

ORIGINAL ARTICLE

Serial analysis of gene expression in a rat lung model of asthma

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Background and objective: The pathogenesis and molecular mechanism underlying asthma remain undetermined. The purpose of this study was to identify genes and pathways involved in the early airway response (EAR) phase of asthma by using serial analysis of gene expression (SAGE).

Methods: Two SAGE tag libraries of lung tissues derived from a rat model of asthma and controls were generated. Bioinformatic analyses were carried out using the Database for Annotation, Visualization and Integrated Discovery Functional Annotation Tool, Gene Ontology (GO) Tree Machine and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis.

Results: A total of 26 552 SAGE tags of asthmatic rat lung were obtained, of which 12 221 were unique tags. Of the unique tags, 55.5% were matched with known genes. By comparison of the two libraries, 186 differentially expressed tags ($P < 0.05$) were identified, of which 103 were upregulated and 83 were downregulated. Using the bioinformatic tools these genes were classified into 23 functional groups, 15 KEGG pathways and 37 enriched GO categories.

Conclusions: The bioinformatic analyses of gene distribution, enriched categories and the involvement of specific pathways in the SAGE libraries have provided information on regulatory networks of the EAR phase of asthma. Analyses of the regulated genes of interest may inform new hypotheses, increase our understanding of the disease and provide a foundation for future research.

Key words: asthma, gene expression profiling, rat, serial analysis of gene expression, Sprague-Dawley, systems biology.

INTRODUCTION

Asthma is a complex syndrome that can involve potentially permanent airway obstruction, airway hyper-responsiveness and multicellular inflammation,^{1,2} which is characterized by recurrent episodes of wheezing, breathlessness, chest tightness and coughing.³ Despite advances in understanding of the pathogen-

esis and in the treatment of asthma,^{4,5} the prevalence, morbidity and mortality of the disease continue to increase.⁶ Consistent with the complex nature of asthma, the patterns of asthma pathogenesis are also complex⁷ and are still only partly understood.⁸ With the deciphering of the human, mouse and rat genomes,⁹⁻¹¹ new approaches to the analysis of complex diseases such as asthma have been developed. The analysis and utilization of this information have the potential to rapidly improve our understanding of the molecular mechanisms underlying asthma.¹²

Gene expression profiling is frequently used as a high throughput tool allowing the simultaneous analysis of large numbers of transcripts. These techniques hold promise for the identification of novel genes and pathways involved in pathogenesis, and are increasingly being used to study complex diseases such as asthma.¹³ Expression profiling techniques

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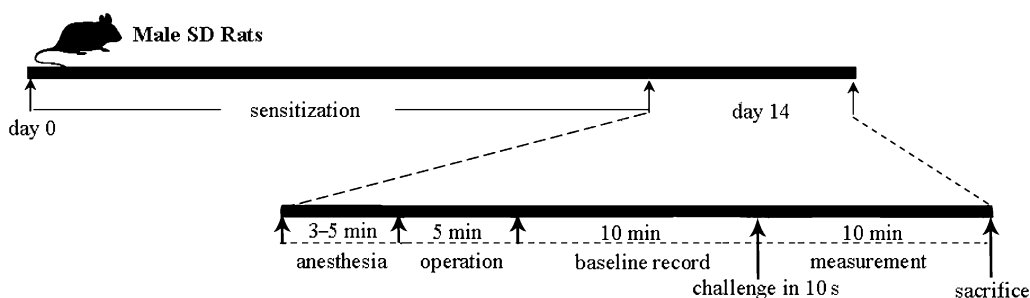


Figure 1 Protocol and measurements for the rat model of allergic asthma. Male Sprague–Dawley rats were sensitized intraperitoneally at day 0 with 1 mg of ovalbumin (OVA) precipitated with 10 mg of aluminium hydroxide gel in 1 mL normal saline. On day 14, the sensitized animals were challenged with 1 mL/kg of 5% OVA in normal saline (5 mg/kg) by injection into the external jugular vein over 10 s. The respiratory parameters of pulmonary resistance, dynamic compliance and respiratory rate were immediately recorded for 10 min. Rats were killed immediately after the measurements. Control rats were sensitized and challenged with normal saline instead of OVA.

include cDNA and oligonucleotide microarray,^{14,15} differential display¹⁶ and subtractive hybridization.¹⁷ Several microarray expression profiling studies using have been performed using asthmatic cells and tissues.^{12,18} However, with few exceptions, these studies have not led to significant advances in the understanding of asthma.^{8,12} Large amounts of high throughput data have not been adequately analysed to extract biologically meaningful data for hypothesis generation and further experimentation.⁸

Serial analysis of gene expression (SAGE), a high-throughput technique similar to microarray, was developed in 1995.¹⁹ It has been applied to the qualitative and quantitative evaluation of gene transcription using short sequences (tags) that correspond to a distinct transcript,^{20,21} and does not require the generation of a hybridization probe for each transcript.²² SAGE has been extensively applied in biological and medical studies, and several SAGE reference databases have been established, including SAGE Genie²³ and SAGEmap,²⁴ which are widely used to determine the gene origin of SAGE tags. However, there has been no report on the use of SAGE in relation to asthma.

In this study, two SAGE tag libraries were generated from lung tissues derived from a rat asthma model. The objectives of this study were to systematically analyse the transcript profile in the model and to gain insight into the molecular mechanisms underlying the pathogenesis of asthma.

METHODS

Animals and sensitization protocol

Pathogen-free, male Sprague–Dawley (SD) rats (4 weeks old, 110–130 g, SLAC Laboratory Animal Co. Ltd., Shanghai, China) were raised in a pathogen-free rodent facility and were provided food and water *ad libitum*. Rats were kept in animal facilities approved by the Shanghai Committee for Accreditation of Laboratory Animal, for at least 1 week before the experiment. The animal experiments conformed to the regulations of the State Science and Technology Commission.

On the day before the experiment (day 0, Fig. 1), SD rats ($n = 8$) were sensitized with an intraperitoneal injection of 1 mg ovalbumin (OVA) (grade V, Sigma, Taufkirchen, Germany), precipitated with 10 mg of aluminium hydroxide gel in 1 mL normal saline. On day 14, the sensitized animals were challenged with 1 mL/kg of 5% OVA in normal saline by injection into the external jugular vein over 10 s. The final challenge dose of OVA was 5 mg/kg. Control rats ($n = 8$) were sensitized and challenged with normal saline instead of OVA.

Measurement of pulmonary function

Airway responsiveness was assessed as an increase in pulmonary resistance (RL), decreases in dynamic compliance (C_{dyn}) and changes in respiratory rate (RR) in response to OVA challenge in anaesthetized, spontaneously breathing rats. The methods of measurement were modified from those of Glaab *et al.*^{25,26}

Construction of the SAGE libraries

Total RNA was extracted separately from the lungs of asthmatic and control rats using Trizol reagent (Invitrogen, Carlsbad, CA, USA). The mRNA was purified from total RNA using the Oligotex mRNA Midi Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's protocol.

Two SAGE libraries were established using the I-SAGE Kit (Invitrogen) according to SAGE protocol v1.0c (<http://www.sagenet.org>).¹⁸ In brief, purified mRNA was bound to oligo(dT) magnetic beads, and cDNA was synthesized with SuperScript II reverse transcriptase using mRNA bound to these beads as the template. cDNAs were digested with the anchoring enzyme *Nla* III and the resulting 3'-cDNAs were split into two pools and ligated with two different 40-bp adapters (A and B) using T₄ DNA ligase. The ligation products were cleaved with the tagging enzyme *Bsm*FI. The magnetic beads were discarded,

and the released linker-tags were blunt-ended with T₄ DNA polymerase and ligated to form 102-bp ditags. The diluted (1:40) ligation products were amplified by PCR with 28 cycles of 95°C for 30 s, 55°C for 1 min, and 70°C for 1 min and the products were analysed by 12% polyacrylamide gel electrophoresis. The PCR products were pooled and digested with *Nla* III to release the ditags, which were then separated from the linkers by 16% polyacrylamide gel electrophoresis. The ditags were then eluted and ligated together to form concatemers, which were subsequently size-fractionated by 8% polyacrylamide gel electrophoresis. The concatemers (500 and 1200 bp long) were isolated and cloned into pZero vector. Clones were analysed by direct PCR using the M13 forward and M13 reverse primers. PCR products >500 bp in size were isolated by 1% agarose gel electrophoresis and sequenced on an ABI 3730 DNA sequencer (Perkin-Elmer Corp., Norwalk, CT, USA).

Annotation of SAGE tags

The sequence data were analysed using SAGE 2000 software, version 4.5 (courtesy of Ken Kinzler and Victor Velculescu, Johns Hopkins University School of Medicine, Baltimore, MD, USA) to quantify tags and identify the corresponding transcripts. The identities of the mRNAs that corresponded to the SAGE tags were determined by interrogation of the SAGEmap databases (www.ncbi.nlm.nih.gov/SAGE/). Double or multi-matched tags were verified by the presence of the last CATG cleavage site in the reference sequence from GenBank.²⁷

To compare these SAGE libraries, each tag number was normalized using SAGE software. Statistical significance was assessed using Monte Carlo analysis, which allowed a large number of comparisons of SAGE data.

Quantitative real-time PCR

Quantitative real-time PCR (qRT-PCR) was performed on an Applied Biosystems 7300 Real-Time PCR System using TOYOBO Realtime PCR Master Mix (Toyobo, Osaka, Japan). The threshold cycle number was determined using SDS v1.4 software and the reactions were performed in triplicate. Total RNA (5 µg) was reverse transcribed to cDNA using the RevertAid First Strand cDNA synthesis kit (Fermentas Inc., Glen Burnie, MD, USA). For qRT-PCR of the cDNA, intron-spanning primer pairs were designed to generate products of 101–150 bp (primer sequences are available in Table S1). The generation of specific PCR products was confirmed by melting curve and gel analysis. The expression ratio was calculated according to the formula $2^{(Rt - Et)} / 2^{(Rn - En)}$ as described previously.²⁸ Transcripts with a twofold increase in expression were considered to be upregulated and those with a 0.5-fold decrease in expression were considered to be downregulated.

Bioinformatic analysis of SAGE tags

Genes differentially expressed ($P < 0.05$) between the two SAGE libraries were functionally annotated and classified using the Database for Annotation, Visualization and Integrated Discovery (DAVID) Functional Annotation Tool (<http://david.abcc.ncifcrf.gov/>),²⁹ a web-based tool that provides integrated solutions for the annotation and analysis of genome-scale datasets derived from high-throughput technologies such as microarray and SAGE.³⁰

To understand the key regulatory processes in asthma, the Gene Ontology (GO) Tree Machine (<http://www.genereg.ornl.gov/gotm/>)³¹ was applied for bioinformatic analysis and visualization of the upregulated and downregulated expression data. GOTree Machine generates a GOTree, a tree-like structure to navigate the GO directed acyclic graph (DAG) for input gene sets, and provides data navigation and visualization. Enrichment statistical analysis was performed to identify the most important GO categories of input gene sets and to suggest their potential biological importance.

