N/A	N/A	Cyclin-dependent kinase 9
<i>Sh2d3c</i>	SH2 domain containing 3C	29,427,821 - 29,461,649	N/A	N/A	SH2 domain-containing Eph receptor-binding protein SHEP1
<i>Tor2a</i>	Torsin family 2, member A	29,463,984 - 29,469,065	N/A	N/A	Similar to torsin family 2, member
<i>Rpl12</i>	Ribosomal protein L12	29,668,696 - 29,671,425	N/A	N/A	Ribosomal protein L12
<i>Slc2a8</i>	Solute carrier family 2, (facilitated glucose transporter), member 8	29,679,965 - 29,689,055	24	SLC2A8, 9q34.13	Carbohydrate transport, fructose transport
<i>Angptl2</i>	Angiopoietin-like 2	29,923,220 - 29,954,051	N/A	N/A	Angiopoietin-related protein-2

Table 10 (cont'd).

Symbol	Gene name	Celera physical map position (bp)	Jackson lab RH map position (cM)	Human homolog name & position	Function & Phenotype
<i>Abi1</i>	v-abl Abelson murine leukemia oncogene 1	N/A			
<i>Lmx1b</i>	LIM homeobox transcription factor 1 beta	30,269,231 - 30,345,093	21	ABL1, 9q34.1	Intracellular signaling cascade, protein kinase activity
<i>Fpgs</i>	Folypolyglutamyl synthetase	N/A	21	LMX1B, 9q34.1	Regulation of transcription, DNA binding, txn factor activity
<i>Nup214</i>	Nucleoporin 214	N/A	21.2	FPGS, 9cen-q34	ATP binding activity, folypolyglutamate synthase activity
<i>Pbx3</i>	Pre B-cell leukemia transcription factor 3	30,869,693 - 31,069,147	21.3	NUP214, 9q34.1	mRNA-nucleus export, protein-nucleus import
<i>Hspa5</i>	Heat shock 70kD protein 5 (glucose-regulated protein)	31,472,569 - 31,476,938	22	PBX3, 9q33-q34	DNA binding, txn factor activity
<i>Fbxw2</i>	F-box and WD-40 domain protein 2	31,505,866 - 31,526,413	22.5	HSPA5, 9q33-q34.1	ATP binding activity, heat shock protein activity
<i>Psm5</i>	Proteasome (prosome, macropain) 26S subunit, non-ATPase, 5	31,552,212 - 31,570,895	N/A	N/A	F-box and WD-40 domain protein 2
<i>Traf1</i>	Tnf receptor-associated factor 1	31,637,577 - 31,656,092	N/A	N/A	Similar to proteasome (prosome, macropain) 26S subunit, non-ATPase, 5
<i>Hc</i>	Hemolytic complement	31,677,653 - 31,755,923	23.5	C5, 9q33-q34	Tnf receptor-associated factor 1
<i>Rab14</i>	RAB14, member RAS oncogene family	31,874,440 - 31,895,504	N/A	N/A	Complement activation, endopeptidase activity
<i>Gsn</i>	Gelsolin	31,951,095 - 32,002,612	24.5	GSN, 9q33	GTPase Rab14 [Homo sapiens]
<i>Epb7</i>	Erythrocyte protein band 7.2	32,008,710 - 32,031,730	24	STOM, 9q34.1	Actin binding activity, actin filament severing activity
					Stomatatin

Table 10 (cont'd).

Symbol	Gene name	Celera physical map position (bp)	Jackson lab RH map position (cM)	Human homolog name & position	Function & Phenotype
<i>Ggla1</i>	Glycoprotein galactosyltransferase alpha 1, 3	32,094,839 - 32,165,347	25	GGTA1, 9q33-q34	Carbohydrate metabolism; Manganese binding activity
<i>Lhx6</i>	LIM homeobox protein 6	32,785,894 - 32,809,002	N/A	N/A	LIM homeobox protein 7
<i>Pigs1</i>	Prostaglandin-endoperoxide synthase 1	32,934,352 - 32,956,085	29	PTGS1, 9q32-q33.3	Prostaglandin biosynthesis, oxidoreductase activity
<i>Pdcl</i>	Phosducin-like	34,034,071 - 34,043,296	N/A	N/A	Phosducin-like
<i>Gpr21</i>	G protein-coupled receptor 21	34,201,678 - 34,204,631	N/A	N/A	Similar to Probable G protein-coupled receptor GPR21
<i>Nek6</i>	NIMA (never in mitosis gene a)-related expressed kinase 6	37,274,367 - 37,309,320	28	NEK6, 9q33.3-q34.11	Protein amino acid phosphorylation; kinase activity
<i>Psmb7</i>	Proteasome (prosome, macropain) subunit, beta type 7	37,309,754 - 37,370,921	27.5	PSMB7, 9q34.11-q34.12	Hydrolyase activity, peptidase activity
<i>Lcn2</i>	Lipocalin 2	N/A	27	LCN2, 9q34	Transport, binding activity, transporter activity
<i>Nr5a1</i>	Nuclear receptor subfamily 5, group A, member 1	37,416,843 - 37,438,684	23.5	NR5A1, 9q33	Regulation of transcription, DNA binding activity, ligand-dependent nuclear receptor activity
<i>Rpl35</i>	Ribosomal protein L35	37,727,322 - 37,730,897	N/A	N/A	Ribosomal protein L35
<i>Ppp6c</i>	Protein phosphatase 6, catalytic subunit	37,920,560 - 37,952,524	N/A	N/A	Protein phosphatase6, catalytic subunit; serine/threonine protein phosphatase catalytic subunit
<i>Lrp1b</i>	Low density lipoprotein-related protein 1B (deleted in tumors)	39,315,045 - 41,349,825	N/A	N/A	Low density lipoprotein receptor related protein LRP1B/LRP-DIT

Table 10 (cont'd).

Symbol	Gene name	Celera physical map position (bp)	Jackson lab RH map position (cM)	Human homolog name & position	Function & Phenotype
<i>Kynu</i>	Kynureninase (L-kynurenine hydrolase)	42,275,607 - 42,403,071	N/A	N/A	Kynureninase (L-kynurenine hydrolase)
<i>Zfx1b</i>	Zinc finger homeobox 1b	43,700,785 - 43,825,578	N/A	N/A	Smad interacting protein 1
<i>Neb</i>	Nebulin	N/A	30	NEB, 2q22	Regulation of actin thin filament length activity
<i>Acvr2</i>	Activin receptor IIA	47,487,131 - 47,573,125	30	ACVR2, 2q22.2-q23.3	Serine/threonine kinase signaling pathway
<i>Kif5c</i>	Kinesin family member 5C	48,292,623 - 48,444,781	32.5	KIF5C, 2q23.3	ATP binding activity, kinesin motor activity
<i>Arhe</i>	Ras homolog gene family, member E	49,814,574 - 49,833,308	N/A	N/A	Small GTP binding protein Rho8
<i>Tnfrsf6</i>	Tumor necrosis factor alpha induced protein 6	50,762,776 - 50,783,057	N/A	N/A	TNF-stimulated gene 6 protein
<i>Cacnb4</i>	Calcium channel, voltage-dependent, beta 4 subunit	51,163,884 - 51,406,380	33.9	CNCB4, 2q22-q31	Calcium ion transport, ATP binding activity
<i>Stam2</i>	Signal transducing adaptor molecule (SH3 domain and ITAM motif) 2	51,425,147 - 51,476,196	N/A	N/A	Signal transducing adaptor molecule 2B [Homo sapiens]
<i>Frbp3</i>	Formin binding protein 3	51,880,092 - 51,932,591	N/A	N/A	Formin binding protein 3, formin binding protein 11
<i>Kcnj3</i>	Potassium inwardly-rectifying channel, subfamily J, member 3	54,211,330 - 54,365,389	N/A	N/A	Potassium inwardly-rectifying channel J3
<i>Nr4a2</i>	Nuclear receptor subfamily 4, group A, member 2	55,867,857 - 55,874,963	34.5	NR4A2, 2q22-q23	Neurogenesis, regulation of tbn

Table 10 (cont'd).

Symbol	Gene name	Celera physical map position (bp)	Jackson lab RH map position (cM)	Human homolog name & position	Function & Phenotype
<i>Gpd2</i>	Glycerol phosphate dehydrogenase 2, mitochondrial	55,980,470 - 56,113,189	33	GDP2, 2q24.1	Electron transport, glycerol-3-phosphate metabolism
<i>Acrv1</i>	Activin A receptor, type 1	57,203,024 - 57,294,262	N/A	N/A	Transforming growth factor beta type I
<i>Axot</i>	Axotrophin	58,919,763 - 58,943,391	N/A	N/A	Axotrophin
<i>Pla2g1br</i>	Phospholipase A2, group IB, pancreas receptor	59,114,845 - 59,248,845	N/A	N/A	Similar to phospholipase A2, group IB, pancreas receptor; PLA2-I
<i>Itgb6</i>	Integrin beta 6	59,297,394 - 59,419,858	34	ITGB6, 2q24.3	Cell adhesion, cell-matrix adhesion
<i>Rbms1</i>	RNA binding motif, single stranded interacting protein 1	59,450,009 - 59,660,398	N/A	N/A	Similar to RNA binding motif, single stranded interacting protein 1
<i>Tbr1</i>	T-box brain gene 1	60,506,542 - 60,516,088	32	TBR1, 2q24	ATP binding activity, transcription factor activity
<i>Dpp4</i>	Dipeptidylpeptidase 4	61,023,615 - 61,102,356	35	DPP4, 2q24.3	Aminopeptidase activity, dipeptidase activity
<i>Fap</i>	Fibroblast activation protein	61,191,100 - 61,264,176	36	FAP, 2q23	Electron transport, proteolysis
<i>Gcg</i>	Glucagon	N/A	36	GCG, 2q36-q37	Hormone activity
<i>Gca</i>	Grancalcin	61,354,551 - 61,386,726	N/A	N/A	Grancalcin
<i>Kcnh7</i>	Potassium voltage-gated channel, subfamily H (eag-related), member 7	61,395,434 - 61,577,776	N/A	N/A	Potassium channel protein erg3
<i>Fign</i>	Fidgetin	62,663,225 - 62,783,581	34	FIGN, 2q24.3	ATP binding activity, nucleotide binding activity

Table 10 (cont'd).

Symbol	Gene name	Celera physical map position (bp)	Jackson lab RH map position (cM)	Human homolog name & position	Function & Phenotype
<i>Grb14</i>	Growth factor receptor bound protein 14	63,583,161 - 63,693,422	N/A	N/A	Growth factor receptor bound protein 14
<i>Scn3a</i>	Sodium channel, voltage-gated, type III, alpha polypeptide	64,170,622 - 64,176,102	36	SCN3A, 2q24	ATP binding activity, cation channel activity
<i>Scn2a</i>	Sodium channel, voltage-gated, type II, alpha polypeptide	N/A	36	CSN2A1, 2q22-q23	Apoptosis, neurogenesis, Sodium channel activity
<i>Scn1a</i>	Sodium channel, voltage-gated, type I, alpha polypeptide	64,952,233 - 65,030,497	36	SCN1A, 2q24.3	Cation channel activity, ion channel activity
<i>Scn9a</i>	Sodium channel, voltage-gated, type IX, alpha polypeptide	N/A	36	SCN9A, 2q24	Cation channel activity, Sodium channel activity
<i>B3galt1</i>	UDP-Gal:betaGlcNAc beta 1,3-galactosyltransferase, polypeptide 1	66,780,835 - 66,796,532	N/A	N/A	UDP-Gal:betaGlcNAc beta 1,3-galactosyltransferase-I
<i>Stk39</i>	Serine/threonine kinase 39, STE20/SPS1 homolog (yeast)	66,879,901 - 67,140,584	N/A	N/A	Pancreatic serine threonine kinase
<i>Iiga6</i>	Integrin alpha 6	N/A	38	ITGA6, 2q31.1	Cell adhesion to receptor activity, receptor activity
<i>Abcb11</i>	ATP-binding cassette, sub-family B (MDR/TAP), member 11	67,904,511 - 68,008,374	38.4	ABCB11, 2q24	ATP binding cassette (ABC) transporter activity
<i>Lrp2</i>	Low density lipoprotein receptor-related protein 2	N/A	40	LRP2, 2q24-q31	Vitamin metabolism; receptor activity
<i>Sp5</i>	Trans-acting transcription factor 5	69,140,845 - 69,143,344	40	N/A	Trans-acting transcription factor 5

Table 11. GATA-3 expression in lungs.

