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Abstract
This study was conducted to compare the gene expression profiles, after*Eimeria maxima* infection, between 2 *B*-complex congenic lines of Fayoumi chickens that display differences in disease resistance and innate immunity against avian coccidiosis using cDNA microarray. When compared with uninfected controls using a cutoff of >2.0-fold alteration ($P < 0.05$), M5.1 demonstrated altered expression of 1 (downregulated), 12 (6 up, 6 down), and 18 (5 up, 13 down) mRNA at 3, 4, and 5 d postinfection, respectively. In the M15.2 line, altered expression was observed in 6 (3 up, 3 down), 29 (11 up, 18 down), and 32 (8 up, 24 down) transcripts at the 3 time points, compared with uninfected controls. Comparison of the expression levels between M5.1 and M15.2 chickens after *E. maxima* infection revealed alterations in 32 (10 up, 22 down), 98 (43 up, 55 down), and 92 (33 up, 59 down) mRNA at the 3 time points. Functional analysis using gene ontology categorized the genes exhibiting the different expression patterns between 2 chicken lines into several gene ontology terms including immunity and defense. In summary, transcriptional profiles showed that more gene expression changes occurred with *E. maxima* infection in the M15.2 than the M5.1 line. The most gene expression differences between the 2 chicken lines were exhibited at d 4 and 5 after *E. maxima* infection. These results demonstrate that differential gene expression patterns associated with the host genetic difference in coccidiosis resistance provide insights into the host protective immune mechanisms and present a rational basis to target specific genes and gene products to bolster host defenses against avian coccidiosis.

Keywords
coccidiosis, Fayoumi, complementary DNA microarray, intraepithelial lymphocyte

Comments
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Gene expression profiles of two *B*-complex disparate, genetically inbred Fayoumi chicken lines that differ in susceptibility to *Eimeria maxima*

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ABSTRACT This study was conducted to compare the gene expression profiles, after *Eimeria maxima* infection, between 2 *B*-complex congenic lines of Fayoumi chickens that display differences in disease resistance and innate immunity against avian coccidiosis using cDNA microarray. When compared with uninfected controls using a cutoff of >2.0-fold alteration ($P < 0.05$), M5.1 demonstrated altered expression of 1 (downregulated), 12 (6 up, 6 down), and 18 (5 up, 13 down) mRNA at 3, 4, and 5 d postinfection, respectively. In the M15.2 line, altered expression was observed in 6 (3 up, 3 down), 29 (11 up, 18 down), and 32 (8 up, 24 down) transcripts at the 3 time points, compared with uninfected controls. Comparison of the expression levels between M5.1 and M15.2 chickens after *E. maxima* infection revealed alterations in 32 (10 up, 22 down), 98 (43 up, 55 down), and 92 (33 up, 59 down) mRNA at the 3 time points. Functional analysis using gene ontology categorized the genes exhibiting the different expression patterns between 2 chicken lines into several gene ontology terms including immunity and defense. In summary, transcriptional profiles showed that more gene expression changes occurred with *E. maxima* infection in the M15.2 than the M5.1 line. The most gene expression differences between the 2 chicken lines were exhibited at d 4 and 5 after *E. maxima* infection. These results demonstrate that differential gene expression patterns associated with the host genetic difference in coccidiosis resistance provide insights into the host protective immune mechanisms and present a rational basis to target specific genes and gene products to bolster host defenses against avian coccidiosis.

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INTRODUCTION

Avian coccidiosis is caused by infection with protozoa belonging to the genus *Eimeria* and presents a significant concern to the poultry industry due to its detrimental influence on production efficiency affecting survivability, nutrient absorption, growth rate, and egg production of poultry (Lillehoj and Li, 2004; Lillehoj et al., 2007). Until now, disease control strategies against avian coccidiosis have mostly depended on prophylactic chemotherapy and vaccination, but both methods have serious drawbacks (Min et al., 2005). Genetic selection for disease resistance and immune responses can lead to a reduction of drug use and risk of vaccination in commercial poultry production. However, information about the chicken immune system at the molecular level is very limited.

In recent years, the chicken EST database has reached such a level that analysis of the sequence data is feasible. There are now more than 580,000 chicken EST sequences from a wide variety of tissues and developmental stages publicly available (http://www.tigr.org/tdb/tgi/). Therefore, it is now possible to ascribe provisional identities and functions to genes on the basis of sequence comparison. High-throughput genomic analyses have suggested a path toward increasing identification of transcriptional regulations involved in the immune response (Degen et al., 2006). Large-scale gene expression profiles of host responses to infection could provide enormous information on the interactions between pathogens and the host immune system. The microarray technique is an appropriate tool for characterizing the biology of immunologic processes and immune-related diseases (van der Pouw Kraan et al., 2004). In chicken, several microarray studies have been performed for the infectious diseases such as Marek's

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disease (Levy et al., 2005; Sarson et al., 2006; Heidari et al., 2008; Sarson et al., 2008), West Nile virus (Groves et al., 2008), *Salmonella* (van Hemert et al., 2006, 2007), avian influenza virus (Degen et al., 2006; Groves et al., 2008; Zaffuto et al., 2008), avian infectious bronchitis virus (Dar et al., 2005), and *Eimeria* species (Min et al., 2003).

The Fayoumi breed, which originated in Egypt, was imported to the United States in 1954 primarily because of its reported resistance to the avian leukosis. Subsequently, it was shown to develop a robust protective response against *Eimeria tenella* (Pinard-Van Der Laan et al., 1998). Derived from the original Fayoumi breed, the M5.1 and M15.2 congenic pair lines are highly inbred and genetically distant from broiler and Leghorn lines. They share an identical genetic background but differ in the haplotype on chromosome 16 carrying the MHC (Zhou and Lamont, 1999, 2003). In a previous study, we compared the typical disease parameters of avian coccidiosis, BW gain and fecal oocyst shedding, and transcriptional expression of cytokine genes between the Fayoumi M5.1 and M15.2 lines after oral infection with *Eimeria maxima* oocysts. The results demonstrated that line M5.1 is more resistant against *E. maxima* than M15.2 and showed differential expression of immune-related cytokines between the 2 genetically disparate chicken lines after infection.

The present study was conducted to compare gene expression profiles between these 2 chicken congenic lines, which show a difference in disease resistance to coccidiosis, using our avian intestinal intraepithelial lymphocyte cDNA microarray (**AVIELA**; Kim et al., 2008). The AVIELA is specific for mucosal transcriptional investigations including immune response and was constructed using an intraepithelial lymphocyte (**IEL**) cDNA library of *Eimeria*-infected chickens (Min et al., 2005). Because IEL are the primary immune effector cells in the gut and play a critical role in eliciting protective immunity to enteric pathogens, the results contribute to comprehensive understanding of the innate immune responses in the chicken gut stimulated by *E. maxima* infection.

MATERIALS AND METHODS

Birds and Experimental Eimeria Infection

Chicks were bred and maintained in the Poultry Genetics Program at Iowa State University (Ames). All matings were carried out by artificial insemination to ensure pedigree accuracy and *B*-complex genotypes were confirmed by serological testing. Day-old chicks were air-shipped to the Animal and Natural Resources Institute. Chickens either remained uninfected or were orally inoculated at 4 wk of age with 1.0×10^4 sporulated oocysts/chicken of *E. maxima*. All experiments were approved by the Animal and Natural Resources Institute Institutional Animal Care and Use Committee.

RNA Extraction and Aminoallyl-Labeled RNA Preparation

After euthanization of the birds, jejunal intestines were taken from 5 birds in each infected or noninfected group at 0, 3, 4, and 5 d postinfection (**dpi**). Intestines were cut longitudinally and washed 3 times with ice-cold Hanks' balanced salt solution containing 100 U/mL of penicillin and 100 mg/mL of streptomycin (Sigma, St. Louis, MO). The mucosal layer was carefully scraped using a surgical scalpel and IEL were isolated from the jejunum as described previously (Lee et al., 2007). Total RNA was isolated from a constant number of IEL (5.0×10^7) using Trizol (Invitrogen, Carlsbad, CA) and purified using the RNeasy Mini RNA Purification Kit (Qiagen, Valencia, CA; Wu et al., 2004). In preliminary experiments, we determined that equal cell numbers and equal amounts of total RNA were obtained per unit volume of gut mucosa (D. K. Kim, unpublished data).