The DAVID Functional Annotation Tool was also used for the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of the differentially expressed genes between the two SAGE libraries. The threshold of EASE (the Expression Analysis Systematic Explorer) score is a modified fisher exact P -value for gene-enrichment analysis, which ranges from 0 to 1. The meaningful pathways were determined by the default setting (EASE score ≤ 0.1).

Statistical analysis

Statistical analysis of RL, Cdyn and RR between asthmatic and control rats was performed using Student's t -test. Statistical analysis of the significance of the two SAGE libraries was performed using Monte Carlo analysis. The enrichments of GOTree Machine were statistically significant as determined by the hypergeometric test.³¹

RESULTS

Pulmonary function measurements after OVA challenge

The allergen-specific early airway response (EAR) to OVA in sensitized rats showed significant increases in RL and significant decreases in simultaneously measured Cdyn and RR compared with control rats, indicating an allergen-specific EAR to OVA. The most prominent alteration was for RL with a maximum increase of 0.236 ± 0.119 kPa/mL/s ($P < 0.001$ vs control) at 3 min after challenge, while Cdyn showed a maximum decrease of -2.410 ± 9.274 mL/kPa ($P < 0.001$ vs control) at 5 min after challenge, and RR showed a maximum decrease of 26.634 ± 25.294 ($P < 0.05$ vs control) at 2 min after OVA challenge.

Table 1 Tag frequencies and percentage distribution of unique tags from the two SAGE libraries

Tag copies	1	2–4	5–9	10–99	>100
Asthma	9209 (75.4%)	2273 (18.6%)	446 (3.6%)	279 (2.3%)	15 (0.1%)
Control	9525 (74.0%)	2493 (19.4%)	547 (4.3%)	279 (2.2%)	13 (0.1%)

SAGE, serial analysis of gene expression.

Table 2 Summary of serial analysis of gene expression data for the two libraries

	Total tags	Unique tags	Genes matched	Expressed sequence tags matched	Unmatched
Asthma	26 552	12 221	55.5%	36.2%	8.3%
Control	28 284	12 857	54.1%	38.5%	7.4%

General analysis of SAGE libraries

The two lung SAGE libraries were deposited in the SAGEmap database at the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/geo>). Accession numbers GSM45195 and GSM119459. A total of 26 552 tags from the asthmatic rat lung library, consisting of 12 221 unique tags, were obtained and analysed. Of these unique tags, 75.4% were single copies, 18.6% comprised 2–4 copies, 3.6% had 5–9 copies, 2.3% had 10–99 copies, and only 0.1% of the tags had more than 100 copies (Table 1). Comparison of these 12 221 SAGE tags in asthmatic rat lung with the SAGE database showed that 55.5% of the tags were matched with known genes, 36.2% with expressed sequence tags (EST), and 8.3% had no match (Table 2).

Comparison with the SAGE data from the control rat lungs, showed that 186 unique tags were differentially expressed ($P < 0.05$), including 147 genes, 14 EST and 25 unmatched sequences (the gene list is available in Table S2). Among the differentially expressed tags, 103 tags were upregulated and 83 tags were downregulated. Twenty tags were expressed only in asthmatic rat lungs and 30 tags were expressed only in control rat lungs.

Confirmation of SAGE results by qRT-PCR

To partly confirm the expression profiles in the two SAGE libraries, nine differentially expressed genes of interest were chosen and their expression levels were evaluated by qRT-PCR. The four upregulated genes were dual specificity phosphatase 1 (Dusp1, Rn.98260), S100 calcium-binding protein A9 (S100A9) (Rn.6703), similar to metallothionein-2 (MT-2, Rn.115549) and c-fos (Rn.103750). The five downregulated genes were procollagen, type VI, α -2 (Col6a2, Rn.128903), heat shock protein 8 (Hspa8, Rn.120392), elastin (Rn.54384), RAB7 (Rn.1425) and Mapk9 (Rn.9910). The expression of each transcript was compared with the SAGE data as shown in Figure 2. The results indicated that the expression profiles of

representative genes by qRT-PCR analysis corresponded to the SAGE profiles, and this was judged to validate use of the quantitative data for further bioinformatic analysis.

Functional annotation of the differentially expressed genes

For the functional annotation of the differentially expressed genes, 147 known genes and 14 EST with a UniGene ID were assigned by applying the DAVID Functional Annotation Tool. Among the 161 UniGenes, 106 were functionally classified into 23 groups using default settings (medium classification stringency) and all GO terms for biological processes. Among the 23 groups, eight with enrichment scores equal to or higher than 1 are shown in Figure 3.

In order to assess the functional differences between the upregulated and downregulated genes, GOTree Machine was applied to the analysis. By means of DAG for visualizing enriched gene categories, 91 upregulated and 70 downregulated known genes with a UniGene ID were uploaded. The upregulated genes were enriched in 25 categories related to terms such as response to stimulus (18 genes), physiological defence response (7 genes), antigen processing and presentation (3 genes), chemotaxis (4 genes), DNA modification (2 genes), biosynthesis (16 genes) (See Fig. S1a). The downregulated genes were enriched in 12 categories, including muscle development (4 genes), synapse organization and biogenesis (2 genes), acetylcholine receptor signalling, muscarinic pathway (2 genes), musculoskeletal movement (2 genes) (See Fig. S1b). The differences in the biological processes between the upregulated and downregulated genes may be related to the hyperimmunity of asthma and feedback regulation at the onset of asthma.

KEGG pathway analysis of the differentially expressed genes

In order to understand the higher-order functional significance of the differentially expressed genes,

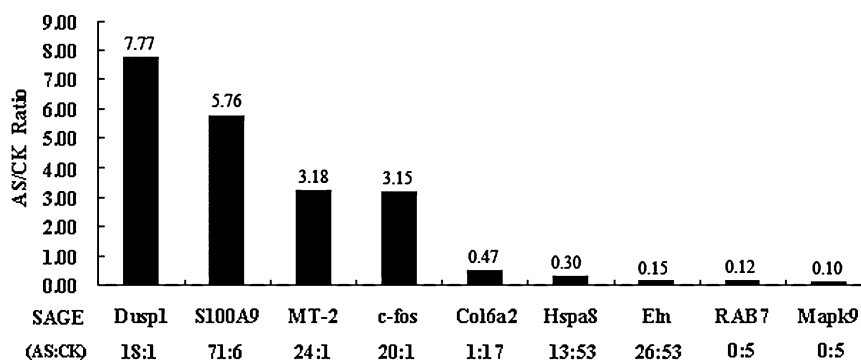


Figure 2 Real-time PCR (qRT-PCR) confirmation of differentially regulated genes of interest as predicted by serial analysis of gene expression (SAGE). qRT-PCR was performed on selected genes as described in *Methods*. The corresponding SAGE tag copies are indicated below the qRT-PCR data for each gene. GAPDH was used as the reference gene. Dusp1, dual specificity phosphatase 1; S100A9, S100 calcium-binding protein A9 (calgranulin B); MT-2, metallothionein-2; c-fos, c-fos FBJ murine osteosarcoma viral oncogene homologue; Col6a2: procollagen, type VI, α -2; Hspa8, heat shock protein 8; Eln, elastin; RAB7, RAB7, member RAS oncogene family; Mapk9, Mapk9 stress-activated protein kinase α -II; AS, SAGE tag copies in asthmatic rats; CK, SAGE tag copies in control rats.

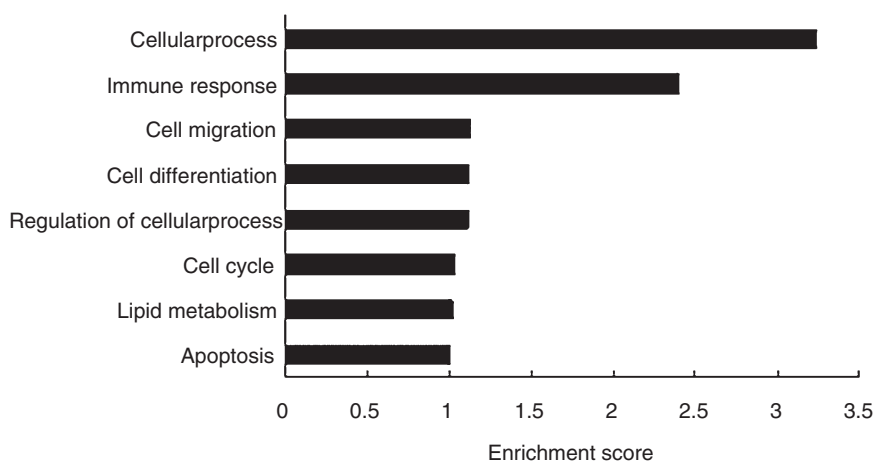


Figure 3 Eight functional groups of differentially expressed genes ($P < 0.05$) with an enrichment score ≥ 1 . Expression Analysis Systematic Explorer (EASE) score is an alternative name for Fisher's exact statistical test in the Database for Annotation, Visualization and Integrated Discovery system, referring to one-tailed Fisher's exact probability values used for gene-enrichment analysis. The biological significance of gene groups is ranked on the basis of overall EASE scores for all enriched annotation terms. The complete gene list for each group is available in Table S3.

KEGG pathway analysis was performed by applying the DAVID Annotation Tool. Among the 161 differentially expressed genes with a UniGene ID, 50 genes were involved in 45 pathways in the rat. Fifteen pathways (without ribosome), including at least three differentially expressed genes are listed in Table 3. The meaningful pathways, as measured by an EASE score ≤ 0.1 , were focal adhesion, the mitogen-activated protein kinase (MAPK) signalling pathway and the B-cell receptor signalling pathway. By means of DAVID Functional Annotation Clustering analysis with custom classification stringency, three main functional groups were identified. Group 1 included focal adhesion and the wntless (WNT) signalling pathway. Group 2 included MAPK signalling, B-cell receptor signalling, Toll-like receptor signalling and

T-cell receptor signalling pathways. Group 3 included extracellular matrix (ECM)-receptor interaction and cell communication.

