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Role of the Maternal Liver in Lactating Mice

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by

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Dedication:

This work is dedicated to my husband, Nicholas, and children, Alexandria, Cameron, and Katrina, who continue to inspire me every day. This work is also dedicated to my mother, Evelyn A. Camba, whose early demise made me realize how precious our time is with our friends and loved ones.

ABSTRACT OF THE DISSERTATION

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Doctor of Philosophy,
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Dr. Frances M. Sladek, Chairperson

Lactation brings about numerous maternal physiological and metabolic changes in order to sustain viable offspring. The liver plays a role in meeting the high energetic and metabolic needs of lactation as it is a key player in various biological processes that include lipid metabolism and homeostasis. A growing body of evidence suggests that the metabolic and physiological changes that lactation brings to the nursing mother results in a maternal profile that lowers the incidence of metabolic diseases. In this study, we examine *in vivo* and gene expression profiling data of fasted, age-matched virgin (V), non-lactating (NL), and lactating (L) C57Bl/6 mice in an attempt to understand the role the maternal liver plays in lactation in a global context. Our model allows us to examine the long term effects of lactation, since both the lactating and non-lactating mice have previously nursed pups. *In vivo*

results highlight maternal adaptations to lactation that include rapid weight loss, increased liver weight, increased plasma triglyceride levels, and decreased hepatic triglyceride storage. Gene expression profiling reveals molecular signatures of different classes of genes that may explain the resulting maternal adaptations to lactation. Our model suggests a highly metabolically active liver that plays a role in supplying the energy that the lactating mammary gland needs, by synthesizing triglycerides and secreting them into the blood stream, thus making them available to the lactating mammary gland.

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Introduction

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Background: Pregnancy and Lactation

The ultimate goal of reproduction is the survival of off-spring that are reproductively viable (Hurley ; Buhimschi 2004) . The processes involved, pregnancy and lactation, are closely linked. Pregnancy provides a safe, warm, and sterile environment that promotes the growth and development of the fetus in utero. Lactation is an adaptation that allows the mother to continue the maternal-fetal metabolic relationship that was established and nurtured throughout pregnancy outside of the womb. Lactation includes breast development (mammogenesis) which occurs during pregnancy; milk synthesis and secretion (lactogenesis 1 and 2) which starts at mid-pregnancy (lactogenesis 1) and is triggered at parturition (lactogenesis 2); and the maintenance of milk production (galactopoesis) which occurs due to suckling by the baby (Hartmann et al. 1996). Not only do mammogenesis and lactogenesis coincide with pregnancy, but the hormones that regulate pregnancy regulate lactation as well (Hadley 1996).

Studies have shown that lactation benefits infants in various ways: providing protection against infection (Cochi et al. 1986; Pabst and Spady 1990; Alho et al. 1990; Lucas and Cole 1990; Lerman, Slepon, and Cohen 1994; Holberg et al. 1991), illnesses and morbidity (Koutras and Vigorita 1989; Wright et al. 1989); promoting infant survival (Habicht, DaVanzo, and Butz 1986); providing protection against allergies (Chandra, Puri, and Hamed 1989; Merrett et al. 1988); enhancing development and intelligence (Lucas et al. 1992; Morrow-Tlucak, Haude, and

Ernhart 1988); decreasing risk factors for diseases such as diabetes mellitus (Samuelsson, Johansson, and Ludvigsson 1993; Mayer et al. 1988), childhood cancer (Shu et al. 1999; Davis, Savitz, and Graubard 1988), Hodgkin's disease (Schwartzbaum et al. 1991), and juvenile rheumatoid arthritis (Mason et al. 1995), to name a few. As an added bonus, lactation provides benefits for mothers as well: delays fertility (Diaz 1983; Shirkie 1982), decreases the risk for cancers (McTiernan and Thomas 1986; Schneider 1987; Newcomb and Trentham-Dietz 2000), ameliorates gestational diabetes (Davies et al. 1989), and promotes postpartum weight loss (Kramer et al. 1993). Successful reproduction, therefore, requires successful lactation which is vital to the growth, development, and survival of mammals (Holman and Grimes 2003; Buhimschi 2004).

Pregnancy and lactation bring about many physiological and metabolic changes due to the increasing nutrient demands of the growing fetus (Beers and Berkow 1999). Increasing levels of estrogen and progesterone, along with the metabolic demands of the fetus, placenta, and uterus, bring on early changes in pregnancy (Ciliberto and Marx 1998). Those changes include glucose and lipid metabolism (Samsioe and Gustafson 1975), blood chemistries (Knopp et al. 1985; Chiang A-N et al. 1995), blood volume (Beers and Berkow 1999), metabolite excretion (Scott, Chakraborty, and Marks 1986), serum protein levels (Dube et al. 1977), cardiac output (Everson 1998), biliary lipid metabolism and gall bladder function (Everson et al. 1982; Kern et al. 1981). Starting at mid-pregnancy, changes in the mother brought on by pregnancy are anatomical in nature (Ciliberto and Marx 1998) and

are due to the growth/expansion of the fetus, placenta, uterus, and mammary glands (Allen 2002). The net results are effects on the cardiovascular, urinary , respiratory , gastrointestinal, and hepatobilliary systems (Ciliberto and Marx 1998). The high metabolic rate of the lactating breast demands much higher nutrient requirements for the mother compared to her pregnant nutrient requirements (Hartmann et al. 1996; Allen 2002). Besides providing the nutritional and immunological needs of the infant, lactation also provides the emotional support and connection that are also needed for the survival of the newborn (Holman and Grimes 2003).

