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Original Article

Profiling of remote skeletal muscle gene changes resulting from stimulation of atopic dermatitis disease in NC/Nga mouse model

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ABSTRACT Although atopic dermatitis (AD) is known to be a representative skin disorder, it also affects the systemic immune response. In a recent study, myoblasts were shown to be involved in the immune regulation, but the roles of muscle cells in AD are poorly understood. We aimed to identify the relationship between mitochondria and atopy by genome-wide analysis of skeletal muscles in mice. We induced AD-like symptoms using house dust mite (HDM) extract in NC/Nga mice. The transcriptional profiles of the untreated group and HDM-induced AD-like group were analyzed and compared using microarray, differentially expressed gene and functional pathway analyses, and protein interaction network construction. Our microarray analysis demonstrated that immune response-, calcium handling-, and mitochondrial metabolism-related genes were differentially expressed. In the Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology pathway analyses, immune response pathways involved in cytokine interaction, nuclear factor-kappa B, and T-cell receptor signaling, calcium handling pathways, and mitochondria metabolism pathways involved in the citrate cycle were significantly upregulated. In protein interaction network analysis, chemokine family-, muscle contraction process-, and immune response-related genes were identified as hub genes with many interactions. In addition, mitochondrial pathways involved in calcium signaling, cardiac muscle contraction, tricarboxylic acid cycle, oxidation-reduction process, and calcium-mediated signaling were significantly stimulated in KEGG and Gene Ontology analyses. Our results provide a comprehensive understanding of the genome-wide transcriptional changes of HDM-induced AD-like symptoms and the indicated genes that could be used as AD clinical biomarkers.

INTRODUCTION

Atopic dermatitis (AD) is a common problem that affects many people from infancy and is caused by a variety of allergens, including environmental factors and foods [1,2]. AD is accompanied by intractable itching—a common symptom of systemic diseases—including peripheral and central nervous system itch and pruritoceptive components, such as skin dryness [3]. Under the conditions of central sensitization, painful irritation is usually perceived as itching [4]. Severe itching may also cause a secondary infection. As such, itching is a major part of the pain disorder.

Recent studies have suggested that myoblasts can act as antigen-presenting cells in the immune response in muscles, and have been proposed as potential neoantigens in the inflammatory

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response [5-7]. Myoblasts are considered active participants in the immune response and secrete or regulate the expression of various cytokines, chemokines, and cell adhesion molecules such as interleukin 6 (Il6), Il2, Cd4, and Cd8 [7-9]. For example, the airway smooth muscle can modulate immune responses through cell surface receptor-mediated recognition of various molecules such as Il6, Il8, and Il11 [10,11].

Allergen-induced mitochondrial dysfunction and increased production of reactive oxygen species (ROS) have been demonstrated in a variety of allergic rhinitis and AD cases [12]. ROS are byproducts of mitochondrial respiration, which play roles in antibacterial immune signaling and phagocyte bactericidal activity [13]. Furthermore, increased mitochondrial numbers and densities were observed in patients with severe asthma [14,15]. This is consistent with a previous study demonstrating that allergen-induced early asthmatic responses are associated with glycolysis, Ca^{2+} binding, and mitochondrial activity [16], and a genome-wide study of allergic rhinitis that suggested the involvement of a mitochondrial pathway [17]. In addition, the development of atopic allergic diseases in children is strongly associated with genetics [18], particularly maternal atopic history [19-21]. Furthermore, sequence changes in the mitochondria, which are maternally inherited, are related to the pathogenesis of asthma and AD [12,22]. Thus, recent approaches to treating allergic disease target the mitochondria [23], and we anticipate that this may also be an effective strategy for treating AD.

AD is known to be a representative skin disorder, but it also affects the systemic immune response [24,25]. In a recent study, myoblasts were shown to be involved in the immune response as antigen presenting cells; however, other roles of muscle cells in AD are poorly understood. In this study, we used a murine model of atopic dermatitis (NC/Nga) that was induced by house dust mite (HDM) extract to measure changes in skeletal muscle. Specifically, we performed a genome-wide transcriptional analysis, microarray analysis, and quantitative reverse transcription-polymerase chain reaction (qRT-PCR) to measure changes in gene expression and determine differences in protein interaction networks. In addition, we measured the mitochondrial DNA copy number to investigate the mitochondrial changes caused by AD induction.

METHODS

Animals and groups

Nine-week-old female NC/Nga mice weighing 23–27 g were purchased from Central Lab Animal Inc. (Seoul, Korea) and allowed to acclimate for 2 weeks before the experiments were initiated. The mice were divided into the AD-induced group and the no treatment group (n = 2/group). Mice were housed in a standard, controlled environment: 22 ± 3°C, 50 ± 10% humidity, 12-h light-dark cycle, and ventilation 10–15 times/h with a wind velocity of 10–20 cm/s. All animal studies were conducted in accordance with the guidelines for animal testing and were approved by the institutional Ethics Committee of the Chung-Ang University, Korea (approval no. 201800012).

Induction of AD

AD was induced using Biostir AD cream containing *Dermatophagoides farinae* extract (Central Lab Animal Inc.) following the manufacturer's instructions. Briefly, the hair behind the ears and backs of the mice was removed using scissors and a razor, and 150 μl of 4% sodium dodecyl sulfate solution was evenly dispersed on the shaved skin to create a barrier rupture. The solution was dried using a hair dryer (cold air setting) for 2–3 h, and 100 mg of the cream was uniformly applied on the shaved skin. Biostir AD was applied twice per week for 8 weeks. Before the second treatment, the hair was shaved if it had grown back. Mice were sacrificed under anesthesia and blood samples were collected from the abdominal aorta. Gluteal muscles were rapidly removed and washed at least three times in phosphate-buffered saline.

Microarray

The Affymetrix Whole-transcript Expression array was used according to the manufacturer's protocol (GeneChip Whole Transcript [WT] PLUS reagent kit; Affymetrix, Santa Clara, CA, USA). Complementary DNA was reverse-transcribed from the mouse gluteal muscle tissue using the GeneChip WT amplification kit (Affymetrix) following the manufacturer's instructions. The sense complementary DNA was then fragmented and biotin-labeled with terminal deoxynucleotidyl transferase using the GeneChip WT Terminal labeling kit (Affymetrix). Approximately 5.5 μg of labeled DNA target was hybridized to the Affymetrix GeneChip mouse 2.0 ST Array (Affymetrix) at 45°C for 16 h. Hybridized arrays were washed and stained on a GeneChip Fluidics Station 450 and scanned on a GCS3000 Scanner (Affymetrix). Fluorescent signal values were measured using the Affymetrix GeneChip Command Console software (Affymetrix).

Determination of a differentially expressed genes (DEGs) interaction network

A protein-protein interaction network analysis was performed to determine the protein-protein interactions between DEGs that had been identified by the microarray analysis. DEGs with fold-change values < 1.5 and p-values < 0.05 were analyzed by Search Tool for the Retrieval of Interacting Genes (STRING) version 10.5 (http://www.string-db.org/) using the highest confidence minimum interaction score of 0.900.

Measurement of mitochondrial DNA copy number

Total DNA was extracted from the mouse gluteal muscle using the QIAamp DNA mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. NADH dehydrogenase 4 (*Nd4*), D-loop, cytochrome *c* oxidase I (*Cox1*), and glyceraldehyde 3-phosphate dehydrogenase (*Gapdh*) were amplified by real-time PCR using a LightCycler 2.0 (Roche, Mannheim, Germany). Each 10-μl reaction contained 2 mM $MgCl_2$, 0.5 μM of each primer, 1x Light-Cycler DNA Master SYBR Green I (Roche), and 15 ng of DNA template. All primer sequences are listed in Table 1. The reaction conditions were as follows: denaturation (95°C for 10 min), amplification for 35 cycles (95°C for 10 sec; 62°C for 10 sec; 72°C for 10 sec), a melting curve program (65°C to 95°C with a heating rate of 0.1°C/sec), and a cooling step (40°C). The results are expressed as the ratio of mtDNA to gDNA and compared as previously described [26].

Raw data preparation and statistical analysis

The data were summarized and normalized with the robust multi-average method implemented in Affymetrix Power Tools. Gene levels were used to perform the DEG analysis. Statistically significant fold changes were determined using the local pooled error test. The false discovery rate (FDR) was controlled using the Benjamini-Hochberg algorithm. For each DEG set, hierarchical cluster analysis was performed using complete linkage and Euclidean distance as a measure of similarity. Gene-Enrichment and Functional Annotation analysis for the list of significant genes was performed using Gene Ontology (GO; http://geneontology.org) and Kyoto Encyclopedia of Genes and Genomes (KEGG; http://kegg.jp). All data analysis and visualization of DEGs was conducted using R 3.0.2 (www.r-project.org). Statistical significance between the control group and experimental group was determined using Student's t-test, Bonferroni correction, and the FDR. In all cases, p-values < 0.05 were considered significant.

Table 1. The primer sequences and annealing temperatures for PCR

Gene symbol	Primer sequence	Annealing temperature (°C)	Product size (bp)
Nd4	ATAGCCACATGATGACTGATAGC TGCCGCGTTGGGTGGTAA	58	190
D-loop	AGCTACTCCCCACCACCAG TGACGGCTATGTTGATGAAAGT	60	128
Cox1	GAGCAATCCAGGTCGGTTTC CTTACGCAATTTCCTGGCTCTG	55	184
Gapdh	TGCTTCACCACCTTCTTGAT TGGAAAGCTGTGGCGTGAT	58	217

RESULTS

Measurement of gene expression changes in skeletal muscle from the murine HDM-induced AD model by microarray analysis

Microarray analysis using mouse gluteal muscle total mRNA was performed for the control and the AD model groups. A total of 35,240 transcripts were detected, and 2,394 DEGs were detected between the two conditions—1591 genes were up-regulated and 803 were down-regulated (FC > 1.5) (Fig. 1A). In comparing the two groups, 421 genes were significantly dysregulated with FC values > 1.5 and p-values < 0.05, and 416 genes were significantly dysregulated with FC values > 2 and p-values < 0.05 (Fig. 1B). The significantly dysregulated 421 DEGs are listed in Supplementary Table 1. The top 30 differentially expressed up-regulated and down-regulated genes between the two conditions and their p-values are listed in Tables 2 and 3, respectively.

KEGG analysis of the significant pathways altered in AD

The significantly regulated pathways were determined according to the functions and interactions of DEGs based on the KEGG database. The 2,394 DEGs significantly stimulated by the

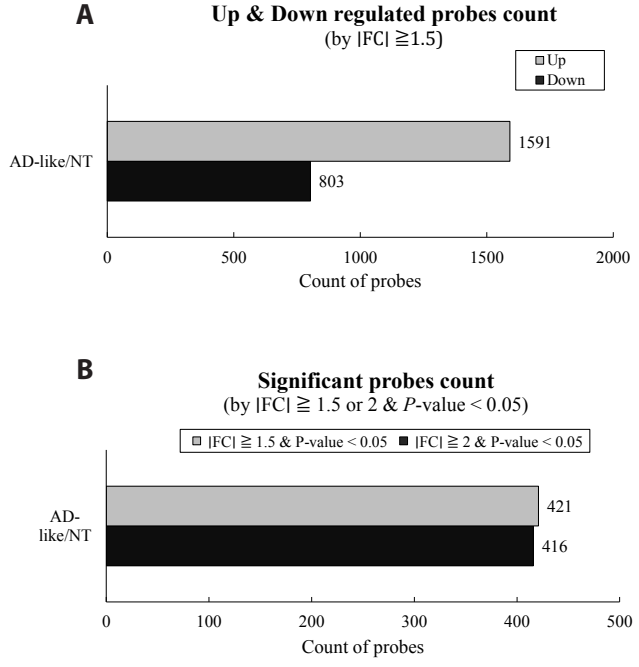


Fig. 1. Regulated probes through house dust mite-induced atopic dermatitis (AD)-like in microarray analysis by fold change (FC) and p-value. (A) Up- and downregulated probes count compared between the AD-like (Itch) group and non-treated (NT) group (|FC| < 2). (B) Significantly regulated probes count compared between the Itch group and NT group (|FC| < 1.5 and 2 with p-value < 0.05).

Table 2. Top 30 up-regulated genes identified by microarray analysis

Gene name	Gene symbol	Fold-change	p-value
Immunoglobulin heavy chain (V3609N non-productive)	*Igh-V3609N*	37.1754	0
Glycosylation dependent cell adhesion molecule 1	*Glycam1*	35.4278	0
Myosin, heavy polypeptide 7, cardiac muscle, beta	*Myh7*	25.3377	0
Immunoglobulin kappa variable 5-48	*Igkv5-48*	19.2719	0
Immunoglobulin kappa variable 6-17	*Igkv6-17*	18.7449	0
Immunoglobulin kappa chain variable 4-70	*Igkv4-70*	18.6879	0
Immunoglobulin kappa variable 4-57-1	*Igkv4-57-1*	16.2358	0
Immunoglobulin kappa variable 10-96	*Igkv10-96*	16.2052	0
Immunoglobulin kappa variable 6-23	*Igkv6-23*	15.8888	0
Cysteine and glycine-rich protein 3	*Csrp3*	15.6094	0
Protein tyrosine phosphatase, receptor type, C	*Ptprc*	14.9040	0
Immunoglobulin kappa joining 1	*Igkj1*	14.6315	0
Immunoglobulin heavy chain (X24 family)	*Igh-VX24*	14.2970	0
Immunoglobulin kappa chain variable 4-72	*Igkv4-72*	12.9740	0
CD3 antigen, gamma polypeptide	*Cd3g*	12.8406	0
Apolipoprotein L 7c	*Apol7c*	12.1598	0
Immunoglobulin heavy variable 3-2	*Ighv3-2*	11.8548	0
Immunoglobulin heavy variable 1-34	*Ighv1-34*	11.7469	0
Troponin C, cardiac/slow skeletal	*Tnnc1*	11.2839	0
T cell receptor alpha constant	*Trac*	11.1193	0
Immunoglobulin kappa variable 3-7	*Igkv3-7*	10.9758	0
CD53 antigen	*Cd53*	10.4683	0
Immunoglobulin kappa variable 3-12	*Igkv3-12*	10.4207	0
Immunoglobulin kappa variable 1-135	*Igkv1-135*	10.0585	0
Chemokine (C-X-C motif) ligand 13	*Cxcl13*	9.5213	0
Trans-2,3-enoyl-coa reductase-like	*Tecrl*	9.3244	0
Complement receptor 2	*Cr2*	9.2697	0
Membrane-spanning 4-domains, subfamily A, member 1	*Ms4a1*	8.6954	0
Atpase, Ca++ transporting, cardiac muscle, slow twitch 2	*Atp2a2*	8.3273	0
Immunoglobulin kappa variable 6-14	*Igkv6-14*	8.3243	6.26188E-11

AD-like condition were correlated with the Database for Annotation, Visualization, and Integrated Discovery and the KEGG pathway mapper to determine the pathways stimulated by AD-like. Based on KEGG pathway analysis, 136 signaling pathways were identified, including cytokine-cytokine receptor interactions, metabolic, nuclear factor (NF)-kappa B signaling, chemokine signaling, B cell receptor signaling, cell adhesion molecules, and T cell receptor signaling ($p < 0.001$). The top 20 significantly regulated pathways are shown in Fig. 2, including cytokine-cytokine receptor interactions, metabolic pathways, cancer-associated pathways, NF-kappa B signaling, chemokine signaling pathways, leukocyte transendothelial migration, hematopoietic cell lineage, B cell receptor signaling pathway, tuberculosis, cell adhesion molecules, T cell receptor signaling, phagosome, regulation of actin cytoskeleton, Th17 cell differentiation, phosphoinositide-3-kinase (PI3K)-Akt signaling pathway, toxoplasmosis, human T-cell lymphotropic virus type I infection, nucleotide-binding oligomerization domain (NOD)-like receptor signaling pathway, Janus kinase-signal transducers and activators of transcription (Jak-STAT) signaling pathway, and measles.

