ABSTRACT

Title of Document: IDENTIFICATION OF A SINGLE NUCLEOTIDE POLYMORPHISM ASSOCIATED WITH ADIPOSITY FOLLOWING TRANSCRIPTIONAL PROFILING OF GENE EXPRESSION IN THE ANTERIOR PITUITARY GLAND

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Although the anterior pituitary secretes three hormones that affect metabolism and body fat stores, a comprehensive analysis of pituitary gene expression associated with body fat has not been performed. This research used cDNA microarrays to investigate pituitary gene expression in two chicken lines that were selected for low and high body fat (Lean and Fat). RNA was extracted from pituitaries at 1, 3, 5, and 7 weeks of age. 386 genes that showed significant differences in expression levels by line or in the line-by-age interaction were analyzed further. Differentially expressed genes between lines are potential candidates as genetic markers for high and low potential for body fat accumulation. One such candidate, the lysophosphatidic acid (LPA) receptor-1 (LPAR1), was identified as a potential marker, being differentially expressed between the 2 lines at the early ages. Genomic DNA from the Fat and Lean F_0 generation was sequenced

upstream of the LPAR1 coding region. A SNP consisting of a T to C transversion that introduces a GATA-1 transcription factor binding site was identified in the Lean line (Fisher's Exact Test, $p \leq 0.001$). The fattest and leanest animals of both sexes in the back-crossed F_2 generation (n=48 each) were genotyped by allele-specific PCR, and an association was present between the genotype and phenotype (generalized linear model, $p \leq 0.05$). Expression of GATA transcription factors in mice inhibits differentiation of preadipocytes into mature adipocytes. LPAR1 also inhibits differentiation of preadipocytes in mice, and LPAR1 knock-out mice become significantly fatter than wild-type mice. A SNP that introduces a GATA site in the promoter of LPAR1 could up-regulate its expression in the Lean line, and increased LPA signaling could then inhibit preadipocyte differentiation. Conversely, loss of the GATA binding site could explain decreased levels of LPAR1 expression and attenuated inhibition of adipocyte maturation in the Fat line.

IDENTIFICATION OF A SINGLE NUCLEOTIDE POLYMORPHISM ASSOCIATED WITH ADIPOSITY FOLLOWING TRANSCRIPTIONAL PROFILING OF GENE EXPRESSION IN THE ANTERIOR PITUITARY GLAND

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Chapter 1: Introduction

The aim of this research was to identify genes expressed in neuroendocrine tissues that regulate body fat composition in domestic chickens using cDNA microarrays. Specifically, the research focused on the identification of differentially expressed genes in the anterior pituitary gland of fat and lean chicken lines and analysis of candidate genes to be used for marker-assisted selection of leaner chickens in the future.

Protein is a major class of nutrient and the primary source of amino acids. Chicken meat provides a high quality complete source of protein. Commercial broiler chickens have been selected for increased body mass (*i.e.* muscle) and rapid growth rate (Havenstein, Ferket et al. 2003). Unfortunately, this selection has also increased body fat deposition (Deeb and Lamont 2002), along with other undesirable traits like decreased reproductive performance, increased skeletal muscle abnormalities, ascites, and fatty liver and kidney syndrome (Griffin and Goddard 1994; Julian 2005). Excessive fat deposition is an undesirable from a production and consumer standpoint, but traditionally commercial selection against this highly heritable trait has not been practiced, due to the cost and labor involved in slaughtering and dissecting animals in breeding assays (Lagarrigue, Pitel et al. 2006). A means of genetically selecting for leanness would be of great commercial value.

In addition, it is likely that any genes involved in body composition phenotype in the chicken have human orthologs, and so may play a role in understanding obesity—a major health concern in humans, now and in the future. Obesity has been described as a rising epidemic in the developed world, especially in the United States, where the prevalence of overweight and obese individuals is increasing. Approximately 60 % of

the population in the United States is considered overweight or obese [body mass index (BMI, body weight in kilograms over the square of height in meters) greater than 25.0 and 30.0, respectively] (Hedley, Ogden et al. 2004). With this increase in the prevalence of obesity, there has been a concomitant increase in the incidence of other diseases, such as diabetes, hypertension, and cardiovascular disease (Muoio and Newgard 2006).

The fact that the chicken is a warm-blooded vertebrate species that can produce large numbers of offspring quickly, which allows generation of fairly large pedigrees of animals in a relatively short time, makes it a valuable genetic resource and experimental model. Most domestic farm animals take months to years to reach reproductive age and produce relatively small numbers of offspring. The chicken also offers the advantage, after egg deposition, of being free of maternal-fetal nutritional and hormonal interactions, which can affect body composition in mammals later in life (Oken and Gillman 2003).

Chapter 2: Literature Review

Overview of involvement of pituitary hormonse in regulating body composition

Body composition is determined by a complex interaction between environmental, hormonal, genetic, behavioral, and nutritional factors. The pituitary produces (at least) three hormones that exert major effects on growth, body composition, and metabolism: growth hormone (GH), pro-opiomelanacortin (POMC), and thyroid-stimulating hormone (TSH). These hormones are produced by somatotrophs, corticotrophs, and thyrotrophs, respectively. Genes involved in the expression and regulation of these hormones, especially during the developmental period during which adipocytes undergo differentiation, will be of particular interest with regard to understanding neuroendocrine regulation of adiposity.

Overview of regulation of pituitary hormone secretion

The endocrine system, along with the nervous system, regulates and integrates the functions of the various tissues and cells that make up multicellular organisms. The heart of this system is comprised of the hypothalamus-pituitary-target tissue axes. The classic model of the endocrine system involves the production of hypophysiotrophic factors by neurons in the hypothalamus whose axons project to the median eminence (ME), where the released factors enter the capillaries of the hypophysial-portal system and are transported to the anterior pituitary, integrating the central nervous system with the endocrine system. Hypothalamic factors generally either stimulate the biosynthesis and release of pituitary hormones, or inhibit their synthesis or release. The hormones travel

through the systemic circulation to reach their target organs, where they initiate the physiological effects, such as the secretion of the target organ's own hormones, which then can travel back through the circulation to the hypothalamus or pituitary to down-regulate the trophic factors and hormones in a negative feedback loop. The hypothalamus-pituitary-target axes and their feedback loops provide mechanisms by which an organism maintains homeostasis.

Hypothalamic factors

There are several peptides produced by the hypothalamus that exert trophic effects on the pituitary gland. The chicken GH-releasing hormone (GHRH) gene has been cloned (McRory, Parker et al. 1997). Three different transcripts were isolated, GHRH 1-46, GHRH 1-43, and GHRH 33-46. The full length chicken GHRH peptide is less than 50% identical to mouse and human GHRH. Interestingly, the GHRH gene also encodes for pituitary adenylate cyclase-activating polypeptide (PACAP) whereas GHRH and PACAP are encoded by different genes in mammals. It has been posited that, like mammals, the GHRH-producing neurons are located in the arcuate nucleus, since lesions in this area reduced GH secretion (Harvey, Fraser et al. 1991).

The preprothyrotropin-releasing hormone (TRH) cDNA has been recently cloned (Vandenborne, Roelens et al. 2005); TRH is a three amino acid peptide (pyro-Glu-His-Pro amine) produced in the paraventricular nucleus (PVN) and in the lateral hypothalamus (Geris, D'Hondt et al. 1999; Vandenborne, Roelens et al. 2005) as a 26 kDa prohormone and is processed into active TRH by the actions of prohormone convertase (PC) 1/3 and PC2 (Perello, Friedman et al. 2006).

Chicken ghrelin is a recently described 26-amino acid protein that acts as a GH-secretagoue *in vivo* (Kaiya, Van Der Geyten et al. 2002). In the hypothalamus, ghrelin immunoreactivity was not found in the arcuate nucleus as in mammals, but in the anterior medial hypothalamus (Ahmed and Harvey 2002).

Somatotropin-release inhibitory factor (SRIF) has been found in the hypothalamus of chickens (Geris, Meeussen et al. 2000). It is also found in other hypothalamic nuclei that do not project to the ME, and in the brainstem and other peripheral tissues (gonads, thyroid, and intestine).

Corticotropin-releasing hormone (CRH) has been cloned in the chicken (Vandenborne, De Groef et al. 2005). Like mammalian CRH, it is a 41 amino acid peptide found in the paraventricular nucleus (Jozsa, Vigh et al. 1984).

Growth hormone

GH is necessary for normal growth after hatching (King and Scanes 1986). GH is a 22 kDa glycoprotein hormone synthesized by somatotrophs in the caudal lobe of the chicken anterior pituitary. A cDNA for chicken GH has been sequenced (Lamb, Galehouse et al. 1988). GH expression and secretion are stimulated by GH-releasing hormone (GHRH) (Scanes and Harvey 1984), thyrotropin-releasing hormone (TRH) in embryos and young birds (Van As, Careghi et al. 2004), and ghrelin (Baudet and Harvey 2003), and inhibited by somatotropin-release inhibiting factor (SRIF) (Spencer, Harvey et al. 1986). These hypothalamic factors exert their effects by binding to receptors on the cell surface of the somatotrophs. Receptor binding and activation initiates signaling cascades of second messenger molecules that mediate changes in enzyme activity and gene expression.

The GHRH receptor (GHRH-R) is a member of the G protein-coupled receptor superfamily. Ligand binding causes the exchange of guanosine diphosphate (GDP) for guanosine triphosphte (GTP) and the disassociation of the cytoplasmic $G\alpha_s$ subunit, which activates adenylyl cyclase, causing an increase of cAMP levels (Gaylinn 2002). The increase in cAMP in turn activates protein kinase A (PKA), whose catalytic subunits, after dissociation from their regulatory subunits, travel to the nucleus to phosphorylate their target proteins (*e.g.* cAMP response element binding protein) which mediate genomic responses. The $G\alpha_s$ subunit has a GTPase activity and signaling stops when GTP is hydrolyzed to GDP. There is also evidence that GHRH can activate the mitogen-activated protein kinase (MAPK) pathway independently of PKA signaling and that this is mediated by the $G\beta\gamma$ subunits of the receptor (Pombo, Zalvide et al. 2000). In mammals, the major effect of GHRH is the stimulation of the synthesis and release of GH (Mayo, Miller et al. 2000). The GHRH receptor has recently been cloned in chickens that binds to human GHRH and increases cAMP accumulation, but only weakly responds to recombinant chicken GHRH, suggesting that GHRH may not be the endogenous ligand for this receptor (Porter, Ellestad et al. 2006; Toogood, Harvey et al. 2006; Wang, Wang et al. 2006). Chicken GHRH has been cloned and has less than 50% amino acid identity with rat and human GHRH (McRory, Parker et al. 1997).

The TRH receptor has been cloned in chickens (Sun, Millar et al. 1998). Ligand binding to the TRH receptor causes the activation of membrane-bound phospholipase C (PLC) via the GTP-bound $G_{q/11}$ subunit; PLC in turn catalyzes the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) into the second messenger molecules diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP_3) (Song and Hinkle 2005). IP_3

binds to an endoplasmic receptor and causes the release of Ca^{++} from the endoplasmic reticulum (ER) in the cytosol. Free cytosolic Ca^{++} binds to the kinase calmodulin, which then can phosphorylate its targets, elicting its celluar effects. DAG also acts as a second messenger in that it activates protein kinase C (PKC), a phospholipid- and Ca^{++}-dependent serine/threonine kinase (Kiley, Parker et al. 1991). TRH receptor signaling can also activate the MAPK pathway (Ohmichi, Sawada et al. 1994).

The ghrelin receptor also belongs to the G protein-coupled receptor family (Tanaka, Miyazaki et al. 2003). As with TRH receptor, ligand binding to the ghrelin receptor results in the activation of the PLC and PKC signaling pathways (Richards, Poch et al. 2006). Ghrelin receptor mRNA expression has been detected in the pituitary (Tanaka, Miyazaki et al. 2003).

There are 5 SRIF receptor subtypes that are members of the G protein-coupled receptor family. SRIF receptor inhibits the activity of adenylyl cyclase via its $G\alpha_i$ subunit; it also stimulates the activity of phosphotyrosine phosphatase, and interacts with the MAPK pathway (Patel 1999). Inhibition of GH-release in birds by SRIF is mediated primarily through the SRIF receptor subtype 2, and to a lesser extent the subtype 5 (Bossis and Porter 2001). SRIF-binding sites are present on the membranes of pituitary cells (Harvey, Attardo et al. 1990). There are 2 forms of biologically active SRIF that have been described in birds, a 14-amino acid peptide that is identical to SRIF-14 in mammals, and a 28-amino acid peptide that differs by one residue (Hasegawa, Miyamoto et al. 1984).

GH secretion is pulsatile in nature and exerts its effect through the GH receptor (Vasilatos-Younken, Cravener et al. 1988). A sex-linked dwarf strain of chickens,

which have a truncated GH receptor that does not bind GH, have a phenotype of adult weight that is 30-40 % lower than normal (Burnside, Liou et al. 1992). The chicken GH receptor has been cloned (Burnside, Liou et al. 1991). The GH receptor is a member of the cytokine receptor superfamily. Upon ligand binding, the GH receptor dimerizes and recruits the intracellular tyrosine kinase, Janus 2 (JAK2). Two JAK2 molecules bind to the membrane proximal cytoplasmic domains of the dimerized GH receptors and undergo autophosphorylation; they then phosphorylate tyrosine residues on the GH receptor. These phosphorylated tyrosines serve as docking sites for protein with *src* homology 2 (SH2) domains. The classic proteins that are phosphorylated by the activated JAK2-receptor complexes are the signal transducers and activators of transcription (STAT) transcription factors. Phosphorylated STATs form dimers, translocate to the nucleus, and bind to regulatory regions in responsive genes. Activated GH receptor also has been shown to phosphorylate other proteins such as insulin receptor substrate-1 (IRS-1), *src* homology- containing protein (SHC), phosphidylinositol 3' phosphate kinase (PI-3), and MAPKs (Zhou, Wang et al. 2005).

GH exerts its effects throughout the body, but it mediates most of its actions through production of insulin-like growth factors (IGFs) by the liver. IGFs are structurally related to insulin and play a role in a variety of anabolic and metabolic processes (reviewed in McMurtry, Francis et al. 1997). GH has been shown to increase release of IGF-1 from chicken hepatocytes *in vitro* (Houston and O'Neill 1991). When GH is administered to birds in a pusatile fashion that mimics endogenous release, circulating IGF-1 levels are much greater than in birds administered GH continuously (Vasilatos-Younken, Cravener et al. 1988). The IGF-1 levels in the sex-linked dwarf

chicken that lack a functional GH receptor are lower than normal chickens despite having higher levels of circulating GH (Vasilatos-Younken, Dunnington et al. 1997). Circulating levels of IGF-1 were significantly higher in a line of chickens selected for increased breast muscle and decreased fatness than in a control line (Tesseraud, Pym et al. 2003). Injection of IGF-1 increases lean growth and feed efficiency and decreases carcass fat (Tomas, Pym et al. 1998). The chicken IGF-1 gene maps to 165.5 cM on chromosome 1; interestingly, this is near a QTL for body weight (160 cM, confidence interval 114 to 180 cM; (Sewalem, Morrice et al. 2002) and a QTL for abdominal fat weight (150 cM, confidence interval 100 to 182 cM; (Ikeobi, Woolliams et al. 2002). A SNP in the IGF-1 promoter has recently been described that is associated with significant differences in percentage of abdominal fat weight in chickens (broiler-Leghorn and broiler-Fayoumi crosses) (Zhou, Mitchell et al. 2005). IGF-I exerts a negative feedback effect on the pituitary, decreasing synthesis of GH (Buonomo, Lauterio et al. 1987; Scanes, Proudman et al. 1999).

GH also has metabolic effects. GH increases lipolysis *in vitro* in adipose tissue (Harvey, Scanes et al. 1977) and this effect can be blocked with a GH antagonist (Campbell, Chen et al. 1993). *In vivo*, injection with recombinant chicken GH increases free fatty acid levels in the blood (Hall, Cheung et al. 1987). A week-long pulsatile administration of GH decreased fat pad size and lipogenesis in young birds (Rosebrough, McMurtry et al. 1991).

GH also affects levels of circulating thyroid hormones (TH), which are essential in the development of the embryo, hatchability, thermoregulation, growth, and metabolism (reviewed in (McNabb 2000)). The thyroid gland secretes thyroxine (T_4)

into the circulation, which is then deiodinated at the outer ring via Type I 5'-deiodinase, primarily in the chicken liver, to the active metabolite 3,5,3'-triiodothyronine (T_3), which is the preferred ligand of the 2 major isoforms of thyroid hormone receptor (TRα and TRβ) (Decuypere, Van As et al. 2005). Inner ring deiodination of T_4 and T_3 by Type III 5-deiodinases leads to the formation of the inactive metabolites reverse 3,3',5'-triiodothyronine (rT_3) and 3,3'-diiodothyronine (T_2), respectively. Chicken GH does not affect Type I 5'-deiodinase activity but does inhibit Type III 5-deiodinase activity, which leads to higher levels of circulating T_3 (Darras, Rudas et al. 1993).

T_3 in turn feeds back to inhibit secretion of TRH-stimulated growth hormone (Harvey 1990). T_3 has also been shown to down regulate TRH-binding sites on pituitary membranes (Harvey and Baidwan 1990). GH synthesis is increased in birds made hypothyroid by methimazole (MMI) injection, indicating that TH inhibit the production of GH (Denver and Harvey 1991). In the same study, treatment with T_3 inhibited the release of GH in response to TRH.

Thyroid-stimulating hormone

TSH stimulates the production of thyroid hormones, which are important for normal growth, development, and metabolism. Thyrotrophs produce TSH, a heterodimer of two glycoproteins, an α-subunit which is shared with follicle stimulating hormone (FSH) and luteinizing hormone (LH), and a hormone specific β-subunit. Chicken TSHβ has been cloned (Gregory and Porter 1997). Although there is no chicken TSHβ antibody available--and therefore no direct measurements of TSH in plasma, the report that TRH administration increases levels of T_4 in the blood (Thommes and Hylka 1978), and a subtractive assay (where LH and FSH specific β-subunit levels were subtracted

from total levels of α-subunit to obtain an estimate of TSHβ) (Berghman, Darras et al. 1993) that indicated increase of TSHβ in response to TRH administration, support that TSH is upregulated and secreted in response to TRH. TSH has also been shown to be released by ovine corticotrophin-releasing hormone (CRH) (Geris, Kotanen et al. 1996), and to be inhibited by SRIF (Lam, Harvey et al. 1986) (Lam, Harvey et al. 1986), as inferred by alterations in levels of thyroid hormones.

CRH receptor types I (Yu, Xie et al. 1996) and II (DeGroef, Grommen et al. 2004) have been cloned in chickens. The CRH receptors are members of the G protein-coupled receptor family and activate adenylyl cyclase, presumably through their $G\alpha_s$ subunit. However, in chickens the Type I receptor mRNA and TSHβ peptide do not colocalize (DeGroef, Geris et al. 2003), indicating the involvement of the Type II receptor in TSH secretion. Treatment with Type II receptor-specific agonists and antagonist indicates that it is the Type II receptor that mediates CRF-induced TSH secretion (De Groef, Goris et al. 2003). The target organ of TSH action is the thyroid gland, where it stimulates the growth of the gland and the production and release of thyroxine (T_4). The general mechanism of T_4 action is conversion into active triiodothyronine (T_3) by peripheral deiodinases in thyroid hormone target tissues (*e.g.* liver, see above).

TSH mediates its effects through its receptor, which has recently been cloned (Grommen, Taniuchi et al. 2006). TSH receptor in a G protein-coupled receptor and treatment with bovine TSH increases intracellular cAMP levels in transfected HEK cells, suggesting the presence of a $G\alpha_s$ subunit. TSH receptor mRNA is found in the thyroid, and in several other tissues, including brain, pineal gland, and retina.

Thyroid hormones are required for normal growth. In a study involving an induction of either a hyperthyroid or hypothyroid state by feeding TH or MMI, respectively, an increase in fatness and a decrease in growth was seen with MMI treatment, and TH decreased both fatness and growth (Decuypere, Buyse et al. 1987). Thyroid hormone receptors are members of the nuclear receptor superfamily. Receptors in the family share structural similarities; they possess an amino-terminal domain, a DNA-binding domain, a hinge region, and a hormone binding domain. The nuclear receptor superfamily is made up of 3 classes: Type I receptors bind steroid hormones, Type II bind THs, retinoic acids, and vitamin D_3, and Type III receptors are orphan receptors (Mendelson 2004). TRs, in general, bind to DNA in an unliganded state and repress transcription at positively regulated thyroid hormone response elements (TREs); at negatively regulated TREs, THs activate or derepress gene transcription (Decuypere, Van As et al. 2005).

Thyroid hormones exert negative feedback on thyrotrophs, as goitrogens—substances that inhibit thyroid hormone production—increase thyrotroph number and pituitary TSH content (Sharp, Chiasson et al. 1979; Thommes, Martens et al. 1983; Decuypere, Buyse et al. 1987), and TSHβ mRNA (Muchow, Bossis et al. 2005).

Pro-opiomelanocortin

The pituitary also regulates adrenocortical function and the production of glucocorticoids. Chicken POMC has been cloned; it is a 251 amino acid peptide with 9 proteolytic cleavage sites (Takeuchi, Teshigawara et al. 1999). POMC is a precursor protein that is produced by corticotrophs, and through proteolytic cleavage produces many peptide hormones—most notably adrenocorticotropic hormone (ACTH), β-

endorphin, and α- and β-melanocyte- stimulating hormone (MSH). ACTH is the major hormone produced by the avian pituitary corticotrophs and has been localized to the cephalic lobe of the anterior pituitary (Hayashi, Imai et al. 1991).

Not much is known about the regulation of the hypothalamic-pituitary-adrenal (HPA) axis in birds. In mammals, CRH stimulates secretion of ACTH from the pituitary, which in turn stimulates the production of glucocorticoids and mineralocorticoids in the adrenal gland. Glucocorticoids then feed back to the hypothalamus to down regulate CRH transcription and mRNA stability (Ma, Camacho et al. 2001). Adrenalectomy increased ACTH synthesis and secretion in rats (Spinedi, Giacomini et al. 1991).

In chickens, corticosterone (CORT) is the major glucocorticoid. Dexamethasone (DEX) has been shown to suppress secretion of ACTH *in vivo* (Herold, Brezinschek et al. 1992). Ovine CRH has been shown to stimulate the release of ACTH in chicken pituitary cells, and DEX inhibited basal and stimulated ACTH release (Carsia, Weber et al. 1986). Glucocorticoid negative feedback is also suggested by the lower levels of circulating ACTH and higher levels of CORT in chickens fed protein-restricted diets (Carsia, Weber et al. 1988). CORT injection decreases levels of CRH precursor mRNA and CORT plasma levels in embryonic and 9-day old chicks (Vandenborne, De Groef et al. 2005). Levels of CRH peptide in the hypothalamus, and levels of CRH receptor-1 and POMC mRNA levels in the pituitary were not affected. However, culture of pituitary cells with CORT decreased levels of POMC mRNA.

Glucocorticoids and mineralocorticoids are steroids that exert their effects through Type I nuclear hormone receptors. In general, steroid hormone receptors are not bound to DNA in their unliganded state. Glucocorticoid receptor is localized in the

cytoplasm bound to heat shock proteins. Upon ligand binding, the heat shock proteins dissociate and the receptor localizes to the nucleus, where it dimerizes and binds DNA to exert transcriptional effects.

Daily injections of CORT in 26-day old broilers increases abdominal fat pad size, liver size, and liver fat content (Bartov, Jensen et al. 1980). This same effect (injections starting on day 28) was seen (Buyse, Decuypere et al. 1987) in 2 lines of chickens selected for abdominal fat weight based on selection for levels of very low density lipoproptein (VLDL) (Whitehead and Griffin 1984).

Deposition of body fat in birds

Adipose tissue, the main fat depot in the body, functions to store excess energy in the form of triglycerides and to release fatty acids during energy deficit. In birds a major storage depot of adipose tissue is the abdominal fat pad (Hood 1982). The abdominal fat pad provides a good estimator of overall body fat in adipose tissues, since they are highly correlated (Cahaner, Nitsan et al. 1986). Unlike mammals, adipocytes contribute little to the synthesis of new fatty acids found in adipose tissue in birds (Griffin, Guo et al. 1992). The source of the majority of the fatty acids found in adipose tissue come from lipids in the plasma (Griffin 1993). Since commercial chickens are fed diets low in lipids, the liver synthesizes most of the lipids that are utilized by the body (Hermier 1997). Adipose tissue growth can occur by formation of new adipocytes from clonal expansion of differentiating preadipocytes derived from mesenchymal stem cells (hyperplasia) and/or an increase in adipocyte size via the uptake of lipids (hypertrophy) (Kacsoh 2000). In young broilers (up to 14 weeks) adipose tissue growth mainly occurs via hyperplasia, after that further growth chiefly occurs by hypertrophy of the adipocytes (Hood 1982).

The major processes that regulate growth of adipose tissue are: 1) hepatic lipogenesis, 2) transport of lipids from the liver to the adipose tissue, and 3) uptake of triglycerides by the adipocytes (Hermier 1997).

As reviewed in (Nir, Nitzan et al. 1988; Nelson and Cox 2000); the diet is a source of carbohydrate that provides the substrate for the formation of acetyl-CoA. Acetyl-CoA is converted into malonyl-CoA by acetyl-CoA carboxylase. After a meal, insulin stimulates the activity of the enzyme fatty acid synthase (FAS), which incorporates acetyl-CoA and malonyl Co-A into palmitate. Palmitate is a 16-carbon fatty acid that is incorporated into triglycerides (although other fatty acids formed by elongation and desaturation are used, too). In the liver triglycerides are assembled, along with apolipoproteins apoB-100 (the major protein), apoC-I, apoC-II, apoC-III, and apo-E and cholesterol into VLDLs and secreted into the circulation. When the VLDL come in contact with lipoprotein lipase associated with the cell surface of the adipocytes, the enzyme is activated by apoC-II, and free fatty acids are hydrolyzed from triglycerides and are taken up into the adipocytes where they are reesterified back into triglycerides for storage.

Fat and Lean lines of chickens

Two lines of chickens have been genetically selected that exhibit significant differences in body fat accumulation. The lean and fat chicken lines (LL and FL, respectively) were created at the Institute Nationale Reserches Agrinomique (INRA), Nouzilly, France, and the F_0-F_2 generations were first described in 1980 (Leclercq, Blum et al. 1980). The selection criterion was based on the proportion of body fat in males at 9

weeks of age. Generation of the fat and lean broiler strains until the end of selection (F_7) was as described previously (Leclercq, Blum et al. 1980; Leclercq 1988).

The F_{-1} generation consisted of a blending of 6 different strains of broiler chickens with 23 males bred with 68 females. Six strains were used to capture as many broiler-type alleles as possible. Four sons per hen were sacrificed at 9 weeks of age and abdominal fat pad weights were measured. Birds have little intramuscular fat compared to mammals but maintain a large abdominal fat pad depot. Each of the F_0 families was classified as fat line or lean line depending on the deviation (positive or negative) from the linear regression between ratio of abdominal fat:body weight with body weight (Figure 1).

Progeny from the 20 hens whose sacrificed sons had the highest deviations from the regression line (above line for fat line, under for lean line) were used to breed the F_1 generation. Since deviations from the regression line were used for selection, the body fat:body weight ratio was not correlated with body weight, and mean body weights of the two lines were similar. After F_0, the proportion of abdominal fat was not correlated with body weight within each line and so selection was based on abdominal fat proportion and not the deviation between the fat proportion and body weight. Four sons per hen were sacrificed and from the 15 hens whose progeny had the highest or lowest ratios the sires and dams of the F_2 generation were selected. The progeny of 14-15 dams per line (4 sacrificed males per dam at 9 weeks) that had the highest or lowest ratios of abdominal fat: body weight were used to produce the subsequent generations with care taken not to cross full or half sibs. Selection was continued for 7 generations, with 14 sires and 5 hens per sire in each line for the F_0 and F_1 generations and then 15 sires and 5 dams per

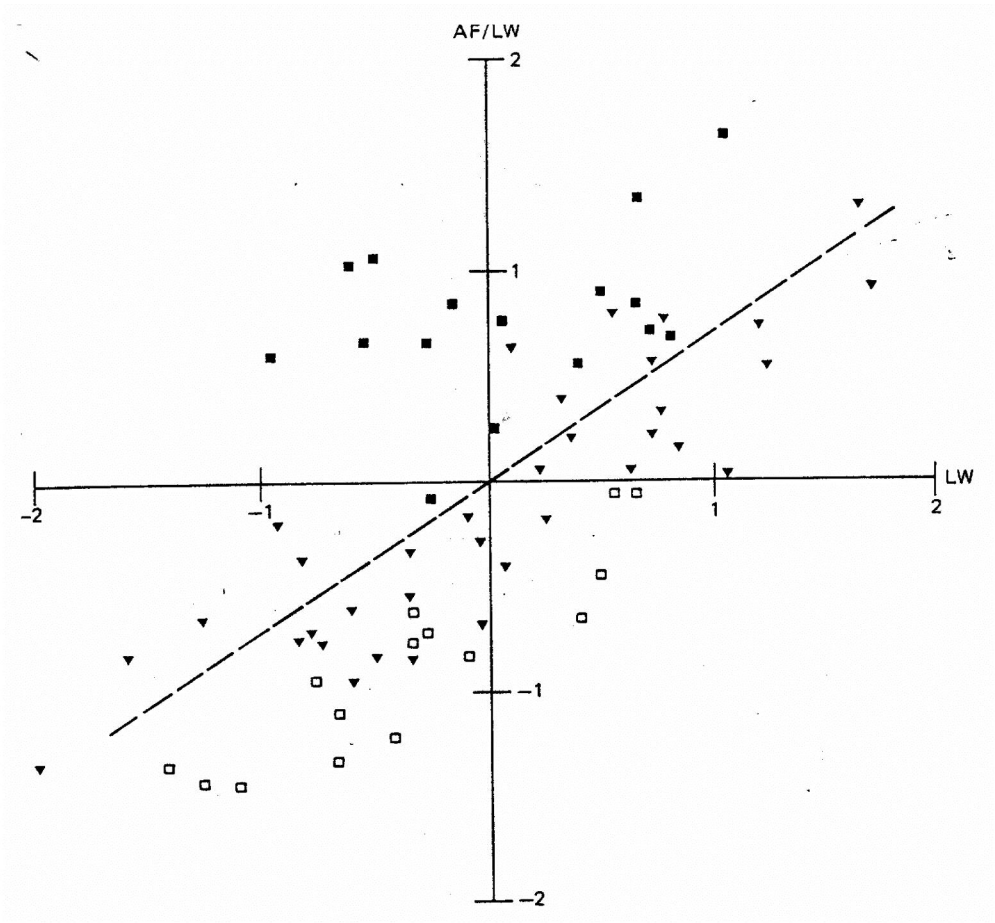

Figure 1. Relationship between abdominal fat to live weight ratio (AF/LW) and live weight (LW) of 9-week-old broilers (F_0). Each symbol represents mean of 4 sons per dam:■, families kept to create the fat line; □, families kept to create the lean line; ▼, discarded families (Figure 2.1, Leclercq 1988)

sire in each line for generations F_2 through F_7. Realized heritabilities were calculated for proportion of abdominal fat from F_0 to F_1 and F_0 to F_2 and although heritabilities were reduced over three generations, they still were considered high (h^2 = 0.67 and 0.57, respectively) by Leclercq (1988). Selection was stopped after F_7, and representative birds (one son per male and one daughter per hen) were kept for the continuance of the two lines.

The difference between lines in fat pad:body weight ratio at nine weeks remained constant through F_{10}, the last generation examined (Leclercq 1988). Differences in abdominal fat ratio between the two lines were seen as early as 4 weeks of age by the F_2 generation, and by the end of selection (F_7) the fat line had a ratio of abdominal fat:body weight that was more than three times greater than the lean line. Metabolizable energy intake was not significantly different between the lines (Leclercq and Saadoun 1982). These two strains have now been sustained for more than twenty-five years after selection was discontinued and still maintain the difference in abdominal body fat.

Since the creation of the LL and FL, several differences between the two lines have been noted. For example, i*n vivo* fatty acid synthesis is greater in the FL than the LL, and FL livers are heavier (Saadoun and Leclercq 1983). The LL is more susceptible to depressed growth when fed a low protein diet (Leclercq 1983). The FL birds always have lower blood glucose levels than the LL, whether they are fed or fasted; conversely, the LL always have lower levels of triglycerides, especially in a fed state (Leclercq, Hermier et al. 1984). There are no significant differences in levels of cholesterol or free fatty acids. The FL hens are lighter than LL hens at the onset of the laying phase and produce lighter eggs but the yolk percentage of the egg is higher in FL hens, indicating

that yolk production may be superior in the FL (Leclercq, Kouassi-Kouakou et al. 1985). The effect of CORT injection on various metabolic parameters was tested, and differing sensitivity to CORT does not seem to be involved in fattening (Saadoun, Simon et al. 1987). When the birds are fed a diet with high enough fat to suppress liver lipogenesis, there are still differences in body composition (Saadoun and Leclercq 1987; Griffin 1993). The activity of delta 9-desaturase, the rate-limiting enzyme in the synthesis of unsaturated fatty acids, was 45% higher in the FL (Legrand, Mallard et al. 1987). The differences between the lines are not due to differing lipolytic activity of the abdominal adipose tissue (Lemarchal, Beaunez et al. 1988). In a fasted state, the FL has lower blood glucose than LL, but levels of insulin, T_3, T_4, and CORT are not different (Saadoun, Simon et al. 1988). When fed, insulin and T_4 are increased in the FL, and T_3 is slightly higher in the LL. After refeeding, glucose and insulin were lower in the FL; T_3 was lower and T_4 was higher in the FL. To see if thyroid hormone differences were involved, T_3 was added to the diet of the FL to bring T_3 levels up to LL levels and only a small change in body composition was observed, suggesting that the higher T_3 levels in the LL only account for a small difference in the phenotype (Leclercq, Guy et al. 1988). The FL has higher LPL activity in their adipose tissue, but this is due to hyperplasia and hypertrophy of the adipocytes and not increased intrinsic LPL activity (Hermier, Quignard-Boulange et al. 1989). The difference in fatness is not accounted for by an increase in the number or kinase activity of insulin receptors (Simon, Chevalier et al. 1991). Utilization of dietary protein is more efficient in the LL (Leclercq and Guy 1991). mRNA levels of apoAI [a major component of high density lipoprotein (HDL)] were found to be significantly different between lines and correlated with abdominal tissue

weight (Douaire, Fur et al. 1992). GH levels are slightly higher in LL with no difference in GH binding to liver membranes (Buyse, Vanderpooten et al. 1994). Increased activation of insulin receptor pathways after feeding could be responsible for increased liver lipogenesis in the FL (Dupont, Chen et al. 1999). The FL has higher levels of IGF-I and IGF-II in both fed and fasted states (Beccavin, Chevalier et al. 2001). Malic enzyme, ATP citrate-lyase, and (as previously shown) ApoAI genes, which are involved in lipogenesis in the liver, have greater mRNA levels and transcription rates in the FL (Daval, Lagarrigue et al. 2000). In short, much work has been done with the FL and LL birds. However, the underlying genetic basis for the differences in abdominal fat is not known. A summary of metabolic differences between LL and FL is presented in Table 1 (Leclercq 1988).

14K cDNA Microarrays

DNA microarrays allow the quantification of expression levels for thousands of genes simultaneously. The construction of the cDNA libraries and the production of the Del-Mar 14K Chicken Integrated Systems Microarray have been described in detail (Cogburn, Wang et al. 2003; Cogburn, Wang et al. 2004; Carre, Wang et al. 2006). The Del-Mar 14K Chicken Integrated Systems Microarrays were printed from cDNA libraries created from metabolic tissues (liver and fat), somatic tissues (skeletal muscle and growth plate), reproductive tissues (oviduct, ovaries, and testes), and neuroendocrine tissues (pituitary, hypothalamus, and pineal). These tissues were chosen for their agricultural and biological importance. All of the publicly available (as of March 1, 2003) chicken expressed sequence tags (ESTs; ~407000) were assembled into 33949 contigs using the CAP3 software program (Huang and Madan 1999). The ESTs from the tissue specific

libraries were incorporated into these contigs. Contigs were then identified by their highest scoring BLASTX and BLASTN returns. cDNA clones from the libraries were amplified by PCR and printed onto glass slides. The Del-Mar 14K Chicken Integrated Systems Microarray contains 19200 spots and 14053 of these represent unique cDNA. In addition to the cDNAs from the tissue specific libraries, 387 60-mer oliogonucleotide probes for specific genes were printed, along with 72 quality control spots. The quality control spots are salmon sperm DNA, which has been included for an estimation of background hybridization, and 8 housekeeping genes: β-tubulin, TEF1α, β-actin, pre-mRNA splicing factor, GAPDH, dynactin, Na^+/K^+ ATPase, and sodium pump 3 (printed in 8 replicate spots each). The composition of the microarray is summarized in Table 2.

Chapter 3: Identification of a single nucleotide polymorphism associated with adiposity following transcriptional profiling of gene expression in the anterior pituitary gland

Introduction

Body composition is determined by complex interactions between environmental, hormonal, genetic, behavioral, and nutritional factors. Commercial broiler chickens have been selected for increased muscle mass and rapid growth (Havenstein, Ferket et al. 2003). Unfortunately, selection for these desirable commercial traits has also led to a concomitant increase of undesirable traits such as ascites, decreased reproductive performance, increased skeletal muscle abnormalities, and fatty liver syndrome (Griffin and Goddard 1994; Havenstein, Ferket et al. 2003; Julian 2005). Excessive fat deposition is an undesirable trait from a production standpoint due to inefficient feed conversion and from a consumer viewpoint due to the increasing demand for leaner meats. In commercial industry it is not economically feasible to select against this highly heritable trait due to the high cost and labor involved in slaughtering and dissecting animals in breeding assays (Lagarrigue, Pitel et al. 2006). Identification of genetic markers for the quantitative trait would be of great commercial value. Additionally, with the incidence of obesity in humans reaching epidemic proportions—approximately 60% of Americans are considered overweight or obese (Hedley, Ogden et al. 2004)—identification of genes involved in development of adiposity in chickens will likely implicate their human orthologs in obesity. This could be especially helpful, considering the relationship of obesity with other diseases that are on the rise, such as diabetes, hypertension, and heart disease (Muoio and Newgard 2006).

