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Differentially expressed genes in the liver of lean and fat chickens

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ABSTRACT. This study aimed to investigate gene expression in the chicken liver for lean and fat broiler lines. Birds used in this study were 2 and 4 weeks of age; they were derived from the 14th generation of Northeast Agricultural University broiler lines, which were divergently selected based on abdominal fat content. Chicken Genome Arrays were used to screen differentially expressed genes in the liver tissue from lean and fat birds. At 2 and 4 weeks of age, 770 and 452 genes were differentially expressed between the 2 lines, respectively. The differentially expressed genes were involved in Wnt, insulin signaling,

and cell cycle pathways. At 2 and 4 weeks, 42 shared, differentially expressed genes were revealed by the analysis. We speculate that these genes might regulate chicken lipid metabolism.

Key words: Chicken; Lean and fat broiler lines; Chicken liver; Gene expression profile

INTRODUCTION

Chickens, widely raised farm animals, are excellent animal models for genetic selection/evolutionary research studies. The excessive accumulation of lipids in the adipose tissue of chickens is a major problem in the broiler industry. Unlike mammals, little or no fatty acid synthesis occurs in chicken adipose tissue; the liver is the main tissue of fatty acid synthesis in chickens (Griffin et al., 1992; Cui et al., 2012).

In our previous study, Chicken Genome Arrays were developed to construct gene expression profiles and screen differentially expressed genes in the liver of lean and fat broiler lines at different developmental stages. Our results indicate that 4 weeks of age is a more important stage for chicken liver lipogenesis, and abdominal fat weight (AFW) and percentage of abdominal fat (AFP) are significantly different from those at 2 weeks of age (Wang et al., 2010). In order to better understand gene expression in the chicken liver, gene expression profiles of the liver at 2 and 4 weeks of age were investigated. This study will help to elucidate the molecular mechanisms of lipid metabolism in chickens and contribute to related research in other species.

MATERIAL AND METHODS

Animals

The birds utilized in the current study were obtained from the 14th generation population of the Northeast Agricultural University broiler lines, which were divergently selected based on abdominal fat content (Guo et al., 2011). Birds were kept in similar environmental conditions and had access to feed and water. The G0 generation of the 2 lines came from the same grandsire line originating from the Arbor Acres breed.

Sample preparation

Birds were slaughtered at 2 and 4 weeks of age. The 6 birds used in present study were chosen based on AFP: 3 had high AFP and 3 had low AFP. Total RNA was isolated from the livers using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and quantified by spectrophotometry. Messenger RNA (mRNA) was isolated using the Oligotex mRNA Mini-Kit (Qiagen, Valencia, CA, USA). Complementary DNA (cDNA) was prepared by oligo-dT-primed reverse transcription (Affymetrix, Santa Clara, CA, USA). cRNA probes were prepared using an IVT Labeling Kit (Affymetrix). The microarrays were prepared using the GeneChip® Hybridization, Wash, and Stain Kit (Affymetrix). The Chicken Genome Arrays,

with comprehensive coverage of >38,000 probe sets representing 32,773 transcripts, were created by Affymetrix, Inc.

Statistical analysis

Differentially expressed genes were identified from normalized data using the significance analysis of microarrays (SAM) algorithm (Tusher et al., 2001) and the *t*-test (Forrester and Ury, 1969); data were obtained using the procedure of SAS. According to the SAM algorithm, differentially expressed genes are identified based on the expression differences among the sample groups and the consistency of those differences; a score is assigned to each gene on the basis of a change in its expression level relative to the standard deviation of repeated measurements for that gene. Differences were considered highly significant at $P < 0.01$ and significant at $P < 0.05$.

RESULTS

A P value of 0.05 was used as the threshold for significantly different expression levels. At 2 and 4 weeks of age, 770 and 452 genes were differentially expressed between the 2 lines, respectively. The differentially expressed genes were analyzed by Gene Ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways.

The 770 differentially expressed genes at 2 weeks of age were enriched for 4 significant GO terms ($P < 0.05$), including ribonucleoprotein complex, structural constituent of ribosome, ribonucleoprotein binding, and establishment of RNA localization functional category. They were mainly annotated to 11 significant pathways by KEGG analysis ($P < 0.05$), including ribosome, base excision repair, spliceosome, n-glycan biosynthesis, Wnt signaling pathway, Toll-like receptor signaling pathway, SNARE interactions in vesicular transport, histidine metabolism, protein export, amino sugar and nucleotide sugar metabolism, and insulin signaling pathway.

Differentially expressed genes at 4 weeks of age were significantly enriched for only one GO term (extracellular matrix; $P = 0.0581$). They were annotated to 9 significant pathways by KEGG analysis, including cell adhesion molecules, spliceosome, tight junction, notch signaling pathway, cell cycle, intestinal immune network for IgA production, nucleotide excision repair, pentose and glucuronate interconversions, and one carbon pool by folate.