DISCUSSION

Recent studies have used a global analysis approach, including whole genome scans in the field of complex diseases, such as asthma.³² A number of microarray experiments have described expression profiles derived from human asthmatic lung tissue, mouse airway tissue or from key cell types associated with asthma, and some novel genes that are potentially involved in the pathogenesis of asthma were identified, including ADAM8, small proline rich protein 2,

Table 3 Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis of 15 pathways with differentially expressed tags ($P < 0.05$)

KEGG pathway	UniGene ID	Gene description	SAGE tag copies (asthma : control)
Focal adhesion	Rn.774	Laminin, β -2	3:12
	Rn.53801	Procollagen, type IV, α -1	1:8
	Rn.22279	Cyclin d1	0:5
	Rn.71224	Harvey rat sarcoma oncogene, subgroup r (predicted)	7:1
	Rn.9910	MAPK 9	0:5
	Rn.2863	Ras-related c3 botulinum substrate 2	5:0
	Rn.93714	Jun oncogene	8:2
	Rn.22518	Caveolin	47:30
	Rn.72459	Vinculin (predicted)	3:14
MAPK signalling pathway	Rn.128903	Procollagen, type VI, α -2	1:17
	Rn.98260	Dual specificity phosphatase 1	18:1
	Rn.103750	Fbj murine osteosarcoma viral oncogene homologue	21:1
	Rn.71224	Harvey rat sarcoma oncogene, subgroup r (predicted)	7:1
	Rn.120392	Heat shock protein 8	31:53
	Rn.9954	Transforming growth factor, β -receptor II	2:11
	Rn.9910	MAPK 9	0:5
	Rn.93714	Jun oncogene	8:2
	Rn.10000	Nuclear receptor subfamily 4, group a, member 1	34:6
B-cell receptor signalling pathway	Rn.2863	Ras-related c3 botulinum substrate 2	5:0
	Rn.103750	Fbj murine osteosarcoma viral oncogene homologue	21:1
	Rn.71224	Harvey rat sarcoma oncogene, subgroup r (predicted)	7:1
Toll-like receptor signalling pathway	Rn.93714	Jun oncogene	8:2
	Rn.2863	Ras-related c3 botulinum substrate 2	5:0
	Rn.103750	Fbj murine osteosarcoma viral oncogene homologue	21:1
	Rn.9910	MAPK 9	0:5
ECM receptor interaction	Rn.93714	Jun oncogene	8:2
	Rn.2863	Ras-related c3 botulinum substrate 2	5:0
	Rn.774	Laminin, β -2	3:12
	Rn.53801	Procollagen, type IV, α -1	1:8
WNT signalling pathway	Rn.2163	Agrin	1:7
	Rn.128903	Procollagen, type VI, α -2	1:17
	Rn.22279	Cyclin d1	0:5
	Rn.9910	MAPK 9	0:5
Cell communication	Rn.93714	Jun oncogene	8:2
	Rn.2863	Ras-related c3 botulinum substrate 2	5:0
	Rn.44161	Lamin a	7:0
	Rn.774	Laminin, β -2	3:12
Leucocyte transendothelial migration	Rn.53801	Procollagen, type IV, α -1	1:8
	Rn.128903	Procollagen, type VI, α -2	1:17
	Rn.105888	Similar to claudin-18a1.2	10:26
	Rn.2863	Ras-related c3 botulinum substrate 2	5:0
Tight junction	Rn.3036	Guanine nucleotide binding protein, α inhibiting 2	15:30
	Rn.72459	Vinculin (predicted)	3:14
	Rn.105888	Similar to claudin-18a1.2	10:26
	Rn.5812	α -spectrin 2	7:17
Regulation of actin cytoskeleton	Rn.71224	Harvey rat sarcoma oncogene, subgroup r (predicted)	7:1
	Rn.3036	Guanine nucleotide binding protein, α inhibiting 2	15:30
	Rn.2605	Thymosin, β -4	217:145
	Rn.71224	Harvey rat sarcoma oncogene, subgroup r (predicted)	7:1
Adherens junction	Rn.2863	Ras-related c3 botulinum substrate 2	5:0
	Rn.72459	Vinculin (predicted)	3:14
	Rn.9954	Transforming growth factor, β -receptor II	2:11
	Rn.2863	Ras-related c3 botulinum substrate 2	5:0
IgE receptor signalling pathway	Rn.72459	Vinculin (predicted)	3:14
	Rn.71224	Harvey rat sarcoma oncogene, subgroup r (predicted)	7:1
	Rn.9910	MAPK 9	0:5
Antigen processing and presentation	Rn.2863	Ras-related c3 botulinum substrate 2	5:0
	Rn.5892	MHC, class II, dm β	5:0
	Rn.120392	Heat shock protein 8	31:53
T-cell receptor signalling pathway	Rn.1868	β -2 microglobulin	46:26
	Rn.103750	Fbj murine osteosarcoma viral oncogene homologue	21:1
	Rn.71224	Harvey rat sarcoma oncogene, subgroup r (predicted)	7:1
Axon guidance	Rn.93714	Jun oncogene	8:2
	Rn.71224	Harvey rat sarcoma oncogene, subgroup r (predicted)	7:1
	Rn.2863	Ras-related c3 botulinum substrate 2	5:0
	Rn.3036	Guanine nucleotide binding protein, α inhibiting 2	15:30

ECM, extracellular matrix; MAPK, mitogen-activated protein kinase; WNT, wingless.

trefoil factor 2, interferon- γ and fatty acid binding proteins. However, relatively few studies have exploited these findings to discover new pathways that may be involved in the pathogenesis of asthma.⁸ Similar to microarray, SAGE can provide quantitative information on gene expression, but unlike microarray, which is a closed high-throughput method that enables the measurement of a large and predetermined set of known genes or sequences, SAGE is an open high-throughput expression profiling technique that allows unbiased assessment of virtually all polyadenylated transcripts in a single sample.³³ Therefore, comparative analysis of genome-wide expression profiles generated by means of SAGE is another powerful approach to gaining insight into asthma. In order to get a systematic understanding of the mechanism of asthma, two SAGE tag libraries were developed from lung tissue obtained in a rat asthma model and extensive bioinformatic analysis was performed.

Comparison of gene expression patterns from SAGE and microarray analysis

The findings from the present study were similar to previous reports of microarray studies in asthma. Complement factor 5 (C5) was identified by microarray as a susceptibility locus for allergen-induced airway hyperresponsiveness in a murine model of asthma,³⁴ and plays an important protective role in asthma by suppressing inflammatory responses and Th2 effector functions.³⁵ Another complement component, C7, played a central role in the membrane attack complex cascade and bound to the C345C module of C5 with the factor I modules.³⁶ In the present study, complement component 7 (Rn.33787) was significantly upregulated, indicating that C7 may interact with C5 or other components to regulate the pathogenesis of asthma.

Zimmermann *et al.*³⁷ showed that asthmatic responses in mice involved sequential induction of 4.7% of the tested genome and 36 genes induced at the early time point (3 h), including S100A9, S100A8, CD14, CD83 and C-type lectin, were associated with pathogen recognition and initiation of the immune response. Similarly, the present data showed that expression of S100A9, Fc receptor, IgG, low affinity III (Fc γ 3, Rn.6050), CD24 antigen (CD24, Rn.6007), Ig heavy chain (α -polypeptide, Rn.102149) and rat Ig-active kappa-chain mRNA VJC-region from immunocytoma IR2 (Rn.82721) was upregulated at the EAR phase of asthma in rats. The results from SAGE not only confirm the results of previous microarray experiments (although there were differences in the models and the time course of the experiments), but also provide more information on changes in the expression of gene families.

Genes with marked changes in expression levels

Of the 186 differentially expressed unique tags, the genes for MT-2, c-fos, Dusp1, Egr1 and S100A9 were

the most upregulated with expression being increased >10-fold. The genes for Col6a2, CD24, matrix gla protein (MGP, Rn.2379) were the most downregulated with expression being decreased over 10-fold. These changes in gene expression may affect the immune response or other biological processes relevant to asthma.

MT-2 is ubiquitously expressed in all eukaryotes, including plants, yeast, worms, flies and vertebrates. The gene for MT-2 may be coordinately regulated and highly inducible by a variety of agents, including heavy metals, reactive oxygen species, UV radiation, glucocorticoids and LPS.³⁸ Recent studies demonstrated that MT-2 was not only involved in maintaining homeostasis of essential metals and metal detoxification, but was also implicated in a range of physiological processes, including scavenging of reactive oxidant species.³⁹ In our SAGE library, the expression of MT-2 was highly increased. Considering its ability to counteract oxidative damage,⁴⁰ MT-2 may play a protective role in the EAR phase of asthma, and may be a potential candidate antioxidant drug for asthma and inflammation that warrants further research.

Col6a2, a precursor of collagen, is synthesized in fibroblasts and subsequently converted into collagen (a major component of ECM in the lungs) *in vivo* by a proteolytic mechanism.⁴¹ MGP plays a role in lung growth and development,⁴² is known to be synthesized in type II cells of the lung and is also found in fetal fibroblasts, where its expression was stimulated by dexamethasone.⁴³ Elastin, a major component of the ECM in the airways, is disrupted by the high levels of active elastase produced in the asthmatic lung.⁴⁴ The simultaneous decrease in the expression of Col6a2, MGP and elastin may reduce the cohesiveness of the connective tissue, permeabilize the walls of vessels and facilitate the migration of inflammatory cells in the EAR phase of asthma.

Gene classification using DAVID

From the DAVID classification analysis (Fig. 3), it was observed that the term 'cellular process' was assigned the highest EASE score (3.24) among the differentially expressed genes. In this category, the gene named potassium large conductance calcium-activated channel (Kcnma1, also known as MaxiK, Rn.30616) was expressed at significantly higher levels ($P = 0.025$) in asthmatic rat lungs than in control rat lungs. MaxiK is functionally coupled to voltage-dependent Ca^{2+} channels (VDCC), an association in which MaxiK acts as a negative feedback for VDCC activation and Ca^{2+} entry into the cell.⁴⁵ The MaxiK α subunit may associate with multiple signalling proteins through its C-terminus depending on the stimulus.⁴⁶ The biological characteristics of this gene may suggest a role as a signal transducer in the pathophysiological processes of asthma. Elevated expression of inflammatory factors such as Egr1, S100A9, connective tissue growth factor (Ctgf, Rn.17145) and vascular early response gene protein (Verge, Rn.87679) may lead to contraction of the airways. When the MaxiK channel was activated by Ca^{2+} spikes⁴⁷ or an increase in cAMP,⁴⁸

it formed complexes with the caveolin, G protein-coupled receptors, c-Src and focal adhesion kinase. These mixed signals resulted in cascade-like activation of the MAPK and focal adhesion pathways, which play a crucial role in processing and integrating extracellular signals leading to airway inflammation and remodelling.⁴⁹

The term 'immune response' (EASE score 2.4) was also significant in the DAVID classification analysis. This term encompassed 16 genes, including early growth response 1 (Egr1, Rn.9096), S100A9, Fcgr3 and CD24. Egr1 activates the transcription of target genes, the products of which are required for mitogenesis and differentiation, by recognizing and binding to the DNA sequence 5'-CGCCCCCGC-3'.⁵⁰ Egr1 is sensitive to alterations in growth factors and cytokines and to mechanical stimulation.⁵¹ Genomic linkage analysis has shown that Egr1 is located in the same chromosomal region as genes regulating serum IgE levels and airway hyperresponsiveness.⁵² S100A9 is a small calcium-binding protein that is highly expressed in neutrophils and monocytes.⁵³ It is present at high levels in the extracellular milieu during inflammatory conditions but its extracellular function remains unclear.⁵⁴ Inhibition of the S100A8 and S100A9 proteins prior to intranasal allergen challenge in mice resulted in preservation of all the characteristics of the asthma phenotype, but there was a significant reduction in the number of eosinophils in BAL fluid.⁵⁵

Important genes as revealed by DAG analysis

Directed acyclic graph analysis provided a global assessment of the importance of various biological processes that are potentially relevant to asthma. The DAG upregulated genes indicate that immune response, as represented by 'antigen processing and presentation', 'response to stimulus', etc., is of importance. The DAG downregulated genes suggest the importance of enrichment categories such as 'acetylcholine receptor signalling, muscarinic pathway' and 'musculoskeletal movement'. These DAG analyses therefore suggest the involvement of various biological processes in the EAR phase of asthma.