Experimental strains of chickens have been developed by genetic selection for body fat that allow for investigation into the genetic and metabolic mechanisms involved in adiposity. Lines of lean and fat chickens (LL and FL, respectively) were developed by selection for abdominal fat pad weight in males at 9 weeks of age (Leclercq, Blum et al. 1980). The FL has a ratio of abdominal fat weight: body weight that is almost 4-fold higher than the LL. The difference in adiposity between the two lines appears by four weeks of age and can not be accounted for by differences in feed consumption or use of metabolizable energy (Leclercq 1988).

The adenohypophysis, or anterior pituitary gland, is comprised of five major cell types characterized by the hormones they synthesize and secrete. Three of these cell types: somatotrophs, corticotrophs, and thyrotrophs, produce hormones—growth hormone (GH), pro-opiomelanocortin [POMC and its derivative adrenocorticotropin (ACTH)], and thyrotropin (TSH), respectively—that exert major effects on growth, body composition, and metabolism. Differences in the production and secretion of these hormones may be involved in the difference in adiposity that is manifested between the Lean and Fat lines of chickens.

DNA microarrays now allow the simultaneous analysis of expression levels of thousands of genes in a single assay (Duggan, Bittner et al. 1999), and DNA microarrays have been used to identify differentially expressed genes involved in signaling pathways in the pituitary (Ma, Qi et al. 2005). The Del-Mar 14K Chicken Integrated Systems Microarray (GEO accession no. GPL1731) contains 14053 unique cDNAs from multiple tissue specific cDNA libraries (Cogburn, Wang et al. 2003; Cogburn, Wang et al. 2004; Carre, Wang et al. 2006). Of the cDNAs contained in the microarray, the neuroendocrine

cDNA library contributed 5929 of these cDNAs, and these neuroendocrine cDNAs have already been used in our laboratory to profile gene expression patterns during pituitary development by microarray analysis (Porter and Ellestad 2005; Ellestad, Carre et al. 2006). In recent years, there has been a dramatic increase in the available tools for chicken genomics, including the completion of the draft of the chicken genome (Hillier, Miller et al. 2004). The chicken genome is about 1 billion base pairs in sequence containing 20000-23000 genes. Most genetic markers are polymorphic sequences of DNA that have a known locus. Examples of markers are known genes (first generation Type I markers) and much shorter polymorphic segments like microsatellites or variable number tandem repeats (second generation Type II markers) (Emara and Kim 2003). The most common genetic marker is the single nucleotide polymorphism (SNP) and they are considered the basis of third generation genetic maps (Wang, Fan et al. 1998). It is estimated that the chicken genome contains 2.8 million SNPs (Wong, Liu et al. 2004). SNPs can be found anywhere in the genome, but SNPs that are located in the promoter regions of differentially expressed genes will be of particular interest due to the fact that they may alter transcriptional machinery binding sites.

A combined functional genomic and bioinformatic approach was taken in the present study to analyze differential gene expression in the anterior pituitary between the LL and FL at 1-, 3-, 5-, and 7-weeks of age (the time frame during which adiposity becomes significantly different) and to identify polymorphisms such as SNPs in the flanking regions of differentially expressed genes that could be used as genetic markers for adiposity. The objective of this research was to identify a genetic marker that could be used to genotype chickens for this trait.

Materials and methods

Animals

The Fat line and Lean line chickens were produced by inseminating hens with pooled semen from each line (eight pools; seven hens per semen pool). Each hen's eggs were marked and incubated; chicks were sexed, wing-banded, and vaccinated against Marek's disease at hatch. Males (87 Fat, and 102 Lean) were reared together in 4.4 x 3.9 meter floor pens under a standard heat program. The birds were fed *ad libitum* a mashed diet for the first few days, a pelleted starter diet for the first three weeks and then a growing diet up to week 11; water was freely available. Light cycles were 24 hours for the first two days and then 14 hours light/10 hours dark.

Pituitary glands from 8 birds in each line (1 bird per semen pool/ different hen) were extracted at weeks 1, 3, 5, and 7, snap-frozen in liquid nitrogen, and stored at -75°C. Birds were weighed and blood was drawn before sacrifice by cervical dislocation. Fat pad was excised and weighed, and other tissues were collected (hypothalamus, breast muscle, liver, and abdominal fat) for use in other studies.

The F_0 generation used to produce the F_2 intercross consisted of thirty animals: four males and thirteen females from the Lean line, and five males and eight females from the Fat line. Fat males were bred to Lean females and *vice versa* to produce the F_1 generation. Five males and fifty females of the F_1 generation animals were kept to produce the F_2 generation. Three of the F_1 males had Fat sires and Lean dams; two had Lean sires and Fat dams. Thirty of the F1 females were from Fat sires and Lean dams; twenty females were from Lean sires and Fat dams. Six hundred thirty-seven F_2 animals (332 females and 305 males) were able to provide biometric data. The F_0 and the F_2

intercross generations were reared under the same conditions and sacrificed at 9 weeks of age. Blood was drawn and body weight and abdominal fat pad weight were measured.

RNA extraction and amplification

Total RNA was extracted from individual chicken pituitaries using Qiagen RNeasy mini-prep kits according to the manufacturer's protocols and quantified by absorbance at 260 nm. RNA quality was assessed using the Agilent Bioanalyzer (Agilent Technologies, Palo Alto, CA) at the University of Maryland Microarray Core Facility and replacement samples were extracted if the RNA was of low quality. A drawback of using the chicken pituitary as the tissue of interest for microarray analysis is its small size and therefore low content of RNA. To ensure sufficient quantities of RNA for microarray analysis, a variation of the Eberwine procedure was used to generate amplified RNA (aRNA) (Van Gelder, von Zastrow et al. 1990; Luo, Salunga et al. 1999; Porter and Ellestad 2005). A reverse transcriptase (Superscript II, Invitrogen, Carlsbad, CA) was used with a poly-dT primer with a 5' T7 RNA polymerase promoter sequence (5'-GGCCAGTGAATTGTAATACGACTCACTATAGGGAGGCGGT$_{24}$-3'; Affymetrix, Santa Clara, CA) to transcribe 0.5 μg of total RNA into first strand cDNA. After RNaseH digestion, DNA polymerase I synthesized the second strand using the digested RNA as primers and the first strand as template; DNA ligase joined the second strand fragments together, and T4 polymerase polished any single stranded overhangs to form blunt-ended double-stranded cDNA. The cDNA was then extracted with phenol-chloroform in a phase-lock centrifuge tube (Eppendorf, Westbury, NY), washed in a Microcon-30 (Millipore, Billerica, MA) spin column, and dried down in a vacuum centrifuge.

In vitro transcription of aRNA off of the cDNA was performed using Ambion's (Austin, TX) T7 MEGAscript kit as per the manufacturer's directions. Amplified RNA was then phenol-chloroform extracted using a phase-lock centrifuge tube (Eppendorf) and purified by centrifugation through a Spin Column-30 (Sigma, St. Louis, MO). The aRNA was then quantified by absorbance at 260 nm and with the RiboGreen RNA Quantification kit (Molecular Probes), and visualized by ethidium bromide staining after electrophoresis in an agarose-formaldehyde gel.

Note: The RNA amplification procedure has been previously validated in our laboratory. Pooled total RNA and RNA amplified from that pool were hybridized in replicate to 5K Chicken Neuroendocrine System microarrays (GEO accession no. 1744), and the mean $\log_2$-transformed raw pixel intensities from each spot were found to be highly correlated (r^2=0.96) between the total RNA and the aRNA (Ellestad and Porter 2005).

Labeling and hybridization of microarrays

The labeling and hybridization of 1μg of target aRNA to the Del-Mar 14K Chicken Integrated Systems microarrays (GEO accession no. 1731) was performed by the Microarray Core Facility, Center for Biosystems Research at the University of Maryland, College Park. Target aRNA (1μg) was reversed transcribed using random primers into cDNA containing a dTTP analog that has a reactive amino allyl group [5-(3-aminoallyl)-dUTP] (Ambion). After purification, cDNA was labeled with the ester form of the fluorophores Cy3 and Cy5 (Amersham, Piscataway, NJ), which link to the amino allyl group.

cDNA from each of the pituitaries was labeled with Cy3, and a pooled reference sample was labeled with Cy5. The pooled reference sample was created from equal amounts of all of the aRNA samples from the experiment. The labeling reactions were purified to remove unincorporated dye, hybridized to the microarrays overnight at 42 °C, and then washed with increasingly stringent sodium citrate saline solutions. After washing, slides were scanned by the Facility's 418 confocal laser (Affymetrix) at 550 nm for Cy3 and 650 nm for Cy5. For each slide, a TIFF image file was generated for each fluorophore and saved.

Analysis of microarrays

The Institute for Genomic Research (TIGR) makes available a suite of free software for the analysis of microarray data (Saeed, Sharov et al. 2003). The TIFF files were loaded into Spotfinder (version 2.2.4), an image processing program, to visualize the overlaid hybridization scans and to quantify pixel intensities of the spots. The software creates a grid that overlays each spot on the slide within a square cell. The grid for the 14K slides consists of forty-eight 20x20-cell blocks arranged in 4 columns by 12 rows; this grid arrangement matches the printing pattern of the spots. For each hybridization, the Cy3 scan was loaded into channel A and the Cy5 scan into channel B. The slides were analyzed using the *Otsu thresholding* algorithm option with the spot size parameters set at a minimum of *3* and a maximum of *22.5*. The *quality control filter* was used and the *flagged values* and *raw data* were kept. The data generated by Spotfinder analysis were saved as *MEV* files and exported into Microarray data Analysis System (MIDAS; version 2.18), TIGR's data normalization software, using the *data directory* mode. This allowed normalization of all the data in a single processing step. Parameters

that were applied were *using flags* and *background checking* for both channels; signal-to-noise ratio threshold was set to *3.0*. These parameters rejected flagged spots that were saturated, not detected, malformed, or had a background greater than the spot intensity; the background checking parameter keeps only the spots whose intensities are 3 times the background for both channels. Spots that failed to pass these criteria were excluded from downstream analysis. Cy3 spot intensities were normalized by block with the *LOWESS* algorithm using a smoothing parameter of *0.33*, and normalization was followed by *standard deviation regularization* by *block* and then by *slide* using Cy 5 (pooled aRNA) as the reference channel. The output was saved as MEV files.

Statistical analysis

Two-way analysis of variance of the normalized data using Statistical Analysis System software version 8.02 (SAS Institute, Cary, NC) was used to identify differentially expressed spots and to trim spots that did not exhibit sufficient changes to be of proximate interest. Only spots with at least 2 replicates for all ages in both lines were examined. The $\log_2$ ratio of normalized Cy3: raw Cy5 was analyzed to detect significant differences ($p<0.05$) between lines, among ages, and interactions between lines and ages. Spots that did not show any significant differences were excluded from further analysis. The next criterion for further analysis was that the fold-spread of the least squares means of a spot across all ages and lines must be ≥ 0.68 ($\log_2$ ratio). The final trimming step excluded any spots that did not have intensities greater than the 8 salmon sperm DNA control spots on each array for both Cy3 and Cy5. Three hundred and eighty-six genes were kept for further analysis.

Cluster analysis

GeneCluster 2 software (http://www.broad.mit.edu/cancer/software/genecluster2/gc2.html) was used to cluster and visualize genes with similar expression patterns by self-organizing maps (SOMs) analysis. SOMs use a clustering algorithm that imposes partial structure on a dataset while also reflecting some of the natural structure of the dataset by an iterative classification of data points into nodes, or clusters, which are easy to visually interpret (Tamayo, Slonim et al. 1999). The fat and lean lines were analyzed separately. Genes were classified into 30 clusters with the geometry of the nodes being a 6x5 grid using the default parameters with the exception that the number of iterations was increased to 500,000. To simplify confirmation of gene expression patterns by qRT-PCR, 7 expression profiles were defined as follows: 1) gene expression always greater in the Fat line than Lean line, 2) gene expression always greater in the Lean line than Fat line, 3) early age gene expression (weeks 1 and 3) greater in Lean line than Fat line, 4) late age gene expression (weeks 5 and 7) greater in Lean line than Fat line, 5) early age gene expression greater in Fat line than Lean line, 6) late age gene expression greater in Fat line than Lean line, and 7) no difference in gene expression between the 2 lines.

Marker analysis

GeneCluster 2 software was also used to identify "marker" genes whose up or down regulation is most correlated with the Fat or Lean lines. Twenty-five markers per line were determined at 1-, 3-, and 5-weeks of age using both the signal to noise ratio $[(\mu_a-\mu_b)/(\sigma_a+\sigma_b)]$ and the t-test statistic $[(\mu_a-\mu_b)/\sqrt{(\sigma_a^2+\sigma_b^2)}]$ as the distance metric (μ is the mean per class and σ is the standard deviation per class). Genes with missing replicates were not included in the analysis.

Verification of gene expression

Expression profiles of microarray gene expression were confirmed by 2-step quantitative reverse transcription PCR (qRT-PCR). Before qRT-PCR, the gel picture of the PCR product that was spotted on the microarray was inspected to ascertain whether PCR amplification of the cDNA library clone produced a clean, single band. Since a DNAse digestion was not performed on the RNA extracted from the pituitaries, genomic contamination in the qRT PCR reaction was a concern. To design PCR primers, the sequence of the EST clone that was printed on the microarray (http://www.chickest.udel.edu/Cogburn_CAP3_DB) was BLASTed against the chicken genome using ENSEMBL. After confirming that the sequence was within an EST or mRNA, the entire expressed sequence was used to design primer pairs that spanned at least one intron. The lack of a single sharp melting peak of the PCR product indicates probable genomic contamination (although it could be due to alternatively spliced cDNAs). Two genes from each of the 7 expression profiles were verified by qRT-PCR. qRT-PCR for the 14 genes on each of the 32 total RNA samples and a no reverse transcriptase negative control reaction were performed in duplicate. The RNA for the no enzyme control was from the pooled reference total RNA sample. The first step of the qRT-PCR was performed as for the first step of the RNA amplification procedure above except that oligo-dT primer (5'-CGGAATTCTTTTTTTTTTTTTTTTTTTV-3', Sigma Genosys, Houston, TX) was used. The cDNA from these reactions, along with a water negative control, were quantified by real time-PCR using Qiagen's Quantitect SYBR Green PCR kits. This required 28 specific primer pairs that were designed using Primer Express software (version 2.0, Applied BioSystems). The parameters for primer design

were a primer length of *18-30* nucleotides spaced *115-130* base pairs apart, a G/C content of *40-60%*, and a melting temperature of *58-60* °C. The primers were generally targeted to the 3' end of the gene sequence due to the fact that dT-primed reverse transcription preferentially transcribes mRNA sequences localized in the 3' end. The real-time 2-step PCR was done in an iCycler thermocycler (BioRad). One microliter of cDNA was used as template, and primers were at a final concentration of 300nM. Thermocycler parameters were an initial enzyme activation incubation for 15 minutes at 95 °C, 40 cycles of denaturation at 95 °C for 10 seconds then annealing and extension for 45 seconds at 55 °C, and a final denaturation at 95 °C and extension step at 55 °C for one minute each. Primer sequences used are given in Table 1 in the appendix.

Identification of markers for genes of interest

Twelve genes whose expression patterns were confirmed by qRT-PCR were chosen for further analysis. The DNA sequences of the microarray clones for each of the 12 genes were BLASTed against the chicken genome to determine genomic location using ENSEMBL. ENSEMBL *GeneSeqView* was used to display SNPs in the genomic sequence within 5000 base pairs (bp) upstream of the first exon. Primers were designed that would generate PCR products of about 1000 bp in length containing as many SNPs as possible. Genomic DNA was phenol-chloroform extracted from ~100 µl of blood taken from F_0 animals. Seventeen primer pairs were designed for the 12 genes. The parameters for primer design were a primer length of *18-30* nucleotides spaced *500-1000* base pairs apart, a G/C content of *40-60%*, and a melting temperature of *58-60* °C. One microliter of genomic DNA (not quantified) was used as starting template, and primers were at a concentration of 200 nM in the reaction. Thermocycler parameters were initial

denaturation of 3 minutes at 95 °C; 35 cycles of denaturation at 95 °C for 1 minute, annealing at 55 °C for 1 minute, and then extension for 1 minute at 72 °C; and a final extension step at 72 °C for 7 minutes each. PCR was used to amplify genomic DNA from 22 F_0 animals (4 males and 7 females from each line). Primer sequences used are given in Table 2 in the appendix.

Genomic sequencing

PCR products from the 17 reactions per 22 F_0 animals were submitted in 96-well format to the High-Throughput Genomics Unit (HTGU), Department of Genome Sciences, University of Washington for sequencing using the forward primers. The PCR reactions were subjected to clean-up using exonuclease/ shrimp alkaline phosphatase by HTGU and sequenced using Applied Biosystems' (Foster City, CA) BigDye terminator v3.1 Cycle sequencing kit and a 3730xl DNA analyzer (Applied Biosystems). SNPs were identified by assembling the sequences into contigs using the ContigExpress feature in the Vector NTI software package (Invitrogen).

Locked nucleic acid primer genotyping

Allele specific primers were designed with a locked nucleic acid nucleotide at the 3' end that was specific for a T/C SNP found 718 bp upstream of the first exon of lysophosphatidic acid receptor-1 at position 32846318 on the Z chromosome. The antisense primer sequence For the T SNP was TCTAAGATTTGAACTGGGA*A*, and the antisense primer sequence for the C SNP was TCTAAGATTTGAACTGGGA*G*; the sense primer sequence for both reactions was GGCAATGAAACACAACTGAGTGAAA. PCR was performed using Promega

(Madison, WI) Green GoTaq master mix; 1µl of PCR product from genomic DNA diluted 1:100 was used as a template, and primers were at a final concentration of 200nM. Thermocycler parameters were an initial denaturation step at 95 °C for 3 minutes; 35 cycles (32 cycles for the C-specific primer) of denaturation at 95 °C for 40 seconds, annealing at 52.5 °C (55 °C for the C-specific primer) for 20 seconds, and then extension for 40 seconds at 72 °C; and a final extension step at 72 °C for 7 minutes each. Genotype was determined by the presence or absence of SNP specific PCR amplicons after electrophoresis in an agarose gel. Forty-eight of the leanest and fattest F_2 animals of both sexes were genotyped (final n=189) and a generalized mixed model (PROC GLIMMIX, SAS) was used to test whether an association between genotype and the phenotypic tails of the population was present.

Results

Microarray analysis

Gene expression profiles in the anterior pituitary were characterized using cDNA microarrays representing greater than 14,000 genes. Pituitaries were extracted from Fat and Lean birds at 1-, 3-, 5-, and 7- weeks of age and total RNA isolated and amplified. Four replicate samples for each strain and age were labeled and hybridized to the Del-Mar 14K Chicken Integrated Systems microarrays (GEO accession no. GPL1731) for a total of 32 samples. The raw data was first subjected to LOWESS normalization using MIDAS and then each slide was subjected to standard deviation regularization by block within slide and the across all slides. Two-way ANOVA (SAS) of $\log_2$ ratios was used to detect significant ($p<0.05$) differences by line, age, and the line-by age interaction. There were 1150 significantly different genes between the 2 lines and 339 of these genes

exhibited greater than 0.68-fold differences in their $\log_2$ ratios (highest group mean at least 160% of the lowest group mean). One thousand four hundred twenty nine genes were significantly different by age and of these, 583 exhibited fold changes greater than 0.68 in the $\log_2$ ratio. There were 145 genes that significantly differed in their line-by age interaction, and 62 of these exhibited greater than 0.68-fold differences. Since it is known that gene expression changes with age, only the 386 genes with significant differences by line or for line-by-age interaction with at least a 0.68-fold change in $\log_2$ ratio and $n \geq 2$ for each experimental group were kept for further analysis (Tables 3 and 4).

At 1 week of age, mRNA levels of 59 genes were up-regulated in the Lean line (Table 5) and 58 genes were up-regulated in the Fat line (Table 6). At 3 weeks of age, 41 genes were up-regulated in the Lean line (Table 7) and 61 genes up-regulated in the Fat line (Table 8). At 5 weeks of age, 60 genes were up-regulated in the Lean line (Table 9) and 64 genes up-regulated in the Fat line (Table 10).

Cluster analysis

GeneCluster 2 software was used to cluster and visualize genes with similar expression patterns by self-organizing maps (SOMs) analysis. Genes were classified into 30 clusters with the geometry of the nodes being a 6x5 grid. The Lean and Fat lines were analyzed separately. Three genes which represent different expression patterns between the Lean and Fat lines have been identified by microarray spot number (Figure 2).

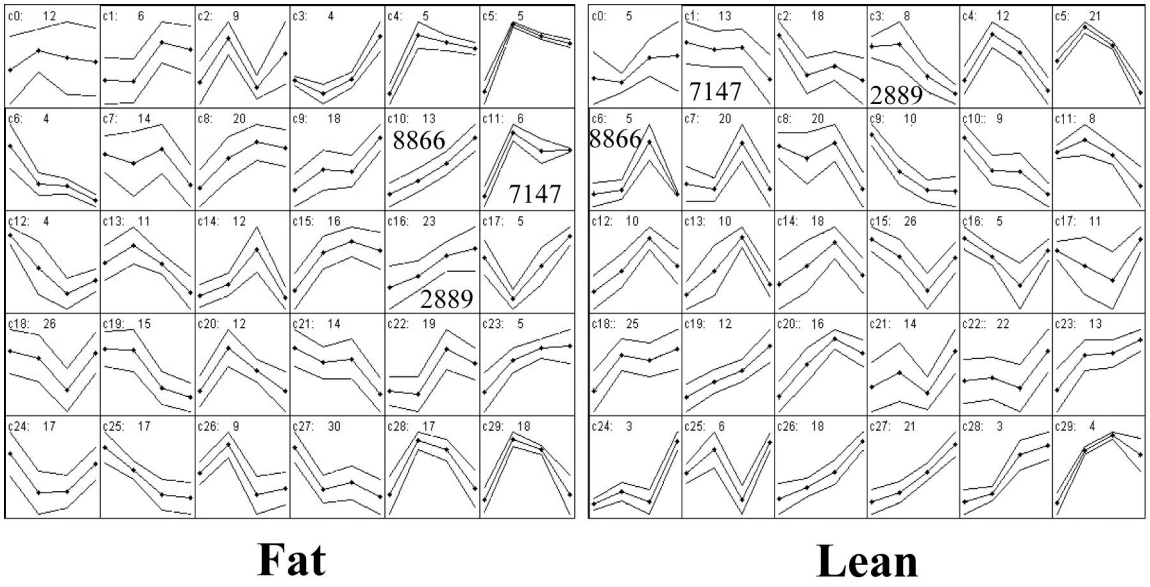

Figure 2. Self-Organizing Map analysis of differentially expressed genes in the Lean (right) and Fat (left) chicken lines. Y-axis is relative gene expression; x-axis is age. Clusters which contain genes that represent different expression patterns between the Lean and Fat lines are identified by the gene's microarray spot number.

Redundancy was apparent in the clusters. To simplify confirmation of gene expression patterns by qRT-PCR, 7 expression profiles were defined using genes that had differences in gene expression between the 2 lines as follows: 1) gene expression always greater in the Fat line than Lean line (Figure 3); 2) gene expression always greater in the Lean line than Fat line (Figure 4); 3) early age gene expression (weeks 1 and 3) greater in Lean line than Fat line (Figure 5); 4) late age gene expression (weeks 5 and 7) greater in Lean line than Fat line (Figure 6); 5) early age gene expression greater in Fat line than Lean line (Figure 7); 6) late age gene expression greater in Fat line than Lean line (Figure 8); and 7) no difference in gene expression between the 2 lines (Figure 9).

Although GH, POMC, and TSH were not significantly different in the microarray analysis, qRT-PCR was performed for those genes (Figure 10).

Identification of marker genes

Genecluster 2 software was used to identify the 25 genes most correlated with each strain at 1-, 3- , and 5-weeks of age using both the signal-to-noise ratio and the t-test as the distance metric between the means of the genes in the 2 classes (Lean and Fat). Results are present in Tables 11-16.

A total of 12 genes were chosen as candidate genes (Table 17). The 12 genes were either up-regulated (0.68-fold $\log_2$ ratios) or were identified in the marker analysis at weeks 1 and 3. The sole exception was Ubiquinone biosynthesis monooxgenase COQ6 (GEO no. 44.3.14), which looked to be highly up-regulated in the Lean line as determined by qRT-PCR. Gene expression levels for eight of the candidate genes were already verified by qRT-PCR (see figures above). In addition to Ubiquinone biosynthesis monooxgenase COQ6, Aldo-keto reductase (GEO no. 5.17.9), Leptin receptor

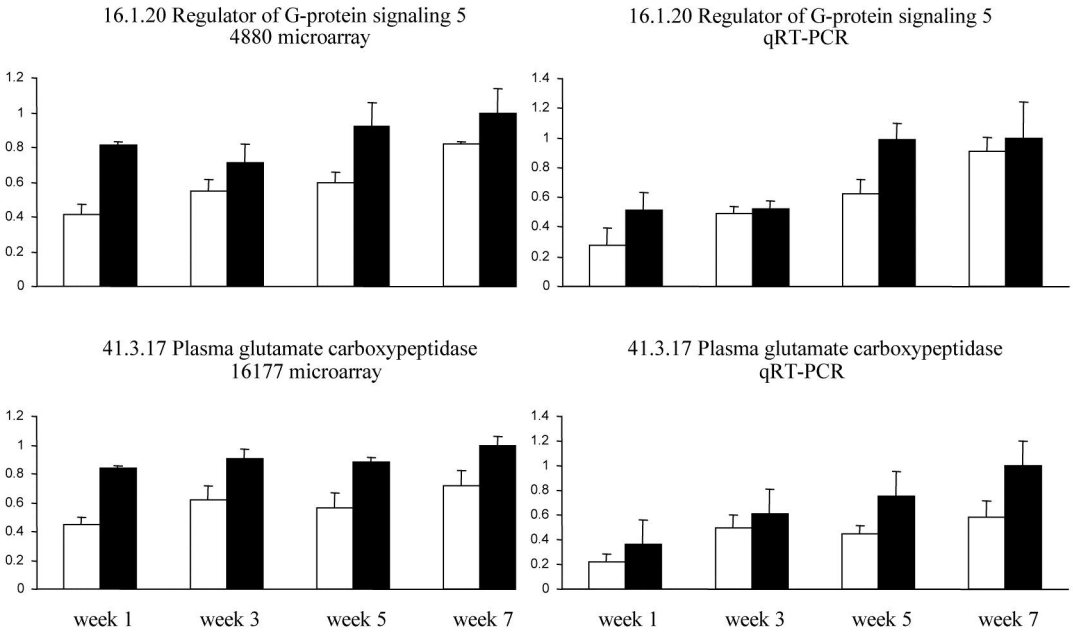

Figure 3. Gene expression always greater in the Fat line than Lean line. Relative gene expression levels were quantified by microarray (left) and real-time PCR (right). White bars are Lean; Black bars are Fat. Expression levels were normalized to the highest mean for each gene. GEO accession no. GPL 1731 ID and ENSEMBL BLAST hit name are given. Mean ± SEM of four replicate experiments. Y-axis is the relative expression level normalized to the highest mean.

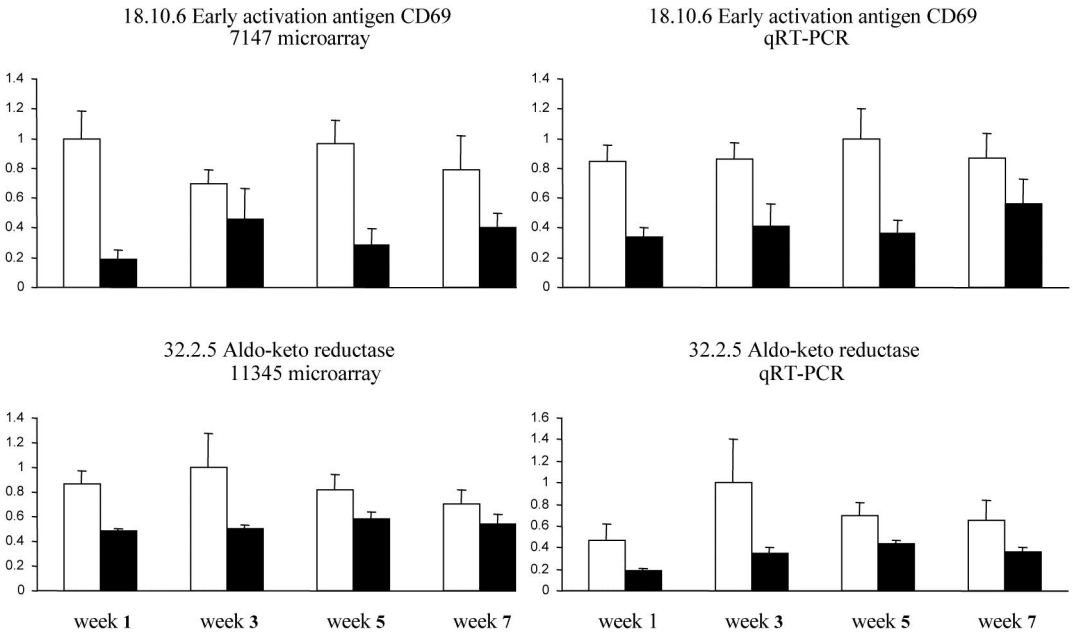

Figure 4. Gene expression always greater in the Lean line than Fat line. Relative gene expression levels were quantified by microarray (left) and qRT-PCR (right). White bars are Lean line; Black bars are Fat line. See Figure 3 for further details.

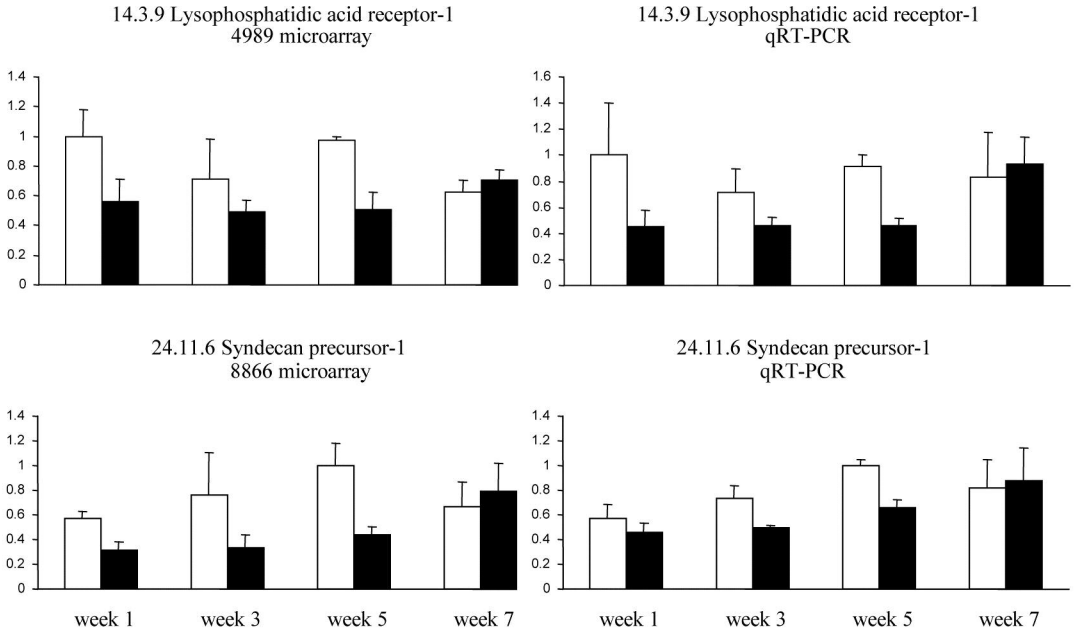

Figure 5. Early age gene expression (weeks 1 and 3) greater in Lean line than Fat line. Relative gene expression levels were quantified by microarray (left) and qRT-PCR (right). White bars are Lean line; Black bars are Fat line. See Figure 3 for further details.

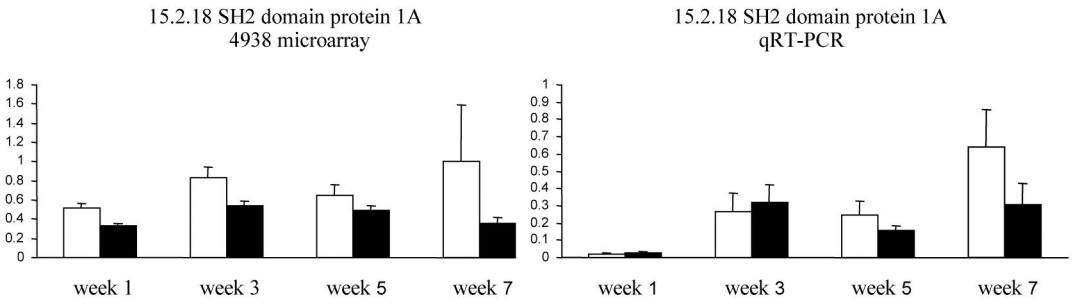

Figure 6. Late age gene expression (weeks 5 and 7) greater in Lean line than Fat line. Relative gene expression levels were quantified by microarray (left) and qRT-PCR (right). White bars are Lean line; Black bars are Fat line. See Figure 3 for further details.

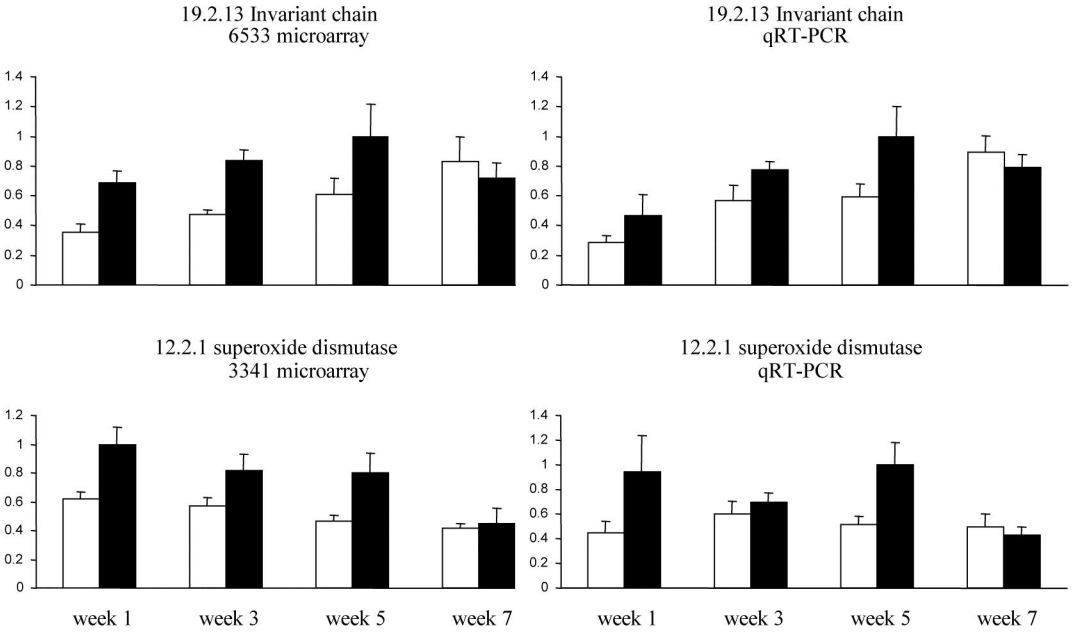

Figure 7. Early age gene expression greater in Fat line than Lean line. Relative gene expression levels were quantified by microarray (left) and qRT-PCR (right). White bars are Lean line; Black bars are Fat line. See Figure 3 for further details.

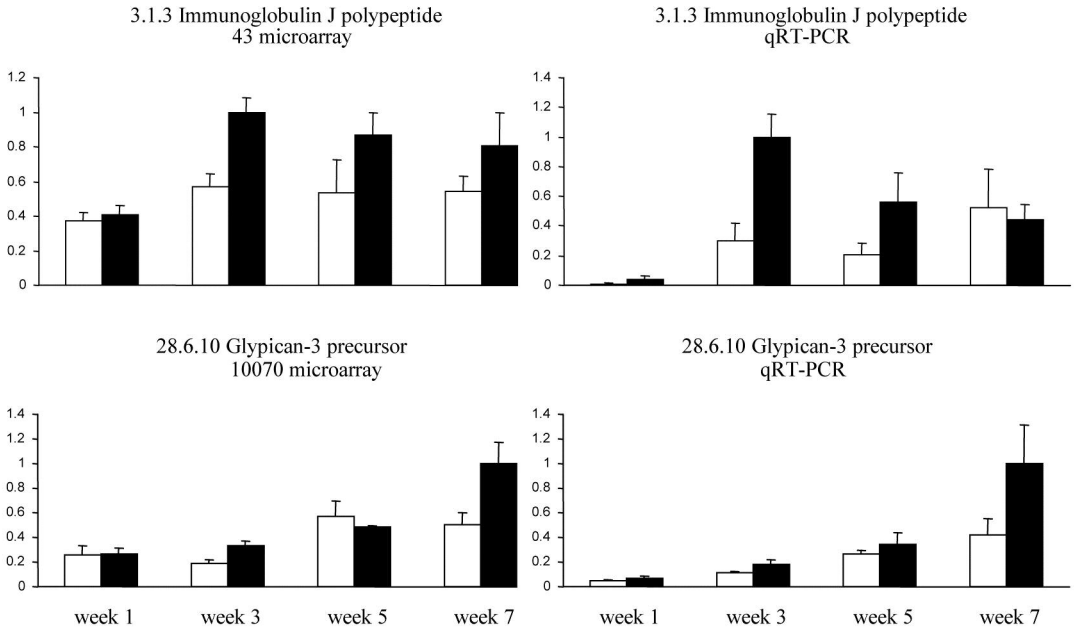

Figure 8. Late age gene expression greater in Fat line than Lean line. Relative gene expression levels were quantified by microarray (left) and qRT-PCR (right). White bars are Lean line; Black bars are Fat line. See Figure 3 for further details.

