



Analysis of Gene Expression Responses to a *Salmonella* Infection in Rugao Chicken Intestine Using GeneChips

D. Q. Luan, G. B. Chang, Z. W. Sheng, Y. Zhang, W. Zhou, Z. Z. Li¹, Y. Liu² and G. H. Chen*

College of Animal Science and Technology, Yangzhou University, Yangzhou 225009, China

ABSTRACT: Poultry products are an important source of *Salmonella enterica*. An effective way to reduce food poisoning due to *Salmonella* would be to breed chickens more resistant to infection. Unfortunately host responses to *Salmonella* are complex with many factors involved. To learn more about responses to *Salmonella* in young chickens of 2 wk old, a cDNA Microarray containing 13,319 probes was performed to compare gene expression profiles between two chicken groups under control and *Salmonella* infected conditions. Newly hatched chickens were orally infected with *S. enterica* serovar Enteritidis. Since the intestine is one of the important barriers the bacteria encounter after oral inoculation, intestine gene expression was investigated at 2 wk old. There were 588 differentially expressed genes detected, of which 276 were known genes, and of the total number 266 were up-regulated and 322 were down-regulated. Differences in gene expression between the two chicken groups were found in control as well as *Salmonella* infected conditions indicating a difference in the intestine development between the two chicken groups which might be linked to the difference in *Salmonella* susceptibility. The differential expressions of 4 genes were confirmed by quantitative real-time PCR and the results indicated that the expression changes of these genes were generally consistent with the results of GeneChips. The findings in this study have lead to the identification of novel genes and possible cellular pathways, which are host dependent. (**Key Words** : Rugao Chickens, *Salmonella*, GeneChips, Intestine Tissues, Gene Expression Profile, Quantitative Real-time PCR)

INTRODUCTION

The chicken is an important model organism that bridges the evolutionary gap between mammals and other vertebrates (Hillier et al., 2004). Research on chickens (*Gallus gallus*) has had a significant impact on fundamental biology. The domestic chicken also provides a major protein source from meat and eggs for most human populations throughout the world. Its economic importance has made it the focus of numerous research projects, including a recent effort to sequence the entire chicken genome (Wicker et al., 2005). Chicken should be used to study *Salmonella* susceptibility, because in addition to its impact on human health, salmonellosis in young chickens results in economic losses for the poultry industry,

amounting to approximately US\$64 million to US\$114 million annually in the United States (Bryan and Doyle, 1995).

Salmonella enterica is one of the most common causes of food poisoning in humans, mostly caused by poultry products infected by *S. enterica* serovars Typhimurium or Enteritidis (Rabsch et al., 2001). Following oral ingestion, *Salmonella* colonize the intestines and invade the intestinal mucosa. In addition to the enteric disease, humans *Salmonella* serovars Typhimurium and Enteritidis are also capable of causing severe systemic disease in newly hatched chicks and in birds under extreme stress conditions. The infection seldom causes mortality in birds more than 1 month old (Suzuki, 1994).

In young chickens infection with *Salmonella* leads to diarrhea and intestinal lesions and to an influx of heterophils into the gut accompanied by inflammation and damage to villi (Barrow et al., 1987). In contrast to other species, no genome-wide expression profiles in the chicken in response to *Salmonella* have been measured. Therefore several processes in the chicken host in response to *Salmonella* may be unidentified. Furthermore validations of the invitro gene expression observations by *in vivo* data are

* Corresponding Author : G. H. Chen. Tel: +86-51487979206, Fax: +86-51487979075, E-mail: ghchen@yzu.edu.cn

¹ Crop Cultivation and Farming System, Huazhong Agricultural University, Wuhan 430070, China.

² Institute of Agricultural Resources and Environment Research/ Engineering Research Center for Digital Agriculture, Jiangsu Academy of Agricultural Sciences, Nanjing 210014, China.

Received June 10, 2011; Accepted September 1, 2011

scarce. In addition it is not known why some chicken lines are more susceptible to *Salmonella* infections than others (Barrow et al., 1987; Guillot et al., 1995).

Host gene expression responses to a *Salmonella* infection have widely been studied. One of the methods to investigate gene expression responses is the use of GeneChips that allow the analysis of the expression of a large number of genes in a single experiment. Indeed, GeneChips have been used to study gene expression responses to *Salmonella* (Rosenberger et al., 2001). With this approach it was found that in human epithelial cells cultures *Salmonella typhimurium* induce a classical proinflammatory gene expression pathway with up regulation of several cytokines, kinases and transcription factors (Eckmann et al., 2000; Zeng et al., 2003).