Mice#	Treat	Hour	Strain	Gata3 Ct	18s Ct	Gata3 (ng)	Gata3 18s (ng)	Ave. Gata3 (ng)	Ave. 18s (ng)	Gata3/18s	Note	Group Ave.	SEM	Normalizer	SEM1
1	PBS	24	C3H/HeJ	31.18	17.887	0.457	0.57	0.422	0.713	0.591		0.748	0.099	0.939698	0.124
1	PBS	24	C3H/HeJ	31.298	17.438	0.426	0.753								
1	PBS	24	C3H/HeJ	31.477	17.307	0.383	0.816								
2	PBS	24	C3H/HeJ	29.888	16.456	0.986	1.38	1.735	1.909	0.909					
2	PBS	24	C3H/HeJ	28.109	15.321	2.848	2.782								
2	PBS	24	C3H/HeJ	29.337	16.252	1.37	1.566								
3	PBS	24	C3H/HeJ	30.532	17.014	0.672	0.978	1.153	1.108	1.04					
3	PBS	24	C3H/HeJ	29.337	16.505	1.37	1.339								
3	PBS	24	C3H/HeJ	29.281	16.967	1.416	1.007								
4	PBS	24	C3H/HeJ	x	x	x	x	x	x	x	Delete				
4	PBS	24	C3H/HeJ	x	x										
4	PBS	24	C3H/HeJ	x	x										
5	PBS	24	C3H/HeJ	30.325	16.53	0.76	1.319	1.076	2.091	0.515					
5	PBS	24	C3H/HeJ	29.213	15.474	1.475	2.531								
5	PBS	24	C3H/HeJ	29.876	15.545	0.993	2.423								
6	PBS	24	C3H/HeJ	31.054	17.209	0.492	0.867	0.889	1.298	0.685					
6	PBS	24	C3H/HeJ	29.595	15.996	1.175	1.834								
6	PBS	24	C3H/HeJ	29.866	16.693	0.999	1.192								
7	PBS	24	A/J	30.094	16.811	0.872	1.109	1.116	1.28	0.872		1.029	0.206	1.292714	0.259
7	PBS	24	A/J	29.587	16.32	1.18	1.501								
7	PBS	24	A/J	29.432	16.643	1.294	1.23								
8	PBS	24	A/J	31.058	16.38	0.491	1.447	0.773	1.768	0.437					
8	PBS	24	A/J	30.266	16.15	0.787	1.667								
8	PBS	24	A/J	29.798	15.708	1.041	2.191								

Table 11 (cont'd).

Mice#	Treat	Hour	Strain	Gata3 Ct	18s Ct	Gata3 (ng)	18s (ng)	Ave. Gata3 (ng)	Ave. 18s (ng)	Gata3/18s	Note	Group Ave.	SEM	Normalizer	SEM1
9	PBS	24	A/J	31.547	17.068	0.367	0.946	0.511	0.887	0.576					
9	PBS	24	A/J	30.702	17.253	0.607	0.844								
9	PBS	24	A/J	30.841	17.203	0.559	0.87								
10	PBS	24	A/J	28.164	16.322	2.756	1.499	1.779	1.157	1.537					
10	PBS	24	A/J	29.773	17.119	1.056	0.917								
10	PBS	24	A/J	29.158	16.891	1.524	1.055								
11	PBS	24	A/J	28.608	15.558	2.115	2.403	1.845	1.736	1.063					
11	PBS	24	A/J	29.355	16.577	1.355	1.281								
11	PBS	24	A/J	28.649	16.297	2.064	1.523								
12	PBS	24	A/J	30.172	18.116	0.833	0.495	0.658	0.39	1.686					
12	PBS	24	A/J	31.108	18.637	0.477	0.359								
12	PBS	24	A/J	30.55	18.839	0.665	0.317								
13	OVA	24	C3H/HeJ	30.008	15.806	0.918	2.062	0.779	1.789	0.435		0.512	0.041	0.643216	0.052
13	OVA	24	C3H/HeJ	30.554	16.155	0.663	1.662								
13	OVA	24	C3H/HeJ	30.336	16.175	0.755	1.642								
14	OVA	24	C3H/HeJ	28.556	14.618	2.182	4.294	1.669	3.682	0.453					
14	OVA	24	C3H/HeJ	28.923	14.671	1.753	4.156								
14	OVA	24	C3H/HeJ	29.746	15.433	1.073	2.596								
15	OVA	24	C3H/HeJ	28.488	14.818	2.272	3.795	2.188	3.396	0.644					
15	OVA	24	C3H/HeJ	28.034	15.156	2.978	3.08								
15	OVA	24	C3H/HeJ	29.406	15.038	1.315	3.313								
16	OVA	24	C3H/HeJ	29.191	15.238	1.494	2.928	1.29	2.252	0.573					
16	OVA	24	C3H/HeJ	29.582	16.107	1.184	1.712								
16	OVA	24	C3H/HeJ	29.57	15.764	1.192	2.116								

Table 11 (cont'd).

Mice#	Treat	Hour	Strain	Gata3 Ct	18s Ct	Gata3 (ng)	18s (ng)	Ave. Gata3 (ng)	Ave. 18s (ng)	Gata3/18s	Note	Group Ave.	SEM	Normalizer	SEM1
17	OVA	24	C3H/HeJ	30.621	16.507	0.637	1.338	0.493	1.086	0.454					
17	OVA	24	C3H/HeJ	31.093	16.76	0.481	1.144								
17	OVA	24	C3H/HeJ	31.581	17.39	0.36	0.775								
18	OVA	24	C3H/HeJ	33.147	24.961	0.141	0.007	0.081	0.008	10.675	Delete				
18	OVA	24	C3H/HeJ	35.695	24.554	0.031	0.009								
18	OVA	24	C3H/HeJ	34.28	25.168	0.072	0.006								
19	OVA	24	A/J	31.057	17.135	0.491	0.908	0.641	0.898	0.714		0.495	0.062	0.621859	0.078
19	OVA	24	A/J	29.995	17.063	0.925	0.949								
19	OVA	24	A/J	31.009	17.267	0.506	0.837								
20	OVA	24	A/J	31.482	17.109	0.382	0.922	0.31	0.806	0.384					
20	OVA	24	A/J	31.843	17.264	0.308	0.838								
20	OVA	24	A/J	32.259	17.657	0.24	0.658								
21	OVA	24	A/J	31.408	16.598	0.399	1.264	0.367	0.959	0.383					
21	OVA	24	A/J	31.528	17.252	0.371	0.844								
21	OVA	24	A/J	31.713	17.408	0.332	0.767								
22	OVA	24	A/J	31.592	16.486	0.357	1.355	0.335	0.946	0.354					
22	OVA	24	A/J	32.05	17.101	0.272	0.927								
22	OVA	24	A/J	31.506	17.93	0.376	0.556								
23	OVA	24	A/J	31.267	17.465	0.434	0.74	0.389	0.605	0.643					
23	OVA	24	A/J	31.506	17.93	0.376	0.556								
23	OVA	24	A/J	31.595	18.042	0.357	0.518								
24	OVA	24	A/J	31.75	17.372	0.325	0.784	0.338	0.69	0.489					
24	OVA	24	A/J	31.878	17.523	0.301	0.714								
24	OVA	24	A/J	31.459	17.881	0.387	0.573								

Table 11 (cont'd).

Mice#	Treat	Hour	Strain	Gata3 Ct	18s Ct	Gata3 (ng)	18s (ng)	Ave. Gata3 (ng)	Ave. 18s (ng)	Gata3/18s	Note	Group Ave.	SEM	Normalizer	SEM1
25	PBS	48	C3H/HeJ	34.178	18.386	0.275	0.386	0.234	0.318	0.737		0.966	0.072	1.213568	0.09
25	PBS	48	C3H/HeJ	34.293	18.807	0.26	0.306								
25	PBS	48	C3H/HeJ	35.171	19.099	0.168	0.261								
26	PBS	48	C3H/HeJ	32.749	17.926	0.558	0.496	0.527	0.55	0.958					
26	PBS	48	C3H/HeJ	32.205	17.472	0.731	0.635								
26	PBS	48	C3H/HeJ	34.061	17.842	0.291	0.519								
27	PBS	48	C3H/HeJ	33.156	18.486	0.456	0.365	0.391	0.372	1.052					
27	PBS	48	C3H/HeJ	33.297	17.958	0.426	0.487								
27	PBS	48	C3H/HeJ	34.069	19.089	0.29	0.263								
28	PBS	48	C3H/HeJ	33.507	18.135	0.384	0.442	0.382	0.427	0.895					
28	PBS	48	C3H/HeJ	33.399	18.28	0.405	0.409								
28	PBS	48	C3H/HeJ	33.642	18.186	0.359	0.43								
29	PBS	48	C3H/HeJ	32.375	17.687	0.672	0.565	0.537	0.426	1.26					
29	PBS	48	C3H/HeJ	33.036	18.583	0.484	0.346								
29	PBS	48	C3H/HeJ	33.164	18.475	0.455	0.367								
30	PBS	48	C3H/HeJ	34.742	19.407	0.208	0.221	0.188	0.21	0.893					
30	PBS	48	C3H/HeJ	35.082	19.466	0.176	0.214								
30	PBS	48	C3H/HeJ	35.034	19.619	0.18	0.197								
31	PBS	48	A/J	33.58	18.797	0.37	0.308	0.297	0.334	0.889		0.781	0.037	0.981156	0.046
31	PBS	48	A/J	34.474	19.009	0.238	0.274								
31	PBS	48	A/J	34.11	18.226	0.284	0.421								
32	PBS	48	A/J	32.721	17.876	0.566	0.509	0.4	0.511	0.782					
32	PBS	48	A/J	33.53	17.478	0.379	0.633								
32	PBS	48	A/J	34.337	18.357	0.254	0.392								

Table 11 (cont'd).

Mice#	Treat	Hour	Strain	Gata3 Ct	18s Ct	Gata3 (ng)	18s (ng)	Ave. Gata3 (ng)	Ave. 18s (ng)	Gata3/18s	Note	Group Ave.	SEM	Normalizer	SEM1
33	PBS	48	A/J	35.106	19.475	0.174	0.213	0.17	0.209	0.817					
33	PBS	48	A/J	35.277	19.576	0.16	0.201								
33	PBS	48	A/J	35.054	19.486	0.178	0.212								
34	PBS	48	A/J	33.655	18.451	0.356	0.372	0.278	0.336	0.828					
34	PBS	48	A/J	34.622	18.559	0.221	0.351								
34	PBS	48	A/J	34.312	18.939	0.257	0.285								
35	PBS	48	A/J	34.403	18.254	0.246	0.414	0.296	0.397	0.744					
35	PBS	48	A/J	33.906	18.264	0.315	0.412								
35	PBS	48	A/J	33.835	18.482	0.326	0.366								
36	PBS	48	A/J	33.712	17.428	0.346	0.65	0.357	0.571	0.626					
36	PBS	48	A/J	33.596	17.863	0.367	0.513								
36	PBS	48	A/J	33.641	17.736	0.359	0.55								
37	OVA	48	C3H/HeJ	33.444	18.677	0.396	0.329	0.325	0.41	0.793		0.982	0.1	1.233668	0.126
37	OVA	48	C3H/HeJ	34.169	18.329	0.276	0.398								
37	OVA	48	C3H/HeJ	33.979	17.895	0.304	0.504								
38	OVA	48	C3H/HeJ	33.43	18.51	0.398	0.36	0.392	0.408	0.96					
38	OVA	48	C3H/HeJ	32.945	18.365	0.507	0.39								
38	OVA	48	C3H/HeJ	34.218	18.011	0.27	0.473								
39	OVA	48	C3H/HeJ	34.538	19.346	0.23	0.228	0.259	0.205	1.263					
39	OVA	48	C3H/HeJ	34.148	20.025	0.279	0.158								
39	OVA	48	C3H/HeJ	34.239	19.344	0.267	0.229								
40	OVA	48	C3H/HeJ	40	33.061	0.015	0	0.139	0.101	1.378	Delete				
40	OVA	48	C3H/HeJ	40	33.308	0.015	0								
40	OVA	48	C3H/HeJ	33.491	18.83	0.387	0.303								

Table 11 (cont'd).

Mice#	Treat	Hour	Strain	Gata3 Ct	18s Ct	Gata3 (ng)	18s (ng)	Ave. Gata3 (ng)	Ave. 18s (ng)	Gata3/18s	Note	Group Ave.	SEM	Normalizer	SEM1
41	OVA	48	C3H/HeJ	40	32.194	0.015	0	0.032	0	162.034	Delete				
41	OVA	48	C3H/HeJ	40	31.872	0.015	0								
41	OVA	48	C3H/HeJ	37.082	32.88	0.065	0								
42	OVA	48	C3H/HeJ	33.187	17.989	0.449	0.479	0.444	0.488	0.91					
42	OVA	48	C3H/HeJ	33.353	17.923	0.414	0.496								
42	OVA	48	C3H/HeJ	33.101	17.952	0.469	0.489								
43	OVA	48	A/J	34.151	18.167	0.279	0.435	0.372	0.486	0.766		1.027	0.147	1.290201	0.185
43	OVA	48	A/J	33.387	17.873	0.407	0.51								
43	OVA	48	A/J	33.275	17.864	0.43	0.513								
44	OVA	48	A/J	37.158	19.069	0.063	0.266	0.089	0.272	0.328	Delete				
44	OVA	48	A/J	36.22	18.807	0.1	0.306								
44	OVA	48	A/J	36.115	19.217	0.105	0.245								
45	OVA	48	A/J	34.136	18.427	0.281	0.377	0.316	0.362	0.873					
45	OVA	48	A/J	33.649	18.006	0.357	0.474								
45	OVA	48	A/J	33.944	19.305	0.309	0.234								
46	OVA	48	A/J	34.321	18.411	0.256	0.38	0.298	0.391	0.761					
46	OVA	48	A/J	34.412	18.24	0.245	0.418								
46	OVA	48	A/J	33.465	18.438	0.392	0.375								
47	OVA	48	A/J	34.102	19.33	0.286	0.23	0.263	0.215	1.224					
47	OVA	48	A/J	34.643	19.499	0.218	0.21								
47	OVA	48	A/J	34.107	19.551	0.285	0.204								
48	OVA	48	A/J	33.491	18.83	0.387	0.303	0.387	0.256	1.509					
48	OVA	48	A/J	33.056	18.893	0.48	0.292								
48	OVA	48	A/J	34.044	19.844	0.294	0.174								

Table 11 (cont'd).