The Role of Hormones During Pregnancy and Lactation: Progesterone, Estrogen, Prolactin, and Growth Hormone

Hormones play an important role in orchestrating pregnancy and lactation. The levels of hormones, however, differ between species. In humans, progesterone prepares the endometrium for implantation (Itskovitz and Hodgen 1988; Okada et al. 2003), plays some role in maternal immune tolerance of the fetus (Okada et al. 2003; Siiteri et al. 1977; Stites and Siiteri 1983; Szekeres-Bartho 2002), and prepares the breasts for lactation by increasing ductal branching (Fuchs 1986; Soares and Talamantes 1982). Progesterone levels increase throughout pregnancy and antagonize uterine smooth muscle contractions, contributing the maintenance of the pregnancy (Pepe and Albrecht 1995; Hadley 1996). Lower levels of progesterone at term allows parturition and signal the end of pregnancy (Hadley 1996). In the mice and rats, however, the estrous cycle is brief. In order for

pregnancy to occur, progesterone is needed to produce a pseudopregnancy, and then move on to pregnancy.

Another important hormone in pregnancy is estrogen. Placental estrogen increases throughout gestation (Oakey 1970). Estrogen plays a major role in the increased biosynthesis of progesterone (Pepe and Albrecht 1995). It also contributes to the circulatory changes that occur during pregnancy (Perrot-Applanat 1999), neovascularization (angiogenesis) of the fetus (Pepe and Albrecht 1995; Cullinan-Bove and Koos 1993; Presta 1988), and increases ductal elongation in the breasts, thereby contributing to breast development for lactation (Pepe and Albrecht 1995). In addition, rising estrogen levels toward the end of pregnancy enhance uterine excitability which causes a cascade of events that lead to the initiation of parturition.

In addition to progesterone and estrogen, a third hormone also plays a pivotal role in breast development and lactation - - prolactin (PRL) (Freeman et al. 2000; Naylor et al. 2005). PRL belongs to the prolactin/growth hormone/placental lactogen family (Horseman and Yu-Lee 1994). PRL affects mammogenesis (mammary gland development) where it is absolutely required for the development of secretory alveoli, lactogenesis (milk synthesis), and galactopoiesis (milk secretion maintenance) (Freeman et al. 2000). The initial step in PRL action is binding to a specific membrane-bound receptor, prolactin receptor (PRLR) (Bole-Feysot et al. 1998).

PRL concentrations increase as gestation progresses due to the influence of increasing concentrations of estrogen on pituitary PRL production (Hadley 1996) in humans. In mice, however, prolactin levels are low during mid-pregnancy, which are then increased by gestation day 18 (Swartz, Ogren, and Talamantes 1986). In humans, progesterone increases PRL levels by reducing hypothalamic dopamine, an inhibitor of PRL gene expression (Rakoff and Yen 1978; Sprangers, Brenner, and Bethea 1989). Meanwhile, estrogen and progesterone levels decrease dramatically at parturition while increased concentrations of PRL are maintained (Allen 2002). This rapid decrease of progesterone in addition to high plasma concentrations of PRL trigger lactogenesis (Kuhn 1969; Buhimschi 2004). In rats, progesterone levels decrease during the last days of pregnancy, while estrogen and progesterone levels increase right before parturition. Studies have also shown that suckling is an important stimulus for prolactin release which affects the maintenance of lactation once it has been established (galactopoiesis) (Howie et al. 1980; Delvoye, Demaegd, and Delogne-Desnoeck 1977).

A fourth hormone that plays a role in pregnancy and lactation is growth hormone (GH) (Hull and Harvey 2002) . GH also belongs to the prolactin/growth hormone/placental lactogen family. It is activated by ligand binding to growth hormone recptor (Goffin and Kelly 1997). There is a progressive increase in circulating GH in rodents during the second half of pregnancy (Kishi, Hirashiba, and Hasegawa 1991), which then declines right before parturition. The rise in GH during pregnancy may be caused by rising levels in estrogen (Jahn, Rastrilla, and

Deis 1993) and placental lactogens (Kishi, Hirashiba, and Hasegawa 1991). In rats, circulating GH levels rise during parturition and remain elevated for several days afterward (Carlsson, Eden, and Jansson 1990). Circulating levels of GH are also increased during lactation (Etherton and Bauman 1998) . Several studies have shown that circulating GH levels are increased with suckling (Wehrenberg and Gaillard 1989; Rushen, Foxcroft, and De Passille 1993; Schams et al. 1994). The increased levels of circulating GH promotes mammary development and milk production (Hull and Harvey 2001). In rats, prolactin promotes mammary gland development and milk production.

Pregnancy, Lactation, and Disease

The demands of pregnancy on the mother makes her organs work harder. As a result, gestational syndromes may develop when the organ system cannot meet the increased demands of the pregnancy. Some researchers have suggested that by unmasking less than optimal organ systems, pregnancy can act as a maternal stress test and predict a woman's health in later life (Williams 2003). For example, pregnancy can temporarily trigger metabolic conditions that can exacerbate pre-existing undiagnosed conditions and trigger diseases that can re-emerge later in life as the woman ages (Williams 2003; Sattar and Greer 2002; Seely and Solomon 2003). Some of the diseases include insulin resistance and hypertension (pre-eclampsia) (Redman, Sacks, and Sargent 1999; Sibai, Sarinoglu, and Mercer 1992; von Dadelszen, Magee, and Roberts 2003), cardiovascular disease (Bosio et al. 1999;

Martin et al. 1999), hypercoagulation leading to thrombosis (Greer 1999; van Walraven et al. 2003), and diabetes mellitus (Kuhl 1991). Liver ailments that can develop during pregnancy and then lead to serious liver disease include haemolysis elevated liver enzymes, and low platelets (HELLP) syndrome and acute fatty liver of pregnancy (AFLP) (Rahman and Wendon 2002). If either of these conditions is left untreated, it can be fatal.

Recently, there has been great concern about the growing number of people that may develop metabolic syndrome. Metabolic syndrome includes risk factors that increase the chances of developing cardiovascular disease and type 2 diabetes (Love-Gregory et al. 2008; Klein, Klein, and Lee 2002; Malik et al. 2004). Such risk factors include visceral obesity, insulin resistance, diabetes, hypertension, and dyslipidemia (Chan, Barrett, and Watts 2004; Ginsberg and Stalenhoef 2003). Some of the metabolic changes that occur with pregnancy may put the mother at risk for metabolic syndrome. For example, the weight gain that accompanies pregnancy may lead to obesity and increase the chances of developing metabolic syndrome after pregnancy (Gunderson et al. 2009; Jain et al. 2007; Davis and Olson 2009). In addition, the development of gestational diabetes may also increase the risk for developing maternal metabolic syndrome (Pirkola et al. 2009; Retnakaran 2009).