The aminoallyl-labeled RNA from jejunum IEL from each group was prepared using the Amino Allyl Message Amp II aRNA Amplification Kit ('t Hoen et al., 2003) according to the protocol of the manufacturer (Ambion, Austin, TX). Briefly, first strand cDNA was prepared by reverse transcription from 2.0 μg of RNA using a modified oligo-dT primer containing a T7 RNA polymerase binding site on the 5′ end, followed by second strand cDNA synthesis. The double-stranded cDNA generated from first and second cDNA synthesis were transcribed to aminoallyl-labeled RNA using T7 RNA polymerase with aminoallyl-uridine triphosphate. Two 20-μg aliquots of each aminoallyl-RNA sample were fluorescently labeled with Alexa Fluor 555 or Alexa Fluor 647 (Invitrogen) according to the instructions of the manufacturer and labeled RNA were column-purified using the RNA Amplification Kit (Ambion; 't Hoen et al., 2003). Concentration and labeling efficiencies of RNA were determined spectrophotometrically.

Microarray Hybridization

Both of the RNA, labeled with 2 different fluorescent dyes, from the noninfected control birds and the treated birds were hybridized to the AVIELA microarray. The AVIELA was constructed with 10,162 spot elements from the previously prepared IEL clones (Min et al., 2005) and the immune-related genes from lipopolysaccharide (**LPS**)-activated macrophage (HD11) cDNA library (Min et al., 2003) as well as the direct PCR clones of several cytokines and chemokines (Min et al., 2005).

A total of 12 microarrays were used in this study. According to the reference design (McShane et al., 2003) with dye swap, 4 values were obtained for each time point (3, 4, and 5 dpi) in a chicken line, 2 on 1 slide and 2 on the dye-swapped slide because all elements are duplicated on the AVIELA. Each treatment group was normalized with the noninfected control of the

Table 1. The oligonucleotide primers used in the quantitative real-time PCR

Gene	Description	Left primer	Right primer	Product size (bp)	GenBank no.	Reference
APOA4	Apolipoprotein A-IV	3′-CACTGTGCTCTGGAGGTACT-5′	3′-CTGCTGGATCTGCTTCTTC-5′	229	CD736338	Min et al. (2005)
CD36	CD36 molecule (thrombospondin receptor)	3′-ATGGAGTTTACCAGACCAGT-5′	3′-GCTCAGACCTTCAACATCA-5′	257	CD738626	Min et al. (2005)
FYN	FYN oncogene related to SRC, FGR, YES	3′-CTGACTTTGGATTAGCAAGG-5′	3′-CCAAGACTTCACGGTTATTC-5′	210	CF075134	Min et al. (2005)
NRAS	Neuroblastoma RAS viral (v-ras) oncogene homolog	3′-CTCAACAGCAACGAAGATG-5′	3′-CACCAAGAGATATGACTGCTC-5′	270	CD726890	Min et al. (2005)

same line. Two-color microarray hybridizations were performed using HybIt hybridization buffer (Telechem International, Sunnyvale, CA) in ArrayIt reaction cassettes (Telechem International) at 50°C overnight. After hybridization, the slides were rinsed in 0.5 × saline sodium citrate (**SSC**), 0.01% SDS at room temperature, and washed once for 15 min in 0.2 × SSC, 0.2% SDS at 50°C, 3 times for 1 min in 0.2 × SSC at room temperature, and 3 times for 1 min in distilled water at room temperature. Each sample had a repeated hybridization using the alternate fluorescent dye between the infected treatment and uninfected control.

Microarray Scanning and Image Analysis

Images were acquired by laser confocal scanning using a ScanArray Lite microarray analysis system (Perkin-Elmer, Boston, MA) at a resolution of 10 μm. A 16-bit TIFF image was generated for each channel (Alexa Fluor 555 and Alexa Fluor 647). The scanned microarray images for each channel were overlaid and quantified to determine the fluorescent intensities of the 2 dyes for each spot by using the ScanArray Express version 3.0 software (Perkin-Elmer). Spots were detected using an adaptive circle algorithm in the ScanArray program and all spots were visually confirmed.

Microarray Data Analysis

The MIDAS 2.19 software of the TM4 microarray analysis package (http://www.tigr.org) was used to qualify and normalize image analysis data. Briefly, each of median spot intensity (Alexa Fluor 555 or Alexa Fluor 647) was determined by subtracting the median local background from the median signal intensity values and then flag information was applied to filter out bad spots. Genes missing more than 50% of their values because of a bad signal or noise ratio were removed from further analysis. Two-step normalization was performed (Sioson et al., 2006): total intensity and block locally weighted regression and smoothing scatter plots methods, followed by SD regularization of block and slide SD. Information on the microarray has been submitted into the Minimum Information About a Microarray Experiment online (http://www.mged.org/Workgroups/MIAME/). The accession number for the array is A-MEXP-1487.

The qualified and normalized array data were inputted into GeneSpring GX 7.3 software (Silicon Genetics, Redwood, CA) for statistical and fold-change analyses. To generate signal ratio, signal channel values (infected group) were divided by control channel values (control group). The significantly differentially expressed genes were filtered using the Volcano Plot method (Jin et al., 2001) built by comparing a chicken line with itself or between 2 chicken lines at the same dpi. The modulated elements were defined by 2-fold differences and a *P*-value cutoff ($P < 0.05$) by parametric test.

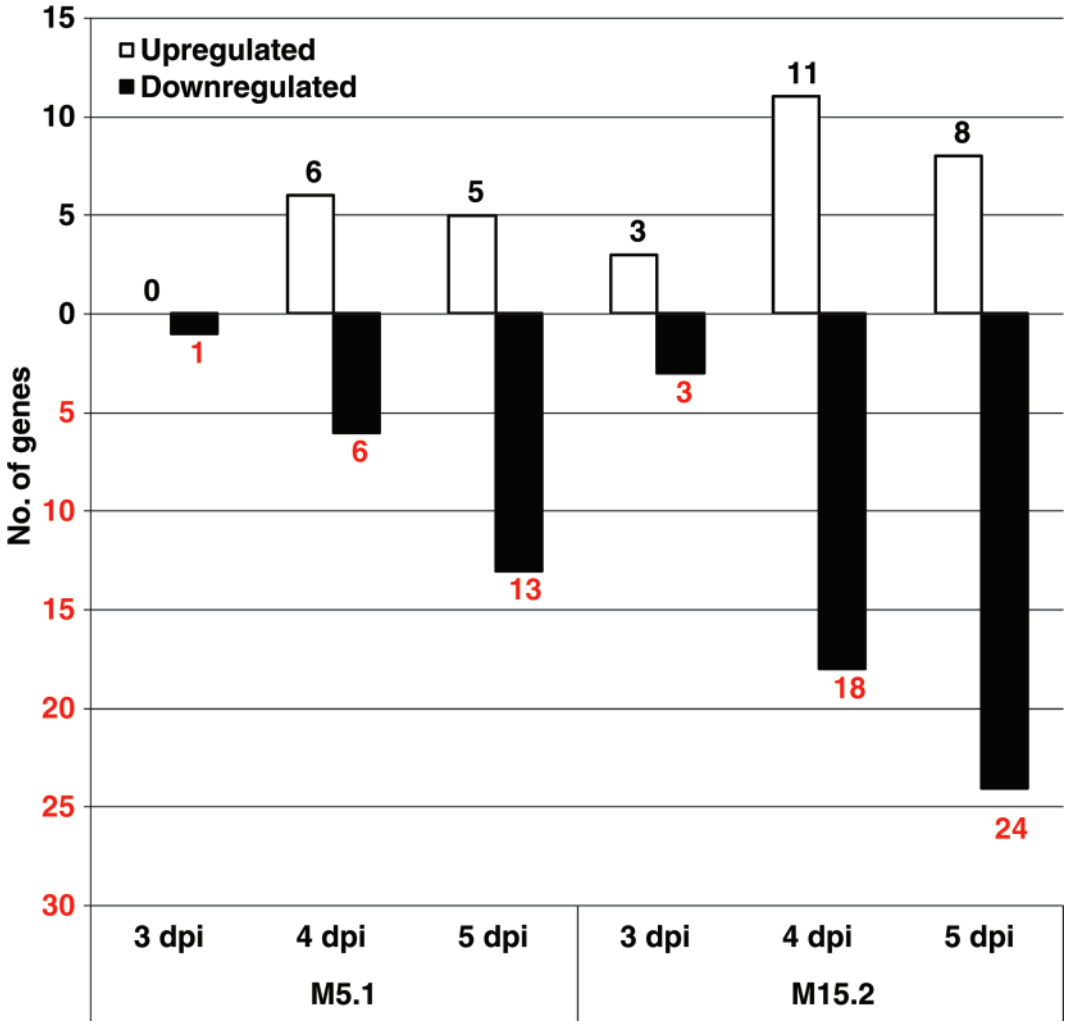

Figure 1. Number of transcripts on the avian intestinal intraepithelial lymphocyte cDNA microarray (AVIELA) showing the alteration of the expression levels in M5.1 and M15.2 lines with >2.0-fold changes after *Eimeria maxima* infection compared with uninfected controls. dpi = days postinfection.

