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Expression profiling of the solute carrier gene family in chicken intestine from the late embryonic to early post-hatch stages

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Summary

Intestinal development during late embryogenesis and early post-hatch has a long-term influence on digestive and absorptive capacity in chickens. The objective of this research was to obtain a global view of intestinal solute carrier (SLC) gene family member expression from late embryogenesis until 2 weeks post-hatch with a focus on SLC genes involved in uptake of sugars and amino acids. Small intestine samples from male chicks were collected on embryonic days 18 (E18) and 20 (E20), day of hatch and days 1, 3, 7 and 14 post-hatch. The expression profiles of 162 SLC genes belonging to 41 SLC families were determined using Affymetrix chicken genome microarrays. The majority of SLC genes showed little or no difference in level of expression during E18–D14. A number of well-known intestinal transporters were upregulated between E18 and D14 including the amino acid transporters *rBAT*, *y^+LAT-2* and *EAAT3*, the peptide transporter *PepT1* and the sugar transporters *SGLT1*, *GLUT2* and *GLUT5*. The amino acid transporters *CAT-1* and *CAT-2* were downregulated. In addition, several glucose and amino acid transporters that are novel to our understanding of nutrient absorption in the chicken intestine were discovered through the arrays (*SGLT6*, *SNAT1*, *SNAT2* and *AST*). These results represent a comprehensive characterization of the expression profiles of the SLC family of genes at different stages of development in the chicken intestine and lay the ground work for future nutritional studies.

Keywords chicken, intestinal development, microarray, nutrient transporter, solute carrier.

Introduction

At hatch, chicks must transition from nutrition based on a lipid-rich yolk to exogenous carbohydrate-rich feeds (Uni *et al.* 1998). To support the needs of growth and development, the chicken small intestine undergoes dramatic changes in morphology and function in the immediate post-hatch period. With these dramatic developmental changes, hundreds of genes involved in intestinal development, growth regulation and nutrient transport are likely to exhibit changes in expression.

Changes in intestinal digestive enzyme and nutrient transporter expression and activity correlate with the morphological changes that are observed during the later stages of incubation into the first week post-hatch. During embryological development, the expression of many intestinal nutrient transporters can be detected, and at hatch the presence of a large spectrum of transporters with various substrate specificities ensures efficient assimilation of nutrients from the diet. Activities of many brushborder membrane digestive enzymes and nutrient transporters rise during the early post-hatch period in response to increasing needs to digest and absorb the large quantity of nutrients for supporting growth and metabolism (Nitsan *et al.* 1991; Uni *et al.* 1999, 2003b; Sklan & Noy 2000; Gilbert *et al.* 2007). Expression of digestive enzymes and nutrient transporters may be influenced by a variety of factors, including nutrition during the post-hatch period (Batal & Parsons 2002; Chen *et al.* 2005). Currently, little is known about the developmental pattern of gene expression for many of these transporters in the chicken, including the ones that have been well characterized in mammalian species.

Solute carriers (SLC) play an important role in capturing luminal nutrients in the chick small intestine. As of June

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2007, the human SLC gene family included 45 families and 351 transporter genes, which encode active and passive transporters, ion-coupled transporters and exchangers (http://www.bioparadigms.org/slc/menu.asp). Expression studies of several chicken SLC genes, digestive enzymes and regulatory factors, such as the *peptide transporter* (*PepT1*), *sodium-glucose transporter* (*SGLT1*), *fructose transporter* (*GLUT5*), *aminopeptidase N* (*APN*) and transcription factors *caudal homeobox domain A* and *B* (*CDXA* and *CDXB*) were studied using northern blot or real-time PCR (Barfull *et al.* 2002; Geyra *et al.* 2002; Chen *et al.* 2005; Gilbert *et al.* 2007). The expression of these genes is likely to influence the development of intestinal digestive and absorptive functions *in ovo* or immediately post-hatch. Although much research has been conducted on individual SLC genes, a comprehensive analysis of all members of the SLC gene family series has not been conducted in the small intestine of the chicken.

DNA microarrays represent a powerful tool to analyse global gene expression and thus provide a means to examine differential gene expression in the small intestine of chicks. In recent years, a number of EST libraries from different chicken tissues have been constructed (Boardman *et al.* 2002; Carre *et al.* 2006; Cogburn *et al.* 2007). Chicken microarrays based on these EST libraries were generated by different groups (reviewed in Cogburn *et al.* 2007), including a chicken jejunum cDNA microarray (Van Hemert *et al.* 2003), the 13K mixed-tissue microarray (Burnside *et al.* 2005) and several custom DNA microarrays (Carre *et al.* 2006). Van Hemert *et al.* (2003, 2004, 2006) reported the use of DNA microarrays for profiling chicken intestinal genes in response to a *Salmonella* infection in slow- and fast-growing lines and malabsorption syndrome in different broiler lines. An avian macrophage-specific cDNA microarray containing 4906 unique gene elements was used to elucidate the transcriptional response of macrophages to three avian protozoan pathogens (Dalloul *et al.* 2007). Several single and multiple chicken tissue (not including intestine) DNA microarrays for global gene expression profiling have been constructed (Cogburn *et al.* 2003; Carre *et al.* 2006).

In this study, we determined the expression profiles of 162 SLC genes in the chick small intestine from late embryogenesis (E18) to 14 days post-hatch (D14). We identified genes that appear to be most responsive to developmental changes and described changes in transporters not previously reported to be expressed in the chick small intestine. Information gained from this research will be useful in improving our understanding of the processes associated with nutrient assimilation in the chick small intestine during the transition from late embryonic development to the early post-hatch period. Target genes analysed in this study may provide a focus for future studies aimed at enhancing absorption of particular nutrients in the intestine.

Materials and methods

Animals and tissue collection

Chickens used in the present experiment were Aviagen commercial broilers. Two genetically selected lines of Aviagen broilers (line A and line B) were used in the study and their characteristics are described in Gilbert *et al.* (2007). These lines originated from a single genetic stock, but have since been selected on diets that differ in amino acid concentration, which has led to differences in growth rate. In general, when fed a standard commercial corn-soy diet, line A is the faster-growing line, and nutritional perturbations accentuate the differences in growth between the two lines. Only males were evaluated in this experiment because their rapid rate of growth would increase the likelihood of detecting significant developmental changes. Furthermore, we have observed a difference in the expression profiles of nutrient transporters between males and females (C. Mott, unpublished results). Eggs were obtained from Aviagen Inc. Day-of-hatch chicks were randomly assigned to heated floor pens with wood shavings and kept under 24-h light. After hatch, birds were given *ad libitum* access to a corn-soy-based diet that was formulated to contain 3060 kcal/kg ME and 20.0% CP. On embryonic days 18 (E18) and 20 (E20), day of hatch (DOH) and days 1, 3, 7 and 14 post-hatch, chicks were killed and the small intestine was collected. The three segments of the intestine, duodenum, jejunum and ileum, were collected individually at E20, DOH and days 1, 3, 7 and 14 post-hatch. Due to the difficulty in handling the soft tissue, the entire small intestine was collected at E18. Intestinal segments were rinsed with ice-cold phosphate-buffered saline, minced with a razor blade, frozen as aliquots in liquid nitrogen and stored at −80 °C.

DNA extraction and PCR-based sexing

DNA was isolated from liver samples using the DNeasy Tissue Kit (Qiagen). The sex of the birds was determined by PCR using primers for *tyrosinase* as a positive control (forward primer, 5′-TCGAGAGGCATAATAATGCATCCA-3′; reverse primer, 5′-AGAGCTTGCTGAGGAAGGAGTG-3′), and primers for a sequence on the W chromosome (forward primer, 5′-CTGTGATAGAGACCGCTGTGC-3′; reverse primer, 5′-CAACGCTGACACTTCCGATGT-3′; R. Okimoto, personal communication). The PCR products were analysed on a 1.0% agarose gel. All samples contained the 400-bp *tyrosinase*-specific band and female birds were identified by the presence of the 1200-bp W-specific band, whereas male birds lacked this band.

RNA isolation

Total RNA was isolated for both microarray and real-time PCR studies. Tissue samples were ground in a mortar and

pestle under liquid nitrogen. Total RNA samples from intestinal segments of 12 male birds at each time point were isolated using the RNeasy Mini Kit (Qiagen). RNA samples were quantified by UV spectrophotometry ($A_{260/280}$), evaluated for quality by electrophoresis through a 1.0% agarose gel containing formaldehyde and analysed on an Agilent Bioanalyzer. Each sample of total RNA was separated into two parts: one part of the sample was used for real-time PCR and the other part was used for making a total intestinal RNA pool. The RNA pool for microarrays was reconstructed by combining duodenum, jejunum and ileum RNA in a proportion similar to the tissue weight. Equal amounts of total RNA from three individual chicks were then combined to create a pooled sample that was used for hybridization to one microarray.

Microarray

The Affymetrix GeneChip Chicken Genome Array was chosen for this study because it provided comprehensive coverage of 32 773 transcripts corresponding to over 28 000 chicken genes. Annotation was from the Affymetrix database. The samples were prepared and the array hybridized according to the Affymetrix GeneChip Expression Analysis Technical Manual. Arrays were scanned using an Affymetrix GeneChip(R) Scanner (GCS) 3000. Four samples of pooled RNA (three birds/pool) were hybridized for each time point. Thus, a total of 28 arrays were used for the seven time points (E18, E20, DOH, D1, D3, D7 and D14). The four samples per time point included two pools of line A birds and two pools of line B birds. Because the expression profiles of only 10/162 SLC genes showed two- to fourfold differences between lines, the data were combined and the focus of the study became development-specific changes in expression rather than expression differences between lines. For all 10 SLC genes, parallel up- or downregulation was observed in lines A and B, but the magnitude of the E18–E14 ratio was different. The DNA microarray data are available at the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/) through GEO Series accession number GSE8495.