On the other side of this equation, there is a growing body of evidence that shows an inverse relationship between lactation and metabolic syndrome (Gunderson et al. 2007). This may be due to the metabolic changes that occur with lactation. For example, nursing women were found to have lower risk for type 2 diabetes (Stuebe

et al. 2005) and cardiovascular disease (Kallio et al. 1992). Other studies suggest that this maternal health benefit against metabolic syndrome may also have long term effects (Ram et al. 2008; Schwarz et al. 2009; Gunderson 2008).

The Liver

The liver is the largest and most metabolically complex organ in humans (Schiff, Sorrell, and Maddrey 2003; Sherlock and Dooley 2002; Worman 1999; Zakim and Boyer 2003; Samson ; Worobetz et al. ; www.siemed.edu/~dking2/erg/liver.htm 2002). The liver plays an important role in glucose homeostasis. The liver stabilizes glucose levels by glycogenesis (the formation of glycogen from glucose), glycogenolysis (the formation of glucose from glycogen), and gluconeogenesis (the formation of glucose from certain amino acids, lactate, or glycerol). The liver also synthesizes plasma proteins including albumins and globulins such as fibrinogen, prothrombin II, blood coagulation factors V, VII, IX, and X. In addition, the liver breaks down amino acids to urea, and in the process detoxifies ammonia. The liver takes up fatty acids and converts these into triglycerides which it then packages with cholesterol, phospholipids and apoprotein into lipoproteins. The liver also synthesizes cholesterol. Bile salts are a product of cholesterol catabolism which also occurs in the liver.

Role of the Liver During Pregnancy and Lactation

The liver plays an important role during pregnancy and lactation (Bauman 2000; Bell and Bauman 1997; Herath et al. 2004). It provides for the metabolic needs of

the mother and fetus and acts as a detoxification center for both. Nonetheless, despite the critical role of the liver in these important physiological processes, there are no published studies about the role of liver enriched transcription factors (LETF), such as HNF4α, during pregnancy or lactation. HNF4α has been directly linked to diseases such as Maturity Onset Diabetes of the Young 1 (MODY 1) and hemophilia (Ryffel 2001; Sladek and Seidel 2001; Yamagata et al. 1996; Gupta and Kaestner 2004). HNF4α regulates glucose metabolism in the liver as well as the expression of blood coagulation factors VII-X; both are critical to pregnancy, as mentioned above. Other target genes for HNF4α are the apolipoproteins Apo A1; Apo A2; Apo A4; Apo B; Apo C1; and Apo C3, the protein components of high density lipoprotein (HDL, Apo A1), low density lipoprotein (LDL, Apo B), which transport cholesterol and triglycerides throughout the body. Studies have shown that hyperlipidemia, an increase in triglycerides and cholesterol, is common in pregnancy (Chiang A-N et al. 1995). Levels of HDL and LDL cholesterol and triglycerides are important in gestational diabetes and in cardiovascular disease. Low levels of HDL, high levels of LDL, and high levels of triglycerides indicative of cardiovascular disease (Cekmen et al. 2003). In addition, HNF4α activates the expression of many cytochrome P450 genes that carry out steroid, drug, and xenobiotic metabolism important in detoxification (Sladek and Seidel 2001). There could also be a role for HNF4α in the kidney during pregnancy since it, along with hypoxia inducible factor (HIF), up regulates the erythropoietin (*EPO*) gene whose product stimulates the red blood cell production required for the increased blood

volume that occurs during pregnancy (Samsioe and Gustafson 1975; Zhu, Jackson, and Bunn 2002). Lactation, as mentioned earlier, imposes heavy demands on the mother, especially the liver (Widdowson 1976; Bauman 2000). HNF4α may also have links to prolactin (PRL), an important hormone of lactation, since it is a positive regulator of the prolactin receptor (*PRLR*) gene (Moldrup et al. 1996). Given that HNF4α increases expression of the *PRLR*, and lactation has been shown to provide beneficial effects on glucose and lipid metabolism in women with gestational diabetes (Kjos et al. 1993; Davies et al. 1989), it is not unreasonable to propose that HNF4α may play a role in pregnancy and lactation.

Role of the maternal liver in lactation?

The purpose of this study is to globally characterize the changes that occur in the maternal liver with lactation. We hypothesize that the demands of lactation cause differential expression of genes in the liver. We will utilize bioinformatics in order to tease out molecular signatures of differentially up regulated and down regulated genes as a result of lactation and connect them with maternal adaptations that occur in order to meet the demands of the lactating mammary gland and the suckling pups. At the same time, we will also be looking at liver enriched transcription factors, such as HNF4α, and the role they might play in lactation.

HNF4α is a Member of the Nuclear Receptor Superfamily

Background on Nuclear Receptors

Nuclear receptors (NR) are ligand-activated transcription factors involved in diverse biological and physiological functions such as reproduction, differentiation, development, metabolism, metamorphosis, and homeostasis (Escriva, Delaunay, and Laudet 2000). They are also therapeutic targets for human diseases such as cancer, obesity, diabetes, and heart disease (Gronemeyer, Gustafsson, and Laudet 2004; Nettles and Greene 2005; Sladek 2003). These nuclear receptors constitute a superfamily whose members share a highly conserved DNA binding domain (DBD) as well as a hydrophobic ligand binding domain (LBD). The DBD contains two zinc fingers and binds specific DNA sequences known as hormone response elements (HRE). Nuclear receptors bind DNA as heterodimers, homodimers, or monomers (Mangelsdorf and Evans 1995) .