Of the differentially expressed genes, 42 were common in birds belonging to the 2 age groups (Table 1). In comparison to fat chickens, 18 genes were upregulated and 16 were downregulated (**Table S1**) in lean chickens at 2 and 4 weeks of age, 4 genes were upregulated at 2 weeks and downregulated at 4 weeks, and 4 genes were downregulated at 2 weeks and upregulated at 4 weeks. The upregulated/downregulated genes were analyzed by functional classification and KEGG pathway. The 18 upregulated genes were enriched for 2 significant GO terms, deaminase activity and macromolecular complex, and the 16 downregulated genes were enriched for one significant GO term, ribonucleoprotein binding. These common genes were not involved in any significant pathways via KEGG analysis.

Table 1. Summary of common differentially expressed genes between 2 chicken lines at 2 and 4 weeks.

Probe ID	2 weeks		4 weeks			Symbol	Description
	P values	Fold-change (lean/fat)	P values	Fold-change (lean/fat)	Gene ID		
Gga.8932.1.S1_at	0.0138	0.188	0.018	0.2487	395105	CRHR2	Corticotropin releasing hormone receptor 2
Gga.15984.1.S1_at	0.0138	0.2098	0.0121	0.249	422512	CCNG2	Cyclin G2
Gga.12241.1.S1_s_at	0.0289	0.418	0.0377	0.5572	423147	PPFIA1	Protein tyrosine phosphatase, receptor type, f polypeptide (PTPRF), interacting protein (liprin)
GgaAffx.12334.1.S1_at	0.0305	0.4223	0.0199	0.196	423142	ORAOV1	Oral cancer overexpressed 1
Gga.9610.1.S1_at	0.0175	0.4527	0.0375	2.1327	423633	BMS1	BMS1 homolog, ribosome assembly protein (yeast)
GgaAffx.23472.1.S1_at	0.0181	0.4695	0.0276	2.6805	428437	CHN2	Chimerin (chimaerin) 2
Gga.6296.1.S1_at	0.0453	0.4939	0.0038	0.2977	416314	RCJMB04_11a23	SAR1 homolog B (*Saccharomyces cerevisiae*)
GgaAffx.23081.2.S1_s_at	0.014	0.5032	0.0046	0.4625	421399	PLEKHH2	Pleckstrin homology domain containing, family H (with MyTH4 domain) member 2
GgaAffx.6136.4.A1_at	0.0301	0.5243	0.0174	0.787	426868	GOLGB1	Golgin B1, golgi integral membrane protein
Gga.1739.1.S1_s_at	0.0259	0.5566	0.0368	0.6973	428256	ZNF259	Zinc finger protein 259
Gga.8676.1.S1_at	0.0134	0.5755	0.0162	0.4689	423269	TMEM229B	Chromosome 14 open reading frame 83
Gga.2330.1.S1_at	0.0016	0.6182	0.0218	0.6042	415822	LOC415822	Similar to conserved hypothetical protein
GgaAffx.12269.1.S1_at	0.0182	0.6416	0.0437	0.6393	418246	RCJMB04_11o5	Similar to LOC150383 protein
Gga.8512.1.S1_at	0.038	0.646	0.0084	0.6504	426454	GPR155	G protein-coupled receptor 155
Gga.20035.1.S1_at	0.0016	0.6668	0.0335	0.7752	424463	DPH5	DPH5 homolog (*S. cerevisiae*)
GgaAffx.4428.2.S1_s_at	0.0416	0.6673	0.0325	0.7319	424898	ATP13A3	ATPase type 13A3
Gga.10227.1.S1_at	0.045	0.7051	0.0485	1.3956	422529	SGMS2	Sphingomyelin synthase 2
GgaAffx.11945.1.S1_s_at	0.0137	0.7422	0.0223	0.8286	419140	EIF6	Eukaryotic translation initiation factor 6
Gga.15389.1.S1_s_at	0.0389	0.7556	0.005	1.2659	422259	TRMT12	tRNA methyltransferase 12 homolog (*S. cerevisiae*)
Gga.3130.4.S1_x_at	0.0318	0.8648	0.0331	0.6441	395784	CLIP1	CAP-GLY domain containing linker protein 1
Gga.16190.1.S1_at	0.0203	1.1076	0.0167	1.2108	427530	LOC427530	Similar to THAP domain containing 11
Gga.74.1.S1_at	0.0342	1.2164	0.0333	1.8619	374129	LMO2	LIM domain only 2 (rhombotin-like 1)
Gga.3121.1.S1_a_at	0.0117	1.2216	0.0061	1.4038	768445	NTAN1	N-terminal asparagine amidase
Gga.9040.1.S1_s_at	0.0165	1.2348	0.0183	1.1696	426265	RCJMB04_1p22	Splicing factor, arginine/serine-rich 7, 35 kDa
Gga.5732.2.S1_at	0.0391	1.2405	0.0376	1.1655	418063	FBXO7	F-box protein 7
Gga.5269.1.S1_at	0.0064	1.3464	0.0211	1.4223	420356	MRPL13	Mitochondrial ribosomal protein L13
Gga.10454.1.S1_at	0.0397	1.3859	0.0042	2.5847	425117	LOC425117	Hypothetical gene supported by BX932111
Gga.8444.3.S1_s_at	0.0132	1.4125	0.0479	1.1856	419617	RCJMB04_23a7	Chromosome 1 open reading frame 149
Gga.870.2.S1_a_at	0.0155	1.462	0.0442	0.7079	396516	FGFR1	Fibroblast growth factor receptor 1
Gga.3282.2.S1_a_at	0.0216	1.4794	0.0426	0.7272	769996	MRPS16	Mitochondrial ribosomal protein S16
Gga.8130.1.S1_at	0.0415	1.5355	0.0343	0.8462	419166	SGK2	Serum/glucocorticoid regulated kinase 2
Gga.11908.1.S1_at	0.0296	1.6966	0.0435	0.7194	420659	CMC1	COX assembly mitochondrial protein homolog (*S. cerevisiae*)
Gga.4599.1.S1_at	0.0024	1.716	0.0076	1.7447	424436	MRPS14	Mitochondrial ribosomal protein S14
Gga.11601.1.S1_at	0.0478	1.7593	0.0024	1.1099	426014	CPSF7	Cleavage and polyadenylation specific factor 7, 59 kDa
Gga.4516.2.S1_s_at	0.0404	1.7989	0.0001	2.0526	422635	11-Sep	Septin 11
GgaAffx.12359.1.S1_at	0.0205	1.8475	0.0372	1.6943	419053	POLD3	Polymerase (DNA-directed), delta 3, accessory subunit
GgaAffx.3297.1.S1_at	0.0168	1.8978	0.0121	2.0147	416528	LMF1	Lipase maturation factor 1
Gga.10593.1.S1_s_at	0.0184	1.9274	0.034	1.4932	417433	RGS9	Regulator of G-protein signaling 9
Gga.2513.1.S1_at	0.0474	1.9897	0.0222	1.4502	395726	FTCD	Formiminotransferase cyclodeaminase
Gga.11782.1.S1_at	0.0409	2.0251	0.0297	1.276	427121	CAST	Calpastatin
GgaAffx.24337.1.S1_at	0.0165	2.1817	0.0393	1.7851	426357	C1orf77	Chromosome 1 open reading frame 77
Gga.5830.1.S1_at	0.0352	4.9266	0.0171	1.3089	418484	RWDD2B	RWD domain containing 2B