The top 30 pathways except for disease-related pathways, and the top 10 genes for each pathway with their respective p-values are shown in Supplementary Table 2. The significantly regulated pathways included cytokine-cytokine receptor interaction, metabolic, NF-kappa B signaling, chemokine signaling, leukocyte transendothelial migration, hematopoietic cell lineage, B cell receptor signaling, cell adhesion molecules, T cell receptor signaling, phagosome, regulation of actin cytoskeleton, Th17 cell differentiation, PI3K-Akt signaling pathway, NOD-like receptor signaling pathway, Jak-STAT signaling pathway, primary immunodeficiency, osteoclast differentiation, antigen processing and presentation, Th1 and Th2 cell differentiation, intestinal immune network for immunoglobulin A production, endocytosis, natural killer cell-mediated cytotoxicity, mitogen-activated protein kinase signaling pathway, Toll-like receptor signaling pathway, cardiac muscle contraction, adrenergic signaling in cardiomyocytes, hypertrophic cardiomyopathy, cyclic adenosine monophosphate signaling pathway, tumor necrosis factor signaling pathway, and calcium signaling ($p < 0.001$).

Specific to cytokine-cytokine receptor interaction pathways, the analysis showed that *Cxcl13*, interleukin 2 receptor gamma chain (*Il2rg*), *Cd4*, *Il7r*, chemokine ligand 5 (*Ccl5*), lymphotoxin B (*Ltb*), *Ccl22*, *Lep*, *Cd27*, and chemokine receptor 7 (*Ccr7*) were significantly altered in the AD model ($p = 2.7E-36$). Regarding

Table 3. Top 30 down-regulated genes identified by microarray analysis

Gene name	Gene symbol	Fold-change	p-value
Casein alpha s1	*Csn1s1*	–7.6527	2.26399E-11
Casein beta	*Csn2*	–7.3311	7.34889E-11
Mucin 15	*Muc15*	–3.7743	0.00035
Casein kappa	*Csn3*	–3.7083	0.00035
Fc fragment of igg binding protein	*Fcgbp*	–3.6153	0.00010
Casein alpha s2-like A	*Csn1s2a*	–3.2033	0.00587
Lactotransferrin	*Ltf*	–3.0503	0.00373
Myosin binding protein H	*Mybph*	–3.0011	0.00001
Phosphoenolpyruvate carboxykinase 1, cytosolic	*Pck1*	–2.8493	0.00015
Actin, alpha, cardiac muscle 1	*Actc1*	–2.7027	0.00001
Cadherin 4	*Cdh4*	–2.6432	0.00077
Leptin	*Lep*	–2.6211	0.00106
Angiopoietin-like 4	*Angptl4*	–2.6158	0.00290
Amine oxidase, copper containing 3	*Aoc3*	–2.6102	0.00098
Phospholipase A2, group VII (platelet-activating factor acetylhydrolase, plasma)	*Pla2g7*	–2.6038	0.00103
Keratin 18	*Krt18*	–2.6004	0.02743
Thyroid hormone responsive	*Thrsp*	–2.5871	0.00116
Glycogen synthase 2	*Gys2*	–2.5275	0.01502
Lipocalin 2	*Lcn2*	–2.5197	0.00591
Microtubule associated monooxygenase, calponin and LIM domain containing 2	*Mical2*	–2.5104	0.00136
Prolactin receptor	*Prlr*	–2.4817	0.07528
Sine oculis-related homeobox 2	*Six2*	–2.4166	0.03186
Chloride intracellular channel 6	*Clic6*	–2.3845	0.14195
Orosomucoid 1	*Orm1*	–2.3769	0.01169
Lectin, galactose binding, soluble 12	*Lgals12*	–2.3761	0.00568
Protein kinase, AMP-activated, gamma 3 non-catatlytic subunit	*Prkag3*	–2.3624	0.00590
Serine (or cysteine) peptidase inhibitor, clade B, member 9e	*Serpinb9e*	–2.2775	0.25753
Synuclein, gamma	*Sncg*	–2.2739	0.01138
Transmembrane protease, serine 2	*Tmprss2*	–2.2438	0.13079
Calcium channel, voltage-dependent, gamma subunit 1	*Cacng1*	–2.2426	0.01124

metabolic pathways, the following genes were significantly altered: UDP-glucose ceramide glucosyltransferase (*Ugcg*), phosphoenolpyruvate carboxykinase 1 cytosolic (*Pck1*), ADP-ribosyltransferase 2b (*Art2b*), mannosidase 1 alpha (*Man1a*), amine *Aoc3*, phospholipase A2 group 7 (*Pla2g7*), heparinase (*Hpse*), phospholipase C gamma 2 (*Plcg2*), inositol polyphosphate-4-phosphatase type 2 (*Inpp4b*), and *Fasn* (p = 7.5E-28).

GO function analysis of the significant pathways altered in AD

GO function analysis was used to determine the main gene functions affected by HDM treatment. An interaction network of significant GO terms was assembled into a GO map to determine the prominent functional categories. The GO functions of the DEGs were determined according to categories that included biological process, molecular functions, and cellular components. In the biological process category, single-organism, cellular, biological regulation, single-organism cellular, single-organism developmental, developmental, single-multicellular organism, anatomical structure development, and multicellular organismal processes were identified (Fig. 3A). In the cellular component category, extracellular, organelle, membrane-bound, and cytoplasmic function were identified (Fig. 3B). In the molecular component category, protein binding, protein dimerization activity, catalytic activity, anion and cation binding, and metal ion binding functions were identified (Fig. 3C).

The 30 significantly regulated pathways, their p-values, and FDRs are listed in Table 4. Single-organism process, cellular process, biological regulation, single-organism cellular process, cell part, cell, single-organism developmental process, extracellular region, developmental process, organelle, extracellular region part, regulation of biological process, single-multicellular organism process, anatomical structure development, protein binding, multicellular organismal process, binding, multicellular organismal development, system development, membrane-bounded organelle, response to stimulus, small molecule metabolic process, lipid metabolic process, regulation of multicellular organismal process, regulation of cellular process, organ development, negative regulation of biological process, intracellular part, muscle structure development, and cytoplasmic part were significantly altered in response to HDM treatment ($p < 0.001$).

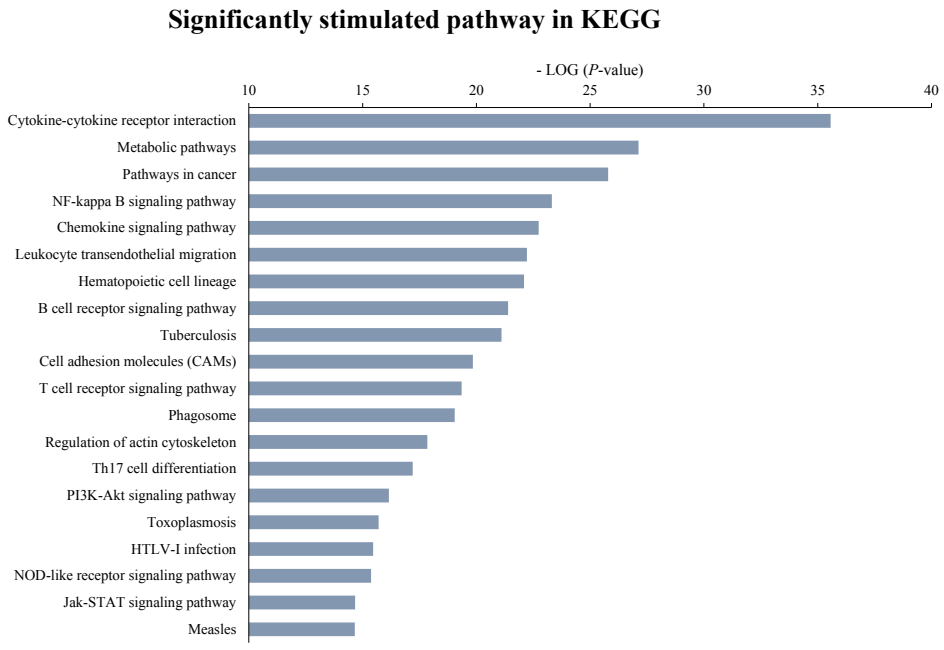


Fig. 2. The top 20 most significantly stimulated pathways in Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis by house dust mite-induced atopy dermatitis-like. The p-value is expressed in log format. NF, nuclear factor; PI3K, phosphoinositide-3-kinase; HTLV-I, human T-cell lymphotropic virus type I; NOD, nucleotide-binding oligomerization domain; Jak-STAT, Janus kinase-signal transducers and activators of transcription.

Correlation of DEGs in interaction network analysis

Based on the GO and KEGG pathway analysis, the protein interaction analysis was performed to determine the hub genes involved in HDM-induced AD-like in the NC/Nga mouse. We used the STRING database of interactions to reveal a putative protein association network based on the microarray data [27]. Network analysis was performed on 187 significant DEGs, 98 of which were found in STRING ($p < 0.01$, $|FC| < 1.5$). The interaction relationship network of genes is shown in Supplementary Fig. 1. The hub genes, *Cxcl5*, *Cxcr13*, *Ccl5*, *Ccl21c*, *Ccr5*, *Ccr7*, actinin alpha 2 (*Actn2*), actin alpha cardiac muscle 1 (*Actc1*), troponin I skeletal slow 1 (*Tnni1*), tropomyosin 3 gamma (*Tpm3*), myosin light polypeptide 3 (*Myl3*), myosin heavy polypeptide 7 cardiac muscle beta (*Myh7*), myosin binding protein C slow-type (*Mybpc1*), troponin T1 skeletal slow (*Tnnt1*), troponin C cardiac/slow skeletal (*Tnnc1*), *Cd3d*, *Cd3e*, *Cd3g*, *Cd4*, *Cd74*, histocompatibility 2 class II antigen A alpha (*H2-aa*), *H2-ab1*, *H2-dma*, cathepsin S (*Ctss*), and protein tyrosine phosphatase non-receptor type 6 (*Ptpn6*), had more than 10 interactions and were located in the center of the network. The chemokines, *Cxcl5, Cxcr13, Ccl5, Ccl21c, Ccr5,* and *Ccr7*, were included [28], as well as genes involved in muscle contraction, such as *Actn2, Actc1, Tnni1, Tpm3, Myl3, Myh7, Mybpc1, Tnnt1, Tnnc1,* and *Tnni1* [29]. The immune and inflammatory response-associated genes, *Cd3d, Cd3e, Cd3g, Cd4, H2-aa, H2-ab1, Cd74, H2-dma, Ctss,* and *Ptpn6*, were also included in the network [30].

Mitochondrial function-related gene effects in response to AD

Based on the KEGG and GO analyses shown in Supplementary Tables 2 and Table 4, respectively, the pathways involved in mitochondrial metabolism, such as calcium signaling pathway (KEGG:04020), cardiac muscle contraction (KEGG:04260), tricarboxylic acid cycle (KEGG:0020), oxidation-reduction process (GO:0055114), calcium-mediated signaling (GO:0019722), and mitochondrion (GO:0005739) were significantly stimulated by AD-like. The DEGs in these pathways involved in mitochondrial function are listed in Table 5. Phospholipase C, delta 4 (*Plcd4*), *Plcg2*, *Tnnc1*, PTK2 protein tyrosine kinase 2 beta (*Ptk2b*), and protein kinase C beta (*Prkcb*), which are associated with calcium signaling, were significantly stimulated (FDR:0.001338772) by KEGG analysis. For cardiac muscle contraction, *Atp2a2, Tnnc1, Tpm2, Tpm3, Tpm4, Actc1, Myh2, Myh3, Myh4,* and *Myh7* were also significantly stimulated (FDR:2.86478E-09). In the tricarboxylic acid cycle, *Pck1*, pyruvate dehydrogenase beta (*Pdhb*), pyruvate carboxylase (*Pcx*), ATP citrate lyase (*Acly*), isocitrate dehydrogenase 2 (*Ldh2*), and dihydrolipoamide dehydrogenase (*Dlb*) were significantly stimulated (FDR:0.001761341). Acetyl-coenzyme A acyltransferase 2 (*Acaa2*), solute carrier family 25 member 25 (*Slc25a25*), cytochrome c oxidase subunit VIIIa (*Cox8a*), cytochrome b-245, beta polypeptide (*Cybb*), *Fasn*, mitochondrial amidoxime reducing component 1 (*Marc1*), extracellular superoxide dismutase 3 (*Sod3*), *Acly*, and uncoupling protein 1 (*Ucp1*), which are mitochondrial metabolism-related genes, were significantly stimulated based on the microarray analysis. The pathways related to mitochondrial function were selected based on previous studies [31,32].