There are several groups of receptors within this superfamily based on protein dimerization, the structure of the cognate DNA binding site, and intracellular localization (Jiang et al. 1995) (Fig. 1.1). Group I receptors are bound to heat shock proteins (HSP) in the cytoplasm or in the nucleus in the absence of a ligand. In the presence of a ligand, the receptors are disassociated from the HSP and are translocated to the nucleus, form predominantly homodimers and bind DNA elements composed of direct repeats. Examples of Group I receptors are: the steroid hormone receptors, glucocorticoid receptor (GR), estrogen receptor (ER), and progesterone receptor (PR). Group II receptors exist primarily in the nucleus

and bind DNA either as monomers, homodimers, or heterodimers. They have a preference to form heterodimers on DNA, particularly with RXRα. They bind DNA elements composed of direct repeats, with the exception of certain thyroid hormone response elements, which are indirect repeats. Examples of Group II receptors are retinoid acid receptor (RAR), retinoid X receptor (RXR), vitamin D receptor (VDR), and peroxisome proliferators-activated receptor (PPAR). Group III receptors exist as monomers in solution and are found in both the nucleus and the cytoplasm. They bind extended half-sites as monomers, although at least two members (NGFI-B and NURR1) also form heterodimers with RXRα. Examples of Group III receptors are NGFI-B, FTZ-F1, stroidogenic factor 1 (SF-1), RORa1, and NURR1. Finally, Group IV has the following characteristics: it is similar to Group II in terms of its amino acid sequence, preference for direct repeat (DR) binding sites, and nuclear localization. It is also similar to Group I receptors: it exists in solution as a stable homodimer and binds DNA exclusively as a homodimer. However, it is not complexed with HSP in the absence of a ligand. Currently, Group IV has one member, HNF4.

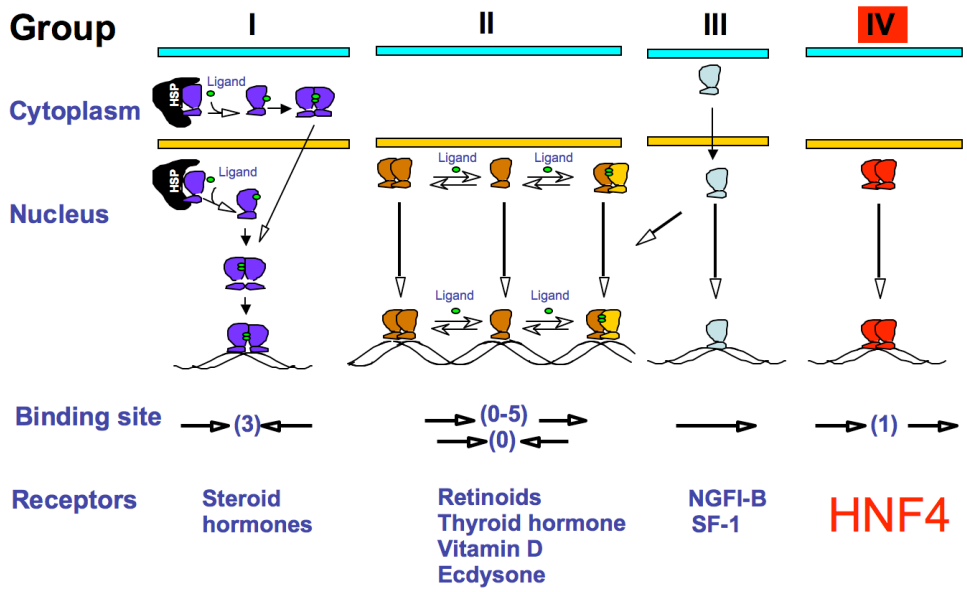

Figure 1.1: The nuclear receptor superfamily
Members of the nuclear receptor superfamily can be grouped into several classes based on their ligand-binding, dimerization, and DNA-binding properties. Shown are different classes and representative receptors for each group. Reprinted from Jiang G and Sladek FM, 1995, Microbiology and Cellular Biology, 15(9): 5131-5143, with permission from the American Society for Microbiology.

Nuclear Receptors as Transcription Factors: Structure, Function, Mechanism of Transactivation

Structure and Function

As transcription factors, nuclear receptors control the expression of genes spatially and temporally in response to a wide range of developmental, physiological, and environmental cues. In order to understand how nuclear receptors work, one must take a closer look at their structure (Fig.1.2). Nuclear receptors have several domains (Mangelsdorf and Evans 1995; Mangelsdorf et al. 1995; Escriva, Delaunay, and Laudet 2000; Schrem, Klempnauer, and Borlak 2002; Robinson-Rechavi, Escriva Garcia, and Laudet 2003; Germain et al. 2003;

Giguere 1999). These domains are: the A/B domain with ligand-independent activation function, the DNA binding domain (DBD) , the hinge domain, the ligand binding domain (LBD), and the C-terminal domain.

The N-terminal or A/B domain is the least conserved and is highly variable in terms of length and primary sequence. The A/B domain typically contains an activation function (AF-1) that mediates ligand-independent transactivation. The A/B region can activate transcription constitutively when linked to a heterologous, DBD , (e.g., the Gal 4 DBD)(Germain et al. 2003). It can interact directly with steroid receptor coactivators (SRCs) to enhance the activity of the recptor complex (Giguere 1999; Onate et al. 1998). It is subject to alternative splicing and differential promoter usage, and to posttranslational events such as phosphorylation (Germain et al. 2003). The AF-1 displays cell, DNA binding domain (DBD), and promoter specificity.

The DNA-binding domain (DBD) is the most highly conserved region (Robinson-Rechavi, Escriva Garcia, and Laudet 2003; Germain et al. 2003; Giguere 1999; Escriva, Delaunay, and Laudet 2000; Glass 1994). It contains two zinc fingers encoded by 66 to 70 amino acid residues and a carboxy-terminal extension (CTE) that spans ~ 25 residues (Giguere 1999). The CTE provides a protein-DNA interface and a protein-protein interface (Rastinejad, Evilia, and Lu 1995; Zhao et al. 1998). The DBD contains several sequence elements: P (proximal), D (distal), T, and A boxes. These sequence elements contribute to response element specificity and dimerization. In addition, these sequence

elements make contact with the DNA backbone and residues flanking the DNA core recognition sequence.