Mice#	Treat	Hour	Strain	Gata3 Ct	18s Ct	Gata3 (ng)	Gata3 18s (ng)	Ave. Gata3 (ng)	Ave. 18s (ng)	Gata3/18s	Note	Group Ave.	SEM	Normalizer	SEM1
49	PBS	72	C3H/HeJ	33.044	17.827	0.397	0.765	0.234	0.736	0.318		0.413	0.048	0.518844	0.06
49	PBS	72	C3H/HeJ	34.46	18.483	0.155	0.522								
49	PBS	72	C3H/HeJ	34.498	17.507	0.151	0.921								
50	PBS	72	C3H/HeJ	33.219	19.067	0.353	0.372	0.268	0.451	0.594					
50	PBS	72	C3H/HeJ	34.02	18.586	0.207	0.492								
50	PBS	72	C3H/HeJ	33.784	18.599	0.242	0.488								
51	PBS	72	C3H/HeJ	33.923	18.502	0.221	0.517	0.328	0.642	0.511					
51	PBS	72	C3H/HeJ	32.819	17.863	0.461	0.749								
51	PBS	72	C3H/HeJ	33.45	18.078	0.303	0.661								
52	PBS	72	C3H/HeJ	34.359	17.919	0.165	0.725	0.245	0.774	0.317					
52	PBS	72	C3H/HeJ	33.916	17.791	0.222	0.781								
52	PBS	72	C3H/HeJ	33.237	17.714	0.349	0.816								
53	PBS	72	C3H/HeJ	34.152	18.719	0.19	0.455	0.226	0.533	0.424					
53	PBS	72	C3H/HeJ	33.398	18.261	0.314	0.594								
53	PBS	72	C3H/HeJ	34.27	18.395	0.175	0.55								
54	PBS	72	C3H/HeJ	34.188	18.043	0.185	0.674	0.186	0.597	0.313					
54	PBS	72	C3H/HeJ	34.494	18.418	0.151	0.542								
54	PBS	72	C3H/HeJ	33.91	18.324	0.223	0.573								
55	PBS	72	A/J	32.41	19.627	0.605	0.269	0.339	0.435	0.78		0.486	0.126	0.610553	0.158
55	PBS	72	A/J	33.107	18.81	0.38	0.432								
55	PBS	72	A/J	33.475	18.788	0.298	0.437								
56	PBS	72	A/J	34.203	19.105	0.183	0.364	0.143	0.36	0.397					
56	PBS	72	A/J	35.759	19.251	0.065	0.334								
56	PBS	72	A/J	34.233	19.022	0.18	0.382								

Table 11 (cont'd).

Mice#	Treat	Hour	Strain	Gata3 Ct	18s Ct	Gata3 (ng)	18s (ng)	Ave. Gata3 (ng)	Ave. 18s (ng)	Gata3/18s	Note	Group Ave.	SEM	Normalizer	SEM1
57	PBS	72	A/J	34.736	18.433	0.129	0.538	0.104	0.502	0.208					
57	PBS	72	A/J	35.742	18.961	0.066	0.396								
57	PBS	72	A/J	34.863	18.323	0.118	0.573								
58	PBS	72	A/J	34.443	18.233	0.156	0.604	0.123	0.539	0.229					
58	PBS	72	A/J	34.988	18.574	0.109	0.495								
58	PBS	72	A/J	35.04	18.496	0.105	0.518								
59	PBS	72	A/J	33.01	18.907	0.406	0.408	0.406	0.424	0.958					
59	PBS	72	A/J	33.523	19.224	0.288	0.34								
59	PBS	72	A/J	32.626	18.476	0.524	0.524								
60	PBS	72	A/J	34.386	18.279	0.162	0.588	0.219	0.635	0.345					
60	PBS	72	A/J	33.907	18.058	0.223	0.669								
60	PBS	72	A/J	33.616	18.111	0.271	0.648								
61	OVA	72	C3H/HeJ	34.021	19.948	0.207	0.223	0.174	0.317	0.549		0.443	0.085	0.556533	0.107
61	OVA	72	C3H/HeJ	34.811	19.112	0.122	0.362								
61	OVA	72	C3H/HeJ	34.129	19.099	0.193	0.365								
62	OVA	72	C3H/HeJ	33.243	17.777	0.348	0.787	0.351	0.767	0.458					
62	OVA	72	C3H/HeJ	33.307	17.637	0.333	0.854								
62	OVA	72	C3H/HeJ	33.139	18.078	0.372	0.661								
63	OVA	72	C3H/HeJ	30.521	18.823	2.127	0.429	3.925	0.394	9.963	Delete				
63	OVA	72	C3H/HeJ	32.807	19.209	0.465	0.343								
63	OVA	72	C3H/HeJ	28.323	18.897	9.183	0.411								
64	OVA	72	C3H/HeJ	34.313	19.293	0.171	0.326	0.2	0.292	0.686					
64	OVA	72	C3H/HeJ	33.569	19.31	0.28	0.323								
64	OVA	72	C3H/HeJ	34.504	19.927	0.15	0.226								

Table 11 (cont'd).

Mice#	Treat	Hour	Strain	Gata3 Ct	18s Ct	Gata3 (ng)	18s (ng)	Ave. Gata3 (ng)	Ave. 18s (ng)	Gata3/18s	Note	Group Ave.	SEM	Normalizer	SEM1
65	OVA	72	C3H/HeJ	39.289	23.055	0.006	0.037	0.018	0.053	0.33					
65	OVA	72	C3H/HeJ	38.691	22.432	0.009	0.053								
65	OVA	72	C3H/HeJ	36.59	21.921	0.037	0.071								
66	OVA	72	C3H/HeJ	37.382	21.131	0.022	0.112	0.021	0.11	0.192					
66	OVA	72	C3H/HeJ	37.305	21.09	0.023	0.115								
66	OVA	72	C3H/HeJ	37.699	21.283	0.018	0.103								
67	OVA	72	A/J	33.84	18.124	0.234	0.643	0.455	0.695	0.655		0.955	0.25	1.199749	0.314
67	OVA	72	A/J	32.572	17.838	0.543	0.76								
67	OVA	72	A/J	32.452	18.024	0.588	0.682								
68	OVA	72	A/J	33.991	19.671	0.211	0.262	0.183	0.231	0.795					
68	OVA	72	A/J	33.853	20.002	0.232	0.216								
68	OVA	72	A/J	35.009	20.017	0.107	0.214								
69	OVA	72	A/J	34.856	18.845	0.119	0.423	0.187	0.455	0.411					
69	OVA	72	A/J	33.839	18.508	0.234	0.515								
69	OVA	72	A/J	34.01	18.834	0.209	0.426								
70	OVA	72	A/J	39.187	22.378	0.007	0.054	0.039	0.051	0.763					
70	OVA	72	A/J	36.581	22.652	0.038	0.046								
70	OVA	72	A/J	35.606	22.456	0.072	0.052								
71	OVA	72	A/J	33.15	19.835	0.37	0.238	0.457	0.213	2.15					
71	OVA	72	A/J	33.195	19.851	0.359	0.236								
71	OVA	72	A/J	32.317	20.476	0.644	0.164								
72	OVA	72	A/J	36.621	22.02	0.037	0.067	0.059	0.061	0.954					
72	OVA	72	A/J	36.345	22.324	0.044	0.056								
72	OVA	72	A/J	35.193	22.175	0.095	0.061								

Table 11 (cont'd).

Mice#	Treat	Hour	Strain	Gata3 Ct	18s Ct	Gata3 (ng)	18s (ng)	Ave. Gata3 (ng)	Ave. 18s (ng)	Gata3/18s	Note	Group Ave.	SEM	Normalizer	SEM1
79	PBS	6	C3H/HeJ	32.606	19.052	0.483	0.325	0.266	0.331	0.806		0.796	0.077	1	0.097
79	PBS	6	C3H/HeJ	34.104	19.262	0.196	0.287								
79	PBS	6	C3H/HeJ	34.92	18.789	0.12	0.38								
80	PBS	6	C3H/HeJ	33.226	18.826	0.333	0.372	0.305	0.334	0.915					
80	PBS	6	C3H/HeJ	33.169	19.188	0.344	0.3								
80	PBS	6	C3H/HeJ	33.776	19.031	0.239	0.329								
81	PBS	6	C3H/HeJ	33.359	19.152	0.307	0.306	0.336	0.305	1.101					
81	PBS	6	C3H/HeJ	33.216	19.001	0.335	0.335								
81	PBS	6	C3H/HeJ	33.073	19.346	0.365	0.273								
82	PBS	6	C3H/HeJ	34.003	19.078	0.208	0.32	0.266	0.371	0.716					
82	PBS	6	C3H/HeJ	33.662	18.84	0.256	0.369								
82	PBS	6	C3H/HeJ	33.227	18.605	0.332	0.424								
83	PBS	6	C3H/HeJ	33.871	18.531	0.226	0.443	0.264	0.428	0.618					
83	PBS	6	C3H/HeJ	33.259	18.365	0.326	0.489								
83	PBS	6	C3H/HeJ	33.758	18.922	0.241	0.351								
84	PBS	6	C3H/HeJ	34.156	19.54	0.19	0.243	0.164	0.264	0.62					
84	PBS	6	C3H/HeJ	34.682	19.644	0.138	0.229								
84	PBS	6	C3H/HeJ	34.409	19.077	0.163	0.32								
85	PBS	6	A/J	32.286	19.766	0.586	0.213	0.479	0.265	1.808		0.868	0.29	1.090452	0.364
85	PBS	6	A/J	32.292	18.999	0.584	0.335								
85	PBS	6	A/J	33.585	19.512	0.268	0.247								
86	PBS	6	A/J	33.409	19.565	0.298	0.24	0.422	0.246	1.714					
86	PBS	6	A/J	33.161	19.602	0.346	0.234								
86	PBS	6	A/J	32.19	19.404	0.621	0.264								

Table 11 (cont'd).

Mice#	Treat	Hour	Strain	Gata3 Ct	18s Ct	Gata3 (ng)	18s (ng)	Ave. Gata3 (ng)	Ave. 18s (ng)	Gata3/18s	Note	Group Ave.	SEM	Normalizer	SEM1
87	PBS	6	A/J	35.117	18.789	0.106	0.38	0.091	0.425	0.215					
87	PBS	6	A/J	35.481	18.504	0.085	0.45								
87	PBS	6	A/J	35.555	18.522	0.082	0.445								
88	PBS	6	A/J	33.954	18.288	0.215	0.512	0.148	0.392	0.376					
88	PBS	6	A/J	35.264	18.971	0.097	0.341								
88	PBS	6	A/J	34.771	19.055	0.131	0.324								
89	PBS	6	A/J	34.592	19.049	0.146	0.326	0.253	0.356	0.709					
89	PBS	6	A/J	33.848	18.89	0.229	0.358								
89	PBS	6	A/J	32.993	18.767	0.383	0.385								
90	PBS	6	A/J	35.412	18.994	0.089	0.336	0.117	0.305	0.386					
90	PBS	6	A/J	34.548	19.458	0.15	0.255								
90	PBS	6	A/J	35.015	19.069	0.113	0.322								
91	OVA	6	C3H/HeJ	33.345	18.198	0.31	0.54	0.273	0.457	0.596		1.017	0.265	1.277638	0.333
91	OVA	6	C3H/HeJ	33.917	18.801	0.219	0.377								
91	OVA	6	C3H/HeJ	33.461	18.487	0.289	0.455								
92	OVA	6	C3H/HeJ	34.237	18.404	0.181	0.478	0.267	0.416	0.643					
92	OVA	6	C3H/HeJ	33.331	18.634	0.312	0.417								
92	OVA	6	C3H/HeJ	33.348	18.914	0.309	0.353								
93	OVA	6	C3H/HeJ	30.855	18.64	1.389	0.415	1.799	0.407	4.425	Delete				
93	OVA	6	C3H/HeJ	31.67	18.735	0.85	0.392								
93	OVA	6	C3H/HeJ	29.492	18.651	3.158	0.412								
94	OVA	6	C3H/HeJ	32.943	18.785	0.395	0.381	0.331	0.319	1.038					
94	OVA	6	C3H/HeJ	33.016	19.153	0.378	0.306								
94	OVA	6	C3H/HeJ	33.91	19.37	0.22	0.269								

Table 11 (cont'd).

Mice#	Treat	Hour	Strain	Gata3 Ct	18s Ct	Gata3 18s (ng)	Ave. Gata3 18s (ng)	Gata3/18s	Note	Group Ave.	SEM	Normalized ¹	SEM ¹
95	OVA	6	C3H/HeJ	34.766	21.01	0.131	0.102	0.202	0.1	2.033			
95	OVA	6	C3H/HeJ	34.058	21.109	0.201	0.096						
95	OVA	6	C3H/HeJ	33.545	21.015	0.274	0.101						
96	OVA	6	C3H/HeJ	33.533	19.539	0.276	0.243	0.229	0.295	0.775			
96	OVA	6	C3H/HeJ	34.03	18.741	0.205	0.391						
96	OVA	6	C3H/HeJ	34.034	19.489	0.204	0.251						
97	OVA	6	A/J	35.789	22.014	0.071	0.056	0.117	0.065	1.794	0.302	1.243719	0.379
97	OVA	6	A/J	34.268	21.622	0.178	0.071						
97	OVA	6	A/J	35.174	21.659	0.103	0.069						
98	OVA	6	A/J	36.628	20.067	0.043	0.178	0.042	0.135	0.312			
98	OVA	6	A/J	37.279	20.788	0.029	0.116						
98	OVA	6	A/J	36.218	20.849	0.055	0.112						
99	OVA	6	A/J	34.576	19.347	0.147	0.273	0.145	0.26	0.558			
99	OVA	6	A/J	34.632	19.271	0.143	0.285						
99	OVA	6	A/J	34.596	19.69	0.146	0.223						
100	OVA	6	A/J	35.657	19.396	0.077	0.265	0.098	0.242	0.404			
100	OVA	6	A/J	35.039	19.807	0.112	0.208						
100	OVA	6	A/J	35.14	19.469	0.105	0.254						
101	OVA	6	A/J	32.461	18.933	0.528	0.349	0.303	0.357	0.848			
101	OVA	6	A/J	35.284	18.672	0.096	0.407						
101	OVA	6	A/J	33.49	19.111	0.284	0.314						
102	OVA	6	A/J	35.023	20.06	0.113	0.179	0.311	0.153	2.026			
102	OVA	6	A/J	34.679	20.478	0.139	0.139						
102	OVA	6	A/J	32.037	20.444	0.681	0.142						

Table 11 (cont'd).

