Transcriptional profiling of hypothalamus during development of adiposity in genetically selected fat and lean chickens

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1Department of Animal and Avian Sciences and 2Neuroscience and Cognitive Science Program, University of Maryland, College Park, Maryland; 3Institut National de la Recherche Agronomique (INRA), UR83 Recherches Avicoles, Nouzilly, France; 4Department of Animal and Food Sciences, University of Delaware, Newark, Delaware; and 5Department of Poultry Science, University of Georgia, Athens, Georgia

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Byerly MS, Simon J, Cogburn LA, Le Bihan-Duval E, Duclos MJ, Aggrey SE, Porter TE. Transcriptional profiling of hypothalamus during development of adiposity in genetically selected fat and lean chickens. Physiol Genomics 42: 157–167, 2010. First published April 6, 2010; doi:10.1152/physiolgenomics.00029.2010.—The hypothalamus integrates peripheral signals to regulate food intake, energy metabolism, and ultimately growth rate and body composition in vertebrates. Deviations in hypothalamic regulatory controls can lead to accumulation of excess body fat. Many regulatory genes involved in this process remain unidentified, and comparative studies may be helpful to unravel evolutionarily conserved mechanisms controlling body weight and food intake. In the present study, divergently selected fat (FL) and lean (LL) lines of chickens were used to characterize differences in hypothalamic gene expression in these unique genetic lines that develop differences in adiposity without differences in food intake or body weight. Hypothalamic transcriptional profiles were defined with cDNA microarrays before and during divergence of adiposity between the two lines. Six differentially expressed genes identified in chickens are related to genes associated with control of body fat in transgenic or knockout mice, supporting the importance of these genes across species. We identified differences in expression of nine genes involved in glucose metabolism, suggesting that alterations in hypothalamic glycolysis might contribute to differences in levels of body fat between genotypes. Expression of the sweet taste receptor (TAS1R1), which in mammals is involved in glucose sensing and energy uptake, was also higher in FL chickens, suggesting that early differences in glucose sensing might alter the set point for subsequent body composition. Differences in expression of genes associated with tumor necrosis factor (TNF) signaling were also noted. In summary, we identified alterations in transcriptional and metabolic processes within the hypothalamus that could contribute to excessive accumulation of body fat in FL chickens in the absence of differences in food intake, thereby contributing to the genetic basis for obesity in this avian model.

microarray; obesity; glycolysis; glucose sensor; genetic susceptibility; tumor necrosis factor-α

IN HUMANS AND OTHER VERTEBRATES, differences in body composition and development of an obese phenotype are controlled by genetics as well as environmental factors (11, 37). The hypothalamus maintains whole body energy homeostasis by regulating energy expenditure, integrating metabolic processes, and influencing food intake (2, 20). In most experimental models, it is difficult to dissociate genes within the hypothalamus that regulate the development of adiposity from genes that also regulate food intake, because these alterations often occur simultaneously. Divergently selected fat (FL) and lean (LL) lines of chicken provide a unique experimental model in which excessive adiposity has been dissociated from alterations in food intake. These lines have been selected for extremes in fatness or leanness at similar body weights and without altered food intake (17, 25, 50). It has been hypothesized that these two phenotypes are the results of polygenic changes (24) leading to discrete changes in metabolism (reviewed in Ref. 43). We reported previously (7) that important genes known to regulate metabolism in mammals are differentially expressed in the hypothalamus of FL and LL chickens, supporting the usefulness of this unique genetic model for studies aimed at identifying the underlying genetic basis for differences in the propensity to accumulate body fat among individuals.

After completion of genomic sequencing and development of high-throughput genomic tools for functional exploration, the chicken is now one of the premier National Institutes of Health-sponsored model organisms for biomedical research (http://www.nih.gov/science/models/gallus/). These genetic tools and unique genetic lines have not been used for identification of genes that function within the hypothalamus to control metabolic rate and adiposity. Currently, there are more than 600 genes identified to be associated with obesity (mostly in humans and mice), and new candidate genes are still emerging (35, 37). Here, we utilized a high-throughput transcriptional screen to identify novel candidate genes and biological pathways associated with differences in adiposity. In this study, gene expression patterns within the hypothalamus of FL and LL chickens were determined with cDNA microarrays. Our goal was to identify genes that were differentially expressed before and during the divergence of adiposity. The gene expression data were then clustered by using self-organizing maps (SOMs) to identify 1) biologically relevant patterns in the data set, 2) phenotypic marker genes distinguishing fat and lean animals, and 3) neighborhood marker genes centered around specific genes of interest that have been identified in transgenic or knockout mice. Gene expression results were then subjected to pathway analysis to identify putative genetic interactions predicted by the data set. Our results indicate that two novel pathways in the hypothalamus are divergently expressed between the lines before observed differences in adiposity. These pathways contain genes involved in glucose sensing and metabolism and genes associated with tumor necrosis factor (TNF) signaling.
MATERIALS AND METHODS