The hinge region is variable in terms of length and primary sequence. It is located between the DBD and the LBD and serves as a "hinge" between these two domains. The hinge region is very flexible, allowing the DBD and LBD to adopt several different conformations without creating steric hindrance. It allows the DBD to rotate 180^{o} so that some receptors bind as dimers to both direct and inverted HREs (Glass 1994). Studies have shown that the hinge region may serve as a docking site for corepressor proteins (Horlein et al. 1995; Chen and Evans 1995). In addition, the hinge region contains the nuclear localization signal (NLS) (Ylikomi et al. 1992; Germain et al. 2003; Robinson-Rechavi, Escriva Garcia, and Laudet 2003).

The ligand binding domain (LBD) is the largest domain and is moderately conserved (Escriva, Delaunay, and Laudet 2000; Robinson-Rechavi, Escriva Garcia, and Laudet 2003; Germain et al. 2003; Giguere 1999). It is highly structured with a variable primary sequence and a conserved secondary structure of 12 helices. The LBD is a multifunctional domain that mediates ligand binding, dimerization, interaction with heat shock proteins, nuclear localization, transactivation functions, and repression. A highly conserved helix (helix 12 or AF-2) is found at the carboxy terminal end of the LBD. The AF-2 is responsible for ligand-dependent transactivation by recruiting coactivators.

The C-terminus or F-domain has little evolutionary conservation (Germain, Altucci, and al. 2003) . Although its function is unknown, studies have shown that it might play a role in coactivator recruitment to the LBD (E domain) and in determining the specificity of the LBD coactivator interface (Germain et al. 2003; Peters and Khan 1999; Sladek et al. 1999). It has been suggested that, because this domain may affect antagonist action, it fine-tunes the molecular events associated with the transcriptional properties of the LBD (Germain et al. 2003; Montano et al. 1995; Nichols, Rientjes, and Stewart 1998).

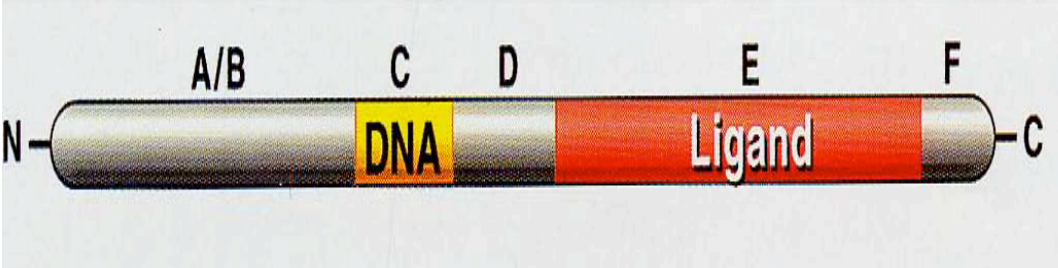

Fig 1.2 : Structure of a typical nuclear receptor
Nuclear receptors have the following domains from left to right: the least conserve N-terminal or A/B domain; the highly conserved DNA binding domain (DBD) or C domain; the variable hinge-region or D domain; the moderately conserved ligand binding domain (LBD) or E-domain; and the variable C-terminal domain or F-domain. This research was originally published in The Journal of Biological Chemistry. Olefsky JM. Journal of Biological Chemistry. 2001; 276(40):36863-36864. © the American Society for Biochemistry and Molecular Biology

Mechanism of Transactivation

Nuclear receptors act in three steps: repression, derepression, and transcription activation (Robinson-Rechavi, Escriva Garcia, and Laudet 2003; Giguere 1999) (Fig. 1.3) (Bastien and Rochette-Egly 2004). Chromatin structure plays an important role in gene activation at all three stages (Schrem,

Klempnauer, and Borlak 2002; Wolffe 1997). Chromatin displays basal levels of histone acetylation and transcription in the absence of a receptor. In the absence of a ligand, many nuclear receptors are transcriptional silencers (Chen and Evans 1995; Baniahmad, Kohne, and Renkawitz 1992). Apo receptors (unliganded receptors) recruit a co-repressor complex with histone deacetylase activity (HDAC) to keep the chromatin in a repressed state (Nagy et al. 1997; Heinzel et al. 1997; Wong et al. 1998). Ligand-binding leads to a conformational change in the LBD, in which helix 12 (AF-2) changes position. (Brzozowski et al. 1997; Wagner et al. 1995). The repressor complex dissociates from the receptor and the receptor is then free to interact with coactivator complexes which include p300/CBP and p/CAF (p300/CBP-associated factor), both of which have been shown to possess histone acetylase activity (HAT). This results in chromatin decondensation and derepression (Ogryzko et al. 1996; Lanz et al. 1999; Robinson-Rechavi, Escriva Garcia, and Laudet 2003). Although a permissive chromatin environment is required for efficient gene expression (Schrem, Klempnauer, and Borlak 2002), it alone is not sufficient for transcriptional activation. In addition to chromatin modulation, interactions between nuclear receptors and the general transcription machinery are required to regulate gene expression (Wong, Shi, and Wolffe 1997) Transcription occurs as a result of the dissociation of the HAT complex and the assembly and recruitment of a second coactivator complex (TRAP/DRIP/ARC/ often referred

to as the mediator complex) which then makes contact with the basal transcription machinery(Rachez and Freedman 2001).