Bioinformatic Analysis

All sequence data files were obtained from National Center for Biotechnology Information (**NCBI**). The IEL cDNA elements used to create the IEL cDNA microarray were mapped to the chicken genome reference assembly (version 2.1) and reference RNA and protein sequences (formatted database for BLAST, May 2006) using NCBI BLAST (version 2.2.13). The criteria for the acceptance of BLAST search results were alignment length $\geq$100 nucleotides and e-value $\leq 1e^{-100}$ for DNA sequences and alignment length $\geq$30 amino acids and e-value $\leq 1e^{-10}$ for protein sequences. Entrez gene data and Homologene data (October 2007) from NCBI were used to gather gene information [identification number (**ID**), symbol, and name]. Gene Ontology (**GO**) annotations were extracted from gene to GO data in NCBI and 7,878 elements were annotated in a total of 10,162 spots on AVIELA. To analysis pathway information, chicken Entrez gene ID >2.0-fold differentially expressed between M5.1 and M15.2 were mapped to Homologene ID (locus link ID) for human because a large portion of these sequences have not defined their function in chicken yet. The mapped gene ID were used for analyses of pathway classification from the PANTHER database (http://www.pantherdb.org). Having annotation derived from orthologous human genes means that cross-species comparisons between chicken and human array data may be possible (Smith et al., 2006).

Quantitative Reverse Transcription-PCR

To confirm gene expression changes observed by microarray analysis, quantitative real-time PCR (**QRT-PCR**) was performed as described (Hong et al., 2006). Equivalent amounts of same RNA samples used for microarray hybridizations were reverse-transcribed using the StrataScript first strand synthesis system (Stratagene, La Jolla, CA). Amplification and detection were carried out with the Mx3000P system and Brilliant SYBR Green QRT-PCR master mix (Stratagene). Standard curves were generated using base-10 logarithm-diluted standard RNA and levels of individual transcripts were normalized to those of glyceraldehyde

3-phosphate dehydrogenase by the Q-gene program (Muller et al., 2002).

For the calculation of fold changes between control and treated group, the normalized cycle threshold values of target gene to glyceraldehyde 3-phosphate dehydrogenase were calibrated to the relevant control line values. Oligonucleotide primers for QRT-PCR are listed in Table 1. Each analysis was performed in triplicate.

RESULTS

Gene Expression Profile Induced by E. maxima Infection

In this study, differential expression levels of transcripts caused by *E. maxima* infection in intestinal IEL of M5.1 and M15.2 line chickens were analyzed using AVIELA microarray.

When compared with uninfected controls and using a cutoff of >2.0-fold differential expression between infected and noninfected, M5.1 demonstrated altered expression of 1 (downregulated), 12 (6 up, 6 down), and 18 (5 up, 13 down) mRNA at 3, 4, and 5 dpi, respectively. In the case of the M15.2 line, altered expression was observed in 6 (3 up, 3 down), 29 (11 up, 18 down), and 32 (8 up, 24 down) transcripts at the 3 time points compared with uninfected controls (Figure 1). The genes with differential expression after infection and identified with common gene names mapped using human gene ID are listed in Table 2 and 3. The numbers of the genes annotated among >2.0-fold altered ones are 1, 8, and 17 in M5.1 and 5, 24, and 22 in M15.2 at each times point, respectively.

Comparing birds of line M5.1 with M15.2 after *E. maxima* infection revealed differential expression in 32 (10 upregulated, 22 downregulated), 98 (43 up, 55 down), and 92 (33 up, 59 down) mRNA, using a cutoff of >2.0-fold at the 3 time points (Figure 2). The >2.0-fold altered genes between the 2 lines at 3 time points examined are shown in Table 4, 5, and 6, respectively.

Gene Ontology Annotation and Bioinformatics Analysis

The elements that corresponded to human gene ID and that were differentially modulated >2.0-fold between lines M5.1 and M15.2 at any of the time points after *E. maxima* infection were distributed in the branch of biological process according to the GO index using the PANTHER databases (Mi et al., 2005). In the branches of biological process, two of the largest parts were involved in the categories of protein metabolism and modification (14 up, 11 down), and nucleoside, nucleotide, and nucleic acid metabolism (9 up, 5 down) in both up- and downregulated genes in M5.1 compared with M15.2 (Figure 3). Generally, a larger number of

Table 2. The differentially expressed genes identified using the correspondent identity name of human genes after *Eimeria maxima* infection in Fayoumi M5.1 line compared with uninfected controls (>2.0-fold changes)

GenBank no.	Normalized ratio	*P*-value	Gene symbol[1]	Description
3 d postinfection				
CF074949	0.3	0.03	HAS2	Hyaluronan synthase 2
4 d postinfection				
CD738831	9.7	0.04	PGAM1	Phosphoglycerate mutase 1 (brain)
CD726890	9.6	0.05	NRAS	Neuroblastoma RAS viral (v-ras) oncogene homolog
CF075017	3.0	0.03	IL13RA1	Interleukin 13 receptor, α 1
CD732030	2.1	0.00	LOC423827	Similar to TUBA
CF075109	0.4	0.01	LOC419815	Splicing factor, arginine/serine-rich 3
CD739019	0.3	0.03	QTRTD1	Queuine tRNA-ribosyltransferase domain containing 1
CD728022	0.3	0.03	LOC423895	Similar to glycerol-3-phosphate acyltransferase, mitochondrial
CD736338	0.3	0.02	APOA4	Apolipoprotein A-IV
5 d postinfection				
CD736627	11.0	0.04	RARRES1	Retinoic acid receptor responder (tazarotene induced) 1
CF075017	4.3	0.01	IL13RA1	Interleukin 13 receptor, α 1
CD737602	3.2	0.03	PPP3CB	Protein phosphatase 3 (formerly 2B) catalytic subunit β isoform
CD737613	2.8	0.04	LOC770135	Similar to smallest subunit of ubiquinol-cytochrome c reductase
CF075075	2.4	0.03	EIF4H	Eukaryotic translation initiation factor 4H
CF075109	0.5	0.03	LOC419815	Splicing factor, arginine/serine-rich 3
CD731060	0.5	0.02	LOC769871	Similar to transcriptional adaptor 1 (HFI1 homolog, yeast)-like
CF074926	0.5	0.02	LOC777308	Similar to eukaryotic translation initiation factor 3 p42 subunit
CF075105	0.4	0.04	LOC749246	Similar to ribosomal protein S9
CD740249	0.4	0.04	LOC419390	Similar to enhancer of split-related protein-7
CF075134	0.4	0.03	FYN	FYN oncogene related to SRC, FGR, YES
CD737563	0.3	0.05	LOC423450	Hypothetical LOC423450
CD728772	0.3	0.04	Chro.40465	4034766 1GAL - chicken intestinal lymphocyte *Gallus gallus* cDNA clone 1GAL_66B17 5′, mRNA sequence
CD729740	0.2	0.03	TSR1	TSR1, 20S rRNA accumulation, homolog (*Saccharomyces cerevisiae*)
CD729636	0.2	0.01	c-fps	Similar to c-fps proto oncogene
CD729635	0.2	0.04	KCNAB2	Potassium voltage-gated channel, shaker-related subfamily, β member 2
CD739894	0.2	0.03	LOC419427	Similar to RIKEN cDNA 9430015G10

[1]Gene names for human mapped by chicken Entrez gene identification numbers..