Animals and tissue preparation. Divergently selected fat (FL) and lean (LL) chicken lines developed at the Institut National de la Recherche Agronomique (INRA, U83 Recherches Avicoles) were used in this study (25). These chickens have been selected for either high or low abdominal fat at similar body weight at 9 wk of age for 7 generations. The samples used in the present experiment were described previously (7). Briefly, male FL and LL chickens were reared in a floor pen (4.4 × 3.9 m) together to eliminate environmental differences. They were given ad libitum access to feed and water throughout the study with a conventional pelleted starter diet (0–3 wk, 3,125 kcal/kg metabolizable energy and 20.9% protein) or a grower pellet diet (3–11 wk, 3,025 kcal/kg metabolizable energy and 17.9% protein). The light-dark cycle was 24 h of light for the first 2 days after hatch and then 14 h of light and 10 h of dark thereafter. Animals were killed at 1, 3, 5, and 7 wk of age (n = 4 for each age and group), with the hypothalamus being immediately dissected, snap frozen in liquid nitrogen, and stored at −80°C until further processing. Abdominal fat, which is the predominant site for storage of fat and highly correlated to total body fat in the chicken (8, 21, 34), was dissected and weighed as an index of fattening. All procedures were conducted according to protocols approved by the Institutional Animal Care and Use Committees at INRA, the University of Delaware, and the University of Maryland.

Microarray processing and data analysis. Individual tissue samples (n = 4 per genotype and age group) were homogenized and total RNA extracted with RNeasy Midi kits (Qiagen, Valencia, CA) according to the manufacturer’s protocol, as described previously (7). Extracted RNA was quantified with UV absorbance (260/280), and integrity was verified with a Bioanalyzer (Agilent Technologies, Palo Alto, CA). Total RNA samples were analyzed with the Del-Mar 14K Chicken Integrated Systems microarrays, using a reference RNA hybridization design as described previously by our group (16, 36). A single microarray was used in a reference RNA design for hybridization of four biological replicates of FL and LL chickens at four ages (1, 3, 5, and 7 wk of age) per genotype and age group (n = 4 per group). The 14K-CIS microarrays were used in a reference RNA design for hybridization of four biological replicates of FL and LL chickens at four ages (1, 3, 5, and 7 wk of age). A negative control was included to measure genomic DNA contamination by performing the reverse transcription reaction with no reverse transcriptase added. Transcript levels were quantified by using 2 μl of diluted cDNA (1:200) in a 20-μl PCR reaction using SYBR Green real-time quantitative PCR master mix [2 × PCR buffer, 0.12 μl Taq polymerase, 400 nM dNTPs, 40 nM fluorescein (Invitrogen), and SYBR Green I Nucleic Acid Gel Stain (Invitrogen) diluted 1:10,000] and analyzed with the iCycler iQ Real-Time PCR Detection System (Bio-Rad, Hercules, CA). A three-step PCR cycle was used with an initial denaturation at 95°C for 3 min followed by 40 cycles of 94°C for 45 s, 60°C for 45 s, and 72°C for 45 s, which was followed by a melting curve analysis. PCR products were verified for the appropriate size by gel electrophoresis.