Mitochondrial DNA copy number

We performed mitochondrial copy number analysis via qRT-PCR using gluteal muscle isolated from the NC/Nga mice. Mi-

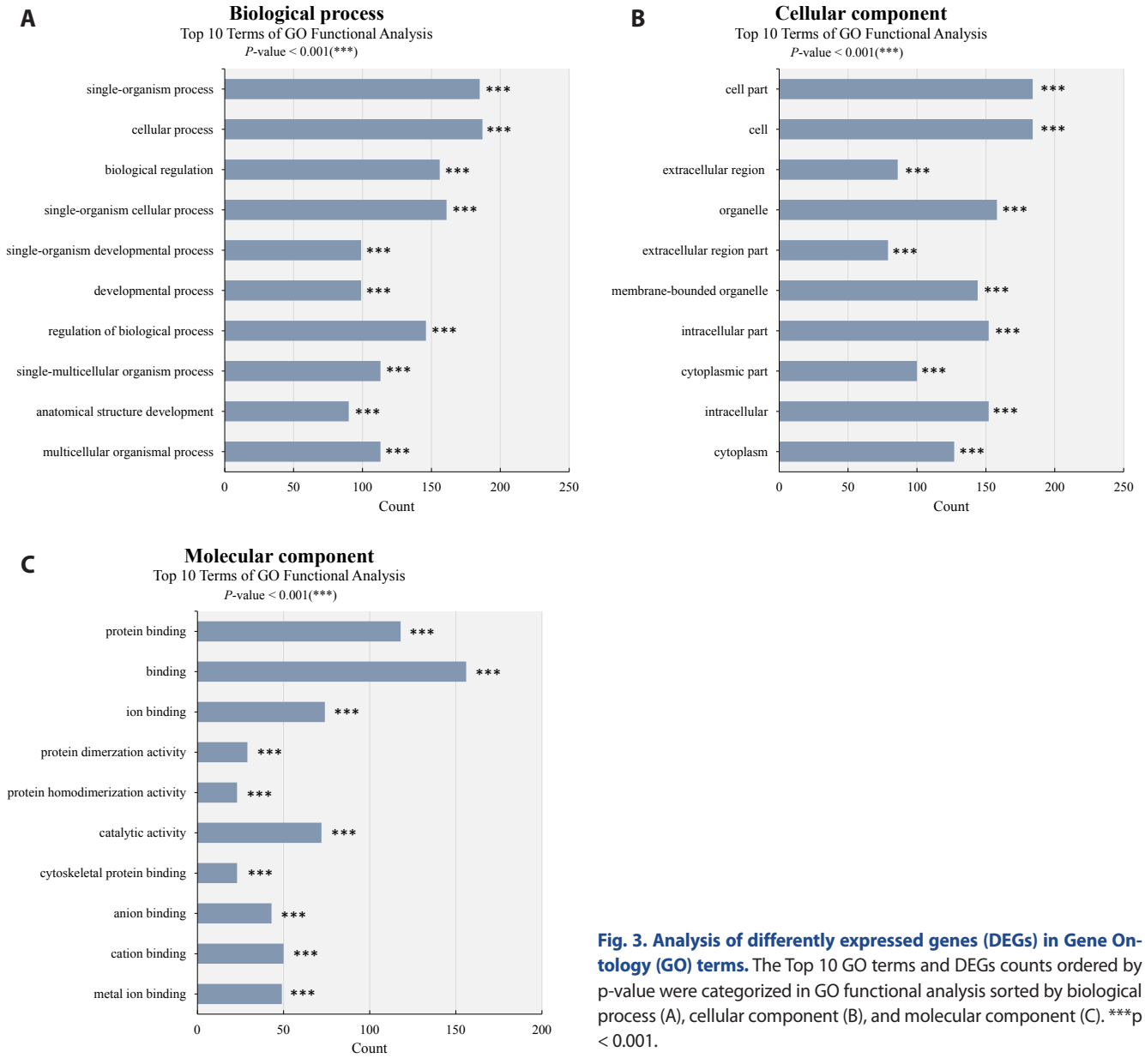


Fig. 3. Analysis of differently expressed genes (DEGs) in Gene Ontology (GO) terms. The Top 10 GO terms and DEGs counts ordered by p-value were categorized in GO functional analysis sorted by biological process (A), cellular component (B), and molecular component (C). ***p < 0.001.

tochondrial copy number measurements were performed three times and averaged. *Nd4*, *D-loop*, and *Cox1* of mtDNA and *18S* of gDNA were intercompared. The results revealed that mtDNA copy numbers were not significantly different between the two groups ($p < 0.01$) (data not shown).

DISCUSSION

Immunoglobulin E measurements were performed to confirm that HDM-induced AD-like. We found that the AD-like group had increased immunoglobulin E levels compared to the control group (data not shown), which was consistent with a previous study indicating that HDM induces AD [33]. In addition, we found that the expression of immune response-associated genes, cytokines, immunoglobulins, and CDs, were up-regulated in the microarray analysis. Thus, HDM successfully induced AD-dependent responses in the gluteal muscle.

Microarray analysis was performed to determine mRNA expression changes in response to HDM-induced AD-like, which identified 421 genes and 136 pathways that were significantly altered. The top 30 significantly regulated pathways identified by KEGG and GO analyses primarily identified immune response-related genes and pathways. Production of cytokines and responses to these molecules are important for regulating immune and inflammatory processes. Chemokines (*Ccl5, Ccl8, Ccl22, Cxcl12, Cxcl13, Ccr7*) and interleukins (*Il7r, Il2rg*) were significantly up-regulated in response to AD-like, which is consistent

Table 4. Top 30 significantly enriched terms identified by Gene Ontology (GO) analysis

GO top 30 term	GO ID	p-value	FDR
Single-organism process	GO:0044699	1.77302E-27	8.2747E-24
Cellular process	GO:0009987	5.07549E-23	1.18436E-19
Biological regulation	GO:0065007	7.69318E-23	1.1968E-19
Single-organism cellular process	GO:0044763	4.1294E-21	3.85806E-18
Cell part	GO:0044464	4.13334E-21	3.85806E-18
Cell	GO:0005623	6.66731E-21	4.5343E-18
Single-organism developmental process	GO:0044767	7.40289E-21	4.5343E-18
Extracellular region	GO:0005576	7.77254E-21	4.5343E-18
Developmental process	GO:0032502	1.2359E-20	6.40885E-18
Organelle	GO:0043226	1.73527E-20	8.0985E-18
Extracellular region part	GO:0044421	2.10197E-20	8.91807E-18
Regulation of biological process	GO:0050789	4.9866E-20	1.93937E-17
Single-multicellular organism process	GO:0044707	8.37892E-20	3.00803E-17
Anatomical structure development	GO:0048856	4.06517E-19	1.35515E-16
Protein binding	GO:0005515	6.31605E-19	1.89788E-16
Multicellular organismal process	GO:0032501	6.50655E-19	1.89788E-16
Binding	GO:0005488	1.85569E-18	5.09442E-16
Multicellular organismal development	GO:0007275	7.09561E-18	1.83973E-15
System development	GO:0048731	8.29342E-18	2.03713E-15
Membrane-bounded organelle	GO:0043227	1.0162E-17	2.3713E-15
Response to stimulus	GO:0050896	2.7812E-17	6.18089E-15
Small molecule metabolic process	GO:0044281	4.80876E-17	1.02011E-14
Lipid metabolic process	GO:0006629	9.70601E-17	1.96948E-14
Regulation of multicellular organismal process	GO:0051239	1.11721E-16	2.1725E-14
Regulation of cellular process	GO:0050794	1.92497E-16	3.59353E-14
Organ development	GO:0048513	5.15211E-16	9.24803E-14
Negative regulation of biological process	GO:0048519	7.10469E-16	1.22806E-13
Intracellular part	GO:0044424	7.75892E-16	1.29325E-13
Muscle structure development	GO:0061061	1.4181E-15	2.28216E-13
Cytoplasmic part	GO:0044444	1.52592E-15	2.37382E-13

FDR, false discovery rate.

with previous studies [34,35]. In addition, we showed that AD-related phenotype genes, *Il18, Il4ra, Il13ra1, Tlr1, Tlr7, Tlr8, Tlr13, Fcer1a, Fcer2a,* and *Spink5l* were upregulated in the microarray analysis [36-38]. *Hspa1a, Stat1, Adam13,* and *Adam23* were also upregulated and may be useful inflammatory markers [39-42]. We have also studied other genes considered to play integral roles in AD-like. We predicted *Igh-v3609n, Glycam1, Igkv5-48, Igkv6-17, Igkj1, Apol7c, Trac, Cdh4, Thrsp, Gys2,* and *Lgals12* to play an important role in the AD pathway by microarray and protein-protein interaction network analysis.

KEGG and GO pathway analyses showed that the pathways represented not only immune response processes but also mitochondrial function. We identified the pathways and genes affected by mitochondria in atopic manifestations and provided a list of mitochondrial-related genes that could be targeted for future treatment of AD-like symptoms. The KEGG pathway analysis showed that the pathways related to calcium signaling, cAMP signaling pathway, cardiac muscle contraction, and citrate cycle were significantly stimulated. The GO pathway analysis showed that the significantly altered genes were related to metabolic process, oxidation-reduction, calcium-mediated signaling, and mitochondrion. Changes in calcium signaling and mitochondrial function-related signaling pathways indicated that AD induction was associated with altered mitochondrial function. Genes involved in the metabolic pathway were *Lep*, *Fasn*, *Aoc3*, *Acly*, *Pck1*, *Ucp1* and ATPase Na^+/K^+ transporting beta 1 polypeptide (*Atp1b1*). Especially, *Acly*, *Pck1*, *Ucp1*, *Atp1b1* also have an essential role in the ATP synthase process [43-46].

We performed mtDNA copy number measurements to quantitatively observe the mitochondrial changes in HDM-induced AD-like group via qRT-PCR. As the number of mitochondria increases, mitochondrial functional outputs such as oxygen consumption and ATP production increase. Therefore, it is necessary to determine the function of individual mitochondria by dividing functional output by number of mitochondria. Our results demonstrated that mitochondria copy number did not change in the AD-like group compared to that of the control group. The changes in mitochondrial functional genes are only expected and a numerical increase does not occur.

We performed protein interaction network analysis to iden-

Table 5. Differentially expressed genes in pathways associated with mitochondrial function by Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) analysis

Pathway name	Gene name	Map ID	Gene symbol	Gene ID	Fold-change	p-value
Metabolic pathway		KEGG:01100				7.50528E-28
	UDP-glucose ceramide glucosyltransferase		*Ugcg*	22234	3.4298	2.20686E-05
	Phosphoenolpyruvate carboxykinase 1, cytosolic		*Pck1*	18534	–2.8493	0.000149194
	ADP-ribosyltransferase 2b		*Art2b*	11872	4.1334	0.000436275
	Mannosidase 1, alpha		*Man1a*	17155	2.6758	0.00092214
	Amine oxidase, copper containing 3		*Aoc3*	11754	–2.6102	0.000982147
	Phospholipase A2, group VII (platelet-activating factor acetylhydrolase, plasma)		*Pla2g7*	27226	–2.6038	0.001028025
	Heparanase		*Hpse*	15442	2.7003	0.002399199
	Phospholipase C, gamma 2		*Plcg2*	234779	2.7840	0.002576994
	Inositol Polyphosphate-4-phosphatase, type II		*Inpp4b*	234515	2.6277	0.003007459
	Fatty acid synthase		*Fasn*	14104	–2.2376	0.004242857
MAPK signaling pathway		KEGG:04010				2.13015E-11
	Protein kinase C, beta		*Prkcb*	18751	4.7940	8.43315E-08
	RAS-related C3 botulinum substrate 2		*Rac2*	19354	2.5858	0.002214744
	Calcium channel, voltage-dependent, gamma subunit 1		*Cacng1*	12299	–2.2426	0.011240559
	RAS related protein 1b		*Rap1b*	215449	2.1587	0.021951174
	TAO kinase 3		*Taok3*	330177	2.0617	0.091109254
	Nuclear receptor subfamily 4, group A, member 1		*Nr4a1*	15370	–1.8287	0.122795267
	RAS-related protein-1a		*Rap1a*	109905	1.8729	0.13788196
	Colony stimulating factor 1 receptor		*Csf1r*	12978	1.8421	0.200175322
	Transforming growth factor, beta 1		*Tgfb1*	21803	1.8611	0.200327384
	Fibroblast growth factor 1		*Fgf1*	14164	1.8555	0.206473803
Cardiac muscle contraction		KEGG:04260				5.07804E-10
	Troponin C, cardiac/slow skeletal		*Tnnc1*	21924	11.2839	0
	Myosin, heavy polypeptide 7, cardiac muscle, beta		*Myh7*	140781	25.3377	0
	ATPase, Ca^{++} transporting, cardiac muscle, slow twitch 2		*Atp2a2*	11938	8.3273	0
	Myosin, light polypeptide 3		*Myl3*	17897	5.7018	4.96498E-10
	Tropomyosin 3, gamma		*Tpm3*	59069	4.0864	8.07949E-08
	Actin, alpha, cardiac muscle 1		*Actc1*	11464	–2.7027	7.15757E-06
	ATPase, Na^+/K^+ transporting, beta 1 polypeptide		*Atp1b1*	11931	3.0179	1.01881E-05
	Calcium channel, voltage-dependent, gamma subunit 1		*Cacng1*	12299	–2.2426	0.011240559
	Tropomyosin 2, beta		*Tpm2*	22004	1.8086	0.067947903
	ATPase, Na^+/K^+ transporting, beta 2 polypeptide		*Atp1b2*	11932	–1.7850	0.123829945
cAMP signaling pathway		KEGG:04024				3.53222E-09
	ATPase, Ca^{++} transporting, cardiac muscle, slow twitch 2		*Atp2a2*	11938	8.3273	0
	ATPase, Na^+/K^+ transporting, beta 1 polypeptide		*Atp1b1*	11931	3.0179	1.01881E-05
	RAS-related C3 botulinum substrate 2		*Rac2*	19354	2.5858	0.002214744
	Vav 1 oncogene		*Vav1*	22324	2.8148	0.002719009
	Rho-associated coiled-coil containing protein kinase 1		*Rock1*	19877	2.3129	0.0088963
	Coagulation factor II (thrombin) receptor		*F2r*	14062	2.2565	0.020547361
	RAS related protein 1b		*Rap1b*	215449	2.1587	0.021951174

Table 5. Continued

Pathway name	Gene name	Map ID	Gene symbol	Gene ID	Fold-change	p-value
	Thyroid stimulating hormone receptor		*Tshr*	22095	–2.2073	0.038586986
	ATPase, Na^+/K^+ transporting, beta 2 polypeptide		*Atp1b2*	11932	–1.7850	0.123829945
	RAS-related protein-1a		*Rap1a*	109905	1.8729	0.13788196
Calcium signaling pathway		KEGG:04020				0.00059103
	Troponin C, cardiac/slow skeletal		*Tnnc1*	21924	11.2839	0
	ATPase, Ca^{++} transporting, cardiac muscle, slow twitch 2		*Atp2a2*	11938	8.3273	0
	Protein kinase C, beta		*Prkcb*	18751	4.7940	8.43315E-08
	PTK2 protein tyrosine kinase 2 beta		*Ptk2b*	19229	2.7416	0.002350974
	Phospholipase C, gamma 2		*Plcg2*	234779	2.7840	0.002576994
	Coagulation factor II (thrombin) receptor		*F2r*	14062	2.2565	0.020547361
	Adrenergic receptor, beta 3		*Adrb3*	11556	–2.0731	0.136087707
	Phospholipase C, delta 4		*Plcd4*	18802	–1.8712	0.149640841
	Phosphorylase kinase gamma 1		*Phkg1*	18682	–1.5328	0.544630124
	Cysteinyl leukotriene receptor 1		*Cysltr1*	58861	1.6576	0.904170421
Citrate cycle (TCA cycle)		KEGG:00020				0.000801145
	Phosphoenolpyruvate carboxykinase 1, cytosolic		*Pck1*	18534	–2.8493	0.000149194
	ATP citrate lyase		*Acly*	104112	–2.0581	0.047110874
	Pyruvate carboxylase		*Pcx*	18563	–1.7369	0.294660312
	Isocitrate dehydrogenase 2 ($NADP^+$), mitochondrial		*Idh2*	269951	1.6504	0.41356214
	Dihydrolipoamide dehydrogenase		*Dld*	13382	1.5054	0.528579466
	Pyruvate dehydrogenase (lipoamide) beta		*Pdhb*	68263	1.5258	0.581213336
Metabolic process		GO:0008152				8.20872E-15
	Myosin, heavy polypeptide 7, cardiac muscle, beta		*Myh7*	140781	9.2396	0
	Troponin C, cardiac/slow skeletal		*Tnnc1*	21924	4.9515	1.56067E-10
	Protein tyrosine phosphatase, receptor type, C		*Ptprc*	19264	1.6772	1.58413E-09
	Cysteine and glycine-rich protein 3		*Csrp3*	13009	4.8441	6.54947E-09
	Complement receptor 2		*Cr2*	12902	1.5179	1.3569E-07
	Sarcolipin		*Sln*	66402	4.7706	7.86542E-05
	Ankyrin repeat domain 23		*Ankrd2*	56642	4.6069	0.000111618
	TATA box binding protein (Tbp)-associated factor, RNA polymerase I, D		*Taf1d*	75316	3.5900	0.000287478
	Fatty acid synthase		*Fasn*	14104	–3.1209	0.00094772
	Egl-9 family hypoxia-inducible factor 3		*Egln3*	112407	3.0094	0.001295714
	Trans-2,3-enoyl-CoA reductase-like		*Tecrl*	243078	4.3562	0.001949038
	ATP citrate lyase		*Acly*	104112	–2.7872	0.006506317
Oxidation-reduction process		GO:0055114				1.13754E-08
	Fatty acid synthase		*Fasn*	14104	–3.1209	0.00094772
	Egl-9 family hypoxia-inducible factor 3		*Egln3*	112407	3.0094	0.001295714
	Trans-2,3-enoyl-coa reductase-like		*Tecrl*	243078	4.3562	0.001949038
	Stearoyl-Coenzyme A desaturase 1		*Scd1*	20249	–2.3497	0.082571436
	Stearoyl-Coenzyme A desaturase 2		*Scd2*	20250	–2.0047	0.35203592
	Aldehyde dehydrogenase 1 family, member L1		*Aldh1l1*	107747	–1.6721	1
	Liver glycogen phosphorylase		*Pygl*	110095	–1.6774	1
	Phosphogluconate dehydrogenase		*Pgd*	110208	–1.6719	1
	Adiponectin, C1Q and collagen domain containing		*Adipoq*	11450	–2.4945	1
	Amine oxidase, copper containing 3		*Aoc3*	11754	–2.9607	1