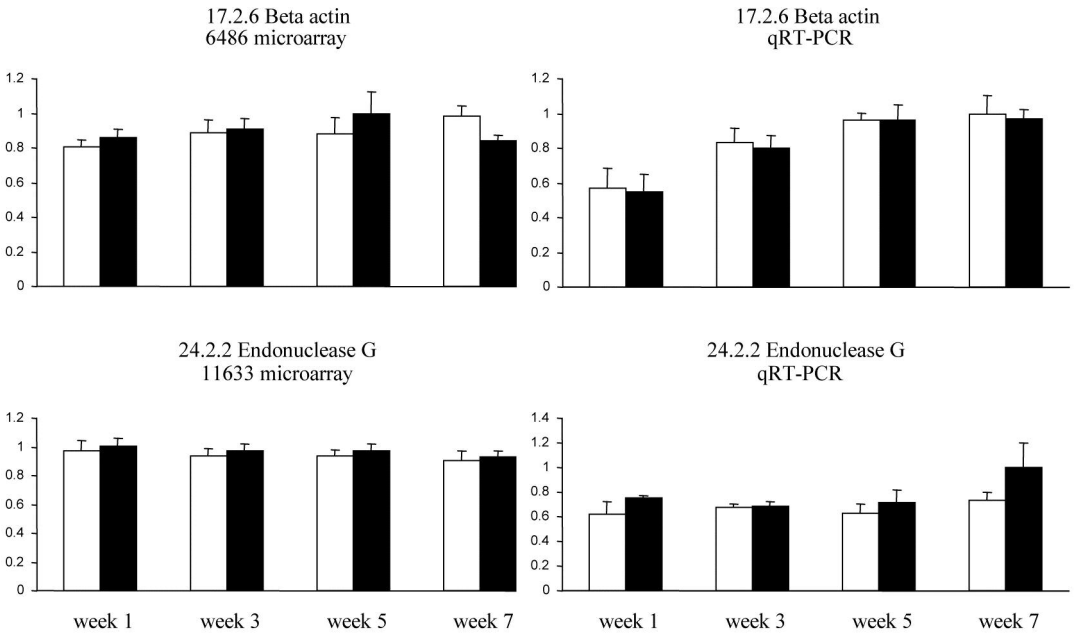

Figure 9. No difference in gene expression between the two lines. Relative gene expression levels were quantified by microarray (left) and qRT-PCR (right). White bars are Lean line; Black bars are Fat line. See Figure 3 for further details.

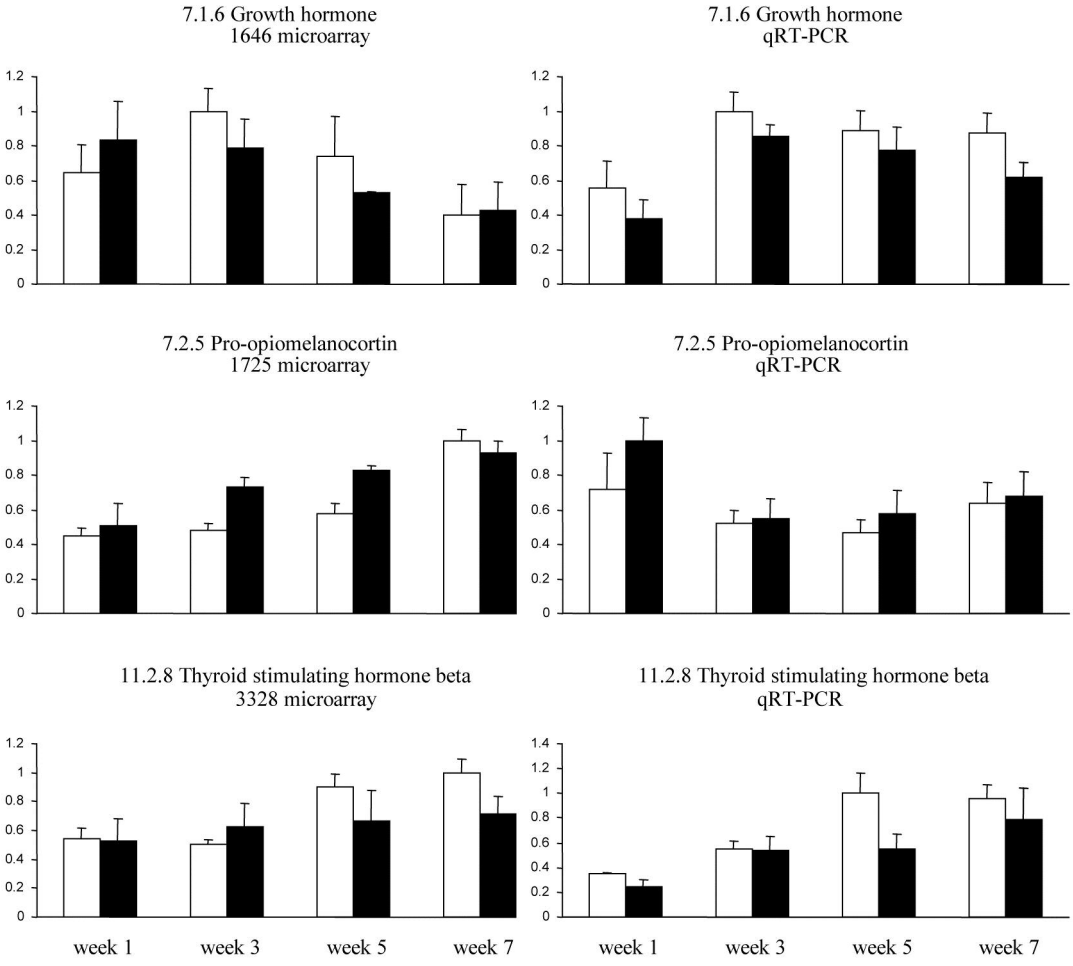

Figure 10. Gene expression profiles of growth hormone, pro-opiomelanocortin, and thyroid stimulating hormone beta as determined by microarray and qRT-PCR. Relative gene expression levels were quantified by microarray (left) and qRT-PCR (right). White bars are Lean line; Black bars are Fat line. See Figure 3 for further details.

overlapping transcript (GEO no. 15.3.6), and Clusterin (GEO no. 20.8.17) were chosen as candidates and expression profiles for these candidates were also verified by qRT-PCR (Figure 11).

Identification and genotyping of SNPs

Genomic sequences of the candidate genes were located within the chicken genome using the online database ENSEMBL (http://www.ensembl.org/Gallus_gallus/index.html), which also shows the location of known SNPs (Figure 12).

Regions of up to a 1000 bp containing known SNPs located within 5000 bp upstream of the first exon of 12 genes whose expression patterns were verified by qRT-PCR were chosen for sequencing to identify polymorphisms (*e.g.* SNPs). Although regulatory elements may be located throughout a gene, the upstream region was chosen as a systematic approach to identifying polymorphisms. The sole exception was Leptin receptor overlapping transcript, which was sequenced in the 3' untranslated region of the mRNA that contained numerous SNPs.

Seventeen genomic regions of the 12 candidate genes were amplified by PCR performed on genomic DNA from the F_0 generation (4 males and 7 females from each line), and the products were sequenced by the High-Throughput Genomics Unit (HTGU), Department of Genome Sciences, Univ. of Washington. The sequences that were obtained were assembled into contigs using Vector NTI software and examined for SNPs (see Figure 13 for a representative example). A SNP had to be detected in at least 4

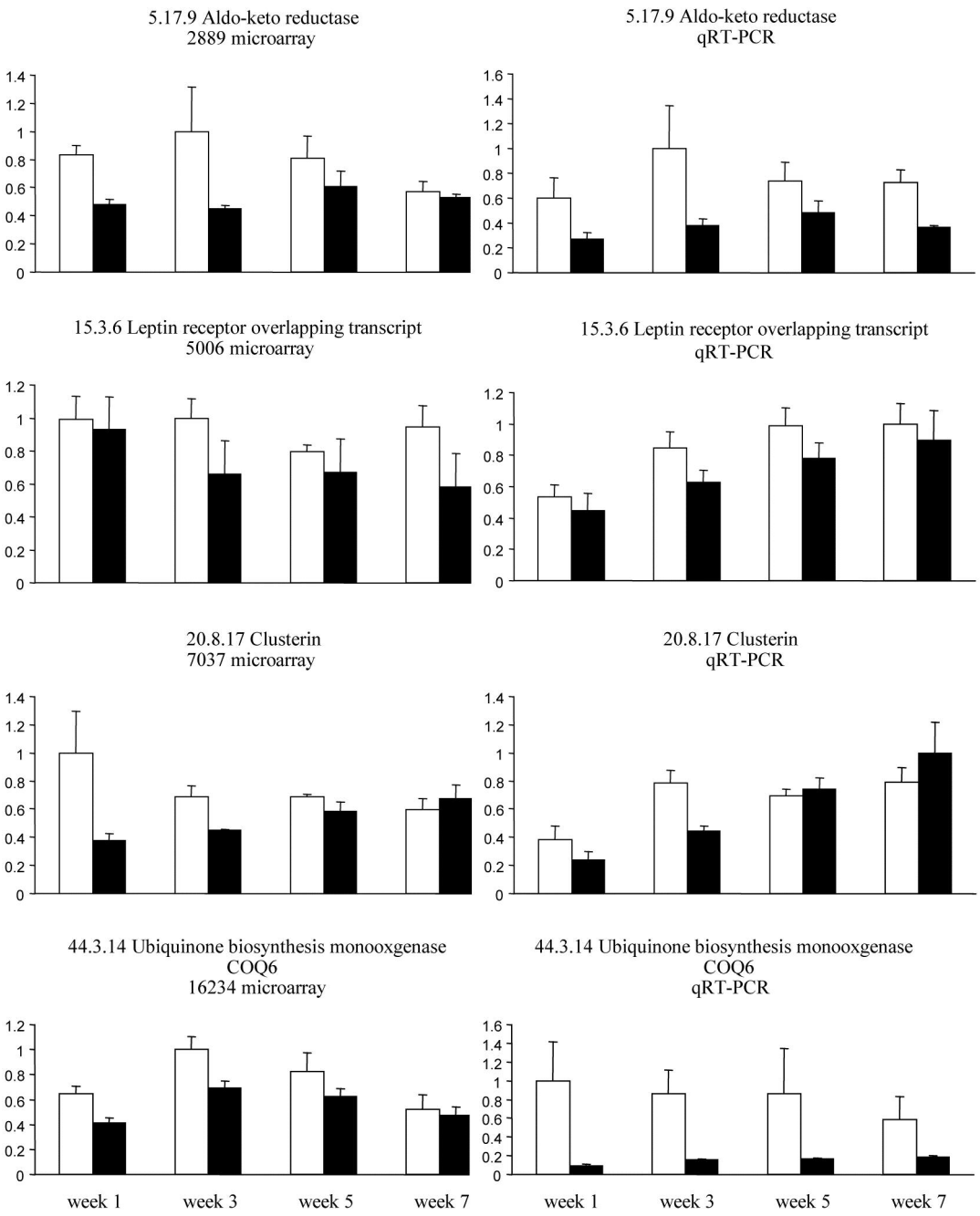

Figure 11. Gene expression profiles of aldo-keto reductase, leptin receptor overlapping transcript, clusterin, and ubiquinone biosynthesis monooxgenase COQ6 as determined by microarray (left) and real-time PCR (right). White bars are Lean; Black bars are Fat. See legend to Figure 3 for further details.

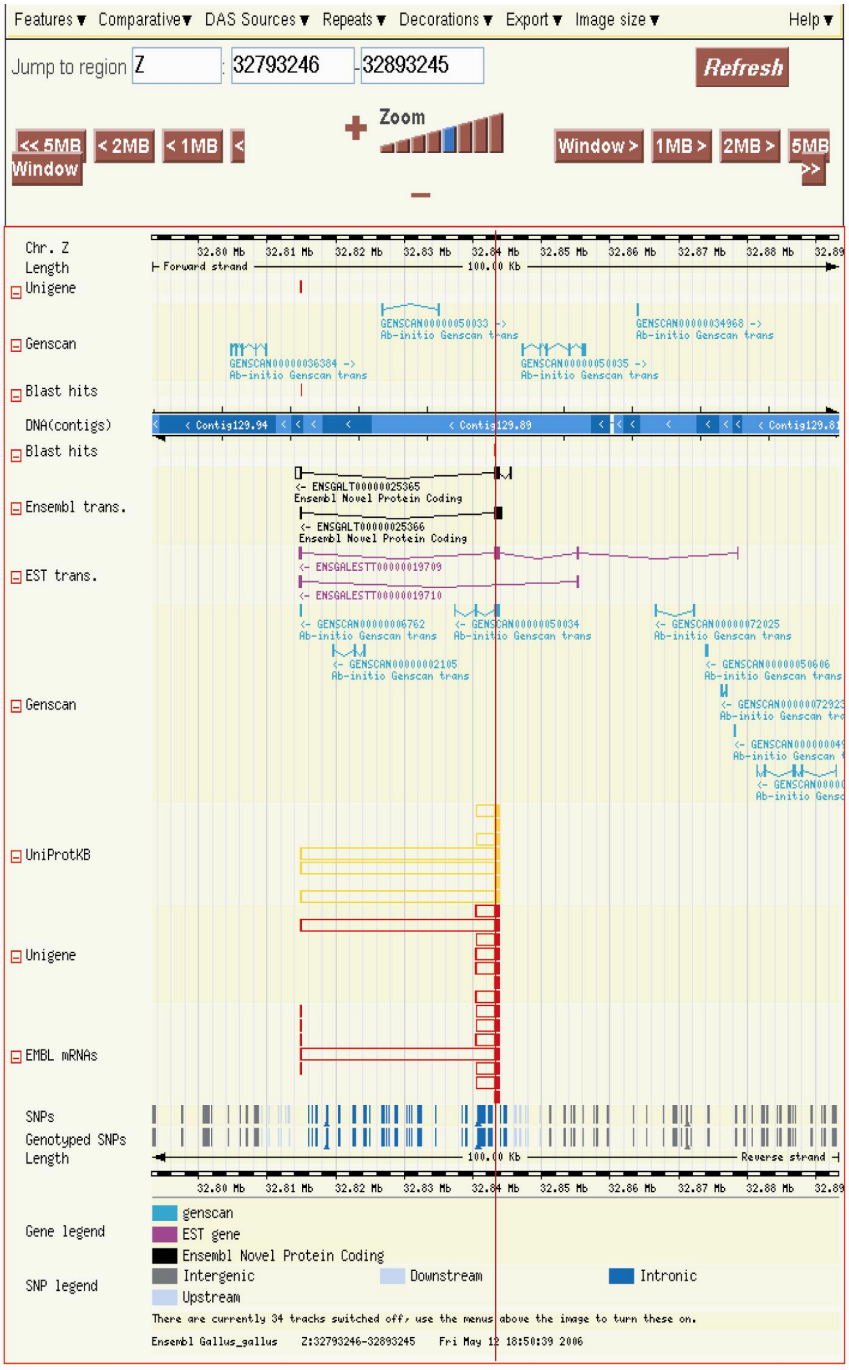

Figure 12. Screen capture of the genomic location of LPAR-1 from ENSEMBL Chicken *ContigView*. Genomic distance is indicated by the black and white bars at the top and bottom of the screen capture. Known SNPs are represented by vertical bars near bottom of screen capture.

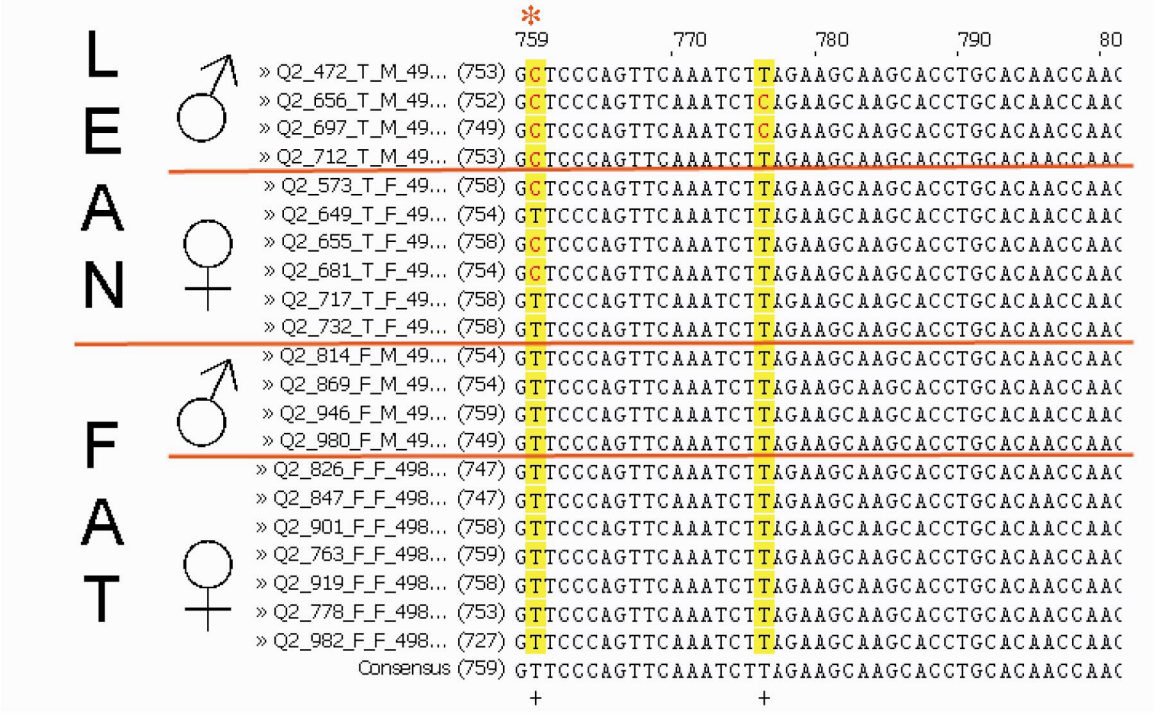

Figure 13. Identification of a SNP in the lysophosphatidic acid receptor-1 5' upstream region. Sequences were assembled into contigs using Vector NTI software. Asterisk highlights the location of the SNP.

animals within a line to be considered for further analysis. There were 11 SNPs in 5 genes identified that met this criterion (Table 18).

Confirmation of sequencing results

LPAR-1 was chosen to be further investigated as a candidate marker due to the fact that it had a large difference in SNP frequency in the F_0 generation between the two lines and it is involved with cell differentiation, including that of adipocytes (Pages, Girard et al. 2000). The frequency of the T/C SNP was significantly different between the 2 lines ($p \leq 0.001$, Fisher's Exact Test). To confirm that the sequencing results were accurate, PCR using allele-specific locked nucleic acid (LNA) primers was performed. PCR primers with LNA at their 3'-ends are highly specific and can be used to discriminate between two SNPs (Johnson, Haupt et al. 2004). Genotype was determined by the presence or absence of an allele-specific PCR band using the SNP-specific LNA primers (Figure 14).

The allele-specific PCR confirmed the results of the sequencing performed by HTGU. The Genotypes of the 22 F_0 birds are presented in Table 19. The sequence chromatograms indicated that birds 472 and 712 were heterozygous for the T/C SNP, and this was confirmed by PCR.

Identification of potential transcription factor binding sites

The SNP-specific consensus sequences returned for the LPAR-1 were searched for vertebrate transcription factor binding sites using the TRANSFAC (version 1.3) website (http://mbs.cbrc.jp/research/db/TFSEARCH.html; default score threshold = 85.0). TRANSFAC is a searchable database that identifies *cis*-acting elements and their

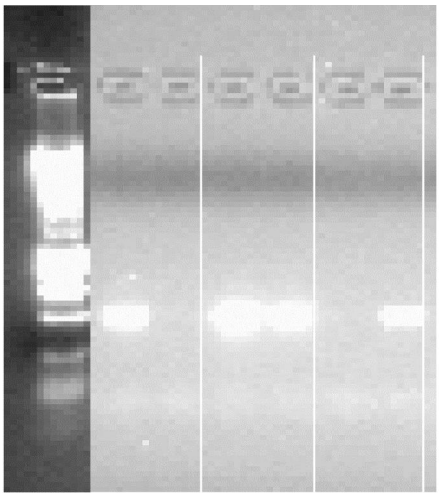

Figure 14. Confirmation of genotyping by Locked Nucleic Acid Primer PCR. Genotype was determined by the presence or absence of a primer-specific PCR product. All 3 possible genotypes are shown.

trans-acting factors (Heinemeyer, Wingender et al. 1998). The T-SNP consensus sequence did not return any transcription factor binding sites in the location of the SNP; however the C-SNP introduced a putative GATA-1 binding site (score = 87.3) (Figure 15). When the search score threshold cutoff is lowered to 75.0, an additional putative GATA-2 binding site is introduced in the same sequence (score = 79.4) (data not shown).

Genotyping the F_2 generation for the T/C SNP

The F_0 Lean and Fat lines were intercrossed to produce a F_2 generation, and abdominal fat percentage was measured at 9 weeks (n=637). The F_2 generation has a normal distribution of abdominal body fat percentage (data not shown). The animals in the tails of the abdominal body percentage distribution (48 leanest males, 48 leanest females, 48 fattest males, and 48 fattest females) were genotyped by LNA PCR (Figure 16; Table 20). Since the C-SNP introduces a putative GATA transcription factor binding site, animals were categorized into C-SNP positive (C/C and C/T) or negative (TT) genotypes (Table 21).

A binomial generalized linear mixed model with a logit link function (PROC GLIMMIX; SAS) was used to test the association of genotypes with the tails of the distributions. PROC GLIMMIX "fits statistical models to data with correlations or nonconstant variability and where the response is not necessarily normally distributed" (SAS Institute 2005). There was a significant association between the C-SNP negative genotype (TT) and the fat tail ($p < 0.05$, n = 189).

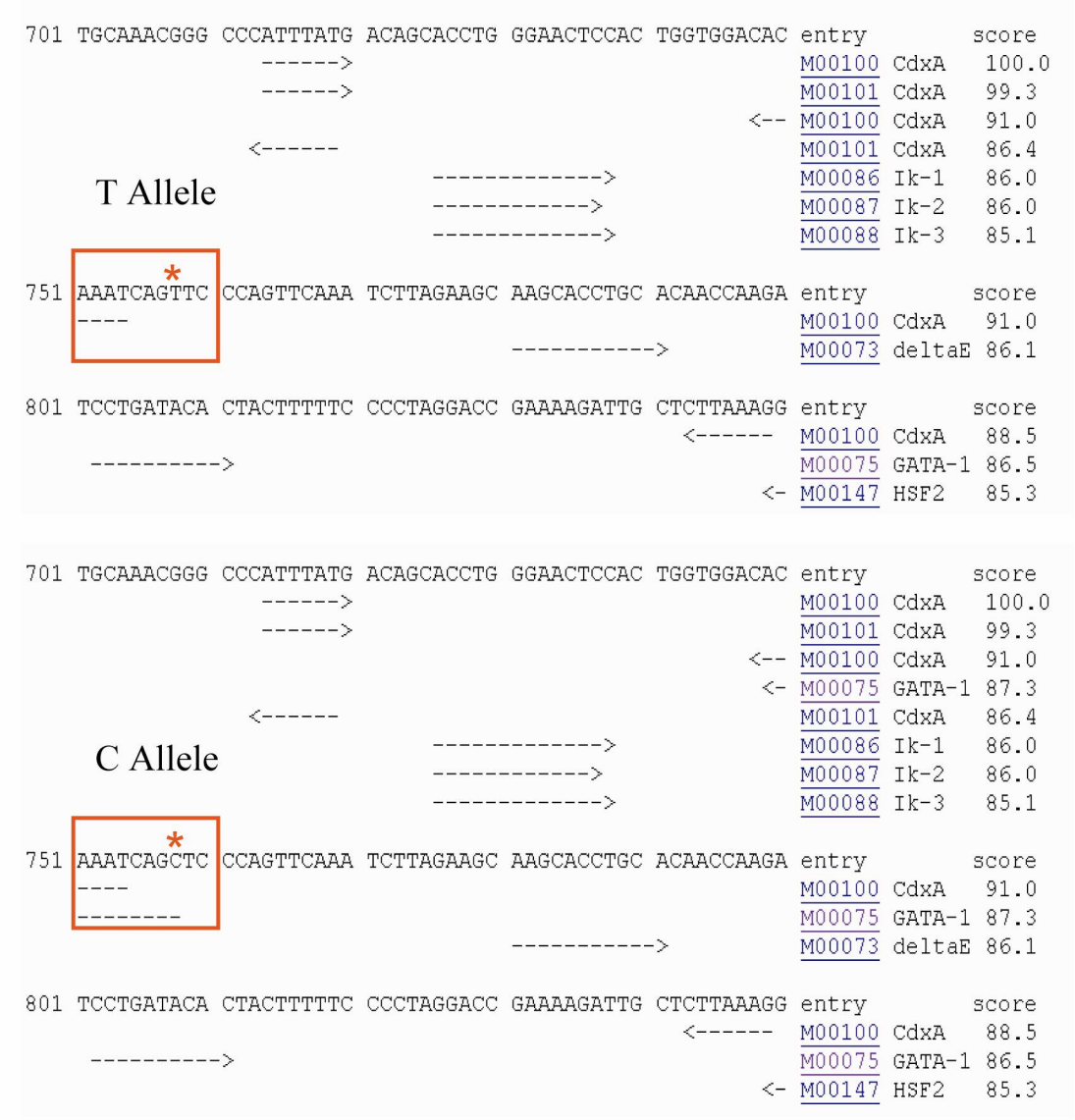

Figure 15. A T/C SNP introduces a GATA-1 binding site. Screen capture of the results from the transcription factor binding site search using TRANSFAC. The asterisk indicates the location of the SNP. The vertebrate transcription factor matrix was used, and the default score threshold (85.0) was applied.

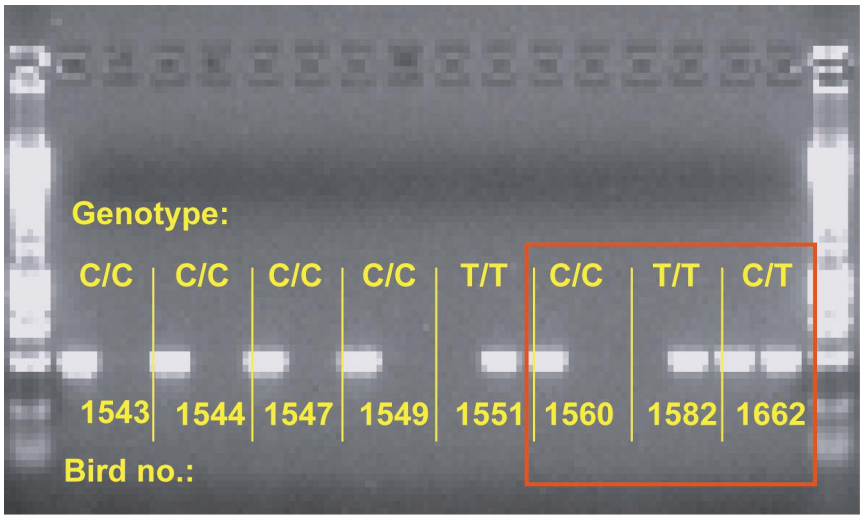

Figure 16. Genotyping of the F2 generation. Genotype was determined by the presence or absence of a primer-specific PCR product. All three possible genotypes are highlighted in box.

Discussion

The goal of this research was to identify polymorphisms in the chicken genome that were associated with adiposity. The Del-Mar 14K Chicken Integrated Systems microarrays were used to identify differentially expressed genes in the anterior pituitary gland of two lines of chickens that have been genetically selected for low and high adiposity. Over a thousand genes were identified as being significantly different by microarray analysis and nearly four hundred of these exhibited large enough fold changes in expression that they were examined further. Since microarrays measure relative gene expression, a bioinformatics approach was taken to identify polymorphisms in regions upstream of the first exon (potential *cis*-regulatory sites) that might affect transcription of differentially expressed genes. It is known that there are SNPs which are significantly different in frequency between the FL and LL (Fotouhi, Karatzas et al. 1993). DNA sequences of differentially expressed genes were BLASTed against the chicken genome to determine their genomic location and to identify known SNPs within 5000 bp of their first exon. Genomic DNA that contained putative SNPs upstream of twelve differentially expressed candidate genes was amplified by PCR and sequenced. Eleven SNPs in five genes were identified as potential candidate markers. The frequency of a SNP in the LPAR-1 upstream region was significantly different between the LL and FL, and this SNP introduced a potential GATA transcription factor binding site. The phenotypic tails of a F_2 generation created by intercrossing the LL and FL lines were genotyped for the SNP, and a significant association was found between the genotype and phenotypic tails.

The first objective of this project was to identify genes that were differentially expressed between the Lean and Fat lines. Gene expression profiles were obtained by

analyzing levels of mRNA in the pituitary gland over a time course during which adiposity becomes significantly different between the two lines. The difference in adiposity appears by 4 weeks of age (Leclercq, Blum et al. 1980), so pituitaries were collected on ages before, during, and after this divergence in adiposity in order to identify differentially expressed genes that may be involved in the development of body composition. After gene expression levels were quantified, ANOVA was used to identify genes which had significant differences in expression. Over a thousand genes were significantly different by line, and more than three hundred were different in their line-by-age interaction (many genes were significant by both line and line-by- age interaction. Almost fifteen hundred genes were significantly different by age, but since our primary goal was to identify differences between the lines, we did not explore these further. ANOVA uses the variances about means to determine significant differences. Two means might be very similar in magnitude, but if the variance about the mean is small enough, the means can be statistical different; conversely, two means may be quite different in magnitude but not be significant if their variances are large. To narrow down the list of genes to be investigated further, genes that did not exhibit greater than 0.68-fold differences in their mean $\log_2$ ratios were filtered out. This cut-off value was chosen so that there were not an overwhelming number of genes to analyze, but not to be so stringent that potentially important genes were lost. Each gene also had to have at least two replicates per age as to limit the influence of any outliers. Three hundred eighty six genes fulfilled these prerequisites for further analysis.

Next, genes were classified according to whether they were up or down regulated in a line at 1-, 3-, and 5-weeks of age. Week seven was not examined because the

difference in adiposity has already occurred by that time. Disappointingly, none of the expression levels of the three major pituitary hormones—GH, POMC, and TSH—involved in body composition was significantly different between the LL and FL in the microarray analysis.

All 386 genes were organized in 30 clusters of expression patterns in a 6x5 configuration using SOMs analysis. The 6x5 configuration was chosen (after evaluation of all geometric node configurations of 12 to 36 clusters) to minimize within cluster variation and between cluster redundancy. However, redundancy was still apparent among the clusters, so instead 7 simpler expression profiles were chosen to verify microarray results. Six profiles with different patterns were chosen, along with a seventh profile that exhibited no difference between lines. Genes were classified into the seven profiles based on their microarray expression patterns. To verify expressions patterns of genes within these profiles, qRT-PCR was performed and relative gene expression was quantified and compared to the microarray results. Following the primer design procedures used in the present study resulted in single melt curves in at least 85% of the qRT-PCR performed, indicating a single PCR product. Approximately 70% of the microarray expression profiles were verified by qRT-PCR.

The GeneCluster2 software allowed for the identification of markers for the LL and FL. The marker identification program has been used to classify cancer types using gene expression levels as determined by microarray (Golub, Slonim et al. 1999). The same approach was used to classify genes as markers of the FL or LL. A problematic drawback of this approach was the fact that replicates with missing values were not able

to be analyzed by the software. Still, it was useful to identify which of the genes that had full replicates were potential markers for each line.

In order to expedite the verification of the 7 different microarray expression patterns and verification of candidate gene profiles, genes were chosen that were differentially expressed or were identified as markers and that also fit into one of the six changing profiles. This allowed the process of verifying general microarray expression profiles and confirming expression patterns of candidate genes to be consolidated into one step. Eight candidate marker genes were also used to verify microarray expression profiles. With the goal of examining 12 genes as candidates, three more genes were chosen that were either differentially expressed or identified as markers. GEO no. 32.2.5 and GEO no. 5.17.9 are both aldo-keto reductase ESTs that were assembled into the same contig by the CAP3 program. As an internal control for the microarray, the expression profiles of aldo-keto reductase [GEO no. 32.2.5; Figure 4 and GEO no. 5.17.9; Figure 11] were confirmed by qRT-PCR using different primer pairs. The one gene that was not differentially expressed or a marker was putative ubiquinone biosynthesis monooxgenase COQ6 (GEO no. 44.3.14). This gene was chosen as a candidate based on its qRT-PCR profile, which indicated it was highly expressed in the LL compared to FL. It turns out that the DNA printed in the spot is not COQ6 (the clone was amplified from the library and sequenced). The primers were designed for COQ6 and so, although the microarray profile was not verified, this appeared to be an interesting gene.

The 12 candidate genes are listed in Table 16, and their functions are briefly summarized below. Immunoglobulin (Ig) J polypeptide (GEO no. 3.1.3; microarray 43; Accession no. BAA83785) encodes the peptide that links polymeric IgG molecules

together (Takahashi, Iwase et al. 2000). Aldo-keto reductase (GEO no. 5.17.9; microarray no. 2889; Accession no. CAC40811) is an enzyme that functions to oxidize alcohols and reduce retinoids (Crosas, Cederlund et al. 2001). This is a potentially interesting gene because Crosas *et al.*speculate that the enzyme functions as a metabolic barrier to alcohols and aldehydes present in foodstuffs. Extracellular superoxide dismutase 3 (SOD3) (GEO no. 12.2.1; microarray no. 3341; Accession no. NP_003093) is a secreted enzyme that eliminates oxygen radicals in the plasma. SOD3 has been shown to provide protection from oxidative stress in birds (Altan, Pabuccuoglu et al. 2003). Regulator of G-protein signaling 5 (GEO no. 16.1.20; microarray no. 4880; O15539) is a member of a family of proteins that modulate G protein-coupled receptor signaling by increasing the rate of GTP hydrolysis (Zhong and Neubig 2001). This protein is interesting considering that most of the hypothalamic trophic factors bind to G protein-coupled receptors in the pituitary. Lysophosphatidic acid receptor-1 (GEO no. 14.3.9; microarray no. 4989; NP_446388) is an especially promising candidate gene given that signaling through the receptor inhibits adipocyte differentiation (Pages, Daviaud et al. 2001; Simon, Daviaud et al. 2005). Leptin receptor overlapping transcript (GEO no. 15.3.6; microarray no. 5006; Accession no. AAF63411) is an alternate transcript of the leptin promoter that encodes for a protein structurally unrelated to the leptin receptor isoforms (Bailleul, Akerblom et al. 1997). Leptin is an important regulator of energy homeostasis; leptin deficient *ob/ob* mice and leptin receptor deficient *db/db* rats exhibit hyperphagia, decreased energy expenditure, insulin resistance, and obesity (Jazet, Pijl et al. 2003). It is possible that leptin receptor expression, and therefore response to leptin, is regulated by transcription of the alternate transcript.

Clusterin (GEO no. 20.8.17; microarray no. 7037; Accession no. AAD17257) is also know as apolipoprotein J, and as a apolipoprotein it is involved in lipid transport in the plasma and it is a marker for HDL (de Silva, Stuart et al. 1990). A gene that expresses a protein involved in lipid transport is interesting, since the triglycerides in the adipose tissue in chickens are synthesized in the liver and transported in the plasma. Early activation antigen CD69 (GEO no. 18.10.7; microarray 7147; gene 17.5; Accession no. M88072) is a transmembrane protein belonging to the major histocompatability (MHC) family (Bernot, Zoorob et al. 1994). Syndecan-1 (GEO no. 24.11.6; microarray 8866; Accession no. AAA60605) and glypican-3 (GEO no. 28.6.10; microarray 10070; Accession no. 004475) are both heparan sulfate proteoglycans that are involved in lipoprotein clearance and metabolism (Kolset and Salmivirta 1999). Again, genes that encode proteins that are involved in the transport and uptake of triglycerides would be of interest in adipocyte hypertrophy. Blood plamsa glutamate carboxypeptidase (GEO no. 41.3.17; microarray no. 16177; Accession no. AAD31418) is a circulating enzyme that may be involved in the hydrolysis of circulating peptides (Gingras, Richard et al. 1999). Similar to Putative ubiquinone biosynthesis monooxgenase COQ6 (GEO no. 44.3.14; microarray no. 16234; Accession no. XP_126972) is a peripheral membrane protein associated with the matrix of the mitochondria that is involved in the biosynthesis of coenzyme Q in yeast (Gin, Hsu et al. 2003). COQ6 is potentially interesting as a candidate gene due to its role in aerobic respiration.

Seventeen genomic regions of the 12 genes were sequenced; sixteen regions were upstream of the first exon, with 2 upstream regions for aldo-keto reductase, LPAR-1, COQ6, SOD 3, and syndecan-1 sequenced. Leptin receptor gene related-protein was

sequenced in the 3' untranslated region as that contained numerous SNPs that may affect mRNA stability. Not all of the sequencing was successful, but the contigs that were assembled demonstrated 11 SNPs in 5 genes showing promising differences in SNP frequencies between the lines: SOD3, LPAR-1, aldo-keto reductase, glypican, and syndecan (Table 17). Glypican and syndecan are interesting because they are involved in lipoprotein transport. Aldo-keto reductase may be involved in regulating the expression of GH, TSH, and ACTH in the pituitary (Angioni, Lania et al. 2005). SOD3 and LPAR-1 contained SNPs upstream that were highly significant in association between the lines ($p < 0.001$). SOD3 (genomic location: chromosome 4; 73,901,427-73,902,577) is an especially interesting candidate because it is located within a known Quantitative Trait Loci (QTL) for abdominal body fat on chromosome 4 (flanking markers MCW240-LEI073, position 201-243 cM, genomic location 68517805-80227860) (Lagarrigue, Pitel et al. 2006). However, LPAR-1 was chosen for further investigation due to the fact that it is directly involved in regulating adipocyte differentiation (Pages, Daviaud et al. 2001; Simon, Daviaud et al. 2005). LPA is a phospholipid found in the serum and is produced by the hydrolysis of cell surface phosphatidic acid by phospholipase A_2 or lysophosphatidylcholine by autotaxin/lysophospholipase D (Guo, Kasbohm et al. 2006). Many biological effects are mediated by LPA through multiple G-protein coupled receptor pathways which include stimulation of PLC, inhibition of adenyly cyclase, and Ras-MAPK (Moolenaar, van Meeteren et al. 2004). For example, LPA reduces triglyceride uptake and expression of lipogenesis genes in preadipocytes; removal of LPA from culture medium induces preadipocytes to differentiate. LPAR-1 knockout mice become significantly fatter than wild-type mice but without any difference in food

intake—a similar situation to what is seen in the FL and LL. LPAR-1 is close to, but not included within a known QTL for abdominal fat on chromosome Z (flanking markers LEI0111-LEI0075, position 127 cM, genomic location 22950349-31399653) (Ikeobi, Woolliams et al. 2002). The C-SNP at genomic position 32846269 on chromosome Z introduces a GATA transcription factor binding site. The highest TRANSFAC score was for GATA-1 (87.3), an overlapping GATA-2 site was identified when the threshold score was lowered (79.4), and there is also an adjacent GATA-3 site (77.8). GATA transcription factors are involved in cellular differentiation. GATA-1 is best known for its role in erythropoiesis (Leonard, Lim et al. 1993). GATA transcription factors are know regulators of adipocyte differentiation (Tong, Dalgin et al. 2000). Defective GATA-2 and GATA-3 expression is associated with obesity. GATA-2 and GATA-3 expression inhibits preadipocyte terminal differentiation through suppression of peroxisome proliferator-activated receptor γ (PPARγ). PPARγ is a member of the nuclear receptor superfamily and plays an important role in adipogenesis and lipogenesis, upregulating genes involved in fat uptake and lipogenesis (Kersten 2002). The fattest and leanest animals were genotyped by allele-specific PCR for the T/C SNP in the upstream region of the LPAR-1, and a significant ($p < 0.05$) association was found between the GATA negative genotype (TT) and the fattest animals.