Resistance to systemic salmonellosis in the chicken is a polygenic phenomenon as demonstrated in a number of studies (Lamont, 1998; Kramer et al., 2003a; Malek and Lamont, 2003; Malek et al., 2004; Swaggerty et al., 2004). Studies in chickens have also revealed considerable differences between lines in levels of colonization of *Salmonella* of the gastro-muscle tract and responses to vaccination (Lamont et al., 2002; Liu et al., 2003). Susceptibility to *Salmonella* caecal carrier state was investigated in chickens 1 to 6 wk of age. A down-regulation of IFN-g was found in the caecal tonsils of susceptible chickens compared to the resistant animals (Sadeyen et al., 2004). So far, only candidate genes have been investigated for their possible role in the chicken response to a *Salmonella* infection, but a whole genome approach might give more insight into the genetic aspects of the host response. Gene expression array technology is a powerful tool that has already been used to expand the understanding of host-pathogen interactions. A number of reports have been published about host transcriptional responses to bacterial infection using gene arrays (Rosenberger et al., 2001). However, the effect of *Salmonella* on host gene expression using GeneChips is so far not studied in chickens. In this study, the response on a *Salmonella* infection in relation to the genetic background of the host is studied.

In the present study, we describe the gene expression response in the intestine of young chickens after a *Salmonella* infection. The gene expression differences found with the GeneChips were confirmed using quantitative reverse transcription RT-PCR. Two different chicken groups were also used and the results were compared with each other to determine the role of the genetic background in the host response.

MATERIALS AND METHODS

Chips

Every chip purchased from America Agilent Company

had 43,604 points in which every 3 points represent 3 repeats of one gene. Negative controls and housekeeping genes were removed, it had 13,319 probe sequences of chicken.

Experimental animals

The following study was approved by the Institute of poultry, China Academy of Agricultural Sciences in accordance with the Dutch regulations on animal experimentation, eight full sib family of Rugao chickens (China native breeds, Jiangsu province) were used in the present study. The chickens were free of *Salmonella* by detecting faecal samples. Four were for group A, whereas the other four were for Group B.

Experimental infection

Eight 14-d old chickens of each group (A and B) were randomly divided into 2 groups. The birds were determined to be free of *Salmonella* when hatching. Of the chicken, four chickens of 14-day old were orally inoculated with 0.2 ml of a bacterial suspension containing 105 CFU *S. serovar* Enteritidis phage type 4 (nalidixic acid resistant). Four chickens of the control group were inoculated with 0.2 ml saline. The four chickens of each group were sacrificed 24 h post infection. For detection of *S. serovar* Enteritidis a cloacal swab was taken and after overnight enrichment it was spread on brilliant green agar+100 ppm nalidixic acid for *Salmonella* determination (37°C, 18-24 h). Pieces of the jejunum were snap frozen in liquid nitrogen and stored at -70°C.

RNA isolation and total RNA purification

Pieces of the jejunum were crushed under liquid nitrogen, 50 to 100 mg tissues of the different chicks were used to isolate total RNA using TRIzol reagent (Agilent), according to instructions of the manufacturer with an additional step. The homogenized tissue samples were resuspended in 1 ml of TRIzol Reagent using a syringe and 21 gauge needle and passing the lysate through 10 times. After homogenisation, insoluble material was removed from the homogenate by centrifugation at 12,000×g for 10 min at 4°C. Total RNA of intestine was isolated by Ribonucleic Acid Analysis, 2100 (Agilent) and was then purified by QIAGEN RNeasy Kit, according to the introductions of Qiagen.

Preparation of DNA probe and fluorescein mark

cRNA samples were purified and marked with kit constructed by QIAGEN, which were synthesized from mRNA by two reverse transcriptions. The concentration and the purity of each sample was analyzed at OD260 nm and OD280 nm with ultraviolet spectrophotometer by Cy3-marker, the content of cRNA was adjusted, the molecular concentration of fluorescence was analyzed, the content of

Cy3 at 552 nm was tested and the incorporation efficiency was calculated.

cRNA fragmentation and GeneChip hybridization

The mixed fragmentation liquor was prepared with Cy3 cRNA 875 ng, 10 X Blocking Agilent 11 µl, 25 X Fragmentation Buffer 2.2 µl Nuclease-free water X µl, then incubated in 60°C water for fragmentation for 30 min, 55 µl 2X GEx Hybridization Buffer was added and 100 µl of which was taken on the chip and then they were rollingly hybridized at 65°C, at 10 rpm, for 17 h.

Washing, scanning, GeneChip data normalization and analysis

The chip was dislodged into lotion 1 for washing for 1 min, and then put into lotion 2 for washing for 1 min (37°C). Then the chip was scanned in an Agilent scanner with a resolution of 5 µm, and the scanner automatically scanned the chip to 100% and 10% PMT for each, respectively.

The 8 GeneChips, four sets from each group were scanned and data was quantified using the Agilent scanner and Genespring software. Spot intensities were calculated, using the median value of all spots for further analysis. Poor-quality spots (flag = 0) were filtered from the raw data of each array before analysis, followed by background subtraction of median intensity signals (F532 median-B532 median) and then rescaled by global normalization (Quantile) and different functional classifications were divided by Gene Ontology (GO). Net intensities less than 500 from the normalized data were removed and the remaining signals are transformed logarithmically. Statistically significant differential gene expression was characterized from four sets of GeneChip data derived from each group, using the unpaired t-test. Because the ratio on the array is expressed in a log₂ scale, a ratio of >2.00 or <0.50 corresponds to a more than twofold up- or down-regulation respectively. To compare with the quantitative PCR, the log₂ ratios were transformed to normal ratios. In order to select significant gene expression changes, genes showing statistically different (p<0.01) expression levels, representing two-fold or greater were selected and processed for further analysis by quantitative RT-PCR to validate the result.