Mice#	Treat	Hour	Strain	Gata3 Ct	18s Ct	Gata3 (ng)	Gata3 18s (ng)	Ave. Gata3 (ng)	Ave. 18s (ng)	Gata3/18s	Note	Group Ave.	SEM	Normalizer	SEM1
103	PBS	12	C3H/HeJ	34.121	19.038	0.32	0.352	0.307	0.352	0.872		0.954	0.125	1.198492	0.157
103	PBS	12	C3H/HeJ	33.885	19.02	0.366	0.356								
103	PBS	12	C3H/HeJ	34.663	19.058	0.235	0.348								
104	PBS	12	C3H/HeJ	34.135	19.379	0.318	0.288	0.452	0.303	1.492					
104	PBS	12	C3H/HeJ	33.189	19.266	0.544	0.308								
104	PBS	12	C3H/HeJ	33.361	19.241	0.493	0.312								
105	PBS	12	C3H/HeJ	34.15	19.51	0.315	0.266	0.237	0.208	1.139					
105	PBS	12	C3H/HeJ	34.692	20.289	0.232	0.168								
105	PBS	12	C3H/HeJ	35.293	20.073	0.165	0.191								
106	PBS	12	C3H/HeJ	35.081	19.988	0.186	0.201	0.19	0.242	0.784					
106	PBS	12	C3H/HeJ	34.787	19.677	0.219	0.241								
106	PBS	12	C3H/HeJ	35.304	19.404	0.164	0.284								
107	PBS	12	C3H/HeJ	35.571	19.796	0.141	0.225	0.173	0.243	0.712					
107	PBS	12	C3H/HeJ	34.693	19.552	0.231	0.26								
107	PBS	12	C3H/HeJ	35.493	19.656	0.147	0.244								
108	PBS	12	C3H/HeJ	33.386	18.366	0.486	0.534	0.387	0.535	0.724					
108	PBS	12	C3H/HeJ	33.854	18.094	0.373	0.616								
108	PBS	12	C3H/HeJ	34.225	18.608	0.302	0.454								
109	PBS	12	A/J	34.389	19.574	0.275	0.257	0.229	0.233	0.983		0.657	0.102	0.825377	0.128
109	PBS	12	A/J	34.752	19.877	0.224	0.214								
109	PBS	12	A/J	35.053	19.769	0.189	0.229								
110	PBS	12	A/J	35.747	20.114	0.127	0.186	0.129	0.21	0.613					
110	PBS	12	A/J	35.384	19.711	0.156	0.237								
110	PBS	12	A/J	36.114	19.937	0.103	0.207								

Table 11 (cont'd).

Mice#	Treat	Hour	Strain	Gata3 Ct	18s Ct	Gata3 (ng)	18s (ng)	Ave. Gata3 (ng)	Ave. 18s (ng)	Gata3/18s	Note	Group Ave.	SEM	Normalizer	SEM1
111	PBS	12	A/J	35.483	20.013	0.148	0.198	0.114	0.235	0.486					
111	PBS	12	A/J	36.334	19.568	0.091	0.257								
111	PBS	12	A/J	36.115	19.626	0.103	0.249								
112	PBS	12	A/J	33.251	19.126	0.525	0.334	0.25	0.261	0.958					
112	PBS	12	A/J	35.919	19.584	0.115	0.255								
112	PBS	12	A/J	36.017	20.052	0.109	0.193								
113	PBS	12	A/J	35.955	19.194	0.113	0.321	0.159	0.366	0.435					
113	PBS	12	A/J	35.387	19.048	0.156	0.35								
113	PBS	12	A/J	34.872	18.713	0.209	0.427								
114	PBS	12	A/J	36.575	19.124	0.079	0.335	0.139	0.299	0.465					
114	PBS	12	A/J	36.003	19.691	0.11	0.239								
114	PBS	12	A/J	34.716	19.191	0.228	0.322								
115	OVA	12	C3H/HeJ	35.318	19.501	0.162	0.268	0.204	0.299	0.683		1.032	0.248	1.296482	0.312
115	OVA	12	C3H/HeJ	35.074	19.472	0.186	0.273								
115	OVA	12	C3H/HeJ	34.456	19.016	0.265	0.357								
116	OVA	12	C3H/HeJ	34.6	18.182	0.244	0.584	0.268	0.485	0.552					
116	OVA	12	C3H/HeJ	34.089	18.356	0.326	0.527								
116	OVA	12	C3H/HeJ	34.672	19.074	0.234	0.345								
117	OVA	12	C3H/HeJ	36.925	21.127	0.065	0.102	0.191	0.092	2.065					
117	OVA	12	C3H/HeJ	34.013	21.391	0.341	0.088								
117	OVA	12	C3H/HeJ	35.283	21.409	0.166	0.087								
118	OVA	12	C3H/HeJ	33.773	19.744	0.39	0.232	0.37	0.253	1.462					
118	OVA	12	C3H/HeJ	34.344	19.498	0.282	0.268								
118	OVA	12	C3H/HeJ	33.57	19.558	0.438	0.259								

Table 11 (cont'd).

Mice#	Treat	Hour	Strain	Gata3 Ct	18s Ct	Gata3 (ng)	18s (ng)	Ave. Gata3 (ng)	Ave. 18s (ng)	Gata3/18s	Note	Group Ave.	SEM	Normalizer1	SEM1
119	OVA	12	C3H/HeJ	34.42	19.367	0.27	0.29	0.24	0.279	0.861					
119	OVA	12	C3H/HeJ	34.087	19.419	0.327	0.281								
119	OVA	12	C3H/HeJ	35.79	19.513	0.124	0.266								
120	OVA	12	C3H/HeJ	34.29	18.99	0.291	0.362	0.305	0.536	0.569					
120	OVA	12	C3H/HeJ	33.931	17.731	0.357	0.763								
120	OVA	12	C3H/HeJ	34.439	18.508	0.267	0.482								
121	OVA	12	A/J	34.46	19.756	0.264	0.23	0.227	0.257	0.881		1.037	0.313	1.302764	0.393
121	OVA	12	A/J	40	32.567	Delete	Delete								
121	OVA	12	A/J	35.054	19.402	0.189	0.284								
122	OVA	12	A/J	35.81	20.063	0.123	0.192	0.111	0.217	0.512					
122	OVA	12	A/J	35.916	20.047	0.116	0.194								
122	OVA	12	A/J	36.27	19.523	0.094	0.264								
123	OVA	12	A/J	38.959	32.46	x	x	x	x	x	Delete				
123	OVA	12	A/J	40	31.11										
123	OVA	12	A/J	38.206	32.539										
124	OVA	12	A/J	34.613	19.145	0.242	0.331	0.198	0.297	0.666					
124	OVA	12	A/J	35.617	19.425	0.137	0.28								
124	OVA	12	A/J	34.827	19.433	0.214	0.279								
125	OVA	12	A/J	33.705	20.092	0.406	0.189	0.402	0.178	2.261					
125	OVA	12	A/J	33.602	20.303	0.43	0.167								
125	OVA	12	A/J	33.874	20.202	0.369	0.177								
126	OVA	12	A/J	34.674	19.584	0.234	0.255	0.228	0.263	0.865					
126	OVA	12	A/J	34.644	19.386	0.238	0.287								
126	OVA	12	A/J	34.856	19.629	0.211	0.248								

Table 12. GATA-3 expression in tracheobronchial lymph nodes

Mice	Treatment group	Gata3 Ct	Gata3 (ng)	Ave.Gata3 (ng)	18s Ct (ng)	Ave.18s (ng)	Gata3/18s Ave.	Group	SEM	Normalizer ¹	SEM ¹
1, 2, 3	C3H/HeJ, 24hr, PBS	29.346	1.62	1.64	18.83	0.45	3.652	3.364	0.288	1.626845	0.139
1, 2, 3	C3H/HeJ, 24hr, PBS	29.3	1.66		18.84	0.448					
4, 5, 6	C3H/HeJ, 24hr, PBS	30.454	0.897	0.95	19.66	0.296	3.076				
4, 5, 6	C3H/HeJ, 24hr, PBS	30.246	1.003		19.49	0.322					
7, 8, 9	A/J, 24hr, PBS	28.727	2.253	2.197	17.96	0.705	3.069	2.815	0.254	1.361245	0.123
7, 8, 9	A/J, 24hr, PBS	28.824	2.14		17.89	0.727					
10, 11, 12	A/J, 24hr, PBS	28.289	2.846	2.696	17.1	1.089	2.561				
10, 11, 12	A/J, 24hr, PBS	28.498	2.546		17.24	1.017					
13, 14, 15	C3H/HeJ, 24hr, OVA	29.819	1.259	1.331	17.35	0.961	1.376	1.477	0.101	0.714225	0.049
13, 14, 15	C3H/HeJ, 24hr, OVA	29.616	1.403		17.32	0.974					
16, 17, 18	C3H/HeJ, 24hr, OVA	28.639	2.362	2.322	16.32	1.624	1.578				
16, 17, 18	C3H/HeJ, 24hr, OVA	28.703	2.282		16.73	1.319					
19, 20, 21	A/J, 24hr, OVA	28.248	2.909	3.1	16.37	1.583	1.882	2.238	0.355	1.082211	0.172
19, 20, 21	A/J, 24hr, OVA	28.016	3.292		16.22	1.711					
22, 23, 24	A/J, 24hr, OVA	27.934	3.439	3.486	16.79	1.28	2.593				
22, 23, 24	A/J, 24hr, OVA	27.883	3.534		16.6	1.409					
25, 26, 27	C3H/HeJ, 48hr, PBS	28.22	2.953	3.143	17	1.147	2.654	3.495	0.841	1.690235	0.407
25, 26, 27	C3H/HeJ, 48hr, PBS	27.992	3.334		16.88	1.221					
28, 29, 30	C3H/HeJ, 48hr, PBS	28.366	2.732	2.903	18.1	0.653	4.336				
28, 29, 30	C3H/HeJ, 48hr, PBS	28.144	3.075		18.01	0.686					
31, 32, 33	A/J, 48hr, PBS	28.164	3.042	2.772	17.13	1.072	2.732	2.679	0.052	1.295762	0.025
31, 32, 33	A/J, 48hr, PBS	28.531	2.502		17.36	0.958					
34, 35, 36	A/J, 48hr, PBS	28.188	3.003	2.738	17.1	1.094	2.627				
34, 35, 36	A/J, 48hr, PBS	28.553	2.472		17.29	0.991					
37, 38, 39	C3H/HeJ, 48hr, OVA	27.062	5.474	5.586	15.3	2.731	1.895	2.002	0.107	0.968116	0.052
37, 38, 39	C3H/HeJ, 48hr, OVA	26.987	5.697		15.02	3.164					
40, 41, 42	C3H/HeJ, 48hr, OVA	27.981	3.354	3.478	16.25	1.685	2.109				

Table 12 (cont'd).