The quantitative reverse transcription-PCR (qRT-PCR) data were normalized with geNorm software and methods (51). Briefly, the data were first transformed to a ΔC value by subtracting the sample threshold cycle (Ct) value from the sample with the highest expression level in order to control for amplification efficiency. The ΔΔCv was then calculated by normalizing gene expression to two housekeeping genes, β-actin (ACTB) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The formula used for the normalization is the same as previously described (51). The same 32 RNA samples used for microarray analysis were used for qRT-PCR to confirm expression patterns of 13 selected genes. Differences in expression of individual genes were tested with a two-way (line by week) analysis of variance (ANOVA) with a PDIIFF post hoc analysis to identify differences between the groups at different ages (SAS v.8.02, SAS Institute). Values reported are means ± SE, and P < 0.05 was required to reach statistical significance. All data for both qRT-PCR and microarray analyses were presented with the mean data point equal to 1, to place both data sets on the same scale for graphic presentation. Although the microarray and qRT-PCR data were placed on the same relative scale, absolute quantitative comparisons should not be made between the qRT-PCR and microarray data sets since the fold changes were determined with different normalization parameters and different techniques.

1 The online version of this article contains supplemental material.
RESULTS

General features of phenotypes and hypothalamic gene expression in FL and LL chickens. Body weight, abdominal fat weight, and percent abdominal fat are presented in Table 1. Abdominal fat pad weight and percentage were similar at 1 wk but diverged thereafter, being 2.5 times greater in FL than in LL at 3 wk of age. This difference continued through 11 wk.

Of the 14,053 genes represented on the microarrays, expression of 715 genes was significantly different by line or for the line-by-age interaction. Of these, 420 genes were differentially expressed by line (without any line-by-age interaction) and 331 genes were significant for line-by-age interactions by a two-way ANOVA (P < 0.05). The MIAME-compliant microarray data were deposited in the NCBI Gene Expression Omnibus as Series GSE10052. Means, P values, cDNA clone names, highest BlastX hits, corresponding human protein identification numbers, and HUGO gene symbols are provided for each differentially expressed transcript in Supplemental Table S2.

Differentially expressed genes were analyzed with GeneCluster to identify sets of genes that discriminate between FL and LL. This analysis indicated that many genes were consistently downregulated or upregulated in FL versus LL during development (Supplemental Fig. S1). Genes differentially expressed between FL and LL were clustered with SOMs to identify clusters related to the divergence of the fat and lean phenotypes (Fig. 1). Each graph represents the mean level of genes within the cluster, with expression levels normalized to have mean = 0 and SD = 1 across time points (48). The data are presented in a 3 × 6 SOM, which was the smallest number of clusters without substantial redundancy of profiles. Every cluster but two (clusters 4 and 14) exhibited striking differences in expression profiles during development between FL and LL. The expression patterns for 11 genes (belonging to 6 clusters), plus GAPDH and ACTB, were confirmed by qRT-PCR analysis (Table 2). Results for six of these are presented in Fig. 2. The remaining seven are presented below. In general, results from the microarray and qRT-PCR were in agreement. However, results from the two analyses were more similar to one another at week 1 than at later ages. The basis for the differences observed between the microarray results and the qRT-PCR results is not clear. One possibility is that the two different normalization strategies employed for the two techniques, LOWESS and block normalization for the microarray data and geNorm for the qRT-PCR data, contributed to the apparent differences between the two approaches. Overall, we consider qRT-PCR more robust and less prone to systematic errors.

Expression profiles and cell type specificity of genes during divergence of adiposity. As previously observed (46), differences in adiposity began to develop by 3 wk of age in FL relative to LL (Table 1). Our goal was to identify genes that were differentially expressed before and during the divergence of adiposity. Our rationale was that these genes could contribute to the difference in abdominal fat accumulation between FL and LL birds. Criteria for upregulated (FL > LL) or downregulated (FL < LL) genes were as follows: 1) gene expression was significantly different by line or by line by age (P < 0.05, 760 genes total) and 2) at the age of interest there must be no overlapping error bars between the data points, as presented in the SOM cluster data (Fig. 1). More genes were differentially expressed between the lines at week 1 than at any other age. Nine clusters representing 427 genes were upregulated in FL at 1 wk (Fig. 1; clusters 0, 1, 2, 6, 7, 8, 12, 13, and 14). Eight clusters representing 325 genes were downregulated in FL at 1 wk (Fig. 1; clusters 4, 5, 9, 10, 11, 15, 16, and 17), while cluster 3 had no difference between the lines at this age. With the onset of adiposity differences at week 3, fewer genes exhibited upregulated (Fig. 1; clusters 12, 13, 14, 16, and 17; n = 321 genes) or downregulated (Fig. 1; clusters 1, 2, 3, and 10; n = 144 genes) expression patterns in FL relative to LL (Table 3). By week 7, only 160 genes were upregulated in FL, while 223 genes were downregulated. A more detailed analysis by biological process is provided in Supplemental Table S3. In short, the greatest number of genes differentially expressed in the hypothalamus was found at week 1, before divergence of abdominal fat weight.