Quantitative real-time RT-PCR

Oligonucleotide primers were designed to amplify a fragment containing sequences from two adjacent exons in order to avoid contamination with genomic DNA. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was considered to be a stably expressed housekeeping gene and was used as an internal reference gene. The primer pairs were used to analyze gene expression and the size of product are shown in Table 1.

Four genes were selected from all differently expressed genes for further investigation of their expression. Real-time quantitative RT-PCR was used to evaluate these. The reaction was performed in a volume of 20 µl and carried out in an ABI Prism 7500 Sequence Detection System (Applied Biosystems, Foster City, CA, USA) (Applied Biosystems) programmed to conduct one cycle at 95°C for 30 s, 40 cycles at 95°C for 15 s and 60°C for 34 s. The dissociation curves were analyzed for each PCR reaction to detect and eliminate possible primer-dimer artifacts. Results (fold changes) were expressed as $2^{-\Delta\Delta Ct}$ with $\Delta\Delta Ct = (Ct_{ij} - Ct_{GAPDHj}) - (Ct_{i1} - Ct_{GAPDH1})$, where Ct_{ij} and Ct_{GAPDHj} are the Ct for gene i and for GAPDH in a sample (named j), and Ct_{i1} and Ct_{GAPDH1} are the Ct in sample 1, expressed as the standard (Bourneuf, 2006).

RESULTS AND ANALYSIS

Clinical symptoms of the *Salmonella* infection

After 24 h oral inoculation the chicks were sacrificed. The cloacal swabs of the control animals were free of *Salmonella*, while the cloacal swabs of all *Salmonella* inoculated chicks were positive. Furthermore, the *Salmonella* infected birds from both groups already showed signs of systemic disease as *Salmonella* were detectable in the intestine.

Microarray data analysis

Differential expression genes of intestine to a Salmonella infection and GO analysis: There were 588 differentially expressed genes detected, of which 276 were known genes, and of the total number 266 were up-regulated and 322 were down-regulated. Different functional classifications were divided by Gene Ontology (GO) into those which were involved in immune system process,

Table 1. Primer pairs used to analyze gene expression by quantitative RT-PCR, and size of product

Gene	Forward primer ^a	Reverse primer ^a	Size (bp)
THBS1	TTCAAGCGGAGTAACTGGCGTAG	TGTGCAGTGGCACAAATAGCAA	95
KIT	CCCTTGACAGAGACCCACATTC	TCTGTAGCATTGCCTTGAGTGGA	122
FGF10	CTGTAGAAATTGGAGTTGTGGCAGT	GCCTTCCATTGTGCTTCCAAT	180
CCL1	AAACTGCTCCCGCAGAGCTATTA	TTGGTGGCAGCTCACGTTG	135
GAPDF	GAGAAATTGTGCGTGACATCA	CCTGAACCTCTCATTGCCA	152

^a Sequences of oligonucleotides are indicated from 5' to 3' end.

Table 2. Part of up-regulated genes differentially expressed ($p < 0.01$) between group A and B

Catalog	GenBank ID	Definition	Fold change
Immune system process	CR353090	<i>Gallus gallus</i> finished cDNA, clone ChEST217f14(TLR5)	5.84
	XM_421205	Thrombospondin-1 Fragment (THBS1)	4.63
	D13225	<i>Gallus gallus</i> v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog (KIT)	2.35
	NM_204696	<i>Gallus gallus</i> fibroblast growth factor 10 (FGF10)	8.34
	Z11961	<i>Gallus gallus</i> B-cell CLL/lymphoma 2 (BCL2)	2.01
	L49204	<i>Gallus gallus</i> bone morphogenetic protein receptor, type IA (BMPRI1A)	2.00
	Transporter activity	CR391341	<i>Gallus gallus</i> finished cDNA, clone ChEST685m12(ATP6V0A1)
XM_425129		<i>Gallus gallus</i> potassium voltage-gated channel, subfamily G, member 4 (KCNG4)	13.91
Metabolic process	BX935092	<i>Gallus gallus</i> T-box 22 (TBX22)	3.08
	CR353567	Prolyl 4-hydroxylase subunit beta	2.64
Cellular process	X68073	<i>Gallus gallus</i> v-myc myelocytomatosis viral oncogene homolog (avian) (MYC)	3.01
	AJ719659	<i>Gallus gallus</i> elongation factor RNA polymerase II (ELL)	4.75
Biological regulation	NM_204297	<i>Gallus gallus</i> hypoxia-inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor) (HIF1A)	2.24
	X56772	<i>Gallus gallus</i> secreted phosphoprotein 1 (SPP1)	10.9
Growth	U26645	<i>Gallus gallus</i> BCL2-like 1 (BCL2L1)	2.50
	CR387077	<i>Gallus gallus</i> ciliary neurotrophic factor (CNTF)	2.21
Other	AJ719685	<i>Gallus gallus</i> transforming, acidic coiled-coil containing protein 3 (TACC3)	2.27
	AY299454	<i>Gallus gallus</i> ghrelin/obestatin preprohormone (GHRL)	2.25