Quantitative real-time PCR

Primers for the seven genes chosen for verification by real-time PCR were designed based on published sequences in GenBank and are shown in Table 1. The RNA samples used for RT-PCR are the same samples from individual birds that made up the pools. The RNA samples were reverse-transcribed in parallel in a 20-μl reaction volume using the High Capacity cDNA Archive Kit (Applied Biosystems). Real-time PCR was performed with the ABI 7300 system using SYBR Green PCR Core Reagents (Applied Biosystems). PCR was performed under the following conditions: 50 °C for 10 min and 40 cycles of 95 °C for 1 min and 60 °C for 1 min. The absolute quantification method was used to determine the number of RNA molecules as described in Gilbert *et al.* (2007).

Data analyses

Signal values of all probe sets derived from Affymetrix *.cel files were analysed by ArrayAssist (Iobion, Inc.). To calculate the signal intensities, a modified version of robust multiple array (RMA) normalization (Irizarry *et al.* 2003; Wu *et al.* 2004) was used, which adjusts for background and normalizes and log-transforms the values. We obtained GCRMA data from the ArrayAssist analyses, and entered those into the GENESPRING software (Version 7.2, Silicon Genetics). Analysis of variance was used for differentially expressed genes based on Welch ANOVA ($P < 0.05$) and then probe set lists were filtered using the fold-change analysis provided by GENESPRING. In total, 21 comparisons were performed between different time points to identify differentially expressed genes. We then selected all SLC genes from the entire list of expressed genes to focus our analysis on genes involved in nutrient transport. For genes that showed a

Table 1 Sequences of real-time PCR primers used for selected genes.

Gene name	GenBank ID	Forward primer[1]	Reverse primer[1]
SGLT1 (*SLC5A1*)	XM_415247	5′-GCCATGGCCAGGGCTTA-3′	5′-CAATAACCTGATCTGTGCACCAGTA-3′
SGLT6 (*SLC5A11*)	XM_414862	5′-GGCATGGTTATTCCTTCCCA-3′	5′-GTTTCTGCAGGTACTCCGGC-3′
PepT1 (*SLC15A1*)	NM_204365	5′-CCCCTGAGGAGGATCACTGTTGGCAGTT-3′	5′-CAAAAGAGCAGCAGCAACGA-3′
NHE2 (*SLC9A2*)	XM_416918	5′-TGCCAACTCGTCTTTTCTTTGA-3′	5′-GTGCCCACCACGGCATA-3′
SNAT1 (*SLC38A1*)	XM_416048	5′-CAGAGGATTTGGGCTTCCCT-3′	5′-GATGACCAATGGGATGCTCAC-3′
SNAT2 (*SLC38A2*)	NM_001030741	5′-TGGGTCCATAAAAAGCATAATTCA-3′	5′-GCATTCAGGAAATCGTAACATCC-3′
AST (*SLC17A5*)	NM_001031086	5′-ATGCGCAGGAGAATGGCTT-3′	5′-TCAGCAATTTGCCCAGACAG-3′

SGLT1, *sodium/glucose cotransporter family, member 1*; *SGLT6*, *sodium/glucose cotransporter family, member 6*; *PepT1*, *proton oligopeptide cotransporter family, member 1*; *NHE2*, *sodium/hydrogen exchanger family, member 2*; *SNAT1*, *sodium-coupled neutral amino acid transporter family, member 1*; *SNAT2*, *sodium-coupled neutral amino acid transporter family, member 2*; *AST*, *anion/sugar transporter family, member 5*.
[1]Primers were designed with PRIMER EXPRESS software (Applied Biosystems).

twofold or greater change from E18 to D14, the false discovery rate (FDR) was controlled to be <0.1. To understand the relationship between the samples and genes, the K-MEANS CLUSTERING algorithm was used based on average linkage using the standard correlation in GENESPRING.

Results

Expression profiling of SLC genes

In our study, 162 SLC transporter genes belonging to 41 families were expressed during E18–D14 in the chicken intestine. Fifty-nine of these 162 SLC transporters showed at least a twofold difference in expression ($P < 0.01$). According to the Affymetrix database (http://www.affymetrix.com/analysis/index.affx), a total of 229 SLC genes were found in the chicken genome based on a comparison of chicken genomic sequences with 351 human gene records (http://www.bioparadigms.org/slc/menu.asp). Of the 229 genes, 162 were expressed in our arrays. Because genes exhibiting similar expression patterns may be involved in the same biological processes or regulated by shared mechanisms (Marcotte *et al.* 1999), we grouped the 162 expressed members of the SLC gene family into six clusters by K-MEANS CLUSTER analysis (Fig. 1). The majority of the transporters (84/162) clustered into set 4, which showed little or no developmental changes in expression from E18 to D14. Thirty-one and 11 SLC genes clustered into set 1 and set 5 respectively and showed an increase in expression after E18. Five SLC genes in set 6 showed an increase in expression of at least twofold from E18 to DOH or D1 and then declined. Fifteen SLC genes clustered into set 3, of which a number of these genes showed a transient peak of expression at DOH. The 16 SLC genes that clustered in set 2 showed downregulation. All 162 SLC genes are listed in Table 2 and are categorized based on their cluster set and then within the cluster set, the genes are listed using their standard SLC nomenclature. The fold changes in expression of the SLC genes are shown for the embryonic period (E18–DOH) and the post-hatch period (DOH–D14). A positive

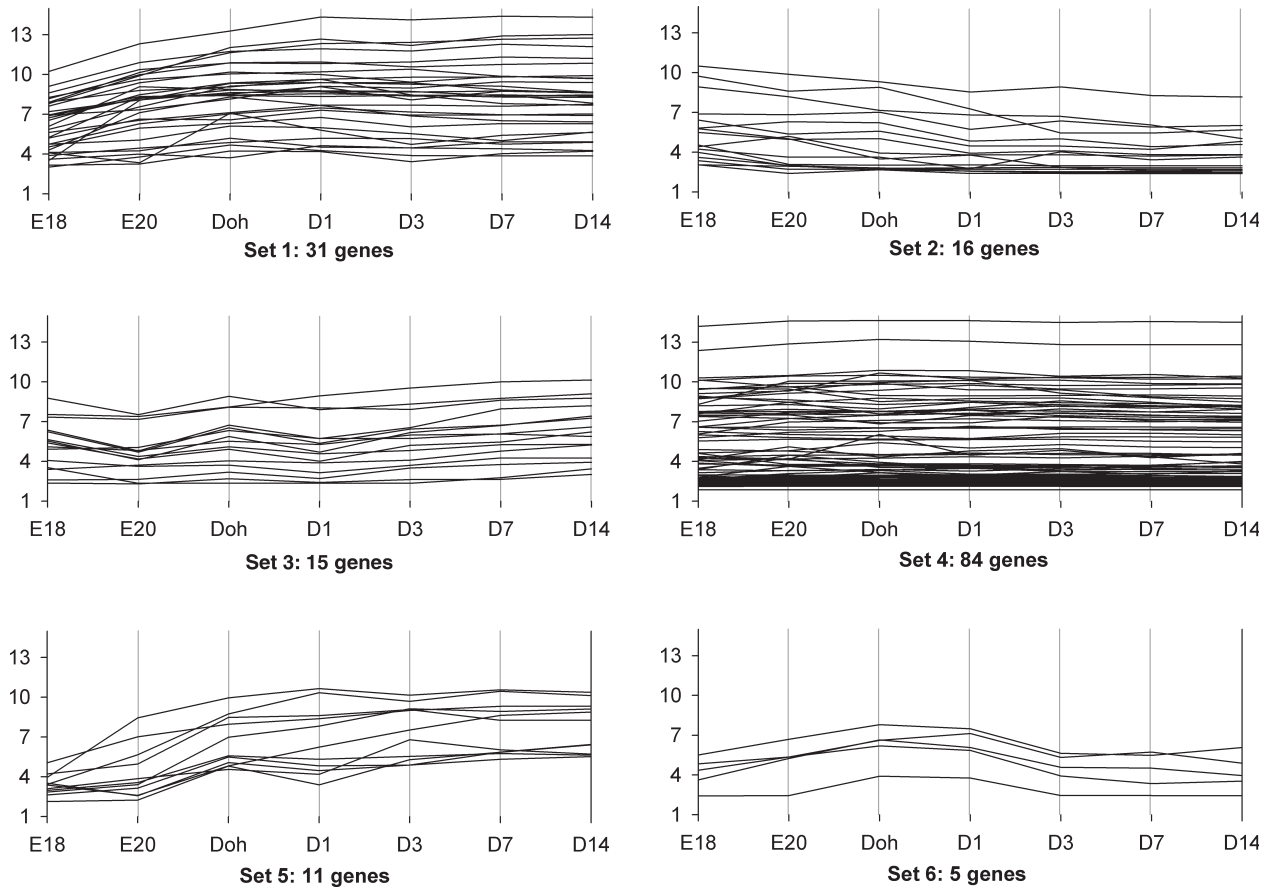

Figure 1 Gene expression profiles of SLC transporters. There were 162 expressed SLC transporter genes clustered into six sets by K-MEANS CLUSTER analysis. The *y*-axis is log-transformed chip hybridization data after normalization. The expression patterns of SLC transporter genes in set 4 have no significant correlation with development of the chick small intestine. SLC transporter genes in sets 1 and 5 increased and those in set 2 decreased during the period of intestinal development. Genes in set 6 increased prior to day of hatch/D1, decreased to D3 and then remained stable after D3. Genes in set 3 showed a complex pattern of up- and downregulation.