Table 5. Continued

Pathway name	Gene name	Map ID	Gene symbol	Gene ID	Fold-change	p-value
Calcium-mediated signaling		GO:0019722				1.81205E-05
	ATPase, Ca^{++} transporting, cardiac muscle, slow twitch 2		*Atp2a2*	11938	3.7937	3.52028E-09
	Calsequestrin 2		*Casq2*	12373	2.5131	0.082571436
	ATPase, Na^+/K^+ transporting, beta 1 polypeptide		*Atp1b1*	11931	1.9500	0.773408074
	Neural cell adhesion molecule 1		*Ncam1*	17967	1.5694	1
	Homer homolog 2 (Drosophila)		*Homer2*	26557	1.5143	1
	LIM and cysteine-rich domains 1		*Lmcd1*	30937	1.7912	1
	Regulator of calcineurin 1		*Rcan1*	54720	1.6215	1
	Transmembrane protein 100		*Tmem100*	67888	1.6551	1
Mitochondrion		GO:0005739				0.000173505
	Troponin C, cardiac/slow skeletal		*Tnnc1*	21924	4.9515	1.56067E-10
	Fatty acid synthase		*Fasn*	14104	–3.1209	0.00094772
	ATP citrate lyase		*Acly*	104112	–2.7872	0.006506317
	DNA-damage-inducible transcript 4		*Ddit4*	74747	–1.9880	0.923075132
	Acetyl-Coenzyme A carboxylase alpha		*Acaca*	107476	–1.8971	1
	Aldehyde dehydrogenase 1 family, member L1		*Aldh1l1*	107747	–1.6721	1
	Cell death-inducing DNA fragmentation factor, alpha subunit-like effector A		*Cidea*	12683	–1.6864	1
	Cytochrome c oxidase subunit via polypeptide 1		*Cox6a1*	12861	–1.6562	1
	Cytochrome c oxidase subunit viiia		*Cox8a*	12868	–1.7344	1
	Solute carrier family 25 (mitochondrial carrier, citrate transporter), member 1		*Slc25a1*	13358	–2.0698	1

tify the hub genes in 187 DEGs, which were screened in GO and KEGG analysis. The protein interaction network of the target gene can reveal the protein-protein interaction of HDM-induced AD-like in NC/Nga mice. Hub proteins identified were included in the chemokine family, muscle contraction process, inflammatory response, and immune response. Several hub proteins in the protein interaction networks might be associated with an AD-like-related pathway, such as *Cxcl13, Cd4, Il7,* and *CCr7* in cytokine interactions (Supplementary Fig. 1).

We identified the association between AD and mitochondrial functions at the gene level and determined the stimulated genes and pathways. In summary, our study deepens our understanding of the genome-wide transcriptional changes of HDM-induced AD-like symptoms in the skeletal muscle of NC/Nga mice, providing to the discovery of genes that could be used as AD clinical biomarkers.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

SUPPLEMENTARY MATERIALS

Supplementary data including two tables and one figure can be found with this article online at http://pdf.medrang.co.kr/paper/pdf/Kjpp/Kjpp2019-23-05-10-s001.pdf.

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Supplementary Table 1. The significant differentially expressed genes in response to atopic dermatitis (AD) based on the microarray analysis (p < 0.05)

Gene symbol	Gene description	Fold-change	p-value
Ptprc	Protein tyrosine phosphatase, receptor type, c	14.904024	0
Cr2	Complement receptor 2	9.269651	0
Myh2	Myosin, heavy polypeptide 2, skeletal muscle, adult	5.214414	0
Igh-V3609N	Immunoglobulin heavy chain (v3609n non-productive)	37.175365	0
Igh-VX24	Immunoglobulin heavy chain (x24 family)	14.296995	0
Ighv3-2	Immunoglobulin heavy variable 3-2	11.854764	0
Ighv1-34	Immunoglobulin heavy variable 1-34	11.746874	0
Ighv1-62-1	Immunoglobulin heavy variable 1-62-1	7.392181	0
Tnnc1	Troponin c, cardiac/slow skeletal	11.283909	0
Trac	T cell receptor alpha constant	11.119307	0
Myh7	Myosin, heavy polypeptide 7, cardiac muscle, beta	25.337723	0
Apol7c	Apolipoprotein L 7c	12.159820	0
Glycam1	Glycosylation dependent cell adhesion molecule 1	35.427801	0
Ankrd2	Ankyrin repeat domain 2 (stretch responsive muscle)	7.473013	0
Ms4a1	Membrane-spanning 4-domains, subfamily A, member 1	8.695355	0
Cd53	CD53 antigen	10.468298	0
Myoz2	Myozenin 2	7.686757	0
Bank1	B cell scaffold protein with ankyrin repeats 1	6.687630	0
Gm13304	Predicted gene 13304	7.324321	0
Gm13304	Predicted gene 13304	7.324321	0
Gm13304	Predicted gene 13304	7.183739	0
Gm13304	Predicted gene 13304	7.324321	0
Gm1987	Predicted gene 1987	7.250950	0
LOC100041504	C-C motif chemokine 21c	6.578415	0
Gm13304	Predicted gene 13304	7.324321	0
Gm13304	Predicted gene 13304	7.183739	0
Gm1987	Predicted gene 1987	8.018598	0
Cxcl13	Chemokine (C-X-C motif) ligand 13	9.521342	0
Tecrl	Trans-2,3-enoyl-coa reductase-like	9.324394	0
Atp2a2	ATPase, Ca++ transporting, cardiac muscle, slow twitch 2	8.327278	0
Igkv1-135	Immunoglobulin kappa variable 1-135	10.058547	0
Igkv6-17	Immunoglobulin kappa variable 6-17	18.744884	0
Igkv3-12	Immunoglobulin kappa variable 3-12	10.420662	0
Igkv3-7	Immunoglobulin kappa variable 3-7	10.975806	0

Igkj1	Immunoglobulin kappa joining 1	14.631543	0
Igk-V21	Immunoglobulin kappa chain variable 21 (V21)	6.750012	0
Igkv6-23	Immunoglobulin kappa variable 6-23	15.888819	0
Igkv10-96	Immunoglobulin kappa variable 10-96	16.205201	0
Igkv4-72	Immunoglobulin kappa chain variable 4-72	12.974032	0
Igkv4-70	Immunoglobulin kappa chain variable 4-70	18.687932	0
Igkv4-62	Immunoglobulin kappa variable 4-62	7.552373	0
Igkv4-57-1	Immunoglobulin kappa variable 4-57-1	16.235839	0
Igkv5-48	Immunoglobulin kappa variable 5-48	19.271941	0
Csrp3	Cysteine and glycine-rich protein 3	15.609420	0
Sln	Sarcolipin	8.089946	0
Cd3g	CD3 antigen, gamma polypeptide	12.840638	0
Sell	Selectin, lymphocyte	8.134169	1.91259E-13
Il2rg	Interleukin 2 receptor, gamma chain	6.883322	1.91259E-13
Ctss	Cathepsin S	6.263244	3.60017E-13
Jchain	Immunoglobulin joining chain	8.232525	3.60017E-13
Timd4	T cell immunoglobulin and mucin domain containing 4	7.174956	5.2964E-13
Igkv6-13	Immunoglobulin kappa variable 6-13	8.012570	1.55894E-12
Lmod2	Leiomodin 2 (cardiac)	4.899943	2.0401E-12
Igk-V1	Immunoglobulin kappa chain variable 1 (V1)	7.993681	2.13117E-12
Cd4	CD4 antigen	7.546538	2.13117E-12
Il7r	Interleukin 7 receptor	7.950139	3.06014E-12
Tnnt1	Troponin T1, skeletal, slow	5.871320	3.32395E-12
Pyhin1	Pyrin and HIN domain family, member 1	7.756684	5.96728E-12
Iglv2	Immunoglobulin lambda variable 2	7.651804	6.17046E-12
Tcrb-J	T cell receptor beta, joining region	5.278160	1.31784E-11
Csn1s1	Casein alpha s1	–7.652706	2.26399E-11
H2-Ea-ps	Histocompatibility 2, class II antigen E alpha, pseudogene	4.982432	2.5564E-11
Igkv4-68	Immunoglobulin kappa variable 4-68	6.247916	3.63044E-11
Igkv6-14	Immunoglobulin kappa variable 6-14	8.324335	6.26188E-11
Csn2	Casein beta	–7.331127	7.34889E-11
Ighv9-2	Immunoglobulin heavy variable V9-2	7.341882	7.98299E-11
Ms4a6b	Membrane-spanning 4-domains, subfamily A, member 6B	7.197196	1.07542E-10
Igkv19-93	Immunoglobulin kappa chain variable 19-93	6.199621	1.66024E-10
Tnni1	Troponin I, skeletal, slow 1	5.503445	3.53957E-10
Myl3	Myosin, light polypeptide 3	5.701751	4.96498E-10
Cd209b	CD209b antigen	5.034680	7.14709E-10
Igh-V7183	Immunoglobulin heavy chain (v7183 family)	4.423643	1.26823E-09

Stk17b	Serine/threonine kinase 17b (apoptosis-inducing)	4.588190	1.61325E-09
Cd52	CD52 antigen	6.049832	1.64447E-09
Ly86	Lymphocyte antigen 86	5.631171	2.60724E-09
Dock10	Dedicator of cytokinesis 10	4.793701	3.78062E-09
Gm8995	Predicted gene 8995	5.256454	4.04914E-09
Ighg	Immunoglobulin heavy chain (gamma polypeptide)	5.338132	1.16695E-08
Egln3	Egl-9 family hypoxia-inducible factor 3	4.062182	1.19759E-08
Traj35	T cell receptor alpha joining 35	6.705570	1.24724E-08
Mybpc1	Myosin binding protein c, slow-type	2.901020	1.25686E-08
Slfn2	Schlafen 2	4.487121	1.41127E-08
Lcp1	Lymphocyte cytosolic protein 1	4.184079	2.97426E-08
Myom3	Myomesin family, member 3	4.359900	3.44233E-08
Epsti1	Epithelial stromal interaction 1 (breast)	4.864798	3.85288E-08
Selplg	Selectin, platelet (p-selectin) ligand	4.860636	4.06416E-08
Smtnl1	Smoothelin-like 1	3.995871	5.26356E-08
Tpm3	Tropomyosin 3, gamma	4.086413	8.07949E-08
Prkcb	Protein kinase c, beta	4.793967	8.43315E-08
Rnu3a	U3A small nuclear RNA	3.675371	1.90929E-07
Ighv1-75	Immunoglobulin heavy variable 1-75	4.139883	2.35356E-07
Pax5	Paired box 5	4.831680	3.18778E-07
Igkv4-86	Immunoglobulin kappa variable 4-86	4.246679	4.38824E-07
Ccl5	Chemokine (C-C motif) ligand 5	3.784414	4.82844E-07
Ighv1-62-2	Immunoglobulin heavy variable 1-62-2	4.397474	4.84557E-07
Ighv1-71	Immunoglobulin heavy variable 1-71	4.397474	4.84557E-07
Traj40	T cell receptor alpha joining 40	6.083957	5.07824E-07
Bin2	Bridging integrator 2	4.498675	7.82775E-07
Cybb	Cytochrome b-245, beta polypeptide	3.860613	8.36122E-07
Igkj3	Immunoglobulin kappa joining 3	4.200977	9.03313E-07
Casq2	Calsequestrin 2	3.742455	1.09762E-06
Iglv1	Immunoglobulin lambda variable 1	4.595462	1.58174E-06
LOC101055672	Nuclear body protein SP140-like	3.820298	1.62019E-06
Aspn	Asporin	3.594734	1.80859E-06
LOC102633783	Sp110 nuclear body protein-like	3.957996	1.88054E-06
Lyz1	Lysozyme 1	3.689218	2.53437E-06
Arhgap15	Rho GTPase activating protein 15	4.197892	2.65577E-06
Rnu3b1	U3B small nuclear RNA 1	2.943474	3.12093E-06