Mice	Treatment group	Gata3 Ct	Gata3 (ng)	Ave.Gata3 (ng)	18s Ct	18s (ng)	Ave.18s (ng)	Gata3/18s Ave.	Group	SEM	Normalizer ¹	SEM ¹
40, 41, 42	C3H/HeJ, 48hr, OVA	27.847	3.602		16.33	1.614						
43, 44, 45	A/J, 48hr, OVA	27.462	4.423	4.431	15.95	1.962	2.053	2.159	2.284	0.125	1.104335	0.06
43, 44, 45	A/J, 48hr, OVA	27.455	4.439		15.78	2.143						
46, 47, 48	A/J, 48hr, OVA	27.013	5.619	5.894	15.58	2.375	2.447	2.408				
46, 47, 48	A/J, 48hr, OVA	26.838	6.168		15.46	2.519						
49, 50, 51	C3H/HeJ, 72hr, PBS	28.609	2.4	2.449	18.64	0.497	0.516	4.749	4.475	0.273	2.164362	0.132
49, 50, 51	C3H/HeJ, 72hr, PBS	28.533	2.499		18.5	0.534						
52, 53, 54	C3H/HeJ, 72hr, PBS	27.169	5.171	5.28	16.97	1.166	1.257	4.202				
52, 53, 54	C3H/HeJ, 72hr, PBS	27.091	5.39		16.69	1.347						
55, 56, 57	A/J, 72hr, PBS	28.487	2.561	2.53	18.09	0.657	0.675	3.746	3.701	0.045	1.789879	0.022
55, 56, 57	A/J, 72hr, PBS	28.533	2.499		17.99	0.694						
58, 59, 60	A/J, 72hr, PBS	29.62	1.4	1.557	19.01	0.412	0.426	3.656				
58, 59, 60	A/J, 72hr, PBS	29.241	1.713		18.88	0.44						
61, 62, 63	C3H/HeJ, 72hr, OVA	26.448	7.594	6.99	15.27	2.78	2.661	2.627	2.249	0.378	1.087659	0.183
61, 62, 63	C3H/HeJ, 72hr, OVA	26.773	6.386		15.44	2.542						
64, 65, 66	C3H/HeJ, 72hr, OVA	26.927	5.883	5.435	15.16	2.947	2.904	1.871				
64, 65, 66	C3H/HeJ, 72hr, OVA	27.237	4.987		15.21	2.861						
67, 68, 69	A/J, 72hr, OVA	27.357	4.678	5.163	15.46	2.524	2.748	1.879	2.12	0.241	1.025396	0.117
67, 68, 69	A/J, 72hr, OVA	27.003	5.649		15.14	2.971						
70, 71, 72	A/J, 72hr, OVA	25.881	10.27	8.969	14.6	3.919	3.798	2.361				
70, 71, 72	A/J, 72hr, OVA	26.431	7.663		14.72	3.677						
79, 80, 81	C3H/HeJ, 6hr, PBS	30.723	0.778	0.819	19.25	0.364	0.374	2.187	2.068	0.119	1	0.058
79, 80, 81	C3H/HeJ, 6hr, PBS	30.534	0.86		19.14	0.385						
82, 83, 84	C3H/HeJ, 6hr, PBS	29.917	1.195	1.186	18.16	0.636	0.609	1.949				
82, 83, 84	C3H/HeJ, 6hr, PBS	29.944	1.178		18.33	0.582						
85, 86, 87	A/J, 6hr, PBS	29.771	1.292	1.252	17.7	0.802	0.794	1.577	1.61	0.033	0.778659	0.016
85, 86, 87	A/J, 6hr, PBS	29.89	1.212		17.74	0.786						

Table 12 (cont'd).

Mice	Treatment group	Gata3 Ct	Gata3 (ng)	Ave.Gata3 (ng)	18s Ct	18s (ng)	Ave.18s (ng)	Gata3/18s Ave.	Group SEM	Normalizor1	SEM1
88, 89, 90	A/J, 6hr, PBS	27.603	4.103	3.907	15.5	2.474	2.377	1.643			
88, 89, 90	A/J, 6hr, PBS	27.791	3.711		15.66	2.28					
91, 92, 93	C3H/HeJ, 6hr, OVA	32.736	0.266	0.287	20.12	0.233	0.24	1.197	1.361	0.658153	0.08
91, 92, 93	C3H/HeJ, 6hr, OVA	32.455	0.309		20	0.248					
94, 95, 96	C3H/HeJ, 6hr, OVA	27.558	4.202	4.063	15.33	2.691	2.664	1.525			
94, 95, 96	C3H/HeJ, 6hr, OVA	27.687	3.923		15.37	2.636					
97, 98, 99	A/J, 6hr, OVA	27.593	4.125	4.335	17.41	0.93	0.939	4.618	4.067	1.966985	0.266
97, 98, 99	A/J, 6hr, OVA	27.411	4.545		17.38	0.947					
100, 101, 102	A/J, 6hr, OVA	26.422	7.7	7.844	15.86	2.056	2.231	3.517			
100, 101, 102	A/J, 6hr, OVA	26.353	7.988		15.55	2.406					
103, 104, 105	C3H/HeJ, 12hr, PBS	28.345	2.762	2.945	16.69	1.346	1.393	2.114	1.986	0.960559	0.062
103, 104, 105	C3H/HeJ, 12hr, PBS	28.112	3.128		16.56	1.441					
106, 107, 108	C3H/HeJ, 12hr, PBS	27.003	5.649	5.414	15.13	2.982	2.913	1.859			
106, 107, 108	C3H/HeJ, 12hr, PBS	27.166	5.179		15.23	2.843					
109, 110, 111	A/J, 12hr, PBS	27.206	5.07	4.932	15.31	2.721	2.869	1.719	1.349	0.652354	0.179
109, 110, 111	A/J, 12hr, PBS	27.311	4.794		15.11	3.017					
112, 113, 114	A/J, 12hr, PBS	29.353	1.614	1.511	16.54	1.451	1.543	0.979			
112, 113, 114	A/J, 12hr, PBS	29.61	1.407		16.31	1.636					
115, 116, 117	C3H/HeJ, 12hr, OVA	27.755	3.783	3.816	15.34	2.682	2.498	1.528	1.61	0.082	0.778443
115, 116, 117	C3H/HeJ, 12hr, OVA	27.723	3.848		15.63	2.313					
118, 119, 121	C3H/HeJ, 12hr, OVA	27.119	5.31	5.096	15.18	2.913	3.013	1.692			
118, 119, 120	C3H/HeJ, 12hr, OVA	27.277	4.881		15.05	3.113					
121, 122, 123	A/J, 12hr, OVA	27.339	4.723	5.031	15.38	2.634	2.741	1.836	1.903	0.920427	0.033
121, 122, 123	A/J, 12hr, OVA	27.109	5.339		15.22	2.848					
124, 125, 126	A/J, 12hr, OVA	26.428	7.675	7.317	14.64	3.826	3.712	1.971			
124, 125, 126	A/J, 12hr, OVA	26.612	6.958		14.76	3.599					

Table 13. GATA-3 expression in spleens

Mice#	Treat	Hour	Strain	Gata3 Ct	18s Ct	Ave. Gata3 Ct	Ave. Gata3 (ng)	Ave. 18s Ct (ng)	Gata3/18s	Note	Group Ave.	SEM	Normalizer	SEM1
1	PBS	24	C3H/HeJ	28.452	15.518	28.443	3.359	15.764	17.373	0.193	0.214	0.016	0.3074713	0.023
1	PBS	24	C3H/HeJ	28.433	16.009									
2	PBS	24	C3H/HeJ	28.577	15.998	28.666	3.028	16.034	14.723	0.206				
2	PBS	24	C3H/HeJ	28.754	16.069									
3	PBS	24	C3H/HeJ	28.191	15.761	28.182	3.793	15.759	17.427	0.218				
3	PBS	24	C3H/HeJ	28.172	15.756									
4	PBS	24	C3H/HeJ	40	40	40.000	x	40	x	x	Delete			
4	PBS	24	C3H/HeJ	40	40									
5	PBS	24	C3H/HeJ	27.502	14.435	27.516	5.171	14.964	28.361	0.182				
5	PBS	24	C3H/HeJ	27.529	15.493									
6	PBS	24	C3H/HeJ	28.497	16.351	28.470	3.317	16.341	12.198	0.272				
6	PBS	24	C3H/HeJ	28.443	16.33									
7	PBS	24	A/J	28.355	17.801	27.982	4.163	17.874	4.766	0.873	0.437	0.102	0.6278736	0.147
7	PBS	24	A/J	27.608	17.946									
8	PBS	24	A/J	28.833	16.039	28.499	3.273	15.906	15.920	0.206				
8	PBS	24	A/J	28.164	15.773									
9	PBS	24	A/J	27.911	15.844	27.963	4.199	15.866	16.320	0.257				
9	PBS	24	A/J	28.015	15.887									
10	PBS	24	A/J	28.198	17.652	28.175	3.805	17.346	6.588	0.578				
10	PBS	24	A/J	28.151	17.039									
11	PBS	24	A/J	28.572	17.113	28.731	2.937	17.089	7.709	0.381				
11	PBS	24	A/J	28.89	17.065									
12	PBS	24	A/J	28.82	17.009	29.052	2.530	17.070	7.800	0.324				
12	PBS	24	A/J	29.284	17.131									

Table 13 (cont'd).

Mice#	Treat	Hour	Strain	Gata3 Ct	18s Ct	Ave. Gata3 Ct	Ave. Gata3 (ng)	Ave. 18s Ct (ng)	Gata3/18s	Note	Group Ave.	SEM	Normalizer	SEM1
13	OVA	24	C3H/HeJ	26.675	15.105	26.943	6.750	15.084	26.350	0.256	0.234	0.032	0.3362069	0.046
13	OVA	24	C3H/HeJ	27.21	15.063									
14	OVA	24	C3H/HeJ	28.048	15.108	28.029	4.073	15.196	24.609	0.166				
14	OVA	24	C3H/HeJ	28.009	15.283									
15	OVA	24	C3H/HeJ	28.984	x	28.864	2.761	x	x	Delete				
15	OVA	24	C3H/HeJ	28.744	x									
16	OVA	24	C3H/HeJ	28.73	16.011	28.531	3.224	15.884	16.141	0.200				
16	OVA	24	C3H/HeJ	28.332	15.756									
17	OVA	24	C3H/HeJ	28.762	16.922	28.674	3.016	16.909	8.609	0.350				
17	OVA	24	C3H/HeJ	28.586	16.896									
18	OVA	24	C3H/HeJ	27.662	15.322	27.594	4.985	15.150	25.305	0.197				
18	OVA	24	C3H/HeJ	27.526	14.978									
19	OVA	24	A/J	28.021	16.352	28.217	3.732	16.439	11.483	0.325	0.412	0.093	0.591954	0.134
19	OVA	24	A/J	28.412	16.526									
20	OVA	24	A/J	28.051	16.816	28.035	4.061	16.780	9.317	0.436				
20	OVA	24	A/J	28.018	16.744									
21	OVA	24	A/J	27.098	15.09	26.926	6.802	15.409	21.597	0.315				
21	OVA	24	A/J	26.754	15.727									
22	OVA	24	A/J	27.549	17.465	27.454	5.321	17.431	6.253	0.851				
22	OVA	24	A/J	27.359	17.396									
23	OVA	24	A/J	27.437	16.068	27.317	5.671	15.869	16.285	0.348				
23	OVA	24	A/J	27.197	15.67									
24	OVA	24	A/J	28.399	15.634	28.225	3.718	15.628	18.884	0.197				
24	OVA	24	A/J	28.05	15.621									

Table 13 (cont'd).

Mice#	Treat	Hour	Strain	Gata3 Ct	18s Ct	Ave. Gata3 Ct	Ave. Gata3 (ng)	Ave. 18s Ct (ng)	Gata3/18s	Note	Group Ave.	SEM	Normalizer	SEM1
25	PBS	48	C3H/HeJ	26.923	17.248	26.972	5.019	17.328	4.048	1.240	0.611	0.136	0.8778736	0.195
25	PBS	48	C3H/HeJ	27.02	17.408									
26	PBS	48	C3H/HeJ	27.36	15.433	27.232	4.390	15.430	12.459	0.352				
26	PBS	48	C3H/HeJ	27.104	15.426									
27	PBS	48	C3H/HeJ	27.391	16.822	27.550	3.727	16.867	5.320	0.701				
27	PBS	48	C3H/HeJ	27.709	16.911									
28	PBS	48	C3H/HeJ	28.134	16.431	27.907	3.103	16.490	6.651	0.467				
28	PBS	48	C3H/HeJ	27.679	16.548									
29	PBS	48	C3H/HeJ	28.443	17.117	28.392	2.418	17.127	4.559	0.530				
29	PBS	48	C3H/HeJ	28.34	17.137									
30	PBS	48	C3H/HeJ	26.402	15.033	26.673	5.854	15.061	15.502	0.378				
30	PBS	48	C3H/HeJ	26.943	15.088									
31	PBS	48	A/J	27.145	14.783	27.201	4.462	14.921	16.837	0.265	0.824	0.207	1.183908	0.297
31	PBS	48	A/J	27.256	15.059									
32	PBS	48	A/J	29.385	15.497	28.648	2.120	15.360	12.987	0.163				
32	PBS	48	A/J	27.91	15.222									
33	PBS	48	A/J	27.353	17.718	27.585	3.661	17.719	3.212	1.140				
33	PBS	48	A/J	27.817	17.719									
34	PBS	48	A/J	27.681	17.727	27.727	3.404	17.639	3.368	1.011				
34	PBS	48	A/J	27.772	17.55									
35	PBS	48	A/J	26.456	16.381	26.596	6.090	16.492	6.641	0.917				
35	PBS	48	A/J	26.735	16.603									
36	PBS	48	A/J	26.564	17.007	26.393	6.761	17.090	4.662	1.450				
36	PBS	48	A/J	26.221	17.172									

Table 13 (cont'd).

Mice#	Treat	Hour	Strain	Gata3 Ct	18s Ct	Ave. Gata3 Ct	Ave. Gata3 (ng)	Ave. 18s Ct (ng)	Gata3/18s	Note	Group Ave.	SEM	Normalizer	SEM1
37	OVA	48	C3H/HeJ	27.471	16.343	27.482	3.861	16.403	7.000	0.552	0.398	0.054	0.5718391	0.078
37	OVA	48	C3H/HeJ	27.492	16.463									
38	OVA	48	C3H/HeJ	26.695	14.392	26.453	6.553	14.360	23.472	0.279				
38	OVA	48	C3H/HeJ	26.211	14.328									
39	OVA	48	C3H/HeJ	28.06	16.43	27.693	3.464	16.409	6.976	0.497				
39	OVA	48	C3H/HeJ	27.325	16.388									
40	OVA	48	C3H/HeJ	27.705	15.108	27.803	3.273	15.030	15.785	0.207				
40	OVA	48	C3H/HeJ	27.901	14.952									
41	OVA	48	C3H/HeJ	27.367	16.318	27.821	3.242	16.402	7.007	0.463				
41	OVA	48	C3H/HeJ	28.275	16.485									
42	OVA	48	C3H/HeJ	28.058	16.433	28.047	2.887	16.316	7.370	0.392				
42	OVA	48	C3H/HeJ	28.035	16.199									
43	OVA	48	A/J	26.769	16.074	26.954	5.066	16.195	7.918	0.640	0.809	0.120	1.1623563	0.172
43	OVA	48	A/J	27.138	16.316									
44	OVA	48	A/J	27.475	19.815	27.318	4.200	19.750	0.965	4.352				
44	OVA	48	A/J	27.161	19.684									
45	OVA	48	A/J	27.449	16.605	27.563	3.703	16.685	5.925	0.625				
45	OVA	48	A/J	27.677	16.764									
46	OVA	48	A/J	26.622	16.81	26.496	6.410	16.912	5.180	1.237				
46	OVA	48	A/J	26.37	17.013									
47	OVA	48	A/J	27.277	16.575	27.352	4.127	16.519	6.538	0.631				
47	OVA	48	A/J	27.427	16.462									
48	OVA	48	A/J	28.143	17.613	27.961	3.017	17.673	3.300	0.914				
48	OVA	48	A/J	27.779	17.733									

Table 13 (cont'd).