Table 2 Expression change of 162 solute carrier (SLC) superfamily members in chicken intestine.[1]

Cluster[2]	Affymetrix ID[3]	Gene nomenclature[4]	Gene description[5]	Aliases[6]	Fold change DOH/E18[7]	Fold change D14/DOH[8]	*P*-value[9]
1	*GgaAffx.6267.1.S1_at*	*SLC3A1*	*Solute carrier family 3 (activator of cystine, dibasic and neutral amino acid transport), member 1*	*CSNU1, D2H, RBAT, NBAT*	6.15	1.29	1.66E-05
1	*Gga.9352.1.S1_at*	*SLC4A7*	*Solute carrier family 4, sodium bicarbonate cotransporter, member 7*	*NBC3, NBC2, SBC2*	3.45	−1.26	5.67E-07
1	*Gga.8635.1.S1_at*	*SLC5A1*	*Solute carrier family 5 (sodium/glucose cotransporter), member 1*	*SGLT1*	13.36	2.16	4.89E-07
1	*GgaAffx.26670.1.S1_at*	*SLC5A11*	*Solute carrier family 5 (sodium/glucose cotransporter), member 11*	*KST1, SMIT2, SGLT6*	6.84	−2.06	3.91E-05
1	*GgaAffx.2235.1.S1_at*	*SLC6A8*	*Solute carrier family 6 (neurotransmitter transporter, creatine), member 8*	*CRTR, CT1*	4.84	−1.09	9.53E-05
1	*GgaAffx.8389.2.S1_s_at*	*SLC6A19*	*Solute carrier family 6 (neutral amino acid transporter), member 19*	*B^0AT1*	18.70	1.97	3.50E-05
1	*Gga.9956.1.S1_at*	*SLC7A6*	*Solute carrier family 7 (cationic amino acid transporter, y^+ system), member 6*	*y^+LAT-2*	6.54	−1.02	4.26E-07
1	*GgaAffx.26296.1.S1_at*	*SLC7A9*	*Solute carrier family 7 (cationic amino acid transporter, y^+ system), member 9*	*$b^{0,+}AT$*	4.74	1.27	1.13E-03
1	*Gga.1222.2.S1_s_at*	*SLC9A3R1*	*Solute carrier family 9 (sodium/hydrogen exchanger), member 3 regulator 1*	*NHERF, EBP50*	2.69	1.04	2.48E-05
1	*Gga.17193.1.S1_at*	*SLC9A6*	*Solute carrier family 9 (sodium/hydrogen exchanger), member 6*	*NHE6*	1.95	1.01	4.14E-03
1	*GgaAffx.12657.1.S1_at*	*SLC12A4*	*Solute carrier family 12 (potassium/chloride transporters), member 4*	*KCC1*	1.09	1.45	2.37E-03
1	*Gga.4106.1.S1_at*	*SLC15A1*	*Solute carrier family 15 (oligopeptide transporter), member 1*	*PEPT1*	8.37	2.05	3.32E-06
1	*GgaAffx.22425.1.S1_s_at*	*SLC16A5*	*Solute carrier family 16, member 5 (monocarboxylic acid transporter 6)*	*MCT6*	16.11	−3.71	1.67E-05
1	*Gga.10061.1.S1_s_at*	*SLC16A6*	*Solute carrier family 16, member 6 (monocarboxylic acid transporter 7)*	*MCT7*	2.12	−1.21	4.79E-03
1	*GgaAffx.12447.1.S1_at*	*SLC16A9*	*Solute carrier family 16, member 9 (monocarboxylic acid transporter 9)*	*MCT9*	3.42	1.08	7.08E-04
1	*GgaAffx.11943.1.S1_s_at*	*SLC17A5*	*Solute carrier family 17 (anion/sugar transporter), member 5*	*AST, SD*	2.20	1.83	8.29E-05
1	*GgaAffx.25719.2.S1_s_at*	*SLC19A3*	*Solute carrier family 19, member 3*		2.11	−1.01	1.65E-02
1	*Gga.3329.1.S1_at*	*SLC20A2*	*Solute carrier family 20 (phosphate transporter), member 2*	*PiT-2*	2.59	1.37	6.36E-03
1	*Gga.15388.1.S1_at*	*SLC24A6*	*Solute carrier family 24 (sodium/potassium/calcium exchanger), member 6*	*FLJ22233*	3.40	−1.02	3.11E-07
1	*Gga.1208.1.S1_at*	*SLC25A16*	*Solute carrier family 25 (mitochondrial carrier; Graves disease autoantigen), member 16*	*GP*	5.94	−1.41	2.85E-05

Table 2 Continued.[1]

Cluster[2]	Affymetrix ID[3]	Gene nomenclature[4]	Gene description[5]	Aliases[6]	Fold change DOH/E18[7]	Fold change D14/DOH[8]	*P*-value[9]
1	*Gga.9566.2.S1_s_at*	*SLC25A37*	*Solute carrier family 25, member 37*	*HT015*	3.13	−1.75	2.02E-03
1	*GgaAffx.26597.1.S1_s_at*	*SLC26A6*	*Solute carrier family 26, member 6*		56.49	−1.64	1.79E-04
1	*Gga.1737.1.S1_at*	*SLC27A4*	*Solute carrier family 27 (fatty acid transporter), member 4*	*FATP4*	9.65	−1.38	1.01E-06
1	*Gga.13557.1.S1_at*	*SLC33A1*	*Solute carrier family 33 (acetyl-CoA transporter), member 1*	*AT-1*	2.82	−1.13	1.20E-05
1	*Gga.216.1.S2_at*	*SLC34A2*	*Solute carrier family 34 (sodium phosphate), member 2*		7.06	−3.17	1.99E-06
1	*Gga.9848.1.S1_at*	*SLC35E1*	*Solute carrier family 35, member E1*	*FLJ14251*	2.83	1.54	4.46E-04
1	*Gga.2836.1.S1_s_at*	*SLC37A2*	*Solute carrier family 37 (glycerol-3-phosphate transporter), member 2*	*Ci2*	2.59	−1.37	3.57E-03
1	*GgaAffx.25485.1.S1_at*	*SLC40A1*	*Solute carrier family 40 (iron-regulated transporter), member 1*	*Ferroportin*	7.86	−2.71	1.14E-04
1	*Gga.9305.1.S1_at*	*SLC41A1*	*Solute carrier family 41, member 1*		3.85	−1.30	2.27E-05
1	*Gga.15714.1.S1_at*	*SLC43A2*	*Solute carrier family 43, member 2*	*LAT-4*	8.85	−1.03	3.69E-04
1	*Gga.1783.1.S1_at*	*SLCO2B1*	*Solute carrier organic anion transporter family, member 2B1*	*OATP2B1*	2.31	2.38	3.89E-06
2	*Gga.249.1.S1_at*	*SLC6A2*	*Solute carrier family 6 (neurotransmitter transporter, noradrenalin), member 2*	*NET1, NAT1*	−2.45	−1.13	3.91E-02
2	*GgaAffx.10939.1.S1_at*	*SLC7A1*	*Solute carrier family 7 (cationic amino acid transporter, y^+ system), member 1*	*CAT-1, REC1, ATRC1, ERR*	−1.58	−1.00	2.78E-01
2	*Gga.10093.1.S1_at*	*SLC7A2*	*Solute carrier family 7 (cationic amino acid transporter, y^+ system), member 2*	*CAT-2(A or B), TEA*	−3.43	−4.39	2.20E-05
2	*Gga.13519.1.S1_s_at*	*SLC7A5*	*Solute carrier family 7 (cationic amino acid transporter, y^+ system), member 5*	*LAT1*	−1.53	1.01	1.32E-01
2	*Gga.5602.2.S1_s_at*	*SLC12A1*	*Solute carrier family 12 (sodium/potassium/chloride transporters), member 1*	*BSC1*	−2.25	−2.23	8.66E-07
2	*GgaAffx.21935.1.S1_at*	*SLC16A12*	*Solute carrier family 16, member 12 (monocarboxylic acid transporter 12)*	*MCT12*	−2.88	−1.02	2.17E-02
2	*Gga.3555.1.S1_at*	*SLC16A3*	*Solute carrier family 16, member 3 (monocarboxylic acid transporter 4)*	*MCT4*	−1.75	1.01	4.08E-01
2	*GgaAffx.6129.1.S1_at*	*SLC16A7*	*Solute carrier family 16, member 7 (monocarboxylic acid transporter 2)*	*MCT2*	−1.17	−1.29	2.21E-01
2	*GgaAffx.5856.1.S1_s_at*	*SLC18A2*	*Solute carrier family 18 (vesicular monoamine), member 2*	*SVMT, VAT2, SVAT, MAT*	−1.40	−1.10	1.69E-02
2	*Gga.12077.1.S1_at*	*SLC18A3*	*Solute carrier family 18 (vesicular acetylcholine), member 3*	*VACHT*	−4.87	−1.79	4.02E-06
2	*GgaAffx.3745.1.S1_s_at*	*SLC22A13*	*Solute carrier family 22 (organic cation transporter), member 13*	*ORCTL3*	−1.78	−9.25	4.65E-05
2	*GgaAffx.11224.2.S1_s_at*	*SLC25A32*	*Solute carrier family 25, member 32*		1.32	−3.13	1.25E-04
2	*GgaAffx.6403.1.S1_at*	*SLC29A1*	*Solute carrier family 29 (nucleoside transporters), member 1*	*ENT1*	−1.38	−2.31	9.56E-05
2	*GgaAffx.24539.1.S1_at*	*SLC35F1*	*Solute carrier family 35, member F1*		−1.28	−1.23	4.38E-02

Table 2 Continued.[1]