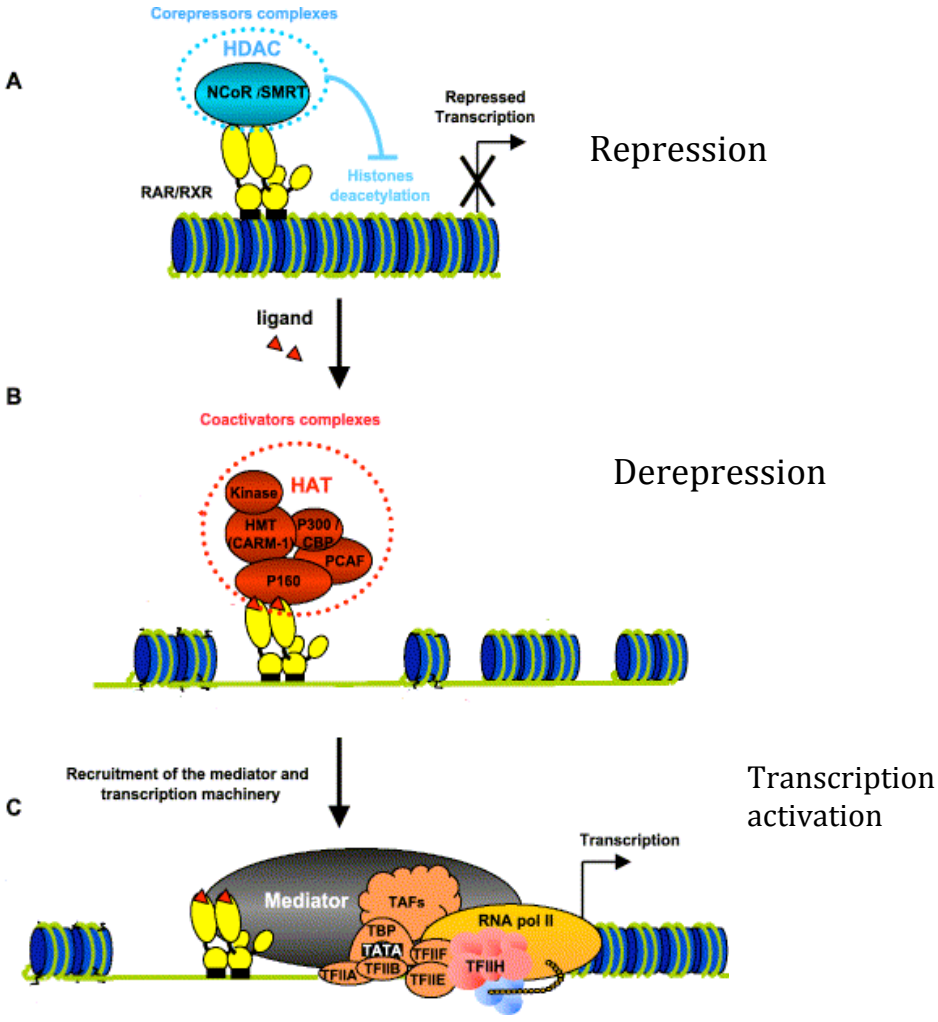

Figure 1.3: Mechanism of Nuclear Receptors
Nuclear receptors act in three major steps: repression, depression, and transcription activation.
A: Apo-receptors recruit a co-repressor complex with histone deacetylase activity (HDAC) to keep chromatin condensed (repression).
B: Upon ligand-bindng, the co-repressor complex dissociates from the receptor allowing it to associate with co-activator complexes with histone acetylase activity (HAT) and results in chromatin decondenstion (derepression).
C: Dissociation of HAT complex and recruitment of a second co-activator complex (mediator) which makes contact with the basal transcription machinery and ultimately results in transcription.
Adapated and reprinted from Bastein Jand Rochett-Egly C, Nuclear retinoid receptors and the transcription of retinoid target genes, Gene 328: 1-16, with permission from Elsevier.

Background

As reviewed by Sladek, FM (Sladek and Seidel 2001; Bolotin, Schnabl, and Sladek 2009), HNF4 (NR2A1) was originally detected as an activity in crude liver nuclear extracts that bound DNA elements required for the transcription of transthyretin (TTR) and apolipoprotein C3 (ApoC3), two liver- specific genes (Costa, Grayson, and Darnell 1989). Subsequent purification and cloning of HNF4 indicated that it was a member of the nuclear receptor superfamily because it exhibited a high degree of sequence similarity to the DBD and LBD of other nuclear receptors (Sladek et al. 1990). Its amino acid sequence is most similar to that of the retinoid X alpha receptor (RXRα), to the chick ovalbumin upstream promoter-transcription factor (COUP-TF) I and apolipoprotein regulatory protein-1 (ARP-1). HNF4 is highly conserved and has been cloned from rat, human, mouse, frog, bovine, chimp, and other species. The original HNF4 gene was renamed HNF4 alpha (HNF4α) when two other related genes were cloned from Xenopus—HNF4β and HNF4γ. There are two HNF4 genes in human and mouse—HNF4α and HNF4γ. HNF4α was originally described as a liver specific transcription factor, and highly expressed in the liver. In addition, it is expressed in the kidney, intestine, pancreas, stomach, and colon in mammals, and also in functionally equivalent organs in invertebrates such as the fat body, malpighian tubules, and gut, respectively.

HNF4α plays an important role in the regulation of hundreds of genes involved in numerous metabolic pathways including glucose, fatty acid, cholesterol, and xenobiotic drug metabolism (Odom et al. 2004; Waxman and Holloway 2009; Gupta and Kaestner 2004; Bolotin et al. 2009). It is one of several transcription factors essential for liver-specific gene expression. Since HNF4α controls the expression of another transcription factor important in the regulation of several hepatic genes (HNF1), HNF4α establishes a transcriptional hierarchy (Miura and Tanaka 1993; Kuo et al. 1992). HNF4α also plays an important role in embryogenesis, for example, disruption of murine HNF4α leads to embryonic lethality (Chen et al. 1994; Duncan et al. 1994; Holewa et al. 1996). In addition, it plays an important role in cell differentiation (Spath and Weiss 1997; Suaud et al. 1997).

HNF4a splice variants and tissue distribution

The human HNF4α gene consists of at least 12 exons and spans 30 kb (Furuta et al. 1997; Bolotin, Schnabl, and Sladek 2009) and maps to human chromosome 20 q 12-13.1 between PLCG1 and D20S17 (Argyrokastritis et al. 1997). The HNF4α gene potentially encodes nine isoforms (HNF4α1 through HNF4α9), several of which were previously identified by cloning (Sladek and Seidel 2001) (Fig 1.4). HNF4α isoforms arise from alternative splicing and alternate promoter (proximal promoter P1 and distal promoter P2) usage (Fig.