transporter activity, metabolic process, cellular process, in response to the *Salmonella* infection in the intestine as biological regulation and growth. In the part of up and different stages of immune responses in both chicken down regulated genes differentially expressed, TLR5, groups, while CCL1, IL8, CD79B and CCL5 were THBS1, KIT, and FGF10 were identified to be up-regulated identified to be down-regulated, and are shown in Table 2

Table 3. Part of down-regulated genes differentially expressed ($p < 0.01$) between group A and B

Catalog	GenBank ID	Definition	Fold change
Immune system process	CD727090	<i>Gallus gallus</i> chemokine (C-C motif) ligand 1 (CCL1)	0.45
	Y14971	<i>Gallus gallus</i> interleukin 8 (IL8)	0.31
	CR523013	<i>Gallus gallus</i> CD79b molecule, immunoglobulin-associated beta (CD79B)	0.14
	AF006742	<i>Gallus gallus</i> lymphotactin (LOC395914)	0.42
	TC278181	chemokine (C-C motif) ligand 5 (CCL5)	0.41
	BX931161	Linker for activation of T-cells family member 2 (LAT2)	0.40
	Transporter activity	U15685	<i>Gallus gallus</i> solute carrier family 16, member 8 (monocarboxylic acid transporter 3) (SLC16A8)
X17700		<i>Gallus gallus</i> kinate binding protein (LOC396300)	0.44
Metabolic process	X54200	<i>Gallus gallus</i> phosphoribosylglycinamide formyltransferase, phosphoribosylglycinamide synthetase, phosphoribosylaminoimidazole synthetase (GART)	0.44
	AB011803	<i>Gallus gallus</i> SRY (sex determining region Y)-box 3 (SOX3)	0.39
Cellular process	X12608	<i>Gallus gallus</i> growth hormone 1 (GH1)	0.31
	X03805	<i>Gallus gallus</i> estrogen receptor 1 (ESR1)	0.45
Biological regulation	U31223	<i>Gallus gallus</i> activin A receptor, type IIB (ACVR2B)	0.39
	CR352407	<i>Gallus gallus</i> 18C15 mRNA, complete cds	0.19
Growth	U31223	<i>Gallus gallus</i> activin A receptor, type IIB (ACVR2B)	0.39
	CR387719	Myosin regulatory light chain 2A, cardiac muscle isoform (MLC-2A)	0.43
Other	BX932509	<i>Gallus gallus</i> finished cDNA, clone ChEST876c21(TSNARE1)	0.39
	XM_415099	<i>Gallus gallus</i> similar to peptide(SLC15A4)	0.43

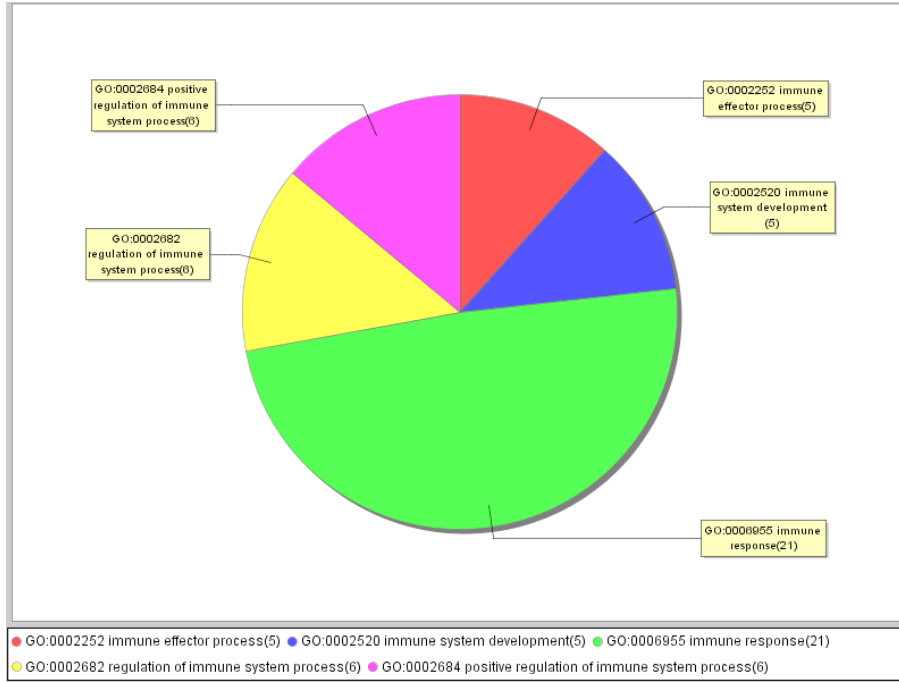


Figure 1. Pie chart of differential genes in immune system process.

and 3. Meanwhile, some genes among all these differentially expressed genes that had no annotation in GenBank were screened out and were presumed to be unknown new genes (Tables 2 and 3), in which there were 24 genes that were associated with immune system process, 201 with metabolic process and 300 with cellular process and so on. The distributions of related differentially expressed genes about the immune system process are shown in Figure 1.