Mice#	Treat	Hour	Strain	Gata3 Ct	18s Ct	Ave. Gata3 Ct	Ave. Gata3 (ng)	Ave. 18s Ct (ng)	Gata3/18s	Note	Group Ave.	SEM	Normalizer	SEM1
49	PBS	72	C3H/HeJ	27.154	15.129	27.104	6.031	15.298	13.471	0.448	0.642	0.071	0.9224138	0.102
49	PBS	72	C3H/HeJ	27.054	15.467									
50	PBS	72	C3H/HeJ	26.115	15.872	26.143	9.695	15.699	10.685	0.907				
50	PBS	72	C3H/HeJ	26.171	15.525									
51	PBS	72	C3H/HeJ	27.475	16.533	27.501	4.958	16.508	6.689	0.741				
51	PBS	72	C3H/HeJ	27.526	16.483									
52	PBS	72	C3H/HeJ	27.03	15.484	26.890	6.703	15.603	11.295	0.593				
52	PBS	72	C3H/HeJ	26.75	15.721									
53	PBS	72	C3H/HeJ	26.042	14.395	26.057	10.119	14.507	21.294	0.475				
53	PBS	72	C3H/HeJ	26.071	14.618									
54	PBS	72	C3H/HeJ	27.053	15.614	26.871	6.768	15.834	9.879	0.685				
54	PBS	72	C3H/HeJ	26.688	16.054									
55	PBS	72	A/J	26.528	15.894	26.457	8.302	15.612	11.236	0.739	1.004	0.205	1.4425287	0.295
55	PBS	72	A/J	26.386	15.629									
56	PBS	72	A/J	28.583	16.669	28.182	3.541	16.500	6.720	0.527				
56	PBS	72	A/J	27.78	16.331									
57	PBS	72	A/J	27.267	16.735	27.383	5.256	16.867	5.436	0.967				
57	PBS	72	A/J	27.498	16.998									
58	PBS	72	A/J	26.18	16.858	26.109	9.860	16.684	6.042	1.632				
58	PBS	72	A/J	26.038	16.51									
59	PBS	72	A/J	27.24	15.957	27.204	5.740	15.732	10.483	0.548				
59	PBS	72	A/J	27.168	15.506									
60	PBS	72	A/J	26.99	17.327	26.869	6.773	17.308	4.212	1.608				
60	PBS	72	A/J	26.748	17.288									

Table 13 (cont'd).

Mice#	Treat	Hour	Strain	Gata3 Ct	18s Ct	Ave. Gata3 Ct	Ave. Gata3 (ng)	Ave. 18s Ct	Ave. 18s (ng)	Gata3/18s	Note	Group Ave.	SEM	Normalizer	SEM1
61	OVA	72	C3H/HeJ	26.701	15.84	26.962	6.471	15.907	9.471	0.683		0.679	0.072	0.9755747	0.103
61	OVA	72	C3H/HeJ	27.222	15.974										
62	OVA	72	C3H/HeJ	27.283	16.478	27.704	4.485	16.557	6.502	0.690					
62	OVA	72	C3H/HeJ	28.124	16.636										
63	OVA	72	C3H/HeJ	26.946	15.444	27.079	6.106	15.495	12.023	0.508					
63	OVA	72	C3H/HeJ	27.212	15.545										
64	OVA	72	C3H/HeJ	28.15	16.303	27.964	3.944	16.234	7.838	0.503					
64	OVA	72	C3H/HeJ	27.777	16.165										
65	OVA	72	C3H/HeJ	27.149	16.523	26.978	6.420	16.549	6.534	0.983					
65	OVA	72	C3H/HeJ	26.806	16.574										
66	OVA	72	C3H/HeJ	28.72	16.94	28.767	2.652	17.504	3.761	0.705					
66	OVA	72	C3H/HeJ	28.814	18.067										
67	OVA	72	A/J	28.221	17.395	28.326	3.298	17.425	3.935	0.838		0.837	0.058	1.2025862	0.083
67	OVA	72	A/J	28.43	17.455										
68	OVA	72	A/J	27.173	17.183	27.242	5.635	16.923	5.263	1.071					
68	OVA	72	A/J	27.31	16.662										
69	OVA	72	A/J	28.692	17.5	28.535	2.974	17.588	3.581	0.830					
69	OVA	72	A/J	28.378	17.676										
70	OVA	72	A/J	28.68	17.563	28.229	3.459	17.462	3.852	0.898					
70	OVA	72	A/J	27.778	17.361										
71	OVA	72	A/J	27.704	16.576	27.527	4.895	16.449	6.922	0.707					
71	OVA	72	A/J	27.349	16.322										
72	OVA	72	A/J	27.202	16.297	27.255	5.599	16.138	8.286	0.676					
72	OVA	72	A/J	27.307	15.979										

Table 13 (cont'd).

Mice#	Treat	Hour	Strain	Gata3 Ct	18s Ct	Ave. Gata3 Ct	Ave. Gata3 (ng)	Ave. 18s Ct (ng)	Gata3/18s	Note	Group Ave.	SEM	Normalizer	SEM1
79	PBS	6	C3H/HeJ	27.632	15.499	27.599	4.275	15.554	7.078	0.604	0.696	0.092	1	0.132
79	PBS	6	C3H/HeJ	27.566	15.609									
80	PBS	6	C3H/HeJ	29.189	18.048	29.142	1.886	18.056	1.665	1.133				
80	PBS	6	C3H/HeJ	29.094	18.064									
81	PBS	6	C3H/HeJ	27.51	15.817	27.556	4.375	15.796	6.154	0.711				
81	PBS	6	C3H/HeJ	27.601	15.775									
82	PBS	6	C3H/HeJ	28.796	16.322	28.904	2.140	16.469	4.170	0.513				
82	PBS	6	C3H/HeJ	29.012	16.616									
83	PBS	6	C3H/HeJ	27.13	14.972	27.127	5.491	15.024	9.619	0.571				
83	PBS	6	C3H/HeJ	27.124	15.075									
84	PBS	6	C3H/HeJ	26.546	14.543	26.386	8.134	14.548	12.668	0.642				
84	PBS	6	C3H/HeJ	26.226	14.552									
85	PBS	6	A/J	27.599	15.013	27.453	4.620	14.980	9.867	0.468	1.089	0.254	1.5646552	0.365
85	PBS	6	A/J	27.306	14.946									
86	PBS	6	A/J	29.465	15.815	28.960	2.077	15.686	6.558	0.317				
86	PBS	6	A/J	28.455	15.557									
87	PBS	6	A/J	27.812	17.707	27.884	3.676	17.754	1.983	1.854				
87	PBS	6	A/J	27.955	17.801									
88	PBS	6	A/J	26.517	16.162	26.587	7.312	15.950	5.629	1.299				
88	PBS	6	A/J	26.657	15.738									
89	PBS	6	A/J	28.058	16.248	27.651	4.159	16.394	4.355	0.955				
89	PBS	6	A/J	27.244	16.54									
90	PBS	6	A/J	27.453	16.91	27.236	5.182	16.951	3.156	1.642				
90	PBS	6	A/J	27.019	16.991									

Table 13 (cont'd).

Mice#	Treat	Hour	Strain	Gata3 Ct	18s Ct	Ave. Gata3 Ct	Ave. Gata3 (ng)	Ave. 18s Ct (ng)	Gata3/18s	Note	Group Ave.	SEM	Normalizer	SEM
91	OVA	6	C3H/HeJ	30.005	15.263	29.249	1.782	15.367	7.886	0.226	1.005	0.186	1.4439655	0.267
91	OVA	6	C3H/HeJ	28.492	15.471									
92	OVA	6	C3H/HeJ	27.016	16.314	27.215	5.242	16.143	5.035	1.041				
92	OVA	6	C3H/HeJ	27.413	15.972									
93	OVA	6	C3H/HeJ	27.325	16.272	27.290	5.036	16.277	4.661	1.080				
93	OVA	6	C3H/HeJ	27.255	16.281									
94	OVA	6	C3H/HeJ	28.237	16.611	28.147	3.197	16.671	3.711	0.862				
94	OVA	6	C3H/HeJ	28.056	16.73									
95	OVA	6	C3H/HeJ	27.021	16.176	26.985	5.920	16.190	4.901	1.208				
95	OVA	6	C3H/HeJ	26.949	16.203									
96	OVA	6	C3H/HeJ	27.864	17.341	27.578	4.323	17.230	2.685	1.610				
96	OVA	6	C3H/HeJ	27.292	17.119									
97	OVA	6	A/J	27.894	19.138	28.033	3.397	19.141	0.889	3.821	2.126	0.500	3.0545977	0.718
97	OVA	6	A/J	28.171	19.144									
98	OVA	6	A/J	27.339	16.181	27.514	4.473	16.157	4.994	0.896				
98	OVA	6	A/J	27.688	16.133									
99	OVA	6	A/J	29.764	20.266	29.562	1.510	19.954	0.556	2.715				
99	OVA	6	A/J	29.359	19.641									
100	OVA	6	A/J	27.468	16.841	27.493	4.522	16.809	3.425	1.320				
100	OVA	6	A/J	27.518	16.777									
101	OVA	6	A/J	27.344	16.131	27.383	4.795	16.191	4.888	0.981				
101	OVA	6	A/J	27.421	16.257									
102	OVA	6	A/J	27.472	18.14	27.573	4.335	18.315	1.434	3.023				
102	OVA	6	A/J	27.673	18.489									

Table 13 (cont'd).

Mice#	Treat	Hour	Strain	Gata3 Ct	18s Ct	Ave. Gata3 Ct	Ave. Gata3 (ng)	Ave. 18s Ct	Ave. 18s (ng)	Gata3/18s	Note	Group Ave.	SEM	Normalizer	SEM1
103	PBS	12	C3H/HeJ	25.326	15.88	26.275	6.269	15.82	12.348	0.508		0.951	0.233	1.3663793	0.335
103	PBS	12	C3H/HeJ	27.223	15.756										
104	PBS	12	C3H/HeJ	23.605	21.297	23.932	20.619	20.86	0.641	32.166	Delete				
104	PBS	12	C3H/HeJ	24.259	20.417										
105	PBS	12	C3H/HeJ	24.545	16.739	24.570	14.908	16.51	8.228	1.812					
105	PBS	12	C3H/HeJ	24.595	16.28										
106	PBS	12	C3H/HeJ	24.53	14.75	24.501	15.441	14.87	21.535	0.717					
106	PBS	12	C3H/HeJ	24.472	14.991										
107	PBS	12	C3H/HeJ	24.346	15.056	24.542	15.126	14.76	22.958	0.659					
107	PBS	12	C3H/HeJ	24.737	14.467										
108	PBS	12	C3H/HeJ	23.211	14.708	23.394	27.103	14.58	25.591	1.059					
108	PBS	12	C3H/HeJ	23.577	14.445										
109	PBS	12	A/J	26.79	20.375	26.407	5.861	20.47	0.805	7.280	Delete	1.276	0.136	1.83333333	0.195
109	PBS	12	A/J	26.024	20.565										
110	PBS	12	A/J	25.288	16.697	25.333	10.119	16.72	7.272	1.391					
110	PBS	12	A/J	25.377	16.743										
111	PBS	12	A/J	26.412	16.936	26.141	6.711	16.97	6.272	1.070					
111	PBS	12	A/J	25.869	17.008										
112	PBS	12	A/J	24.611	16.154	24.731	13.737	15.94	11.501	1.194					
112	PBS	12	A/J	24.851	15.724										
113	PBS	12	A/J	25.326	17.197	25.376	9.900	17.14	5.675	1.744					
113	PBS	12	A/J	25.425	17.088										
114	PBS	12	A/J	25.547	16.474	25.794	8.005	16.52	8.166	0.980					
114	PBS	12	A/J	26.04	16.571										

Table 13 (cont'd).