Table 3. The differentially expressed genes identified using the correspondent identity name of human genes after *Eimeria maxima* infection in Fayoumi M15.2 line compared with uninfected controls (>2.0-fold changes)

GenBank	Normalized ratio	*P*-value	Gene symbol[1]	Description
3 d postinfection				
CF074761	2.8	0.03	TNRC6B	Trinucleotide repeat containing 6B
CF074848	2.3	0.03	RCJMB04_1k19	Similar to RasGAP-activating-like protein 1
CD733250	2.0	0.03	ANKRD10	Ankyrin repeat domain 10
CD740095	0.5	0.02	LOC483748	Similar to Finkel-Biskis-Reilly murine sarcoma virus ubiquitously expressed
CD729173	0.5	0.02	PPIF	Peptidylprolyl isomerase F (cyclophilin F)
4 d postinfection				
CF074761	3.6	0.01	TNRC6B	Trinucleotide repeat containing 6B
CD733216	2.6	0.00	LOC426427	Similar to KIAA0556 protein
CD733250	2.5	0.02	ANKRD10	Ankyrin repeat domain 10
CF074943	2.4	0.03	LOC770561	Archain 1
CD728893	2.3	0.01	RASSF2	Ras association (RalGDS/AF-6) domain family 2
CD738017	2.2	0.05	LOC422421	Similar to FLJ38482 protein
CD738198	2.2	0.04	LOC770246	Adaptor-related protein complex 2, μ 1 subunit
CF074848	2.1	0.04	RCJMB04_1k19	Similar to RasGAP-activating-like protein 1
CF074844	2.0	0.04	RPS5	Ribosomal protein S5
CD729173	0.5	0.04	PPIF	Peptidylprolyl isomerase F (cyclophilin F)
CD735567	0.4	0.03	PIGX	Phosphatidylinositol glycan anchor biosynthesis, class X
CD736593	0.4	0.03	LOC776552	Similar to MADML
CD726866	0.4	0.00	LOC415975	Similar to brefeldin-resistant Arf-GEF 2b
CD733075	0.4	0.01	ZW10	ZW10, kinetochore associated, homolog (*Drosophila*)
CD736356	0.4	0.03	LOC422525	Similar to RIKEN cDNA 2310008M10
CD731124	0.4	0.04	BDNF	Brain-derived neurotrophic factor
CD734663	0.4	0.03	CDK5RAP2	CDK5 regulatory subunit associated protein 2
CD728901	0.4	0.05	BTK	Bruton agammaglobulinemia tyrosine kinase
CD740484	0.4	0.02	ADIPOR2	Adiponectin receptor 2
CD738626	0.3	0.02	CD36	CD36 molecule (thrombospondin receptor)
CD738977	0.3	0.01	LOC426644	Similar to KIAA1815 protein
CD735013	0.3	0.03	LOC415791	Similar to carbonic anhydrase VII
CD732103	0.3	0.01	RCJMB04_30g17	Similar to β-hexosaminidase α chain precursor (N-acetyl-β-glucosaminidase) (β-N-acetylhexosaminidase) (hexosaminidase A)
CD734891	0.3	0.02	Rad23a	RAD23a homolog (*Saccharomyces cerevisiae*)
5 d postinfection				
CD738017	2.6	0.02	LOC422421	Similar to FLJ38482 protein
CF074761	2.5	0.03	TNRC6B	Trinucleotide repeat containing 6B
CF074848	2.5	0.02	RCJMB04_1k19	Similar to RasGAP-activating-like protein 1
CD737969	2.5	0.03	TCERG1	Transcription elongation regulator 1
CD740789	2.3	0.01	LOC420675	Similar to KIAA0096
CD734282	2.2	0.05	LOC424735	Similar to STAG1 variant protein
CD728144	2.1	0.02	LOC423091	Similar to transient receptor potential cation channel, subfamily M, member 5
CF074926	0.5	0.01	LOC777308	Similar to eukaryotic translation initiation factor 3 p42 subunit
CD726837	0.5	0.05	LOC418899	Similar to regulatory factor X-associated protein
CD736696	0.5	0.03	TXNL5	Thioredoxin-like 5
CD731350	0.4	0.00	RCP9	Calcitonin gene-related peptide-receptor component protein
CD735013	0.4	0.03	LOC415791	Similar to carbonic anhydrase VII
CD733203	0.4	0.04	LOC416956	Similar to KIAA0645 protein
CD729173	0.4	0.01	PPIF	Peptidylprolyl isomerase F (cyclophilin F)
CD734891	0.4	0.04	Rad23a	RAD23a homolog (*S. cerevisiae*)
CD733404	0.3	0.02	LOC408047	Thymosin β 4
CD728880	0.3	0.05	PTCD3	Pentatricopeptide repeat domain 3
CD736593	0.3	0.01	LOC776552	Similar to MADML
CD728901	0.3	0.01	BTK	Bruton agammaglobulinemia tyrosine kinase
CD735567	0.3	0.00	PIGX	Phosphatidylinositol glycan anchor biosynthesis, class X
CD738626	0.3	0.00	CD36	CD36 molecule (thrombospondin receptor)
CD732103	0.2	0.01	RCJMB04_30g17	Similar to β-hexosaminidase α chain precursor (N-acetyl-β-glucosaminidase) (β-N-acetylhexosaminidase) (hexosaminidase A)

[1]Gene names for human mapped by chicken Entrez gene identification numbers.

genes upregulated in M5.1 (M5.1 > M15.2) were classified in each GO index than downregulated genes (M5.1 < M15.2) except apoptosis and electron transport. In the 3 terms of GO, cell structure and motility (8 up, 1 down), intracellular protein traffic (5 up, 1 down), and signal transduction (12 up, 3 down), the considerable differences were shown between the number of up- (M5.1 > M15.2) and downregulated (M5.1 < M15.2) genes in M5.1.

In the GO classification for the >2.0-fold altered genes between the M5.1 and M15.2 lines, 7 transcripts are grouping into the category of immunity and defense. Among those genes, 4 upregulated genes including growth factor receptor-bound protein 2 (**GRB2**),

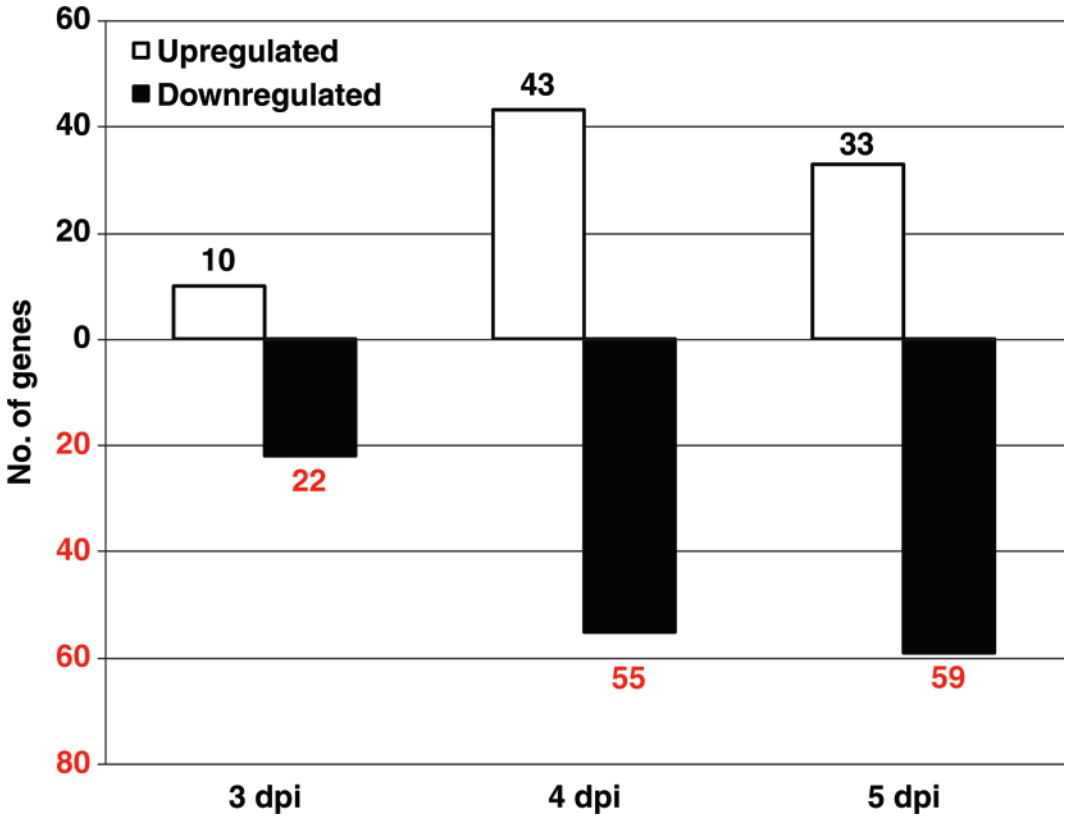

Figure 2. Number of transcripts on the avian intestinal intraepithelial lymphocyte cDNA microarray (AVIELA) showing the alteration of the expression levels between M5.1 and M15.2 lines with >2.0-fold changes after *Eimeria maxima* infection. dpi = days postinfection.