Cluster dendrogram of differential expressed genes :

The results of the samples and related differentially expressed genes in this study were respectively classified and clustered by Heatmap function of SAS system are shown in Figure 2, in which the abscissa is a sample cluster, namely, 2 experimental groups with 4 in each, whereas the vertical axis is differentially expressed gene clusters. The 588 differentially expressed genes were divided into two major categories, which are shown in Figure 2a, the right side of which is the corresponding probe numbers of differentially expressed genes, and different colors

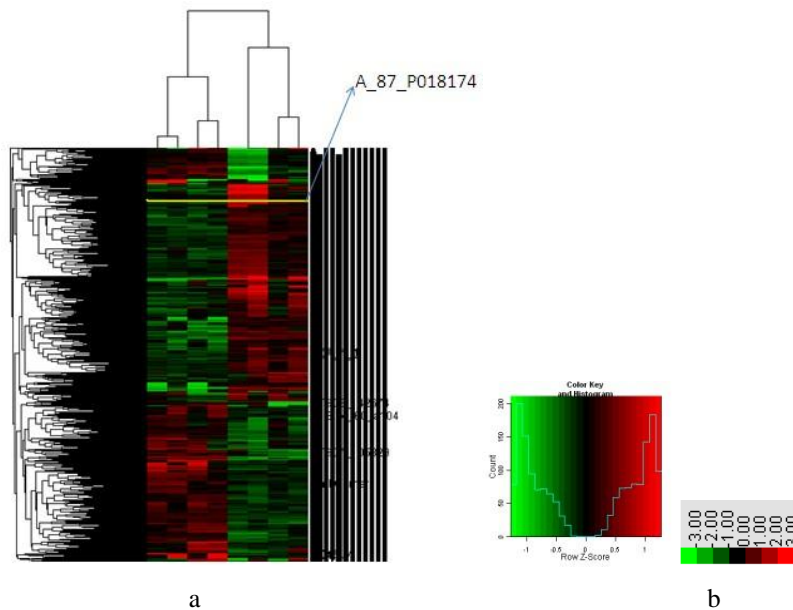


Figure 2. a: Differentially expressed genes cluster dendrogram of group A and B. b: Colorbar scale.

represented the expression strength of the genes in tissues. The color bar is shown in Figure 2b, the concrete expression of a gene in different samples could be seen by using TreeView software, and the gene expression could also be analyzed according to the classification of samples. For example, the differentially expressed gene that corresponded to the probe No. A_87_P018174 was toll-like receptor 5 (TLR5), the expression of which in group A was lower than that in group B from the Figure.

Quantitative RT-PCR analysis of differentially expressed genes: We used quantitative RT-PCR to confirm the differentially expressed genes detected by our microarrays in chicken tissue (n = 4 chickens). We performed quantitative real-time PCR for:

Thrombospondin-1 Fragment (THBS1), Gallus gallus v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog (KIT), Gallus gallus fibroblast growth factor 10 (FGF10) and Gallus gallus chemokine (C-C motif) ligand 1 (CCL1). All the real-time RT-PCR fold differences were in complete correspondence with the microarray data. This positive correlation suggests that our microarray data is an accurate reflection of levels of gene expression present in the intestine tissue taken from the two chicken groups (Table 4).

DISCUSSION

So far few articles are published studying gene expression profiles in the chicken and each study used a different array platform. This makes it difficult to interpret and compare results from different experiments. Recently the first draft genome sequence of the red jungle fowl, *G. gallus* has become available (International Chicken Genome Sequencing Consortium, 2004).

The intestine is a complex system to study *in vivo* due to the different cell types and multiple functions. To highlight the responses after a *Salmonella* infection in cellular context the gene expression in the whole intestine of 14-d old chickens was investigated with GeneChips as is reviewed (Amundson et al., 2001). This array contains a comprehensive coverage of 13,319 probes used to construct gene expression profiles in order to screen differentially