Among the DAG downregulated genes, an interesting gene, agrin, was included in the categories 'ECM organization and biogenesis', 'acetylcholine receptor signalling, muscarinic pathway' and 'synapse organization and biogenesis'. Agrin is best known as a crucial organizer of postsynaptic differentiation at the neuromuscular junction, but it has recently also been implicated as a co-stimulatory molecule in the immunological synapse and the organization of the cytoskeleton.⁵⁶ However, the expression of agrin was decreased in the SAGE library from asthmatic lung, suggesting that agrin may disperse the aggregation of molecules in the immunological synapse,⁵⁷ including the T-cell receptor and IgE receptor.

Pathways important in the EAR phase of asthma

Pathway-level visualization of genomics data provides a valuable means for systems biology capture of

the properties of cellular activities. MAPK cascades, which include extracellular signal-regulated protein kinase (ERK), p38 MAPK and c-Jun N-terminal kinase (JNK) pathways, contribute to the amplification and specificity of transmitted signals,⁵⁸ and play discrete yet complementary roles in accentuating allergic airway inflammation.⁵⁹ In the SAGE libraries, nine MAPK components showed altered expression: Dusp1 (Rn.98260), Nr4a1 (Rn.10000), Tgfb2 (Rn.9954), Rac (Rn.2863), Hspa8 (Rn.120392), Mapk9 (Rn.9910), c-fos (Rn.103750), Jun (Rn.93714) and Rras_predicted (Rn.71224).

Dusp1 is expressed in many haemopoietic and epithelial cell types, and is upregulated in response to oxidative stress, hypoxia, heat shock, LPS and in various malignancies.⁶⁰ It participates in immune system function, metabolic homeostasis, the regulation of cellular stress responses, and plays a key role in the regulation of physiological outputs of signalling through the ERK, JNK and p38 MAPK pathways.⁶¹ Expression of the Dusp1 gene was significantly elevated in this study, demonstrating that Dusp1 may function as a negative regulator to alleviate and counteract the OVA challenge signals transmitted by the MAPK pathway. The Dusp1 gene is therefore a potential target for the development of novel anti-inflammatory drugs.⁶²

The c-fos oncogene, which is one of the activator protein-1 (AP-1) family members, is also referred to as 'immediate-early gene' and 'early response proto-oncogene' and directly couples intracellular signals initiated by various external mitogenic and toxic stimuli to regulate the expression of genes involved in cell proliferation and differentiation, inflammation, immune responses, etc.⁶³ The MAPK signal transduction pathway uses AP-1 as a convergence point to regulate expression of various genes. In this study, c-fos was significantly upregulated 10 min after OVA challenge, indicating that it may play a central role in regulating gene transcription in immunological processes of the EAR phase of asthma, through the MAPK pathways.

In summary, this study provided a systematic profile of gene expression in lung and asthma in a rat model and a comprehensive *in silico* analysis of SAGE data. The analyses of genes of interest, including MaxiK, S100A9, Egr1, MT-2, Col6a2, MGP, elastin, ICAM-2, agrin, Dusp1 and c-fos, informed regulatory mechanisms in the EAR phase of asthma and provide a foundation for further *in vitro* and *in vivo* studies.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1 The enriched Gene Ontology (GO) categories in biological processes as visualized by directed acyclic graph (DAG) analysis. Categories shown in red were enriched, while those shown in black were not enriched. (a) DAG visualization of 25 enriched GO categories for the upregulated genes. (b) DAG visualization of 12 enriched GO categories for the downregulated genes. The complete gene list of enriched categories is available in Table S4.

Table S1 The intron-spanning primer pairs of genes of quantitative real-time PCR confirmation. The primer pairs of nine differentially expressed genes of interest (Dusp1, S100A9, MT-2, c-fos, Col6a2, Hspa8, Eln, RAB7 and Mapk9) and the reference gene (GAPDH) were listed for Figure 2.

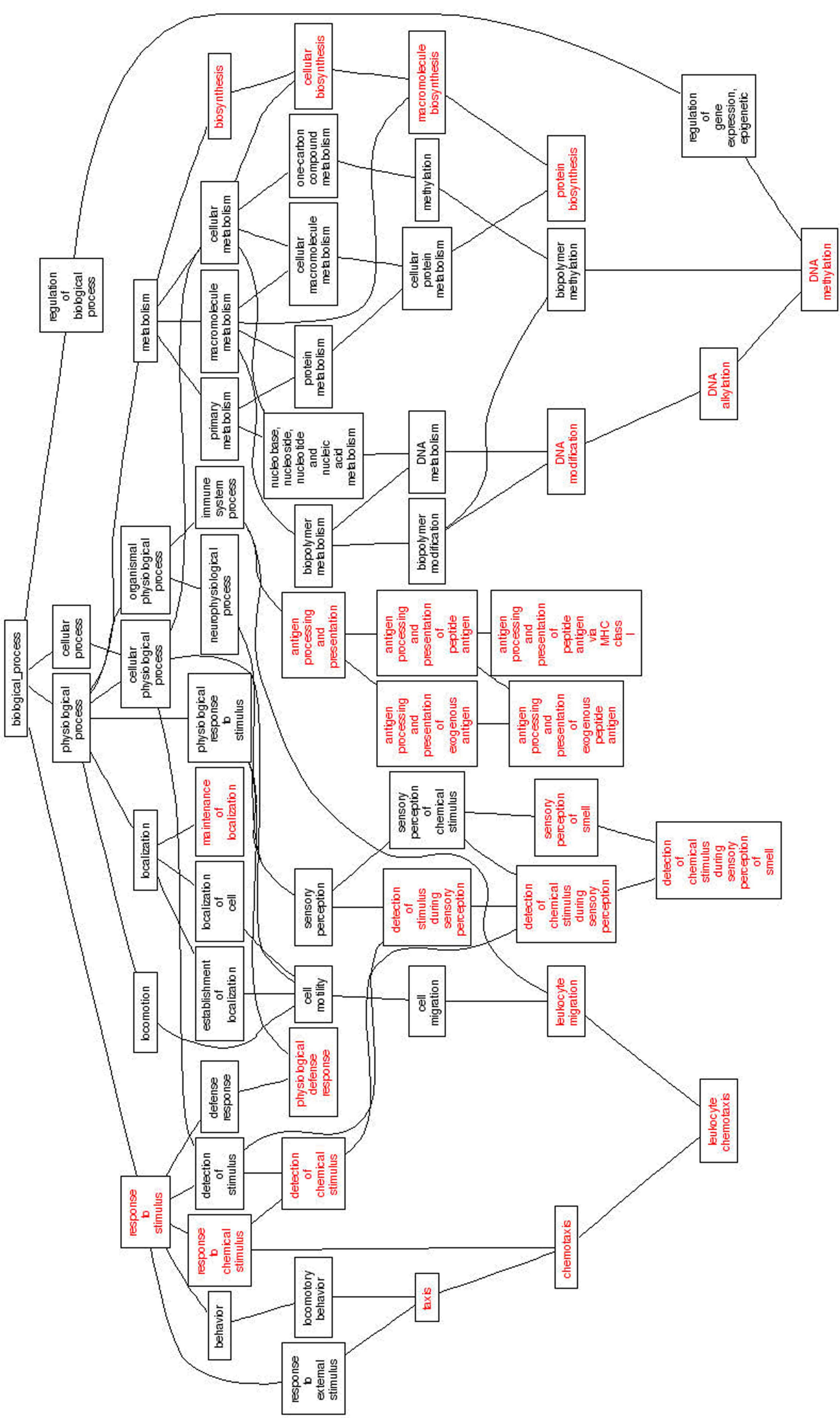
Table S2 The details of the 186 differentially expressed tags ($P < 0.05$) between the libraries of asthmatic and control rat lungs. After the comparison with the SAGE data between the two libraries, 186 differentially expressed