It has not been shown that the LPAR-1 is regulated by GATA transcription factors, but it is tempting to speculate that it might be. GATA-2 and GATA-3 transcripts are expressed in the mouse pituitary and presumably in the chicken also (Charles, Saunders et al. 2006). Although the LPAR-1 SNP was found in an analysis of pituitary gene expression, the same SNP may regulate differential expression in adipocytes or

hepatocytes. It is unknown at the present time whether this is true. A GATA transcription factor binding site might upregulate the expression of LPAR-1, and increased signaling through the receptor may inhibit the maturation of preadipocytes into adipocytes.

An analysis of the differentially expressed genes by Gene Ontology annotation [BioRag (Bioresource for array genes) at www.biorag.org] indicated that the two largest categories of biological functions were signal transduction and DNA-dependent regulation of transcription. This is not surprising considering the pituitary's central role in integrating the hypothalamus and the target tissue axes. The pituitary must respond to hypothalamic trophic factors and then regulate the production and release its own hormones. Although there were no significant differences between the lines in expression of GH, TSHβ, and POMC message, this does not mean these hormones do not play a role in the differences in adiposity between the lines. On a physiological level, quantities of message produced are not necessarily indicative of amount of protein. For example, in the bacterium *Desulfovibrio vulgaris* mRNA abundance accounts for less than 30% of the variation in protein abundance (Nie, Wu et al. 2006). There are many processes that affect the amount of hormone that would actually be secreted into systemic circulation. Even after secretion into the blood, the availability and uptake of hormones are affected by binding proteins and metabolism and/or clearance by the liver or kidneys. GH levels in the blood are hard to interpet due to its pulsatile secretion. There is no direct assay for chicken TSHβ because there is no homologous antibody. Chicken ACTH is measureable, but it has not been measured in the FL and LL. Thus, a lack of significant differences in expression levels of GH, TSHβ, and POMC does not mean that

there are no differences with regard to the physiological effects of these hormones in the FL and LL. The genes on the Del-Mar 14K Integrated Systems microarrays represent about half the genes in the chicken genome. It is possible that there are non-represented genes differentially expressed in the pituitary that play roles in regulating adiposity.

Quantitative traits, such as adiposity, are caused by an unknown number of known and unknown genes exerting known and unknown effects. It is possible that the SNP that introduces the GATA factor binding site upstream of the LPAR-1 gene may increase its expression, which in turn makes the preadipocyte more resistant to differentiation; conversely, loss of the binding site may make preadipocytes more susceptible to differentiation. This difference in differentiation disposition may be one factor regulating body composition in chickens. LPAR-1 therefore may be useful as one marker to be used in marker-assisted selection against adiposity.

Chapter 4: Future directions

The approach taken was successful in identifying a polymorphism upstream of the first exon of the LPAR-1 gene that was significantly associated with adiposity. The entire F_2 generation of chickens should be genotyped for the T/C SNP by allele-specific PCR. Knowing the genotypes of all the animals would allow for more powerful statistical tests for associations between phenotype and genotype.

The higher levels of LPAR-1 mRNA in the pituitary do not necessarily mean a higher numbers of cell surface receptors. Since the pituitary is an endocrine gland that secretes hormones, the physiological effects of LPA treatment on hormonal gene expression, production, and secretion could be investigated by qRT-PCR, immunocytochemistry, and radioimmunoassay, respectively, in each chicken line. Chicken pituitaries could be cultured with differing doses of LPA to determine what effects it has on global gene expression using microarrays. LPA could also be injected *in ovo* and its effects investigated.

To investigate the function of LPAR-1 *in vivo*, tissue-specific LPAR-1 Floxed mice could be created. The LPAR-1 gene could be flanked by LoxP sites using homologous recombination. CRE expression could be placed under control of tissue-specific promoters. To investigate LPA-1 conditional knockout in the pituitary, CRE could be expressed behind the α-glycoprotein subunit. In muscle or liver, CRE could be expressed behind creatine kinase promoter or albumin promoter, respectively. There are a substantial number of tissue and developmental stage CRE mice available; a searchable online database is available (CreXMice; http://nagy.mshri.on.ca/PubLinks/indexmain.php#). Once the LPAR-1 gene or any other

gene of interest is Floxed, it can be conditionally knocked out in a variety of tissues and developmental stages by crossing with the CRE mouse strain of interest.

The T/C SNP introduced a high scoring GATA-1 binding site according to the TRANSFAC analysis, but the same site also binds to GATA-2 *in silico*, which is expressed in the pituitary. On a molecular level, does GATA-2 bind to the upstream transcription factor site? Chromatin immunoprecipitation assays could be performed, and if there is no antibody against chicken GATA-2, one could be produced. This would test whether GATA-2 binds to that region of the genomic DNA. If GATA-2 does bind, its effects on transcription could be investigated by cloning the upstream region into a luciferase expression vector and treating transfected cells with LPA. LPAR agonists and antagonists exist, and their effects could be tested on pituitary cell function *in vitro* or *in vivo* (Gududuru, Zeng et al. 2006). Consequently, these types of experiments could be performed for other candidate genes that are later identified.

The upstream region of superoxide dismutase contains three SNPs that have different frequencies between the FL and LL, one of which is highly significant ($p<0.001$, Fisher's Exact Test). SOD3 is especially interesting since it is located in a known QTL for abdominal body fat. Allele-specific PCR should be performed to confirm the F_0 genotyping for all three SNPs, and then the tails of the F_2 generation should be genotyped. If there are significant associations between the genotypes and the tails, the entire generation should be genotyped. This genotyping also can be done for the other genes that have upstream SNPs that differ significantly between the two lines.

Since these chicken genes have mammalian orthologs, the same genes in mouse, rat, and human can be investigated to see if there are similar SNPs in the upstream

regions. Approximately 1700 bp upstream of the human LPAR-1 first exon is a known SNP that introduces a GATA-3 binding site. The upstream regions of the other genes can be examined similarly for SNPs that introduce or remove transcription factor binding sites. Other potential regulatory regions can also be examined. Are there variations present in the 3' untranslated regions of the mRNAs that might affect message stability? Since microarrays reflect steady state mRNA levels, the levels of actual proteins for genes identified can be examined. This may provide a more accurate picture of the physiological processes that are actually occurring in the cell.

The LPAR-1 marker could be used for animal selection in commercial breeding programs. But complex traits such as adiposity are influenced by many genes. Making the dissection of the genetic architecture of a complex trait like adiposity difficult is the fact that genes do not exert their effects in isolation. Genes interact with other genes in complex networks. Multiple loci that affect a trait can have different effects on that trait depending on epistatic interactions of combinations of alleles at those loci (Carlborg, Jacobsson et al. 2006). Not only do alleles that could be markers for adiposity need to be identified, their relationships with the genetic architecture need to be elucidated in order to more fully understand the regulation of a complex trait like adiposity. As more genes that affect adiposity, and their relationships with other alleles that are involved in adiposity, are identified, the further marker-assisted selection is advanced. A panel of genes known to be involved in regulating adiposity could be developed for easy assay. PCR could be used to genotype potential breeders for a number of genes that are markers for adiposity and be selected against in breeding stocks.

The identification of novel markers for adiposity will provide a means to genetically select and produce leaner chickens, which is beneficial to both to the producer and to the consumer. In addition, genetic markers for adiposity in the chicken may lead to new insight into human obesity.

Appendix

Table1. Primer sequences for qRT-PCR (GEO platform GPL1731 no., gene name, forward sequence, reverse sequence).

GEO no.	Gene name	Forward primer	Reverse primer
3.1.3	IgJ	CCACATCCCCACT CAGAACC	ACCCCCCAGCTCAAT TTCTAC
3.14.9	hypothetical protein LOC421485	ACACGAAGAGTG ATTGGAACAGC	CATCTCCTCCTCCGT GTCTACG
5.17.9	Aldo-keto reductase	GTATTAACACCTG CAGTTTCAGCG	AAGAAGAAAGTGGG ACTCGGC
7.1.6	Growth hormone	TTCAAGAAGGATC TGCACAAGGT	CTCAGATGGTGCAGT TGCTCTCT
7.2.5	Pro-opiomelanocortin	AGGGACCTCAGG GATCATCAA	TGTTCAAGGGCAGGT TGGA
11.2.8	Thyroid stimulating hormone	CTACCCCGTGGCC ATAAGCT	TCTGTGGCTTGGTGC AGTAGTT
12.2.1	Superoxide dismutase	CCAGTGATGGCTG ATAATGAGACT	CTATTTTGGAGCTGG GCTTCA
14.3.9	LPA receptor-1	CAGTTCTGGACCT CGCAGGA	AGCAGATTATGAAA GCACCAAGC
15.2.18	SH2 domain protein 1A	TACGGGACAGCG AGAGCATC	TCTGTCTTGGATACT CGGTAGGTATAAAC
15.3.6	Leptin receptor overlapping transcript	TCTCTCATGAAGT CTCACAGAGGAA	CAATTCAGGCACTTC ACACACA
16.1.20	Regulator of G-protein signaling 5	AGCTCCTGCAGAA CCCCTATG	CACCCAGAACTCGAC GTTCTC
17.2.6	Beta actin	TTCTTTTGGCGCT TGACTCA	GCGTTCGCTCCAACA TGTT
18.10.6	Early activation antigen CD69	ACACGAAGAGTG ATTGGAACAGC	CATCTCCTCCTCCGT GTCTACG
19.2.13	Invariant chain	GGATGACATGCTG GGCAAC	ACTTGTGCATCCAGG TCTCAAA
20.8.17	Clusterin	AAGACCAGCAAG GAGCACCA	TCCAAGGCCAGCTTC ACTG
24.11.6	Syndecan-1 precursor	ATCAGTGCCTTCT CTACAAGCCC	CATGTACGCACAAGT GTTTCTTAGG
24.2.2	Endonuclease G	TATGGAAGGCATC AGAGAACTTGAC	CACGCACAAAGGGA GCAATTA
24.6.19	Pleiotrophin	CCACTGAGGCTGG AAAGAAAGA	TGGGCACACAGACA CTCCAC
28.6.10	Glypican-3 precursor	CCTTGTCATCCAG AATGCTGC	TCCTAAACATGCTGT TGGTGAAGT

32.2.5	Aldo-keto reductase	GGAACCTATGCTG CTGGAGG	TGAACCGGATCAGA ACCTGG
41.3.17	Plasma glutamate carboxypeptidase	AGAAGTTTATGAC ACTGCAGATGGA	TGAGGTCATCACGCA AGCTG
44.3.14	Ubiquinone biosynthesis monooxgenase COQ6	CTTCAACGGGCAA GATCTGG	CACCTATCAAGGAG ACGTTGTGC

Table 2. Primer sequences for the amplification of F_0 genomic DNA. (GEO platform GPL1731 no., gene name, forward sequence, reverse sequence).

GEO no.	Gene name	Forward primer	Reverse primer
3.1.3	IgJ	GGCCAGGGATTCCGAAATT	CCTCTGTGATGCTCCTTCACATTA
5.17.9	Aldo-keto reductase	GAAGTGGTCAGCACACATAGCAG	CCGAGTTTACACGTCCCCTC
5.17.9	Aldo-keto reductase	TTTGCCCAAATGTAAGGAGAGAGGC	GGTCTTTGCTTTTATGGCTCCAGCT
12.2.1	Superoxide dismutase	CTTGTGTGCAGGCTTTGGGGAAA	GAAACAAAACTGTGTGCTATGGGGA
12.2.1	Superoxide dismutase	TAAGGACACACCTTCTTGCTGCTCG	TCTCGGCATAAGAAAAGGGTGAAGA
16.1.20	Regulator of G-protein signaling 5	GCTCAGACCGAGAGGCATCT	GCTCCCCTTCCGACAGCTATATAG
14.3.9	LPA receptor-1	TGAATAGGTGTCGGCTGTAGAAGCA	TGCTCTGCTGGTGTAAAGGATTCTG
14.3.9	LPA receptor-1	TCGTCAGTGCTTGCAGTTCTAAA	TGGAGTAAGGAACCGGACCAA
15.3.6	Leptin receptor overlapping transcript	TGCCTGATCCTGCACGTATC	TCACATCAAGTATTAGTGCACGCA
20.8.17	Clusterin	TGGTGGATTCCCATGTATGCTTTC	CAACCTGACCCTGTCCAATGAAGG
18.10.6	Early activation antigen CD69	GCTCAGTTCAGCGAGGCTCAT	CCCGGCATTACCTCACTGAGA
24.11.6	Syndecan-1 precursor	ACAGTCCCTCATCAGTTATGTAGGC	GGATCCCGTTAGCTACTGTAGGTGT
24.11.6	Syndecan-1 precursor	GTGTCAGCATCCCAGGAACC	AGGACAAGCAGTAGCGCTGC
28.6.10	Glypican-3 precursor	GGAGAAGGGAGAAGCTCTTTGCAAT	AAGAAAAAAGCATTCCTGGAAAGGC
41.3.17	Plasma glutamate carboxypeptidase	CCGTTTGTTCTACAGGTTCAACCA	CAGCTATCTCATTTCTGATGCCTTC
44.3.14	Ubiquinone biosynthesis monooxgenase COQ6	CCAAACACCAGAGCTCCTAAGAC	TGCCAATTGAAACTTGCTAGCA

44.3.14	Ubiquinone biosynthesis monooxgenase COQ6	CCGTTTGTTCTACAGG TTCAACCA	CAGCTATCTCATTTC TGATGCCTTC

Table 3. Differences of metabolism between FL and LL chickens. 0, no difference; +, fat superior to lean; -, fat inferior to lean. (Leclercq 1988).

Energy metabolism	Diff
Basal metabolic rate	0
Maintenance requirement	0
Efficiency of fat and protein gain	0
Body temperature	0
Energy gain as lipids/energy gain	+
Carbohydrate metabolism	
Plasma glucose (fed or starved state)	-
Glucose tolerance test	0+
Liver glycogen	0
Lipid metabolism	
De novo lipogenesis (liver)	+
Plasma free fatty acids (fed or starved)	0+
Plasma VLDL (starved or fed states)	+
Plasma triglyceride (fed state)	+
Plasma HDL	+
Plasma phospholipid	+
LPL per abdominal pad (AF)	+
LPL per adipocytes (AF)	0
Volume per adipocytes (AF)	+
Number of adipocytes (AF)	+
Protein metabolism	
Protein gain/protein ingested	-
Plasma free amino acids	-
Plasma uric acid	0+
Uric acid excretion	+
Hormonal profile	
Insulin (fed state)	0+
Insulin (glucose tolerance test)	+
Insulin (meal test)	-
T_4	+
T_3 (fasted)	0-
T_3 (fed)	-
Corticosterone (fed or fasted)	0
GH	0+
Glucagon	0
Sensitivity to exogenous corticosterone	0
Sensitivity to exogenous insulin	0+

Table 4. Composition of Del-Mar 14K Chicken Integrated Systems Microarray. *Spot category* represents category; *No. of spots* is the number of spots in that category.

Spot category	No. of spots
Microarray	19200
Unique cDNAs	14053
Blanks	971
Oligos	387
Reproductive	2008
Fat	4800
Liver	2635
Muscle	2398
Neuroendocrine	5929
Salmon sperm DNA	8
Pre-mRNA splicing factor	8
Na+/K+ ATPase	8
GAPDH	8
Dynactin	8
B-tubulin	8
TEF1a	8
Sodium pump 3	8
β-actin	8

Table 5. Log_2 ratios of 386 significantly different genes with greater than 0.68-fold differences in log_2 ratios between the highest and lowest mean. *Spot* is the Del-Mar 14K Chicken Integrated Systems microarray individual spot number; *GEO no.* is the GEO accession no. GPL1731 ID; *Lean 1* through *Fat 7* are the normalized log_2 ratios for each line at 1-, 3-, 5-, and 7-weeks of age.

Spot	GEO no.	Lean 1	Lean 3	Lean 5	Lean 7	Fat 1	Fat 3	Fat 5	Fat 7
35	2.1.15	-1.470	-0.502	-1.002	-0.524	-1.019	0.421	0.039	0.011
43	3.1.3	-1.083	-0.475	-0.755	-0.588	-0.970	0.352	0.113	-0.037
159	4.2.19	-0.021	-0.180	-0.307	-0.286	0.323	0.459	0.688	0.196
224	4.3.4	0.003	0.084	-0.300	0.273	-0.172	0.153	0.394	0.036
238	4.3.18	0.892	0.412	0.555	0.430	0.139	-0.130	0.098	0.240
264	2.4.4	-0.099	0.294	0.431	0.200	-0.934	-0.048	-0.187	-0.350
284	3.4.4	-0.340	-0.039	0.374	0.082	-0.722	-0.495	-0.033	-0.059
292	3.4.12	0.856	0.031	-0.098	0.026	-0.046	-0.142	0.085	-0.328
310	4.4.10	-0.193	-0.139	0.202	0.000	-0.592	-0.350	-0.124	-0.068
321	1.5.1	0.942	0.664	0.284	-0.048	0.360	0.371	-0.197	0.021
333	1.5.13	-0.932	-0.414	-0.193	-0.407	-0.449	0.802	0.876	0.282
343	2.5.3	0.906	1.117	1.294	0.673	0.624	0.881	0.577	0.487
408	1.6.8	-0.300	0.325	0.005	-0.776	-0.995	-0.176	-0.362	-0.756
413	1.6.13	0.769	0.439	1.353	0.869	-0.391	-0.226	0.190	0.286
422	2.6.2	0.689	0.029	0.951	0.041	0.478	0.263	-0.221	0.020
455	3.6.15	-0.042	0.348	0.533	0.038	-0.596	-0.949	-0.197	-0.695
501	2.7.1	0.581	0.526	0.394	-0.143	0.231	0.167	0.020	-0.366
608	3.8.8	0.485	0.458	0.922	0.571	0.276	0.427	0.088	0.134
650	1.9.10	-0.301	0.224	0.268	0.310	0.205	0.357	0.316	0.682
689	3.9.9	0.571	0.558	-0.165	-0.247	0.680	1.458	0.658	0.704
701	4.9.1	0.875	1.422	1.355	1.574	0.448	0.382	0.251	0.425
730	1.10.10	0.492	0.701	1.190	0.382	-1.444	-0.345	-0.486	-0.144
744	2.10.4	-0.260	-0.427	-0.211	-0.310	0.165	0.321	-0.247	-0.213
746	2.10.6	-0.608	-0.384	-0.174	0.145	0.033	0.254	0.325	-0.064
771	3.10.11	-0.810	-0.992	-0.387	-0.212	0.201	0.021	-0.234	-0.154
887	1.12.7	0.468	0.651	0.597	0.591	0.510	1.090	0.621	-0.056
1017	3.13.17	0.717	1.342	0.969	0.949	1.473	0.404	1.681	0.524
1018	3.13.18	0.642	0.880	0.441	0.890	-0.303	0.628	0.178	0.161
1037	4.13.17	0.382	0.871	0.487	0.481	0.175	0.354	0.302	0.394
1089	3.14.9	-0.360	0.294	0.301	0.069	-0.102	0.178	0.595	0.690
1123	1.15.3	0.677	0.633	0.324	0.207	0.459	0.240	0.090	-0.063
1185	4.15.5	0.600	0.879	0.872	0.253	-0.104	-0.149	-0.087	0.168
1187	4.15.7	0.956	0.467	0.456	0.482	0.625	0.316	0.168	0.012
1319	2.17.19	-0.945	-0.376	-0.473	-0.172	-0.554	0.392	0.227	0.225
1337	3.17.17	-0.974	-1.337	-0.795	-0.388	-0.448	-0.376	-0.192	-0.336
1507	4.19.7	-0.650	-0.536	-0.562	-0.543	0.236	-0.264	-0.307	-0.250
1602	5.1.2	-0.371	0.007	-0.281	-0.665	-0.998	-0.369	-0.421	-0.855
1639	6.1.19	-0.280	0.321	-0.062	-0.844	-0.978	-0.183	-0.412	-1.027
1650	7.1.10	0.759	1.333	0.633	0.049	0.398	0.079	0.139	0.288

Spot	GEO no.	Lean 1	Lean 3	Lean 5	Lean 7	Fat 1	Fat 3	Fat 5	Fat 7
1652	7.1.12	0.059	0.707	0.697	0.342	-0.673	-0.263	-0.114	0.171
1666	8.1.6	1.197	1.033	1.066	0.611	0.399	0.537	0.211	0.158
1687	5.2.7	0.379	0.390	-0.340	0.824	0.940	1.165	0.824	0.535
1696	5.2.16	0.296	0.444	0.448	0.237	-0.588	-0.595	-0.094	-0.324
1712	6.2.12	-0.213	-0.285	-0.494	-0.300	-0.155	0.226	-0.041	-0.236
1757	8.2.17	-0.181	0.165	-0.020	-0.252	-0.559	-0.245	-0.188	-0.406
1764	5.3.4	0.028	0.288	0.004	-0.776	-0.985	-0.297	-0.444	-0.920
1806	7.3.6	-0.201	0.436	0.004	-0.901	-1.198	-0.213	-0.468	-0.969
1832	8.3.12	0.935	0.715	1.719	0.322	0.144	0.272	-0.157	0.451
1846	5.4.6	0.107	0.259	0.382	0.615	0.107	0.073	-0.126	0.280
1900	7.4.20	0.215	0.001	0.562	0.080	0.025	-0.052	-0.098	0.848
1977	7.5.17	0.330	0.668	0.565	0.108	0.091	0.422	0.249	-0.115
2035	6.6.15	-0.159	0.071	-0.159	-0.884	-1.316	-0.339	-0.290	-1.181
2054	7.6.14	0.988	0.278	-0.044	0.357	0.580	-0.006	-0.260	-0.391
2101	6.7.1	0.528	0.224	0.223	-0.460	-0.214	-0.125	-0.058	-0.982
2141	8.7.1	0.429	0.401	1.417	-0.032	-0.386	-0.033	-0.286	0.568
2145	8.7.5	-0.322	-0.130	-0.359	0.173	0.189	0.162	0.964	-0.161
2296	7.9.16	0.566	0.246	0.868	0.136	0.201	0.383	0.220	0.216
2350	6.10.10	1.795	0.750	1.739	-0.320	0.553	0.943	-0.224	-0.033
2368	7.10.8	-0.337	-0.432	-0.863	-0.522	-0.297	-0.282	-0.041	-0.285
2413	5.11.13	0.573	0.904	0.865	0.864	2.020	0.381	0.840	0.843
2533	7.12.13	0.089	0.267	0.485	0.007	0.226	0.087	-0.830	0.205
2567	5.13.7	0.388	0.751	0.420	2.305	1.752	3.500	1.595	0.762
2569	5.13.9	0.392	0.783	0.826	0.798	0.823	1.274	1.077	0.858
2600	6.13.20	-0.479	-0.249	-0.125	0.036	0.251	0.220	-0.039	0.315
2621	8.13.1	0.205	0.346	0.242	0.409	0.807	-0.045	0.137	0.169
2694	7.14.14	-0.560	-0.515	-0.435	-0.010	-0.187	-0.233	0.039	0.157
2706	8.14.6	1.386	0.314	1.085	-0.264	-1.220	-0.975	-0.798	-0.685
2745	6.15.5	1.053	0.324	0.574	0.154	0.185	-0.022	-0.007	-0.251
2815	5.16.15	0.328	0.267	0.038	0.354	0.086	-0.310	-0.138	-0.365
2851	7.16.11	0.474	0.521	0.971	0.646	0.386	-0.175	0.234	0.122
2854	7.16.14	-0.040	0.236	2.191	0.629	-0.513	-0.973	0.111	-0.253
2858	7.16.18	-0.502	-0.439	-0.216	0.572	-0.502	0.068	0.455	0.692
2889	5.17.9	0.568	0.671	0.466	0.024	-0.221	-0.314	0.075	-0.105
2968	5.18.8	1.108	1.238	2.038	0.543	-0.504	-0.612	0.005	-0.325
3033	8.18.13	0.623	0.892	3.684	0.641	1.509	3.610	1.115	0.614
3221	10.1.1	-3.625	-1.127	-0.637	-0.831	-1.963	1.114	0.369	0.204
3236	10.1.16	0.217	0.144	-0.201	0.091	-0.087	-0.520	-0.316	-0.679
3241	11.1.1	-1.285	-0.758	-0.798	-0.182	-0.174	-0.322	-0.147	-0.093
3242	11.1.2	-0.281	0.338	-0.078	-0.900	-1.032	-0.253	-0.360	-1.066
3260	11.1.20	-0.061	-0.170	-0.274	0.204	-0.337	-0.512	-0.301	-0.322
3270	12.1.10	0.521	0.404	0.219	0.185	0.095	-0.191	0.117	-0.186
3274	12.1.14	0.225	0.177	-0.127	0.149	-0.008	-0.408	-0.344	-0.581
3280	12.1.20	-0.268	0.300	-0.112	-0.881	-0.885	-0.234	-0.414	-0.907
3284	9.2.4	-0.147	0.127	-0.149	-0.599	-0.665	-0.193	-0.353	-0.764
3291	9.2.11	-0.156	0.329	0.081	-0.774	-0.778	-0.105	-0.323	-0.772
3293	9.2.13	-0.133	0.297	0.082	-0.640	-0.609	-0.128	-0.265	-0.658
3341	12.2.1	0.413	0.281	-0.012	-0.179	1.079	0.777	0.738	-0.148
3399	10.3.19	0.327	0.210	-0.216	0.413	0.026	-0.342	-0.381	-0.775
3404	11.3.4	-0.215	0.464	-0.019	-0.930	-1.033	-0.206	-0.403	-1.105
3413	11.3.13	-0.146	0.004	-0.001	0.429	-0.254	-0.263	-0.091	-0.064
3428	12.3.8	-0.161	0.230	0.306	0.329	0.441	0.783	0.351	0.413

Spot	GEO no.	Lean 1	Lean 3	Lean 5	Lean 7	Fat 1	Fat 3	Fat 5	Fat 7
3441	9.4.1	-0.644	-0.938	-1.092	-0.349	-0.259	-0.528	-0.301	-0.584
3451	9.4.11	-0.430	0.022	-0.157	0.037	-0.163	0.196	0.163	0.432
3549	10.5.9	0.615	0.682	0.923	0.477	-0.220	0.204	0.011	0.166
3550	10.5.10	1.389	-0.137	0.922	1.072	0.719	0.213	-0.177	0.070
3623	10.6.3	0.633	0.610	0.462	0.178	0.312	0.241	0.101	-0.093
3642	11.6.2	0.268	0.210	-0.113	0.163	0.034	-0.280	-0.078	-0.451
3645	11.6.5	-0.643	-0.732	-0.064	0.207	-2.594	-1.249	0.066	0.243
3677	12.6.17	-0.632	-0.248	0.476	0.087	-1.628	-1.137	-0.437	-0.060
3759	12.7.19	0.746	0.549	0.567	0.383	0.436	0.215	-0.016	0.314
3818	11.8.18	0.927	0.475	0.579	0.429	0.383	0.580	0.116	0.053
3831	12.8.11	0.057	0.227	0.527	0.697	-0.037	0.140	0.189	0.510
3869	10.9.9	-0.319	-0.124	-0.109	0.038	0.184	0.308	0.385	0.718
3883	11.9.3	0.825	0.478	0.451	0.507	0.454	0.043	0.313	0.107
3940	9.10.20	-0.110	0.240	-0.050	0.299	0.569	0.770	0.395	0.627
4127	11.12.7	-0.223	0.300	-0.227	0.236	0.513	0.686	0.358	0.522
4170	9.13.10	0.093	0.760	0.745	0.922	0.892	0.940	0.912	1.019
4200	10.13.20	0.277	0.600	0.451	1.003	1.130	0.454	0.751	0.697
4247	9.14.7	1.080	0.792	0.848	0.141	0.051	0.714	-0.440	-0.104
4311	12.14.11	-0.955	-0.118	-0.043	0.038	0.243	0.211	1.063	0.102
4352	10.15.12	0.894	-0.041	-0.148	-0.261	-0.169	0.033	0.260	0.023
4354	10.15.14	0.382	0.246	0.825	0.174	0.374	-0.018	-0.129	-0.326
4413	9.16.13	-0.306	-0.416	-0.129	0.579	-0.506	0.228	0.663	0.612
4433	10.16.13	0.316	0.261	-0.017	0.304	0.167	-0.184	-0.177	-0.446
4481	9.17.1	-0.242	-0.200	0.381	0.590	0.215	0.437	0.604	0.650
4649	9.19.9	1.081	0.400	1.266	0.469	0.628	0.374	0.164	0.291
4713	12.19.13	-0.346	-0.704	-0.564	-0.154	-0.011	-0.220	-0.194	-0.028
4811	13.1.11	-0.460	-0.561	-0.031	0.745	0.287	0.560	1.326	0.187
4817	13.1.17	0.366	0.267	0.041	0.235	0.028	-0.297	-0.261	-0.373
4849	15.1.9	0.145	-0.041	-0.581	-0.049	-0.314	-0.086	0.151	-0.286
4880	16.1.20	-0.658	-0.226	-0.104	0.378	0.362	0.117	0.507	0.620
4938	15.2.18	-0.328	0.348	-0.055	-0.173	-0.977	-0.261	-0.433	-0.960
4989	14.3.9	0.947	0.209	0.971	0.290	-0.102	-0.066	-0.090	0.488
5005	15.3.5	-0.208	-0.105	-0.056	0.360	-0.310	-0.544	-0.180	-0.173
5006	15.3.6	0.335	0.361	0.057	0.279	0.138	-0.336	-0.198	-0.522
5007	15.3.7	0.871	1.207	0.843	0.637	0.522	0.669	0.401	0.430
5050	13.4.10	0.971	-0.293	-1.967	0.548	1.496	0.305	0.076	0.869
5073	14.4.13	0.448	0.605	0.440	0.234	0.971	1.945	1.584	0.436
5114	16.4.14	0.471	-0.035	0.052	0.152	-0.080	-0.242	-0.258	0.098
5132	13.5.12	0.405	0.450	0.939	0.503	0.190	0.127	0.177	0.644
5140	13.5.20	0.130	0.255	0.834	0.082	0.381	0.266	0.453	0.318
5155	14.5.15	-0.146	0.397	1.729	1.189	1.653	1.378	4.125	1.094
5193	16.5.13	-2.091	-0.577	-0.674	-0.027	-1.899	1.049	0.465	0.174
5208	13.6.8	0.187	-0.208	0.231	0.458	-0.291	-0.150	-0.100	0.048
5283	13.7.3	0.162	0.289	0.377	0.121	-0.673	-0.689	-0.415	-0.407
5285	13.7.5	0.217	1.345	-0.262	1.172	-0.326	-0.858	-0.213	0.129
5331	15.7.11	1.574	1.807	1.148	0.986	0.702	0.980	0.673	0.853
5353	16.7.13	0.597	0.920	1.645	0.572	0.520	0.750	-0.155	0.589
5430	16.8.10	1.171	1.018	2.544	3.338	0.151	0.910	0.709	0.803
5456	13.9.16	0.144	0.376	0.595	0.333	-0.343	-0.732	0.539	-0.098
5479	14.9.19	-0.452	-0.430	-0.736	-0.414	-0.613	0.232	0.067	0.065
5495	15.9.15	-0.130	0.271	0.276	0.531	0.243	0.482	0.538	0.816
5499	15.9.19	1.240	1.132	1.628	0.470	0.664	0.569	-0.007	0.555

Spot	GEO no.	Lean 1	Lean 3	Lean 5	Lean 7	Fat 1	Fat 3	Fat 5	Fat 7
5532	13.10.12	-0.090	-0.511	-0.287	-0.295	0.405	-0.266	-0.062	0.154
5579	15.10.19	0.932	0.231	0.283	-0.005	0.083	-0.023	-0.139	-0.031
5691	13.12.11	-0.592	-0.268	-0.152	0.277	0.202	0.375	0.496	0.167
5732	15.12.12	0.072	0.955	0.184	0.944	1.840	1.526	1.160	0.968
5781	14.13.1	0.305	0.595	0.760	0.885	-0.161	0.154	0.207	0.643
5845	13.14.5	0.239	0.698	1.229	0.696	0.000	0.576	0.427	0.288
5846	13.14.6	0.724	0.585	1.116	0.702	0.367	0.493	0.288	0.389
5855	13.14.15	-0.568	-1.358	-0.790	-0.381	-0.489	-0.372	0.104	0.298
5862	14.14.2	0.131	0.345	0.510	0.837	0.508	0.755	0.859	0.830
6021	14.16.1	0.617	0.398	0.485	0.379	0.212	0.085	0.268	-0.156
6074	16.16.14	0.311	0.122	-0.219	0.054	0.045	-0.643	-0.869	-0.621
6401	17.1.1	0.252	0.821	0.593	0.537	0.370	0.316	0.116	-0.441
6438	18.1.18	-0.065	0.449	0.495	0.385	0.595	0.162	0.221	0.649
6447	19.1.7	-0.504	0.621	0.175	-1.335	-1.381	-0.357	-0.420	-1.878
6480	20.1.20	0.883	0.622	0.130	-0.180	0.629	0.311	0.121	-0.378
6495	17.2.15	0.530	0.710	0.853	0.858	-0.018	0.495	0.037	0.687
6503	18.2.3	-0.156	0.124	0.414	0.869	0.187	0.383	0.858	0.713
6507	18.2.7	-0.373	-0.273	-0.233	0.095	0.191	0.232	0.309	-0.145
6511	18.2.11	-0.241	0.562	0.505	-0.343	-0.414	0.067	0.012	-0.759
6533	19.2.13	-0.774	-0.314	-0.024	0.400	0.191	0.491	0.640	0.241
6545	20.2.5	0.288	0.266	-0.037	0.148	0.001	-0.411	-0.307	-0.477
6629	20.3.9	-0.793	-0.801	0.092	0.070	-0.327	0.548	-0.113	-0.408
6690	19.4.10	-0.177	-0.070	0.453	0.159	-0.526	-0.239	0.040	0.022
6745	18.5.5	0.344	0.069	-0.075	-0.171	0.564	0.462	0.149	-0.010
6768	19.5.8	0.827	0.702	0.878	0.702	1.144	2.390	1.279	1.073
6794	20.5.14	-0.230	0.298	0.354	0.423	0.213	0.561	0.712	0.502
6840	18.6.20	0.218	0.278	0.444	0.222	-0.622	-0.691	-0.131	-0.234
6849	19.6.9	0.469	0.327	1.265	0.472	0.613	0.554	0.301	0.559
6947	20.7.7	0.062	0.877	0.862	0.114	-0.219	0.466	0.168	-0.198
7037	20.8.17	0.299	-0.064	-0.019	-0.271	-0.918	-0.641	-0.295	-0.102
7110	20.9.10	0.833	0.621	0.593	0.397	0.423	0.163	0.211	0.113
7147	18.10.7	1.437	0.951	1.411	0.843	-0.986	0.245	-0.559	0.033
7157	18.10.17	0.984	1.375	0.945	1.060	0.501	-0.717	-0.269	0.418
7333	19.12.13	0.648	0.554	0.628	-0.003	1.216	2.337	2.284	1.406
7349	20.12.9	-0.457	-1.368	-0.716	-0.106	-0.055	-0.498	0.002	-0.403
7376	17.13.16	0.267	1.054	0.752	0.645	0.627	0.553	0.251	0.568
7388	18.13.8	-0.291	-0.078	0.051	0.379	0.254	0.334	0.483	0.135
7423	20.13.3	-0.020	0.735	0.674	0.679	0.137	0.358	-0.284	0.034
7428	20.13.8	0.442	0.664	1.164	0.663	0.280	0.609	0.384	0.513
7501	20.14.1	0.588	0.714	0.800	0.237	0.426	0.274	0.277	0.028
7517	20.14.17	-0.634	-0.113	-0.245	-0.184	-0.261	-0.130	-0.074	0.191
7570	19.15.10	0.520	0.722	0.631	0.238	0.278	0.292	0.016	0.190
7639	18.16.19	0.256	0.771	0.465	1.050	0.984	0.606	0.871	0.604
7702	18.17.2	0.476	0.891	0.895	1.071	0.062	0.485	0.120	0.906
8048	23.1.8	-0.430	-0.094	0.090	0.060	-0.124	0.461	0.137	0.532
8138	23.2.18	0.436	0.152	-0.092	0.295	0.005	-0.495	-0.209	-0.689
8169	21.3.9	-0.341	0.344	-0.040	-1.172	-1.278	-0.333	-0.538	-1.341
8207	23.3.7	-0.116	-0.132	-0.024	0.397	-0.336	0.157	0.213	0.112
8257	21.4.17	-0.300	-0.211	-0.053	0.319	-0.132	0.224	0.347	0.449
8418	21.6.18	0.693	0.458	0.151	0.064	-0.320	-0.205	-0.016	-0.119
8433	22.6.13	0.019	0.454	0.084	-0.890	-1.075	-0.067	-0.404	-1.054
8438	22.6.18	-0.142	0.205	0.030	0.105	-0.004	0.314	0.825	0.265