The hypothalamus is comprised of neurons and glial subtypes, such as astrocytes and oligodendrocytes. Thus differences in gene expression observed could be attributable to glial rather than neuronal gene expression. To address this issue, we utilized a database for genes specifically expressed in neurons, astrocytes, or oligodendrocytes in mice (9). Genes that were differentially expressed between FL and LL were compared with genes identified for having specific expression in just one cell type. Of the 56 genes found in both data sets, 20 were expressed in neurons, 24 in astrocytes, and 12 in oligodendrocytes (Supplemental Table S4). These findings suggest that neurons, although smaller in number, account for a significant portion of genes that are differentially expressed between FL and LL.

Gene Ontology analysis. GO analysis was used to identify GO biological processes that could contribute to the fat or lean phenotype. Regulation of transcription and signal transduction were GO biological processes that encompassed the largest

<table>
<thead>
<tr>
<th>Table 1. Phenotypic data for FL and LL chickens</th>
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<tr>
<td>Body weight, g</td>
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<td>FL</td>
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<td>LL</td>
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<td>Abdominal fat, g</td>
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<tr>
<td>LL</td>
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<tr>
<td>Abdominal fat/body weight, %</td>
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<tr>
<td>FL</td>
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<td>LL</td>
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Values are means ± SE for n = 8 chickens at ages of 1–11 wk. *P < 0.05 in a 1-way ANOVA comparing fat (FL) and lean (LL) lines of the same age.
Fig. 1. Self-organizing map (SOM) analysis reveals biologically relevant clusters and expression patterns between the fat (FL) and lean (LL) lines. The number of genes included in each cluster is provided at top left; values reported are mean ± SE expression levels for FL (F) and LL (L) by week.
number of genes (112 genes) among all 760 genes differentially expressed between the two lines. However, when only the 108 genes that exhibited \( \geq 1.5 \)-fold differences in expression levels between the lines were considered, transport, metabolic process, signal transduction, and glycolysis (27 genes total) were the most prevalent biological processes represented (Fig. 3). Interestingly, glycolysis was represented by a small number of all significant genes (0.06%) but comprised a larger percentage of genes with \( \geq 1.5 \)-fold differences between FL and LL (4.6%). Although these genes are involved in gluconeogenesis as well, we suggest that in the hypothalamus carbohydrate metabolism is primarily oriented toward glycolysis, since gluconeogenesis has not been demonstrated in the central nervous system. Next, we chose to annotate SOM cluster 17