Table 4. The annotated genes altered with >2.0 changes between Fayoumi M5.1 and M15.2 lines at 3 d post *Eimeria maxima* infection ($P < 0.01$)

GenBank no.	Normalized ratio	Gene symbol[1]	Description
M5.1 > M15.2			
CD726897	5.2	LOC770701	Kelch-like 25 (*Drosophila*)
CF075061	3.4	GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
CD736907	3.3	ADIPOR1	Adiponectin receptor 1
CD728847	2.9	TTL	Tubulin tyrosine ligase
CD735511	2.9	LOC421447	Similar to Ephx1 protein
CD726890	2.7	NRAS	Neuroblastoma RAS viral (v-ras) oncogene homolog
CD729173	2.6	PPIF	Peptidylprolyl isomerase F (cyclophilin F)
CD736353	2.6	LOC419543	Similar to dehydrodolichyl diphosphate synthase
CF074933	2.4	PPP2R5C	Protein phosphatase 2, regulatory subunit B′, γ isoform
CD736260	2.4	GRB2	Growth factor receptor-bound protein 2
CD737334	2.1	LOC424580	Similar to hypothetical protein FLJ10597
CF075002	2.1	RPL5	Ribosomal protein L5
CF075075	2.1	EIF4H	Eukaryotic translation initiation factor 4H
CD729060	2.1	MPST	Mercaptopyruvate sulfurtransferase
CD740122	2.1	PLEKHM1	Pleckstrin homology domain containing, family M (with RUN domain) member 1
CD730633	2.1	ZWILCH	Zwilch, kinetochore associated, homolog (*Drosophila*)
CD731093	2.0	LOC426882	Similar to SMARCD1 protein
M5.1 < M15.2			
CD733216	−2.1	LOC426427	Similar to KIAA0556 protein
CD735777	−2.2	LOC424065	Hypothetical LOC424065
CF075058	−2.1	LOC416900	Similar to MSTP028
CD729397	−2.2	LOC415987	Similar to RIKEN cDNA 1110007C09
CD733805	−2.3	EREG	Epiregulin
CD728146	−2.6	PFL_4320	GGDEF domain protein
CF074920	−3.0	RPL21	Ribosomal protein L21
CD729636	−3.8	c-fps	Similar to c-fps proto oncogene

[1]Gene names for human mapped by chicken Entrez gene identification numbers.

Table 5. The annotated genes altered with >2.0 changes between Fayoumi M5.1 and M15.2 lines at 4 d post *Eimeria maxima* infection ($P < 0.01$)

GenBank no.	Normalized ratio	Gene symbol[1]	Description
M5.1 > M15.2			
CD734677	9.4	USP54	Ubiquitin specific peptidase 54
CD726897	7.5	LOC770701	Kelch-like 25 (*Drosophila*)
CD737602	5.0	PPP3CB	Protein phosphatase 3 (formerly 2B) catalytic subunit β isoform
CD737041	4.4	LCP1	Lymphocyte cytosolic protein 1 (L-plastin)
CD738626	3.5	CD36	CD36 molecule (thrombospondin receptor)
CD739823	3.4	LOC421402	Similar to adenosine triphosphate-binding cassette subfamily G member 8
CD737778	3.4	LOC423730	Catechol-*O*-methyltransferase domain containing 1
CD736563	3.3	PPP2CA	Protein phosphatase 2 (formerly 2A), catalytic subunit, α isoform
CD735620	3.3	RGS2	Regulator of G-protein signaling 2, 24kDa
CD736352	3.2	DNM1L	Dynamin 1-like
CD737576	3.2	HPRT1	Hypoxanthine phosphoribosyltransferase 1
CD735718	3.0	LOC427838	Hypothetical LOC427838
CD735496	2.9	ANKRD43	Ankyrin repeat domain 43
CD735615	2.8	BLMH	Bleomycin hydrolase
CD740791	2.8	LOC431656	Similar to potential phospholipid-transporting ATPase identification number (ATPase class I type 8B member 2) (*Gallus gallus*)
CD735377	2.8	GTL3	Gene trap locus 3 (mouse)
CD737186	2.7	CSNK1G1	Casein kinase 1, γ 1
CD729722	2.7	FRMD4B	FERM domain containing 4B
CD726866	2.6	LOC415975	Similar to brefeldin-resistant Arf-GEF 2b
CD729249	2.6	TOP2A	Topoisomerase (DNA) II α 170kDa
CD737494	2.5	C130052I12RIK|CLPTM1L	CLPTM1-like
CD727217	2.3	LOC416833	Similar to MGC80999 protein
CD734891	2.3	Rad23a	RAD23a homolog (*Saccharomyces cerevisiae*)
CD739023	2.3	RAB10	RAB10, member RAS oncogene family
CD728508	2.3	LOC421939	Similar to V-ATPase C2 subunit
CD738543	2.2	CRYL1	Crystallin, λ 1
CF075038	2.2	DDX6	DEAD (Asp-Glu-Ala-Asp) box polypeptide 6
CD737673	2.1	LOC423737	Similar to Ca^{2+}/calmodulin-dependent protein kinase (EC 2.7.1.123) II γ-E - human
CD729342	2.1	LOC416154	Similar to intestinal 15 kDa protein; I-15P
CD737315	2.1	LOC424093	Similar to MGC83858 protein
CD729537	2.1	SCYL2	SCY1-like 2 (*S. cerevisiae*)
CD736595	2.1	LOC418774	Similar to propionyl-coenzyme A carboxylase, α polypeptide
CD734530	2.0	LOC421055	Similar to band 4.1-like protein 3 (4.1B) (differentially expressed in adenocarcinoma of the lung protein 1) (DAL-1)
M5.1 < M15.2			
CD732036	−2.0	SAE2	SUMO1 activating enzyme subunit 2
CD731958	−2.0	LOC419119	Similar to RP5-977B1.6
CD732691	−2.0	CCDC128	Coiled-coil domain containing 128
CD738642	−2.1	IGLV	Ig light chain variable region
CD733218	−2.1	NUBP2	Nucleotide binding protein 2 (MinD homolog, *Escherichia coli*)
CD734942	−2.1	LOC419439	Weakly similar to NP_796340.2 protein-coupled receptor 157 (*Mus musculus*)
CD740798	−2.1	LOC777558	Similar to HNRPC protein
CD737016	−2.1	LOC395310	Prominin-like protein
CD738651	−2.2	FOS	v-fos FBJ murine osteosarcoma viral oncogene homolog
CD734506	−2.2	P20K	Quiescence-specific protein
CD726889	−2.2	LOC416715	Similar to brain-selective and closely mapped on the counter allele of CMAP in cystatin cluster
CD726888	−2.4	LOC420129	Similar to interferon, γ-inducible protein 30
CF075071	−2.4	P0	Acidic ribosomal phosphoprotein
CD733252	−2.4	CMAS	Cytidine monophosphate N-acetylneuraminic acid synthetase
CD739766	−2.4	LOC423773	Similar to β-microseminoprotein precursor (prostate secreted seminal plasma protein) (prostate secretory protein PSP94) (PSP-94) (seminal plasma β-inhibin) (immunoglobulin-binding factor) (PN44)
CD736633	−2.4	LOC425800	Eukaryotic translation initiation factor 2B, subunit 4 Δ, 67 kDa
CD728109	−2.4	SPCS1	Signal peptidase complex subunit 1 homolog (*S. cerevisiae*)
CD736370	−2.4	LOC426813	GRAM domain containing 3
CD727877	−2.5	EPAS1	Endothelial PAS domain protein 1
CF075156	−2.7	TUBA1C	Tubulin, α 1c
CD740371	−2.7	RBM16	RNA binding motif protein 16
CD736859	−2.7	LOC416104	Cereblon
CF074794	−2.7	WWOX	WW domain containing oxidoreductase
CD728818	−2.8	LOC417458	Similar to cytidine deaminase
CD733216	−2.9	LOC426427	Similar to KIAA0556 protein
CD728075	−2.9	LOC771339	Finished cDNA, clone ChEST567a23
CD734909	−2.9	COPG	Coatomer protein complex, subunit γ
CD735777	−3.0	LOC424065	Hypothetical LOC424065
CF074761	−3.1	TNRC6B	Trinucleotide repeat containing 6B
CD739117	−3.8	LOC424142	Similar to mitochondrial adenosine triphosphate synthase subunit 9
CD732648	−4.0	HNRPAB	Heterogeneous nuclear ribonucleoprotein A/B
CD730156	−5.0	EIF1AX	Eukaryotic translation initiation factor 1A, X-linked
CD727087	−6.1	LOC417268	Similar to solute carrier family 31, member 1

[1]Gene names for human mapped by chicken Entrez gene identification numbers.