Cluster[2]	Affymetrix ID[3]	Gene nomenclature[4]	Gene description[5]	Aliases[6]	Fold change DOH/E18[7]	Fold change D14/DOH[8]	*P*-value[9]
2	*GgaAffx.23019.1.S1_at*	*SLC38A1*	*Solute carrier family 38, member 1*	*SNAT1, ATA1, SAT1*	1.09	−2.01	2.20E-03
2	*Gga.4111.1.S1_at*	*SLC38A2*	*Solute carrier family 38, member 2*	*SNAT2, SAT2, ATA2*	−1.78	−1.70	1.84E-03
3	*Gga.10334.1.S1_s_at*	*SLC1A1*	*Solute carrier family 1 (neuronal/epithelial high affinity glutamate transporter, system Xag), member 1*	*EAAC1, EAAT3*	1.30	2.79	5.53E-07
3	*Gga.10763.1.S1_at*	*SLC6A4*	*Solute carrier family 6 (neurotransmitter transporter, serotonin), member 4*	*5-HTT*	2.67	1.20	4.01E-02
3	*GgaAffx.1584.2.S1_s_at*	*SLC9A9*	*Solute carrier family 9 (sodium/hydrogen exchanger), member 9*	*FLJ35613*	−2.27	2.14	8.00E-03
3	*Gga.12922.1.S1_s_at*	*SLC12A7*	*Solute carrier family 12 (potassium/chloride transporters), member 7*	*KCC4*	−1.39	2.45	1.01E-03
3	*Gga.14178.1.S1_at*	*SLC22A5*	*Solute carrier family 22 (organic cation transporter), member 5*	*OCTN2*	1.58	2.27	5.41E-05
3	*GgaAffx.23736.1.S1_s_at*	*SLC25A17*	*Solute carrier family 25 (mitochondrial carrier; peroxisomal membrane protein, 34kDa), member 17*	*PMP34, ANT1*	1.10	1.14	5.32E-04
3	*Gga.17228.2.S1_s_at*	*SLC25A24*	*Solute carrier family 25 (mitochondrial carrier; phosphate carrier), member 24*	*APC1, SCaMC-1*	1.23	1.66	2.24E-05
3	*Gga.5619.2.S1_a_at*	*SLC30A4*	*Solute carrier family 30 (zinc transporter), member 4*	*ZNT4, Dri27*	1.67	1.62	4.02E-03
3	*Gga.17649.1.S1_s_at*	*SLC30A6*	*Solute carrier family 30 (zinc transporter), member 6*	*ZNT6*	−1.45	1.13	2.36E-03
3	*GgaAffx.9037.1.S1_at*	*SLC30A9*	*Solute carrier family 30 (zinc transporter), member 9*	*ZNT9*	1.33	1.29	8.27E-05
3	*GgaAffx.9673.1.S1_at*	*SLC35A5*	*Solute carrier family 35, member A5*	*UGTre15*	1.25	1.25	2.20E-02
3	*Gga.12099.1.S1_s_at*	*SLC35F5*	*Solute carrier family 35, member F5*		1.27	2.90	3.79E-06
3	*GgaAffx.24879.4.S1_s_at*	*SLC37A1*	*Solute carrier family 37 (glycerol-3-phosphate transporter), member 1*	*G3PP, SPX1*	1.51	1.69	2.23E-02
3	*GgaAffx.6107.1.S1_s_at*	*SLC38A4*	*Solute carrier family 38, member 4*	*SNAT3*	−1.27	1.43	6.06E-02
3	*GgaAffx.8046.1.S1_s_at*	*SLC41A2*	*Solute carrier family 41, member 2*		1.47	4.10	1.73E-07
4	*GgaAffx.5539.1.S1_s_at*	*SLC1A4*	*Solute carrier family 1 (glutamate/neutral amino acid transporter), member 4*	*SATT, ASCT1*	1.14	−1.44	3.56E-03
4	*Gga.14445.1.S1_s_at*	*SLC1A6*	*Solute carrier family 1 (high affinity aspartate/glutamate transporter), member 6*	*EAAT4*	1.03	1.13	2.65E-01
4	*Gga.1040.1.S1_at*	*SLC2A1*	*Solute carrier family 2 (facilitated glucose transporter), member 1*	*GLUT1*	−1.99	−1.35	6.27E-05
4	*Gga.3914.1.S1_at*	*SLC2A8*	*Solute carrier family 2, (facilitated glucose transporter) member 8*	*GLUTX1, GLUT8*	−1.11	−1.45	2.48E-03
4	*GgaAffx.24559.1.S1_s_at*	*SLC2A9*	*Solute carrier family 2 (facilitated glucose transporter), member 9*	*GLUT9*	3.15	−2.68	6.60E-03
4	*Gga.11730.1.S1_at*	*SLC2A10*	*Solute carrier family 2 (facilitated glucose transporter), member 10*	*GLUT10*	−1.31	1.02	2.25E-01
4	*GgaAffx.3775.5.S1_at*	*SLC2A11*	*Solute carrier family 2 (facilitated glucose transporter), member 11*	*GLUT11, GLUT10*	1.02	−1.00	1.97E-02

Table 2 Continued.[1]

Cluster[2]	Affymetrix ID[3]	Gene nomenclature[4]	Gene description[5]	Aliases[6]	Fold change DOH/E18[7]	Fold change D14/DOH[8]	*P*-value[9]
4	*Gga.7988.1.S1_at*	*SLC2A12*	*Solute carrier family 2 (facilitated glucose transporter), member 12*	*GLUT12*	−1.23	−1.03	2.33E-01
4	*Gga.15925.1.S1_at*	*SLC4A1*	*Solute carrier family 4, anion exchanger, member 1 (erythrocyte membrane protein band 3, Diego blood group)*	*AE1, Band3*	−1.29	−1.01	4.36E-01
4	*GgaAffx.20768.1.S1_at*	*SLC4A1AP*	*Solute carrier family 4 (anion exchanger), member 1, adaptor protein*	*Kanadaptin*	1.45	1.05	5.04E-01
4	*GgaAffx.7375.3.S1_at*	*SLC4A4*	*Solute carrier family 4, sodium bicarbonate cotransporter, member 4*	*NBC1, NBC1, NBC2, hhNMC*	1.13	1.19	2.80E-03
4	*GgaAffx.728.1.S1_s_at*	*SLC4A9*	*Solute carrier family 4, sodium bicarbonate cotransporter, member 9*	*AE4*	−1.00	−1.24	6.25E-03
4	*GgaAffx.10205.1.S1_at*	*SLC4A11*	*Solute carrier family 4, sodium bicarbonate transporter-like, member 11*	*dJ794I6.2, BTR1,*	1.09	1.01	3.94E-01
4	*GgaAffx.11044.1.S1_s_at*	*SLC5A7*	*Solute carrier family 5 (choline transporter), member 7*	*CHT, CHT1*	−1.02	−1.03	4.83E-01
4	*GgaAffx.26357.1.S1_at*	*SLC6A1*	*Solute carrier family 6 (neurotransmitter transporter, GABA), member 1*	*GAT1*	1.27	−1.27	1.88E-02
4	*GgaAffx.2436.4.S1_at*	*SLC6A5*	*Solute carrier family 6 (neurotransmitter transporter, glycine), member 5*	*GLYT2, NET1*	1.02	−1.00	2.62E-03
4	*GgaAffx.26559.1.S1_at*	*SLC6A7*	*Solute carrier family 6 (neurotransmitter transporter, L-proline), member 7*	*PROT*	1.03	−1.01	3.72E-01
4	*GgaAffx.3027.1.S1_at*	*SLC6A11*	*Solute carrier family 6 (neurotransmitter transporter, GABA), member 11*	*GAT3*	1.14	1.29	1.84E-02
4	*GgaAffx.8228.8.S1_at*	*SLC6A12*	*Solute carrier family 6 (neurotransmitter transporter, betaine/GABA), member 12*	*BGT-1*	1.01	−1.01	2.97E-01
4	*GgaAffx.8228.5.S1_at*	*SLC6A13*	*Solute carrier family 6 (neurotransmitter transporter, GABA), member 13*	*GAT2*	1.03	1.01	3.51E-02
4	*GgaAffx.23416.2.S1_s_at*	*SLC6A15*	*Solute carrier family 6, member 15*	*B^0AT2, NTT7-3*	−1.01	−1.07	5.67E-01
4	*GgaAffx.26586.7.S1_s_at*	*SLC7A3*	*Solute carrier family 7 (cationic amino acid transporter, y^+ system), member 3*	*CAT-3, ATRC3*	−1.06	−1.14	1.37E-01
4	*Gga.15355.1.S1_at*	*SLC7A4*	*Solute carrier family 7 (cationic amino acid transporter, y^+ system), member 4*	*CAT-4*	1.05	−1.10	1.65E-01
4	*GgaAffx.24805.1.S1_at*	*SLC7A7*	*Solute carrier family 7 (cationic amino acid transporter, y^+ system), member 7*	*y^+LAT-1*	−1.02	−1.00	4.70E-01
4	*GgaAffx.10065.1.S1_at*	*SLC7A13*	*Solute carrier family 7, (cationic amino acid transporter, y^+ system) member 13*	*AGT-1, XAT2*	1.01	−1.00	4.09E-02
4	*GgaAffx.5899.2.S1_s_at*	*SLC7A14*	*Solute carrier family 7 (cationic amino acid transporter, y^+ system), member 14*	*KIAA1613*	−1.01	1.00	1.06E-01
4	*GgaAffx.22620.1.S1_at*	*SLC8A1*	*Solute carrier family 8 (sodium/calcium exchanger), member 1*	*NCX1, NACA, NCE*	−1.08	−1.01	2.75E-01

Table 2 Continued.[1]