<table>
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<tr>
<th>Clone ID</th>
<th>GenBank Accession No.</th>
<th>Gene Name</th>
<th>Cluster</th>
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<tr>
<td>pgp1n.pk005.c24</td>
<td>BI391670</td>
<td>Cytochrome b (CYTB)</td>
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</tr>
<tr>
<td>pgp1n.pk006.h4</td>
<td>BI392043</td>
<td>Elongation factor 1α (EEF1A)</td>
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</tr>
<tr>
<td>pgp1n.pk004.i24</td>
<td>BI394654</td>
<td>Phosphoglycerate mutase (PGAM1)</td>
<td>17</td>
</tr>
<tr>
<td>pgp1n.pk013.i12</td>
<td>BI395062</td>
<td>( \beta )-Ethylmaleimide-sensitive factor attachment protein ( \beta ) (NAPB)</td>
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</tr>
<tr>
<td>pgp2n.pk005.o4</td>
<td>BM427174</td>
<td>5-Tryptophan hydroxylase (TPH1)</td>
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<tr>
<td>pgp1n.pk012.j21</td>
<td>BI393839</td>
<td>Neural enolase (ENO2)</td>
<td>17</td>
</tr>
<tr>
<td>pgp1n.pk006.j9</td>
<td>CD218118</td>
<td>CCAAT/enhancer-binding protein ( \zeta ) (CEBPZ)</td>
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<tr>
<td>pgp1c.pk001.j21</td>
<td>BI390429</td>
<td>Dihydropyrimidinase like protein 2 (DPYSL2)</td>
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<td>pgp2n.pk006.k5</td>
<td>BM427410</td>
<td>Transmembrane protein induced by tumor necrosis factor-( \alpha ) (TMEM120A)</td>
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<td>BM490642</td>
<td>Growth hormone-releasing hormone receptor (GHRHR)</td>
<td>6</td>
</tr>
<tr>
<td>pgp1n.pk010.k1</td>
<td>BI067093</td>
<td>Pitrilysin metalloprotease 1 (PITRM1)</td>
<td>6</td>
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Clone ID is the identification number of the cDNA clone used in the production of the microarray. GenBank accession numbers allow access to partial cDNA sequences. HUGO gene names are provided in parentheses. Cluster number relates to the results presented in Fig. 1. qRT-PCR, quantitative reverse transcription-PCR; SOM, self-organizing map.
with GO terms for biological processes, because this cluster contains two of the genes involved in glycolysis, ENO2 and PGAM1. The 69 genes in cluster 17 were all linked to a GO term. Presented in Supplemental Table S5 are biological processes having at least two genes in a category. Carbohydrate metabolism, inherently related to the glycolytic pathway, contained one additional gene. Interestingly, the genes in cluster 17 were primarily involved with synthesis and metabolism of proteins.

Gene network analysis. The list of differentially expressed genes was initially submitted to Pathway Miner to identify known cellular and metabolic pathways that were differentially expressed between FL and LL (Supplemental Table S6). The pathway with the greatest number of elements represented by differentially expressed genes was again glycolysis, with five genes represented in the pathway. This suggested that genes involved in glycolysis could play a large role within the hypothalamus in driving the difference between the two phenotypes. Results from week 1 were then analyzed by Ingenuity Pathway Analysis (IPA). A total of 528 genes were IPA network eligible from the 715 differentially expressed genes (Supplemental Table S2). Again, a predicted network of genes involved in glucose metabolism was identified (Fig. 4). This network shows an equal number of genes that were either upregulated or downregulated in the hypothalamus of FL compared with LL at 1 wk of age. Interestingly, seven genes involved in glycolysis were downregulated (ALDOC, ENO2, GAPDH, GPI, HK1, PGAM1, and PGK1) whereas two genes were upregulated (GALM and PTGR1) in FL compared with LL (Fig. 4; Supplemental Table S7). Three of these [phosphoglucone isomerase (GPI), phosphoglycerate mutase (PGAM1), and neural enolase (ENO2)] were confirmed by qRT-PCR to be downregulated in FL relative to LL at week 1 (Fig. 5). These genes are involved in catalyzing different steps of glycolysis, which suggests that before the divergence of adiposity FL could have a lower level of glucose metabolism in the hypothalamus relative to LL, as indicated by lower plasma glucose in FL (26). After adiposity differences began to appear at 3 wk of age, this trend reversed. The same genes that were downregulated at week 1 were upregulated at 3 wk in FL relative to LL. This pattern continued at 5 wk, with two genes regulating glycolysis still being upregulated. By 7 wk, when the divergence in adiposity had started to stabilize between the two lines (Table 1), the expression patterns of the glycolytic genes were similar to the patterns observed at 1 wk. qRT-PCR analysis of three glycolytic genes (GPI, PGAM1, and ENO2) confirmed lower expression at 1 wk in FL chickens (Fig. 5). Hypothalamic expression of the gene encoding acid α-glucosidase (GAA) was slightly higher in FL chickens. This enzyme is responsible for breakdown of glycogen to glucose in lysosomes, and mutations in GAA cause Pompe disease (or glycogen storage disease II) (47). Together, these results suggest that genes regulating glycolysis could promote divergence of the fat or lean phenotype.