Table 6. The annotated genes altered with >2.0 changes between Fayoumi M5.1 and M15.2 lines at 5 d post *Eimeria maxima* infection ($P < 0.01$)

GenBank no.	Normalized ratio	Gene symbol[1]	Description
M5.1 > M15.2			
CD736627	8.9	RARRES1	Retinoic acid receptor responder (tazarotene induced) 1
CD735718	4.8	LOC427838	Hypothetical LOC427838
CD736698	4.6	AZIN1	Antizyme inhibitor 1
CD740791	4.3	LOC431656	Similar to potential phospholipid-transporting ATPase identification number (ATPase class I type 8B member 2) (*Gallus gallus*)
CD726890	4.3	NRAS	Neuroblastoma RAS viral (v-ras) oncogene homolog
CD736325	4.2	LOC426107	Similar to T-cell receptor Δ chain
CD731420	3.6	LOC417255	Similar to tubulin, β 8 isoform 2 (*Macaca mulatta*)
CD735845	3.6	LOC424309	Similar to enhancer of polycomb homolog 2
CD731395	3.5	ZA20D2|ZFAND5	Zinc finger, AN1-type domain 5
CD737576	3.5	HPRT1	Hypoxanthine phosphoribosyltransferase 1
CD728880	3.5	PTCD3	Pentatricopeptide repeat domain 3
CD732103	3.5	RCJMB04_30g17	Similar to β-hexosaminidase α chain precursor (N-acetyl-β-glucosaminidase) (β-N-acetylhexosaminidase) (hexosaminidase A)
CD736593	3.3	LOC776552	Similar to MADML
NM_205046	3.2	LOC395914	Lymphotactin
CF074772	3.1	RPL15	Ribosomal protein L15
CD728315	3.1	LOC693257	NK-lysin
CD738700	2.9	LOC768712	TBC1 domain family, member 16
CD733357	2.8	RHOB	ras homolog gene family, member B
CD738626	2.8	CD36	CD36 molecule (thrombospondin receptor)
CD737041	2.7	LCP1	Lymphocyte cytosolic protein 1 (L-plastin)
CD731023	2.6	LOC420135	Similar to myosin IXB
CD730542	2.6	HS6ST1	Heparan sulfate 6-O-sulfotransferase 1
CD727214	2.6	GABPA	GA binding protein transcription factor, α subunit 60kDa
CF075081	2.5	ARPC1B	Actin related protein 2/3 complex, subunit 1B, 41 kDa
CD736565	2.5	LOC429119	Similar to KIAA1585 protein
CD729142	2.5	LOC420097	Hypothetical LOC420097
CD730482	2.5	CBFB	Core-binding factor, β subunit
CD735615	2.5	BLMH	Bleomycin hydrolase
CD728986	2.5	LOC419338	Breast carcinoma amplified sequence 1
CD728235	2.3	RCJMB04_2c12	Similar to KIAA0776 protein
CD736321	2.3	STK38L	Serine/threonine kinase 38-like
XM_418246.2	2.3	LOC420129	Similar to interferon, γ-inducible protein 30 (LOC420129)
CD735602	2.3	ITGB5	Integrin, β 5
CD734166	2.2	LOC425007	Similar to maltase-glucoamylase
CD737055	2.2	AQR	Aquarius homolog (mouse)
NM_001001315	2.2	LOC408047	Thymosin β 4 (LOC408047)
CD734530	2.2	LOC421055	Similar to band 4.1-like protein 3 (4.1B) (differentially expressed in adenocarcinoma of the lung protein 1) (DAL-1)
CD730417	2.2	BAK1	BCL2-antagonist/killer 1
CD732989	2.0	NUP214	Nucleoporin 214 kDa
NM_001001315	2.0	LOC408047	Thymosin β 4 (LOC408047)
CD732701	2.0	LMBRD1	LMBR1 domain containing 1
M5.1 < M15.2			
CD734004	−2.0	LOC426803	Hypothetical gene supported by CR388998
CD729545	−2.1	CCDC134	Coiled-coil domain containing 134
CD729490	−2.2	GPR174	G protein-coupled receptor 174
CD734526	−2.2	LOC423816	Similar to KIAA0940 protein
CD737501	−2.2	DNAJA1	DnaJ (Hsp40) homolog subfamily A member 1
CD736407	−2.2	RNASEH1	Ribonuclease H1
CD738866	−2.3	NDUFA10	NADH dehydrogenase (ubiquinone) 1 α subcomplex, 10, 42 kDa
CD737774	−2.3	RNF13	Ring finger protein 13
CD734283	−2.3	LOC419386	Finished cDNA, clone ChEST495b14
CD739480	−2.3	MON1A	MON1 homolog A (yeast)
CD739166	−2.4	LOC421039	Similar to centrosomal protein 76
CD731792	−2.4	LOC425564	Similar to death receptor 3
CD739782	−2.4	RCJMB04_10d1	Peptidase D
CD740029	−2.5	ACOX1	Acyl-coenzyme A oxidase 1, palmitoyl
CD736633	−2.5	LOC425800	Eukaryotic translation initiation factor 2B, Subunit 4 Δ, 67 kDa
CD738420	−2.7	MFAP3	Microfibrillar-associated protein 3
CD735820	−2.7	LOC426168	Similar to MGC69002 protein
CD736528	−2.9	STK17B	Serine/threonine kinase 17b
CD730156	−3.4	EIF1AX	Eukaryotic translation initiation factor 1A, X-linked
CD730231	−3.4	CTNNB1	Catenin (cadherin-associated protein), β 1, 88 kDa
CD728146	−3.7	PFL_4320	GGDEF domain protein
CD735777	−4.3	LOC424065	Hypothetical LOC424065
CD729636	−4.4	c-fps	Similar to c-fps proto oncogene
CD735687	−5.2	PYGL	Liver glycogen phosphorylase
CD729578	−5.3	LOC430685	Similar to SHC (Src homology 2 domain containing) transforming protein 1
CD729635	−6.3	KCNAB2	Potassium voltage-gated channel, shaker-related subfamily, β member 2

[1]Gene names for human mapped by chicken Entrez gene identification numbers.