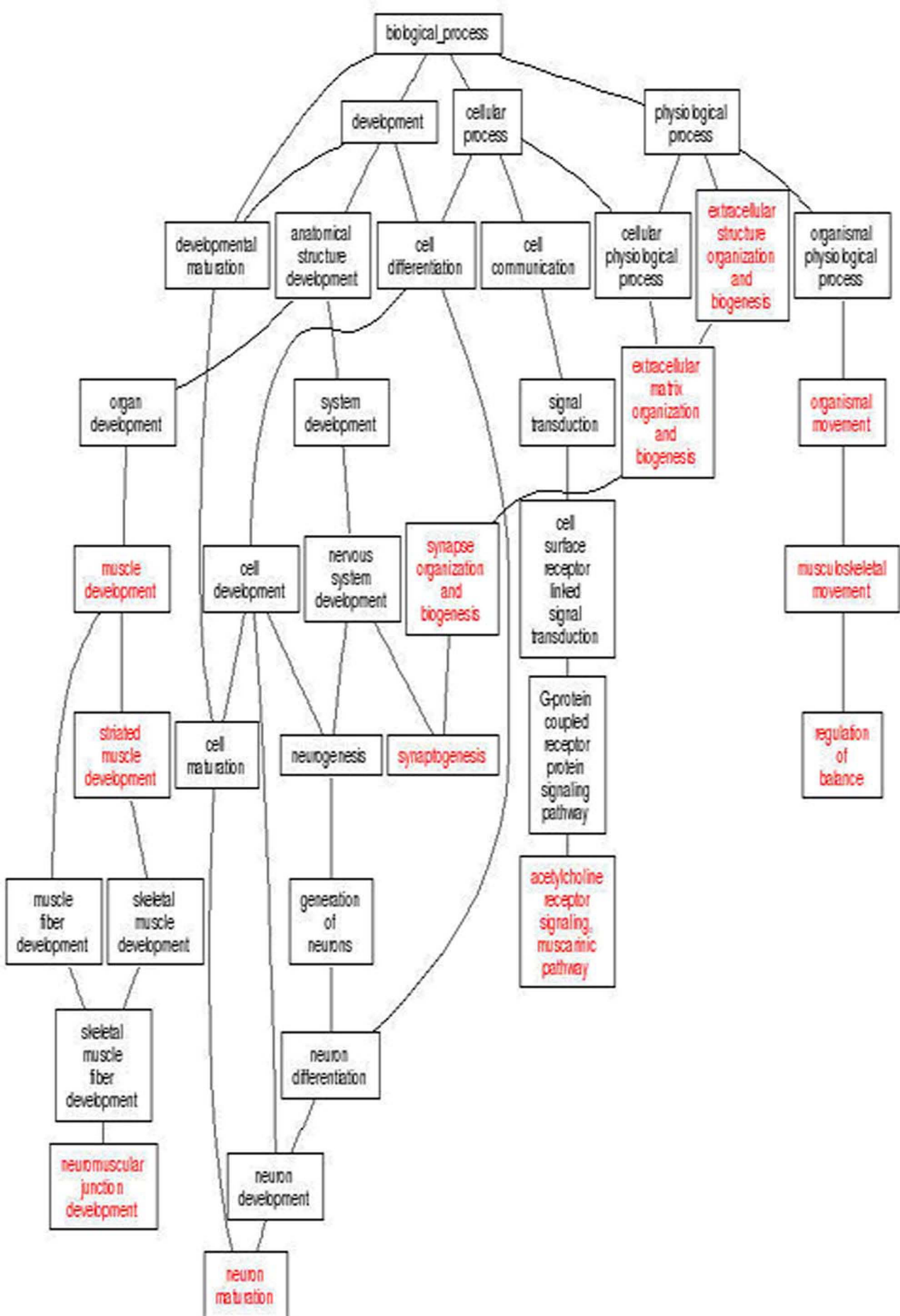
tags ($P < 0.05$) were identified, of which 103 were upregulated and 83 were downregulated. This table includes the tag sequence, tag copies of groups of control and asthma, the fold change, the P -value, the UniGene number and the gene description.

Table S3 The complete gene list of eight functional groups divided via DAVID tools. The complete gene list of eight functional groups with enrichment scores equal to or higher than 1 was shown for Figure 3. It includes the gene groups of cellular process, immune response, cell migration, cell differentiation, regulation of cellular process, cell cycle, lipid metabolism and apoptosis in the separated sheet.

Table S4 The complete gene list of the 37 enriched categories via Gene Ontology (GO) Tree Machine. The complete gene list of the 37 enriched categories was provided for Figure S1a and b. It contains 25 enriched GO categories for the upregulated genes and 12 enriched GO categories for the downregulated genes in the separated sheet.

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Genes	Sequences (5'to3')
Dusp1 forword	TTCAAAGCCCCATCACAACC
Dusp1 reverse	TTGCATTGCTCCTCCCATG
S100A9 forword	ACCCTGAACAAGGCGGAATT
S100A9 reverse	TTTGTGTCCAGGTCCTCCATG
MT-2 forword	TGCAAGAAAAGCTGCTGTTCC
MT-2 reverse	TTACACCATTGTGAGGACGCC
c-fos forword	GAGAAACGGAGAATCCGAAGG
c-fos reverse	GTTGATCTGTCTCCGCTTGGA
Col6a2 forword	ATGGAAGCCAGAACCAGCAAC
Col6a2 reverse	CCACGTGCGAGAAAGAATTGA
Hspa8 forword	TTCCAGCATGGAAAGGTGGA
Hspa8 reverse	CGCATCCCCAATTAATCGTTC
Eln forword	TTCTATGACCACCCCCCCTACT
Eln reverse	GCTGTCTGATTTTCCTTGTCCTG
RAB7 forword	TGGTGTTGGAAAGACATCGCT
RAB7 reverse	TCATCCACCATCACCTCCTTG
Mapk9 forword	CTGGAGCCCAAGGAATTGTTT
Mapk9 reverse	TGCATGCGTTTGGTTCTGAA
GAPDH forword	TCCTGCACCACCAACTGCTTAG
GAPDH reverse	AGTGGCAGTGATGGCATGGACT

Tag sequences	Control	Asthma	Fold*	P Vaule	UniGene	Description
up-regulated (103)						
CACACTAACC	288	433	1.5	0	2283	Lysozyme
TGGGTTGTCT	193	248	1.3	0.001	36610	Tumor protein, translationally-
						controlled 1
TTGGTGAAGG	145	217	1.5	0	2605	Thymosin beta-4
CTAGTCTTTG	134	168	1.3	0.007	34942	Ribosomal protein S29
TCCAATAAAG	94	151	1.6	0	973	Ribosomal protein, large, P1
TCAGGCTGCC	84	120	1.4	0.002	54447	Ferritin, heavy polypeptide 1
GCACGGAAT	79	103	1.3	0.018	34429	Ribosomal protein L17
GGATTTGGCC	52	70	1.3	0.03	976	Ribosomal protein, large P2
GGAAGCCTCC	42	56	1.3	0.049	95653	Similar to osteoclast inhibitory lectin
CTAATATTG	41	116	2.8	0	82721	Rat Ig active kappa-chain mRNA VJC-
						region from immunocytoma IR2
AGGAGGCTAC	38	55	1.4	0.024	108039	Ribosomal protein L14
AACCGCTTTT	35	61	1.7	0.002	2989	Homeobox only domain
CCCAGTTTTTC	35	52	1.5	0.023	107056	Similar to 60S ribosomal protein L26
AAAATGCACA	32	46	1.4	0.04	2267	Beta defensin-2
TCCTATAAAG	30	47	1.6	0.019	22518	Caveolin
GCTACTTCTG	26	46	1.8	0.006	1868	Beta-2 microglobulin
CGGAAGGCGG	25	40	1.6	0.022	1439	Ribosomal protein L36
TCAGAGGGAG	20	53	2.7	0	102149	Immunoglobulin heavy chain (alpha
						polypeptide)
GGTAGCCACT	18	37	2.1	0.004	106034	Ribosomal protein S27a
CCGTGGGTGA	13	26	2.0	0.017	2139	Ribosomal protein S23
TTTGACCTT	13	24	1.8	0.031	17145	Connective tissue growth factor
ATTGTGATTG	12	35	2.9	0.001	36966	Hbb Beta-glo
GATGTGGCTG	12	26	2.2	0.009	3910	Eukaryotic translation elongation factor
						1 beta 2
AGACACTTCC	11	22	2.0	0.027	90546	Anxa2 calpactin I heavy chain
AGGTCGGGTG	10	21	2.1	0.026	92211	Ribosomal protein L13A
TTACTAATTT	10	19	1.9	0.047	107335	Integral membrane protein 2B
TTGGCTGCCC	10	19	1.9	0.047	36102	Ribosomal protein S14
GGAACAGAGG	10	29	2.9	0.001	3867	Putative ISG12(a) protein
CTGTGTGATC	9	42	4.7	0	22047	Similar to hypothetical protein
						MGC6835
TGGACCTAGA	9	33	3.7	0	1997	Cathepsin H
GGCTCAGCCT	8	18	2.3	0.025	84435	Glucose phosphate isomerase
TCTTGCCAGT	7	18	2.6	0.014	43300	Eukaryotic translation elongation factor
						1 gamma
GTTCGTGCCA	7	18	2.6	0.014	2812	Ribosomal protein L35a
TGGGAAATGA	7	18	2.6	0.014	4302	Similar to hypothetical protein
						CLONE24945
CTTTGTTTTG	7	17	2.4	0.025	30616	Potassium large conductance calcium-
						activated channel, subfamily M, alpha

TAACATCTGC	6	71	11.8	0	6703	member 1 S100 calcium-binding protein A9
TAGCTGAAAA	6	34	5.7	0	10000	(calgranulin B) Nuclear receptor subfamily 4, group A,
GCCGTTCTTA	6	19	3.2	0.005	145166	member 1 lineage leukemia mRNA
ATGCTCTGGA	6	18	3.0	0.007	6050	Fc receptor, IgG, low affinity III
CAAAAAATAG	6	14	2.3	0.04	1507	Sulfotransferase family 1A, phenol-
GGGTTTTTAT	5	17	3.4	0.006	145410	preferring, member 1 Nuclease sensitive element binding
GCTGACTCTT	5	15	3.0	0.016	29476	protein 1 Similar to RIKEN cDNA 2700055K07
ATGGCTTAAT	4	18	4.5	0.001	9459	Protein tyrosine phosphatase 4a1
CTGGCCTGAG	4	15	3.8	0.007	150983	Similar to NP_032607.1 antigen
TGCTGCGAAA	4	12	3.0	0.027	22087	Similar to interferon induced
TAGTTTTCCC	4	12	3.0	0.027	105052	transmembrane protein 2 like Slfn2_predicted Schlafen 2
TTCAAAAAAA	4	12	3.0	0.027	115424	Similarity to protein pir:A55388
TTCCCTGTGA	3	11	3.7	0.022	39743	(H.sapiens) RT1 class Ib, locus Aw2
CACCACTGTT	3	10	3.3	0.038	4192	Similar to 60S ribosomal protein L7a
GGATGCCGGG	3	10	3.3	0.038	5950	(Surfeit locus protein 3) Transketolase
GGATATGTGG	2	27	13.5	0	9096	Early growth response 1
GTCCTGAGAG	2	17	8.5	0	82672	Vesicle-associated associated membrane
TCCTCTAGAA	2	14	7.0	0.001	1952	protein 8 Surfactant associated protein B
TGTTGTTGAC	2	9	4.5	0.024	17667	Similar to ankyrin repeat domain 40
GCCAAGCCAT	2	9	4.5	0.024	36770	Clk1 CDC-like kinase 1
GAAGGTTTTA	2	8	4.0	0.043	93714	Jun v-jun sarcoma virus 17 oncogene
GCCACTTAGG	2	8	4.0	0.043	12102	homolog (avian) H13_predicted Histocompatibility 13
TCTTCTCACA	2	8	4.0	0.043	6172	Ribosomal protein L39
ATCCGAAAGA	2	8	4.0	0.043	36614	Ribosomal protein L28
TCAGCATTCA	2	8	4.0	0.043	18560	Secretory leukocyte protease inhibitor
TAACTGACAA	1	25	25.0	0	115549	Similar to Metallothionein-2 (MT-2)
TGGAAGTGA	1	21	21.0	0	103750	(Metallothionein-II) C-fos FBJ murine osteosarcoma viral
TGAGCTGGTC	1	18	18.0	0	98260	oncogene homolog Dual specificity phosphatase 1
AGGGGGAGGG	1	10	10.0	0.004	87679	Vascular early response gene protein
GCTCTGATAT	1	10	10.0	0.004	3285	Diazepam binding inhibitor
TCTGAACAAA	1	7	7.0	0.027	22103	Similar to nucleoside diphosphate linked
TGCCTTCTGT	1	7	7.0	0.027	71224	moiety X-type motif 9 Rras_predicted Harvey rat sarcoma
GTTTTTTAAA	1	7	7.0	0.027	4224	oncogene, subgroup R Ribophorin I