Cluster[2]	Affymetrix ID[3]	Gene nomenclature[4]	Gene description[5]	Aliases[6]	Fold change DOH/E18[7]	Fold change D14/DOH[8]	*P*-value[9]
4	*GgaAffx.22912.1.S1_s_at*	*SLC8A3*	*Solute carrier family 8 (sodium-calcium exchanger), member 3*	*NCX3*	1.02	−1.02	4.48E-01
4	*GgaAffx.606.1.S1_at*	*SLC9A1*	*Solute carrier family 9 (sodium/hydrogen exchanger), member 1 (antiporter, Na+/H+, amiloride sensitive)*	*APNH, NHE1*	−1.16	−1.02	4.82E-01
4	*Gga.9162.1.S1_at*	*SLC9A3R2*	*Solute carrier family 9 (sodium/hydrogen exchanger), member 3 regulator 2*	*SIP-1, TKA-1, NHERF-2, E3KARP*	−2.25	−1.04	4.13E-05
4	*GgaAffx.10747.1.S1_at*	*SLC9A4*	*Solute carrier family 9 (sodium/hydrogen exchanger), member 4*	*NHE4*	1.01	1.01	4.42E-02
4	*Gga.7330.2.S1_a_at*	*SLC9A7*	*Solute carrier family 9 (sodium/hydrogen exchanger), member 7*	*NHE7*	−1.56	1.25	4.20E-02
4	*GgaAffx.12662.1.S1_s_at*	*SLC9A8*	*Solute carrier family 9 (sodium/hydrogen exchanger), member 8*	*KIAA0939, NHE8*	−1.16	−1.02	2.26E-04
4	*Gga.6773.1.S1_at*	*SLC10A4*	*Solute carrier family 10 (sodium/bile acid cotransporter family), member 4*		−1.34	−1.19	7.81E-02
4	*GgaAffx.9310.1.S1_s_at*	*SLC12A2*	*Solute carrier family 12 (sodium/potassium/chloride transporters), member 2*	*NKCC1BSC2*	1.66	1.12	1.28E-01
4	*Gga.2860.1.S1_a_at*	*SLC12A3*	*Solute carrier family 12 (sodium/chloride transporters), member 3*	*TSC*	1.05	−1.04	1.35E-01
4	*GgaAffx.23760.1.S1_s_at*	*SLC12A8*	*Solute carrier family 12 (potassium/chloride transporters), member 8*		1.40	1.14	4.95E-02
4	*Gga.13732.1.S1_at*	*SLC13A4*	*Solute carrier family 13 (sodium/sulphate symporters), member 4*	*SUT-1*	1.02	−1.00	2.22E-01
4	*Gga.12341.1.S1_s_at*	*SLC13A5*	*Solute carrier family 13 (sodium-dependent citrate transporter), member 5*	*NACT*	−1.08	−1.02	6.51E-01
4	*Gga.7955.1.S1_at*	*SLC14A2*	*Solute carrier family 14 (urea transporter), member 2*	*UT1*	−1.02	−1.00	5.43E-01
4	*GgaAffx.1756.2.S1_s_at*	*SLC15A4*	*Solute carrier family 15, member 4*	*PHT1, PTR4*	−1.78	1.14	1.70E-04
4	*GgaAffx.13241.1.S1_at*	*SLC16A1*	*Solute carrier family 16, member 1 (monocarboxylic acid transporter 1)*	*MCT1*	−1.38	1.01	3.32E-04
4	*GgaAffx.24008.1.S1_at*	*SLC16A13*	*Solute carrier family 16, member 13 (monocarboxylic acid transporter 13)*	*MCT13*	1.00	−1.01	9.22E-01
4	*Gga.4821.2.S1_s_at*	*SLC19A1*	*Solute carrier family 19 (folate transporter), member 1*	*FOLT, RFC*	3.38	−2.99	8.03E-06
4	*GgaAffx.22050.6.S1_s_at*	*SLC22A4*	*Solute carrier family 22 (organic cation transporter), member 4*	*OCTN1*	1.02	−1.01	1.74E-02
4	*GgaAffx.9560.2.S1_s_at*	*SLC22A16*	*Solute carrier family 22 (organic cation transporter), member 16*	*FLIPT2, CT2, OCT6*	1.25	−1.41	1.10E-01
4	*GgaAffx.26351.2.S1_s_at*	*SLC24A5*	*Solute carrier family 24, member 5*		−1.26	−1.02	5.80E-01
4	*GgaAffx.11697.1.S1_s_at*	*SLC25A3*	*Solute carrier family 25 (mitochondrial carrier; phosphate carrier), member 3*	*PTP*	1.80	−1.33	4.02E-04

Table 2 Continued.[1]

Cluster[2]	Affymetrix ID[3]	Gene nomenclature[4]	Gene description[5]	Aliases[6]	Fold change DOH/E18[7]	Fold change D14/DOH[8]	*P*-value[9]
4	*GgaAffx.11491.1.S1_s_at*	*SLC25A4*	*Solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 4*	*PEO3, PEO2, ANT1, T1*	1.39	−2.24	1.54E-04
4	*GgaAffx.11870.1.S1_s_at*	*SLC25A6*	*Solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 6*	*ANT3, T3*	1.37	−1.10	7.91E-04
4	*Gga.4188.1.S1_s_at*	*SLC25A13*	*Solute carrier family 25, member 13 (citrin)*	*Citrin*	2.30	−1.07	3.72E-06
4	*Gga.5240.1.S1_s_at*	*SLC25A14*	*Solute carrier family 25 (mitochondrial carrier, brain), member 14*	*BMCP1, UCP5*	1.00	−1.34	1.21E-03
4	*Gga.1685.1.S1_at*	*SLC25A20*	*Solute carrier family 25 (carnitine/acylcarnitine translocase), member 20*	*CACT*	1.48	−1.49	3.32E-02
4	*Gga.11135.2.S1_a_at*	*SLC25A26*	*Solute carrier family 25, member 26*		1.57	−1.17	3.58E-02
4	*GgaAffx.21599.1.S1_at*	*SLC25A28*	*Solute carrier family 25, member 28*	*MRS3/4, MRS4L*	−1.25	1.05	1.75E-01
4	*GgaAffx.12140.1.S1_s_at*	*SLC25A36*	*Solute carrier family 25, member 36*	*FLJ10618*	−1.46	−1.09	1.87E-03
4	*GgaAffx.558.1.S1_s_at*	*SLC26A8*	*Solute carrier family 26, member 8*	*TaT1*	1.04	1.01	2.81E-01
4	*GgaAffx.11883.1.S1_at*	*SLC30A5*	*Solute carrier family 30 (zinc transporter), member 5*	*ZTL1, ZNT5*	1.31	−1.21	2.93E-03
4	*Gga.2148.4.S1_a_at*	*SLC31A1*	*Solute carrier family 31 (copper transporters), member 1*	*CTR1*	2.93	−1.17	3.08E-04
4	*Gga.8731.1.S1_at*	*SLC34A1*	*Solute carrier family 34 (sodium phosphate), member 1*	*NAPI-3*	−1.02	1.02	5.59E-01
4	*Gga.4430.1.S1_a_at*	*SLC35A1*	*Solute carrier family 35 (CMP-sialic acid transporter), member A1*	*CST*	1.27	1.34	5.49E-05
4	*Gga.1484.1.S1_at*	*SLC35B1*	*Solute carrier family 35, member B1*	*UGTREL1*	1.05	−1.01	9.35E-02
4	*Gga.12890.1.S1_s_at*	*SLC35B3*	*Solute carrier family 35, member B3*	*CGI-19, UGTREL6*	−1.04	−1.15	2.71E-01
4	*GgaAffx.22590.3.S1_s_at*	*SLC35B4*	*Solute carrier family 35, member B4*	*YEA4*	1.01	−1.02	1.85E-01
4	*Gga.7831.1.S1_at*	*SLC35C2*	*Solute carrier family 35, member C2*	*OVCOV1*	−1.12	1.62	6.61E-04
4	*Gga.16176.1.S1_at*	*SLC35E2*	*Solute carrier family 35, member E2*	*KIAA0447*	1.02	−1.09	6.87E-01
4	*Gga.5475.1.S1_at*	*SLC35E3*	*Solute carrier family 35, member E3*	*BLOV1*	1.26	−1.00	8.62E-02
4	*GgaAffx.7672.1.S1_at*	*SLC35F3*	*Solute carrier family 35, member F3*		1.04	−1.25	3.09E-03
4	*GgaAffx.2712.2.S1_s_at*	*SLC36A1*	*Solute carrier family 36 (proton/amino acid symporter), member 1*	*LYAAT-1, PAT1*	1.09	1.01	2.20E-01
4	*Gga.11234.1.S1_at*	*SLC36A4*	*Solute carrier family 36 (proton/amino acid symporter), member 4*	*PAT4*	1.04	−1.01	3.61E-03
4	*Gga.8894.1.S1_at*	*SLC37A3*	*Solute carrier family 37 (glycerol-3-phosphate transporter), member 3*	*SPX3*	1.83	−1.06	1.96E-04
4	*GgaAffx.36.1.S1_at*	*SLC38A3*	*Solute carrier family 38, member 3*	*SNAT4, ATA3, SAT3, PAAT, NAT3*	1.01	−1.01	7.00E-01
4	*GgaAffx.24145.1.S1_at*	*SLC39A6*	*Solute carrier family 39 (zinc transporter), member 6*	*LIV-1*	1.02	−1.01	1.47E-04
4	*GgaAffx.7796.1.S1_at*	*SLC39A8*	*Solute carrier family 39 (zinc transporter), member 8*	*BIGM103*	1.14	−1.10	1.22E-02
4	*GgaAffx.12788.1.S1_s_at*	*SLC39A9*	*Solute carrier family 39 (zinc transporter), member 9*		1.56	1.14	7.15E-06
4	*GgaAffx.22358.1.S1_s_at*	*SLC39A10*	*Solute carrier family 39 (zinc transporter), member 10*		−1.06	1.02	7.23E-02
4	*GgaAffx.26205.1.S1_s_at*	*SLC39A11*	*Solute carrier family 39 (metal ion transporter), member 11*		1.71	−1.29	6.02E-02