Rnu3b1	U3B small nuclear RNA 1	2.943474	3.12093E-06
Rnu3b4	U3B small nuclear RNA 4	2.943474	3.12093E-06
Rnu3b4	U3B small nuclear RNA 4	2.943474	3.12093E-06
Igkv1-117	Immunoglobulin kappa variable 1-117	4.924544	3.13745E-06
Lilrb4a	Leukocyte immunoglobulin-like receptor, subfamily B, member 4A	4.048956	3.20735E-06
Cd22	CD22 antigen	4.356516	3.20735E-06
Fcmr	Fc fragment of IgM receptor	4.248151	3.72714E-06
Ctse	Cathepsin e	3.722766	3.93616E-06
Dock2	Dedicator of cyto-kinesis 2	3.698615	4.06357E-06
Sp100	Nuclear antigen Sp100	3.476896	5.93746E-06
Cd79a	CD79A antigen (immunoglobulin-associated alpha)	3.830705	6.00136E-06
Mybph	Myosin binding protein H	–3.001139	6.62407E-06
Ikzf1	IKAROS family zinc finger 1	4.316494	6.71261E-06
Igkv4-59	Immunoglobulin kappa variable 4-59	5.134593	7.04713E-06
Agbl1	ATP/GTP binding protein-like 1	3.555864	7.04713E-06
Traj21	T cell receptor alpha joining 21	4.012677	7.15757E-06
Actc1	Actin, alpha, cardiac muscle 1	–2.702651	7.15757E-06
Lbh	Limb-bud and heart	3.365440	8.28182E-06
Mpeg1	Macrophage expressed gene 1	3.577967	8.91416E-06
Igkv4-50	Immunoglobulin kappa variable 4-50	4.836053	9.4246E-06
Atp1b1	ATPase, Na+/K+ transporting, beta 1 polypeptide	3.017942	1.01881E-05
H2-M2	Histocompatibility 2, m region locus 2	4.329454	1.07827E-05
Cmah	Cytidine monophospho-n-acetylneuraminic acid hydroxylase	3.766801	1.11673E-05
Ikzf3	IKAROS family zinc finger 3	4.393939	1.131E-05
Ralgps2	Ral GEF with PH domain and SH3 binding motif 2	3.866063	1.21918E-05
Igkv4-55	Immunoglobulin kappa variable 4-55	3.617229	1.35831E-05
Igh-VJ558	Immunoglobulin heavy chain (J558 family)	4.327954	1.59541E-05
Gm24411	Predicted gene, 24411 [Source:MGI Symbol;Acc:MGI:5454188]	3.536678	1.7598E-05
Igkv10-94	Immunoglobulin kappa variable 10-94	4.260507	1.95558E-05
Actn2	Actinin alpha 2	2.493989	2.06104E-05
Cd180	CD180 antigen	4.626464	2.20686E-05
Ugcg	UDP-glucose ceramide glucosyltransferase	3.429754	2.20686E-05
Lyz2	Lysozyme 2	3.057866	2.4385E-05
Ighv2-9	Immunoglobulin heavy variable 2-9	3.281249	2.9176E-05
Ighv1-62-3	Immunoglobulin heavy variable 1-62-3	3.879713	3.12877E-05
Gm10717	Predicted gene 10717 [Source:MGI	–3.067450	3.26443E-05

	Symbol;Acc:MGI:3642031]		
Ltb	Lymphotoxin b	3.352646	3.66469E-05
Itgb2	Integrin beta 2	3.275341	3.75636E-05
Igkv4-73	Immunoglobulin kappa variable 4-73	3.521268	4.11459E-05
Lgi1	Leucine-rich repeat lgi family, member 1	4.068198	4.34605E-05
P2ry10	Purinergic receptor p2y, g-protein coupled 10	4.712475	4.36156E-05
Gm24119	Predicted gene, 24119 [source:mgi symbol;acc:mgi:5453896]	2.975052	4.79831E-05
Ptpn22	Protein tyrosine phosphatase, non-receptor type 22 (lymphoid)	4.013887	4.79831E-05
Ighv1-69	Immunoglobulin heavy variable 1-69	4.604007	4.84353E-05
Pip4k2a	Phosphatidylinositol-5-phosphate 4-kinase, type II, alpha	3.246961	4.87833E-05
Nckap1l	NCK associated protein 1 like	3.319015	5.04094E-05
Ptpn6	Protein tyrosine phosphatase, non-receptor type 6	3.204957	5.88237E-05
2310075C17 Rik	RIKEN cDNA 2310075C17 gene	2.996244	6.34617E-05
Gm23444	Predicted gene, 23444 [Source:MGI Symbol;Acc:MGI:5453221]	2.415221	7.79215E-05
Gm24497	Predicted gene, 24497 [Source:MGI Symbol;Acc:MGI:5454274]	2.415221	7.79215E-05
Gm25939	Predicted gene, 25939 [Source:MGI Symbol;Acc:MGI:5455716]	2.415221	7.79215E-05
Gm23444	Predicted gene, 23444 [Source:MGI Symbol;Acc:MGI:5453221]	2.415221	7.79215E-05
Gm10719	Predicted gene 10719 [Source:MGI Symbol;Acc:MGI:3641690]	–2.964225	7.79215E-05
Traj12	T cell receptor alpha joining 12	3.441697	7.89626E-05
Snord13	Small nucleolar RNA, C/D box 13	2.759410	9.10405E-05
LOC100041034	Sp110 nuclear body protein pseudogene	3.049790	9.77277E-05
Fcgbp	Fc fragment of IgG binding protein	–3.615312	9.98493E-05
Lcp2	Lymphocyte cytosolic protein 2	3.075050	0.000100617
Rnu12	RNA U12, small nuclear	2.843770	0.000104358
Klhl6	Kelch-like 6	3.495927	0.000112613
Ankrd1	Ankyrin repeat domain 1 (cardiac muscle)	2.872695	0.000122828
Cd74	CD74 antigen	2.826957	0.000130769
Ighv1-7	Immunoglobulin heavy variable V1-7	3.206801	0.000133321
Pck1	Phosphoenolpyruvate carboxykinase 1, cytosolic	–2.849344	0.000149194
Ighm	Immunoglobulin heavy constant mu	4.420838	0.000152748

Gm25360	Predicted gene, 25360 [Source:MGI Symbol;Acc:MGI:5455137]	2.381078	0.000194401
Ccl22	Chemokine (C-C motif) ligand 22	3.350961	0.000217941
Taf1d	TATA box binding protein (Tbp)-associated factor, RNA polymerase I, D	2.917272	0.00022316
Cd79b	CD79B antigen	3.397656	0.000261267
Gm25813	Predicted gene, 25813 [Source:MGI Symbol;Acc:MGI:5455590]	2.511858	0.000261267
Gm23927	Predicted gene, 23927 [Source:MGI Symbol;Acc:MGI:5453704]	2.771314	0.000269543
Kctd12	Potassium channel tetramerisation domain containing 12	2.919315	0.00027062
LOC102636558	Component of Sp100-rs-like	3.322214	0.000275081
Hspb7	Heat shock protein family, member 7 (cardiovascular)	2.676810	0.000321287
Traj38	T cell receptor alpha joining 38	3.641670	0.000322536
Iglv3	Immunoglobulin lambda variable 3	3.908649	0.000332243
Trbj1-1	T cell receptor beta joining 1-1	3.891955	0.000347005
Muc15	Mucin 15	–3.774315	0.000352101
Csn3	Casein kappa	–3.708305	0.000352101
Igkv12-46	Immunoglobulin kappa variable 12-46	3.499551	0.00040599
Gm10719	Predicted gene 10719 [Source:MGI Symbol;Acc:MGI:3641690]	–3.235211	0.00041192
Stap1	Signal transducing adaptor family member 1	3.754589	0.000431204
Art2b	ADP-ribosyltransferase 2b	4.133389	0.000436275
Itgal	Integrin alpha L	3.076969	0.000441262
Igkv8-24	Immunoglobulin kappa chain variable 8-24	4.110603	0.000540103
Gm9800	Predicted gene 9800 [Source:MGI Symbol;Acc:MGI:3710580]	2.606294	0.00054366
Cpa3	Carboxypeptidase A3, mast cell	2.769480	0.00054366
Snora3	Small nucleolar RNA, H/ACA box 3	2.565402	0.000596982
H2-DMb2	Histocompatibility 2, class II, locus Mb2	3.155919	0.000690586
Traj27	T cell receptor alpha joining 27	3.768081	0.000724914
Cdh4	Cadherin 4	–2.643226	0.000769449
Ighm	Immunoglobulin heavy constant mu	2.609150	0.000803644
Igkv4-69	Immunoglobulin kappa variable 4-69	3.795987	0.000832927
Man1a	Mannosidase 1, alpha	2.675837	0.00092214
Aoc3	Amine oxidase, copper containing 3	–2.610181	0.000982147
Ms4a4c	Membrane-spanning 4-domains, subfamily A, member 4C	3.569248	0.000983903

Stat4	Signal transducer and activator of transcription 4	3.533126	0.000984191
Dock11	Dedicator of cytokinesis 11	2.789961	0.000984191
Pla2g7	Phospholipase A2, group VII (platelet-activating factor acetylhydrolase, plasma)	–2.603766	0.001028025
Lep	Leptin	–2.621096	0.0010592
Cytip	Cytohesin 1 interacting protein	3.446782	0.001074213
Slamf6	SLAM family member 6	3.275000	0.001092862
Enah	Enabled homolog (Drosophila)	2.646976	0.00111811
Ighv7-3	Immunoglobulin heavy variable 7-3	3.895045	0.001134759
Thrsp	Thyroid hormone responsive	–2.587099	0.0011567
Slfn9	Schlafen 9	3.464999	0.0012256
Lef1	Lymphoid enhancer binding factor 1	3.183492	0.001229218
Btla	B and T lymphocyte associated	3.413153	0.001258718
Mical2	Microtubule associated monooxygenase, calponin and LIM domain containing 2	–2.510413	0.001358841
Siglec1	Sialic acid binding Ig-like lectin 1, sialoadhesin	2.928395	0.00144171
Eml4	Echinoderm microtubule associated protein like 4	2.781995	0.00167963
Ighv1-19	Immunoglobulin heavy variable V1-19	3.367330	0.001695767
Actr3	ARP3 actin-related protein 3	2.488222	0.001781265
Tcrb-J	T cell receptor beta, joining region	3.080586	0.001795145
Cd27	CD27 antigen	3.275897	0.001810382
Gbp4	Guanylate binding protein 4	2.815205	0.001955207
Cd84	CD84 antigen	2.883708	0.001965417
BE692007	Expressed sequence BE692007	3.687109	0.002064728
Fhl1	Four and a half LIM domains 1	2.551490	0.002097917
Rac2	RAS-related C3 botulinum substrate 2	2.585754	0.002214744
Fcer2a	Fc receptor, IgE, low affinity II, alpha polypeptide	3.349603	0.002259893
Srgn	Serglycin	2.498791	0.002340615
Ccr7	Chemokine (C-C motif) receptor 7	2.873163	0.002340615
Ptk2b	PTK2 protein tyrosine kinase 2 beta	2.741604	0.002350974
Hpse	Heparanase	2.700338	0.002399199
Traj20	T cell receptor alpha joining 20	3.597588	0.002402298
Ms4a6c	Membrane-spanning 4-domains, subfamily A, member 6C	3.251015	0.002402298
Apbb1ip	Amyloid beta (A4) precursor protein-binding, family B, member 1 interacting protein	2.773581	0.002402298
Igkv15-103	Immunoglobulin kappa chain variable 15-103	2.746025	0.002439022
Tespa1	Thymocyte expressed, positive selection associated 1	3.448371	0.002505782
Plcg2	Phospholipase C, gamma 2	2.783982	0.002576994

Vav1	Vav 1 oncogene	2.814786	0.002719009
Gm18853	GTPase, very large interferon inducible 1 pseudogene	2.520037	0.002847865
Serpinb1a	Serine (or cysteine) peptidase inhibitor, clade B, member 1a	2.742953	0.002883344
Angptl4	Angiopoietin-like 4	–2.615778	0.002902851
Igsf6	Immunoglobulin superfamily, member 6	2.910930	0.002999457
Trim59	Tripartite motif-containing 59	3.038133	0.002999564
Mb	Myoglobin	2.275329	0.003007459
Lmcd1	LIM and cysteine-rich domains 1	2.343687	0.003007459
Inpp4b	Inositol polyphosphate-4-phosphatase, type II	2.627672	0.003007459
Gm10267	Predicted gene 10267	3.319325	0.003018581
Ccdc88a	Coiled coil domain containing 88A	2.548326	0.003365085
Wdfy4	WD repeat and FYVE domain containing 4	2.708596	0.003365085
Itga4	Integrin alpha 4	2.557386	0.003365085
Cd3e	CD3 antigen, epsilon polypeptide	2.555924	0.00337812
Gm10718	Predicted gene 10718 [Source:MGI Symbol;Acc:MGI:3642028]	–2.415832	0.003535879
Prg4	Proteoglycan 4 (megakaryocyte stimulating factor, articular superficial zone protein)	2.631609	0.00356826
Pydc4	Pyrin domain containing 4	3.311053	0.003585987
Traj30	T cell receptor alpha joining 30	3.432632	0.003659721
Gm8989	Very large inducible GTPase 1 pseudogene	2.457148	0.003666822
Gm24525	Predicted gene, 24525 [Source:MGI Symbol;Acc:MGI:5454302]	2.497172	0.003733774
Ltf	Lactotransferrin	–3.050276	0.003733774
Gm24588	Predicted gene, 24588 [Source:MGI Symbol;Acc:MGI:5454365]	3.162356	0.003830879
Cd96	CD96 antigen	3.492633	0.003834672
Fasn	Fatty acid synthase	–2.237635	0.004242857
Arhgdib	Rho, GDP dissociation inhibitor (GDI) beta	2.615941	0.004321403
Cfh	Complement component factor h	2.397357	0.004412941
Cyth4	Cytohesin 4	2.452180	0.004929348
Cyfip2	Cytoplasmic FMR1 interacting protein 2	2.555021	0.005254131
Sat1	Spermidine/spermine N1-acetyl transferase 1	2.430874	0.005520293
Igh-V7183	Immunoglobulin heavy chain (V7183 family)	3.430788	0.005555156
Lgals12	Lectin, galactose binding, soluble 12	–2.376099	0.005680479
Gm8979	Very large inducible GTPase 1 pseudogene	2.371328	0.005747562
Csn1s2a	Casein alpha s2-like A	–3.203280	0.005874172
Mir5099	MicroRNA 5099	–1.657036	0.005876739

Prkag3	Protein kinase, AMP-activated, gamma 3 non-catatlytic subunit	–2.362444	0.005895163
Lcn2	Lipocalin 2	–2.519697	0.005913239
Igkv5-43	Immunoglobulin kappa chain variable 5-43	3.207857	0.006163646
Akna	AT-hook transcription factor	2.625396	0.006281686
Gm10715	Predicted gene 10715 [Source:MGI Symbol;Acc:MGI:3642376]	–2.351555	0.006347041
Cxcr5	Chemokine (C-X-C motif) receptor 5	2.742678	0.006354229
St3gal5	ST3 beta-galactoside alpha-2,3-sialyltransferase 5	2.379775	0.006500756
Snora7a	Small nucleolar RNA, H/ACA box 7A	2.343346	0.006521848
Igkv4-79	Immunoglobulin kappa variable 4-79	2.546119	0.006547125
Ptpn3	Protein tyrosine phosphatase, non-receptor type 3	2.352541	0.007310712
Dock8	Dedicator of cytokinesis 8	2.459065	0.007723878
Igkv8-21	Immunoglobulin kappa variable 8-21	2.989274	0.008329242
Ifi203	Interferon activated gene 203	2.439668	0.00841883
Rapgef6	Rap guanine nucleotide exchange factor (GEF) 6	2.470144	0.00841883
AI504432	Expressed sequence AI504432	3.251240	0.008540969
Baz1a	Bromodomain adjacent to zinc finger domain 1A	2.446255	0.008631044
Rock1	Rho-associated coiled-coil containing protein kinase 1	2.312855	0.0088963
Fam111a	Family with sequence similarity 111, member A	2.724582	0.0088963
Lyve1	Lymphatic vessel endothelial hyaluronan receptor 1	2.356050	0.009002276
Igkv17-127	Immunoglobulin kappa variable 17-127	3.261849	0.009027763
Arhgap30	Rho GTPase activating protein 30	2.467782	0.009376338
Traj22	T cell receptor alpha joining 22	2.996711	0.010071581
Igkv6-20	Immunoglobulin kappa variable 6-20	2.798250	0.010071581
Cxcr4	Chemokine (C-X-C motif) receptor 4	2.420030	0.010165808
Arhgap25	Rho GTPase activating protein 25	2.625551	0.010440601
6330407A03 Rik	RIKEN cDNA 6330407A03 gene	2.778295	0.010508163
Lrrn1	Leucine rich repeat protein 1, neuronal	2.547011	0.010509001
Gm26440	Predicted gene, 26440 [Source:MGI Symbol;Acc:MGI:5456217]	2.271515	0.010746776
Dpp4	Dipeptidylpeptidase 4	2.436162	0.010785965
F8	Coagulation factor VIII	2.822336	0.01094913
Cacng1	Calcium channel, voltage-dependent, gamma subunit 1	–2.242635	0.011240559
Traj2	T cell receptor alpha joining 2	3.174908	0.011240559
Sncg	Synuclein, gamma	–2.273886	0.011378523
Myh1	Myosin, heavy polypeptide 1, skeletal muscle, adult	1.777057	0.011441903