Mice#	Treat	Hour	Strain	Gata3 Ct	18s Ct	Ave. Gata3 Ct	Ave. Gata3 (ng)	Ave. 18s Ct	Ave. 18s (ng)	Gata3/18s	Note	Group Ave.	SEM	Normalizer	SEM1
115	OVA	12	C3H/HeJ	24.09	15.45	24.522	15.277	15.47	15.173	1.007		1.034	0.095	1.4856322	0.136
115	OVA	12	C3H/HeJ	24.954	15.484										
116	OVA	12	C3H/HeJ	25.562	16.277	25.723	8.297	16.15	10.150	0.817					
116	OVA	12	C3H/HeJ	25.884	16.027										
117	OVA	12	C3H/HeJ	25.214	16.101	24.713	13.867	16.13	10.315	1.344					
117	OVA	12	C3H/HeJ	24.211	16.148										
118	OVA	12	C3H/HeJ	25.694	16.168	25.908	7.554	16.2	9.885	0.764					
118	OVA	12	C3H/HeJ	26.121	16.226										
119	OVA	12	C3H/HeJ	24.246	15.365	24.463	15.746	15.42	15.561	1.012					
119	OVA	12	C3H/HeJ	24.679	15.483										
120	OVA	12	C3H/HeJ	25.571	17.28	26.012	7.165	17.14	5.681	1.261					
120	OVA	12	C3H/HeJ	26.452	17.001										
121	OVA	12	A/J	25.348	17.663	25.736	8.242	17.49	4.643	1.775		0.879	0.304	1.262931	0.437
121	OVA	12	A/J	26.124	17.306										
122	OVA	12	A/J	25.468	14.792	25.251	10.547	14.83	22.001	0.479					
122	OVA	12	A/J	25.034	14.876										
123	OVA	12	A/J	26.116	15.655	26.203	6.503	15.82	12.323	0.528					
123	OVA	12	A/J	26.289	15.988										
124	OVA	12	A/J	25.762	16.445	26.027	7.109	16.23	9.707	0.732					
124	OVA	12	A/J	26.292	16.011										
125	OVA	12	A/J	40	40	40.000	x	40	x	x	Delete				
125	OVA	12	A/J	40	40										
126	OVA	12	A/J	40	40	40.000	x	40	x	x	Delete				
126	OVA	12	A/J	40	40										

BIBLIOGRAPHY

1. Lemanske, R.F., Jr. and W.W. Busse, *6. Asthma*. J Allergy Clin Immunol, 2003. **111**(2 Suppl): p. S502-19.
2. Wills-Karp, M., *Immunologic basis of antigen-induced airway hyperresponsiveness*. Annu Rev Immunol, 1999. **17**: p. 255-81.
3. Lemanske, R.F., Jr. and W.W. Busse, *Asthma*. Jama, 1997. **278**(22): p. 1855-73.
4. CDC, *Asthma Prevalence, Health Care Use, and Mortality, 2000-2001*. 2004.
5. Bel, E.H., *Clinical phenotypes of asthma*. Curr Opin Pulm Med, 2004. **10**(1): p. 44-50.
6. Hargreave, F.E., et al., *Bronchial responsiveness to histamine or methacholine in asthma: measurement and clinical significance*. J Allergy Clin Immunol, 1981. **68**(5): p. 347-55.
7. Yamamoto, Y. and R.B. Gaynor, *Therapeutic potential of inhibition of the NF-kappaB pathway in the treatment of inflammation and cancer*. J Clin Invest, 2001. **107**(2): p. 135-42.
8. Johnson, M., *The beta-adrenoceptor*. Am J Respir Crit Care Med, 1998. **158**(5 Pt 3): p. S146-53.
9. Popescu, F.D., *New asthma drugs acting on gene expression*. J Cell Mol Med, 2003. **7**(4): p. 475-86.
10. Kline, J.N., *DNA therapy for asthma*. Curr Opin Allergy Clin Immunol, 2002. **2**(1): p. 69-73.
11. Los, H., P.E. Postmus, and D.I. Boomsma, *Asthma genetics and intermediate phenotypes: a review from twin studies*. Twin Res, 2001. **4**(2): p. 81-93.
12. Clarke, J.R., et al., *Evidence for genetic associations between asthma, atopy, and bronchial hyperresponsiveness: a study of 8- to 18-yr-old twins*. Am J Respir Crit Care Med, 2000. **162**(6): p. 2188-93.
13. Laitinen, T., et al., *Importance of genetic factors in adolescent asthma: a population-based twin-family study*. Am J Respir Crit Care Med, 1998. **157**(4 Pt 1): p. 1073-8.

14. Duffy, D.L., C.A. Mitchell, and N.G. Martin, *Genetic and environmental risk factors for asthma: a cotwin-control study*. Am J Respir Crit Care Med, 1998. **157**(3 Pt 1): p. 840-5.
15. Marsh, D.G., et al., *Linkage analysis of IL4 and other chromosome 5q31.1 markers and total serum immunoglobulin E concentrations*. Science, 1994. **264**(5162): p. 1152-6.
16. Burchard, E.G., et al., *Association between a sequence variant in the IL-4 gene promoter and FEV(1) in asthma*. Am J Respir Crit Care Med, 1999. **160**(3): p. 919-22.
17. Heinzmann, A., et al., *Genetic variants of IL-13 signalling and human asthma and atopy*. Hum Mol Genet, 2000. **9**(4): p. 549-59.
18. Martinez, F.D., et al., *Linkage of circulating eosinophils to markers on chromosome 5q*. Am J Respir Crit Care Med, 1998. **158**(6): p. 1739-44.
19. Ahmadi, K.R., et al., *Novel association suggests multiple independent QTLs within chromosome 5q21-33 region control variation in total humans IgE levels*. Genes Immun, 2003. **4**(4): p. 289-97.
20. Buckova, D., et al., *Two CD14 promoter polymorphisms and atopic phenotypes in Czech patients with IgE-mediated allergy*. Allergy, 2003. **58**(10): p. 1023-6.
21. Kabesch, M., et al., *Association between polymorphisms in serine protease inhibitor, kazal type 5 and asthma phenotypes in a large German population sample*. Clin Exp Allergy, 2004. **34**(3): p. 340-345.
22. Huang, S.K., P. Zwollo, and D.G. Marsh, *Class II major histocompatibility complex restriction of human T cell responses to short ragweed allergen, Amb a V*. Eur J Immunol, 1991. **21**(6): p. 1469-73.
23. Wilson, A.G., et al., *Effects of a polymorphism in the human tumor necrosis factor alpha promoter on transcriptional activation*. Proc Natl Acad Sci U S A, 1997. **94**(7): p. 3195-9.
24. Hijazi, Z., et al., *High frequency of IgE receptor Fc epsilonRIbeta variant (Leu181/Leu183) in Kuwaiti Arabs and its association with asthma*. Clin Genet, 1998. **53**(2): p. 149-52.
25. Hoffjan, S., D. Nicolae, and C. Ober, *Association studies for asthma and atopic diseases: a comprehensive review of the literature*. Respir Res, 2003. **4**(1): p. 14.
26. Alberts, B.e.a., *Molecular Biology of the Cell (3rd edition)*. 1994.

27. Janeway, C.e.a., *Immunobiology: the immune system in health and disease (5th edition)*. 2001.
28. Ray, A. and L. Cohn, *Th2 cells and GATA-3 in asthma: new insights into the regulation of airway inflammation*. J Clin Invest, 1999. **104**(8): p. 985-93.
29. Stumbles, P.A., et al., *Resting respiratory tract dendritic cells preferentially stimulate T helper cell type 2 (Th2) responses and require obligatory cytokine signals for induction of Th1 immunity*. J Exp Med, 1998. **188**(11): p. 2019-31.
30. Walker, C., et al., *Allergic and nonallergic asthmatics have distinct patterns of T-cell activation and cytokine production in peripheral blood and bronchoalveolar lavage*. Am Rev Respir Dis, 1992. **146**(1): p. 109-15.
31. Constant, S.L. and K. Bottomly, *Induction of Th1 and Th2 CD4+ T cell responses: the alternative approaches*. Annu Rev Immunol, 1997. **15**: p. 297-322.
32. Ho, I.C. and L.H. Glimcher, *Transcription: tantalizing times for T cells*. Cell, 2002. **109 Suppl**: p. S109-20.
33. Pfeiffer, C., et al., *Altered peptide ligands can control CD4 T lymphocyte differentiation in vivo*. J Exp Med, 1995. **181**(4): p. 1569-74.
34. Brogdon, J.L., D. Leitenberg, and K. Bottomly, *The potency of TCR signaling differentially regulates NFATc/p activity and early IL-4 transcription in naive CD4+ T cells*. J Immunol, 2002. **168**(8): p. 3825-32.
35. Ise, W., et al., *Naive CD4+ T cells exhibit distinct expression patterns of cytokines and cell surface molecules on their primary responses to varying doses of antigen*. J Immunol, 2002. **168**(7): p. 3242-50.
36. Rodriguez-Palmero, M., et al., *Triggering of T cell proliferation through CD28 induces GATA-3 and promotes T helper type 2 differentiation in vitro and in vivo*. Eur J Immunol, 1999. **29**(12): p. 3914-24.
37. Inami, M., et al., *CD28 costimulation controls histone hyperacetylation of the IL-5 gene locus in developing Th2 cells*. J Biol Chem, 2004. **[Epub ahead of print]**.
38. Bour-Jordan, H., et al., *CTLA-4 regulates the requirement for cytokine-induced signals in T(H)2 lineage commitment*. Nat Immunol, 2003. **4**(2): p. 182-8.
39. Smits, H.H., et al., *Intercellular adhesion molecule-1/LFA-1 ligation favors human Th1 development*. J Immunol, 2002. **168**(4): p. 1710-6.

40. Barner, M., et al., *Differences between IL-4R alpha-deficient and IL-4-deficient mice reveal a role for IL-13 in the regulation of Th2 responses*. Curr Biol, 1998. **8**(11): p. 669-72.
41. Flood-Page, P., et al., *Anti-IL-5 treatment reduces deposition of ECM proteins in the bronchial subepithelial basement membrane of mild atopic asthmatics*. J Clin Invest, 2003. **112**(7): p. 1029-36.
42. Dong, C. and R.A. Flavell, *Control of T helper cell differentiation--in search of master genes*. Sci STKE, 2000. **2000**(49): p. PE1.
43. Kuo, C.T. and J.M. Leiden, *Transcriptional regulation of T lymphocyte development and function*. Annu Rev Immunol, 1999. **17**: p. 149-87.
44. Decker, T. and A. Meinke, *Jaks, Stats and the immune system*. Immunobiology, 1997. **198**(1-3): p. 99-111.
45. Lopez-Rodriguez, C., et al., *NFAT5, a constitutively nuclear NFAT protein that does not cooperate with Fos and Jun*. Proc Natl Acad Sci U S A, 1999. **96**(13): p. 7214-9.
46. Diehl, S., et al., *Induction of NFATc2 expression by interleukin 6 promotes T helper type 2 differentiation*. J Exp Med, 2002. **196**(1): p. 39-49.
47. Wierenga, E.A., et al., *Evidence for suppressed activity of the transcription factor NFAT1 at its proximal binding element P0 in the IL-4 promoter associated with enhanced IL-4 gene transcription in T cells of atopic patients*. Int Immunol, 1999. **11**(2): p. 297-306.
48. Kerppola, T.K. and T. Curran, *Maf and Nrl can bind to AP-1 sites and form heterodimers with Fos and Jun*. Oncogene, 1994. **9**(3): p. 675-84.
49. Hwang, E.S., I.A. White, and I.C. Ho, *An IL-4-independent and CD25-mediated function of c-maf in promoting the production of Th2 cytokines*. Proc Natl Acad Sci U S A, 2002. **99**(20): p. 13026-30.
50. Kim, J.I., et al., *The transcription factor c-Maf controls the production of interleukin-4 but not other Th2 cytokines*. Immunity, 1999. **10**(6): p. 745-51.
51. Caamano, J. and C.A. Hunter, *NF-kappaB family of transcription factors: central regulators of innate and adaptive immune functions*. Clin Microbiol Rev, 2002. **15**(3): p. 414-29.
52. Baeuerle, P.A. and D. Baltimore, *NF-kappa B: ten years after*. Cell, 1996. **87**(1): p. 13-20.

53. Das, J., et al., *A critical role for NF-kappa B in GATA3 expression and TH2 differentiation in allergic airway inflammation*. Nat Immunol, 2001. **2**(1): p. 45-50.
54. Szabo, S.J., et al., *A novel transcription factor, T-bet, directs Th1 lineage commitment*. Cell, 2000. **100**(6): p. 655-69.
55. Finotto, S., et al., *Development of spontaneous airway changes consistent with human asthma in mice lacking T-bet*. Science, 2002. **295**(5553): p. 336-8.
56. Afkarian, M., et al., *T-bet is a STAT1-induced regulator of IL-12R expression in naive CD4+ T cells*. Nat Immunol, 2002. **3**(6): p. 549-57.
57. Sundrud, M.S., et al., *Genetic reprogramming of primary human T cells reveals functional plasticity in Th cell differentiation*. J Immunol, 2003. **171**(7): p. 3542-9.
58. Fields, P.E., S.T. Kim, and R.A. Flavell, *Cutting edge: changes in histone acetylation at the IL-4 and IFN-gamma loci accompany Th1/Th2 differentiation*. J Immunol, 2002. **169**(2): p. 647-50.
59. Avni, O., et al., *T(H) cell differentiation is accompanied by dynamic changes in histone acetylation of cytokine genes*. Nat Immunol, 2002. **3**(7): p. 643-51.
60. Chakir, H., et al., *T-bet/GATA-3 ratio as a measure of the Th1/Th2 cytokine profile in mixed cell populations: predominant role of GATA-3*. J Immunol Methods, 2003. **278**(1-2): p. 157-69.
61. Yamamoto, M., et al., *Activity and tissue-specific expression of the transcription factor NF-E1 multigene family*. Genes Dev, 1990. **4**(10): p. 1650-62.
62. George, K.M., et al., *Embryonic expression and cloning of the murine GATA-3 gene*. Development, 1994. **120**(9): p. 2673-86.
63. Ko, L.J. and J.D. Engel, *DNA-binding specificities of the GATA transcription factor family*. Mol Cell Biol, 1993. **13**(7): p. 4011-22.
64. Hirasawa, R., et al., *Essential and instructive roles of GATA factors in eosinophil development*. J Exp Med, 2002. **195**(11): p. 1379-86.
65. Pedone, P.V., et al., *The N-terminal fingers of chicken GATA-2 and GATA-3 are independent sequence-specific DNA binding domains*. Embo J, 1997. **16**(10): p. 2874-82.
66. Ahmed, M., et al., *Natural history of GATA1 mutations in Down syndrome*. Blood, 2004. **103**(7): p. 2480-9.