Spot	GEO no.	Lean 1	Lean 3	Lean 5	Lean 7	Fat 1	Fat 3	Fat 5	Fat 7
8479	24.6.19	-1.871	-0.943	0.537	0.882	-0.169	1.221	0.352	2.344
8555	24.7.15	-0.230	0.034	0.169	0.460	0.465	0.639	0.790	0.410
8655	21.9.15	-1.092	-1.167	-0.428	0.347	-0.034	0.352	-0.960	-0.601
8673	22.9.13	-0.535	-0.339	-0.864	-0.094	0.550	0.610	0.311	0.092
8703	24.9.3	0.701	0.655	0.923	0.434	0.804	0.236	0.203	0.610
8851	23.11.11	1.040	0.688	0.619	0.323	2.510	1.201	1.592	0.487
8866	24.11.6	0.723	0.743	2.250	0.784	-0.207	-0.239	0.328	1.043
8876	24.11.16	-0.270	-0.474	-0.604	0.077	-0.221	-0.198	0.090	-0.135
8919	22.12.19	0.307	0.700	0.781	0.613	0.305	-0.035	0.049	1.091
8961	21.13.1	-0.403	-0.307	-0.230	-0.361	0.253	0.113	0.120	0.325
8984	22.13.4	0.176	-0.069	0.049	0.464	0.255	0.340	1.057	0.676
8989	22.13.9	0.972	0.328	0.144	0.315	0.023	-0.238	-0.366	-0.178
9001	23.13.1	0.048	0.774	0.695	0.844	0.987	1.260	1.648	1.418
9071	22.14.11	0.408	0.746	1.665	1.134	1.480	3.187	1.022	1.452
9090	23.14.10	0.220	0.691	1.165	1.130	-0.800	-0.500	0.432	0.972
9447	21.19.7	0.307	0.377	0.306	0.525	-0.099	-1.178	-0.534	0.658
9635	26.1.15	-0.049	0.759	0.150	-0.335	-0.683	-0.068	-0.132	-0.434
9659	27.1.19	-0.637	-0.224	0.416	0.127	0.060	0.815	1.068	0.599
9713	26.2.13	0.350	0.197	-0.067	0.157	0.016	-0.399	-0.218	-0.503
9743	28.2.3	-0.142	-0.198	-0.066	0.292	-0.057	-0.100	0.316	-0.439
9781	26.3.1	-0.206	0.350	0.254	-0.414	-0.748	-0.056	-0.198	-0.569
9843	25.4.3	-1.732	-0.727	-0.922	-0.776	-1.302	0.724	0.148	0.046
9878	26.4.18	0.271	0.203	0.918	0.408	0.992	0.731	1.214	0.529
10038	26.6.18	0.510	0.912	-0.273	1.066	-0.406	-0.161	-0.193	0.146
10040	26.6.20	-0.329	0.375	0.071	-0.914	-1.166	-0.244	-0.410	-0.914
10070	28.6.10	-1.396	-1.429	0.170	-0.037	-0.886	-0.543	0.030	0.991
10098	25.7.18	0.708	0.477	0.839	0.223	-0.637	-0.967	-0.220	-0.387
10099	25.7.19	2.382	1.272	1.702	1.294	1.901	1.314	-0.061	0.255
10168	25.8.8	0.665	1.042	1.171	0.873	0.536	0.643	0.344	0.785
10231	28.8.11	0.441	0.163	0.265	0.246	0.982	0.380	0.383	0.481
10287	27.9.7	0.903	0.535	0.494	0.806	0.536	0.334	0.141	0.690
10322	25.10.2	1.310	0.945	0.919	0.751	0.823	0.688	0.613	0.452
10345	26.10.5	1.193	0.772	0.496	0.575	0.762	0.063	-0.090	-0.095
10348	26.10.8	0.773	0.997	2.768	0.723	0.672	1.174	0.694	0.989
10350	26.10.10	-0.255	-0.047	0.220	0.809	0.526	0.754	0.563	0.950
10438	26.11.18	0.959	1.001	1.677	1.855	3.850	0.665	1.060	0.622
10551	28.12.11	0.890	0.880	0.503	1.102	0.827	0.750	0.150	-0.169
10633	28.13.13	0.645	0.557	0.636	0.315	0.323	-0.265	-0.342	-0.156
10667	26.14.7	-0.075	-0.260	0.140	0.493	-0.761	-0.322	0.291	0.040
10675	26.14.15	-0.455	-0.582	-1.928	-0.274	0.237	-0.557	-0.155	-0.023
10702	28.14.2	-0.696	-0.344	-0.161	0.372	0.112	0.334	0.552	0.166
10767	27.15.7	0.351	0.362	0.378	0.340	0.962	0.272	0.384	0.315
10888	25.17.8	-0.162	-0.487	-0.623	-1.430	0.363	0.355	-0.063	0.079
10937	27.17.17	-1.549	-0.489	-0.393	-0.338	-0.951	0.991	0.401	0.589
10954	28.17.14	0.145	0.584	0.441	0.258	-0.126	0.491	0.103	0.184
11262	32.1.2	0.033	0.240	0.522	0.599	-0.226	-0.075	0.196	0.336
11265	32.1.5	-0.133	0.306	0.558	0.455	-0.377	-0.135	0.163	0.397
11292	29.2.12	-0.249	-0.448	-0.332	0.365	-0.404	0.116	0.406	-0.077
11305	30.2.5	-0.038	0.375	0.233	-0.231	-0.420	-0.039	0.116	-0.318
11309	30.2.9	-0.018	0.093	-0.033	0.312	1.036	0.866	0.680	0.876
11313	30.2.13	-0.629	0.311	0.055	-0.973	-2.536	-0.477	-0.541	-1.205
11345	32.2.5	0.393	0.485	0.288	0.074	-0.428	-0.369	-0.183	-0.293

Spot	GEO no.	Lean 1	Lean 3	Lean 5	Lean 7	Fat 1	Fat 3	Fat 5	Fat 7
11363	29.3.3	-0.885	-0.388	-0.527	-0.351	-1.462	-0.559	-0.523	-0.700
11494	31.4.14	-0.128	0.040	0.301	0.093	-0.392	-0.175	-0.004	0.128
11515	32.4.15	0.393	0.028	0.034	-0.125	-0.125	-0.233	-0.193	-0.421
11523	29.5.3	0.150	0.199	0.304	0.093	-0.427	-0.469	-0.038	-0.162
11530	29.5.10	-0.229	-0.097	-0.115	-0.078	0.225	0.092	0.278	0.550
11560	30.5.20	-0.085	0.600	0.909	0.204	0.587	0.845	-0.047	0.555
11610	29.6.10	-0.042	-0.396	-0.744	0.017	-0.754	-0.766	0.308	-0.554
11622	30.6.2	-0.624	-0.034	0.357	-0.028	-0.474	0.810	0.468	0.389
11649	31.6.9	0.285	-0.097	-0.393	-0.360	0.378	0.063	0.001	-0.211
11797	30.8.17	0.799	0.471	0.477	0.035	0.361	0.025	-0.144	-0.106
11963	31.10.3	1.359	0.840	0.894	0.404	0.575	0.488	0.697	0.517
12097	29.12.17	0.739	0.612	1.082	0.759	0.085	0.373	0.479	0.883
12199	30.13.19	0.803	0.496	0.952	0.788	-0.061	0.911	0.077	-0.328
12217	31.13.17	-0.093	0.068	0.354	0.480	0.622	0.436	0.344	0.873
12255	29.14.15	-0.372	-0.602	-0.060	0.012	-0.308	-0.444	-0.028	0.729
12314	32.14.14	-0.427	-0.394	-0.358	-0.852	0.110	-0.065	-0.275	-0.429
12350	30.15.10	-0.545	-0.171	0.597	0.419	0.295	0.947	0.563	0.752
12915	34.2.15	0.060	0.546	0.303	-0.073	-0.231	0.182	0.064	-0.099
12945	36.2.5	-0.171	0.400	0.089	-0.445	-0.663	-0.039	-0.186	-0.501
12970	33.3.10	-0.289	0.271	0.035	-0.793	-0.924	-0.221	-0.311	-0.873
12975	33.3.15	-0.387	0.026	0.246	0.524	0.098	0.248	0.784	0.790
12976	33.3.16	-0.243	0.238	-0.093	-0.697	-0.915	-0.237	-0.360	-0.846
13006	35.3.6	-0.346	0.183	0.079	-0.599	-1.045	-0.226	-0.404	-0.468
13019	35.3.19	0.256	0.398	0.323	-0.209	0.149	-0.272	-0.398	-0.281
13057	33.4.17	0.471	0.149	0.405	0.314	0.451	0.161	0.569	1.513
13108	36.4.8	-0.490	-0.237	-0.296	-0.019	0.266	0.339	0.134	0.068
13203	33.6.3	0.233	0.341	1.212	0.614	0.486	0.858	1.282	1.115
13212	33.6.12	-0.512	-0.319	-0.324	0.079	-0.033	0.028	0.469	-0.073
13227	34.6.7	0.312	0.217	0.007	0.245	0.044	-0.117	-0.053	-0.388
13304	34.7.4	0.676	0.259	-0.056	0.099	0.118	-0.150	0.426	1.633
13578	35.10.18	1.068	1.203	0.954	1.014	0.863	0.439	0.335	0.923
13779	33.13.19	0.812	0.863	0.860	0.592	0.427	0.297	0.356	-0.004
13876	34.14.16	-0.124	-0.064	-0.127	-0.043	0.695	0.326	-0.310	-0.093
14002	33.16.2	0.107	-0.030	-0.315	-0.017	-0.097	-0.423	-0.473	-0.646
14052	35.16.12	0.474	0.392	0.036	0.382	0.265	-0.265	-0.159	-0.335
14080	36.16.20	0.041	-0.116	-0.211	-0.024	-0.304	-0.759	-0.728	-0.775
14091	33.17.11	0.089	-0.069	0.241	0.124	0.388	0.310	-0.514	0.196
14109	34.17.9	1.126	0.326	0.481	0.269	0.564	0.183	-0.077	0.497
14157	36.17.17	-0.575	-0.144	-0.509	-0.096	-0.262	0.386	0.217	0.195
14236	36.18.16	0.585	0.304	0.735	0.488	0.960	0.836	1.202	0.924
14275	34.19.15	0.641	-0.257	0.095	-0.088	0.526	0.387	0.432	0.865
14490	37.2.10	-0.279	0.238	-0.022	-0.695	-0.887	-0.205	-0.215	-0.824
14515	38.2.15	-0.805	-0.649	-0.251	0.277	-0.021	0.582	-0.116	-0.383
14541	40.2.1	0.361	0.030	-0.081	0.100	0.029	-0.418	-0.306	-0.380
14563	37.3.3	-0.240	0.130	-0.193	-0.425	-0.691	-0.229	-0.306	-0.699
14571	37.3.11	0.438	0.368	0.604	-0.037	0.434	0.693	2.083	0.989
14589	38.3.9	0.749	0.470	0.132	-0.081	0.507	0.276	0.119	-0.320
14596	38.3.16	-0.183	0.224	0.027	-0.549	-0.815	-0.212	-0.313	-0.690
14623	40.3.3	-0.252	-0.444	-0.756	0.311	-0.151	0.081	1.117	0.016
14625	40.3.5	-0.554	-0.246	-0.110	0.211	0.029	0.197	0.298	0.094
14643	37.4.3	-0.172	0.171	-0.164	-0.510	-0.659	-0.177	-0.289	-0.775
14688	39.4.8	-0.540	-0.399	-0.152	0.654	-0.485	0.106	0.426	0.635

Spot	GEO no.	Lean 1	Lean 3	Lean 5	Lean 7	Fat 1	Fat 3	Fat 5	Fat 7
14690	39.4.10	-0.517	-0.343	-0.405	0.180	-0.081	0.133	0.004	0.082
14816	37.6.16	0.438	0.249	0.090	0.057	-0.001	0.021	-0.061	-0.346
14839	38.6.19	0.423	0.097	-0.807	0.073	-0.010	-0.857	0.378	0.255
14871	40.6.11	0.842	0.611	1.458	1.494	1.171	1.678	2.339	1.744
14944	40.7.4	-0.250	0.281	0.462	0.388	-0.267	0.012	0.026	0.040
14955	40.7.15	1.431	1.533	1.954	1.386	3.412	2.425	4.907	2.605
15001	39.8.1	0.703	1.283	1.242	0.901	0.343	0.914	0.692	0.248
15027	40.8.7	0.538	0.633	0.936	0.934	2.534	1.636	0.926	0.533
15125	37.10.5	0.174	0.035	0.028	0.110	0.383	-0.467	-0.521	-0.459
15165	39.10.5	0.414	0.461	0.505	0.701	-0.063	-0.170	0.203	0.261
15188	40.10.8	0.097	0.034	0.808	0.232	0.541	0.632	0.708	0.610
15228	38.11.8	-0.370	-0.052	-0.120	0.457	0.652	0.995	0.615	0.437
15285	37.12.5	0.641	1.316	1.447	0.676	0.564	0.661	0.661	0.790
15370	37.13.10	-0.259	-0.264	-0.176	0.052	-0.060	0.844	0.334	0.668
15379	37.13.19	0.272	0.228	-0.510	0.135	0.702	0.442	0.755	0.040
15429	40.13.9	0.400	0.620	0.920	0.938	0.880	1.674	0.763	0.935
15432	40.13.12	0.020	0.424	0.182	0.057	0.846	0.862	1.576	0.382
15436	40.13.16	-0.341	0.038	0.096	0.527	0.502	0.709	0.687	0.885
15439	40.13.19	0.712	0.731	0.581	0.437	0.312	0.002	0.077	0.513
15471	38.14.11	0.718	0.401	0.595	1.125	-0.654	-0.231	-0.058	0.358
15473	38.14.13	0.583	0.487	-0.431	0.963	1.647	1.007	1.191	0.996
15599	40.15.19	-0.202	0.032	-0.344	-0.929	-0.455	-1.161	-0.636	0.442
15633	38.16.13	0.380	0.264	0.021	0.317	0.046	-0.341	-0.248	-0.472
15831	40.18.11	-0.286	-0.436	-0.658	-0.557	0.125	-0.168	0.004	-0.320
16022	42.1.2	0.476	0.672	0.875	0.817	0.383	0.183	0.368	0.488
16054	43.1.14	-0.806	-0.665	0.227	0.263	-0.269	0.786	-0.167	-0.590
16088	41.2.8	-0.208	0.198	-0.038	-0.537	-0.682	-0.184	-0.245	-0.624
16138	43.2.18	-0.500	-0.473	-0.145	0.419	-0.770	-0.591	-0.273	-0.002
16145	44.2.5	-0.081	0.222	0.391	0.589	0.129	0.369	0.636	0.724
16173	41.3.13	-0.179	0.301	0.252	0.340	0.353	0.400	0.616	0.664
16177	41.3.17	-0.054	0.363	0.171	0.594	0.861	0.970	1.330	1.117
16182	42.3.2	-3.586	-1.109	-0.342	-0.812	-2.463	1.239	0.554	0.216
16213	43.3.13	0.343	0.203	-0.023	0.155	0.132	-0.328	-0.269	-0.478
16234	44.3.14	-0.386	0.244	-0.085	-0.843	-1.038	-0.278	-0.430	-0.905
16255	41.4.15	-0.366	-0.355	-0.232	0.281	0.052	0.480	0.032	-0.164
16294	43.4.14	-4.189	-1.489	-0.357	-2.370	-3.373	0.972	0.542	-0.261
16296	43.4.16	-0.453	-0.321	-0.790	-0.180	-0.229	-0.009	-0.166	-0.015
16323	41.5.3	0.306	-0.332	-0.520	-0.235	1.110	0.237	0.189	-0.444
16376	43.5.16	0.453	0.617	1.162	0.739	0.326	0.562	0.430	0.361
16383	44.5.3	0.363	-0.152	0.439	1.214	0.189	1.366	0.629	0.133
16400	44.5.20	0.333	0.312	-0.084	0.191	0.295	-0.417	-0.294	-0.252
16413	41.6.13	0.477	0.550	0.355	0.398	-0.367	0.080	0.099	0.643
16482	41.7.2	-0.618	-0.462	-0.187	0.126	0.001	0.428	-0.239	-0.411
16488	41.7.8	-1.127	-0.539	-0.325	-0.348	-1.007	0.408	0.083	-0.051
16502	42.7.2	0.554	0.115	0.118	-0.082	0.924	0.239	1.130	2.028
16579	41.8.19	1.091	0.617	0.582	0.403	0.585	0.576	0.221	0.388
16632	44.8.12	0.184	-0.579	-0.253	-0.178	0.333	-0.078	-0.008	0.190
16638	44.8.18	-0.202	0.127	0.235	0.480	0.502	0.464	0.484	0.590
16722	41.10.2	0.775	-0.048	0.745	0.025	0.012	-0.005	-0.007	-0.045
16935	43.12.15	-0.416	-0.339	-0.356	-0.253	-0.075	-0.482	-0.123	0.267
16939	43.12.19	0.221	0.918	0.822	0.663	0.255	0.461	0.385	0.617
17004	43.13.4	0.180	0.092	0.289	-0.251	-0.057	-0.215	-0.305	-0.411

Spot	GEO no.	Lean 1	Lean 3	Lean 5	Lean 7	Fat 1	Fat 3	Fat 5	Fat 7
17076	42.14.16	1.012	1.428	-0.149	0.145	0.189	-0.536	0.165	2.235
17103	44.14.3	-0.241	-0.039	0.029	0.356	0.213	0.359	0.614	0.371
17128	41.15.8	0.613	1.108	0.909	2.642	0.973	1.466	1.259	0.123
17186	44.15.6	0.715	0.566	0.344	0.205	0.592	0.426	0.235	-0.001
17319	42.17.19	-0.174	-0.439	-0.281	0.101	0.196	-0.138	0.028	0.301
17419	43.18.19	1.265	0.772	0.456	0.923	4.589	2.132	0.681	3.966
17438	44.18.18	0.267	0.316	0.695	0.479	-0.068	0.109	0.346	0.366
17440	44.18.20	-0.022	0.111	0.517	0.230	-0.367	-0.204	0.035	0.196
17454	41.19.14	1.617	1.064	1.231	0.892	1.429	1.892	1.678	2.510
17622	46.1.2	-0.132	0.274	0.496	0.331	-0.392	0.047	0.139	0.290
17651	47.1.11	0.093	-0.011	0.674	0.229	0.303	0.776	1.057	0.326
17746	48.2.6	-0.903	-0.642	-0.434	0.130	0.037	0.249	0.212	-0.040
17981	48.5.1	-3.005	-1.109	-0.655	-1.528	-2.757	0.873	0.185	-0.096
17984	48.5.4	0.336	0.659	1.456	0.554	0.452	1.387	0.477	0.777
18016	45.6.16	0.286	0.569	0.193	2.057	0.415	2.282	2.020	4.044
18020	45.6.20	-0.175	0.404	0.264	-0.157	-0.599	0.011	-0.079	-0.274
18126	47.7.6	-0.590	0.406	-1.064	0.402	0.296	1.378	0.272	1.529
18215	47.8.15	0.839	0.577	0.589	0.424	0.374	0.369	0.136	0.298
18264	46.9.4	0.653	0.407	0.416	0.283	0.580	0.302	-0.053	-0.073
18295	47.9.15	-0.463	-0.129	0.030	0.353	0.183	0.361	0.319	0.227
18331	45.10.11	0.104	-0.379	-0.160	0.009	1.654	1.705	0.223	0.461
18439	46.11.19	0.598	0.370	0.074	-0.087	0.652	0.845	0.232	0.643
18472	48.11.12	0.273	0.318	-0.096	0.255	0.967	1.863	0.179	0.595
18476	48.11.16	0.300	0.647	0.192	0.689	1.449	1.106	1.488	1.531
18511	46.12.11	0.879	0.691	0.542	0.357	0.629	0.623	0.468	0.169
18522	47.12.2	0.294	0.238	0.065	0.418	0.605	0.765	0.689	0.326
18533	47.12.13	0.255	1.054	0.918	0.311	0.683	0.877	0.270	0.377
18589	46.13.9	0.771	0.703	1.429	0.671	0.808	1.597	2.237	2.374
18615	47.13.15	0.119	0.366	0.132	0.758	0.403	0.816	0.955	0.997
18634	48.13.14	0.341	0.608	0.502	0.892	1.251	0.715	1.153	0.875
18786	48.15.6	0.344	0.604	0.674	0.444	0.356	0.407	-0.306	0.170
18823	46.16.3	-0.236	-0.055	0.250	-0.006	-0.548	-0.269	-0.188	-0.094
18850	47.16.10	-1.262	-0.424	-0.442	-0.232	-0.850	1.046	0.496	0.361
18991	46.18.11	-0.166	0.331	0.322	-0.068	0.810	0.660	0.623	0.670
19041	45.19.1	0.469	0.485	0.196	0.198	0.904	1.067	0.595	1.143

Table 6. Results of ANOVA. 387 genes were significantly different by line or for the line-by-age interaction and had at least a 0.68-fold difference in their $\log_2$ ratios between lines. *Spot* is the Del-Mar 14K Chicken Integrated Systems microarray individual spot number; *GEO no.* is the GEO accession no. GPL1731 ID; *BlastX* is the highest scoring translated BLAST hit; *line*, *age*, *interaction* are the ANOVA probility values; and *Fold diff* is the difference between the highest and lowest $\log_2$ means.

Spot	GEO no.	BlastX	line	age	interaction	Fold diff
35	2.1.15	gamma-immunoglobulin heavy chain (504 AA) [Gallus gallus]	0.002	0.004	0.723	1.891
43	3.1.3	immunoglobulin J chain [Gallus gallus]	0.003	0.006	0.444	1.435
159	4.2.19	No Hits Found	0.000	0.700	0.477	0.995
224	4.3.4	hypothetical protein BC013949 [Homo sapiens]	0.452	0.483	0.036	0.694
238	4.3.18	TUC-4b [Rattus norvegicus]	0.004	0.377	0.607	1.022
264	2.4.4	unnamed protein product [Mus musculus]	0.020	0.178	0.895	1.365
284	3.4.4	hypothetical protein MGC10731 [Homo sapiens]	0.028	0.014	0.880	1.096
292	3.4.12	Slit1 protein [Gallus gallus]	0.036	0.042	0.079	1.184
310	4.4.10	No Hits Found	0.029	0.036	0.723	0.794
321	1.5.1	No Hits Found	0.009	0.000	0.220	1.139
333	1.5.13	Skin secretory protein XP2 precursor (APEG protein)	0.001	0.021	0.647	1.808
343	2.5.3	No Hits Found	0.044	0.297	0.687	0.806
408	1.6.8	serologically defined colon cancer antigen 28; phosphatidylcholinetransfer protein-like [Mus musculus]	0.015	0.001	0.389	1.320
413	1.6.13	Unknown (protein for MGC:10089) [Homo sapiens]	0.002	0.249	0.774	1.744
422	2.6.2	similar to The KIAA0150 gene product is novel. [Homo sapiens]	0.099	0.127	0.045	1.172
455	3.6.15	No Hits Found	0.000	0.183	0.468	1.483
501	2.7.1	KIAA0789 protein [Homo sapiens]	0.049	0.024	0.985	0.948
608	3.8.8	similar to dJ475N16.3 (novel protein similar to RPL7A (60Sribosomal protein L7A)) [Homo sapiens]	0.006	0.835	0.175	0.833
650	1.9.10	No Hits Found	0.005	0.001	0.236	0.983
689	3.9.9	gag/env fusion protein [Gallus gallus]	0.025	0.207	0.699	1.705
701	4.9.1	prolyl 4-hydroxylase, alpha subunit (EC 1.14.11.2)	0.012	0.884	0.820	1.323
730	1.10.10	No Hits Found	0.003	0.491	0.565	2.634
744	2.10.4	deoxyribonuclease gamma [Xenopus laevis]	0.026	0.503	0.137	0.748
746	2.10.6	lacZ alpha peptide	0.001	0.065	0.015	0.933
771	3.10.11	expressed sequence AA408877; hypothetical protein MGC38511 [Musmusculus]	0.022	0.816	0.286	1.193
887	1.12.7	gene_id:F1D9.26~unknown protein [Arabidopsis thaliana]	0.757	0.009	0.017	1.146
1017	3.13.17	hypothetical protein XP_148064 [Mus musculus]	0.899	0.260	0.025	1.278
1018	3.13.18	No Hits Found	0.024	0.259	0.607	1.193
1037	4.13.17	dJ604K5.1 (15 kDa selenoprotein) [Homo sapiens]	0.050	0.290	0.622	0.696
1089	3.14.9	unnamed protein product [Homo sapiens]	0.050	0.004	0.285	1.050
1123	1.15.3	NADH-ubiquinone oxidoreductase 19 kDa subunit (Complex I-19KD)(CI-19KD) (Complex I-PGIV) (CI-PGIV)	0.006	0.003	0.912	0.741
1185	4.15.5	gene 17.5 protein - chicken	0.002	0.902	0.350	1.029
1187	4.15.7	Frizzled-1 [Gallus gallus]	0.040	0.050	0.880	0.944
1319	2.17.19	EH-domain containing 3; EH domain containing 3 [Homo sapiens]	0.001	0.002	0.708	1.338
1337	3.17.17	similar to hypothetical protein [Homo sapiens] [Mus musculus]	0.010	0.241	0.371	1.145
1507	4.19.7	No Hits Found	0.022	0.776	0.484	0.886
1602	5.1.2	ADP-ribosylation factor 1 GTPase activating protein [Rattusnorvegicus]	0.002	0.001	0.309	1.005
1639	6.1.19	similar to FLJ00237 protein [Homo sapiens]	0.010	0.001	0.689	1.348
1650	7.1.10	cell division cycle control protein 37	0.031	0.297	0.139	1.284

Spot	GEO no.	BlastX	line	age	interaction	Fold diff
1652	7.1.12	transmembrane 4 superfamily member 2; membrane component, xchromosome, surface marker 1; T-cell acute lymphoblasticleukemia associated antigen 1; transmembrane proteinA15; tetraspanin protein; cell surface glycoprotein A15;CD231 antigen; transmembrane 4 superfamily 2b [Homosapiens]	0.000	0.008	0.163	1.380
1666	8.1.6	MAPK-interacting and spindle-stabilizing protein [Homo sapiens]	0.034	0.711	0.944	1.039
1687	5.2.7	No Hits Found	0.013	0.306	0.087	1.504
1696	5.2.16	CD63 antigen	0.000	0.491	0.568	1.042
1712	6.2.12	No Hits Found	0.041	0.509	0.438	0.720
1757	8.2.17	gene_id:F1D9.26~unknown protein [Arabidopsis thaliana]	0.003	0.020	0.573	0.725
1764	5.3.4	unnamed protein product [Homo sapiens]	0.006	0.021	0.407	1.273
1806	7.3.6	Similar to butyrate-induced transcript 1 [Mus musculus]	0.003	0.001	0.272	1.634
1832	8.3.12	Cathepsin D precursor	0.040	0.880	0.257	1.877
1846	5.4.6	No Hits Found	0.009	0.055	0.200	0.741
1900	7.4.20	similar to Amyloid beta A4 precursor protein-binding family B member 2 (Fe65-like protein) [Homo sapiens]	0.837	0.211	0.037	0.946
1977	7.5.17	No Hits Found	0.022	0.005	0.988	0.783
2035	6.6.15	Beta-neoendorphin-dynorphin precursor (Proenkephalin B)(Preprodynorphin) [Contains: Beta-neoendorphin;Dynorphin; Leu-Enkephalin; Rimorphin; Leumorphin]	0.021	0.015	0.292	1.387
2054	7.6.14	hypothetical protein [Homo sapiens]	0.044	0.012	0.747	1.378
2101	6.7.1	DKFZP586B1621 protein [Homo sapiens]	0.013	0.004	0.792	1.510
2141	8.7.1	UDP-Gal:betaGlcNAc beta 1,3-galactosyltransferase 1; beta-3-galt1[Homo sapiens]	0.032	0.538	0.044	1.803
2145	8.7.5	OSJNBa0029H02.25 [Oryza sativa (japonica cultivar-group)]	0.035	0.584	0.055	1.322
2296	7.9.16	3-oxoacid CoA transferase precursor; Succinyl CoA:3-oxoacid CoA transferase; succinyl-CoA:3-ketoacid-CoA transferase precursor [Homo sapiens]	0.068	0.119	0.040	0.732
2350	6.10.10	Neuropeptide Y precursor (NPY)	0.047	0.034	0.066	2.115
2368	7.10.8	No Hits Found	0.021	0.881	0.180	0.822
2413	5.11.13	No Hits Found	0.355	0.295	0.048	1.639
2533	7.12.13	ISOCITRATE DEHYDROGENASE [NADP] CYTOPLASMIC (OXALOSUCCINATE DECARBOXYLASE) (IDH) (NADP+-SPECIFIC ICDH) (IDP)	0.136	0.534	0.031	1.315
2567	5.13.7	chromodomain helicase DNA binding protein 2 [Homo sapiens]	0.087	0.425	0.049	3.113
2569	5.13.9	E74-like factor 1 (ets domain transcription factor) [Rattus norvegicus]	0.047	0.217	0.716	0.882
2600	6.13.20	hypothetical protein MGC10485 [Homo sapiens]	0.022	0.566	0.518	0.793
2621	8.13.1	No Hits Found	0.760	0.114	0.016	0.851
2694	7.14.14	putative protein; protein id: At5g12470.1, supported by cDNA:gi_20268751, supported by cDNA: gi_21281148 [Arabidopsisthaliana]	0.021	0.081	0.871	0.717
2706	8.14.6	No Hits Found	0.002	0.695	0.306	2.606
2745	6.15.5	RIKEN cDNA 1810046J19; expressed sequence AI463380 [Mus musculus]	0.003	0.041	0.643	1.304
2815	5.16.15	No Hits Found	0.031	0.757	0.694	0.719
2851	7.16.11	hypothetical protein; extensin-like; with SH3 Src homology domain [Schizosaccharomyces pombe]	0.027	0.544	0.689	1.146
2854	7.16.14	dJ259A10.1 (ssDNA binding protein (SEB4D)) [Homo sapiens]	0.022	0.138	0.694	3.165
2858	7.16.18	similar to K02B2.3.p [Homo sapiens]	0.024	0.000	0.278	1.194
2889	5.17.9	aldo-keto reductase [Gallus gallus]	0.001	0.566	0.231	0.985
2968	5.18.8	suppressor of fused [Gallus gallus]	0.000	0.355	0.691	2.650
3033	8.18.13	fragile X mental retardation protein	0.644	0.085	0.043	3.071
3221	10.1.1	Ig light chain precursor	0.003	0.000	0.726	4.738
3236	10.1.16	similar to serum amyloid A-activating factor SAF-8 [Homo sapiens]	0.019	0.524	0.570	0.896
3241	11.1.1	adenylate cyclase 2; adenylyl cyclase 2; adenylate cyclase II; adenylyl cyclase; ATP pyrophosphate-lyase; type II adenylate cyclase; 3',5'-cyclic AMP synthetase [Homo sapiens]	0.040	0.449	0.570	1.191
3242	11.1.2	chromosome 11 open reading frame2; chromosome 11 open readingframe2 [Homo sapiens]	0.012	0.001	0.573	1.404
3260	11.1.20	ATP-binding cassette, sub-family B, member 6 [Homo sapiens]	0.028	0.421	0.573	0.715
3270	12.1.10	similar to vesicle-associated calmodulin-binding protein [Musmusculus]	0.024	0.532	0.753	0.712
3274	12.1.14	mel transforming oncogene; ras-associated protein RAB8 [Homo sapiens]	0.013	0.450	0.621	0.806
3280	12.1.20	Synaptogyrin 1	0.033	0.003	0.583	1.207

Spot	GEO no.	BlastX	line	age	interaction	Fold diff
3284	9.2.4	No Hits Found	0.008	0.002	0.633	0.891
3291	9.2.11	PEROXISOMAL FARNESYLATED PROTEIN	0.015	0.001	0.463	1.106
3293	9.2.13	No Hits Found	0.018	0.002	0.568	0.954
3341	12.2.1	superoxide dismutase 3, extracellular [Homo sapiens]	0.002	0.001	0.276	1.257
3399	10.3.19	Dynactin 1 (150 kDa dynein-associated polypeptide) (DP-150) (DAP-150) (p150-glued)	0.041	0.589	0.490	1.189
3404	11.3.4	unnamed protein product [Homo sapiens]	0.007	0.001	0.573	1.568
3413	11.3.13	No Hits Found	0.048	0.127	0.584	0.692
3428	12.3.8	No Hits Found	0.014	0.236	0.236	0.944
3441	9.4.1	Gamma-interferon inducible lysosomal thiol reductase precursor(Gamma-interferon-inducible protein IP-30)	0.021	0.333	0.097	0.833
3451	9.4.11	alpha-2-antiplasmin precursor	0.003	0.002	0.838	0.862
3549	10.5.9	gene 17.5 protein - chicken	0.000	0.587	0.472	1.143
3550	10.5.10	hepatocyte growth factor activator inhibitor precursor; hepatocytegrowth factor activator inhibitor [Homo sapiens]	0.038	0.072	0.233	1.566
3623	10.6.3	2',5'-oligo adenylate synthetase A [Gallus gallus]	0.018	0.086	0.992	0.726
3642	11.6.2	No Hits Found	0.027	0.466	0.372	0.719
3645	11.6.5	LH-beta	0.024	0.000	0.024	2.837
3677	12.6.17	gastrin-releasing peptide precursor splice form III - human	0.014	0.017	0.683	2.104
3759	12.7.19	succinate dehydrogenase Ip subunit [Gallus gallus]	0.022	0.394	0.619	0.761
3818	11.8.18	hepatitis B virus x-interacting protein; hepatitis B virusx-interacting protein (9.6kD); HBx-interacting protein[Homo sapiens]	0.007	0.054	0.168	0.874
3831	12.8.11	sequestosome 1; UBIQUITIN-BINDING PROTEIN P62; phosphotyrosineindependent ligand for the Lck SH2 domain p62 [Homosapiens]	0.023	0.000	0.613	0.734
3869	10.9.9	SH2 domain protein 1A; Duncan disease homolog [Mus musculus]	0.045	0.622	0.979	1.037
3883	11.9.3	DIHYDROPYRIMIDINASE RELATED PROTEIN-2 (DRP-2) (COLLAPSIN RESPONSE MEDIATOR PROTEIN CRMP-62)	0.011	0.137	0.833	0.781
3940	9.10.20	similar to expressed sequence AI649009 [Homo sapiens]	0.014	0.490	0.906	0.880
4127	11.12.7	No Hits Found	0.008	0.274	0.786	0.913
4170	9.13.10	catalase [Canis familiaris]	0.043	0.130	0.293	0.926
4200	10.13.20	No Hits Found	0.221	0.388	0.049	0.853
4247	9.14.7	No Hits Found	0.009	0.124	0.220	1.519
4311	12.14.11	Thymosin beta	0.010	0.120	0.278	2.019
4352	10.15.12	similar to kinesin superfamily protein 1C [Mus musculus]	0.683	0.285	0.029	1.155
4354	10.15.14	NADH-ubiquinone oxidoreductase SGDH subunit, mitochondrialprecursor (Complex I-SGDH) (CI-SGDH)	0.041	0.338	0.439	1.151
4413	9.16.13	No Hits Found	0.023	0.000	0.037	1.169
4433	10.16.13	No Hits Found	0.015	0.351	0.420	0.762
4481	9.17.1	No Hits Found	0.004	0.001	0.265	0.892
4649	9.19.9	DNA mismatch repair protein MSH6 (MutS-alpha 160 kDa subunit) (G/T mismatch binding protein) (GTBP) (GTMBP) (P160)	0.037	0.255	0.258	1.102
4713	12.19.13	similar to RAB7, member RAS oncogene family [Mus musculus]	0.015	0.154	0.775	0.694
4811	13.1.11	Zinc finger protein CKR1	0.017	0.157	0.066	1.886
4817	13.1.17	unnamed protein product [Mus musculus]	0.017	0.623	0.900	0.739
4849	15.1.9	60S RIBOSOMAL PROTEIN L3 (L4)	0.987	0.867	0.046	0.732
4880	16.1.20	Regulator of G-protein signaling 5	0.000	0.004	0.127	1.278
4938	15.2.18	Similar to neural proliferation, differentiation and control gene 1[Mus musculus]	0.032	0.259	0.958	1.325
4989	14.3.9	endothelial differentiation, lysophosphatidic acidG-protein-coupled receptor, 2 [Rattus norvegicus]	0.037	0.688	0.225	1.073
5005	15.3.5	ubiquitin fusion-degradation 1-like protein [Gallus gallus]	0.007	0.035	0.337	0.903
5006	15.3.6	OB-receptor gene related protein [Rattus norvegicus]	0.017	0.566	0.592	0.883
5007	15.3.7	ELL gene (11-19 lysine-rich leukemia gene) [Homo sapiens]	0.013	0.236	0.860	0.806
5050	13.4.10	scavenger-receptor protein [Sus scrofa]	0.032	0.006	0.487	3.463
5073	14.4.13	solute carrier family 29 (nucleoside transporters), member 1 [Homosapiens]	0.032	0.251	0.609	1.711
5114	16.4.14	mixed lineage kinase-related kinase MRK-beta; mixed lineage kinasewith a leucine zipper and a sterile alpha motif; mixedlineage kinase-related kinase [Homo sapiens]	0.003	0.023	0.235	0.729
5132	13.5.12	Unknown (protein for MGC:25768) [Mus musculus]	0.042	0.280	0.158	0.811
5140	13.5.20	aristaless related homeobox; aristaless-related homeobox, X-linked[Homo sapiens]	0.703	0.001	0.024	0.753
5155	14.5.15	No Hits Found	0.019	0.035	0.314	4.271
5193	16.5.13	RIKEN cDNA 1110063G11 [Mus musculus]	0.045	0.001	0.435	3.140