expressed genes of intestine tissues to a *Salmonella* infection in Rugao chicken. The advantage of investigating the gene response *in vivo* above *in vitro* infection in cell lines is that the cellular context is retained. This context is important to identify complex cellular regulation. As expected, genes that were identified to be up-regulated in response to the *Salmonella* infection in the intestine were involved in different stages of the immune responses in both chicken groups, such as TLR5, THBS1, KIT, and FGF10. Toll-like receptor 5 (TLR5) senses flagellin of several bacterial species and activates the innate immune system. The avian TLR repertoire exhibits considerable functional diversity compared to mammalian TLRs and evidence of a functional TLR5 in the avian species is lacking. Keestra et al. (2008) found that chicken TLR5 activation by flagellin of *S. enterica* serovar Typhimurium revealed that chTLR5 consistently yielded stronger responses than human but not mouse TLR5. This species-specific reactivity was not observed with flagellin of serovar Enteritidis. These results show that chickens express a functional TLR5 albeit with different flagellin sensing qualities compared to human TLR5. Tucker et al. (1997) found that TSP-1 is expressed in the spinal cord floor plate, in mesenchyme surrounding the developing paramesonephric duct, at the tips of growing lung bronchioles, in lens and in corneal endothelium. These observations indicate that there is little overlap in the expression patterns of TSP-1 and that it is expressed consecutively during the development of cartilage and neurons. The pattern of TSP-1 expression in vascular tissues and in the floor plate is consistent with possible roles for this glycoprotein in regulating angiogenesis and establishing morphogenetic gradients. Chicken Kit was isolated from a chicken brain cDNA library. The deduced amino acid (aa) sequence of chicken Kit shows 63% identity to mouse Kit, and suggests that chicken Kit shares common structural and functional features with mouse Kit. RNA blot analysis indicated that the expression pattern of the chicken c-kit transcript in chicken organs was similar to that of mouse c-kit in mice, suggesting that chicken Kit has biological roles analogous to those of mouse Kit (Sasaki et al., 1997). In a previous study of Shin et al. (2006), they showed that FGF10 was an early mesenchymal signal that was critically associated with the developmental processes in the proventricular epithelium. Furthermore, virus-mediated Fgf10 over expression results in a hypermorphic epithelial structure and an increase in epithelial cell number. In fibroblasts, wild-type v-Rel induced expression of mip-1beta and nfkb1, while non transforming mutants of v-Rel failed to do so, suggesting a role for these two genes in v-Rel mediated transformation. Finally, these genes are expressed at high levels in cells over expressing wild-type

Table 4. Comparison of GeneChip and real-time RT-PCR analyses

Gene	GeneChip	Real-time RT-PCR	
	Fold change	Fold change	Student T-test (p-value) ^a
THBS1	4.63	4.53	0.027*
KIT	2.35	2.335	0.0097**
FGF10	8.34	8.27	0.042*
CCL1	0.45	0.47	0.031*

^a * p<0.05. ** p<0.01.

and truncated forms of c-Rel, implying that v-Rel transforms, in part, by induction of c-Rel target genes (Petrenko et al., 1997). Excluding the genes involved in the immune system process, using microarray hybridization methods we also isolated a number of other differentially expressed genes. Most of the other genes we identified were related to transporter activity, metabolic process, cellular process, biological regulation and growth (Tables 2 and 3), as defined by the Gene Ontology (Al-Shahrour et al., 2004).

The 588 genes screened by the Agilent microarray were divided into different categories according to their functions, for example, 24 genes associated with immune system process, 201 with metabolic process and 300 with cellular process. Information could be subtle as to the function of each gene and its participation in the relevant biological path. However, GO functional classification reduced the tedious validation of gene function by biological methods and could divide known genes into different functional classifications. GO could also speculate that the 'unknown' genes, some of which sequences had no annotation in GeneBank, may be involved in the same biological process, thus new genes can be discovered, but biological methods will be required to verify them. The expression of different samples of a particular gene and the classification of samples according to gene expression could be studied in Heatmap cluster diagram by the use of Java TreeView software, for example, toll-like receptor 5 (TLR5), the expression of which in group A was lower than that in group B as shown in Figure 2. This suggested that the TLR5 gene in the immune system process showed an ascent trend, indicating that the immune response activity was relatively strengthening in Rugao chicken intestine to a *Salmonella* infection over time. However, the biological process of which needs to be further validated by biological methods. Four differentially expressed genes were selected from our microarray data and analyzed using quantitative RT-PCR to confirm that they were also expressed at different levels in intestine tissue. All the real-time RT-PCR fold differences were in complete correspondence with the microarray data. This positive correlation suggests that our microarray data is an accurate reflection of levels of gene expression present in the intestine tissue taken from the two chicken groups. Further, the high correlation coefficients we achieved between our microarray replicates suggest that the method we used to construct our arrays and carry out microarray hybridization is reliable and highly reproducible.

ACKNOWLEDGEMENTS

This study was supported by National Natural Science Foundation (30972088, 31172199) and Jiangsu Science and Technology Program Support (BE2008359).