Table 2 Continued.[1]

Cluster[2]	Affymetrix ID[3]	Gene nomenclature[4]	Gene description[5]	Aliases[6]	Fold change DOH/E18[7]	Fold change D14/DOH[8]	*P*-value[9]
4	*GgaAffx.13067.1.S1_s_at*	*SLC39A13*	*Solute carrier family 39 (zinc transporter), member 13*		−1.25	1.11	5.67E-01
4	*GgaAffx.7990.1.S1_at*	*SLC39A14*	*Solute carrier family 39 (zinc transporter), member 14*	*ZIP14*	1.00	1.00	1.61E-01
4	*GgaAffx.7232.2.S1_s_at*	*SLC44A5*	*Solute carrier family 44, member 5*	*MGC34032, CTL5*	−1.07	−1.04	6.79E-01
4	*GgaAffx.8368.1.S1_s_at*	*SLCO1C1*	*Solute carrier organic anion transporter family, member 1C1*	*OATP1C1*	1.03	−1.01	5.23E-03
4	*GgaAffx.4294.1.S1_at*	*SLCO3A1*	*Solute carrier organic anion transporter family, member 3A1*	*OATP3A1*	−1.02	1.01	5.83E-01
4	*GgaAffx.11438.1.S1_s_at*	*SLCO4A1*	*Solute carrier organic anion transporter family, member 4A1*	*OATP4A1*	2.03	−1.00	2.27E-02
4	*GgaAffx.24718.2.S1_at*	*SLCO5A1*	*Solute carrier organic anion transporter family, member 5A1*	*OATP5A1*	1.14	−1.01	4.72E-03
5	*Gga.8236.1.S1_at*	*SLC2A2*	*Solute carrier family 2 (facilitated glucose, galactose and fructose transporter), member 2*	*GLUT2*	39.81	2.61	5.60E-06
5	*GgaAffx.25502.1.S1_s_at*	*SLC2A5*	*Solute carrier family 2 (facilitated fructose transporter), member 5*	*GLUT5*	2.74	16.56	8.87E-08
5	*GgaAffx.198.1.S1_at*	*SLC9A2*	*Solute carrier family 9 (sodium/hydrogen exchanger), member 2*	*NHE2*	6.39	3.05	7.32E-08
5	*GgaAffx.10788.1.S1_at*	*SLC10A2*	*Solute carrier family 10 (sodium/bile acid cotransporter family), member 2*	*ASBT, ISBT*	17.69	4.38	5.41E-07
5	*GgaAffx.2278.1.S1_at*	*SLC13A2*	*Solute carrier family 13 (sodium-dependent dicarboxylate transporter), member 2*	*NaDC-1, SDCT1*	19.09	−1.16	2.17E-04
5	*Gga.18316.1.S1_at*	*SLC16A10*	*Solute carrier family 16, member 10 (aromatic amino acid transporter)*	*TAT1*	2.95	2.58	2.02E-05
5	*GgaAffx.7341.1.S1_s_at*	*SLC22A3*	*Solute carrier family 22 (extraneuronal monoamine transporter), member 3*	*OCT3, EMT*	2.76	2.20	6.45E-05
5	*GgaAffx.473.1.S1_at*	*SLC26A9*	*Solute carrier family 26, member 9*		6.15	1.02	4.70E-07
5	*Gga.8627.1.S1_at*	*SLC28A1*	*Solute carrier family 28 (sodium-coupled nucleoside transporter), member 1*	*CNT1*	63.56	1.34	2.15E-08
5	*GgaAffx.6040.2.S1_s_at*	*SLC30A10*	*Solute carrier family 30, member 10*	*ZNT-10*	7.34	1.02	3.77E-04
5	*GgaAffx.12463.1.S1_s_at*	*SLC35A3*	*Solute carrier family 35 (UDP-N-acetylglucosamine (UDP-GlcNAc) transporter), member A3*	*UGTre12*	7.57	2.57	2.17E-05
6	*GgaAffx.2936.1.S1_at*	*SLC7A10*	*Solute carrier family 7, (neutral amino acid transporter, y^+ system) member 10*	*asc-1*	2.84	−2.81	5.70E-05
6	*GgaAffx.25903.3.S1_s_at*	*SLC16A4*	*Solute carrier family 16, member 4 (monocarboxylic acid transporter 5)*	*MCT5*	3.43	−3.33	9.69E-02
6	*Gga.6731.2.S1_a_at*	*SLC23A2*	*Solute carrier family 23 (nucleobase transporters), member 2*	*SVCT2, YSPL2, NBTL2, NCBT1*	8.17	−6.48	2.01E-05

Table 2 Continued.[1]

Cluster[2]	Affymetrix ID[3]	Gene nomenclature[4]	Gene description[5]	Aliases[6]	Fold change DOH/E18[7]	Fold change D14/DOH[8]	*P*-value[9]
6	*Gga.6388.1.S1_s_at*	*SLC25A15*	*Solute carrier family 25 (mitochondrial carrier, ornithine transporter) member 15*	*ORNT1*	3.61	−6.39	4.91E-04
6	*GgaAffx.25186.1.S1_at*	*SLC35F2*	*Solute carrier family 35, member F2*		4.87	−3.31	2.57E-04

[1]This table was sorted by cluster first, then by gene nomenclature.
[2]The K-MEANS CLUSTERING algorithm was performed on all expressed chicken SLC genes based on average linkage using the standard correlation in GENESPRING. Members of the chicken SLC gene family were separated into one of six clusters based on similar expression patterns.
[3]Probeset ID for chicken SLC genes expressed on the Affymetrix gene chip used for the experiment.
[4]SLC identification of the chicken gene based on the human SLC gene nomenclature system that was originally proposed by the Human Genome Organization (HUGO) as a standard way of classifying gene members.
[5]Description of SLC identification. In most cases, substrate information is also included.
[6]Common gene aliases reported in the Affymetrix gene annotation.
[7]Fold change in gene expression based on the ratio of average expression at day of hatch (DOH) to embryonic day 18 (E18). Positive values indicate that gene expression was upregulated while negative values indicate that it was downregulated.
[8]Fold change in gene expression based on the ratio of average expression at day 14 post-hatch (D14) to DOH. Positive values indicate that gene expression was upregulated while negative values indicate that it was downregulated.
[9]One-way ANOVA, main effect of developmental age.

value for DOH/E18 or D14/DOH indicates an increase in expression from E18 to DOH or DOH to D14 respectively whereas a negative value indicates a decrease in expression for these same periods.

Expression profiles of SLC genes that were upregulated

We have highlighted the expression profiles of amino acid, peptide and sugar transporters. Amino acids can be transported across the intestinal epithelia as free amino acids or short peptides. The amino acid transporters *SLC3A1* (*rBAT, heavy chain of dibasic and neutral amino acid transporter*), *SLC6A19* (B^0AT1, *neutral amino acid transporter*), *SLC7A6* ($y^+LAT\text{-}2$, *cationic amino acid transporter, y^+ system*), *SLC7A9* ($b^{0,+}AT$, *cationic amino acid transporter, y^+ system*) and *SLC1A1* (*EAAT3*, *aspartate/glutamate transporter*) were upregulated 7.9-, 36.8-, 6.4-, 6.0- and 3.6-fold respectively from E18 to D14. In the intestine, *EAAT3* is a key transporter because glutamate serves as the primary fuel source for the enterocyte (Wu 1998). The proton-dependent, di- and tri-peptide transporter *SLC15A1* (*PepT1*) was upregulated 17.2-fold from E18 to D14.

The monosaccharide transporters consist of the sodium glucose cotransporter SGLT family, SLC5, and the facilitative GLUT transporter family, SLC2 (Wood & Trayhurn 2003; Wright & Turk 2004). Expression of the sodium glucose cotransporter *SLC5A1* (*SGLT1*), which is the main intestinal glucose transporter post-hatch, was upregulated 28.8-fold from E18 to D14. In contrast, *SLC5A11* (*SGLT6*), which transports glucose and myo-inositol, showed a different pattern of expression that was upregulated during embryogenesis and downregulated post-hatch. The facilitated glucose, galactose and fructose transporter *SLC2A2* (*GLUT2*) and the facilitated fructose transporter *SLC2A5* (*GLUT5*) were upregulated 104- and 45.4-fold respectively from E18 to D14.

Other transcripts that were upregulated greater than fivefold from E18 to D14 include the Na^+/H^+ exchanger *SLC9A2* (*NHE2*), the sodium/bile acid cotransporter *SLC10A2* (*ASBT*), the sodium-dependent dicarboxylate transporter *SLC13A2* (*NaDC-1*), the sodium-coupled nucleoside transporter *SLC28A1* (*CNT-1*), the zinc transporter *SLC30A10* (*ZnT-10*) and the UDP-*N*-acetylglucosamine transporter *SLC35A3*. These transcripts were upregulated 19.5-, 77.5, 16.3-, 85.2-, 7.5- and 19.5-fold respectively from E18 to D14.

Expression profiles of SLC genes that were downregulated

The downregulated transcripts in set 2 included the cationic amino acid transporters of the y^+ system *SLC7A1* (*CAT-1*), *SLC7A2* (*CAT-2*) and *SLC7A5* (*LAT1*). These transcripts decreased 1.6-, 14- and 1.5-fold respectively between E18 and D14. The sodium-coupled neutral amino acid

transporters *SLC38A1* (*SNAT1*) and *SLC38A2* (*SNAT2*) were also downregulated 1.8- and 3.0-fold from E18 to D14.