GCGCCTTCAA	1	7	7.0	0.027	162206	similar to Intercellular adhesion
TGTGGTGGAG	1	7	7.0	0.027	74199	molecule-2 precursor Similar to Ubiquitin carboxyl-terminal
AAGCATAGAA	1	7	7.0	0.027	3578	hydrolase 24 Thioredoxin-like 2
ATTGTGCAGA	1	7	7.0	0.027	266	Ornithine decarboxylase antizyme 1
TAAAATAAAA	1	7	7.0	0.027	6660	Mrpl52_predicted Mitochondrial
TTGTCTTAAA	1	7	7.0	0.027	58420	ribosomal protein L52 Nfkbiz_predicted Nuclear factor of kappa light polypeptide gene enhancer
GCCCCCTTCC	0	8	8.0	0.003	15806	in B-cells inhibitor, zeta Jun-B oncogene
CCAAAGTCTT	0	7	7.0	0.007	44161	Lamin A
GGTCGAGGCA	0	6	6.0	0.013	11944	Cytokine receptor-like protein CYRL
TAAGATTCTT	0	6	6.0	0.013	33807	Cct6a Chaperonin subunit 6a (zeta)
TGGGGAGAAA	0	6	6.0	0.013	22509	Putative ISG12(b) protein
TAAGCAAATC	0	6	6.0	0.013	11148	Similar to Leydig cell tumor 10 kd protein
TGGGAGTAGG	0	5	5.0	0.028	13115	ATP-binding cassette, sub-family A (ABC1), member 2
CTCAGATGCT	0	5	5.0	0.028	17691	Hermansky-Pudlak syndrome 1
TAAATATTGG	0	5	5.0	0.028	5892	homolog (human) Hla-dmb major histocompatibility
TACAAAATAG	0	5	5.0	0.028	105855	complex, class II, DM beta Dennd2d_predicted DENN/MADD
AAGGATGGGA	0	5	5.0	0.028	33787	domain containing 2D Complement component 7
CACCCACCTG	0	5	5.0	0.028	2863	Similar to EN-7 protein
GTTCAGTTTC	2	8	4.0	0.043	114823	EST
GGGGAAAAAA	1	8	8.0	0.016	16261	EST
GTAAGCAAAT	0	8	8.0	0.003	48933	EST
TGCTGAGTAT	0	6	6.0	0.013	203119	EST
GAATTACCTG	0	5	6.0	0.028	153630	EST
GTGACCACGG	41	94	2.3	0		No match
ACCCGCCGGG	7	20	2.9	0.006		No match
CTAACTAGTT	4	14	3.5	0.012		No match
TTGTCTTAGA	4	13	3.3	0.019		No match
CCGACGGGCG	3	11	3.7	0.022		No match
TGGGTGTCTA	2	13	6.5	0.003		No match
TACACTAACC	1	11	11.0	0.002		No match
TCCATAAAGA	0	8	8.0	0.003		No match
ATTTGAGCAG	0	5	5.0	0.028		No match
AATTAGAGCA	0	5	5.0	0.028		No match
TTAATATTTG	0	5	5.0	0.028		No match
CTAATATTGG	0	5	5.0	0.028		No match
Down-regulated (83)						
ATAACACATA	544	348	1.6	0	3658	GPCR family C, group 5, member C

CACGCCTCTC	276	157	1.8	0	107334	Hemoglobin alpha 2 chain
AGAGAGGCAG	64	38	1.7	0.016	12328	Mfap4 Microfibrillar-associated
GAATAATAAA	53	31	1.7	0.025	120392	protein 4 Heat shock protein 8
TCTCACCAAC	53	26	2.0	0.004	54384	Elastin
CTGTACAAGC	50	26	1.9	0.008	112831	Similar to AHNAK
ATAGCTTTCT	39	20	2.0	0.017	2104	Amyloid beta (A4) precursor protein
ATCTCCTGTC	39	4	9.8	0	2379	Matrix gla protein
ATTTGAAATA	30	15	2.0	0.031	3036	GTP-binding protein
TTTTGTAAct	26	10	2.6	0.01	105888	Similar to claudin-18A1.2
TGACCATATT	23	11	2.1	0.045	56603	Amine oxidase, copper containing 3
AGGAAAATTG	20	7	2.9	0.016	86651	Cytochrome P450, subfamily 4B,
TTGCTGTGTG	18	6	3.0	0.019	57349	polypeptide 1 Similar to microtubule-actin
TACAAGACAA	17	7	2.4	0.049	5812	crosslinking factor Alpha-spectrin 2
CCCAATGGCC	17	1	17.0	0	128903	Procollagen, type VI, alpha 2
TTGAAAAAAA	14	5	2.8	0.045	11330	Urate oxidase
TCAGTTTATT	14	4	3.5	0.022	3775	Serinc3 Serine incorporator 3
CAAATGTTTT	14	3	4.7	0.009	72459	Similar to Vinculin
CTACTCGAAT	14	3	4.7	0.009	145219	Membrane-associated ring finger
AAGGAGATGG	13	3	4.3	0.014	1101	(C3HC4) 2 Ribosomal protein L31
TTATTTATGA	13	3	4.3	0.014	98839	Similarity to tissue inhibitor of
CGTTTGTCCG	12	3	4.0	0.025	774	metalloproteinase 3 precursor; Laminin, beta 2
ACAACCTTCCT	12	2	6.0	0.008	98783	GM2 ganglioside activator protein
TCATTGAACA	12	2	6.0	0.008	11349	Trans-golgi network protein 1
TTATGGAATT	12	1	12.0	0.002	6007	CD24 antigen
ATAGAGGCAA	11	2	5.5	0.016	6961	Morf4l2 Mortality factor 4 like 2
TACAATAAAC	11	2	5.5	0.016	7685	Progesterone receptor membrane
TATTCAAATA	11	2	5.5	0.016	9954	component 2 Transforming growth factor, beta
ACAAAATAAA	10	2	5.0	0.024	21931	receptor II Nfe2l1_predicted Nuclear factor,
CAATTAAAAA	9	2	4.5	0.044	101044	erythroid derived 2,-like 1 Xbp1 X-box binding protein 1
CTAATAAATG	9	2	4.5	0.044	102247	Similar to DREV protein
GTGTTTGTTC	9	2	4.5	0.044	1365	Similar to Mylk protein
ACTGAAAAAA	9	2	4.5	0.044	18030	Bone morphogenetic protein 7
TGCCTGCTCC	9	1	9.0	0.013	7405	Basophilic leukemia expressed protein
GGGTTCCTCC	9	1	9.0	0.013	74081	BLES03 Col16a1 Procollagen, type XVI, alpha 1
GTGCTACTCC	8	1	8.0	0.025	53801	Col4a1 Procollagen, type IV, alpha 1
TCGTGGCCCC	8	1	8.0	0.025	11098	H2A histone family, member Y
TTTTTGTAAG	7	1	7.0	0.042	32236	Similar to RIKEN cDNA 4930570C03

TGTTCAAAAA	7	1	7.0	0.042	116828	Spermatogenesis associated 5
TTGACAACGT	7	1	7.0	0.042	15408	Similarity to protein ref:NP_057711.1
AAATAAAGTC	7	1	7.0	0.042	2163	Agrin
GTAGCAGGTT	7	0	7.0	0.01	3896	FXVD domain-containing ion transport
AGACATACTG	7	0	7.0	0.01	3793	regulator 3 Similar to tensin
TGCTCTTTAT	6	0	6.0	0.019	86009	Stard3nl STARD3 N-terminal like
GCTTGCTTCC	5	0	5.0	0.037	1425	RAB7, member RAS oncogene family
TGAGGAACAA	5	0	5.0	0.037	138656	Similar to RIKEN cDNA 1110025F24
CCTCGGGGGC	5	0	5.0	0.037	40243	Similarity to protein pir:S34968 (M.musculus) S34968 fibulin, splice
GTCCAGGAAA	5	0	5.0	0.037	22279	form D precursor - mouse Cyclin D1
TATTCATCAG	5	0	5.0	0.037	17321	Pantothenate kinase 4
TGTTTTGGGG	5	0	5.0	0.037	13788	Chsy1_predicted Carbohydrate
TTACTAACAC	5	0	5.0	0.037	13171	(chondroitin) synthase 1 Tyrosine kinase receptor 1
TTGCTCTTCA	5	0	5.0	0.037	29825	Rasgef1a_predicted Rasgef1a
CATTTCATA	5	0	5.0	0.037	1702	Fibrinogen, gamma polypeptide
GAGTCCTTCC	5	0	5.0	0.037	33218	Syntaxin 4
CTGTGTAAAG	5	0	5.0	0.037	106287	Hypothetical protein MGC15854
CTTTTATTTT	5	0	5.0	0.037	25166	Glutathione S-transferase omega 1
TCCAAAAAAA	5	0	5.0	0.037	9910	Mapk9 stress activated protein kinase
GAATCCAAC	5	0	5.0	0.037	3377	alpha II Ndufb11_predicted NADH dehydrogenase (ubiquinone) 1 beta
GAGAAATAAA	5	0	5.0	0.037	819	subcomplex, 11 Similar to SRD5A2L
GTTGCATAAT	5	0	5.0	0.037	6249	Carnitine acetyltransferase
TTTACAGGGG	5	0	5.0	0.037	36545	Tenascin XA
TATGGCTTTA	46	13	3.5	0	2943	EST
GGAGGGGAAG	12	3	4.0	0.025	22772	EST
TAAATAAAGC	10	2	5.0	0.024	12150	EST
ATCAAATGTA	8	1	8.0	0.025	22807	EST
CAGGCAAAAC	8	1	8.0	0.025	18922	EST
TTCCATTGGT	7	1	7.0	0.042	11902	EST
GTGTCGCATC	5	0	5.0	0.037	45804	EST
TAAGGTTTTT	5	0	5.0	0.037	8299	EST
TTGTCATCAA	5	0	5.0	0.037	13277	EST
ATACTGACAC	252	182	1.4	0.004		No match
GATGCCCCCC	85	28	3.0	0		No match
GAATGACCCT	23	8	2.9	0.009		No match
CTGGGGCATC	11	3	3.7	0.037		No match
GTAGAGGCCG	10	1	10.0	0.008		No match
GAACATAATA	9	1	9.0	0.013		No match
GTGAATTCGG	8	0	8.0	0.005		No match

TTGCGACATT	6	0	6.0	0.019	No match
GGCAGCACA	5	0	5.0	0.037	No match
GCGCGCGTTT	5	0	5.0	0.037	No match
AAAGAAAAAA	5	0	5.0	0.037	No match
TACTGTCAAA	5	0	5.0	0.037	No match
TAATAAGCTT	5	0	5.0	0.037	No match

* The fold was determined by dividing the number of tags between asthma and control group. To avoid division by 0, we used a tag value of 1 for any tag that was not detectable in samples.