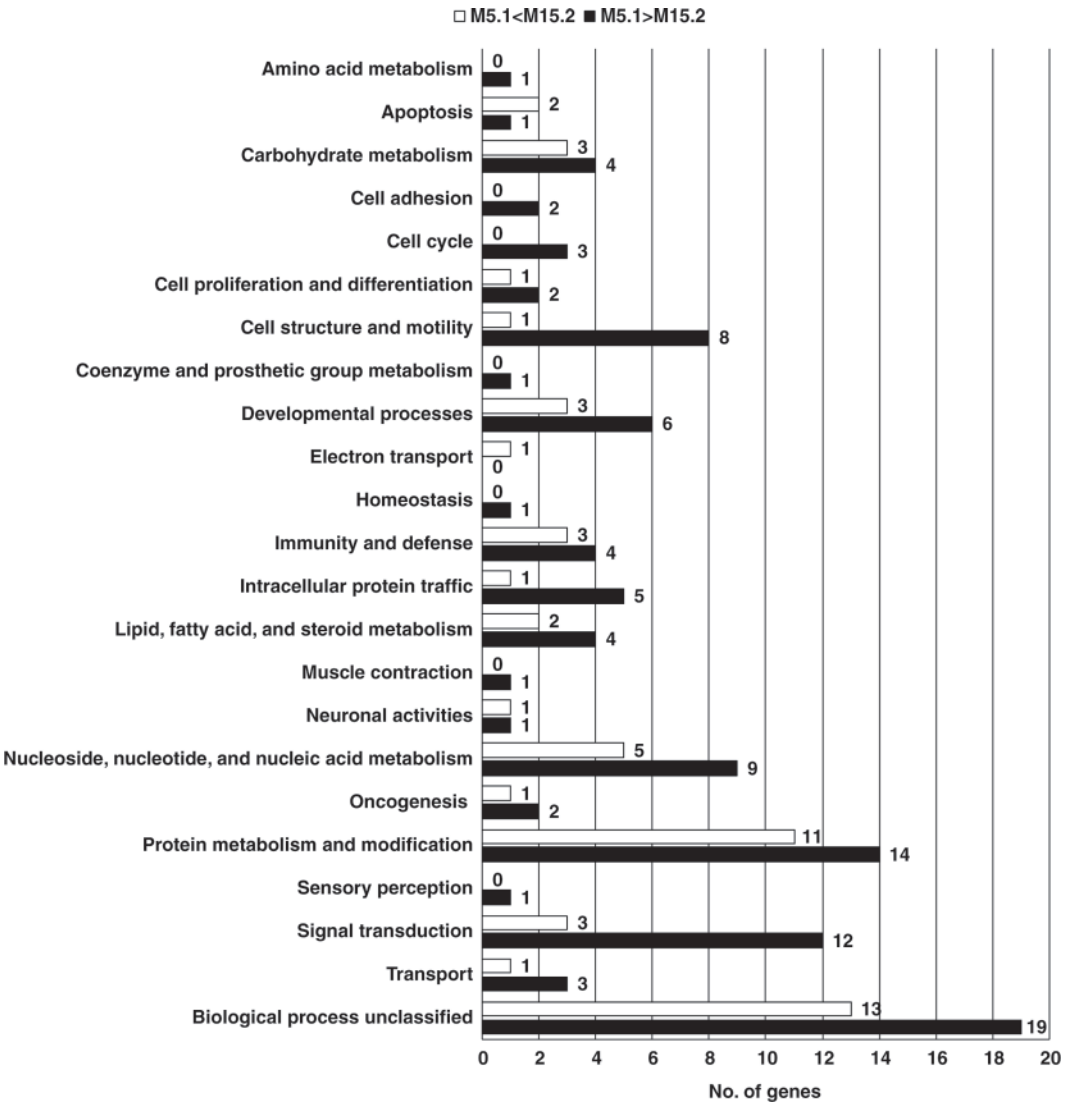

Figure 3. Gene ontology (GO) analysis of genes mapped for human exhibiting >2.0-fold altered expression levels between M5.1 and M15.2 lines during *Eimeria maxima* infection using the GO terms of biological process.

integrin β 5, protein phosphatase 3 catalytic subunit β isoform, peptidylprolyl isomerase F and 1 downregulated gene, DnaJ (Hsp40) homolog subfamily A member 1, are annotated with common gene names for human (Table 7).

QRT-PCR

We selected 4 genes and followed the kinetics of their corresponding transcript levels after *E. maxima* infection. All of the genes selected showed alteration of expression with >2.0-fold change in the normalized data of AVIELA at more than one time point examined ($P < 0.05$). Three of those genes, apolipoprotein A-IV (**APOA4**); FYN oncogene related to SRC, FGR, YES (**FYN**); and neuroblastoma RAS viral oncogene homolog (**NRAS**), are involved in more than one specific pathway by the analysis using GeneSpring GX 7.3 software.

Expression of APOA4 was more repressed in M5.1 than M15.2 at 4 dpi of *E. maxima,* as measured by both AVIELA and QRT-PCR (Figure 4A). Pathway analysis indicated that APOA4 is related with the statin pathway. Statins are highly effective in lowering serum cholesterol concentrations and preventing ischemic heart disease (Pignone et al., 2000). The expression of FYN transcripts was significantly downregulated in AVIELA data and RT-PCR analysis ($P < 0.05$) at 3 dpi in the M5.1 line. In contrast, it was upregulated in the M15.2 line (Figure 4B). The gene FYN is supposed to be involved in several interleukin signaling pathways, integrin signaling pathways, and immune cell receptor pathways. In our study, NRAS was markedly upregulated in M5.1 chickens at 4 dpi, measured by using microarray; however, its expression was only slightly increased as quantified by RT-PCR (Figure 4C). In the analysis using GeneSpring GX 7.3, the epidermal growth factor 1 signaling pathway and mitogen-activated protein ki-

Table 7. The genes classified into the gene ontology (GO) term of immunity and defense with >2.0-fold changes between Fayoumi M5.1 and M15.2 lines using the PANTHER database

Gene ID[1] (human)	Gene name	Gene symbol	Score[2]	Biological process	Pathway
Upregulated					
				Receptor protein tyrosine kinase signaling pathway	Inflammation mediated by chemokine and cytokine signaling pathway→Growth factor receptor-bound protein
				Other immune and defense	PI3 kinase pathway→Grb2 FGF signaling pathway→Grb2 Interleukin signaling pathway→growth factor receptor binding protein 2 T-cell activation→Grb2 Dopamine receptor-mediated signaling pathway→growth factor receptor-bound protein 2
2885	Growth factor receptor-bound protein 2	GRB2	1.00E-110		Insulin/insulin-like growth factor pathway-mitogen-activated protein kinase kinase/mitogen-activated protein kinase cascade→growth factor receptor bound protein 2 Ras pathway→growth factor receptor-bound protein-2 T-cell activation→Gads B-cell activation→Grb2 Angiogenesis→Growth factor Receptor-bound protein 2 Integrin signaling pathway→growth factor receptor bound protein 2 PDGF signaling pathway→growth factor Receptor binding protein 2 EGF receptor signaling pathway→Grb2 Angiogenesis→growth factor receptor-bound protein 2
3693	Integrin, β 5	ITGB5	0.00E+00	Cell adhesion-mediated signaling Cell adhesion Blood clotting Cell motility	Integrin signaling pathway→integrin β Blood coagulation→glycoprotein IIIa
5532	Protein phosphatase 3 (formerly 2B) catalytic subunit β isoform (calcineurin A β)	PPP3CB	0.00E+00	Regulation of carbohydrate metabolism Other polysaccharide metabolism Glycogen metabolism mRNA transcription Protein phosphorylation Translational regulation Other intracellular signaling cascade Stress response Meiosis Cell cycle control Mitosis Homeostasis	B-cell activation→calcineurin T-cell activation→calcineurin Wnt signaling pathway→calcineurin
10105	Peptidylprolyl isomerase F (cyclophilin F)	PPIF	0.00E+00	Protein folding Nuclear transport Immunity and defense	—
Downregulated					
3003	Granzyme K (granzyme 3; tryptase II)	GZMK	0.00E+00	Proteolysis T-cell-mediated immunity Natural killer cell-mediated immunity Granulocyte-mediated immunity Apoptosis	—
10437	Interferon, γ-inducible protein 30	IFI30	0.00E+00	Proteolysis MHCII-mediated immunity	—
3301	DnaJ (Hsp40) homolog subfamily A member 1	DNAJA1	0.00E+00	Protein folding Stress response	—

[1]Homologene identification numbers for human mapped by chicken Entrez gene identification numbers.

[2]The low scores closing with 0 indicate the high level of confidence in the relation between gene and GO term.

nase signaling pathway were searched as NRAS associated pathway. Both pathways play vital roles as important regulators in response to a diverse range of stimuli, such as cytokines, growth factors, neurotransmitters, cellular stress, and cell adherence during development, cellular proliferation, survival, and migration (Orton et al., 2005; Marks et al., 2007). Finally, expression of CD36 was downregulated in the M15.2 line at 4 dpi using both AVIELA and real-time RT-PCR (Figure 4D).

DISCUSSION

We previously reported that 2 genetically disparate Fayoumi chicken lines, M5.1 and M15.2, show differences in susceptibility against avian coccidiosis (Kim et al., 2008). The MHC molecules play important roles in the regulation of the immune response by communicating among different cellular components of the immune system: T cells, B cells, and antigen-presenting cells (Lamont, 1998). Because M5.1 and M15.2 are highly identical congenic lines except the chromosome including MHC, the differentially expressed genes after the *E. maxima* infection might be related with avian immunity. Accordingly, the current study was conducted to analyze the gene expression profiles induced by the infection of *E. maxima* oocysts in these 2 *B*-complex congenic chicken lines by cDNA microarray technique.

Avian intestinal IEL are fabricated with cDNA clones selected from a cDNA library of chicken intestinal IEL of *Eimeria*-infected chickens and LPS-stimulated macrophages (Min et al., 2003, 2005). Intraepithelial lymphocytes play an important role in protective immunity to avian coccidiosis (Lillehoj and Lillehoj, 2000). Lipopolysaccharides, a component of bacteria membrane, provoke an endotoxic shock and induce a strong response in animal immune systems. It causes the secretion of proinflammatory cytokines by a receptor complex composed of LPS-binding protein CD14 and Toll-like receptor 4 in macrophages (Beutler, 2000). Furthermore, there are the selected cytokines and chemokine genes from the direct PCR clones on AVIELA microarray. Therefore, AVIELA is a suitable tool for the host gene expression profiling of the interaction between host and parasite in the chicken gut.

In the current study, the number of genes with >2-fold changes in IEL was increased with time after *Eimeria* infection (Figure 1). The altered gene number was larger in line M15.2 than in line M5.1 birds at any dpi and the latter line is more resistant compared with M15.2. The genes changed in transcriptional regulation are increased after the conditions of coccidiosis and the higher number of differentially expressed genes appeared in the more susceptible line. These data suggest that the greater pathology induces the higher gene expression changes. However, more functional approaches needed to clarify this assumption.