Marker analysis in FL and LL chickens. More than 600 genes have been associated with body weight and adiposity in other species, of which 166 have been linked directly to body composition or metabolism by knockout and transgenic technologies in mice (35). We screened whether any of the genes that were differentially expressed between FL and LL were among the 166 candidate genes validated in mice. From this comparison, we identified 24 genes that were either homologous to or directly related to the 166 genes validated in mice. We then selected four of these [CAAT enhancer binding protein ζ (CEBPZ), a gene induced by TNF-α (TMEM120A), growth hormone-releasing hormone receptor (GHHR), and pitirilysin metallopeptidase 1 (PITRM1)] to confirm by qRT-PCR (Supplemental Table S8; Fig. 6). In each case, gene expression was greater in FL than in LL at 1 wk of age, in agreement with the microarray results. At subsequent ages, levels of each mRNA were similar between lines. To generate a hypothetical model for gene interactions within the hypothalamus, we next performed marker-specific analysis with GeneCluster software. The genes presented in Figs. 5 and 6 were used, one at a time, as a marker for analyzing the microarray data to identify genes whose expression levels are most closely correlated with one another (Supplemental Table S9). Two gene interaction models were developed from correlations among the gene expression patterns (Fig. 7).

Microarray analysis of gene expression patterns shows that TNF receptor-associated factor 1 (TRAF1), which is associated with TNF-α signaling, is linked to other genes that modulate energy metabolism (Fig. 7A). For example, TRAF1 is potentially related to many genes that modulate body composition: 5-tryptophan hydroxylase (TPH1), GHRRH, sweet taste receptor 1 (TAS1R1), and PITRM1. At 1 wk of age, the expression of TASIR1 in the hypothalamus of FL birds was 1.8-fold higher than that of LL chickens. This model also suggests that CEBPZ is associated with TPH1 and TMEM120a gene expression. qRT-PCR analysis verified increased expression in FL

### Table 3. Numbers of differentially expressed genes in FL relative to LL by age

<table>
<thead>
<tr>
<th></th>
<th>Week 1</th>
<th>Week 3</th>
<th>Week 5</th>
<th>Week 7</th>
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<tr>
<td>Upregulated</td>
<td>427 (54%)</td>
<td>321 (40%)</td>
<td>372 (47%)</td>
<td>160 (20%)</td>
</tr>
<tr>
<td>Downregulated</td>
<td>325 (41%)</td>
<td>144 (18%)</td>
<td>266 (34%)</td>
<td>223 (28%)</td>
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Numbers in parentheses are percentage of all significantly differentially expressed genes.

![Top 16 Categories GO Biological Process](108 Genes)

**Fig. 3.** Gene Ontology (GO) and pathway analysis of significant genes. Genes significantly different (P < 0.05) and expressed at 1.5-fold or greater levels between FL and LL animals (108 genes total) were analyzed for GO terms for biological process. Shown are the results for GO terms represented by ≥4 genes.
relative to LL for four of these genes, CEBPZ, TMEM120a, PITRM1, and GHRHR (Fig. 6). We also developed a model for gene interactions in which four of the five glycolytic genes are closely linked (Fig. 7B). Within this model, we also identified dihydropyrimidinase-like 2 (DPYSL2) as a gene that may interact with the glycolytic pathway in the hypothalamus during the divergence of adiposity between FL and LL. qRT-PCR analysis confirmed that PGAM1, ENO2, GPI, and DPYSL2 mRNA levels were reduced in FL relative to LL (Fig. 5).

**DISCUSSION**

Microarray analysis revealed dynamic changes in gene expression within the hypothalamus before and after the development of adiposity in our experimental model of genetically linked “obesity.” More genes were differentially expressed between FL and LL at 1 wk of age than at later ages. This is likely attributable to developmental processes or neural circuitry being established during this age. Considering the

Fig. 4. Gene interaction network in hypothalamus of FL and LL chickens at 1 wk of age. Results for differentially expressed genes were analyzed with Ingenuity Pathway Analysis. The network shown included a number of genes involved in glucose metabolism. Red gene symbols indicate upregulated and green symbols represent downregulated genes in FL relative to LL.
known central role of the hypothalamus in controlling food intake and metabolism (2, 20), this finding also suggests that
differences in establishment of these hypothalamic neural pro-
cesses could contribute to the difference in abdominal fat and
total body fat between the two genetic lines.