1.4) (Eeckhoute et al. 2003; Thomas et al. 2001; Boj et al. 2001; Hansen et al. 2002; Briancon et al. 2004). Alternative splicing is common among nuclear receptors (Gronemeyer and Laudet 1995) . The widespread use of alternative promoters in mammals allows for the differential expression of genes with complex spatio-temporal expression patterns (Landry, Mager, and Wilhelm 2003).

HNF4α promoter P1 initiates transcrips that are expressed in the liver and kidneys (Ihara et al. 2005; Servitja and Ferrer 2004). These transcripts contain exon 1A and give rise to isofoms HNF4α1 through HNF4α3 (Eeckhoute et al. 2003). The most prevalent isoform for the adult liver is HNF4α2 (Hata, Tsukamoto, and Osumi 1992). HNF4α2 and HNF4α3 are alternative splice variants of HNF4α1, differing only in their F domains. HNF4α2 has an insertion of 10 amino acids in the F domain (Hata, Tsukamoto, and Osumi 1992), while HNF4α3 has an F domain with a completely different sequence (Kritis et al. 1996). Promoter P2, on the other hand, initiates transcripts that are predominantly expressed in the pancreatic islet cells (Ihara et al. 2005; Hansen et al. 2002). These transcripts contain exon 1D which result in isoforms HNF4α7 through HNF4α9 (Thomas et al. 2001; Boj et al. 2001).

The predominant tissues for most of the vertebrate isoforms tested are the liver, kidney, intestine, and colon (Nakhei et al. 1998; Kritis et al. 1996; Drewes et al. 1996; Hata, Tsukamoto, and Osumi 1992; Sladek et al. 1990). They are also

detected in the pancreas and the stomach and barely detectable in testis and skeletal muscle (Kritis et al. 1996; Drewes et al. 1996). Studies using reverse transcriptase polymerase chain reaction (RT-PCR) and RNAase protection have shown that HNF4α2 mRNA is more abundant than HNF4α1 in rat, mouse, and human liver and kidney and the human hepatocarcinoma cell line, HepG2 (Chartier et al. 1994; Hata, Tsukamoto, and Osumi 1992; Hata et al. 1995; Sladek and Seidel 2001). Others have shown a lower expression of HNF4α3 in all tissues analyzed (Kritis et al. 1996). Nakhei et al (Nakhei et al. 1998) hypothesized the presence of HNF4α7 in a stem cell population since it is present at high levels in the stomach, absent in kidney, present in a dedifferentiated cell line (F9 cells), and barely detected in other tissues that typically lack HNF4α, namely the ovary, heart, bladder, and brain. Other researchers have detected HNF4α7 and HNF4α8 in embryonic liver and fetal-like hepatoma cells (Torres-Padilla, Fougere-Deschatrette, and Weiss 2001; Torres-Padilla, Sladek, and Weiss 2002) while other studies have shown that HNF4α8 is selectively expressed in pancreatic b cells (Ihara et al. 2005).

The HNF4α isoforms have similarities and differences. Since they have identical DNA binding and dimerization domains, they are all expected to bind the same response elements and to heterodimerize with one another, although neither has been tested in a rigorous fashion (Sladek and Seidel 2001). HNF4α1, HNF4α2, and HNF4α3 activate transcription well in a constitutive fashion while

others (HNF4α7) do not (Nakhei et al. 1998; Kritis et al. 1996; Drewes et al. 1996; Sladek et al. 1990). Sladek et al. (Sladek et al. 1999) have reported that HNF4α2 activates transcription better than HNF4α1 in some cell lines while Suaud et al., (Suaud, Formstecher, and Laine 1999) have reported the opposite in other cell lines. Nakhei et al. (Nakhei et al. 1998) have reported the transactivation of HNF4α7 to be a function of cell type used in transfection assays (Nakhei et al. 1998; Torres-Padilla, Fougere-Deschatrette, and Weiss 2001). Although the significance of the different HNF4α isoforms in human disease is not yet known, the different isoforms will probably play different roles in the liver and other organs (Sladek and Seidel 2001).

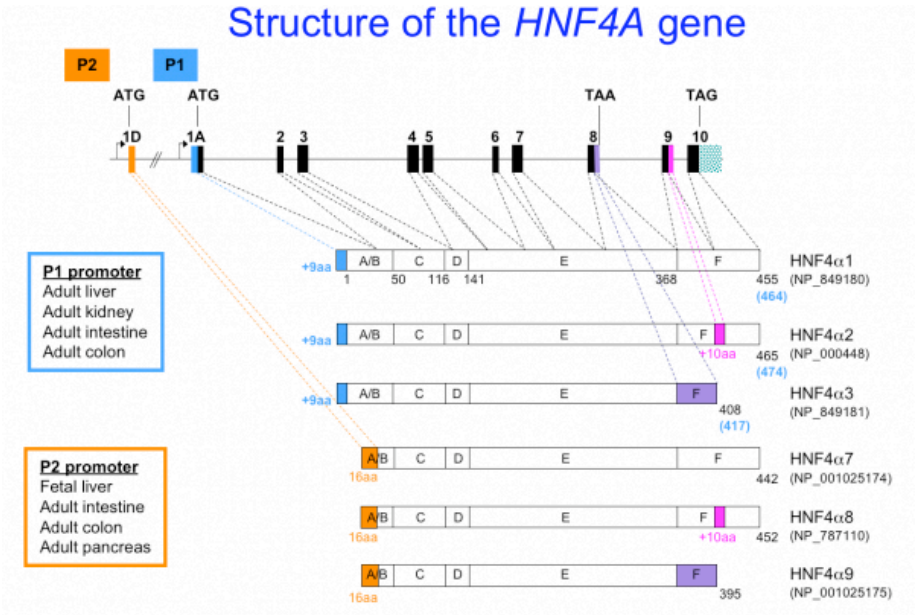

Fig 1.4 Structure of HNF4α gene and HNF4α isoforms.
A schematic representation of the HNF4a gene and its promoters P1 and P2. The proximal promoter P1 drives the expression of the HNF4α1-3 isoforms. The distal promoter, P2, drives HNF4α7-9. Bolotin, E., et. All (Updated November 11, 2009). HNF4α . From the Trascription Factor Encyclopedia. http://www.cisreg.ca/tfa. Accessed December 12, 2009.