Fcrl1	Fc receptor-like 1	2.774110	0.011441903
Gm17757	GTPase, very large interferon inducible 1 pseudogene	2.315671	0.011441903
Orm1	Orosomucoid 1	–2.376931	0.011692644
Bcl11a	B cell CLL/lymphoma 11A (zinc finger protein)	2.791131	0.011820853
Stat1	Signal transducer and activator of transcription 1	2.260442	0.012104993
Ptbp3	Polypyrimidine tract binding protein 3	2.278801	0.012104993
Id2	Inhibitor of DNA binding 2	2.426909	0.012555446
Gm22973	Predicted gene, 22973 [Source:MGI Symbol;Acc:MGI:5452750]	2.609394	0.012571734
Snora74a	Small nucleolar RNA, H/ACA box 74A	2.234853	0.012919013
Hmha1	Histocompatibility (minor) HA-1	2.421129	0.013346186
Tcf7	Transcription factor 7, T cell specific	2.380336	0.01384378
Traj43	T cell receptor alpha joining 43	2.460199	0.014085245
Tnc	Tenascin C	2.536132	0.014119702
Traj36	T cell receptor alpha joining 36	3.175844	0.014229073
Ifi44	Interferon-induced protein 44	2.298990	0.014419417
Gm4759	GTPase, very large interferon inducible 1 pseudogene	3.160680	0.014857094
LOC101055663	Component of Sp100-rs-like	3.163705	0.014909621
Gys2	Glycogen synthase 2	–2.527472	0.015019358
Dnase1l3	Deoxyribonuclease 1-like 3	2.804201	0.015116738
Sim2	Single-minded homolog 2 (Drosophila)	–2.225454	0.015235525
Gpr65	G-protein coupled receptor 65	2.788038	0.015883464
Igkv12-44	Immunoglobulin kappa variable 12-44	2.704946	0.016083404
Aff3	AF4/FMR2 family, member 3	2.413924	0.01616958
Ly6c2	Lymphocyte antigen 6 complex, locus C2	2.323758	0.016384861
Ifi30	Interferon gamma inducible protein 30	2.229639	0.017229862
B3gnt5	UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 5	2.829555	0.017694507
Gm22155	Predicted gene, 22155 [Source:MGI Symbol;Acc:MGI:5451932]	2.212604	0.017815488
Plek	Pleckstrin	2.460634	0.01804354
Spink11	Serine peptidase inhibitor, Kazal type 11	3.049093	0.018146645
Fabp3-ps1	Fatty acid binding protein 3, muscle and heart, pseudogene 1	2.182830	0.018266666
Inpp5d	Inositol polyphosphate-5-phosphatase D	2.507283	0.018856792
Trbv31	T cell receptor beta, variable 31	2.645976	0.019839065
F2r	Coagulation factor II (thrombin) receptor	2.256513	0.020547361
Gm22676	Predicted gene, 22676 [Source:MGI	2.378653	0.020870804

	Symbol;Acc:MGI:5452453]		
Cd8b1	CD8 antigen, beta chain 1	2.698308	0.021186091
Gm20506	Predicted gene 20506	3.068886	0.021451884
Smc4	Structural maintenance of chromosomes 4	2.221293	0.021547268
Rap1b	RAS related protein 1b	2.158666	0.021951174
Ogn	Osteoglycin	2.159092	0.021986785
St6gal1	Beta galactoside alpha 2,6 sialyltransferase 1	2.493384	0.022032063
Pik3ap1	Phosphoinositide-3-kinase adaptor protein 1	2.293355	0.02256013
Elf1	E74-like factor 1	2.206845	0.023460381
LOC102633627	Tropomyosin alpha-4 chain pseudogene	2.185222	0.023653378
Kif21b	Kinesin family member 21B	2.402689	0.024154052
Mir703	MicroRNA 703	2.170541	0.024154052
Pdk4	Pyruvate dehydrogenase kinase, isoenzyme 4	1.917657	0.024332586
Plin1	Perilipin 1	–2.159175	0.024544784
Cnn2	Calponin 2	2.208329	0.024903186
Ighv1-43	Immunoglobulin heavy variable V1-43	2.960016	0.025124966
Lepr	Leptin receptor	2.795265	0.026147544
Syk	Spleen tyrosine kinase	2.351310	0.026443134
Scarna8	Small Cajal body-specific RNA 8	2.319912	0.026611121
Cd69	CD69 antigen	2.838945	0.026809801
Klhl34	Kelch-like 34	2.244789	0.027172552
Krt18	Keratin 18	–2.600385	0.027432055
St8sia4	ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase 4	2.221501	0.027505724
Ptma	Prothymosin alpha	2.126455	0.027538095
Fyb	FYN binding protein	2.831281	0.027815978
Cxcl12	Chemokine (C-X-C motif) ligand 12	2.134569	0.02892272
Smox	Spermine oxidase	–2.137130	0.029459765
Marco	Macrophage receptor with collagenous structure	2.269029	0.02989193
Rassf2	Ras association (RalGDS/AF-6) domain family member 2	2.266938	0.030419582
Aim2	Absent in melanoma 2	2.843504	0.030732924
Tecr	Trans-2,3-enoyl-CoA reductase	–1.998289	0.030878007
Rasal3	RAS protein activator like 3	2.363967	0.031055549
Ccl8	Chemokine (C-C motif) ligand 8	2.296489	0.031137278
Grap2	GRB2-related adaptor protein 2	2.455046	0.031159485
Pdlim1	PDZ and LIM domain 1 (elfin)	2.137582	0.031159485
Zdhhc20	Zinc finger, DHHC domain containing 20	2.250889	0.031858775
Six2	Sine oculis-related homeobox 2	–2.416577	0.031858775

Bach2	BTB and CNC homology 2	2.437268	0.031858775
Ddx60	DEAD (Asp-Glu-Ala-Asp) box polypeptide 60	2.815312	0.031858775
Tmem123	Transmembrane protein 123	2.102186	0.032142539
Rasd2	RASD family, member 2	–2.129021	0.032512135
Gm8369	Predicted gene 8369	2.828927	0.032515215
Gm26384	Predicted gene, 26384 [Source:MGI Symbol;Acc:MGI:5456161]	2.058302	0.032659623
Agpat2	1-acylglycerol-3-phosphate O-acyltransferase 2	–2.113157	0.033084523
Pdcd10	Programmed cell death 10	2.248511	0.033084523
Fchsd2	FCH and double SH3 domains 2	2.200689	0.03419107
Ces1d	Carboxylesterase 1D	–2.199751	0.034210236
Vcam1	Vascular cell adhesion molecule 1	2.205598	0.034362706
Aqp4	Aquaporin 4	2.115348	0.037167255
Slamf7	SLAM family member 7	2.618354	0.03718661
Otub2	OTU domain, ubiquitin aldehyde binding 2	–2.179882	0.03718661
Traj44	T cell receptor alpha joining 44	2.316738	0.03718661
Ifi27l2a	Interferon, alpha-inducible protein 27 like 2A	–2.092931	0.037446662
Tshr	Thyroid stimulating hormone receptor	–2.207281	0.038586986
Mki67	Antigen identified by monoclonal antibody Ki 67	2.328103	0.038586986
Ciita	Class II transactivator	2.261665	0.03868402
Gm10719	Predicted gene 10719 [Source:MGI Symbol;Acc:MGI:3641690]	–2.038729	0.039130352
Gm23947	Predicted gene, 23947 [Source:MGI Symbol;Acc:MGI:5453724]	2.354442	0.039276033
Rspo3	R-spondin 3	2.468535	0.039368285
Laptm5	Lysosomal-associated protein transmembrane 5	2.396087	0.041412858
Snora73b	Small nucleolar RNA, H/ACA box 73b	2.060844	0.042473778
Gimap3	GTPase, IMAP family member 3	2.287592	0.042473778
Mir675	MicroRNA 675	2.058131	0.042473778
Arhgap11a	Rho GTPase activating protein 11A	2.462272	0.042714463
Gpr174	G protein-coupled receptor 174	2.723874	0.044231361
Itk	IL2 inducible T cell kinase	2.515421	0.044394491
Tceb2	Transcription elongation factor B (SIII), polypeptide 2	–1.906908	0.044394491
Gpr18	G protein-coupled receptor 18	2.344280	0.044472279
Gm26175	Predicted gene, 26175 [Source:MGI Symbol;Acc:MGI:5455952]	2.047417	0.044964432
Crlf3	Cytokine receptor-like factor 3	2.182089	0.04540695
Spib	Spi-B transcription factor (Spi-1/PU.1 related)	2.672787	0.045496053

Ap1s2	Adaptor-related protein complex 1, sigma 2 subunit	2.084751	0.045534376
Bdh1	3-hydroxybutyrate dehydrogenase, type 1	2.310980	0.045804464
Gm10801	Predicted gene 10801 [Source:MGI Symbol;Acc:MGI:3641656]	–2.825870	0.045880636
Igkv4-78	Immunoglobulin kappa variable 4-78	2.435157	0.045970345
Spinkl	Serine protease inhibitor, Kazal type-like	2.423152	0.046480226
Cetn3	Centrin 3	2.228163	0.046763599
Phf11b	PHD finger protein 11B	2.192398	0.046763599
P3h2	Prolyl 3-hydroxylase 2	2.194877	0.047016456
Acly	ATP citrate lyase	–2.058096	0.047110874
Tmem71	Transmembrane protein 71	2.234226	0.049568619

Supplementary Table 2. The top 10 genes in pathways associated with atopic dermatitis (AD) identified by Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis

KEGG pathway name	Gene name	Gene symbol	Gene ID	Fold-change	p-value
Cytokine-cytokine receptor interaction					2.70005E-36
	Chemokine (C-X-C motif) ligand 13	*Cxcl13*	55985	9.5213	0
	Interleukin 2 receptor, gamma chain	*Il2rg*	16186	6.8833	1.91259E-13
	CD4 antigen	*Cd4*	12504	7.5465	2.13117E-12
	Interleukin 7 receptor	*Il7r*	16197	7.9501	3.06014E-12
	Chemokine (C-C motif) ligand 5	*Ccl5*	20304	3.7844	4.82844E-07
	Lymphotoxin B	*Ltb*	16994	3.3526	3.66469E-05
	Chemokine (C-C motif) ligand 22	*Ccl22*	20299	3.3510	0.000217941
	Leptin	*Lep*	16846	–2.6211	0.0010592
	CD27 antigen	*Cd27*	21940	3.2759	0.001810382
	Chemokine (C-C motif) receptor 7	*Ccr7*	12775	2.8732	0.002340615
Metabolic					7.50528E-28
	UDP-glucose ceramide glucosyltransferase	*Ugcg*	22234	3.4298	2.20686E-05
	Phosphoenolpyruvate carboxykinase 1, cytosolic	*Pck1*	18534	–2.8493	0.000149194
	ADP-ribosyltransferase 2b	*Art2b*	11872	4.1334	0.000436275
	Mannosidase 1, alpha	*Man1a*	17155	2.6758	0.00092214
	Amine oxidase, copper containing 3	*Aoc3*	11754	–2.6102	0.000982147
	Phospholipase A2, group VII (platelet-activating factor acetylhydrolase, plasma)	*Pla2g7*	27226	–2.6038	0.001028025
	Heparanase	*Hpse*	15442	2.7003	0.002399199
	Phospholipase C, gamma 2	*Plcg2*	234779	2.7840	0.002576994
	Inositol polyphosphate-4-phosphatase, type II	*Inpp4b*	234515	2.6277	0.003007459
	Fatty acid synthase	*Fasn*	14104	–2.2376	0.004242857
NF-kappa B signaling					4.82182E-24
	Protein kinase C, beta	*Prkcb*	18751	4.7940	8.43315E-08
	Lymphotoxin B	*Ltb*	16994	3.3526	3.66469E-05
	Phospholipase C, gamma 2	*Plcg2*	234779	2.7840	0.002576994
	Spleen tyrosine kinase	*Syk*	20963	2.3513	0.026443134
	Chemokine (C-X-C motif) ligand 12	*Cxcl12*	20315	2.1346	0.02892272
	Vascular cell adhesion molecule 1	*Vcam1*	22329	2.2056	0.034362706
	B cell linker	*Blnk*	17060	2.2740	0.07015631
	MALT1 paracaspase	*Malt1*	240354	1.9904	0.098788511
	Baculoviral IAP repeat-containing 3	*Birc3*	11796	1.9096	0.14727599
	DEAD (Asp-Glu-Ala-Asp) box polypeptide 58	*Ddx58*	230073	1.9400	0.158848743