Glycolysis and development of adiposity. Enzymes of gly-
colysis are generally not considered as rate limiting in glucose
utilization, except in specific cells or organs where the first
intracellular step of glucose metabolism is rate limiting and
dependent upon the activity of hexokinase 1 (HK1). In the
hypothalamus of mammals, HK1 is expressed in glucose-
sensitive neurons, where it exerts a major role in sensing glucose, although additional and important elements have also
been proposed (22, 27). Furthermore, glucokinase (GCK) has
been implicated in more direct glucose sensing in the murine
anterior pituitary (53). Here, we have identified differences
between FL and LL chickens in hypothalamic expression of
nine genes encoding enzymes involved in glucose metabolism

Fig. 5. Expression pattern verification for
genes involved in glycolysis. Data expression
patterns were similar for microarray (left) and
qRT-PCR (right). The log scale microarray
data have been converted, which compresses
the data points around the value of 1. Values
reported are means ± SE. *Differential ex-
pression between the lines at the same age
(P < 0.05). Gene symbols are defined in the
text.

Fig. 6. Expression pattern verification for
genes identified by comparison of significant
genes. Expression patterns for FL relative to
LL at 1 wk of age were increased for genes
presented in our model. The log scale mi-
croarray data have been converted, which
compresses the data points around the value
of 1. Values reported are means ± SE. *Dif-
ferential expression between the lines at the
same age (P < 0.05). Gene symbols are
defined in the text.
and glycolysis. Glycolytic gene expression was downregulated in the hypothalamus of FL relative to LL at 1 wk of age, before divergence in adiposity between the lines. In a similar manner, an enhancement in hepatic glycolysis has been associated with a reduction in obesity (52). In contrast, the glycolytic potential of muscle has been reported to be greater in FL than LL animals (42). To our knowledge, no alternative functions besides their role in glycolysis have been proposed for enzymes identified in the present microarray experiment, including ENO2. However, α-enolase is located in multiple subcellular compartments (including the cell surface) and may exert other functions (49). We have identified DPYSL2 as a coregulated gene with four of the genes involved in glycolysis and therefore as a potential upstream regulator or downstream target of glycolysis. DPYSL2 has been previously associated with Alzheimer disease (5, 14) and is known to be involved in development of the nervous system (12). This is the first report associating the function of DPYSL2 with energy metabolism and processes contributing to development of a fat or lean phenotype.

In contrast to the situation that prevails in obese mammals, FL chickens generally exhibit low plasma glucose and high or normal plasma insulin (44). Other studies performed on isolated, perfused pancreas in chickens from the same genetic lines have also hypothesized the presence of a discrete change in the glucose-insulin balance between FL and LL chickens (39). At 61 days of age, differences in glucose levels had not been observed in cerebrospinal fluid in either the fasted or fed state, despite a clear difference in plasma glucose level in the fasted state between FL and LL chickens (45). The decrease in mRNA for glycolytic enzymes in the hypothalamus of FL chickens would most likely not depend upon changes in glucose availability. However, the possibility of a change in the membrane glucose transporter step cannot be excluded. Conversely, a discrete change in glucose metabolism within the hypothalamus, as presently suggested, may generate different peripheral signals.

Our discovery of differential expression of the sweet taste receptor gene (TASIR1) in the hypothalamus of the FL chicken could be of major physiological significance. The sweet taste receptor T1R1 has been recently proposed as a hypothalamic glucose sensor in the mouse (38), where heterodimeric T1R2/ T1R3 senses glucose and T1R1/T1R3 monitors l-amino acid levels in the brain. In mouse small intestine, the T1R taste receptors sense sugar levels and regulate active and facilitated absorption of glucose (30, 32). Two independent computational surveys, which examined either distribution of G protein-coupled receptors in the chicken genome (23) or the evolution and diversity of sweet and bitter taste receptor genes (40), have both shown that chickens lack the TASIR2 gene, which could account for their inability to perceive sweetness. Interestingly, TASIR1 transcripts are also differentially expressed in both liver and abdominal fat samples from FL and LL chickens (L. A. Cogburn, unpublished observations). Increased expression of TASIR1 transcripts in multiple tissues (hypothalamus, liver, and abdominal fat) of FL chickens supports a role of the TASIR1 gene in glucose sensing and utilization in this unique model of genetic obesity.

Homologous genes associated with obesity in knockout or transgenic mice. The present analysis identified several genes previously known to modulate adiposity in mice (37). TNF-α-associated genes were another family of genes that had expression differences between the lines. TNF-α is a cytokine secreted from many cell types, including adipocytes, and has been shown to act on the hypothalamus to suppress appetite (3). We demonstrated upregulation of genes related to TNF-α signaling in LL relative to FL, potentially explaining the decreased adiposity in LL relative to FL.