Spot	GEO no.	BlastX	line	age	interaction	Fold diff
5208	13.6.8	hypothetical protein [Rhodopseudomonas palustris]	0.044	0.171	0.519	0.748
5283	13.7.3	carbamoyl-phosphate synthetase (E.C.6.3.5.5)	0.000	0.725	0.767	1.066
5285	13.7.5	bone morphogenetic protein 1 [Gallus gallus]	0.048	0.544	0.322	2.203
5331	15.7.11	CD47 antigen [Bos taurus]	0.010	0.318	0.544	1.134
5353	16.7.13	unnamed protein product [Mus musculus]	0.033	0.785	0.024	1.800
5430	16.8.10	No Hits Found	0.047	0.424	0.588	3.188
5456	13.9.16	CD63 antigen	0.007	0.033	0.230	1.327
5479	14.9.19	Ig light chain precursor	0.050	0.528	0.449	0.968
5495	15.9.15	prosaposin [Gallus gallus]	0.014	0.004	0.959	0.946
5499	15.9.19	multiple exostoses type II protein EXT2.I [Homo sapiens]	0.033	0.740	0.241	1.635
5532	13.10.12	immunoglobulin-like receptor CHIR-A [Gallus gallus]	0.031	0.118	0.895	0.916
5579	15.10.19	nectin-lke 1 [Mus musculus]	0.003	0.018	0.103	1.072
5691	13.12.11	MHC class II-associated invariant chain [Gallus gallus]	0.001	0.144	0.094	1.088
5732	15.12.12	No Hits Found	0.029	0.750	0.322	1.768
5781	14.13.1	aldose reductase	0.003	0.007	0.854	1.046
5845	13.14.5	No Hits Found	0.030	0.049	0.568	1.228
5846	13.14.6	similar to dJ886K2.3(GALE (UDP-galactose-4-epimerase)) [Homosapiens]	0.018	0.867	0.442	0.828
5855	13.14.15	growth hormone receptor [Gallus gallus]	0.002	0.026	0.291	1.657
5862	14.14.2	Similar to RIKEN cDNA 0610013D04 gene [Homo sapiens]	0.023	0.029	0.557	0.728
6021	14.16.1	RIKEN cDNA 2610034N24 [Mus musculus]	0.028	0.496	0.903	0.772
6074	16.16.14	hypothetical protein XP_148705 [Mus musculus]	0.009	0.126	0.835	1.180
6401	17.1.1	No Hits Found	0.048	0.419	0.339	1.262
6438	18.1.18	D15Wsu75e protein [Mus musculus]	0.372	0.313	0.006	0.714
6447	19.1.7	histocompatibility 13 [Mus musculus]	0.040	0.006	0.962	2.499
6480	20.1.20	stathmin [Gallus gallus]	0.037	0.000	0.644	1.261
6495	17.2.15	leucine-rich, glioma inactivated 1 precursor; epilepsy, partial[Homo sapiens]	0.004	0.061	0.345	0.876
6503	18.2.3	No Hits Found	0.044	0.000	0.219	1.024
6507	18.2.7	translocase of inner mitochondrial membrane 8 homolog A;deafness/dystonia peptide; translocase of innermitochondrial membrane 8 (yeast) homolog A [Homosapiens]	0.001	0.797	0.011	0.682
6511	18.2.11	claudin 10 [Homo sapiens]	0.018	0.001	0.866	1.321
6533	19.2.13	MHC class II-associated invariant chain [Gallus gallus]	0.002	0.057	0.114	1.414
6545	20.2.5	hypothetical protein [Macaca fascicularis]	0.010	0.509	0.735	0.765
6629	20.3.9	No Hits Found	0.231	0.379	0.044	1.349
6690	19.4.10	Transcript Antisense to Ribosomal RNA; Tar1p [Saccharomycescerevisiae]	0.049	0.016	0.843	0.980
6745	18.5.5	No Hits Found	0.014	0.002	0.839	0.735
6768	19.5.8	dJ963K23.4 (continues in dJ1041C10 (AL162615)) [Homo sapiens]	0.036	0.464	0.353	1.688
6794	20.5.14	No Hits Found	0.020	0.011	0.699	0.942
6840	18.6.20	Glycylpeptide N-tetradecanoyltransferase 2 (PeptideN-myristoyltransferase 2) (Myristoyl-CoA:proteinN-myristoyltransferase 2) (NMT 2) (Type IIN-myristoyltransferase)	0.000	0.155	0.470	1.135
6849	19.6.9	KIAA0872 protein [Homo sapiens]	0.422	0.433	0.034	0.964
6947	20.7.7	No Hits Found	0.008	0.002	0.750	1.096
7037	20.8.17	clusterin [Gallus gallus]	0.010	0.817	0.048	1.217
7110	20.9.10	Glutathione reductase, mitochondrial precursor (GR) (GRase)	0.000	0.064	0.924	0.720
7147	18.10.7	gene 17.5 protein - chicken	0.000	0.910	0.232	2.423
7157	18.10.17	No Hits Found	0.018	0.815	0.580	2.092
7333	19.12.13	unnamed protein product [Mus musculus]	0.016	0.624	0.826	2.340
7349	20.12.9	No Hits Found	0.026	0.028	0.106	1.370
7376	17.13.16	No Hits Found	0.125	0.160	0.036	0.803
7388	18.13.8	No Hits Found	0.019	0.272	0.086	0.774
7423	20.13.3	unnamed protein product [Homo sapiens]	0.048	0.443	0.332	1.020
7428	20.13.8	No Hits Found	0.045	0.221	0.268	0.885
7501	20.14.1	3-mercaptopyruvate sulfurtransferase [Homo sapiens]	0.036	0.184	0.796	0.772
7517	20.14.17	serine (or cysteine) proteinase inhibitor, clade F, member 2;plasmin inhibitor alpha 2; alpha 2 antiplasmin; serine(or cysteine) proteinase inhibitor, clade F (alpha-2antiplasmin, pigment epithelium derived factor), member2 [Mus musculus]	0.010	0.005	0.278	0.825
7570	19.15.10	unnamed protein product [Mus musculus]	0.004	0.252	0.261	0.706
7639	18.16.19	CCCH zinc finger protein C3H-3 [Xenopus laevis]	0.345	0.724	0.023	0.794
7702	18.17.2	peptidyl-glycine alpha-amidating monooxygenase precursor (EC 1.14.17.3)	0.041	0.120	0.786	1.009

Spot	GEO no.	BlastX	line	age	interaction	Fold diff
8048	23.1.8	ionized calcium binding adapter molecule 2 [Mus musculus]	0.023	0.047	0.608	0.961
8138	23.2.18	EWS [Homo sapiens]	0.017	0.469	0.537	1.125
8169	21.3.9	hyaluronoglucosaminidase 3 [Homo sapiens]	0.024	0.005	0.709	1.685
8207	23.3.7	unnamed protein product [Mus musculus]	0.950	0.003	0.035	0.733
8257	21.4.17	proline rich protein 2 [Mus musculus]	0.001	0.000	0.402	0.748
8418	21.6.18	calreticulin [Mus musculus]	0.002	0.756	0.143	1.013
8433	22.6.13	No Hits Found	0.020	0.009	0.546	1.530
8438	22.6.18	enoyl-Coenzyme A, hydratase/3-hydroxyacyl Coenzyme A dehydrogenase[Homo sapiens]	0.039	0.091	0.258	0.966
8479	24.6.19	pleiotrophin; osteoblastic cell factor; heparin-binding brainmitogen; heparin-binding growth factor 8; heparin affinregulatory peptide; heparin-binding neurotorphic factor;heparin-binding neurite promoting factor;heparin-binding growth-associated molecule [Musmusculus]	0.043	0.040	0.557	4.215
8555	24.7.15	FLJ00239 protein [Homo sapiens]	0.002	0.268	0.211	1.020
8655	21.9.15	MHC class I antigen [Gallus gallus]	0.342	0.565	0.014	1.519
8673	22.9.13	tetranectin [Gallus gallus]	0.000	0.550	0.326	1.475
8703	24.9.3	basic leucine zipper and W2 domains 1; KIAA0005 gene product; basic leucine-zipper protein BZAP45 [Homo sapiens]	0.076	0.292	0.030	0.720
8851	23.11.11	proline rich protein 2 [Mus musculus]	0.007	0.012	0.287	2.187
8866	24.11.6	syndecan	0.012	0.094	0.169	2.489
8876	24.11.16	No Hits Found	0.059	0.172	0.031	0.694
8919	22.12.19	calcium binding protein P22; Sid470p [Mus musculus]	0.143	0.076	0.031	1.126
8961	21.13.1	proteasome 26S ATPase subunit 4 isoform 1; protease 26S subunit 6;Tat-binding protein 7; MB67 interacting protein [Homosapiens]	0.006	0.990	0.871	0.728
8984	22.13.4	No Hits Found	0.015	0.155	0.219	1.126
8989	22.13.9	No Hits Found	0.048	0.533	0.940	1.338
9001	23.13.1	kelch domain containing 1 [Homo sapiens]	0.046	0.562	0.943	1.601
9071	22.14.11	No Hits Found	0.041	0.296	0.057	2.779
9090	23.14.10	No Hits Found	0.006	0.003	0.495	1.964
9447	21.19.7	Wnt-5a [Gallus gallus]	0.015	0.064	0.144	1.836
9635	26.1.15	similar to hypothetical protein similar to RNA-binding protein lark[Homo sapiens] [Rattus norvegicus]	0.010	0.013	0.409	1.442
9659	27.1.19	hypothetical protein [Rhodobacter sphaeroides]	0.045	0.201	0.937	1.705
9713	26.2.13	No Hits Found	0.024	0.493	0.735	0.854
9743	28.2.3	Cytokine-inducible SH2-containing protein (CIS) (CIS-1) (Suppressor of cytokine signaling) (SOCS)	0.763	0.481	0.033	0.755
9781	26.3.1	probable translational initiation factor; putative translationinitiation factor IF-2(fragment) [Streptomycescoelicolor A3(2)]	0.016	0.008	0.821	1.098
9843	25.4.3	immunoglobulin alpha heavy chain [Gallus gallus]	0.004	0.008	0.670	2.456
9878	26.4.18	PTD009 protein [Homo sapiens]	0.036	0.098	0.687	1.011
10038	26.6.18	No Hits Found	0.042	0.325	0.599	1.472
10040	26.6.20	UV radiation resistance associated gene [Homo sapiens]	0.016	0.003	0.457	1.542
10070	28.6.10	glypican 3 [Homo sapiens]	0.003	0.000	0.099	2.420
10098	25.7.18	No Hits Found	0.000	0.513	0.653	1.806
10099	25.7.19	No Hits Found	0.041	0.053	0.409	2.443
10168	25.8.8	syndecan-2 variant 1 [Gallus gallus]	0.002	0.349	0.089	0.826
10231	28.8.11	laminin, gamma 1 precursor; formerly LAMB2 [Homo sapiens]	0.022	0.041	0.585	0.819
10287	27.9.7	aspartyl aminopeptidase [Homo sapiens]	0.027	0.029	0.811	0.762
10322	25.10.2	Similar to gene trap ROSA 26 antisense, Philippe Soriano [Mus musculus]	0.040	0.216	0.952	0.858
10345	26.10.5	active breakpoint cluster region-related protein isoform a [Homosapiens]	0.011	0.078	0.970	1.288
10348	26.10.8	chromosome 6 open reading frame 4 isoform 1; chromosome 6 open reading frame 6; chromosome 6 open reading frame 5; connection to IKK and SAPK/JNK; NFkB-activating protein ACT1 [Homo sapiens]	0.181	0.149	0.047	2.096
10350	26.10.10	RIKEN cDNA 1200015A22 [Mus musculus]	0.009	0.043	0.478	1.204
10438	26.11.18	ATPase, H+ transporting, lysosomal V0 subunit a isoform 4; vacuolarproton pump 116 kDa accessory subunit; vacuolar protonpump, subunit 2; H(+)-transporting two-sector ATPase,noncatalytic accessory protein 1B; ATPase, H+transporting, lysosomal (vacuolar proton pump)non-catalytic accessory protein 1B; renal tubularacidosis; ATPase, H+ transporting, lysosomal (vacuolarproton pump) non-catalytic accessory protein 2 (38kD)[Homo sapiens]	0.685	0.140	0.030	3.228
10551	28.12.11	No Hits Found	0.039	0.212	0.204	1.270

Spot	GEO no.	BlastX	line	age	interaction	Fold diff
10633	28.13.13	Predicted CDS, cuticle collagen family member [Caenorhabditis elegans]	0.011	0.605	0.731	0.986
10667	26.14.7	No Hits Found	0.038	0.001	0.085	1.254
10675	26.14.15	alpha-1-antiproteinase [Xenopus laevis]	0.037	0.156	0.229	2.165
10702	28.14.2	6-phosphofructo-2-kinase/fructose-2, 6-bisphosphatase [Gallus gallus]	0.002	0.039	0.056	1.248
10767	27.15.7	LYSYL-TRNA SYNTHETASE (LYSINE --TRNA LIGASE) (LYSRS)	0.189	0.048	0.042	0.690
10888	25.17.8	similar to smoothelin isoform c [Homo sapiens] [Mus musculus]	0.011	0.342	0.672	1.793
10937	27.17.17	lysyl oxidase-like 2 [Homo sapiens]	0.000	0.000	0.563	2.540
10954	28.17.14	No Hits Found	0.030	0.002	0.617	0.710
11262	32.1.2	vesicular membrane protein p24; vesicular membrain protein p24[Homo sapiens]	0.002	0.000	0.988	0.825
11265	32.1.5	Platelet-endothelial tetraspan antigen 3 (PETA-3) (GP27) (Membrane glycoprotein SFA-1) (CD151 antigen)	0.003	0.000	0.410	0.934
11292	29.2.12	RNA polymerase II largest subunit	0.199	0.089	0.012	0.854
11305	30.2.5	hypothetical protein MGC4293 [Homo sapiens]	0.016	0.002	0.512	0.794
11309	30.2.9	No Hits Found	0.001	0.820	0.858	1.069
11313	30.2.13	cited2/melanocyte specific gene-related gene 1 MRG1 [Gallus gallus]	0.002	0.001	0.124	2.847
11345	32.2.5	aldo-keto reductase [Gallus gallus]	0.000	0.807	0.504	0.913
11363	29.3.3	ADP-ribosylation factor-like 1 [Rattus norvegicus]	0.019	0.000	0.293	1.111
11494	31.4.14	unnamed protein product [Homo sapiens]	0.024	0.004	0.419	0.692
11515	32.4.15	RIKEN cDNA 1810046J19; expressed sequence AI463380 [Mus musculus]	0.001	0.015	0.583	0.814
11523	29.5.3	unnamed protein product [Mus musculus]	0.011	0.633	0.798	0.773
11530	29.5.10	similar to cytoplasmic cysteine conjugate-beta lyase;glutamine-phenylpyruvate aminotransferase [Homo sapiens][Mus musculus]	0.000	0.254	0.438	0.779
11560	30.5.20	No Hits Found	0.660	0.264	0.027	0.995
11610	29.6.10	No Hits Found	0.473	0.592	0.031	1.075
11622	30.6.2	skeletrophin; dystrophin-like [Mus musculus]	0.037	0.002	0.428	1.435
11649	31.6.9	No Hits Found	0.002	0.000	0.296	0.771
11797	30.8.17	Unknown (protein for IMAGE:3507673) [Homo sapiens]	0.000	0.001	0.370	0.943
11963	31.10.3	similar to ZK792.1b.p [Homo sapiens]	0.046	0.117	0.202	0.955
12097	29.12.17	stromal cell-derived factor 2-like 1 [Mus musculus]	0.041	0.199	0.292	0.997
12199	30.13.19	No Hits Found	0.037	0.595	0.148	1.280
12217	31.13.17	catecholamine-O-methyltransferase [Rattus norvegicus]	0.001	0.011	0.076	0.966
12255	29.14.15	No Hits Found	0.020	0.000	0.062	1.331
12314	32.14.14	No Hits Found	0.004	0.018	0.525	0.962
12350	30.15.10	L-lactate dehydrogenase A chain (LDH-A)	0.003	0.024	0.123	1.492
12915	34.2.15	ADP-ribosylation factor 1 [Homo sapiens]	0.027	0.007	0.652	0.777
12945	36.2.5	Fc fragment of IgG binding protein; IgG Fc binding protein [Homo sapiens]	0.009	0.001	0.512	1.062
12970	33.3.10	RIKEN cDNA 1200007O21 [Mus musculus]	0.019	0.002	0.626	1.194
12975	33.3.15	No Hits Found	0.038	0.013	0.889	1.177
12976	33.3.16	UV radiation resistance associated gene [Homo sapiens]	0.011	0.003	0.578	1.154
13006	35.3.6	unnamed protein product [Mus musculus]	0.030	0.021	0.348	1.229
13019	35.3.19	proteasome (prosome, macropain) 28 subunit, alpha; protease(prosome, macropain) 28 subunit, alpha [Mus musculus]	0.019	0.257	0.303	0.796
13057	33.4.17	hypothetical protein MGC32124 [Homo sapiens]	0.050	0.022	0.037	1.364
13108	36.4.8	complement component factor h; complement factor H related protein 3A4/5G4 [Mus musculus]	0.000	0.438	0.051	0.829
13203	33.6.3	glucosamine-6-phosphate isomerase [Homo sapiens]	0.032	0.004	0.667	1.048
13212	33.6.12	major histocompatibility complex class II beta chain; B-L betachain [Gallus gallus]	0.003	0.160	0.048	0.981
13227	34.6.7	No Hits Found	0.039	0.662	0.599	0.701
13304	34.7.4	rhomboid-related protein [Homo sapiens]	0.217	0.082	0.013	1.783
13578	35.10.18	No Hits Found	0.043	0.644	0.553	0.867
13779	33.13.19	CGI-133 protein [Homo sapiens]	0.002	0.365	0.958	0.867
13876	34.14.16	ALCOHOL DEHYDROGENASE CLASS III (GLUTATHIONE-DEPENDENT FORMALDEHYDE DEHYDROGENASE) (FDH)	0.061	0.043	0.044	1.005
14002	33.16.2	carbonic anhydrase [Mus musculus]	0.011	0.150	0.547	0.753
14052	35.16.12	ADENYLATE KINASE ISOENZYME 1 (ATP-AMP TRANSPHOSPHORYLASE) (AK1)(MYOKINASE)	0.016	0.332	0.579	0.809
14080	36.16.20	No Hits Found	0.007	0.588	0.889	0.817
14091	33.17.11	SEC24 (S. cerevisiae) homolog B; secretory protein 24; Sec24-related protein B; protein transport protein Sec24B [Homo sapiens]	0.994	0.327	0.047	0.902
14109	34.17.9	KIAA0573 protein [Homo sapiens]	0.027	0.001	0.050	1.204

Spot	GEO no.	BlastX	line	age	interaction	Fold diff
14157	36.17.17	Cog4S [Homo sapiens]	0.004	0.068	0.695	0.960
14236	36.18.16	No Hits Found	0.019	0.501	0.991	0.898
14275	34.19.15	No Hits Found	0.026	0.277	0.246	1.122
14490	37.2.10	No Hits Found	0.029	0.003	0.641	1.125
14515	38.2.15	MHC class I histocompatibility antigen B-F alpha chain 2 - chicken	0.086	0.542	0.019	1.386
14541	40.2.1	Protein CGI-100 precursor	0.012	0.157	0.907	0.779
14563	37.3.3	bA207C16.3 (novel protein similar to predicted yeast, plant andworm proteins) [Homo sapiens]	0.006	0.005	0.664	0.829
14571	37.3.11	No Hits Found	0.006	0.021	0.091	2.119
14589	38.3.9	No Hits Found	0.038	0.000	0.688	1.069
14596	38.3.16	No Hits Found	0.008	0.010	0.639	1.039
14623	40.3.3	Lysosomal alpha-mannosidase precursor (Mannosidase, alpha B) (Lysosomal acid alpha-mannosidase) (Laman) (Mannosidase alpha class 2B member 1)	0.029	0.489	0.024	1.873
14625	40.3.5	unnamed protein product [Mus musculus]	0.001	0.016	0.056	0.852
14643	37.4.3	similar to hypothetical protein FLJ20225 [Homo sapiens] [Musmusculus]	0.025	0.012	0.787	0.946
14688	39.4.8	beta-xylosidase B [Clostridium stercorarium]	0.039	0.000	0.261	1.194
14690	39.4.10	type I hair keratin 6; keratin, hair, acidic, 6 [Homo sapiens]	0.012	0.055	0.226	0.697
14816	37.6.16	polymerase (DNA directed), delta 2, regulatory subunit 50kDa; polymerase (DNA directed), delta 2, regulatory subunit (50kD) [Homo sapiens]	0.013	0.137	0.778	0.784
14839	38.6.19	No Hits Found	0.984	0.247	0.024	1.280
14871	40.6.11	procKr2 - chicken (fragment)	0.021	0.070	0.588	1.729
14944	40.7.4	Unknown (protein for MGC:26097) [Homo sapiens]	0.034	0.022	0.637	0.729
14955	40.7.15	attractin [Mesocricetus auratus]	0.013	0.343	0.647	3.522
15001	39.8.1	dJ761I2.1 (enhancer of filamentation (HEF1)) [Homo sapiens]	0.049	0.223	0.959	1.035
15027	40.8.7	solute carrier family 21 (organic anion transporter), member 11 [Homo sapiens]	0.038	0.232	0.031	2.002
15125	37.10.5	repressor protein [Homo sapiens]	0.039	0.100	0.283	0.904
15165	39.10.5	neurofascin - chicken	0.000	0.077	0.702	0.871
15188	40.10.8	similar to latent transforming growth factor beta binding protein 1; latent TGF beta binding protein [Mus musculus]	0.040	0.173	0.421	0.774
15228	38.11.8	Salivary gland secretion 1 [Drosophila melanogaster]	0.004	0.637	0.259	1.366
15285	37.12.5	No Hits Found	0.017	0.088	0.077	0.883
15370	37.13.10	small integral membrane protein of lysosome/late endosome [Gallusgallus]	0.025	0.421	0.609	1.108
15379	37.13.19	lecithin cholesterol acyltransferase [Anas platyrhynchos]	0.025	0.419	0.097	1.265
15429	40.13.9	hypothetical protein [Rhodopseudomonas palustris]	0.027	0.110	0.032	1.274
15432	40.13.12	No Hits Found	0.022	0.485	0.633	1.556
15436	40.13.16	blood plasma glutamate carboxypeptidase precursor;prostate-specific membrane antigen (PSMA) [Homo sapiens]	0.000	0.018	0.599	1.226
15439	40.13.19	serine/threonine kinase 25 (yeast); Ste20-like kinase;serine/threonine kinase 25 (Ste20, yeast homolog); YeastSps1/Ste20-related kinase 1 [Mus musculus]	0.028	0.848	0.376	0.730
15471	38.14.11	No Hits Found	0.007	0.274	0.760	1.779
15473	38.14.13	Similar to Human C219-reactive peptide (L34688) [Homo sapiens]	0.050	0.542	0.490	2.078
15599	40.15.19	GTP-binding protein Rab0 [Rattus norvegicus]	0.734	0.812	0.015	1.603
15633	38.16.13	talin [Gallus gallus]	0.004	0.462	0.625	0.851
15831	40.18.11	No Hits Found	0.006	0.278	0.673	0.784
16022	42.1.2	hypothetical protein XP_161952 [Mus musculus]	0.018	0.519	0.695	0.692
16054	43.1.14	MHC Rfp-Y class I alpha chain [Gallus gallus]	0.525	0.448	0.043	1.592
16088	41.2.8	No Hits Found	0.018	0.004	0.630	0.880
16138	43.2.18	unnamed protein product [Mus musculus]	0.037	0.000	0.719	1.189
16145	44.2.5	No Hits Found	0.012	0.000	0.933	0.804
16173	41.3.13	No Hits Found	0.000	0.003	0.259	0.843
16177	41.3.17	blood plasma glutamate carboxypeptidase precursor;prostate-specific membrane antigen (PSMA) [Homo sapiens]	0.000	0.268	0.507	1.385
16182	42.3.2	Ig light chain precursor	0.005	0.000	0.617	4.824
16213	43.3.13	KIAA1627 protein [Homo sapiens]	0.010	0.206	0.673	0.821
16234	44.3.14	similar to Putative ubiquinone biosynthesis monooxgenase COQ6 (CGI-10) [Mus musculus]	0.030	0.006	0.650	1.283
16255	41.4.15	MHC class I antigen [Gallus gallus]	0.066	0.597	0.027	0.847
16294	43.4.14	Ig gamma chain (clone 36) - chicken (fragment)	0.022	0.002	0.730	5.161
16296	43.4.16	No Hits Found	0.035	0.284	0.706	0.781

Spot	GEO no.	BlastX	line	age	interaction	Fold diff
16323	41.5.3	neuromedin U [Homo sapiens]	0.043	0.013	0.365	1.631
16376	43.5.16	Unknown (protein for IMAGE:3659371) [Homo sapiens]	0.048	0.362	0.442	0.836
16383	44.5.3	No Hits Found	0.594	0.536	0.006	1.518
16400	44.5.20	DEAD-box protein abstrakt; putative RNA helicase [Homo sapiens]	0.026	0.146	0.417	0.750
16413	41.6.13	NIP1 [Homo sapiens]	0.027	0.144	0.067	1.011
16482	41.7.2	MHC class I [Gallus gallus]	0.189	0.672	0.028	1.046
16488	41.7.8	REST corepressor; KIAA0071 protein [Homo sapiens]	0.042	0.006	0.559	1.535
16502	42.7.2	hypothetical protein FLJ11198 [Homo sapiens]	0.047	0.581	0.400	2.111
16579	41.8.19	hypothetical protein MGC2494 [Homo sapiens]	0.036	0.017	0.259	0.869
16632	44.8.12	mitogen-activated protein kinase kinase 1 interacting protein 1;MEK partner 1; MEK binding partner 1 [Homo sapiens]	0.041	0.034	0.797	0.912
16638	44.8.18	No Hits Found	0.021	0.292	0.489	0.792
16722	41.10.2	androgen induced protein [Homo sapiens]	0.045	0.215	0.267	0.823
16935	43.12.15	No Hits Found	0.030	0.056	0.153	0.749
16939	43.12.19	GROWTH HORMONE RECEPTOR PRECURSOR (GH RECEPTOR) (SERUM BINDING PROTEIN)	0.031	0.012	0.191	0.698
17004	43.13.4	Lamin A	0.046	0.304	0.786	0.700
17076	42.14.16	Serum albumin precursor	0.829	0.314	0.035	2.771
17103	44.14.3	NADH dehydrogenase subunit 6 [Gallus gallus]	0.002	0.050	0.236	0.856
17128	41.15.8	similar to hypothetical protein MGC30022 [Homo sapiens] [Musmusculus]	0.360	0.702	0.032	2.519
17186	44.15.6	NONHISTONE CHROMOSOMAL PROTEIN HMG-14A	0.025	0.000	0.945	0.716
17319	42.17.19	No Hits Found	0.010	0.019	0.952	0.739
17419	43.18.19	KIAA1784 protein [Homo sapiens]	0.020	0.165	0.441	4.134
17438	44.18.18	No Hits Found	0.024	0.033	0.830	0.763
17440	44.18.20	Nd1 [Mus musculus]	0.011	0.015	0.522	0.884
17454	41.19.14	hypothetical protein FLJ33903 [Homo sapiens]	0.004	0.815	0.028	1.618
17622	46.1.2	SH3-containing protein SH3GLB2 [Mus musculus]	0.008	0.000	0.530	0.887
17651	47.1.11	KIAA1184 protein [Homo sapiens]	0.022	0.028	0.367	1.068
17746	48.2.6	Similar to guanylate cyclase 1, soluble, alpha 3 [Homo sapiens]	0.000	0.110	0.025	1.153
17981	48.5.1	p5kip2	0.016	0.000	0.525	3.878
17984	48.5.4	similar to Ca<2+>dependent activator protein for secretion; Ca2+-dependent activator protein for secretion [Homo sapiens]	0.899	0.063	0.016	1.120
18016	45.6.16	PROTEIN-TYROSINE PHOSPHATASE X PRECURSOR (R-PTP-X) (M1851)	0.006	0.009	0.402	3.851
18020	45.6.20	RIKEN cDNA 1200007O21 [Mus musculus]	0.000	0.000	0.521	1.003
18126	47.7.6	mitochondrial carrier homolog 1 isoform a [Mus musculus domesticus]	0.023	0.087	0.983	2.593
18215	47.8.15	procollagen-lysine, 2-oxoglutarate 5-dioxygenase 3;procollagen-lysine, 2-oxoglutarate 5-dioxygenase 3v;lysyl hydroxylase 3 [Homo sapiens]	0.038	0.574	0.775	0.703
18264	46.9.4	hypothetical protein MGC19606 [Homo sapiens]	0.040	0.020	0.571	0.726
18295	47.9.15	MHC class II-associated invariant chain [Gallus gallus]	0.012	0.091	0.133	0.824
18331	45.10.11	keratin 18 [Homo sapiens]	0.000	0.012	0.007	2.084
18439	46.11.19	No Hits Found	0.043	0.132	0.480	0.932
18472	48.11.12	tumor necrosis factor receptor	0.020	0.095	0.355	1.959
18476	48.11.16	No Hits Found	0.040	0.970	0.882	1.339
18511	46.12.11	No Hits Found	0.040	0.000	0.719	0.709
18522	47.12.2	CD48 antigen; expressed sequence AI449234 [Mus musculus]	0.008	0.843	0.154	0.699
18533	47.12.13	No Hits Found	0.408	0.001	0.007	0.798
18589	46.13.9	CD38 antigen; CD38 antigen (ADP-ribosyl cyclase / cyclic ADP-ribose hydrolase) [Rattus norvegicus]	0.007	0.079	0.246	1.702
18615	47.13.15	No Hits Found	0.003	0.031	0.468	0.878
18634	48.13.14	No Hits Found	0.018	0.782	0.152	0.910
18786	48.15.6	nucleotide binding protein 2 (MinD homolog, E. coli); nucleotidebinding protein 2 (E.coli MinD like) [Homo sapiens]	0.045	0.630	0.262	0.980
18823	46.16.3	similar to Kelch motif containing protein~data source:Pfam, sourcekey:PF01344, evidence:ISS~putative [Homo sapiens]	0.017	0.040	0.666	0.798
18850	47.16.10	Ig light chain precursor	0.001	0.001	0.370	2.308
18991	46.18.11	unnamed protein product [Mus musculus]	0.035	0.919	0.725	0.976
19041	45.19.1	amyotrophic lateral sclerosis 2 (juvenile) chromosome region,candidate 19; partitioning-defective 3-like [Homosapiens]	0.040	0.786	0.874	0.947

Table 7. Up-regulated genes in the Lean line at 1-week of age. *Spot* is the Del-Mar 14K Chicken Integrated Systems microarray individual spot number; *BlastX* is the highest scoring translated BLAST hit; *Lean 1* and *Fat 1* are the normalized $\log_2$ ratios for each line at 1-week of age; *Fold diff* is the fold change between the Lean and Fat $\log_2$ means. Bolded and italicized Spot ***numbers*** are genes that were chosen as potential candidate genes for further investigation.

Spot	BlastX	Lean 1	Fat 1	Fold diff
2706	No Hits Found	1.386	-1.220	2.606
7147	gene 17.5 protein - chicken	1.437	-0.986	2.423
3645	LH-beta	-0.643	-2.594	1.952
730	No Hits Found	0.492	-1.444	1.936
11313	cited2/melanocyte specific gene-related gene 1 MRG1 [Gallus gallus]	-0.629	-2.536	1.907
2968	suppressor of fused [Gallus gallus]	1.108	-0.504	1.612
15471	No Hits Found	0.718	-0.654	1.372
10098	No Hits Found	0.708	-0.637	1.345
2350	Neuropeptide Y precursor (NPY)	1.795	0.553	1.242
7037	clusterin [Gallus gallus]	0.299	-0.918	1.217
413	Unknown (protein for MGC:10089) [Homo sapiens]	0.769	-0.391	1.160
2035	Beta-neoendorphin-dynorphin precursor (Proenkephalin B)(Preprodynorphin) [Contains: Beta-neoendorphin;Dynorphin; Leu-Enkephalin; Rimorphin; Leumorphin]	-0.159	-1.316	1.157
8433	No Hits Found	0.019	-1.075	1.095
4352	similar to kinesin superfamily protein 1C [Mus musculus]	0.894	-0.169	1.063
4989	endothelial differentiation, lysophosphatidic acidG-protein-coupled receptor, 2 [Rattus norvegicus]	0.947	-0.102	1.049
4247	No Hits Found	1.080	0.051	1.028
5430	No Hits Found	1.171	0.151	1.020
9090	No Hits Found	0.220	-0.800	1.019
8418	calreticulin [Mus musculus]	0.693	-0.320	1.013
1764	unnamed protein product [Homo sapiens]	0.028	-0.985	1.013
1806	Similar to butyrate-induced transcript 1 [Mus musculus]	-0.201	-1.198	0.997
3677	gastrin-releasing peptide precursor splice form III - human	-0.632	-1.628	0.996
8989	No Hits Found	0.972	0.023	0.950
1018	No Hits Found	0.642	-0.303	0.945
8169	hyaluronoglucosaminidase 3 [Homo sapiens]	-0.341	-1.278	0.937
8866	syndecan	0.723	-0.207	0.930
10038	No Hits Found	0.510	-0.406	0.916
292	Slit1 protein [Gallus gallus]	0.856	-0.046	0.901
1696	CD63 antigen	0.296	-0.588	0.884
6447	histocompatibility 13 [Mus musculus]	-0.504	-1.381	0.876
5331	CD47 antigen [Bos taurus]	1.574	0.702	0.872
2745	RIKEN cDNA 1810046J19; expressed sequence AI463380 [Mus musculus]	1.053	0.185	0.868
12199	No Hits Found	0.803	-0.061	0.864

Spot	BlastX	Lean 1	Fat 1	Fold diff
5579	nectin-lke 1 [Mus musculus]	0.932	0.083	0.849
16413	NIP1 [Homo sapiens]	0.477	-0.367	0.845
6840	Glycylpeptide N-tetradecanoyltransferase 2 (PeptideN-myristoyltransferase 2) (Myristoyl-CoA:proteinN-myristoyltransferase 2) (NMT 2) (Type IIN-myristoyltransferase)	0.218	-0.622	0.840
10040	UV radiation resistance associated gene [Homo sapiens]	-0.329	-1.166	0.838
264	unnamed protein product [Mus musculus]	-0.099	-0.934	0.836
3549	gene 17.5 protein - chicken	0.615	-0.220	0.835
5283	carbamoyl-phosphate synthetase (E.C.6.3.5.5)	0.162	-0.673	0.834
17076	Serum albumin precursor	1.012	0.189	0.823
11345	aldo-keto reductase [Gallus gallus]	0.393	-0.428	0.821
3404	unnamed protein product [Homo sapiens]	-0.215	-1.033	0.818
2141	UDP-Gal:betaGlcNAc beta 1,3-galactosyltransferase 1; beta-3-galt1[Homo sapiens]	0.429	-0.386	0.814
1666	MAPK-interacting and spindle-stabilizing protein [Homo sapiens]	1.197	0.399	0.798
1832	Cathepsin D precursor	0.935	0.144	0.791
2889	aldo-keto reductase [Gallus gallus]	0.568	-0.221	0.789
11963	similar to ZK792.1b.p [Homo sapiens]	1.359	0.575	0.785
16722	androgen induced protein [Homo sapiens]	0.775	0.012	0.763
238	TUC-4b [Rattus norvegicus]	0.892	0.139	0.753
3242	chromosome 11 open reading frame2; chromosome 11 open readingframe2 [Homo sapiens]	-0.281	-1.032	0.751
2101	DKFZP586B1621 protein [Homo sapiens]	0.528	-0.214	0.742
1652	transmembrane 4 superfamily member 2; membrane component, xchromosome, surface marker 1; T-cell acute lymphoblasticleukemia associated antigen 1; transmembrane proteinA15; tetraspanin protein; cell surface glycoprotein A15;CD231 antigen; transmembrane 4 superfamily 2b [Homosapiens]	0.059	-0.673	0.732
11610	No Hits Found	-0.042	-0.754	0.712
1185	gene 17.5 protein - chicken	0.600	-0.104	0.704
13006	unnamed protein product [Mus musculus]	-0.346	-1.045	0.699
1639	similar to FLJ00237 protein [Homo sapiens]	-0.280	-0.978	0.698
408	serologically defined colon cancer antigen 28; phosphatidylcholinetransfer protein-like [Mus musculus]	-0.300	-0.995	0.694
10667	No Hits Found	-0.075	-0.761	0.686

Table 8. Up-regulated genes in the Fat line at 1-week of age. *Spot* is the Del-Mar 14K Chicken Integrated Systems microarray individual spot number; *BlastX* is the highest scoring translated BLAST hit; *Lean 1* and *Fat 1* are the normalized $\log_2$ ratios for each line at 1-week of age; *Fold diff* is the fold change between the Lean and Fat $\log_2$ means. Bolded and italicized Spot ***numbers*** are genes that were chosen as potential candidate genes for further investigation.