Chemokine signaling					1.81802E-23
	Chemokine (C-X-C motif) ligand 13	*Cxcl13*	55985	9.5213	0
	Protein kinase C, beta	*Prkcb*	18751	4.7940	8.43315E-08
	Chemokine (C-C motif) ligand 5	*Ccl5*	20304	3.7844	4.82844E-07
	Dedicator of cyto-kinesis 2	*Dock2*	94176	3.6986	4.06357E-06
	Chemokine (C-C motif) ligand 22	*Ccl22*	20299	3.3510	0.000217941
	RAS-related C3 botulinum substrate 2	*Rac2*	19354	2.5858	0.002214744
	Chemokine (C-C motif) receptor 7	*Ccr7*	12775	2.8732	0.002340615
	PTK2 protein tyrosine kinase 2 beta	*Ptk2b*	19229	2.7416	0.002350974
	Vav 1 oncogene	*Vav1*	22324	2.8148	0.002719009
	Chemokine (C-X-C motif) receptor 5	*Cxcr5*	12145	2.7427	0.006354229
Leukocyte transendothelial migration					6.03478E-23
	Protein kinase C, beta	*Prkcb*	18751	4.7940	8.43315E-08
	Cytochrome b-245, beta polypeptide	*Cybb*	13058	3.8606	8.36122E-07
	Integrin beta 2	*Itgb2*	16414	3.2753	3.75636E-05
	Integrin alpha L	*Itgal*	16408	3.0770	0.000441262
	RAS-related C3 botulinum substrate 2	*Rac2*	19354	2.5858	0.002214744
	PTK2 protein tyrosine kinase 2 beta	*Ptk2b*	19229	2.7416	0.002350974
	Phospholipase C, gamma 2	*Plcg2*	234779	2.7840	0.002576994
	Vav 1 oncogene	*Vav1*	22324	2.8148	0.002719009
	Integrin alpha 4	*Itga4*	16401	2.5574	0.003365085
	Rho-associated coiled-coil containing protein kinase 1	*Rock1*	19877	2.3129	0.0088963
Hematopoietic cell lineage					8.0555E-23
	Complement receptor 2	*Cr2*	12902	9.2697	0
	Membrane-spanning 4-domains, subfamily A, member 1	*Ms4a1*	12482	8.6954	0
	CD3 antigen, gamma polypeptide	*Cd3g*	12502	12.8406	0
	CD4 antigen	*Cd4*	12504	7.5465	2.13117E-12
	Interleukin 7 receptor	*Il7r*	16197	7.9501	3.06014E-12
	Histocompatibility 2, class II antigen E alpha, pseudogene	*H2-Ea-ps*	100504404	4.9824	2.5564E-11
	CD22 antigen	*Cd22*	12483	4.3565	3.20735E-06
	Histocompatibility 2, class II, locus Mb2	*H2-DMb2*	15000	3.1559	0.000690586
	Fc receptor, ige, low affinity II, alpha polypeptide	*Fcer2a*	14128	3.3496	0.002259893
	Integrin alpha 4	*Itga4*	16401	2.5574	0.003365085
B cell receptor signaling					4.0307E-22
	Complement receptor 2	*Cr2*	12902	9.2697	0
	Protein kinase C, beta	*Prkcb*	18751	4.7940	8.43315E-08
	CD22 antigen	*Cd22*	12483	4.3565	3.20735E-06

	CD79A antigen (immunoglobulin-associated alpha)	*Cd79a*	12518	3.8307	6.00136E-06
	Protein tyrosine phosphatase, non-receptor type 6	*Ptpn6*	15170	3.2050	5.88237E-05
	CD79B antigen	*Cd79b*	15985	3.3977	0.000261267
	RAS-related C3 botulinum substrate 2	*Rac2*	19354	2.5858	0.002214744
	Phospholipase C, gamma 2	*Plcg2*	234779	2.7840	0.002576994
	Vav 1 oncogene	*Vav1*	22324	2.8148	0.002719009
	Inositol polyphosphate-5-phosphatase D	*Inpp5d*	16331	2.5073	0.018856792
Cell adhesion molecules					1.4218E-20
	Protein tyrosine phosphatase, receptor type, C	*Ptprc*	19264	14.9040	0
	Glycosylation dependent cell adhesion molecule 1	*Glycam1*	14663	35.4278	0
	Selectin, lymphocyte	*Sell*	20343	8.1342	1.91259E-13
	CD4 antigen	*Cd4*	12504	7.5465	2.13117E-12
	Histocompatibility 2, class II antigen E alpha, pseudogene	*H2-Ea-ps*	100504404	4.9824	2.5564E-11
	Selectin, platelet (p-selectin) ligand	*Selplg*	20345	4.8606	4.06416E-08
	CD22 antigen	*Cd22*	12483	4.3565	3.20735E-06
	Histocompatibility 2, M region locus 2	*H2-M2*	14990	4.3295	1.07827E-05
	Integrin beta 2	*Itgb2*	16414	3.2753	3.75636E-05
	Integrin alpha L	*Itgal*	16408	3.0770	0.000441262
T cell receptor signaling					4.43925E-20
	Protein tyrosine phosphatase, receptor type, C	*Ptprc*	19264	14.9040	0
	CD3 antigen, gamma polypeptide	*Cd3g*	12502	12.8406	0
	CD4 antigen	*Cd4*	12504	7.5465	2.13117E-12
	Protein tyrosine phosphatase, non-receptor type 6	*Ptpn6*	15170	3.2050	5.88237E-05
	Lymphocyte cytosolic protein 2	*Lcp2*	16822	3.0751	0.000100617
	Vav 1 oncogene	*Vav1*	22324	2.8148	0.002719009
	CD3 antigen, epsilon polypeptide	*Cd3e*	12501	2.5559	0.00337812
	CD8 antigen, beta chain 1	*Cd8b1*	12526	2.6983	0.021186091
	GRB2-related adaptor protein 2	*Grap2*	17444	2.4550	0.031159485
	IL2 inducible T cell kinase	*Itk*	16428	2.5154	0.044394491
Phagosome					8.9857E-20
	Cathepsin S	*Ctss*	13040	6.2632	3.60017E-13
	Histocompatibility 2, class II antigen E alpha, pseudogene	*H2-Ea-ps*	100504404	4.9824	2.5564E-11
	CD209b antigen	*Cd209b*	69165	5.0347	7.14709E-10
	Cytochrome b-245, beta polypeptide	*Cybb*	13058	3.8606	8.36122E-07
	Histocompatibility 2, M region locus 2	*H2-M2*	14990	4.3295	1.07827E-05
	Integrin beta 2	*Itgb2*	16414	3.2753	3.75636E-05
	Histocompatibility 2, class II, locus Mb2	*H2-DMb2*	15000	3.1559	0.000690586

	Macrophage receptor with collagenous structure	*Marco*	17167	2.2690	0.02989193
	Neutrophil cytosolic factor 1	*Ncf1*	17969	2.1895	0.050281506
	Complement component 3	*C3*	12266	2.0112	0.051314556
Regulation of actin cytoskeleton					1.42596E-18
	Integrin beta 2	*Itgb2*	16414	3.2753	3.75636E-05
	Phosphatidylinositol-5-phosphate 4-kinase, type II, alpha	*Pip4k2a*	18718	3.2470	4.87833E-05
	NCK associated protein 1 like	*Nckap1l*	105855	3.3190	5.04094E-05
	Integrin alpha L	*Itgal*	16408	3.0770	0.000441262
	Enabled homolog (Drosophila)	*Enah*	13800	2.6470	0.00111811
	RAS-related C3 botulinum substrate 2	*Rac2*	19354	2.5858	0.002214744
	Vav 1 oncogene	*Vav1*	22324	2.8148	0.002719009
	Integrin alpha 4	*Itga4*	16401	2.5574	0.003365085
	Cytoplasmic FMR1 interacting protein 2	*Cyfip2*	76884	2.5550	0.005254131
	Rho-associated coiled-coil containing protein kinase 1	*Rock1*	19877	2.3129	0.0088963
Th17 cell differentiation					6.31027E-18
	CD3 antigen, gamma polypeptide	*Cd3g*	12502	12.8406	0
	Interleukin 2 receptor, gamma chain	*Il2rg*	16186	6.8833	1.91259E-13
	CD4 antigen	*Cd4*	12504	7.5465	2.13117E-12
	Histocompatibility 2, class II antigen E alpha, pseudogene	*H2-Ea-ps*	100504404	4.9824	2.5564E-11
	Histocompatibility 2, class II, locus Mb2	*H2-DMb2*	15000	3.1559	0.000690586
	CD3 antigen, epsilon polypeptide	*Cd3e*	12501	2.5559	0.00337812
	Signal transducer and activator of transcription 1	*Stat1*	20846	2.2604	0.012104993
	Interleukin 6 receptor, alpha	*Il6ra*	16194	1.9453	0.127652915
	Transforming growth factor, beta 1	*Tgfb1*	21803	1.8611	0.200327384
	Linker for activation of T cells	*Lat*	16797	1.9337	0.216306508
PI3K-Akt signaling pathway					6.96949E-17
	Interleukin 2 receptor, gamma chain	*Il2rg*	16186	6.8833	1.91259E-13
	Interleukin 7 receptor	*Il7r*	16197	7.9501	3.06014E-12
	Phosphoenolpyruvate carboxykinase 1, cytosolic	*Pck1*	18534	–2.8493	0.000149194
	Integrin alpha 4	*Itga4*	16401	2.5574	0.003365085
	Tenascin C	*Tnc*	21923	2.5361	0.014119702
	Glycogen synthase 2	*Gys2*	232493	–2.5275	0.015019358
	Coagulation factor II (thrombin) receptor	*F2r*	14062	2.2565	0.020547361
	Phosphoinositide-3-kinase adaptor protein 1	*Pik3ap1*	83490	2.2934	0.02256013
	Spleen tyrosine kinase	*Syk*	20963	2.3513	0.026443134
	Prolactin receptor	*Prlr*	19116	–2.4817	0.075280525
NOD-like receptor signaling pathway					4.27592E-16

	Chemokine (C-C motif) ligand 5	*Ccl5*	20304	3.7844	4.82844E-07
	Cytochrome b-245, beta polypeptide	*Cybb*	13058	3.8606	8.36122E-07
	Signal transducer and activator of transcription 1	*Stat1*	20846	2.2604	0.012104993
	Absent in melanoma 2	*Aim2*	383619	2.8435	0.030732924
	Transmembrane protein 173	*Tmem173*	72512	2.1258	0.112315813
	Baculoviral IAP repeat-containing 3	*Birc3*	11796	1.9096	0.14727599
	B cell leukemia/lymphoma 2	*Bcl2*	12043	1.8058	0.277335879
	NLR family, apoptosis inhibitory protein 6	*Naip6*	17952	2.1419	0.374297539
	Baculoviral IAP repeat-containing 2	*Birc2*	11797	1.6539	0.47899828
	Caspase 4, apoptosis-related cysteine peptidase	*Casp4*	12363	1.8859	0.495906206
Jak-STAT signaling pathway					2.14323E-15
	Interleukin 2 receptor, gamma chain	*Il2rg*	16186	6.8833	1.91259E-13
	Interleukin 7 receptor	*Il7r*	16197	7.9501	3.06014E-12
	Protein tyrosine phosphatase, non-receptor type 6	*Ptpn6*	15170	3.2050	5.88237E-05
	Signal transducer and activator of transcription 4	*Stat4*	20849	3.5331	0.000984191
	Leptin	*Lep*	16846	–2.6211	0.0010592
	Four and a half LIM domains 1	*Fhl1*	14199	2.5515	0.002097917
	Signal transducer and activator of transcription 1	*Stat1*	20846	2.2604	0.012104993
	Leptin receptor	*Lepr*	16847	2.7953	0.026147544
	Interleukin 22 receptor, alpha 2	*Il22ra2*	237310	2.7092	0.066101671
	Prolactin receptor	*Prlr*	19116	–2.4817	0.075280525
Primary immunodeficiency					2.85309E-15
	Protein tyrosine phosphatase, receptor type, C	*Ptprc*	19264	14.9040	0
	Interleukin 2 receptor, gamma chain	*Il2rg*	16186	6.8833	1.91259E-13
	CD4 antigen	*Cd4*	12504	7.5465	2.13117E-12
	Interleukin 7 receptor	*Il7r*	16197	7.9501	3.06014E-12
	CD79A antigen (immunoglobulin-associated alpha)	*Cd79a*	12518	3.8307	6.00136E-06
	CD3 antigen, epsilon polypeptide	*Cd3e*	12501	2.5559	0.00337812
	CD8 antigen, beta chain 1	*Cd8b1*	12526	2.6983	0.021186091
	Class II transactivator	*Ciita*	12265	2.2617	0.03868402
	Inducible T cell co-stimulator	*Icos*	54167	2.4137	0.05992872
	B cell linker	*Blnk*	17060	2.2740	0.07015631
Osteoclast differentiation					5.45511E-15
	Leukocyte immunoglobulin-like receptor, subfamily B, member 4A	*Lilrb4a*	14728	4.0490	3.20735E-06
	Lymphocyte cytosolic protein 2	*Lcp2*	16822	3.0751	0.000100617
	Phospholipase C, gamma 2	*Plcg2*	234779	2.7840	0.002576994
	Signal transducer and activator of transcription 1	*Stat1*	20846	2.2604	0.012104993

	Spleen tyrosine kinase	*Syk*	20963	2.3513	0.026443134
	Neutrophil cytosolic factor 1	*Ncf1*	17969	2.1895	0.050281506
	B cell linker	*Blnk*	17060	2.2740	0.07015631
	Fyn proto-oncogene	*Fyn*	14360	1.9686	0.150106114
	Colony stimulating factor 1 receptor	*Csf1r*	12978	1.8421	0.200175322
	Transforming growth factor, beta 1	*Tgfb1*	21803	1.8611	0.200327384
Antigen processing and presentation					7.8513E-15
	Cathepsin S	*Ctss*	13040	6.2632	3.60017E-13
	CD4 antigen	*Cd4*	12504	7.5465	2.13117E-12
	Histocompatibility 2, class II antigen E alpha, pseudogene	*H2-Ea-ps*	100504404	4.9824	2.5564E-11
	Histocompatibility 2, M region locus 2	*H2-M2*	14990	4.3295	1.07827E-05
	CD74 antigen (invariant polypeptide of major histocompatibility complex, class II antigen-associated)	*Cd74*	16149	2.8270	0.000130769
	Histocompatibility 2, class II, locus Mb2	*H2-DMb2*	15000	3.1559	0.000690586
	Interferon gamma inducible protein 30	*Ifi30*	65972	2.2296	0.017229862
	CD8 antigen, beta chain 1	*Cd8b1*	12526	2.6983	0.021186091
	Class II transactivator	*Ciita*	12265	2.2617	0.03868402
	CD8 antigen, alpha chain	*Cd8a*	12525	2.3514	0.112888245
Th1 and Th2 cell differentiation					3.51751E-13
	CD3 antigen, gamma polypeptide	*Cd3g*	12502	12.8406	0
	Interleukin 2 receptor, gamma chain	*Il2rg*	16186	6.8833	1.91259E-13
	CD4 antigen	*Cd4*	12504	7.5465	2.13117E-12
	Histocompatibility 2, class II antigen E alpha, pseudogene	*H2-Ea-ps*	100504404	4.9824	2.5564E-11
	Histocompatibility 2, class II, locus Mb2	*H2-DMb2*	15000	3.1559	0.000690586
	Signal transducer and activator of transcription 4	*Stat4*	20849	3.5331	0.000984191
	CD3 antigen, epsilon polypeptide	*Cd3e*	12501	2.5559	0.00337812
	Signal transducer and activator of transcription 1	*Stat1*	20846	2.2604	0.012104993
	Linker for activation of T cells	*Lat*	16797	1.9337	0.216306508
	Lymphocyte protein tyrosine kinase	*Lck*	16818	2.1659	0.21930709
Intestinal immune network for IgA production					3.6881E-13
	Histocompatibility 2, class II antigen E alpha, pseudogene	*H2-Ea-ps*	100504404	4.9824	2.5564E-11
	Histocompatibility 2, class II, locus Mb2	*H2-DMb2*	15000	3.1559	0.000690586
	Integrin alpha 4	*Itga4*	16401	2.5574	0.003365085
	Chemokine (C-X-C motif) receptor 4	*Cxcr4*	12767	2.4200	0.010165808
	Chemokine (C-X-C motif) ligand 12	*Cxcl12*	20315	2.1346	0.02892272
	Icos ligand	*Icosl*	50723	2.1691	0.052602737
	Inducible T cell co-stimulator	*Icos*	54167	2.4137	0.05992872