Cellular process

Rn.33787 complement component 7
Rn.43300 eukaryotic translation elongation factor 1 gamma
Rn.266 ornithine decarboxylase antizyme 1
Rn.101044 x-box binding protein 1
Rn.98839 synapsin iii
Rn.6961 morf-related gene x
Rn.36614 ribosomal protein l28
Rn.87679 vascular early response gene protein
Rn.1952 surfactant associated protein b
Rn.21931 nuclear factor, erythroid derived 2,-like 1 (predicted)
Rn.9096 early growth response 1
Rn.36610 tumor protein, translationally-controlled 1
Rn.128903 procollagen, type vi, alpha 2
Rn.36966 beta-glo
Rn.103750 fbj murine osteosarcoma viral oncogene homolog
Rn.17145 connective tissue growth factor
Rn.86651 cytochrome p450, family 4, subfamily b, polypeptide 1
Rn.56603 amine oxidase, copper containing 3
Rn.112831 ahnak nucleoprotein (desmoyokin)
Rn.3036 guanine nucleotide binding protein, alpha inhibiting 2
Rn.6703 s100 calcium binding protein a9 (calgranulin b)
Rn.1439 ribosomal protein l36
Rn.22087 interferon induced transmembrane protein 1 (predicted)
Rn.2139 ribosomal protein s23
Rn.98783 gm2 ganglioside activator protein
Rn.3578 thioredoxin-like 2
Rn.44161 lamin a
Rn.9459 protein tyrosine phosphatase 4a1
Rn.6249 carnitine acetyltransferase
Rn.22279 cyclin d1
Rn.3285 diazepam binding inhibitor
Rn.33807 chaperonin subunit 6a (zeta)
Rn.36770 cdc-like kinase 1
Rn.93714 jun oncogene
Rn.1702 fibrinogen, gamma polypeptide
Rn.102149 immunoglobulin heavy chain (alpha polypeptide)
Rn.12328 microfibrillar-associated protein 4
Rn.58420 similar to molecule possessing ankyrin-repeats induced by lipopolysaccharide
Rn.105052 schlafen 2 (predicted)
Rn.17691 hermansky-pudlak syndrome 1 homolog (human)
Rn.92211 ribosomal protein l13a
Rn.1425 rab7, member ras oncogene family
Rn.54384 elastin
Rn.6050 fc receptor, igg, low affinity iii

Rn.13788 carbohydrate (chondroitin) synthase 1 (predicted)
Rn.2104 amyloid beta (a4) precursor protein
Rn.71224 harvey rat sarcoma oncogene, subgroup r (predicted)
Rn.36545 cytochrome p450, subfamily 21a, polypeptide 1
Rn.34942 ribosomal protein s29
Rn.15806 jun-b oncogene
Rn.107334 hemoglobin alpha, adult chain 1
Rn.6172 ribosomal protein l39
Rn.13171 tyrosine kinase with immunoglobulin-like and egf-like domains 1
Rn.98260 dual specificity phosphatase 1
Rn.5892 major histocompatibility complex, class ii, dm beta
Rn.2605 thymosin, beta 4
Rn.30616 potassium large conductance calcium-activated channel, subfamily m, alpha n
Rn.11098 h2a histone family, member y
Rn.84435 glucose phosphate isomerase
Rn.22518 caveolin
Rn.3910 eukaryotic translation elongation factor 1 beta 2 (predicted)
Rn.3775 tumor differentially expressed 1
Rn.13115 atp-binding cassette, sub-family a (abc1), member 2
Rn.2812 ribosomal protein l35a
Rn.11330 urate oxidase
Rn.18030 bone morphogenetic protein 7
Rn.22103 nudix -type motif 9
Rn.1997 cathepsin h
Rn.774 laminin, beta 2
Rn.4224 ribophorin i
Rn.3896 fxyd domain-containing ion transport regulator 3
Rn.3658 g protein-coupled receptor, family c, group 5, member c
Rn.10000 nuclear receptor subfamily 4, group a, member 1
Rn.11349 trans-golgi network protein 1
Rn.1507 sulfotransferase family 1a, phenol-preferring, member 1
Rn.2379 matrix gla protein
Rn.25166 glutathione s-transferase omega 1
Rn.36102 ribosomal protein s14
Rn.6007 cd24 antigen
Rn.2863 ras-related c3 botulinum substrate 2
Rn.973 ribosomal protein, large, p1
Rn.72459 vinculin (predicted)
Rn.145219 similar to 9530046h09rik protein
Rn.108039 ribosomal protein l14
Rn.90546 annexin a2
Rn.2163 agrin
Rn.976 ribosomal protein, large p2
Rn.1101 ribosomal protein l31
Rn.9954 transforming growth factor, beta receptor ii

Rn.9910 mitogen-activated protein kinase 9
Rn.2283 lysozyme
Rn.105888 similar to claudin-18a1.2
Rn.54447 ferritin, heavy polypeptide 1
Rn.53801 procollagen, type iv, alpha 1
Rn.120392 heat shock protein 8
Rn.32236 histone deacetylase 7
Rn.33218 syntaxin 4a (placental)
Rn.74081 procollagen, type xvi, alpha 1
Rn.34429 ribosomal protein l17
Rn.107335 integral membrane protein 2b
Rn.106034 ribosomal protein s27a

; ikappab-zeta (predicted)

number 1

Immune response

Rn.103750 fbj murine osteosarcoma viral oncogene homolog

Rn.56603 amine oxidase, copper containing 3

Rn.58420 similar to molecule possessing ankyrin-repeats induced by lipopolysaccharide

Rn.6007 cd24 antigen

Rn.1868 beta-2 microglobulin

Rn.2283 lysozyme

Rn.101044 x-box binding protein 1

Rn.6703 s100 calcium binding protein a9 (calgranulin b)

Rn.87679 vascular early response gene protein

Rn.54447 ferritin, heavy polypeptide 1

Rn.5892 major histocompatibility complex, class ii, dm beta

Rn.32236 histone deacetylase 7

Rn.6050 fc receptor, igg, low affinity iii

Rn.21931 nuclear factor, erythroid derived 2,-like 1 (predicted)

Rn.1702 fibrinogen, gamma polypeptide

Rn.9096 early growth response 1

ride; ikappab-zeta (predicted)

Cell migration

Rn.2605 thymosin, beta 4

Rn.17145 connective tissue growth factor

Rn.6050 fc receptor, igg, low affinity iii

Rn.2104 amyloid beta (a4) precursor protein

Rn.13171 tyrosine kinase with immunoglobulin-like and egf-like domains 1

Rn.18030 bone morphogenetic protein 7

Rn.72459 vinculin (predicted)

Cell differentiation

Rn.17145 connective tissue growth factor

Rn.22279 cyclin d1

Rn.9954 transforming growth factor, beta receptor ii

Rn.2379 matrix gla protein

Rn.6007 cd24 antigen

Rn.13171 tyrosine kinase with immunoglobulin-like and egf-like domains 1

Rn.18030 bone morphogenetic protein 7

Rn.87679 vascular early response gene protein

Rn.17691 hermansky-pudlak syndrome 1 homolog (human)

Rn.2605 thymosin, beta 4

Rn.32236 histone deacetylase 7

Rn.93714 jun oncogene

Rn.2104 amyloid beta (a4) precursor protein

Rn.9096 early growth response 1

Rn.2163 agrin

Regulation of cellular process

Rn.33787 complement component 7

Rn.22279 cyclin d1

Rn.15806 jun-b oncogene

Rn.13171 tyrosine kinase with immunoglobulin-like and egf-like domains 1

Rn.101044 x-box binding protein 1

Rn.6961 morf-related gene x

Rn.72459 vinculin (predicted)

Rn.87679 vascular early response gene protein

Rn.2605 thymosin, beta 4

Rn.36770 cdc-like kinase 1

Rn.93714 jun oncogene

Rn.1702 fibrinogen, gamma polypeptide

Rn.36610 tumor protein, translationally-controlled 1

Rn.9096 early growth response 1

Rn.22518 caveolin

Rn.103750 fbj murine osteosarcoma viral oncogene homolog

Rn.3775 tumor differentially expressed 1

Rn.17145 connective tissue growth factor

Rn.9954 transforming growth factor, beta receptor ii

Rn.58420 similar to molecule possessing ankyrin-repeats induced by lipopolysaccharide

Rn.105052 schlafen 2 (predicted)

Rn.18030 bone morphogenetic protein 7

Rn.54447 ferritin, heavy polypeptide 1

Rn.1425 rab7, member ras oncogene family

Rn.54384 elastin

Rn.120392 heat shock protein 8

Rn.22087 interferon induced transmembrane protein 1 (predicted)

Rn.32236 histone deacetylase 7

Rn.6050 fc receptor, igg, low affinity iii

Rn.10000 nuclear receptor subfamily 4, group a, member 1

Rn.2104 amyloid beta (a4) precursor protein

Rn.107335 integral membrane protein 2b

; ikappab-zeta (predicted)

Cell cycle

Rn.98260 dual specificity phosphatase 1 RG Rattus norvegicus

Rn.9459 protein tyrosine phosphatase 4a1 RG Rattus norvegicus

Rn.36770 cdc-like kinase 1 RG Rattus norvegicus

Rn.22279 cyclin d1 RG Rattus norvegicus

Rn.120392 heat shock protein 8 RG Rattus norvegicus

Rn.22087 interferon induced transmembrane protein 1 (predicted) RG Rattus norvegicus

Rn.32236 histone deacetylase 7 RG Rattus norvegicus

Rn.93714 jun oncogene RG Rattus norvegicus

Rn.15806 jun-b oncogene RG Rattus norvegicus

Rn.2104 amyloid beta (a4) precursor protein RG Rattus norvegicus

Lipid metabolism

Rn.3285 diazepam binding inhibitor RG Rattus norvegicus

Rn.774 laminin, beta 2 RG Rattus norvegicus

Rn.6249 carnitine acetyltransferase RG Rattus norvegicus

Rn.3775 tumor differentially expressed 1 RG Rattus norvegicus

Rn.1507 sulfotransferase family 1a, phenol-preferring, member 1 RG Rattus norvegicus