Expression profiles of SLC genes that showed complex regulation

Some transcripts showed complex patterns of embryonic upregulation and post-hatch downregulation. These transcripts included the monocarboxylic acid transporter *SLC16A5* (*MCT6*), which was upregulated 16.1-fold from E18 to DOH but was downregulated 3.7-fold post-hatch. Similarly, all five transcripts in set 6, *SLC7A10* (*asc-1, neutral amino acid transporter, y^+ system*), *SLC16A4* (*MCT5, monocarboxylic acid transporter*), *SLC23A2* (*SVCT2, ascorbic acid transporter*), *SLC25A15* (*ORNT1, ornithine transporter*) and *SLC35F2*, showed a similar pattern of upregulation during late embryogenesis and downregulation post-hatch.

Validation of the microarray results by absolute quantification real-time PCR

Although Affymetrix microarrays show high precision and repeatability (Woo *et al.* 2004), array results can be influenced by variations in the manufacturing process, sample preparation and data analysis. To validate the microarray results, we measured mRNA expression of seven SLC transporter genes that were up- or downregulated using real-time PCR and the absolute quantification method. Figure 2 shows a comparison of the results of real-time PCR with the microarray for the sodium glucose cotransporters (*SGLT1* and *SGLT6*), the peptide transporter (*PepT1*), the Na^+/H^+ exchanger (*NHE2*), amino acid transporters (*SNAT1* and *SNAT2*) and the anionic sugar transporter (*AST*). The real-time PCR results show expression in individual intestinal segments compared with pooled intestine analysed by microarray. The mean expression of the three segments showed a similar profile to that of the total intestine determined by microarray. A regression analysis of the microarray data and the average of the three segments was performed; the R^2-value for each gene is shown in Fig. 2. As expected, the real-time PCR results on the individual segments confirmed the microarray results and were able to reveal more information about the spatial distribution of the mRNA. For example, expression of *SGLT6* was greatest in the ileum, intermediate in the jejunum and least in the duodenum, which was not revealed using the entire small intestine sample and microarrays. One of the advantages of the absolute quantification method over the relative quantification method is the ability to determine the number of RNA molecules. The real-time PCR results revealed that *SGLT1*, *SGLT6* and *NHE2* were expressed at a level of tens of thousands of mRNA molecules per nanogram total RNA, while *PepT1* and *SNAT2* were expressed at a level of thousands of molecules per nanogram total RNA. The amino acid transporters *SNAT1* and *AST* were low-abundance transcripts and were expressed at a level of hundreds of mRNA molecules per nanogram total RNA.

Discussion

Chickens are a useful model for studying ontogenetic changes in expression of nutrient transporters because they consume a defined diet within the egg during incubation and can be raised on external feed on the DOH independent of maternal nutrients. After hatch, a chick must shift from a lipid-rich, yolk-based diet to a carbohydrate- and protein-rich feed-based diet. During this time, the intestine must undergo a number of anatomical and physiological changes in response to the consumption of an adult diet and bacterial colonization of the gut (Uni *et al.* 1998). As a result, the expression profiles of the nutrient transporters are likely to change as the availability of specific nutrients changes.

In humans, 351 SLC genes have been identified and grouped into 45 families (Hediger *et al.* 2004, http://www.bioparadigms.org/slc/menu.asp). Of these 351 genes, 229 have been identified in the chicken genome and are present on the Affymetrix array. We found that 162 of these 229 SLC genes (71%), belonging to 41 families, were expressed in the chick intestine at one of the time points examined (E18–D14). This is comparable to the results of Anderle *et al.* (2005), who reported that 76% of the then-known transporters were expressed in the intestine of 8-week-old mice along the anterior–posterior and crypt–villus axes using DNA microarrays. In addition, Anderle *et al.* (2005) reported that a number of the transporters were differentially expressed in the different segments of the small intestine or the colon. In our microarray study, we examined expression in total intestinal samples, so we were unable to detect segmental differences. However, for genes that we verified using real-time PCR and in a parallel study (Gilbert *et al.* 2007), we similarly observed that the expression of some transporters varied among the small intestinal segments. Anderle *et al.* (2005) further compared the mRNA expression of selected transporters using total intestinal samples to that using epithelial cells collected by laser capture microdissection. They concluded that for the examined genes, the expression profiles measured in whole intestine tissue extracts were representative of epithelial cell-only expression. Thus, it is not unreasonable to assume that the same should hold true for expression of the examined SLC genes in our total RNA samples from intestinal extracts.

In the case of both mammals and avian species, high prenatal expression of intestinal brushborder membrane-bound nutrient transporters is likely not required, as the fetus obtains nutrients from the placenta and the chick embryo obtains nutrients from the yolk-sac membrane. Expression of intestinal transporters during gestation and during the last few days of incubation, however, allows the

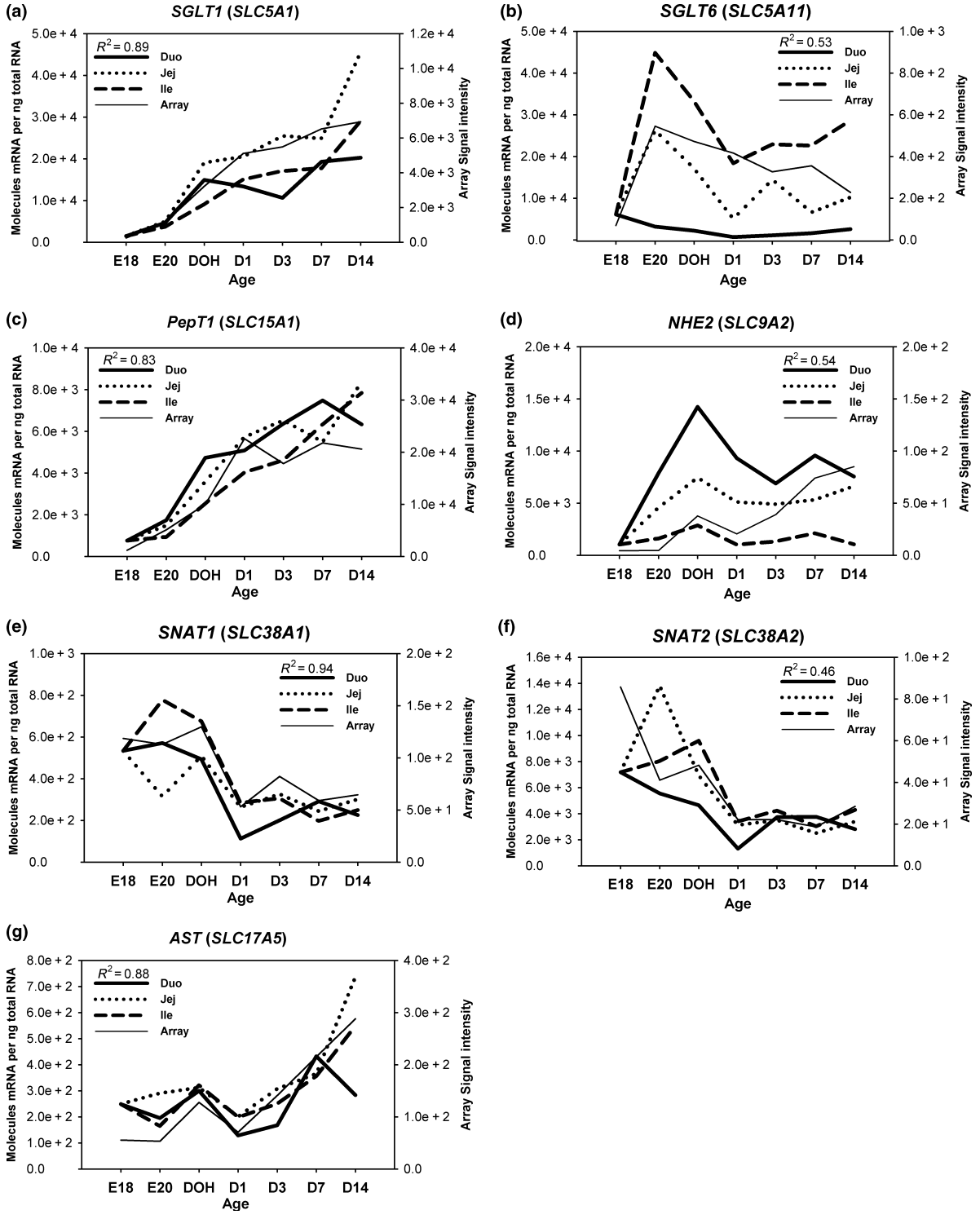

Figure 2 Comparison of microarray and real-time PCR analyses of mRNA abundance of selected up- and downregulated genes. Expression of the monosaccharide transporters *SGLT1* (a) and *SGLT6* (b), the peptide transporter *PepT1* (c), the Na^+/H^+ exchanger *NHE2* (d), the amino acid transporters *SNAT1* (e) and *SNAT2* (f) and the anionic sugar transporter *AST* (g) are shown. The left *y*-axis shows the expressed mRNA copies per nanogram total RNA for the three intestinal segments and the right *y*-axis shows the normalized signal intensity for the microarray hybridization. The *x*-axis represents the 7 days (embryo day 18, E18; embryo day 20, E20; day of hatch, DOH; and days 1, 3, 7 and 14 post-hatch, D1, D3, D7 and D14 respectively) on which intestinal samples were collected. At E18, total intestine was collected and plotted values do not reflect the expression of individual segments at that stage in development. The R^2-value for each gene indicates the relationship between the expression data obtained from microarrays and the average expression data of the three intestinal segments obtained from real-time PCR.

fetus and chick embryo respectively to extract nutrients from swallowed amniotic fluid (Uni & Ferket 2004). Continuous upregulation of brushborder membrane transporters post-hatch may correlate with the maturation of the intestinal mucosa. At hatch, enterocytes are immature and non-polar, lacking a defined brushborder membrane (Geyra *et al.* 2001a). Within the first 24 h, enterocytes become polar and the apical membrane becomes distinct. During the first week, the villi elongate, increase in surface area and become populated by increasing numbers of enterocytes. This increase in absorptive surface area and proportion of mature enterocytes appears to be accompanied by a corresponding enhanced expression of nutrient transporters to maximize absorption of nutrients.