REFERENCES

- Al-Shahrour, F., R. Diaz-Uriarte and J. Dopazo. 2004. FatiGO: a web tool for finding significant associations of Gene Ontology terms with groups of genes. *Bioinformatics* 20:578-580.
- Amundson, S. A., M. Bittner, P. Meltzer, J. Trent and A. J. Fornace Jr. 2001. Physiological function as regulation of large transcriptional programs: the cellular response to genotoxic stress. *Comp. Biochem. Physiol. B.* 129:703-710.
- Barrow, P. A., M. B. Huggins, M. A. Lovell and J. M. Simpson. 1987. Observations on the pathogenesis of experimental *Salmonella typhimurium* infection in chickens. *Res. Vet. Sci.* 42:194-199.
- Bourneuf, E., F. Héroult, C. Chicault, W. Carré, S. Assaf, A. Monnier, S. Mottier, S. Lagarrigue, M. Douaire, J. Mosser and C. Diot. 2006. GeneChip analysis of differential gene expression in the liver of lean and fat chickens. *Gene.* 372: 162-170.
- Bryan, E. D. and M. P. Doyle. 1995. Health risks and consequences of *Salmonella* and campylobacter jejuni in raw meat. *J. Food Prot.* 58:326-344.
- Eckmann, L., J. R. Smith, M. P. Housley, M. B. Dwinell and M. F. Kagnoff. 2000. Analysis by high density cDNA arrays of altered gene expression in human intestinal epithelial cells in response to infection with the invasive enteric bacteria *Salmonella*. *J. Biol. Chem.* 275:14084-14094.
- Hillier, L. W., W. Miller, E. Birney, W. Warren, R. C. Hardison, C. P. Ponting, P. Bork, D. W. Burt, M. A. Groenen, M. E. Delany, J. B. Dodgson, A. T. Chinwalla, P. F. Cliften, S. W. Clifton, K. D. Delehaunty, C. Fronick, R. S. Fulton, T. A. Graves, C. Kremitzki, D. Layman, V. Magrini, J. D. McPherson, T. L. Miner, P. Minx, W. E. Nash, M. N. Nhan, J. O. Nelson, L. G. Oddy, C. S. Pohl, J. Randall-Maher, S. M. Smith, J. W. Wallis, S. P. Yang, M. N. Romanov, C. M. Rondelli, B. Paton, J. Smith, D. Morrice, L. Daniels, H. G. Tempest, L. Robertson, J. S. Masabanda, D. K. Griffin, A. Vignal, V. Fillon, L. Jacobsson, S. Kerje, L. Andersson, R. P. Crooijmans, J. Aerts, J. J. vander Poel, H. Ellegren, R. B. Caldwell, S. J. Hubbard, D. V. Grafham, A. M. Kierzek, S. R. McLaren, I. M. Overton, H. Arakawa, K. J. Beattie, Y. Bezzubov, P. E. Boardman, J. K. Bonfield, M. D. Croning, R. M. Davies, M. D. Francis, S. J. Humphray, C. E. Scott, R. G. Taylor, C. Tickle, W. R. Brown, J. Rogers, J. M. Buerstedde, S. A. Wilson, L. Stubbs, I. Ovcharenko, L. Gordon, S. Lucas, M. M. Miller, H. Inoko, T. Shiina, J. Kaufman, J. Salomonsen, K. Skjoedt, G. K. Wong, J. Wang, B. Liu, J. Wang, J. Yu, H. Yang, M. Nefedov, M. Koriabine, P. J. Dejong, L. Goodstadt, C. Webber, N. J. Dickens, I. Letunic, M. Suyama, D. Torrents, C. von Mering, E. M. Zdobnov, K. Makova, A. Nekrutenko, L. Elnitski, P. Eswara, D. C. King, S. Yang, S. Tyekucheva, A. Radakrishnan, R. S. Harris, F. Chiaromonte, J. Taylor, J. He, M. Rijnkels, S. Griffiths-Jones, A. Ureta-Vidal, M. M. Hoffman, J. Severin, S. M. Searle, A. S. Law, D. Speed, D. Waddington, Z. Cheng, E. Tuzun, E. Eichler, Z. Bao, P. Flicek, D. D. Shteynberg, M. R. Brent, J. M. Bye, E. J. Huckle, S. Chatterji, C. Dewey, L. Pachter, A. Kouranov, Z. Mourelatos, A. G. Hatzigeorgiou, A. H. Paterson, R. Ivarie, M. Brandstrom, E. Axelsson, N. Backstrom, S. Berlin, M. T. Webster, O. Pourquie, A. Reymond, C. Ucla, S. E. Antonarakis, M. Long, J. J. Emerson,