The TRAF1 molecule has been associated with gene expression for the sweet taste receptor (TASIR1), which is also present in the small intestine of mice and is associated with glucose uptake (29). The FL chicken has lower plasma glucose and higher plasma insulin relative to the LL bird (26), with hypothalamic TASIR1 and TRAF1 mRNA levels being lower in FL relative to LL. This suggests that TASIR1 expression in the hypothalamus may be associated with TRAF1 to modulate glucose uptake in the neuron, in a manner similar to that demonstrated in the intestine of mice. TRAF1 was also associated in our present study with GHRHR and TPH1, the rate-limiting enzyme for serotonin synthesis, and indirect relationships have been shown to exist between TNF-α, GHRH, and serotonin production. For example, the antitumor effects of TNF-α were attenuated by blocking serotonin receptors (31). We demonstrate here that decreased levels of TPH1 mRNA correlate with decreased levels of TRAF1 mRNA in the hypothalamus of FL relative to LL birds. Mice with deficits in GHRH signaling were not able to increase mRNA expression for GHRHR or TNFα after virus treatment, suggesting that a direct relationship between the two genes may exist (1).

Our analysis suggests that TMEM120a may interact with CEBPZ, CEBPα and β have been associated with adipocyte differentiation (13), and here we show that hypothalamic CEBPZ expression may be associated with the divergence of
adiposity in FL and LL, with CEBPZ mRNA levels being greater in FL relative to LL. The putative transmembrane protein (TMEM43) could be a peroxisome proliferator-activated receptor γ (PPARγ)-target gene, since response elements for this adipogenic gene have been identified in mammals (33). PITRM1 was also identified in the present study as a candidate gene associated with TRAF1. Interestingly, it has been found that tissue inhibitor of metalloprotease 1 is elevated in obese children, resulting in lower levels of PITRM1 (18). This trend corresponds with the increased levels of PITRM1 mRNA observed in the hypothalamus of FL relative to LL at 1 wk of age. It has also been shown that TNF-α elevates the levels of disintegrin and metalloprotease with thrombospondin motifs 1 (ADAMTS1) (15), which has been directly associated with modulating body composition (41). Collectively, these observations suggest that TNF-α may interact with or regulate expression of PITRM1 to modulate body composition.

Genetic influences. The present study indicates that genotype influences development of adiposity by regulating hypothalamic expression of genes that control deposition of body fat during a critical developmental period. The altered gene expression observed at 1 wk of age, before any differences in adiposity, could modulate neuronal development to regulate the long-term body fat “set point.” Similar developmental aspects of functional neural pathways between hypothalamic nuclei have been shown in the mouse (4). Therefore, we have identified a large set of genes that are differentially regulated after divergent selection of abdominal fat, which could potentially interact to regulate homeostatic mechanisms controlling long-term accumulation of body fat. It should be noted that all of our findings were restricted to levels of mRNA within the hypothalamus. Differences in mRNA levels do not necessarily translate into differences in levels of functional proteins. Furthermore, differences in levels of a specific protein within nervous tissue do not necessarily result in altered function of the neurons. Additional research is necessary to confirm differences in hypothalamic function associated with divergence in adiposity suggested by the present study.

Animal models are useful for identifying novel candidate genes that regulate energy homeostasis. For example, one rat model was bred to be susceptible or resistant to diet-induced obesity (DIO), and this model has been very useful in elucidating interactions between environment and genotype in the development of obesity (28). Here, we utilized a unique animal model to study genotype–regulated differences during development of extremes in adiposity, which are independent of food intake and total body weight. Our findings of differences in hypothalamic gene expression before the onset of differences in adiposity suggest that this chicken model has the potential to help elucidate the underpinnings of genotype-induced human obesity. In addition, the chicken genome has a greater synteny with the human genome than does the mouse (6, 19). In conclusion, we have investigated hypothalamic gene expression patterns in a unique genetic model for obesity and identified novel candidate genes that could contribute to the development of genotype-induced obesity.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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HYPOTHALAMIC GENE EXPRESSION ASSOCIATED WITH ADIPOSITY