Comparing the expression patterns of transcripts between the 2 chicken lines, the number of altered genes

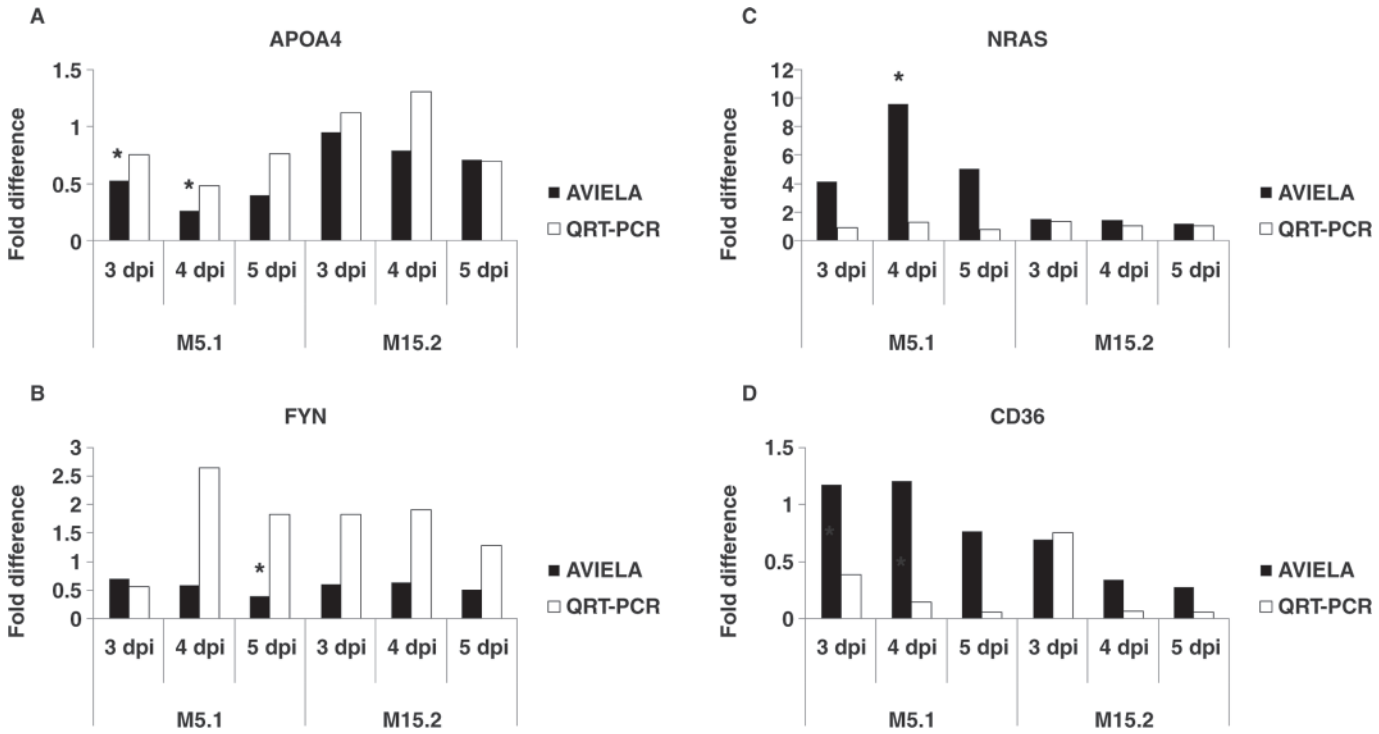

Figure 4. Quantitative analysis of mRNA levels of selected genes by real-time reverse transcription-PCR after *Eimeria maxima* infection at 3, 4, and 5 d postinfection (dpi). (A) Apolipoprotein A-IV (APOA4). (B) FYN oncogene related to SRC, FGR, YES (FYN). (C) Neuroblastoma RAS viral oncogene homolog (NRAS). (D) CD36. $^*P < 0.05$ in the microarray analysis. AVIELA = avian intestinal intraepithelial lymphocyte cDNA microarray; QRT-PCR = quantitative real-time PCR.

was highest at 4 dpi, rather than 5 dpi (Figure 2). This suggests that the transcriptome conversion during the early stage of avian coccidiosis before 4 dpi is crucial for the resistance due to host innate immune system in the chicken gut. Similar results were observed by Min et al. (2003). They monitored transcriptional changes between 1 and 4 d post primary infection with *Eimeria* oocysts in chicken IEL using a cDNA microarray containing 400 chicken genes and showed that the largest changes of transcript number occurred at 4 dpi of primary infection.

Quantitative real-time RT-PCR for the selected genes was performed to confirm the differential expression patterns that we found in AVIELA analysis between the 2 Fayoumi chicken lines. It has been found that APOA4 is an endogenous antiinflammatory protein (Vowinkel et al., 2004) and it shows impaired expression in inflammatory bowel disease mucosa, even in the noninflamed regions (Orso et al., 2007). In mast cells, the absence of FYN and phosphatidylinositol 3-kinase, or the inhibition of p38 mitogen-activated protein kinase activity, demonstrated that they are essential for H_2O_2-driven interleukin-4 production (Frossi et al., 2007). Mitogen-activated protein kinase activity is subject to regulation even in V-raf murine sarcoma viral oncogene homolog B1 (BRAF)/NRAS mutant melanoma cells (Edlundh-Rose et al., 2006). It has been found that CD36 facilitates the uptake of specific lipid molecules and adheres to macromolecules with subsequent transduction of intracellular signals with relevance to inflammation, phagocytosis, and endocytosis (Husemann et al., 2002). The APOA4, FYN, NRAS, and CD36 transcripts showed similar patterns when compared with microarray data in the transcriptional changes between 2 chicken lines, although the normalized values from microarray analysis and QRT-PCR are not perfectly correspondent. It might be caused from the differences of the normalization methods or the fluorescent dyes to detect gene expression between microarray and QRT-PCR, or both. The normalization method using the expression levels of the housekeeping gene is generally applied in QRT-PCR. However, it has been argued to not always be accurate as a negative comparison control (Lee et al., 2002).

In a preliminary analysis, no differential gene expression between the noninfected controls of M5.1 and M15.2 hybridized to AVIELA appeared (D. K. Kim, unpublished data). The differential expressed elements in AVIELA after infection between 2 lines could be highly related with disease resistance, even though the number of genes that changed was narrow.

In the results, 7 differentially altered genes between 2 chicken lines were classified by GO terms of immunity and defense (Table 7). Growth factor receptor-bound protein 2 is an adaptor protein involved in the formation of multiprotein complexes at receptors and adaptor proteins. In T cells, the SH2 domain of GRB2 activates T-cell receptor by binding with phosphorylated tyrosine on the adaptor protein linker of activated T cells (Samelson, 2002) and the GRB2 SH3 domains bring various ligands to the sites of active signaling (Koretzky, 1997). The cDNA sequence of chicken GRB2 was determined in 1993 (Wasenius et al., 1993); however, functional studies have not yet been performed. Integrin, β 5 plays a key role in the regulation of apoptosis of endothelial cells by binding with annexin V, which activates protein kinase C α 2 to stimulate apoptotic events (Cardo-Vila et al., 2003) and mediate phagocytosis of apoptotic cells (Singh et al., 2007). In protein phosphatase 3 catalytic subunit β isoform knockout mice, total peripheral T-cell numbers were significantly reduced and interleukin-2 production in response to phorbol 12-myristate 13-acetate/ionomycin and T-cell receptor cross-linking were defective (Bueno et al., 2002). Lin and Lechleiter (2002) reported that peptidylprolyl isomerase F has a protective role against apoptosis that is mediated by one or more targets other than the adenine nucleotide translocator. The cytosolic chaperone pair of hsp70 and DnaJ (Hsp40) homolog subfamily A member 1 prevents NO-mediated apoptosis upstream of cytochrome c release from mitochondria (Gotoh et al., 2004). Granzyme K triggers rapid cell death by rapid externalization of phosphatidylserine, nuclear morphological changes, and single-stranded DNA nicks (Zhao et al., 2007). The enzyme encoded by interferon, γ-inducible protein 30, is a lysosomal thiol reductase that has an important role in MHC class II-restricted antigen processing (Su et al., 2008).

In conclusion, transcriptional profiling revealed differential expression of genes possessing various functional roles including immunity and defense between 2 *B*-complex disparate, genetically inbred, Fayoumi chicken lines after *Eimeria* infection.

Because of the similarity of genetic background of the lines, and their difference in coccidiosis susceptibility, some of the transcripts altered in this study may be associated with genes controlling coccidiosis disease resistance. Therefore, future studies based on these results will contribute to comprehensive understanding of the innate immune system in the chicken gut and facilitate the development of novel strategies targeting major protective immune pathways important in avian coccidiosis.

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