- E. Betran, I. Dupanloup, H. Kaessmann, A. S. Hinrichs, G. Bejerano, T. S. Furey, R. A. Harte, B. Raney, A. Siepel, W. J. Kent, D. Haussler, E. Eyra, R. Castelo, J. F. Abril, S. Castellano, F. Camara, G. Parra, R. Guigo, G. Bourque, G. Tesler, P. A. Pevzner, A. Smit, L. A. Fulton, E. R. Mardis and R. K. Wilson. 2004. Sequence and comparative analysis of the chicken genome provide unique perspectives on vertebrate evolution. *Nature* 432:695-716.
- International Chicken Genome Sequencing Consortium. 2004. Sequence and comparative analysis of the chicken genome provide unique perspectives on vertebrate evolution. *Nature* 432:695-716.
- Guillot, J. F., C. Beaumont, F. Bellatif, C. Mouline, F. Lantier, P. Colin and J. Protais. 1995. Comparison of resistance of various poultry lines to infection by *Salmonella* enteritidis. *Vet. Res.* 26:81-86.
- Keestra, A. M., M. R. de Zoete, R. A. van Aubel and J. P. van Putten. 2008. Functional characterization of chicken TLR5 reveals species-specific recognition of flagellin. *Mol. Immunol.* 45(5):1298-307.
- Kramer, J., M. Malek and S. J. Lamont. 2003a. Association of twelve candidate gene polymorphisms and response to challenge with *Salmonella* enteritidis in poultry. *Anim. Genet.* 34:339-348.
- Lamont, S. J. 1998. Impact of genetics on disease resistance. *Poult. Sci.* 77:1111-1118.
- Lamont, S. J., M. G. Kaiser and W. Liu. 2002. Candidate genes for resistance to *Salmonella* enteritidis colonization in chickens as detected in a novel genetic cross. *Vet. Immunol. Immunopathol.* 87:423-428.
- Liu, W., M. G. Kaiser and S. J. Lamont, 2003. Natural resistance-associated macrophage protein 1 gene polymorphisms and response to vaccine against or challenge with *Salmonella* enteritidis in young chicks. *Poult. Sci.* 82:259-266.
- Malek, M. and S. J. Lamont. 2003. Association of INOS, TRAIL, TGF-beta2, TGF-beta3, and IgL genes with response to *Salmonella* enteritidis in poultry. *Genet. Sel. Evol.* 35 (Suppl. 1):S99-S111.
- Malek, M., J. R. Hasenstein and S. J. Lamont. 2004. Analysis of chicken TLR4, CD28, MIF, MD-2, and LITAF genes in a *Salmonella* enteritidis resource population. *Poult. Sci.* 83:544-549.
- Petrenko, O., I. Ischenko and P. J. Enrietto. 1997. Characterization of changes in gene expression associated with malignant transformation by the NF-kappaB family member, v-Rel. *Oncogene* 15(14):1671-1680.
- Rabsch, W., H. Tschape and A. J. Baumler. 2001. Non-typhoidal salmonellosis: emerging problems. *Microbes Infect.* 3:237-247.
- Rosenberger, C. M., A. J. Pollard and B. B. Finlay. 2001. Gene array technology to determine host responses to *Salmonella*. *Microbes Infect.* 3:1353-1360.
- Sadeyen, J. R., J. Trotter, P. Velge, J. Marly, C. Beaumont, P. A. Barrow, N. Bumstead and A. C. Lalmanach. 2004. *Salmonella* carrier state in chicken: comparison of expression of immune response genes between susceptible and resistant animals. *Microbes Infect.* 6:1278-1286.
- Sasaki, E., H. Okamura, T. Chikamune, Y. Kanai, M. Watanabe, M. Naito and M. Sakurai. 1993. Cloning and expression of the chicken c-kit proto-oncogene. *Gene.* 128(2):257-261.
- Suzuki, S. 1994. Pathogenicity of *Salmonella* enteritidis in poultry. *Int. J. Food Microbiol.* 21:89-105.
- Wicker, T., J. S. Robertson, S. R. Schulze, F. A. Feltus, V. Magrini, J. A. Morrison, E. R. Mardis, R. K. Wilson, D. G. Peterson, A. H. Paterson and R. Ivarie. 2005. The repetitive landscape of the chicken genome. *Genome Res.* 15:126-136.
- Shin, M., S. Noji, A. Neubüser and S. Yasugi. 2006. FGF10 is required for cell proliferation and gland formation in the stomach epithelium of the chicken embryo. *Dev. Biol.* 294(1): 11-23.
- Swaggerty, C. L., M. H. Kogut, P. J. Ferro, L. Rothwell, I. Y. Pevzner and P. Kaiser. 2004. Differential cytokine mRNA expression in heterophils isolated from *Salmonella*-resistant and -susceptible chickens. *Immunology* 113:139-148.
- Tucker, R. P., C. Hagios, R. Chiquet-Ehrismann and J. Lawler. 1997. *In situ* localization of thrombospondin-1 and thrombospondin-3 transcripts in the avian embryo. *Dev. Dyn.* 208(3):326-337.
- Withanage, G. S., P. Kaiser, P. Wigley, C. Powers, P. Mastroeni, H. Brooks, P. Barrow, A. Smith, D. Maskell and I. McConnell. 2004. Rapid expression of chemokines and proinflammatory cytokines in newly hatched chickens infected with *Salmonella enterica* serovar typhimurium. *Infect. Immun.* 72:2152-2159.
- Zeng, H., A. Q. Carlson, Y. Guo, Y. Yu, L. S. Collier Hyams, J. L. Madara, A. T. Gewirtz and A. S. Neish. 2003. Flagellin is the major proinflammatory determinant of enteropathogenic *Salmonella*. *J. Immunol.* 171:3668-3674.