Spot	BlastX	Lean 1	Fat 1	Fold diff
17419	KIAA1784 protein [Homo sapiens]	1.265	4.589	3.325
10438	ATPase, H+ transporting, lysosomal V0 subunit a isoform 4; vacuolarproton pump 116 kDa accessory subunit; vacuolar protonpump, subunit 2; H(+)-transporting two-sector ATPase,noncatalytic accessory protein 1B; ATPase, H+transporting, lysosomal (vacuolar proton pump)non-catalytic accessory protein 1B; renal tubularacidosis; ATPase, H+ transporting, lysosomal (vacuolarproton pump) non-catalytic accessory protein 2 (38kD)[Homo sapiens]	0.959	3.850	2.892
15027	solute carrier family 21 (organic anion transporter), member 11 [Homo sapiens]	0.538	2.534	1.996
14955	attractin [Mesocricetus auratus]	1.431	3.412	1.980
5155	No Hits Found	-0.146	1.653	1.798
5732	No Hits Found	0.072	1.840	1.768
8479	pleiotrophin; osteoblastic cell factor; heparin-binding brainmitogen; heparin-binding growth factor 8; heparin affinregulatory peptide; heparin-binding neurotorphic factor;heparin-binding neurite promoting factor;heparin-binding growth-associated molecule [Musmusculus]	-1.871	-0.169	1.702
3221	Ig light chain precursor	-3.625	-1.963	1.662
18331	keratin 18 [Homo sapiens]	0.104	1.654	1.550
8851	proline rich protein 2 [Mus musculus]	1.040	2.510	1.469
2413	No Hits Found	0.573	2.020	1.447
2567	chromodomain helicase DNA binding protein 2 [Homo sapiens]	0.388	1.752	1.365
4311	Thymosin beta	-0.955	0.243	1.199
18476	No Hits Found	0.300	1.449	1.149
16182	Ig light chain precursor	-3.586	-2.463	1.122
3241	adenylate cyclase 2; adenylyl cyclase 2; adenylate cyclase II; adenylyl cyclase; ATP pyrophosphate-lyase; type II adenylate cyclase; 3',5'-cyclic AMP synthetase [Homo sapiens]	-1.285	-0.174	1.110
8673	tetranectin [Gallus gallus]	-0.535	0.550	1.085
9071	No Hits Found	0.408	1.480	1.071
15473	Similar to Human C219-reactive peptide (L34688) [Homo sapiens]	0.583	1.647	1.064
8655	MHC class I antigen [Gallus gallus]	-1.092	-0.034	1.058
11309	No Hits Found	-0.018	1.036	1.054
15228	Salivary gland secretion 1 [Drosophila melanogaster]	-0.370	0.652	1.023
4880	Regulator of G-protein signaling 5	-0.658	0.362	1.020

Spot	BlastX	Lean 1	Fat 1	Fold diff
771	expressed sequence AA408877; hypothetical protein MGC38511 [Musmusculus]	-0.810	0.201	1.011
18991	unnamed protein product [Mus musculus]	-0.166	0.810	0.976
6533	MHC class II-associated invariant chain [Gallus gallus]	-0.774	0.191	0.965
17746	Similar to guanylate cyclase 1, soluble, alpha 3 [Homo sapiens]	-0.903	0.037	0.940
9001	kelch domain containing 1 [Homo sapiens]	0.048	0.987	0.939
16177	blood plasma glutamate carboxypeptidase precursor;prostate-specific membrane antigen (PSMA) [Homo sapiens]	-0.054	0.861	0.915
18634	No Hits Found	0.341	1.251	0.910
18126	mitochondrial carrier homolog 1 isoform a [Mus musculus domesticus]	-0.590	0.296	0.886
1507	No Hits Found	-0.650	0.236	0.886
3033	fragile X mental retardation protein	0.623	1.509	0.886
4200	No Hits Found	0.277	1.130	0.853
15436	blood plasma glutamate carboxypeptidase precursor;prostate-specific membrane antigen (PSMA) [Homo sapiens]	-0.341	0.502	0.843
12350	L-lactate dehydrogenase A chain (LDH-A)	-0.545	0.295	0.840
15432	No Hits Found	0.020	0.846	0.826
13876	ALCOHOL DEHYDROGENASE CLASS III (GLUTATHIONE-DEPENDENT FORMALDEHYDE DEHYDROGENASE) (FDH)	-0.124	0.695	0.819
16294	Ig gamma chain (clone 36) - chicken (fragment)	-4.189	-3.373	0.816
10702	6-phosphofructo-2-kinase/fructose-2, 6-bisphosphatase [Gallus gallus]	-0.696	0.112	0.808
16323	neuromedin U [Homo sapiens]	0.306	1.110	0.805
4170	catalase [Canis familiaris]	0.093	0.892	0.799
5691	MHC class II-associated invariant chain [Gallus gallus]	-0.592	0.202	0.795
14515	MHC class I histocompatibility antigen B-F alpha chain 2 - chicken	-0.805	-0.021	0.783
10350	RIKEN cDNA 1200015A22 [Mus musculus]	-0.255	0.526	0.781
13108	complement component factor h; complement factor H related protein 3A4/5G4 [Mus musculus]	-0.490	0.266	0.757
1017	hypothetical protein XP_148064 [Mus musculus]	0.717	1.473	0.756
4811	Zinc finger protein CKR1	-0.460	0.287	0.747
4127	No Hits Found	-0.223	0.513	0.736
2600	hypothetical protein MGC10485 [Homo sapiens]	-0.479	0.251	0.730
7639	CCCH zinc finger protein C3H-3 [Xenopus laevis]	0.256	0.984	0.728
9878	PTD009 protein [Homo sapiens]	0.271	0.992	0.721
12217	catecholamine-O-methyltransferase [Rattus norvegicus]	-0.093	0.622	0.716
16638	No Hits Found	-0.202	0.502	0.704
9659	hypothetical protein [Rhodobacter sphaeroides]	-0.637	0.060	0.697
8555	FLJ00239 protein [Homo sapiens]	-0.230	0.465	0.695
18472	tumor necrosis factor receptor	0.273	0.967	0.694
10675	alpha-1-antiproteinase [Xenopus laevis]	-0.455	0.237	0.692

Table 9. Up-regulated genes in the Lean line at 3-weeks of age. *Spot* is the Del-Mar 14K Chicken Integrated Systems microarray individual spot number; *BlastX* is the highest scoring translated BLAST hit; *Lean 3* and *Fat 3* are the normalized $\log_2$ ratios for each line at 3-weeks of age; *Fold diff* is the fold change between the Lean and Fat $\log_2$ means. Bolded and italicized Spot ***numbers*** are genes that were chosen as potential candidate genes for further investigation.

Spot	BlastX	Lean 3	Fat 3	Fold diff
5285	bone morphogenetic protein 1 [Gallus gallus]	1.345	-0.858	2.20
7157	No Hits Found	1.375	-0.717	2.09
17076	Serum albumin precursor	1.428	-0.536	1.96
2968	suppressor of fused [Gallus gallus]	1.238	-0.612	1.85
9447	Wnt-5a [Gallus gallus]	0.377	-1.178	1.56
10098	No Hits Found	0.477	-0.967	1.44
455	No Hits Found	0.348	-0.949	1.30
2706	No Hits Found	0.314	-0.975	1.29
1650	cell division cycle control protein 37	1.333	0.079	1.25
2854	dJ259A10.1 (ssDNA binding protein (SEB4D)) [Homo sapiens]	0.236	-0.973	1.21
15599	GTP-binding protein Rab0 [Rattus norvegicus]	0.032	-1.161	1.19
9090	No Hits Found	0.691	-0.500	1.19
5456	CD63 antigen	0.376	-0.732	1.11
10038	No Hits Found	0.912	-0.161	1.07
730	No Hits Found	0.701	-0.345	1.05
701	prolyl 4-hydroxylase, alpha subunit (EC 1.14.11.2)	1.422	0.382	1.04
1696	CD63 antigen	0.444	-0.595	1.04
1185	gene 17.5 protein - chicken	0.879	-0.149	1.03
2889	aldo-keto reductase [Gallus gallus]	0.671	-0.314	0.98
8866	syndecan	0.743	-0.239	0.98
5283	carbamoyl-phosphate synthetase (E.C.6.3.5.5)	0.289	-0.689	0.98
6447	histocompatibility 13 [Mus musculus]	0.621	-0.357	0.98
1652	transmembrane 4 superfamily member 2; membrane component, xchromosome, surface marker 1; T-cell acute lymphoblasticleukemia associated antigen 1; transmembrane proteinA15; tetraspanin protein; cell surface glycoprotein A15;CD231 antigen; transmembrane 4 superfamily 2b [Homosapiens]	0.707	-0.263	0.97
6840	Glycylpeptide N-tetradecanoyltransferase 2 (PeptideN-myristoyltransferase 2) (Myristoyl-CoA:proteinN-myristoyltransferase 2) (NMT 2) (Type IIN-myristoyltransferase)	0.278	-0.691	0.97
14839	No Hits Found	0.097	-0.857	0.95
1017	hypothetical protein XP_148064 [Mus musculus]	1.342	0.404	0.94
3677	gastrin-releasing peptide precursor splice form III - human	-0.248	-1.137	0.89
11345	aldo-keto reductase [Gallus gallus]	0.485	-0.369	0.85
5331	CD47 antigen [Bos taurus]	1.807	0.980	0.83
9635	similar to hypothetical protein similar to RNA-binding protein lark[Homo sapiens] [Rattus norvegicus]	0.759	-0.068	0.83

Spot	BlastX	Lean 3	Fat 3	Fold diff
10633	Predicted CDS, cuticle collagen family member [Caenorhabditis elegans]	0.557	-0.265	0.82
11313	cited2/melanocyte specific gene-related gene 1 MRG1 [Gallus gallus]	0.311	-0.477	0.79
6074	hypothetical protein XP_148705 [Mus musculus]	0.122	-0.643	0.77
13578	No Hits Found	1.203	0.439	0.76
8919	calcium binding protein P22; Sid470p [Mus musculus]	0.700	-0.035	0.73
15439	serine/threonine kinase 25 (yeast); Ste20-like kinase;serine/threonine kinase 25 (Ste20, yeast homolog); YeastSps1/Ste20-related kinase 1 [Mus musculus]	0.731	0.002	0.73
16400	DEAD-box protein abstrakt; putative RNA helicase [Homo sapiens]	0.312	-0.417	0.73
10345	active breakpoint cluster region-related protein isoform a [Homosapiens]	0.772	0.063	0.71
7147	gene 17.5 protein - chicken	0.951	0.245	0.71
5006	OB-receptor gene related protein [Rattus norvegicus]	0.361	-0.336	0.70
2851	hypothetical protein; extensin-like; with SH3 Src homology domain [Schizosaccharomyces pombe]	0.521	-0.175	0.70

Table 10. Up-regulated genes in the Fat line at 3-weeks of age. *Spot* is the Del-Mar 14K Chicken Integrated Systems microarray individual spot number; *BlastX* is the highest scoring translated BLAST hit; *Lean 3* and *Fat 3* are the normalized $\log_2$ ratios for each line at 3-weeks of age; *Fold diff* is the fold change between the Lean and Fat $\log_2$ means. Bolded and italicized Spot ***numbers*** are genes that were chosen as potential candidate genes for further investigation.

Spot	BlastX	Lean 3	Fat 3	Fold diff
2567	chromodomain helicase DNA binding protein 2 [Homo sapiens]	0.751	3.500	2.75
3033	fragile X mental retardation protein	0.892	3.610	2.72
16294	Ig gamma chain (clone 36) - chicken (fragment)	-1.489	0.972	2.46
9071	No Hits Found	0.746	3.187	2.44
16182	Ig light chain precursor	-1.109	1.239	2.35
3221	Ig light chain precursor	-1.127	1.114	2.24
8479	pleiotrophin; osteoblastic cell factor; heparin-binding brainmitogen; heparin-binding growth factor 8; heparin affinregulatory peptide; heparin-binding neurotorphic factor;heparin-binding neurite promoting factor;heparin-binding growth-associated molecule [Musmusculus]	-0.943	1.221	2.16
18331	keratin 18 [Homo sapiens]	-0.379	1.705	2.08
17981	p57kip2	-1.109	0.873	1.98
7333	unnamed protein product [Mus musculus]	0.554	2.337	1.78
18016	PROTEIN-TYROSINE PHOSPHATASE X PRECURSOR (R-PTP-X) (M1851)	0.569	2.282	1.71
6768	dJ963K23.4 (continues in dJ1041C10 (AL162615)) [Homo sapiens]	0.702	2.390	1.69
5193	RIKEN cDNA 1110063G11 [Mus musculus]	-0.577	1.049	1.63
18472	tumor necrosis factor receptor	0.318	1.863	1.54
8655	MHC class I antigen [Gallus gallus]	-1.167	0.352	1.52
16383	No Hits Found	-0.152	1.366	1.52
10937	lysyl oxidase-like 2 [Homo sapiens]	-0.489	0.991	1.48
18850	Ig light chain precursor	-0.424	1.046	1.47
16054	MHC Rfp-Y class I alpha chain [Gallus gallus]	-0.665	0.786	1.45
9843	immunoglobulin alpha heavy chain [Gallus gallus]	-0.727	0.724	1.45
17419	KIAA1784 protein [Homo sapiens]	0.772	2.132	1.36
6629	No Hits Found	-0.801	0.548	1.35
5073	solute carrier family 29 (nucleoside transporters), member 1 [Homosapiens]	0.605	1.945	1.34
14515	MHC class I histocompatibility antigen B-F alpha chain 2 - chicken	-0.649	0.582	1.23
333	Skin secretory protein XP2 precursor (APEG protein)	-0.414	0.802	1.22
4811	Zinc finger protein CKR1	-0.561	0.560	1.12
12350	L-lactate dehydrogenase A chain (LDH-A)	-0.171	0.947	1.12
15370	small integral membrane protein of lysosome/late endosome [Gallusgallus]	-0.264	0.844	1.11
14871	procKr2 - chicken (fragment)	0.611	1.678	1.07

Spot	BlastX	Lean 3	Fat 3	Fold diff
15429	hypothetical protein [Rhodopseudomonas palustris]	0.620	1.674	1.05
15228	Salivary gland secretion 1 [Drosophila melanogaster]	-0.052	0.995	1.05
9659	hypothetical protein [Rhodobacter sphaeroides]	-0.224	0.815	1.04
771	expressed sequence AA408877; hypothetical protein MGC38511 [Musmusculus]	-0.992	0.021	1.01
15027	solute carrier family 21 (organic anion transporter), member 11 [Homo sapiens]	0.633	1.636	1.00
5855	growth hormone receptor [Gallus gallus]	-1.358	-0.372	0.99
5155	No Hits Found	0.397	1.378	0.98
18126	mitochondrial carrier homolog 1 isoform a [Mus musculus domesticus]	0.406	1.378	0.97
1337	similar to hypothetical protein [Homo sapiens] [Mus musculus]	-1.337	-0.376	0.96
8673	tetranectin [Gallus gallus]	-0.339	0.610	0.95
16488	REST corepressor; KIAA0071 protein [Homo sapiens]	-0.539	0.408	0.95
35	gamma-immunoglobulin heavy chain (504 AA) [Gallus gallus]	-0.502	0.421	0.92
689	gag/env fusion protein [Gallus gallus]	0.558	1.458	0.90
18589	CD38 antigen; CD38 antigen (ADP-ribosyl cyclase / cyclic ADP-ribose hydrolase) [Rattus norvegicus]	0.703	1.597	0.89
14955	attractin [Mesocricetus auratus]	1.533	2.425	0.89
17746	Similar to guanylate cyclase 1, soluble, alpha 3 [Homo sapiens]	-0.642	0.249	0.89
16482	MHC class I [Gallus gallus]	-0.462	0.428	0.89
10070	glypican 3 [Homo sapiens]	-1.429	-0.543	0.89
7349	No Hits Found	-1.368	-0.498	0.87
11622	skeletrophin; dystrophin-like [Mus musculus]	-0.034	0.810	0.84
10888	similar to smoothelin isoform c [Homo sapiens] [Mus musculus]	-0.487	0.355	0.84
16255	MHC class I antigen [Gallus gallus]	-0.355	0.480	0.84
17454	hypothetical protein FLJ33903 [Homo sapiens]	1.064	1.892	0.83
43	immunoglobulin J chain [Gallus gallus]	-0.475	0.352	0.83
6533	MHC class II-associated invariant chain [Gallus gallus]	-0.314	0.491	0.81
10350	RIKEN cDNA 1200015A22 [Mus musculus]	-0.047	0.754	0.80
17651	KIAA1184 protein [Homo sapiens]	-0.011	0.776	0.79
1687	No Hits Found	0.390	1.165	0.78
11309	No Hits Found	0.093	0.866	0.77
1319	EH-domain containing 3; EH domain containing 3 [Homo sapiens]	-0.376	0.392	0.77
744	deoxyribonuclease gamma [Xenopus laevis]	-0.427	0.321	0.75
17984	similar to Ca<2+>dependent activator protein for secretion; Ca2+-dependent activator protein for secretion [Homo sapiens]	0.659	1.387	0.73

Table 11. Up-regulated genes in the Lean line at 5-weeks of age. *Spot* is the Del-Mar 14K Chicken Integrated Systems microarray individual spot number; *BlastX* is the highest scoring translated BLAST hit; *Lean 5* and *Fat 5* are the normalized $\log_2$ ratios for each line at 5-weeks of age; *Fold diff* is the fold change between the Lean and Fat $\log_2$ means. Bolded and italicized Spot ***numbers*** are genes that were chosen as potential candidate genes for further investigation.

Spot	BlastX	Lean 5	Fat 5	Fold diff
3033	fragile X mental retardation protein	3.684	1.115	2.570
2854	dJ259A10.1 (ssDNA binding protein (SEB4D)) [Homo sapiens]	2.191	0.111	2.081
10348	chromosome 6 open reading frame 4 isoform 1; chromosome 6 open reading frame 6; chromosome 6 open reading frame 5; connection to IKK and SAPK/JNK; NFkB-activating protein ACT1 [Homo sapiens]	2.768	0.694	2.074
2968	suppressor of fused [Gallus gallus]	2.038	0.005	2.033
7147	gene 17.5 protein - chicken	1.411	-0.559	1.970
2350	Neuropeptide Y precursor (NPY)	1.739	-0.224	1.964
8866	syndecan	2.250	0.328	1.922
2706	No Hits Found	1.085	-0.798	1.883
1832	Cathepsin D precursor	1.719	-0.157	1.877
5430	No Hits Found	2.544	0.709	1.835
5353	unnamed protein product [Mus musculus]	1.645	-0.155	1.800
10099	No Hits Found	1.702	-0.061	1.763
2141	UDP-Gal:betaGlcNAc beta 1,3-galactosyltransferase 1; beta-3-galt1[Homo sapiens]	1.417	-0.286	1.703
730	No Hits Found	1.190	-0.486	1.676
5499	multiple exostoses type II protein EXT2.I [Homo sapiens]	1.628	-0.007	1.635
2533	ISOCITRATE DEHYDROGENASE [NADP] CYTOPLASMIC (OXALOSUCCINATE DECARBOXYLASE) (IDH) (NADP+-SPECIFIC ICDH) (IDP)	0.485	-0.830	1.315
4247	No Hits Found	0.848	-0.440	1.288
7157	No Hits Found	0.945	-0.269	1.214
422	similar to The KIAA0150 gene product is novel. [Homo sapiens]	0.951	-0.221	1.172
413	Unknown (protein for MGC:10089) [Homo sapiens]	1.353	0.190	1.164
701	prolyl 4-hydroxylase, alpha subunit (EC 1.14.11.2)	1.355	0.251	1.104
4649	DNA mismatch repair protein MSH6 (MutS-alpha 160 kDa subunit) (G/T mismatch binding protein) (GTBP) (GTMBP) (P160)	1.266	0.164	1.102
3550	hepatocyte growth factor activator inhibitor precursor; hepatocytegrowth factor activator inhibitor [Homo sapiens]	0.922	-0.177	1.099
4989	endothelial differentiation, lysophosphatidic acidG-protein-coupled receptor, 2 [Rattus norvegicus]	0.971	-0.090	1.061
10098	No Hits Found	0.839	-0.220	1.059
18786	nucleotide binding protein 2 (MinD homolog, E. coli); nucleotidebinding protein 2 (E.coli MinD like) [Homo sapiens]	0.674	-0.306	0.980
17984	similar to Ca<2+>dependent activator protein for secretion; Ca2+-	1.456	0.477	0.979

Spot	BlastX	Lean 5	Fat 5	Fold diff
	dependent activator protein for secretion [Homo sapiens]			
10633	Predicted CDS, cuticle collagen family member [Caenorhabditis elegans]	0.636	-0.342	0.978
6849	KIAA0872 protein [Homo sapiens]	1.265	0.301	0.964
1185	gene 17.5 protein - chicken	0.872	-0.087	0.960
7423	unnamed protein product [Homo sapiens]	0.674	-0.284	0.958
11560	No Hits Found	0.909	-0.047	0.956
4354	NADH-ubiquinone oxidoreductase SGDH subunit, mitochondrialprecursor (Complex I-SGDH) (CI-SGDH)	0.825	-0.129	0.954
3677	gastrin-releasing peptide precursor splice form III - human	0.476	-0.437	0.913
3549	gene 17.5 protein - chicken	0.923	0.011	0.912
12199	No Hits Found	0.952	0.077	0.875
1666	MAPK-interacting and spindle-stabilizing protein [Homo sapiens]	1.066	0.211	0.855
9447	Wnt-5a [Gallus gallus]	0.306	-0.534	0.841
608	similar to dJ475N16.3 (novel protein similar to RPL7A (60Sribosomal protein L7A)) [Homo sapiens]	0.922	0.088	0.833
5846	similar to dJ886K2.3(GALE (UDP-galactose-4-epimerase)) [Homosapiens]	1.116	0.288	0.828
10168	syndecan-2 variant 1 [Gallus gallus]	1.171	0.344	0.826
6495	leucine-rich, glioma inactivated 1 precursor; epilepsy, partial[Homo sapiens]	0.853	0.037	0.815
1652	transmembrane 4 superfamily member 2; membrane component, xchromosome, surface marker 1; T-cell acute lymphoblasticleukemia associated antigen 1; transmembrane proteinA15; tetraspanin protein; cell surface glycoprotein A15;CD231 antigen; transmembrane 4 superfamily 2b [Homosapiens]	0.697	-0.114	0.812
5845	No Hits Found	1.229	0.427	0.802
5283	carbamoyl-phosphate synthetase (E.C.6.3.5.5)	0.377	-0.415	0.792
15285	No Hits Found	1.447	0.661	0.787
7428	No Hits Found	1.164	0.384	0.781
7702	peptidyl-glycine alpha-amidating monooxygenase precursor (EC 1.14.17.3)	0.895	0.120	0.775
5132	Unknown (protein for MGC:25768) [Mus musculus]	0.939	0.177	0.762
14091	SEC24 (S. cerevisiae) homolog B; secretory protein 24; Sec24-related protein B; protein transport protein Sec24B [Homo sapiens]	0.241	-0.514	0.754
16722	androgen induced protein [Homo sapiens]	0.745	-0.007	0.752
2851	hypothetical protein; extensin-like; with SH3 Src homology domain [Schizosaccharomyces pombe]	0.971	0.234	0.738
16376	Unknown (protein for IMAGE:3659371) [Homo sapiens]	1.162	0.430	0.733
8919	calcium binding protein P22; Sid470p [Mus musculus]	0.781	0.049	0.732
9090	No Hits Found	1.165	0.432	0.732
455	No Hits Found	0.533	-0.197	0.730
13019	proteasome (prosome, macropain) 28 subunit, alpha; protease(prosome, macropain) 28 subunit, alpha [Mus musculus]	0.323	-0.398	0.721
8703	basic leucine zipper and W2 domains 1; KIAA0005 gene product; basic leucine-zipper protein BZAP45 [Homo sapiens]	0.923	0.203	0.720
343	No Hits Found	1.294	0.577	0.716
6947	No Hits Found	0.862	0.168	0.694

Table 12. Up-regulated genes in the Fat line at 5-weeks of age. *Spot* is the Del-Mar 14K Chicken Integrated Systems microarray individual spot number; *BlastX* is the highest scoring translated BLAST hit; *Lean 5* and *Fat 5* are the normalized $\log_2$ ratios for each line at 5-weeks of age; *Fold diff* is the fold change between the Lean and Fat $\log_2$ means. Bolded and italicized Spot ***numbers*** are genes that were chosen as potential candidate genes for further investigation.

Spot	BlastX	Lean 5	Fat 5	Fold diff
14955	attractin [Mesocricetus auratus]	1.954	4.907	2.954
5155	No Hits Found	1.729	4.125	2.397
5050	scavenger-receptor protein [Sus scrofa]	-1.967	0.076	2.043
14623	Lysosomal alpha-mannosidase precursor (Mannosidase, alpha B) (Lysosomal acid alpha-mannosidase) (Laman) (Mannosidase alpha class 2B member 1)	-0.756	1.117	1.873
18016	PROTEIN-TYROSINE PHOSPHATASE X PRECURSOR (R-PTP-X) (M1851)	0.193	2.020	1.827
10675	alpha-1-antiproteinase [Xenopus laevis]	-1.928	-0.155	1.773
7333	unnamed protein product [Mus musculus]	0.628	2.284	1.656
15473	Similar to Human C219-reactive peptide (L34688) [Homo sapiens]	-0.431	1.191	1.622
14571	No Hits Found	0.604	2.083	1.479
15432	No Hits Found	0.182	1.576	1.394
4811	Zinc finger protein CKR1	-0.031	1.326	1.356
18126	mitochondrial carrier homolog 1 isoform a [Mus musculus domesticus]	-1.064	0.272	1.335
2145	OSJNBa0029H02.25 [Oryza sativa (japonica cultivar-group)]	-0.359	0.964	1.322
18476	No Hits Found	0.192	1.488	1.296
15379	lecithin cholesterol acyltransferase [Anas platyrhynchos]	-0.510	0.755	1.265
14839	No Hits Found	-0.807	0.378	1.186
8673	tetranectin [Gallus gallus]	-0.864	0.311	1.175
2567	chromodomain helicase DNA binding protein 2 [Homo sapiens]	0.420	1.595	1.174
1687	No Hits Found	-0.340	0.824	1.164
16177	blood plasma glutamate carboxypeptidase precursor;prostate-specific membrane antigen (PSMA) [Homo sapiens]	0.171	1.330	1.159
5073	solute carrier family 29 (nucleoside transporters), member 1 [Homosapiens]	0.440	1.584	1.143
5193	RIKEN cDNA 1110063G11 [Mus musculus]	-0.674	0.465	1.139
4311	Thymosin beta	-0.043	1.063	1.106
9843	immunoglobulin alpha heavy chain [Gallus gallus]	-0.922	0.148	1.070
333	Skin secretory protein XP2 precursor (APEG protein)	-0.193	0.876	1.070
11610	No Hits Found	-0.744	0.308	1.052
35	gamma-immunoglobulin heavy chain (504 AA) [Gallus gallus]	-1.002	0.039	1.041
16502	hypothetical protein FLJ11198 [Homo sapiens]	0.118	1.130	1.012
8984	No Hits Found	0.049	1.057	1.008
3221	Ig light chain precursor	-0.637	0.369	1.005
159	No Hits Found	-0.307	0.688	0.995
5732	No Hits Found	0.184	1.160	0.976

Spot	BlastX	Lean 5	Fat 5	Fold diff
8851	proline rich protein 2 [Mus musculus]	0.619	1.592	0.973
9001	kelch domain containing 1 [Homo sapiens]	0.695	1.648	0.954
18850	Ig light chain precursor	-0.442	0.496	0.939
16294	Ig gamma chain (clone 36) - chicken (fragment)	-0.357	0.542	0.900
16182	Ig light chain precursor	-0.342	0.554	0.896
5855	growth hormone receptor [Gallus gallus]	-0.790	0.104	0.894
14871	procKr2 - chicken (fragment)	1.458	2.339	0.881
43	immunoglobulin J chain [Gallus gallus]	-0.755	0.113	0.868
17981	p57kip2	-0.655	0.185	0.841
18615	No Hits Found	0.132	0.955	0.823
689	gag/env fusion protein [Gallus gallus]	-0.165	0.658	0.823
2368	No Hits Found	-0.863	-0.041	0.822
18589	CD38 antigen; CD38 antigen (ADP-ribosyl cyclase / cyclic ADP-ribose hydrolase) [Rattus norvegicus]	1.429	2.237	0.808
5479	Ig light chain precursor	-0.736	0.067	0.804
8438	enoyl-Coenzyme A, hydratase/3-hydroxyacyl Coenzyme A dehydrogenase[Homo sapiens]	0.030	0.825	0.795
10937	lysyl oxidase-like 2 [Homo sapiens]	-0.393	0.401	0.794
13212	major histocompatibility complex class II beta chain; B-L betachain [Gallus gallus]	-0.324	0.469	0.793
4413	No Hits Found	-0.129	0.663	0.793
3441	Gamma-interferon inducible lysosomal thiol reductase precursor(Gamma-interferon-inducible protein IP-30)	-1.092	-0.301	0.791
3341	superoxide dismutase 3, extracellular [Homo sapiens]	-0.012	0.738	0.750
11292	RNA polymerase II largest subunit	-0.332	0.406	0.739
15228	Salivary gland secretion 1 [Drosophila melanogaster]	-0.120	0.615	0.735
4849	60S RIBOSOMAL PROTEIN L3 (L4)	-0.581	0.151	0.732
14157	Cog4S [Homo sapiens]	-0.509	0.217	0.726
7349	No Hits Found	-0.716	0.002	0.718
10702	6-phosphofructo-2-kinase/fructose-2, 6-bisphosphatase [Gallus gallus]	-0.161	0.552	0.713
11309	No Hits Found	-0.033	0.680	0.713
1017	hypothetical protein XP_148064 [Mus musculus]	0.969	1.681	0.712
16323	neuromedin U [Homo sapiens]	-0.520	0.189	0.710
1319	EH-domain containing 3; EH domain containing 3 [Homo sapiens]	-0.473	0.227	0.700
224	hypothetical protein BC013949 [Homo sapiens]	-0.300	0.394	0.694
8876	No Hits Found	-0.604	0.090	0.694

Table 13. The 25 genes with the highest correlation with the Lean line at 1-week of age. Signal-to-noise genes are on the left; t-test is on the right (see materials and methods details). Genes are ranked by correlation. *Spot Chromosome* is the microarray spot number and the chicken chromosomal location of the gene on which the gene has been located. *Gene* is the highest scoring BLAST hit. Bolded and italicized Spot ***numbers*** are genes that were chosen as potential candidate genes for further investigation.

Signal to noise rank	Spot Chromosome	Gene	t-test rank	Spot Chromosome	Gene
1	3284 chr28:3038633-3043633	No Hits Found	1	3284 chr28:3038633-3043633	No Hits Found
2	5114 chr7:18228252-18233252	mixed lineage kinase-related kinase MRK-beta; mixed lineage kinasewith a leucine zipper and a sterile alpha motif; mixedlineage kinase-related kinase [Homo sapiens]	2	5114 chr7:18228252-18233252	mixed lineage kinase-related kinase MRK-beta; mixed lineage kinasewith a leucine zipper and a sterile alpha motif; mixedlineage kinase-related kinase [Homo sapiens]
3	11515 chr unknown	RIKEN cDNA 1810046J19; expressed sequence AI463380 [Mus musculus]	3	11515 chr unknown	RIKEN cDNA 1810046J19; expressed sequence AI463380 [Mus musculus]
4	3291 chr unknown	PEROXISOMAL FARNESYLATED PROTEIN	4	3291 chr unknown	PEROXISOMAL FARNESYLATED PROTEIN
5	12976 chr1_random:953378-958378	UV radiation resistance associated gene [Homo sapiens]	5	12976 chr1_random:953378-958378	UV radiation resistance associated gene [Homo sapiens]
6	15165 chr26:543854-548854	neurofascin - chicken	6	1602 chr20:8506125-8511125	ADP ribosylation factor 1 GTPase activating protein [Rattusnorvegicus]
7	1602 chr20:8506125-8511125	ADP-ribosylation factor 1 GTPase activating protein [Rattusnorvegicus]	7	15165 chr26:543854-548854	neurofascin - chicken
8	*2889* chr1:59101435-59106435	aldo-keto reductase [Gallus gallus]	8	*2889* chr1:59101435-59106435	aldo-keto reductase [Gallus gallus]
9	10098 chrUn:64091188-64096188	No Hits Found	9	10098 chrUn:64091188-64096188	No Hits Found

Signal to noise rank	Spot Chromosome	Gene	t-test rank	Spot Chromosome	Gene
10	8418 chr unknown	calreticulin [Mus musculus]	10	8418 chr unknown	calreticulin [Mus musculus]
11	***11345*** chr1:59101435-59106435	aldo-keto reductase [Gallus gallus]	11	***11345*** chr1:59101435-59106435	aldo-keto reductase [Gallus gallus]
12	4938 chr17:10371245-10376245	Similar to neural proliferation, differentiation and control gene 1[Mus musculus]	12	4938 chr17:10371245-10376245	Similar to neural proliferation, differentiation and control gene 1[Mus musculus]
13	1639 chr17:930032-935032	similar to FLJ00237 protein [Homo sapiens]	13	10040 chr1_random:953378-958378	UV radiation resistance associated gene [Homo sapiens]
14	10040 chr1_random:953378-958378	UV radiation resistance associated gene [Homo sapiens]	14	1639 chr17:930032-935032	similar to FLJ00237 protein [Homo sapiens]
15	18020 chr2:17425573-17430573	RIKEN cDNA 1200007O21 [Mus musculus]	15	18020 chr2:17425573-17430573	RIKEN cDNA 1200007O21 [Mus musculus]
16	2968 chr6:22250526-22255526	suppressor of fused [Gallus gallus]	16	1757 chr unknown	gene_id:F1D9.26~unknown protein [Arabidopsis thaliana]
17	1757 chr unknown	gene_id:F1D9.26~unknown protein [Arabidopsis thaliana]	17	9635 chrUn:127231079-127236079	similar to hypothetical protein similar to RNA-binding protein lark[Homo sapiens] [Rattus norvegicus]
18	9635 chrUn:127231079-127236079	similar to hypothetical protein similar to RNA-binding protein lark[Homo sapiens] [Rattus norvegicus]	18	16413 chr20:2082302-2087302	NIP1 [Homo sapiens]
19	16413 chr20:2082302-2087302	NIP1 [Homo sapiens]	19	9781 chr9:5201122-5206122	probable translational initiation factor; putative translationinitiation factor IF-2(fragment) [Streptomycescoelicolor A3(2)]
20	***8866*** chr3:99779804-99784804	syndecan	20	2968 chr6:22250526-22255526	suppressor of fused [Gallus gallus]
21	9781 chr9:5201122-5206122	probable translational initiation factor; putative translationinitiation factor IF-2(fragment) [Streptomycescoelicolor A3(2)]	21	***8866*** chr3:99779804-99784804	syndecan

Signal to noise rank	Spot Chromosome	Gene	t-test rank	Spot Chromosome	Gene
22	12970 chr2:17425573-17430573	RIKEN cDNA 1200007O21 [Mus musculus]	22	12970 chr2:17425573-17430573	RIKEN cDNA 1200007O21 [Mus musculus]
23	3404 chr unknown	unnamed protein product [Homo sapiens]	23	3404 chr unknown	unnamed protein product [Homo sapiens]
24	14643 chr2:68261415-68266415	similar to hypothetical protein FLJ20225 [Homo sapiens] [Musmusculus]	24	14643 chr2:68261415-68266415	similar to hypothetical protein FLJ20225 [Homo sapiens] [Musmusculus]
25	16088 chr14:15227753-15232753	No Hits Found	25	16088 chr14:15227753-15232753	No Hits Found

Table 14. The 25 genes with the highest correlation with the Fat line at 1-week of age.

See legend to Table 13 for further details.

Signal to noise rank	Spot Chromosome	Gene	t-test rank	Spot Chromosome	Gene
1	18533 chr7:31010095-31015095	No Hits Found	1	18533 chr7:31010095-31015095	No Hits Found
2	***4880*** chr8:3621157-3626157	Regulator of G-protein signaling 5	2	14515 chr16:197578-202578	MHC class I histocompatibility antigen B-F alpha chain 2 - chicken
3	14515 chr16:197578-202578	MHC class I histocompatibility antigen B-F alpha chain 2 - chicken	3	17746 chr4:21373043-21378043	Similar to guanylate cyclase 1, soluble, alpha 3 [Homo sapiens]
4	***16177*** chr2:126336789-126341789	blood plasma glutamate carboxypeptidase precursor;prostate-specific membrane antigen (PSMA) [Homo sapiens]	4	***16177*** chr2:126336789-126341789	blood plasma glutamate carboxypeptidase precursor;prostate-specific membrane antigen (PSMA) [Homo sapiens]
5	17746 chr4:21373043-21378043	Similar to guanylate cyclase 1, soluble, alpha 3 [Homo sapiens]	5	***4880*** chr8:3621157-3626157	Regulator of G-protein signaling 5
6	12217 chr15:1018333-1023333	catecholamine-O-methyltransferase [Rattus norvegicus]	6	6507 chr4:1874433-1879433	translocase of inner mitochondrial membrane 8 homolog A;deafness/dystonia peptide; translocase of innermitochondrial membrane 8 (yeast) homolog A [Homosapiens]
7	6507 chr4:1874433-1879433	translocase of inner mitochondrial membrane 8 homolog A;deafness/dystonia peptide; translocase of innermitochondrial membrane 8 (yeast) homolog A [Homosapiens]	7	12217 chr15:1018333-1023333	catecholamine-O-methyltransferase [Rattus norvegicus]
8	4127 chr8:27691953-27696953	No Hits Found	8	4127 chr8:27691953-27696953	No Hits Found

Signal to noise rank	Spot Chromosome	Gene	t-test rank	Spot Chromosome	Gene
9	16173 chr3:89930838-89935838	No Hits Found	9	16173 chr3:89930838-89935838	No Hits Found
10	13212 chr16:172765-177765	major histocompatibility complex class II beta chain; B-L betachain [Gallus gallus]	10	14625 chr24:5809269-5814269	unnamed protein product [Mus musculus]
11	14625 chr24:5809269-5814269	unnamed protein product [Mus musculus]	11	13108 chr8:2589470-2594470	complement component factor h; complement factor H related protein 3A4/5G4 [Mus musculus]
12	13108 chr8:2589470-2594470	complement component factor h; complement factor H related protein 3A4/5G4 [Mus musculus]	12	8673 chr2:42182389-42187389	tetranectin [Gallus gallus]
13	8673 chr2:42182389-42187389	tetranectin [Gallus gallus]	13	10702 chrUn:150292401-150297401	6-phosphofructo-2-kinase/fructose-2, 6-bisphosphatase [Gallus gallus]
14	10702 chrUn:150292401-150297401	6-phosphofructo-2-kinase/fructose-2, 6-bisphosphatase [Gallus gallus]	14	13212 chr16:172765-177765	major histocompatibility complex class II beta chain; B-L betachain [Gallus gallus]
15	15436 chr2:126336789-126341789	blood plasma glutamate carboxypeptidase precursor;prostate-specific membrane antigen (PSMA) [Homo sapiens]	15	6533 chr13:12087524-12092524	MHC class II-associated invariant chain [Gallus gallus]
16	6533 chr13:12087524-12092524	MHC class II-associated invariant chain [Gallus gallus]	16	15436 chr2:126336789-126341789	blood plasma glutamate carboxypeptidase precursor;prostate-specific membrane antigen (PSMA) [Homo sapiens]
17	16482 chr16:197579-202579	MHC class I [Gallus gallus]	17	18295 chr13:12087524-12092524	MHC class II-associated invariant chain [Gallus gallus]
18	9071 chr1:99390726-99395726	No Hits Found	18	4311 chr4:1831612-1836612	Thymosin beta

Signal to noise rank	Spot Chromosome	Gene	t-test rank	Spot Chromosome	Gene
19	4311 chr4:1831612-1836612	Thymosin beta	19	746 chr unknown	lacZ alpha peptide
20	18295 chr13:12087524-12092524	MHC class II-associated invariant chain [Gallus gallus]	20	***3341*** chr4:73899798-73904798	superoxide dismutase 3, extracellular [Homo sapiens]
21	7376 chr12:6435460-6440460	No Hits Found	21	7376 chr12:6435460-6440460	No Hits Found
22	7388 chr10:19738390-19743390	No Hits Found	22	8555 chrUn:53664049-53669049	FLJ00239 protein [Homo sapiens]
23	***3341*** chr4:73899798-73904798	superoxide dismutase 3, extracellular [Homo sapiens]	23	15228 chr unknown	Salivary gland secretion 1 [Drosophila melanogaster]
24	746 chr unknown	lacZ alpha peptide	24	7388 chr10:19738390-19743390	No Hits Found
25	8555 chrUn:53664049-53669049	FLJ00239 protein [Homo sapiens]	25	11530 chr8:15474057-15479057	similar to cytoplasmic cysteine conjugate-beta lyase;glutamine-phenylpyruvate aminotransferase [Homo sapiens][Mus musculus]

Table 15. The 25 genes with the highest correlation with the Lean line at 3-weeks of age.

See legend to Table 13 for further details.