Rn.1952 surfactant associated protein b RG Rattus norvegicus

Rn.36545 cytochrome p450, subfamily 21a, polypeptide 1 RG Rattus norvegicus

Rn.13115 atp-binding cassette, sub-family a (abc1), member 2 RG Rattus norvegicus

Rn.98783 gm2 ganglioside activator protein RG Rattus norvegicus

Rn.22518 caveolin

Apoptosis

Rn.33787 complement component 7 RG Rattus norvegicus

Rn.3775 tumor differentially expressed 1 RG Rattus norvegicus

Rn.6050 fc receptor, igg, low affinity iii RG Rattus norvegicus

Rn.10000 nuclear receptor subfamily 4, group a, member 1 RG Rattus norvegicus

Rn.36610 tumor protein, translationally-controlled 1 RG Rattus norvegicus

Rn.2104 amyloid beta (a4) precursor protein RG Rattus norvegicus

Rn.107335 integral membrane protein 2b RG Rattus norvegicus

number	term
1	response to stimulus
2	response to chemical stimulus
3	taxis
4	chemotaxis
5	leukocyte chemotaxis
6	detection of chemical stimulus
7	physiological defense response

- 8 leukocyte migration
- 9 detection of stimulus during sensory perception
- 10 detection of chemical stimulus during sensory perception
- 11 detection of chemical stimulus during sensory perception of
- 12 sensory perception of smell
- 13 maintenance of localization
- 14 antigen processing and presentation
- 15 antigen processing and presentation of exogenous antigen
- 16 antigen processing and presentation of exogenous peptide a
- 17 antigen processing and presentation of peptide antigen
- 18 antigen processing and presentation of peptide antigen via M
- 19 DNA modification
- 20 DNA alkylation
- 21 DNA methylation
- 22 biosynthesis

23 cellular biosynthesis

24 macromolecule biosynthesis

25 protein biosynthesis

genes	in detail
114856(Rn.98260)	dual specificity phosphatase 1
116591(Rn.6050)	Fc receptor, IgG, low affinity III
24223(Rn.1868)	beta-2 microglobulin
24330(Rn.9096)	early growth response 1
25211(Rn.2283)	lysozyme
293268(Rn.36966)	olfactory receptor 130 (predicted)
293618(Rn.22087)	interferon induced transmembrane protein 1 (predicted)
304005(Rn.58420)	nuclear factor of kappa light polypeptide gene enhancer in B-
314322(Rn.103750)	FBJ murine osteosarcoma viral oncogene homolog
360647(Rn.162206)	intercellular adhesion molecule 2
366957(Rn.2863)	RAS-related C3 botulinum substrate 2
405071(Rn.36966)	olfactory receptor 131 (predicted)
405912(Rn.36966)	olfactory receptor 129 (predicted)
444983(Rn.87679)	Vascular early response gene protein
56611(Rn.90546)	annexin A2
64032(Rn.17145)	connective tissue growth factor
64389(Rn.2267)	defensin beta 3
94195(Rn.6703)	S100 calcium binding protein A9 (calgranulin B)
114856(Rn.98260)	dual specificity phosphatase 1
116591(Rn.6050)	Fc receptor, IgG, low affinity III
293268(Rn.36966)	olfactory receptor 130 (predicted)
360647(Rn.162206)	intercellular adhesion molecule 2
366957(Rn.2863)	RAS-related C3 botulinum substrate 2
405071(Rn.36966)	olfactory receptor 131 (predicted)
405912(Rn.36966)	olfactory receptor 129 (predicted)
94195(Rn.6703)	S100 calcium binding protein A9 (calgranulin B)
116591(Rn.6050)	Fc receptor, IgG, low affinity III
360647(Rn.162206)	intercellular adhesion molecule 2
366957(Rn.2863)	RAS-related C3 botulinum substrate 2
94195(Rn.6703)	S100 calcium binding protein A9 (calgranulin B)
116591(Rn.6050)	Fc receptor, IgG, low affinity III
360647(Rn.162206)	intercellular adhesion molecule 2
366957(Rn.2863)	RAS-related C3 botulinum substrate 2
94195(Rn.6703)	S100 calcium binding protein A9 (calgranulin B)
116591(Rn.6050)	Fc receptor, IgG, low affinity III
360647(Rn.162206)	intercellular adhesion molecule 2
366957(Rn.2863)	RAS-related C3 botulinum substrate 2
94195(Rn.6703)	S100 calcium binding protein A9 (calgranulin B)
116591(Rn.6050)	Fc receptor, IgG, low affinity III
24223(Rn.1868)	beta-2 microglobulin
25211(Rn.2283)	lysozyme
304005(Rn.58420)	nuclear factor of kappa light polypeptide gene enhancer in B-

314322(Rn.103750)	FBJ murine osteosarcoma viral oncogene homolog
64389(Rn.2267)	defensin beta 3
94195(Rn.6703)	S100 calcium binding protein A9 (calgranulin B)
116591(Rn.6050)	Fc receptor, IgG, low affinity III
360647(Rn.162206)	intercellular adhesion molecule 2
94195(Rn.6703)	S100 calcium binding protein A9 (calgranulin B)
293268(Rn.36966)	olfactory receptor 130 (predicted)
405071(Rn.36966)	olfactory receptor 131 (predicted)
405912(Rn.36966)	olfactory receptor 129 (predicted)
293268(Rn.36966)	olfactory receptor 130 (predicted)
405071(Rn.36966)	olfactory receptor 131 (predicted)
405912(Rn.36966)	olfactory receptor 129 (predicted)
293268(Rn.36966)	olfactory receptor 130 (predicted)
405071(Rn.36966)	olfactory receptor 131 (predicted)
405912(Rn.36966)	olfactory receptor 129 (predicted)
293268(Rn.36966)	olfactory receptor 130 (predicted)
405071(Rn.36966)	olfactory receptor 131 (predicted)
405912(Rn.36966)	olfactory receptor 129 (predicted)
25319(Rn.54447)	ferritin, heavy polypeptide 1
81814(Rn.2605)	thymosin, beta 4
116591(Rn.6050)	Fc receptor, IgG, low affinity III
24223(Rn.1868)	beta-2 microglobulin
294273(Rn.5892)	major histocompatibility complex, class II, DM beta
116591(Rn.6050)	Fc receptor, IgG, low affinity III
294273(Rn.5892)	major histocompatibility complex, class II, DM beta
116591(Rn.6050)	Fc receptor, IgG, low affinity III
294273(Rn.5892)	major histocompatibility complex, class II, DM beta
116591(Rn.6050)	Fc receptor, IgG, low affinity III
24223(Rn.1868)	beta-2 microglobulin
294273(Rn.5892)	major histocompatibility complex, class II, DM beta
116591(Rn.6050)	Fc receptor, IgG, low affinity III
24223(Rn.1868)	beta-2 microglobulin
314322(Rn.103750)	FBJ murine osteosarcoma viral oncogene homolog
83731(Rn.30616)	potassium large conductance calcium-activated channel, sub
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314322(Rn.103750)	FBJ murine osteosarcoma viral oncogene homolog
83731(Rn.30616)	potassium large conductance calcium-activated channel, sub
116591(Rn.6050)	Fc receptor, IgG, low affinity III
124323(Rn.2139)	ribosomal protein S23
140661(Rn.973)	ribosomal protein, large, P1
140662(Rn.976)	ribosomal protein, large P2
25045(Rn.3285)	diazepam binding inhibitor
25347(Rn.6172)	ribosomal protein L39
25348(Rn.34942)	ribosomal protein S29

25404(Rn.22518)	caveolin
25502(Rn.266)	ornithine decarboxylase antizyme 1
25596(Rn.4224)	ribophorin I
291434(Rn.34429)	Ribosomal protein L17
29284(Rn.36102)	ribosomal protein S14
293725(Rn.43300)	eukaryotic translation elongation factor 1 gamma
363241(Rn.3910)	eukaryotic translation elongation factor 1 beta 2 (predicted)
64638(Rn.36614)	ribosomal protein L28
65043(Rn.108039)	ribosomal protein L14
81777(Rn.106034)	ribosomal protein S27a
94195(Rn.6703)	S100 calcium binding protein A9 (calgranulin B)
116591(Rn.6050)	Fc receptor, IgG, low affinity III
124323(Rn.2139)	ribosomal protein S23
140661(Rn.973)	ribosomal protein, large, P1
140662(Rn.976)	ribosomal protein, large P2
25347(Rn.6172)	ribosomal protein L39
25348(Rn.34942)	ribosomal protein S29
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94195(Rn.6703)	S100 calcium binding protein A9 (calgranulin B)

cells inhibitor, zeta (predicted)

cells inhibitor, zeta (predicted)

family M, alpha member 1

family M, alpha member 1

family M, alpha member 1

number	term
1	muscle development
2	striated muscle development
3	neuromuscular junction development
4	synapse organization and biogenesis
5	synaptogenesis
6	neuron maturation
7	acetylcholine receptor signaling, muscarinic pathway
8	extracellular structure organization and biogenesis
9	extracellular matrix organization and biogenesis
10	organismal movement
11	musculoskeletal movement
12	regulation of balance

genes	in detail
25043(Rn.54384)	elastin
25592(Rn.2163)	agrin
25602(Rn.36545)	tenascin XA
54226(Rn.2104)	amyloid beta (A4) precursor protein
25043(Rn.54384)	elastin
25592(Rn.2163)	agrin
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25592(Rn.2163)	agrin
54226(Rn.2104)	amyloid beta (A4) precursor protein
25592(Rn.2163)	agrin
81664(Rn.3036)	guanine nucleotide binding protein, alpha inhibiting 2
25043(Rn.54384)	elastin
25592(Rn.2163)	agrin
361821(Rn.128903)	procollagen, type VI, alpha 2
54226(Rn.2104)	amyloid beta (A4) precursor protein
25043(Rn.54384)	elastin
25592(Rn.2163)	agrin
361821(Rn.128903)	procollagen, type VI, alpha 2
54226(Rn.2104)	amyloid beta (A4) precursor protein
282838(Rn.98783)	GM2 ganglioside activator protein
54226(Rn.2104)	amyloid beta (A4) precursor protein
282838(Rn.98783)	GM2 ganglioside activator protein
54226(Rn.2104)	amyloid beta (A4) precursor protein
282838(Rn.98783)	GM2 ganglioside activator protein
54226(Rn.2104)	amyloid beta (A4) precursor protein