We found that a number of amino acid, peptide and monosaccharide transporters were differentially expressed. We validated seven of these transporters using real-time PCR and the absolute quantification method. Similar expression profiles derived from the DNA microarray and from real-time PCR suggest that the expression profiles for the other genes on the DNA microarrays are likely accurate representations of the overall pattern of expression, i.e. upregulated or downregulated. Similar to what we had observed previously (Gilbert *et al.* 2007), expression of the brushborder membrane transporters such as the sugar transporters *SGLT1* (*SLC5A1*) and *GLUT5* (*SLC2A5*), the peptide transporter *PepT1* (*SLC15A1*) and the amino acid transporters *EAAT3* (*SLC1A1*) and *rBAT* (*SLC3A1*) increased from E18 to D14, demonstrating their importance post-hatch when mucosal surface area increases and the birds adjust to a carbohydrate- and protein-based diet.

In contrast, the basolateral membrane transporters such as the cationic amino acid transporters *CAT-1* (*SLC7A1*) and *CAT-2* (*SLC7A2*), and the branched chain and aromatic amino acid transporter *LAT1* (*SLC7A5*) decreased from E18 to D14, indicating that they play an important role during late embryogenesis. These results are not unexpected because during embryological development the chick relies on the yolk for nourishment via the yolk-sac membrane (Speake *et al.* 1998) and obtains nutrients for the intestinal cells through the basolateral surface from the bloodstream. It is not until several days before hatch when the yolk sac is internalized into the body cavity and amniotic fluid is swallowed, that transporter function at the brushborder membrane becomes important for luminal nutrient assimilation (Moran 2007). Not all basolateral amino acid transporters, however, were downregulated. The basolateral transporter y^+LAT2 (*SLC7A6*) was upregulated 6.5-fold from E18 to DOH and then remained unchanged from DOH to D14, indicating that it plays an important role in transport of essential cationic amino acids during late embryogenesis and the post-hatch period.

As the chick adjusts its metabolic machinery to process a carbohydrate- and protein-based diet at hatch, transporters associated with monosaccharide uptake will likely change dramatically. Coordinate regulation of the brushborder and basolateral membrane monosaccharide transporters is essential to maintain a controlled flow of monosaccharides from the lumen to the bloodstream. As we have shown previously (Gilbert *et al.* 2007) and in the current microarray study, expression of the primary intestinal glucose transporter *SGLT1*, the fructose transporter *GLUT5*, the basolateral glucose/fructose transporter *GLUT2* and the glucose transporter *SGLT5* were upregulated from E18 to D14. From the microarray data, we identified *SGLT6* as an additional highly expressed intestinal transporter. Real-time PCR verification of *SGLT1* and *SGLT6* revealed that the two transcripts were present at approximately the same level of 20 000 mRNA molecules per nanogram total RNA but showed different developmental and spatial expression patterns. Expression of *SGLT1* was low during embryogenesis, rose continuously to D14 and was expressed in all three segments of the small intestine with greater expression in the jejunum than the duodenum or ileum. In contrast, *SGLT6* expression was highest at E20, gradually declined with age and was expressed highest in the ileum and at very low levels in the duodenum. Wright & Turk (2004) suggested, in their review of the SLC5 family of sodium glucose cotransporters, that *SGLT6* is probably the intestinal low-affinity glucose transporter hinted at in previous studies, with ubiquitous expression in mammals. We had previously reported relatively high ileal expression and developmental changes of *SGLT5* in chickens (Gilbert *et al.* 2007), which in mammals is described as exclusively a renal glucose transporter (Wright & Turk 2004; Zhao *et al.* 2005). These findings suggest that in the chick, the mechanism of glucose assimilation may be more complex than previously thought.

The predominantly jejunal or ileal expression of the monosaccharide transporters *SGLT1*, *SGLT5* and *SGLT6* are consistent with the expression of disaccharidase activity. Disaccharidase activity was shown to be lowest in the proximal small intestine and highest in the mid- and distal segments in broiler chicks during the first 2 weeks post-hatch (Uni *et al.* 1998). Carbohydrate digestion begins in the proximal small intestine through the action of pancreatic amylase, and digestible end products from starch are not made available until further down the digestive tract, paralleling expression and activity of disaccharidases and monosaccharide transporters.

Other types of sugar transporters were also developmentally regulated. The transporter *AST* (*SLC17A5*), also known as sialin, is a lysosomal membrane transporter of anionic sugars such as sialic acid. Within the intestine, sialin expression was observed not only in the enteric nervous system neurons, but also in epithelial cells (Yarovaya *et al.* 2005). In the intestine, sialin may be particularly important where the mucus layer protecting the epithelial cells

primarily comprises mucins, a group of glycoproteins with sialic acid modifications, secreted by goblet cells (Uni *et al.* 2003a). With age post-hatch, the number of goblet cells increases proportionally to the number of enterocytes, and goblet cell density increases more rapidly in the jejunum and ileum compared with the duodenum (Uni *et al.* 2003a), similar to our observed expression pattern of *AST*.

Another example of coordinate regulation involved the peptide transporter *PepT1* and the Na^+/H^+ exchanger *NHE2*. Peptide transporter PepT1 transports di- and tripeptides in a proton-dependent mechanism across the brushborder membrane into intestinal cells (Chen *et al.* 2002; Daniel & Kottra 2004). The transporter NHE2 is an intestinal brushborder membrane protein that exchanges one Na^+ for one H^+, maintaining the transmembrane proton gradient (Zachos *et al.* 2005). Donowitz *et al.* (1998) demonstrated that *NHE2* is expressed on the brushborder membrane of ileal and colonic epithelial cells in chickens, contributing in both tissues to Na^+/H^+ exchange. In rats, *NHE2* is expressed in the greatest quantities in the stomach, small intestine and colon (Bookstein *et al.* 1997). In humans, *NHE2* is expressed in both the small intestine and colon, and within the small intestine is uniformly expressed between jejunum and ileum (Dudeja *et al.* 1996). We observed that *PepT1*, in contrast to the free amino acid transporters, is expressed at greater quantities in the duodenum and jejunum compared with the ileum. A similar decreasing gradient of expression from proximal to distal small intestine was seen for *NHE2*. Furthermore, *PepT1* and *NHE2* showed coordinate temporal expression, which was low during late embryogenesis, rose at DOH and continued rising post-hatch. In rats, *PepT1* and *NHE2* are expressed in the small intestine after birth but with different developmental patterns (Miyamoto *et al.* 1996; Collins *et al.* 1998; Shen *et al.* 2001; Rome *et al.* 2002).

We observed parallel expression of two closely related amino acid transporters, *SNAT1* and *SNAT2*. Both are System A-type transporters that mediate the Na^+-dependent uptake of small, zwitterionic amino acids (Mackenzie & Erickson 2004). Both transporters were expressed at the greatest levels prior to hatch, decreased from DOH to D1 and remain relatively constant thereafter. Although *SNAT2* is ubiquitously expressed throughout the body with low levels detected in human small intestine by northern blot, expression of *SNAT1* is detected predominantly in the nervous system and is not detectable in the small intestine (Wang *et al.* 2000). We observed approximately 10-fold greater quantities of *SNAT2* as compared with *SNAT1* in the small intestine. In the developing rat, expression of the SNAT1 protein increased dramatically from E14 to E21, after which expression decreased to negligible quantities after birth (Weiss *et al.* 2005). This may explain why *SNAT1* was undetected in human small intestine. Weiss *et al.* (2005) suggested that changes in amino acid metabolizing enzymes during fetal and neonatal development in response to changes in dietary substrate may account for the changes in expression of transporters.

Recent advances in avian nutritional technology, including development of an *in ovo* method for administering nutrients to the developing embryo (Foye *et al.* 2007), have demonstrated that changes in growth of the bird can be modulated prehatch. Our data demonstrate that many changes in expression of intestinal nutrient transporters are occurring during this time and may provide insight into formulating the ideal nutritional supplement to the developing embryo. Understanding the expression profile of nutrient transporters post-hatch could lead to improved feed efficiency during the important early post-hatch period, as a number of studies have demonstrated that early nutrition has a profound impact on the overall lifetime performance of the bird (Lilja 1983; Geyra *et al.* 2001b).

In summary, this study represents the expression profiling of 162 members of the SLC gene family in the small intestine of chicks from E18 to D14. We found that the majority of the SLC genes showed little or no difference in level of expression between E18 and D14. A number of nutrient transporters were upregulated between E18 and D14 including the amino acid transporters *rBAT*, *y^+LAT-2* and *EAAT3*, the peptide transporter *PepT1* and the sugar transporters *SGLT1*, *GLUT2* and *GLUT5*. In contrast, a number of the amino acid transporters (*CAT1*, *CAT2*, *SNAT1* and *SNAT2*) were downregulated. We identified multiple transporters not previously characterized or detected in the chicken intestine (*SNAT1*, *SNAT2*, *SGLT6* and *AST*). Determining the precise expression profiles of the complete array of nutrient transporter genes may lead to the development of better feed formulations that more closely match nutrient availability with nutrient uptake capacity.

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