Signal to noise rank	Spot Chromosome	Gene	t-test rank	Spot Chromosome	Gene
1	8919 chr5:21809645-21814645	calcium binding protein P22; Sid470p [Mus musculus]	1	8919 chr5:21809645-21814645	calcium binding protein P22; Sid470p [Mus musculus]
2	5456 chr unknown	CD63 antigen	2	5456 chr unknown	CD63 antigen
3	11305 chrUn:44205720-44210720	hypothetical protein MGC4293 [Homo sapiens]	3	11305 chrUn:44205720-44210720	hypothetical protein MGC4293 [Homo sapiens]
4	18020 chr2:17425573-17430573	RIKEN cDNA 1200007O21 [Mus musculus]	4	18020 chr2:17425573-17430573	RIKEN cDNA 1200007O21 [Mus musculus]
5	6840 chr2:19914025-19919025	Glycylpeptide N-tetradecanoyltransferase 2 (PeptideN-myristoyltransferase 2) (Myristoyl-CoA:proteinN-myristoyltransferase 2) (NMT 2) (Type IIN-myristoyltransferase)	5	1764 chr2:117437722-117442722	unnamed protein product [Homo sapiens]
6	1652 chr1:106456173-106461173	transmembrane 4 superfamily member 2; membrane component, xchromosome, surface marker 1; T-cell acute lymphoblasticleukemia associated antigen 1; transmembrane proteinA15; tetraspanin protein; cell surface glycoprotein A15;CD231 antigen; transmembrane 4 superfamily 2b [Homosapiens]	6	3404 chr unknown	unnamed protein product [Homo sapiens]
7	1764 chr2:117437722-117442722	unnamed protein product [Homo sapiens]	7	12915 chr2:1905524-1910524	ADP ribosylation factor 1 [Homo sapiens]
8	3404 chr unknown	unnamed protein product [Homo sapiens]	8	2851 chrUn:107564314-107569314	hypothetical protein; extensin-like; with SH3 Src homology domain [Schizosaccharomyces pombe]

Signal to noise rank	Spot Chromosome	Gene	t-test rank	Spot Chromosome	Gene
9	12915 chr2:1905524-1910524	ADP-ribosylation factor 1 [Homo sapiens]	9	1652 chr1:106456173-106461173	transmembrane 4 superfamily member 2; membrane component, xchromosome, surface marker 1; T-cell acute lymphoblasticleukemia associated antigen 1; transmembrane proteinA15; tetraspanin protein; cell surface glycoprotein A15;CD231 antigen; transmembrane 4 superfamily 2b [Homosapiens]
10	5283 chr3:102673416-102678416	carbamoyl-phosphate synthetase (E.C.6.3.5.5)	10	3242 chr5:1805559-1810559	chromosome 11 open reading frame2; chromosome 11 open readingframe2 [Homo sapiens]
11	2851 chrUn:107564314-107569314	hypothetical protein; extensin-like; with SH3 Src homology domain [Schizosaccharomyces pombe]	11	5283 chr3:102673416-102678416	carbamoyl-phosphate synthetase (E.C.6.3.5.5)
12	***7037*** chr3:103190761-103195761	clusterin [Gallus gallus]	12	1696	CD63 antigen
13	3242 chr5:1805559-1810559	chromosome 11 open reading frame2; chromosome 11 open readingframe2 [Homo sapiens]	13	8989 chr unknown	No Hits Found
14	8989 chr unknown	No Hits Found	14	6840 chr2:19914025-19919025	Glycylpeptide N-tetradecanoyltransferase 2 (PeptideN-myristoyltransferase 2) (Myristoyl-CoA:proteinN-myristoyltransferase 2) (NMT 2) (Type IIN-myristoyltransferase)
15	1696	CD63 antigen	15	1806 chr10:18733610-18738610	Similar to butyrate-induced transcript 1 [Mus musculus]
16	10098 chrUn:64091188-64096188	No Hits Found	16	455 chr unknown	No Hits Found
17	1806 chr10:18733610-18738610	Similar to butyrate-induced transcript 1 [Mus musculus]	17	1832 chr5:11687981-11692981	Cathepsin D precursor

Signal to noise rank	Spot Chromosome	Gene	t-test rank	Spot Chromosome	Gene
18	455 chr unknown	No Hits Found	18	***7037*** chr3:103190761 -103195761	clusterin [Gallus gallus]
19	1832 chr5:11687981-11692981	Cathepsin D precursor	19	10098 chrUn:6409118 8-64096188	No Hits Found
20	12945	Fc fragment of IgG binding protein; IgG Fc binding protein [Homo sapiens]	20	12945	Fc fragment of IgG binding protein; IgG Fc binding protein [Homo sapiens]
21	3293 chr24:2483844-2488844	No Hits Found	21	3293 chr24:2483844-2488844	No Hits Found
22	9781 chr9:5201122-5206122	probable translational initiation factor; putative translationinitiation factor IF-2(fragment) [Streptomycescoelicolo r A3(2)]	22	9781 chr9:5201122-5206122	probable translational initiation factor; putative translationinitiation factor IF-2(fragment) [Streptomycescoelicolo r A3(2)]
23	6480 chr23:2812501-2817501	stathmin [Gallus gallus]	23	6480 chr23:2812501-2817501	stathmin [Gallus gallus]
24	16939 chrZ:6629404-6634404	GROWTH HORMONE RECEPTOR PRECURSOR (GH RECEPTOR) (SERUM BINDING PROTEIN)	24	16939 chrZ:6629404-6634404	GROWTH HORMONE RECEPTOR PRECURSOR (GH RECEPTOR) (SERUM BINDING PROTEIN)
25	16088 chr14:15227753 -15232753	No Hits Found	25	5781 chrUn:1050314 82-105036482	aldose reductase

Table 16. The 25 genes with the highest correlation with the Fat line at 3-weeks of age.

See legend to Table 13 for further details.

Signal to noise rank	Spot Chromosome	Gene	t-test rank	Spot Chromosome	Gene
1	13108 chr8:2589470-2594470	complement component factor h; complement factor H related protein 3A4/5G4 [Mus musculus]	1	13108 chr8:2589470-2594470	complement component factor h; complement factor H related protein 3A4/5G4 [Mus musculus]
2	14157 chr11:1660833-1665833	Cog4S [Homo sapiens]	2	15228 chr unknown	Salivary gland secretion 1 [Drosophila melanogaster]
3	15228 chr unknown	Salivary gland secretion 1 [Drosophila melanogaster]	3	14157 chr11:1660833-1665833	Cog4S [Homo sapiens]
4	6533 chr13:12087524-12092524	MHC class II-associated invariant chain [Gallus gallus]	4	6533 chr13:12087524-12092524	MHC class II-associated invariant chain [Gallus gallus]
5	4413 chr2:142050069-142055069	No Hits Found	5	4413 chr2:142050069-142055069	No Hits Found
6	10702 chrUn:150292401-150297401	6-phosphofructo-2-kinase/fructose-2, 6-bisphosphatase [Gallus gallus]	6	6507 chr4:1874433-1879433	translocase of inner mitochondrial membrane 8 homolog A;deafness/dystonia peptide; translocase of innermitochondrial membrane 8 (yeast) homolog A [Homosapiens]
7	6507 chr4:1874433-1879433	translocase of inner mitochondrial membrane 8 homolog A;deafness/dystonia peptide; translocase of innermitochondrial membrane 8 (yeast) homolog A [Homosapiens]	7	746 chr unknown	lacZ alpha peptide
8	17746 chr4:21373043-21378043	Similar to guanylate cyclase 1, soluble, alpha 3 [Homo sapiens]	8	10702 chrUn:150292401-150297401	6-phosphofructo-2-kinase/fructose-2, 6-bisphosphatase [Gallus gallus]

Signal to noise rank	Spot Chromosome	Gene	t-test rank	Spot Chromosome	Gene
9	746 chr unknown	lacZ alpha peptide	9	17746 chr4:21373043-21378043	Similar to guanylate cyclase 1, soluble, alpha 3 [Homo sapiens]
10	2858 chr6:10548697-10553697	similar to K02B2.3.p [Homo sapiens]	10	2858 chr6:10548697-10553697	similar to K02B2.3.p [Homo sapiens]
11	5691 chr13:12087524-12092524	MHC class II-associated invariant chain [Gallus gallus]	11	5691 chr13:12087524-12092524	MHC class II-associated invariant chain [Gallus gallus]
12	8555 chrUn:53664049-53669049	FLJ00239 protein [Homo sapiens]	12	771 chr unknown	expressed sequence AA408877; hypothetical protein MGC38511 [Musmusculus]
13	771 chr unknown	expressed sequence AA408877; hypothetical protein MGC38511 [Musmusculus]	13	10937 chr22:1220693-1225693	lysyl oxidase-like 2 [Homo sapiens]
14	6629 chr10:50475-55475	No Hits Found	14	18850 chr15:7886528-7891528	Ig light chain precursor
15	10937 chr22:1220693-1225693	lysyl oxidase-like 2 [Homo sapiens]	15	8555 chrUn:53664049-53669049	FLJ00239 protein [Homo sapiens]
16	18850 chr15:7886528-7891528	Ig light chain precursor	16	8673 chr2:42182389-42187389	tetranectin [Gallus gallus]
17	8673 chr2:42182389-42187389	tetranectin [Gallus gallus]	17	6629 chr10:50475-55475	No Hits Found
18	8876 chr unknown	No Hits Found	18	12350 chr5:10115074-10120074	L-lactate dehydrogenase A chain (LDH-A)
19	18295 chr13:12087524-12092524	MHC class II-associated invariant chain [Gallus gallus]	19	***43*** chr4:50551449-50556449	immunoglobulin J chain [Gallus gallus]
20	12350 chr5:10115074-10120074	L-lactate dehydrogenase A chain (LDH-A)	20	35 chrUn:98785321-98790321	gamma-immunoglobulin heavy chain (504 AA) [Gallus gallus]
21	***43*** chr4:50551449-50556449	immunoglobulin J chain [Gallus gallus]	21	18295 chr13:12087524-12092524	MHC class II-associated invariant chain [Gallus gallus]
22	18615 chr4:4866948-4871948	No Hits Found	22	18615 chr4:4866948-4871948	No Hits Found

Signal to noise rank	Spot Chromosome	Gene	t-test rank	Spot Chromosome	Gene
23	35 chrUn:98785321-98790321	gamma-immunoglobulin heavy chain (504 AA) [Gallus gallus]	23	3221 chr15:7872806-7877806	Ig light chain precursor
24	14688 chrUn:32887624-32892624	beta-xylosidase B [Clostridium stercorarium]	24	16323 chr4:65516303-65521303	neuromedin U [Homo sapiens]
25	3221 chr15:7872806-7877806	Ig light chain precursor	25	14688 chrUn:32887624-32892624	beta-xylosidase B [Clostridium stercorarium]

Table 17. The 25 genes with the highest correlation with the Lean line at 5-weeks of age.

See legend to Table 13 for further details.

Signal to noise rank	Spot Chromosome	Gene	t-test rank	Spot Chromosome	Gene
1	6840 chr2:19914025-19919025	Glycylpeptide N-tetradecanoyltransferase 2 (PeptideN-myristoyltransferase 2) (Myristoyl-CoA:proteinN-myristoyltransferase 2) (NMT 2) (Type IIN-myristoyltransferase)	1	6840 chr2:19914025-19919025	Glycylpeptide N-tetradecanoyltransferase 2 (PeptideN-myristoyltransferase 2) (Myristoyl-CoA:proteinN-myristoyltransferase 2) (NMT 2) (Type IIN-myristoyltransferase)
2	1652 chr1:106456173-106461173	transmembrane 4 superfamily member 2; membrane component, xchromosome, surface marker 1; T-cell acute lymphoblasticleukemia associated antigen 1; transmembrane proteinA15; tetraspanin protein; cell surface glycoprotein A15;CD231 antigen; transmembrane 4 superfamily 2b [Homosapiens]	2	1652 chr1:106456173-106461173	transmembrane 4 superfamily member 2; membrane component, xchromosome, surface marker 1; T-cell acute lymphoblasticleukemia associated antigen 1; transmembrane proteinA15; tetraspanin protein; cell surface glycoprotein A15;CD231 antigen; transmembrane 4 superfamily 2b [Homosapiens]
3	1900 chr4:69278703-69283703	similar to Amyloid beta A4 precursor protein-binding family B member 2 (Fe65-like protein) [Homo sapiens]	3	1900 chr4:69278703-69283703	similar to Amyloid beta A4 precursor protein-binding family B member 2 (Fe65-like protein) [Homo sapiens]
4	1846 chr1:49813466-49818466	No Hits Found	4	5283 chr3:102673416-102678416	carbamoyl-phosphate synthetase (E.C.6.3.5.5)
5	*4989* chrZ:32841437-32846437	endothelial differentiation, lysophosphatidic acidG-protein-coupled receptor, 2 [Rattus norvegicus]	5	1846 chr1:49813466-49818466	No Hits Found
6	5283 chr3:102673416-102678416	carbamoyl-phosphate synthetase (E.C.6.3.5.5)	6	17622 chr17:5374732-5379732	SH3-containing protein SH3GLB2 [Mus musculus]
7	17622 chr17:5374732-5379732	SH3-containing protein SH3GLB2 [Mus musculus]	7	*4989* chrZ:32841437-32846437	endothelial differentiation, lysophosphatidic acidG-protein-coupled receptor, 2 [Rattus norvegicus]

Signal to noise rank	Spot Chromosome	Gene	t-test rank	Spot Chromosome	Gene
8	18823 chr9:8783038-8788038	similar to Kelch motif containing protein~data source:Pfam, sourcekey:PF01344, evidence:ISS~putative [Homo sapiens]	8	11494 chr12:11799407-11804407	unnamed protein product [Homo sapiens]
9	11494 chr12:11799407-11804407	unnamed protein product [Homo sapiens]	9	18823 chr9:8783038-8788038	similar to Kelch motif containing protein~data source:Pfam, sourcekey:PF01344, evidence:ISS~putative [Homo sapiens]
10	6690 chrUn:40752630-40757630	Transcript Antisense to Ribosomal RNA; Tar1p [Saccharomycescerevisiae]	10	6690 chrUn:40752630-40757630	Transcript Antisense to Ribosomal RNA; Tar1p [Saccharomycescerevisiae]
11	7570 chr10:4570711-4575711	unnamed protein product [Mus musculus]	11	7570 chr10:4570711-4575711	unnamed protein product [Mus musculus]
12	5132 chr14:3093148-3098148	Unknown (protein for MGC:25768) [Mus musculus]	12	10954 chr9:13238621-13243621	No Hits Found
13	10954 chr9:13238621-13243621	No Hits Found	13	310 chr unknown	No Hits Found
14	310 chr unknown	No Hits Found	14	5132 chr14:3093148-3098148	Unknown (protein for MGC:25768) [Mus musculus]
15	18020 chr2:17425573-17430573	RIKEN cDNA 1200007O21 [Mus musculus]	15	14080 chr20:455094-460094	No Hits Found
16	14080 chr20:455094-460094	No Hits Found	16	18020 chr2:17425573-17430573	RIKEN cDNA 1200007O21 [Mus musculus]
17	5499 chr5:19038250-19043250	multiple exostoses type II protein EXT2.I [Homo sapiens]	17	1037 chr8:16253747-16258747	dJ604K5.1 (15 kDa selenoprotein) [Homo sapiens]
18	1977 chr4:54163164-54168164	No Hits Found	18	1977 chr4:54163164-54168164	No Hits Found
19	1037 chr8:16253747-16258747	dJ604K5.1 (15 kDa selenoprotein) [Homo sapiens]	19	12915 chr2:1905524-1910524	ADP ribosylation factor 1 [Homo sapiens]
20	284 chr unknown	hypothetical protein MGC10731 [Homo sapiens]	20	11265 chr5:13156299-13161299	Platelet-endothelial tetraspan antigen 3 (PETA-3) (GP27) (Membrane glycoprotein SFA-1) (CD151 antigen)
21	12915 chr2:1905524-1910524	ADP ribosylation factor 1 [Homo sapiens]	21	5499 chr5:19038250-19043250	multiple exostoses type II protein EXT2.I [Homo sapiens]

Signal to noise rank	Spot Chromosome	Gene	t-test rank	Spot Chromosome	Gene
22	11265 chr5:131562 99-13161299	Platelet-endothelial tetraspan antigen 3 (PETA-3) (GP27) (Membrane glycoprotein SFA-1) (CD151 antigen)	22	284 chr unknown	hypothetical protein MGC10731 [Homo sapiens]
23	6849 chr unknown	KIAA0872 protein [Homo sapiens]	23	5353 chr13:125001 79-12505179	unnamed protein product [Mus musculus]
24	5353 chr13:125001 79-12505179	unnamed protein product [Mus musculus]	24	16400 chr13:910358 4-9108584	DEAD-box protein abstrakt; putative RNA helicase [Homo sapiens]
25	408 chr unknown	serologically defined colon cancer antigen 28; phosphatidylcholinetransfer protein-like [Mus musculus]	25	5140 chr unknown	aristaless related homeobox; aristaless-related homeobox, X-linked[Homo sapiens]

Table 18. The 25 genes with the highest correlation with the Fat line at 5-weeks of age.

See legend to Table 13 for further details.

Signal to noise rank	Spot Chromosome	Gene	t-test rank	Spot Chromosome	Gene
1	4413 chr2:142050069 -142055069	No Hits Found	1	4413 chr2:142050069- 142055069	No Hits Found
2	15379 chr11:1016279- 1021279	lecithin cholesterol acyltransferase [Anas platyrhynchos]	2	15379 chr11:1016279- 1021279	lecithin cholesterol acyltransferase [Anas platyrhynchos]
3	333 chr unknown	Skin secretory protein XP2 precursor (APEG protein)	3	11530 chr8:15474057- 15479057	similar to cytoplasmic cysteine conjugate-beta lyase;glutamine-phenylpyruvate aminotransferase [Homo sapiens][Mus musculus]
4	11530 chr8:15474057- 15479057	similar to cytoplasmic cysteine conjugate-beta lyase;glutamine-phenylpyruvate aminotransferase [Homo sapiens][Mus musculus]	4	333 chr unknown	Skin secretory protein XP2 precursor (APEG protein)
5	6507 chr4:1874433- 1879433	translocase of inner mitochondrial membrane 8 homolog A;deafness/dystoni a peptide; translocase of innermitochondrial membrane 8 (yeast) homolog A [Homosapiens]	5	6507 chr4:1874433-1879433	translocase of inner mitochondrial membrane 8 homolog A;deafness/dystonia peptide; translocase of innermitochondrial membrane 8 (yeast) homolog A [Homosapiens]
6	8673 chr2:42182389- 42187389	tetranectin [Gallus gallus]	6	*4880* chr8:3621157-3626157	Regulator of G-protein signaling 5
7	*4880* chr8:3621157- 3626157	Regulator of G-protein signaling 5	7	8673 chr2:42182389- 42187389	tetranectin [Gallus gallus]
8	11649 chrUn:39203353 -39208353	No Hits Found	8	11649 chrUn:39203353- 39208353	No Hits Found
9	8257	proline rich protein 2 [Mus musculus]	9	8257 chr unknown	proline rich protein 2 [Mus musculus]

Signal to noise rank	Spot Chromosome	Gene	t-test rank	Spot Chromosome	Gene
10	14688 chrUn:32887624-32892624	beta-xylosidase B [Clostridium stercorarium]	10	746 chr unknown	lacZ alpha peptide
11	746 chr unknown	lacZ alpha peptide	11	14688 chrUn:32887624-32892624	beta-xylosidase B [Clostridium stercorarium]
12	8207 chr unknown	unnamed protein product [Mus musculus]	12	8207 chr unknown	unnamed protein product [Mus musculus]
13	10937 chr22:1220693-1225693	lysyl oxidase-like 2 [Homo sapiens]	13	10937 chr22:1220693-1225693	lysyl oxidase-like 2 [Homo sapiens]
14	159 chr13:2385579-2390579	No Hits Found	14	14625 chr24:5809269-5814269	unnamed protein product [Mus musculus]
15	*43* chr4:50551449-50556449	immunoglobulin J chain [Gallus gallus]	15	*43* chr4:50551449-50556449	immunoglobulin J chain [Gallus gallus]
16	14625 chr24:5809269-5814269	unnamed protein product [Mus musculus]	16	10702 chrUn:150292401-150297401	6-phosphofructo-2-kinase/fructose-2, 6-bisphosphatase [Gallus gallus]
17	10702 chrUn:150292401-150297401	6-phosphofructo-2-kinase/fructose-2, 6-bisphosphatase [Gallus gallus]	17	159 chr13:2385579-2390579	No Hits Found
18	17746 chr4:21373043-21378043	Similar to guanylate cyclase 1, soluble, alpha 3 [Homo sapiens]	18	17746 chr4:21373043-21378043	Similar to guanylate cyclase 1, soluble, alpha 3 [Homo sapiens]
19	8984 chr8:21373067-21378067	No Hits Found	19	5691 chr13:12087524-12092524	MHC class II-associated invariant chain [Gallus gallus]
20	35 chrUn:98785321-98790321	gamma-immunoglobulin heavy chain (504 AA) [Gallus gallus]	20	18850 chr15:7886528-7891528	Ig light chain precursor
21	18850 chr15:7886528-7891528	Ig light chain precursor	21	13108 chr8:2589470-2594470	complement component factor h; complement factor H related protein 3A4/5G4 [Mus musculus]
22	13108 chr8:2589470-2594470	complement component factor h; complement factor H related protein 3A4/5G4 [Mus musculus]	22	3451 chr19:5082563-5087563	alpha-2-antiplasmin precursor

Signal to noise rank	Spot Chromosome	Gene	t-test rank	Spot Chromosome	Gene
23	6503 chr20:9356312-9361312	No Hits Found	23	6503 chr20:9356312-9361312	No Hits Found
24	9843 chrUn:44832176-44837176	immunoglobulin alpha heavy chain [Gallus gallus]	24	5862 chrZ_random:227861-232861	Similar to RIKEN cDNA 0610013D04 gene [Homo sapiens]
25	5691 chr13:12087524-12092524	MHC class II-associated invariant chain [Gallus gallus]	25	35 chrUn:98785321-98790321	gamma-immunoglobulin heavy chain (504 AA) [Gallus gallus]

Table 19. Candidate genes chosen for sequencing.

Spot	GEO no.	BLAST X
43	3.1.3	Immunoglobulin J polypeptide, linker protein for immunoglobulin alpha and mu polypeptides
2889	5.17.9	aldo-keto reductase
3341	12.2.1	Extracellular superoxide dismutase [Cu-Zn] precursor (EC 1.15.1.1) (EC-SOD)
4880	16.1.20	Regulator of G-protein signaling 5
4989	14.3.9	Lysophosphatidic acid receptor Edg-2 (LPA receptor 1) (LPA-1)
5006	15.3.6	leptin receptor overlapping transcript
7037	20.8.17	clusterin (complement lysis inhibitor, SP-40,40, sulfated glycoprotein 2, testosterone-repressed prostate message 2, apolipoprotein J)
7147	18.10.6	Early activation antigen CD69 (Early T-cell activation antigen p60) (GP32/28) (Leu-23) (MLR-3) (EA1) (BL-AC/P26) (Activation inducer molecule) (AIM)
8866	24.11.6	Syndecan-1 precursor (SYND1) (CD138 antigen)
10070	28.6.10	Glypican-3 precursor (Intestinal protein OCI-5) (GTR2-2) (MXR7)
16177	41.3.17	Plasma glutamate carboxypeptidase
16234	44.3.14	Ubiquinone biosynthesis monooxgenase COQ6

Table 20. Identification of SNPs in candidate genes. Spot is the microarray spot ID, Gene is the gene name of highest scoring BLASTX hit. Sequence identifies the SNP, Genome Location is Chromosome: bp to bp. Lean and Fat contain the ratio of animals with each SNP.

Spot	Gene	Sequence	Gemonic Location	Lean	Fat
3341	Superoxide dismutase	TCCGTGTGCTT(C/T)GGTGGAGGAC	4:73905577 to 73905596	10C:0T	3C:7T
3341	Superoxide dismutase	AATAATAACCT(A/G)AATGATCTAA	4:73905410 to 73905431	3A:7G	9G:1A
3341	Superoxide dismutase	TTTCTACATTA(C/T)GTATTTAGAT	4:73905368 to 73905389	2C:8T	9C:1T
4989	LPA receptor-1	GGTGGACACAAATCAG(T/C)TCCCA GTTCAAATCTT	Z:32846253 to 32846285	3T:7C	10T:0C
2889	Aldo keto reductase	AAAGAATTCAATCCA(A/T)AATACA GAATTATGG	1:59105862 to 59105892	1A:10T	9A:2T
2889	Aldo keto reductase	TCAGCACACATA(G/A)CAGCTGTTG AAATG	1:59105582 to 59105608	6G:5A	10G:1A
10070	Glypican	CTGAATGTTCCCCT(A/C)TGGAAAT ACAGCCC	4:3774193 to 3774221	6A:5C	11A:0C
10070	Glypican	CAGCAAGCAGTCCTG(T/C)TGTACG ACTGCATG	4:3774280 to 3774309	5T:6C	11T:0C
8866	Syndecan	TTCTCCTTTAACCAGA(G/C)CAGTT CCCTGATCTG	3:99791473 to 99791504	5G:6C	11G:0C
8866	Syndecan	AATGGCTCCCCAGGG(C/T)GGTGGG CACAGCTCC	3:99791109 to 99791139	4C:7T	9C:2T
8866	Syndecan	CAGCTCCGAGCTGCC(G/A)GAGCTC AAGGAGCA	3:99791086 to 99791115	5G:6A	11G:0A

Table 21. SNP-specific genotypes of the F_0 birds. Bird ID no., Line, Sex, and Genotype are given.

Bird no.	Line	Sex	Genotype
472	Lean	Male	C/T
573	Lean	Female	C/C
649	Lean	Female	T/T
655	Lean	Female	C/C
656	Lean	Male	C/C
681	Lean	Female	C/C
695	Lean	Female	C/C
697	Lean	Male	C/C
712	Lean	Male	C/T
717	Lean	Female	T/T
732	Lean	Female	T/T
763	Fat	Female	T/T
778	Fat	Female	T/T
814	Fat	Male	T/T
826	Fat	Female	T/T
847	Fat	Female	T/T
869	Fat	Male	T/T
901	Fat	Female	T/T
919	Fat	Female	T/T
946	Fat	Male	T/T
980	Fat	Male	T/T
982	Fat	Female	T/T

Table 22. Genotypes of the animals in the tails of the body fat percentage distribution. Bird ID, genotype as determine by LNA PCR, sex, tail, body weight at sacrifice, weight of abdominal fat pad, body fat percentage.

Bird no.	Genotype	Sex	Tail	Body weight at 9 weeks (g)	Abdominal fat pad weight (g)	Fat yield (%)
1292	CT	Male	Lean	2388	12.16	0.509
1916	TT	Male	Lean	2191	15.73	0.718
1764	TT	Male	Lean	1855	17.25	0.930
1190	CT	Male	Lean	2237	22.90	1.024
1302	CC	Male	Lean	2135	27.19	1.274
1535	CC	Female	Lean	1912	26.43	1.382
1202	TT	Male	Lean	2323	32.49	1.399
1864	CC	Male	Lean	2427	35.32	1.455
1531	TT	Male	Lean	2314	34.12	1.475
1193	TT	Female	Lean	1726	25.57	1.481
1547	CC	Male	Lean	2307	35.38	1.534
51986	CT	Male	Lean	1958	30.57	1.561
51896	TT	Male	Lean	3089	48.39	1.567
51970	CT	Male	Lean	2133	33.60	1.575
1663	CT	Male	Lean	1996	31.52	1.579
1739	TT	Female	Lean	1559	24.73	1.586
1743	CT	Male	Lean	2396	38.33	1.600
1430	TT	Male	Lean	2263	36.52	1.614
1815	TT	Female	Lean	1943	32.40	1.668
1359	TT	Male	Lean	2143	35.80	1.671
51981	CT	Male	Lean	2755	46.39	1.684
1465	TT	Male	Lean	2420	41.16	1.701
1963	CC	Male	Lean	2531	43.24	1.708
1194	TT	Female	Lean	1696	29.03	1.712
1549	CC	Male	Lean	2563	44.34	1.730
51997	CT	Male	Lean	2358	41.62	1.765
1662	CT	Male	Lean	2851	50.96	1.787
1551	TT	Female	Lean	1948	35.61	1.828
1962	CT	Male	Lean	2546	46.80	1.838
1423	CT	Male	Lean	2346	43.28	1.845
1650	CT	Male	Lean	2420	44.82	1.852
1504	TT	Male	Lean	2580	48.25	1.870
1530	CT	Male	Lean	2344	44.22	1.887
1568	CC	Male	Lean	2314	43.66	1.887
51977	CC	Female	Lean	1874	35.37	1.887
1753	CT	Male	Lean	2685	50.74	1.890

Bird no.	Genotype	Sex	Tail	Body weight at 9 weeks (g)	Abdominal fat pad weight (g)	Fat yield (%)
1539	TT	Female	Lean	2018	38.17	1.891
1639	TT	Male	Lean	2704	51.29	1.897
1495	TT	Male	Lean	2189	41.78	1.909
1528	CC	Female	Lean	1309	25.03	1.912
1543	CC	Female	Lean	2033	38.91	1.914
1942	TT	Male	Lean	2374	45.55	1.919
1494	TT	Female	Lean	1651	31.68	1.919
1757	CT	Male	Lean	2604	50.50	1.939
1678	CC	Male	Lean	2180	42.67	1.957
1164	TT	Male	Lean	2448	48.24	1.971
1500	TT	Male	Lean	2329	46.08	1.979
1859	CC	Male	Lean	2452	48.52	1.979
1399	TT	Male	Lean	1938	38.44	1.983
1401	TT	Male	Lean	1892	37.63	1.989
1571	CC	Male	Lean	2569	51.39	2.000
1973	CC	Male	Lean	1928	38.57	2.001
1965	TT	Female	Lean	1857	37.39	2.013
1805	TT	Male	Lean	2450	49.39	2.016
1200	TT	Male	Lean	2276	46.55	2.045
1924	TT	Male	Lean	2430	49.92	2.054
51987	CC	Female	Lean	2085	42.92	2.059
1715	CT	Male	Lean	2426	50.10	2.065
1589	CC	Male	Lean	2550	52.95	2.076
1869	CC	Male	Lean	2234	46.42	2.078
1703	TT	Female	Lean	1525	31.80	2.085
1282	CC	Male	Lean	2596	54.17	2.087
1582	TT	Female	Lean	1817	39.22	2.159
1544	CC	Female	Lean	2084	44.99	2.159
1668	CC	Female	Lean	1863	40.74	2.187
1676	CC	Female	Lean	1872	41.22	2.202
1802	TT	Female	Lean	1322	29.84	2.257
1675	CC	Female	Lean	1477	33.52	2.269
1971	TT	Female	Lean	1748	39.73	2.273
51933	TT	Female	Lean	2289	52.96	2.314
1348	TT	Female	Lean	1683	39.14	2.326
51916	TT	Female	Lean	1846	43.17	2.339
1346	TT	Female	Lean	1718	40.58	2.362
1384	TT	Female	Lean	1679	39.66	2.362
1115	CT	Female	Lean	2349	55.59	2.367
1221	TT	Female	Lean	1953	46.40	2.376
1163	TT	Female	Lean	1639	39.10	2.386
1677	TT	Female	Lean	1921	45.99	2.394
1457	TT	Female	Lean	2139	53.38	2.496

Bird no.	Genotype	Sex	Tail	Body weight at 9 weeks (g)	Abdominal fat pad weight (g)	Fat yield (%)
1950	TT	Female	Lean	1833	46.13	2.517
1533	TT	Female	Lean	1792	45.42	2.535
1964	TT	Female	Lean	2166	54.96	2.537
1553	TT	Female	Lean	1893	48.24	2.548
1161	TT	Female	Lean	1790	46.08	2.574
1588	.	Female	Lean	1790	46.39	2.592
1470	TT	Female	Lean	1398	36.33	2.599
1310	CC	Female	Lean	1409	36.68	2.603
1959	TT	Female	Lean	1699	44.29	2.607
1987	TT	Female	Lean	1715	45.05	2.627
1651	.	Female	Lean	1754	46.25	2.637
1199	CC	Female	Lean	2083	55.17	2.649
1156	TT	Female	Lean	1724	45.77	2.655
1824	CC	Female	Lean	2007	53.49	2.665
51950	TT	Female	Lean	1943	52.13	2.683
1649	TT	Female	Lean	1925	51.73	2.687
1212	CC	Female	Lean	1774	47.81	2.695
51886	TT	Male	Fat	2719	95.43	3.510
1503	TT	Male	Fat	2092	73.64	3.520
1667	CT	Male	Fat	2290	80.85	3.531
1814	TT	Male	Fat	2659	94.49	3.554
1511	TT	Male	Fat	2498	89.66	3.589
1852	CC	Male	Fat	2796	100.65	3.600
1480	TT	Male	Fat	2541	91.71	3.609
1449	CT	Male	Fat	2508	90.83	3.622
1940	TT	Male	Fat	2070	75.35	3.640
1762	CC	Male	Fat	2967	108.10	3.643
1467	CT	Male	Fat	2540	92.76	3.652
1437	TT	Male	Fat	2475	90.46	3.655
51893	TT	Male	Fat	2779	101.65	3.658
51888	TT	Male	Fat	2832	104.20	3.679
1801	TT	Male	Fat	2332	86.59	3.713
1182	CC	Male	Fat	2222	83.10	3.740
1779	TT	Male	Fat	2790	104.95	3.762
1055	TT	Male	Fat	2549	95.89	3.762
1958	TT	Male	Fat	1801	68.65	3.812
51877	CT	Male	Fat	2876	110.23	3.833
1821	TT	Male	Fat	2602	101.08	3.885
1478	TT	Male	Fat	2573	100.28	3.897
1429	CC	Male	Fat	2478	97.05	3.916
1914	TT	Male	Fat	2539	99.47	3.918
1045	TT	Male	Fat	2606	103.48	3.971
1358	TT	Male	Fat	2380	95.65	4.019

Bird no.	Genotype	Sex	Tail	Body weight at 9 weeks (g)	Abdominal fat pad weight (g)	Fat yield (%)
51900	TT	Male	Fat	2840	114.47	4.031
1464	TT	Male	Fat	2549	103.49	4.060
1825	CC	Male	Fat	2466	100.87	4.090
1696	TT	Male	Fat	2604	106.59	4.093
51879	CT	Male	Fat	2956	121.96	4.126
1717	CT	Male	Fat	2546	105.24	4.134
51942	CT	Male	Fat	3022	125.34	4.148
1823	CC	Male	Fat	2099	87.80	4.183
51982	CC	Male	Fat	2815	118.13	4.196
1169	TT	Male	Fat	1957	82.24	4.202
51946	TT	Male	Fat	3021	127.47	4.219
1096	TT	Male	Fat	2301	98.37	4.275
1217	CC	Male	Fat	2046	87.83	4.293
1862	CC	Male	Fat	2424	105.21	4.340
1440	TT	Male	Fat	2101	91.99	4.378
1923	TT	Male	Fat	1990	88.15	4.430
1442	TT	Male	Fat	2543	117.87	4.635
1466	CT	Male	Fat	2668	125.05	4.687
1172	TT	Female	Fat	1926	90.44	4.696
1585	TT	Female	Fat	2024	95.55	4.721
1101	TT	Female	Fat	1764	83.35	4.725
1778	TT	Female	Fat	1987	93.95	4.728
1718	TT	Female	Fat	1922	91.08	4.739
1364	.	Female	Fat	2176	103.12	4.739
51940	CC	Female	Fat	2299	109.03	4.742
51954	CC	Female	Fat	2233	106.10	4.751
1139	TT	Female	Fat	1933	92.69	4.795
1436	CT	Male	Fat	2894	139.46	4.819
1927	TT	Female	Fat	1929	93.02	4.822
1137	TT	Female	Fat	2066	99.87	4.834
1949	TT	Female	Fat	1762	85.50	4.852
1912	CC	Female	Fat	1947	94.55	4.856
1438	CT	Male	Fat	2361	114.78	4.861
1796	TT	Female	Fat	2211	107.70	4.871
1443	TT	Female	Fat	2125	103.57	4.874
51889	TT	Female	Fat	2208	107.76	4.880
1167	TT	Female	Fat	2149	105.14	4.893
51880	CC	Female	Fat	2230	109.37	4.904
51911	TT	Male	Fat	2970	146.64	4.937
51910	TT	Female	Fat	2213	109.61	4.953
1697	TT	Female	Fat	2045	101.39	4.958
1756	CC	Female	Fat	2321	115.22	4.964
1656	TT	Female	Fat	2219	110.38	4.974

Bird no.	Genotype	Sex	Tail	Body weight at 9 weeks (g)	Abdominal fat pad weight (g)	Fat yield (%)
1352	TT	Female	Fat	1903	94.92	4.988
1141	TT	Female	Fat	2069	103.40	4.998
51901	TT	Female	Fat	1841	92.47	5.023
51985	TT	Female	Fat	2265	114.28	5.045
51904	TT	Female	Fat	2062	104.79	5.082
1413	TT	Female	Fat	2262	115.45	5.104
1724	TT	Female	Fat	1990	101.71	5.111
1768	TT	Female	Fat	2192	112.12	5.115
51913	TT	Female	Fat	2183	111.93	5.127
51891	TT	Female	Fat	2227	114.32	5.133
1979	TT	Female	Fat	1790	92.49	5.167
1693	TT	Female	Fat	1928	101.90	5.285
1694	TT	Female	Fat	2316	122.64	5.295
1166	TT	Female	Fat	2095	111.14	5.305
1340	TT	Female	Fat	1955	103.77	5.308
1357	TT	Male	Fat	2713	148.87	5.487
1560	CC	Female	Fat	2121	117.22	5.527
1103	CC	Female	Fat	2044	113.01	5.529
51960	CC	Female	Fat	2278	127.43	5.594
1863	TT	Female	Fat	1634	91.69	5.611
1933	TT	Female	Fat	2072	116.39	5.617
51927	TT	Female	Fat	2283	128.62	5.634
1713	CC	Female	Fat	2086	117.72	5.643
51871	CC	Female	Fat	2122	120.38	5.673
1812	TT	Female	Fat	2154	123.81	5.748
51968	TT	Female	Fat	2138	136.25	6.373
1695	TT	Female	Fat	2278	145.27	6.377

Table 23. Number of animals from the tails of the abdominal body fat percentage. The CC/CT genotypes contain the GATA transcription factor binding site.

Genotype	CC/CT	TT
Lean tail animals	43	51
Fat tail animals	29	66

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