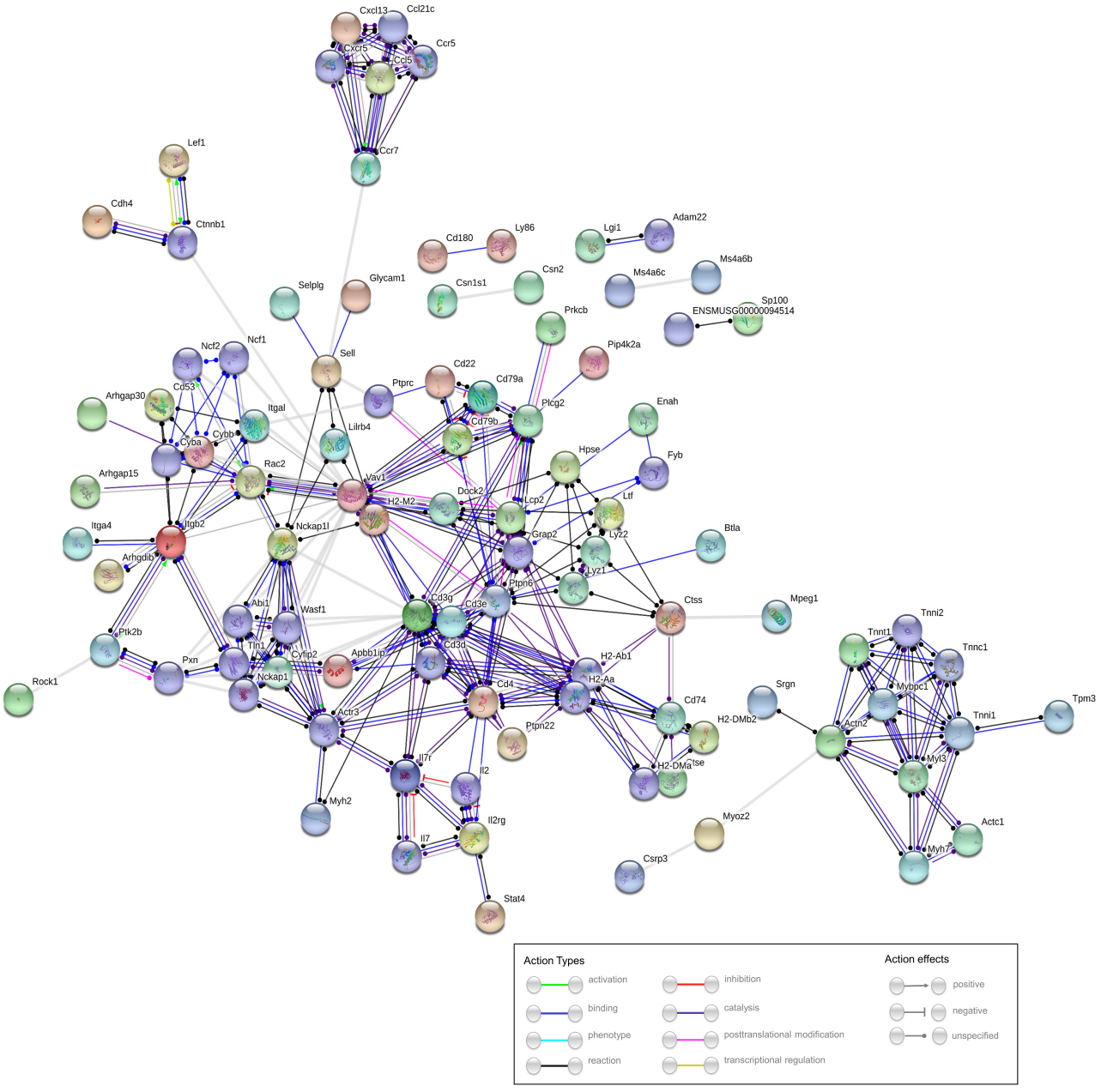
	Transforming growth factor, beta 1	*Tgfb1*	21803	1.8611	0.200327384
	Tumor necrosis factor (ligand) superfamily, member 13b	*Tnfsf13b*	24099	1.8448	0.419152817
	Integrin beta 7	*Itgb7*	16421	1.9667	0.432113252
Endocytosis					3.19463E-12
	Interleukin 2 receptor, gamma chain	*Il2rg*	16186	6.8833	1.91259E-13
	Histocompatibility 2, M region locus 2	*H2-M2*	14990	4.3295	1.07827E-05
	Cytohesin 4	*Cyth4*	72318	2.4522	0.004929348
	Chemokine (C-X-C motif) receptor 4	*Cxcr4*	12767	2.4200	0.010165808
	Promyelocytic leukemia	*Pml*	18854	2.0533	0.092163371
	Capping protein (actin filament) muscle Z-line, alpha 1	*Capza1*	12340	1.8703	0.175747236
	Histocompatibility 2, Q region locus 8	*H2-Q8*	15019	1.8444	0.193624046
	Interleukin 2 receptor, beta chain	*Il2rb*	16185	2.0470	0.28469515
	Heat shock protein 1-like	*Hspa1l*	15482	1.8149	0.338946578
	Pleckstrin and Sec7 domain containing 4	*Psd4*	215632	2.1234	0.346751731
Natural killer cell mediated cytotoxicity					5.20152E-12
	Protein kinase C, beta	*Prkcb*	18751	4.7940	8.43315E-08
	Integrin beta 2	*Itgb2*	16414	3.2753	3.75636E-05
	Protein tyrosine phosphatase, non-receptor type 6	*Ptpn6*	15170	3.2050	5.88237E-05
	Lymphocyte cytosolic protein 2	*Lcp2*	16822	3.0751	0.000100617
	Integrin alpha L	*Itgal*	16408	3.0770	0.000441262
	RAS-related C3 botulinum substrate 2	*Rac2*	19354	2.5858	0.002214744
	PTK2 protein tyrosine kinase 2 beta	*Ptk2b*	19229	2.7416	0.002350974
	Phospholipase C, gamma 2	*Plcg2*	234779	2.7840	0.002576994
	Vav 1 oncogene	*Vav1*	22324	2.8148	0.002719009
	Spleen tyrosine kinase	*Syk*	20963	2.3513	0.026443134
MAPK signaling pathway					2.13015E-11
	Protein kinase C, beta	*Prkcb*	18751	4.7940	8.43315E-08
	RAS-related C3 botulinum substrate 2	*Rac2*	19354	2.5858	0.002214744
	Calcium channel, voltage-dependent, gamma subunit 1	*Cacng1*	12299	–2.2426	0.011240559
	RAS related protein 1b	*Rap1b*	215449	2.1587	0.021951174
	TAO kinase 3	*Taok3*	330177	2.0617	0.091109254
	Nuclear receptor subfamily 4, group A, member 1	*Nr4a1*	15370	–1.8287	0.122795267
	RAS-related protein-1a	*Rap1a*	109905	1.8729	0.13788196
	Colony stimulating factor 1 receptor	*Csf1r*	12978	1.8421	0.200175322
	Transforming growth factor, beta 1	*Tgfb1*	21803	1.8611	0.200327384
	Fibroblast growth factor 1	*Fgf1*	14164	1.8555	0.206473803
Toll-like receptor signaling pathway					2.15417E-11
	Chemokine (C-C motif) ligand 5	*Ccl5*	20304	3.7844	4.82844E-07

	Signal transducer and activator of transcription 1	*Stat1*	20846	2.2604	0.012104993
	Toll-like receptor 1	*Tlr1*	21897	2.3613	0.135077993
	Toll-like receptor 7	*Tlr7*	170743	2.2462	0.24098673
	Thymoma viral proto-oncogene 3	*Akt3*	23797	1.7957	0.257333103
	FBJ osteosarcoma oncogene	*Fos*	14281	–1.7423	0.345977149
	Chemokine (C-X-C motif) ligand 9	*Cxcl9*	17329	1.6956	0.468421758
	Lymphocyte antigen 96	*Ly96*	17087	1.8624	0.532812844
	Caspase 8	*Casp8*	12370	1.6464	0.55520391
	Mitogen-activated protein kinase kinase 2	*Map2k2*	26396	–1.5503	0.696438063
Cardiac muscle contraction					5.07804E-10
	Troponin C, cardiac/slow skeletal	*Tnnc1*	21924	11.2839	0
	Myosin, heavy polypeptide 7, cardiac muscle, beta	*Myh7*	140781	25.3377	0
	Atpase, Ca++ transporting, cardiac muscle, slow twitch 2	*Atp2a2*	11938	8.3273	0
	Myosin, light polypeptide 3	*Myl3*	17897	5.7018	4.96498E-10
	Tropomyosin 3, gamma	*Tpm3*	59069	4.0864	8.07949E-08
	Actin, alpha, cardiac muscle 1	*Actc1*	11464	–2.7027	7.15757E-06
	Atpase, Na+/K+ transporting, beta 1 polypeptide	*Atp1b1*	11931	3.0179	1.01881E-05
	Calcium channel, voltage-dependent, gamma subunit 1	*Cacng1*	12299	–2.2426	0.011240559
	Tropomyosin 2, beta	*Tpm2*	22004	1.8086	0.067947903
	Atpase, Na+/K+ transporting, beta 2 polypeptide	*Atp1b2*	11932	–1.7850	0.123829945
Adrenergic signaling in cardiomyocytes					1.10527E-09
	Troponin C, cardiac/slow skeletal	*Tnnc1*	21924	11.2839	0
	Myosin, heavy polypeptide 7, cardiac muscle, beta	*Myh7*	140781	25.3377	0
	Atpase, Ca++ transporting, cardiac muscle, slow twitch 2	*Atp2a2*	11938	8.3273	0
	Myosin, light polypeptide 3	*Myl3*	17897	5.7018	4.96498E-10
	Tropomyosin 3, gamma	*Tpm3*	59069	4.0864	8.07949E-08
	Actin, alpha, cardiac muscle 1	*Actc1*	11464	–2.7027	7.15757E-06
	Atpase, Na+/K+ transporting, beta 1 polypeptide	*Atp1b1*	11931	3.0179	1.01881E-05
	Calcium channel, voltage-dependent, gamma subunit 1	*Cacng1*	12299	–2.2426	0.011240559
	Tropomyosin 2, beta	*Tpm2*	22004	1.8086	0.067947903
	Phosphoinositide-3-kinase, catalytic, gamma polypeptide	*Pik3cg*	30955	2.2336	0.07771979
Hypertrophic cardiomyopathy (HCM)					1.25338E-09
	Troponin C, cardiac/slow skeletal	*Tnnc1*	21924	11.2839	0
	Myosin, heavy polypeptide 7, cardiac muscle, beta	*Myh7*	140781	25.3377	0
	Atpase, Ca++ transporting, cardiac muscle, slow twitch 2	*Atp2a2*	11938	8.3273	0

	Myosin, light polypeptide 3	*Myl3*	17897	5.7018	4.96498E-10
	Tropomyosin 3, gamma	*Tpm3*	59069	4.0864	8.07949E-08
	Actin, alpha, cardiac muscle 1	*Actc1*	11464	–2.7027	7.15757E-06
	Integrin alpha 4	*Itga4*	16401	2.5574	0.003365085
	Protein kinase, AMP-activated, gamma 3 non-catatlytic subunit	*Prkag3*	241113	–2.3624	0.005895163
	Calcium channel, voltage-dependent, gamma subunit 1	*Cacng1*	12299	–2.2426	0.011240559
	Tropomyosin 2, beta	*Tpm2*	22004	1.8086	0.067947903
cAMP signaling pathway					3.53222E-09
	ATPase, Ca++ transporting, cardiac muscle, slow twitch 2	*Atp2a2*	11938	8.3273	0
	ATPase, Na+/K+ transporting, beta 1 polypeptide	*Atp1b1*	11931	3.0179	1.01881E-05
	RAS-related C3 botulinum substrate 2	*Rac2*	19354	2.5858	0.002214744
	Vav 1 oncogene	*Vav1*	22324	2.8148	0.002719009
	Rho-associated coiled-coil containing protein kinase 1	*Rock1*	19877	2.3129	0.0088963
	Coagulation factor II (thrombin) receptor	*F2r*	14062	2.2565	0.020547361
	RAS related protein 1b	*Rap1b*	215449	2.1587	0.021951174
	Thyroid stimulating hormone receptor	*Tshr*	22095	–2.2073	0.038586986
	ATPase, Na+/K+ transporting, beta 2 polypeptide	*Atp1b2*	11932	–1.7850	0.123829945
	RAS-related protein-1a	*Rap1a*	109905	1.8729	0.13788196
TNF signaling pathway					3.27002E-08
	Chemokine (C-C motif) ligand 5	*Ccl5*	20304	3.7844	4.82844E-07
	Vascular cell adhesion molecule 1	*Vcam1*	22329	2.2056	0.034362706
	Baculoviral IAP repeat-containing 3	*Birc3*	11796	1.9096	0.14727599
	TNF receptor-associated factor 1	*Traf1*	22029	2.0830	0.20412442
	Thymoma viral proto-oncogene 3	*Akt3*	23797	1.7957	0.257333103
	FBJ osteosarcoma oncogene	*Fos*	14281	–1.7423	0.345977149
	Tumor necrosis factor receptor superfamily, member 1b	*Tnfrsf1b*	21938	1.7873	0.439799373
	Interleukin 18 receptor 1	*Il18r1*	16182	2.0032	0.454499375
	Caspase 3	*Casp3*	12367	1.8752	0.467106772
	Baculoviral IAP repeat-containing 2	*Birc2*	11797	1.6539	0.47899828
Calcium signaling pathway					0.00059103
	Troponin C, cardiac/slow skeletal	*Tnnc1*	21924	11.2839	0
	Atpase, Ca++ transporting, cardiac muscle, slow twitch 2	*Atp2a2*	11938	8.3273	0
	Protein kinase C, beta	*Prkcb*	18751	4.7940	8.43315E-08
	PTK2 protein tyrosine kinase 2 beta	*Ptk2b*	19229	2.7416	0.002350974
	Phospholipase C, gamma 2	*Plcg2*	234779	2.7840	0.002576994
	Coagulation factor II (thrombin) receptor	*F2r*	14062	2.2565	0.020547361
	Adrenergic receptor, beta 3	*Adrb3*	11556	–2.0731	0.136087707

Phospholipase C, delta 4	*Plcd4*	18802	–1.8712	0.149640841
Phosphorylase kinase gamma 1	*Phkg1*	18682	–1.5328	0.544630124
Cysteinyl leukotriene receptor 1	*Cysltr1*	58861	1.6576	0.904170421

NF, nuclear factor; PI3K, phosphoinositide-3-kinase; NOD, nucleotide-binding oligomerization domain; Jak-STAT, Janus kinase-signal transducers and activators of transcription; IgA, immunoglobulin A; MAPK, mitogen-activated protein kinase; TNF, tumor necrosis factor.



Supplementary Fig. 1. Protein-protein interaction network analysis of differentially expressed genes (DEGs) by house dust mite-induced atopy dermatitis-like. Protein interaction network was identified for 98 DEGs obtained from microarray analysis results via Search Tool for the Retrieval of Interacting Genes version 10.5 (http://www.string-db.org/).