P value: methods based on conventional *t*-tests provide the probability (P) that a difference in gene expression occurred by chance. Fold-change greater than 1.0 indicates that the gene was upregulated in lean *vs* fat; fold-change of less than 1.0 indicates that the gene was downregulated in lean *vs* fat.

DISCUSSION

Genome expression analysis provides a broad, unbiased survey of the transcriptome. Bourneuf et al. (2006) identified differentially expressed genes in lean and fat chickens and suggested that the mechanisms of expression and regulation of lipogenic genes could be involved in the ontogenesis of fatness in chickens. In our previous study, we found that the AFW and AFP were significantly different at 2 weeks of age, and 4-week-old broilers displayed the exuberant capability of lipogenesis (Wang et al., 2010). We identified significant pathways and speculated that these pathways, especially the Wnt, insulin signaling, and cell cycle pathways, have important effects on chicken lipid metabolism. The Wnt pathway is essential for the early development of eukaryotic organisms and is involved in cell proliferation, differentiation, and oncogenesis (Kitazoe et al., 2010). Lipid peroxidation products activate the canonical Wnt pathway through oxidative stress (Zhou et al., 2011). The insulin signaling pathway controls the synthesis and accumulation of lipids in the mammalian liver. Insulin-resistant states might lead to abnormal triglyceride deposition in the liver (Leavens and Birnbaum, 2011). Lipids play indispensable roles in cell proliferation, cell differentiation, and organ morphogenesis; these processes are intimately associated with cell cycle progression (Donnelly et al., 1999). Cell cycle progression and neutral lipid turnover appear to be linked (Long et al., 2012). With this in mind, we hypothesize that the 3 pathways play important roles in chicken lipid metabolism.

In summary, our research provided a set of enriched functional pathways and genes that regulate adiposity in chickens, contributing resources to further study the molecular mechanisms of lipid metabolism and fatness variability in lean and fat chicken lines.

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Supplementary material

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