67. Fujiwara, Y., et al., *Functional overlap of GATA-1 and GATA-2 in primitive hematopoietic development*. Blood, 2004. **103**(2): p. 583-5.
68. Zhang, H., et al., *GATA-4 regulates cardiac morphogenesis through transactivation of the N-cadherin gene*. Biochem Biophys Res Commun, 2003. **312**(4): p. 1033-8.
69. Kiiveri, S., et al., *Differential expression of GATA-4 and GATA-6 in fetal and adult mouse and human adrenal tissue*. Endocrinology, 2002. **143**(8): p. 3136-43.
70. Krasinski, S.D., et al., *Differential activation of intestinal gene promoters: functional interactions between GATA-5 and HNF-1 alpha*. Am J Physiol Gastrointest Liver Physiol, 2001. **281**(1): p. G69-84.
71. Ko, L.J., et al., *Murine and human T-lymphocyte GATA-3 factors mediate transcription through a cis-regulatory element within the human T-cell receptor delta gene enhancer*. Mol Cell Biol, 1991. **11**(5): p. 2778-84.
72. Oosterwegel, M., et al., *Expression of GATA-3 during lymphocyte differentiation and mouse embryogenesis*. Dev Immunol, 1992. **3**(1): p. 1-11.
73. Nesbit, M.A., et al., *Characterisation of GATA3 mutations in the hypoparathyroidism, deafness and renal dysplasia (HDR) syndrome*. J Biol Chem, 2004. [Epub ahead of print].
74. Van Esch, H., et al., *GATA3 haplo-insufficiency causes human HDR syndrome*. Nature, 2000. **406**(6794): p. 419-22.
75. Zhang, D.H., et al., *Transcription factor GATA-3 is differentially expressed in murine Th1 and Th2 cells and controls Th2-specific expression of the interleukin-5 gene*. J Biol Chem, 1997. **272**(34): p. 21597-603.
76. Pai, S.Y., M.L. Truitt, and I.C. Ho, *GATA-3 deficiency abrogates the development and maintenance of T helper type 2 cells*. Proc Natl Acad Sci U S A, 2004. **101**(7): p. 1993-8.
77. Lee, H.J., et al., *GATA-3 induces T helper cell type 2 (Th2) cytokine expression and chromatin remodeling in committed Th1 cells*. J Exp Med, 2000. **192**(1): p. 105-15.
78. Takemoto, N., K. Arai, and S. Miyatake, *Cutting edge: the differential involvement of the N-finger of GATA-3 in chromatin remodeling and transactivation during Th2 development*. J Immunol, 2002. **169**(8): p. 4103-7.

79. Asnagli, H., M. Afkarian, and K.M. Murphy, *Cutting edge: Identification of an alternative GATA-3 promoter directing tissue-specific gene expression in mouse and human*. J Immunol, 2002. **168**(9): p. 4268-71.
80. Gregoire, J.M. and P.H. Romeo, *T-cell expression of the human GATA-3 gene is regulated by a non-lineage-specific silencer*. J Biol Chem, 1999. **274**(10): p. 6567-78.
81. Lakshmanan, G., et al., *Localization of distant urogenital system-, central nervous system-, and endocardium-specific transcriptional regulatory elements in the GATA-3 locus*. Mol Cell Biol, 1999. **19**(2): p. 1558-68.
82. Ho, I.C., et al., *Human GATA-3: a lineage-restricted transcription factor that regulates the expression of the T cell receptor alpha gene*. Embo J, 1991. **10**(5): p. 1187-92.
83. Marine, J. and A. Winoto, *The human enhancer-binding protein Gata3 binds to several T-cell receptor regulatory elements*. Proc Natl Acad Sci U S A, 1991. **88**(16): p. 7284-8.
84. Ting, C.N., et al., *Transcription factor GATA-3 is required for development of the T-cell lineage*. Nature, 1996. **384**(6608): p. 474-8.
85. Zheng, W. and R.A. Flavell, *The transcription factor GATA-3 is necessary and sufficient for Th2 cytokine gene expression in CD4 T cells*. Cell, 1997. **89**(4): p. 587-96.
86. Siegel, M.D., et al., *Activation of the interleukin-5 promoter by cAMP in murine EL-4 cells requires the GATA-3 and CLE0 elements*. J Biol Chem, 1995. **270**(41): p. 24548-55.
87. Zhang, D.H., L. Yang, and A. Ray, *Differential responsiveness of the IL-5 and IL-4 genes to transcription factor GATA-3*. J Immunol, 1998. **161**(8): p. 3817-21.
88. Ranganath, S., et al., *GATA-3-dependent enhancer activity in IL-4 gene regulation*. J Immunol, 1998. **161**(8): p. 3822-6.
89. Takemoto, N., et al., *Cutting edge: chromatin remodeling at the IL-4/IL-13 intergenic regulatory region for Th2-specific cytokine gene cluster*. J Immunol, 2000. **165**(12): p. 6687-91.
90. Yamashita, M., et al., *Identification of a conserved GATA3 response element upstream proximal from the interleukin-13 gene locus*. J Biol Chem, 2002. **277**(44): p. 42399-408.

91. Ferber, I.A., et al., *GATA-3 significantly downregulates IFN-gamma production from developing Th1 cells in addition to inducing IL-4 and IL-5 levels*. Clin Immunol, 1999. **91**(2): p. 134-44.
92. Ouyang, W., et al., *Inhibition of Th1 development mediated by GATA-3 through an IL-4-independent mechanism*. Immunity, 1998. **9**(5): p. 745-55.
93. Usui, T., et al., *GATA-3 suppresses Th1 development by downregulation of Stat4 and not through effects on IL-12Rbeta2 chain or T-bet*. Immunity, 2003. **18**(3): p. 415-28.
94. Nakamura, Y., et al., *Gene expression of the GATA-3 transcription factor is increased in atopic asthma*. J Allergy Clin Immunol, 1999. **103**(2 Pt 1): p. 215-22.
95. Nakamura, Y., et al., *Upregulation of the transcription factor GATA-3 in upper airway mucosa after in vivo and in vitro allergen challenge*. J Allergy Clin Immunol, 2000. **105**(6 Pt 1): p. 1146-52.
96. Pandolfi, P.P., et al., *Targeted disruption of the GATA3 gene causes severe abnormalities in the nervous system and in fetal liver haematopoiesis*. Nat Genet, 1995. **11**(1): p. 40-4.
97. Smith, V.M., et al., *GATA-3 dominant negative mutant. Functional redundancy of the T cell receptor alpha and beta enhancers*. J Biol Chem, 1995. **270**(4): p. 1515-20.
98. Zhang, D.H., et al., *Inhibition of allergic inflammation in a murine model of asthma by expression of a dominant-negative mutant of GATA-3*. Immunity, 1999. **11**(4): p. 473-82.
99. Finotto, S., et al., *Treatment of allergic airway inflammation and hyperresponsiveness by antisense-induced local blockade of GATA-3 expression*. J Exp Med, 2001. **193**(11): p. 1247-60.
100. Murphy, K.M. and S.L. Reiner, *The lineage decisions of helper T cells*. Nat Rev Immunol, 2002. **2**(12): p. 933-44.
101. Kurata, H., et al., *Ectopic expression of activated Stat6 induces the expression of Th2-specific cytokines and transcription factors in developing Th1 cells*. Immunity, 1999. **11**(6): p. 677-88.
102. Ouyang, W., et al., *Stat6-independent GATA-3 autoactivation directs IL-4-independent Th2 development and commitment*. Immunity, 2000. **12**(1): p. 27-37.
103. Ranganath, S. and K.M. Murphy, *Structure and specificity of GATA proteins in Th2 development*. Mol Cell Biol, 2001. **21**(8): p. 2716-25.

104. Kimura, M., et al., *Regulation of Th2 cell differentiation by mel-18, a mammalian polycomb group gene*. Immunity, 2001. **15**(2): p. 275-87.
105. Miaw, S.C., et al., *ROG, repressor of GATA, regulates the expression of cytokine genes*. Immunity, 2000. **12**(3): p. 323-33.
106. Zhou, M., et al., *Friend of GATA-1 represses GATA-3-dependent activity in CD4+ T cells*. J Exp Med, 2001. **194**(10): p. 1461-71.
107. Kurata, H., et al., *Friend of GATA is expressed in naive Th cells and functions as a repressor of GATA-3-mediated Th2 cell development*. J Immunol, 2002. **168**(9): p. 4538-45.
108. Hwang, E.S., A. Choi, and I.C. Ho, *Transcriptional regulation of GATA-3 by an intronic regulatory region and fetal liver zinc finger protein 1*. J Immunol, 2002. **169**(1): p. 248-53.
109. Komine, O., et al., *The Runx1 transcription factor inhibits the differentiation of naive CD4+ T cells into the Th2 lineage by repressing GATA3 expression*. J Exp Med, 2003. **198**(1): p. 51-61.
110. Gorelik, L., P.E. Fields, and R.A. Flavell, *Cutting edge: TGF-beta inhibits Th type 2 development through inhibition of GATA-3 expression*. J Immunol, 2000. **165**(9): p. 4773-7.
111. Chen, C.H., et al., *Cyclic AMP activates p38 mitogen-activated protein kinase in Th2 cells: phosphorylation of GATA-3 and stimulation of Th2 cytokine gene expression*. J Immunol, 2000. **165**(10): p. 5597-605.
112. Yamagata, T., et al., *Acetylation of GATA-3 affects T-cell survival and homing to secondary lymphoid organs*. Embo J, 2000. **19**(17): p. 4676-87.
113. Lander, E.S., *Mapping complex genetic traits in humans*. Genome Analysis: A Practical Approach, 1988. **Chapter 8**: p. 171-189.
114. Lander, E.S. and N.J. Schork, *Genetic dissection of complex traits*. Science, 1994. **265**(5181): p. 2037-48.
115. Larsen, G., Colasurdo, GN, *Animal models of asthma*. The Lung: Scientific Foundations (2nd edition), 1997. **Chapter 95**: p. 1315-1331.
116. Ewart, S.L., et al., *Airway hyperresponsiveness to acetylcholine: segregation analysis and evidence for linkage to murine chromosome 6*. Am J Respir Cell Mol Biol, 1996. **14**(5): p. 487-95.

117. Ewart, S.L., et al., *Quantitative trait loci controlling allergen-induced airway hyperresponsiveness in inbred mice*. Am J Respir Cell Mol Biol, 2000. **23**(4): p. 537-45.
118. Ewart, S., R. Levitt, and W. Mitzner, *Respiratory system mechanics in mice measured by end-inflation occlusion*. J Appl Physiol, 1995. **79**(2): p. 560-6.
119. Karp, C.L., et al., *Identification of complement factor 5 as a susceptibility locus for experimental allergic asthma*. Nat Immunol, 2000. **1**(3): p. 221-6.
120. Dodgson, J.B., H.H. Cheng, and R. Okimoto, *DNA marker technology: a revolution in animal genetics*. Poult Sci, 1997. **76**(8): p. 1108-14.
121. Risch, N.J., *Searching for genetic determinants in the new millennium*. Nature, 2000. **405**(6788): p. 847-56.
122. Wetsel, R.A., D.T. Fleischer, and D.L. Haviland, *Deficiency of the murine fifth complement component (C5). A 2-base pair gene deletion in a 5'-exon*. J Biol Chem, 1990. **265**(5): p. 2435-40.
123. Lincoln SE, D.M., Lander ES, *Constructing genetic maps with MAPMAKER/EXP 3.0*. Whitehead Institute Technical Report (3rd edition), 1992.
124. Lincoln SE, D.M., Lander ES, *Mapping genes controlling quantitative traits with MAPMAKER/QTL 1.1*. Whitehead Institute Technical Report (2nd edition), 1992.
125. Van Ooijen JW, V.R., *JoinMap version 3.0, software for the calculation of genetic linkage maps*. Plant Research International, Wageningen, the Netherlands, 2001.
126. Lander, E. and L. Kruglyak, *Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results*. Nat Genet, 1995. **11**(3): p. 241-7.
127. Wade, C.M., et al., *The mosaic structure of variation in the laboratory mouse genome*. Nature, 2002. **420**(6915): p. 574-8.
128. Abdi, K., *IL-12: the role of p40 versus p75*. Scand J Immunol, 2002. **56**(1): p. 1-11.
129. Biosystems, A., *TaqMan Universal PCR Master Mix protocol*. 2001.
130. Biosystems, A., *User Bulletin #2, ABI Prism 7700 Sequence Detection Systems*. 2001.
131. Dabbagh, K., et al., *IL-4 induces mucin gene expression and goblet cell metaplasia in vitro and in vivo*. J Immunol, 1999. **162**(10): p. 6233-7.

132. Ooijen, J.W.v., M.P. Boer, R.C. Jansen & C. Maliepaard, *MapQTL 4.0: Software for the calculation of QTL positions on genetic maps (user manual)*. Plant Research International, Wageningen, the Netherlands, 2000.

MICHIGAN STATE UNIVERSITY LIBRARIES



3 1293 02551 6901