HNF4α and its ligand

HNF4α was originally considered to be an orphan receptor because it activated transcription in a constitutive fashion – i.e., in the absence of exogenous ligands in a variety of cell types as well as in vitro (Sladek et al. 1999; Sladek et al. 1990; Malik and Karathanasis 1996). This suggested that either the putative ligand is present in all cells or it is not required for transactivation by HNF4α. A study in 1998 proposed fatty acylcoenzyme A thioesters were not shown to act as a as ligands for HNF4α (Hertz et al. 1998). Since these thioesters are important in pathways that are regulated by HNF4α such as glucose and fatty acid metabolism (Gurr and Harwood 1991), it would make sense for them to act as ligands for HNF4α. A ligand, in the classical sense, binds in the hydrophobic pocket in the LBD which results in a conformational change that removes co-repressors and recruits co-activators (Glass and Rosenfeld 2000). However, the fatty acyl CoA thioesters were not shown to act as a traditional ligand: They did not alter the conformation of HNF4, decrease binding to a co-repressor, or increase binding to a co-activator (Bogan et al. 2000; Ruse, Privalsky, and Sladek 2002). Recent findings show that HNF4α expressed in mammalian cells preferentially binds linoleic acid, an essential fatty acid from the diet, and that this binding is reversible in vivo (Yuan et al. 2009). However, a classical function for Linoleic acid in HNF4α function has not yet been demonstrated.

HNF4α and disease

HNF4α regulates many essential genes related to transport and metabolism of nutrients such as amino acids, lipids, vitamins, and glucose (Bolotin, Schnabl, and Sladek 2009). In addition, HNF4α regulates genes involved in the regulation of serum proteins such as blood coagulation factors, erythropoietin, and anti-thrombin III. HNF4α has also been directly linked to human diseases such as hemophilia B Leyden, a chromosome linked recessive bleeding disorder, and maturity onset diabetes of the young (MODY), an inherited form of non-insulin independent diabetes (Sladek and Seidel 2001; Yamagata et al. 1996; Bolotin, Schnabl, and Sladek 2009). HNF4α is also known to play a critical role in apolipoprotein gene regulation. The apolipoproteins ApoAI, and ApoB are the protein components of high density lipoproteins (HDL) and low density lipoproteins (LDL) respectively. HDL and LDL are important carriers of cholesterol and are linked to atherosclerosis (Barth and Arntzenius 1991), cardiovascular disease (Ballantyne, Arroll, and Shepherd 2005) and diabetes (Taskinen 2005) . Other HNF4α target genes potentially link HNF4α to cancer; they include: hepatitis B virus (HBV) (Tang and McLachlan 2001; Zheng, Li, and Ou 2004), cytochrome p450, and acylcoenmzyme A oxidase (ACO) (Sladek and Seidel 2001; Bolotin, Schnabl, and Sladek 2009).

Liver –Specific Gene Expression

The adult liver is predominantly composed of parenchymal cells or hepatocytes that carry out most of the specialized functions of the liver (Cereghini 1996). The transcription of several hepatic genes is activated during liver development and is later fine-tuned due to extracellular stimulation (Shiojiri, Lemire, and Fausto 1991; Cascio and Zaret 1991; Schmid and Schulz 1990). The transcription rate of genes encoding liver-specific proteins is higher in hepatocytes compared to other cell types (Powell et al. 1984). Studies have shown that the synthesis of these liver-specific proteins is regulated primarily at the level of transcription initiation (Derman et al. 1981; Aran, Cassuto, and Reshef 1995).

There are six families of liver-enriched transcription factors (LETF): HNF-1, HNF-3, HNF-4, HNF-6, C/EBP, and BZIP. The LETFs are a heterogeneous class of evolutionarily conserved transcription factors required for cellular differentiation and metabolism and are not exclusively expressed in the liver although their tissue distribution is limited (Cereghini 1996; Schrem, Klempnauer, and Borlak 2002; Sladek and Darnell 1992).

Studies of these transcription factors have led to the hypothesis that the cooperation of these factors and ubiquitous transactivating factors is necessary and possibly even sufficient for the maintenance of liver specific gene expression (Hayashi et al. 1999). For example, studies of specific promoter elements show that HNFs act in various combinations to direct cell-specific transcription during cellular differentiation (Duncan et al. 1998).

Earlier studies have pointed to a transcriptional hierarchy involved in maintaining the hepatic phenotype, where HNF4α is an upstream regulator of HNF1α (Kuo et al. 1992). Later studies established a positive autoregulation loop between HNF4α and HNF1α (Bailly et al. 2001; Spath and Weiss 1997, 1998). It has been shown that the HNF4α promoter contains HNF1α and HNF3α binding sites but lacks a TATA box (Taraviras et al. 1994). Studies by Duncan et al (Duncan et al. 1998) have shown that HNF3β positively regulates the expression of HNF4α and HNF1α and their downstream targets. Hence, a more elaborate picture is emerging: the liver enriched transcription factors are connected not just functionally but in a complex regulatory network that responds to signals to ultimately regulate gene expression (Sladek and Seidel 2001). For example, in the model propsed by Hatzis and Taliandis (Hatzis and Talianidis 2001), multiple interdependent regulatory pathways control the expression of HNF1 and HNF4α in a stringent manner (Fig. 1.5). This maintains balanced levels of HNF1α and HNF4α in the cell. During the early stages of liver development, HNF1β and GATA6 synergistically activate HNF4α. During the later stages, an increase in HNF1α levels, along with a decrease in HNF1β levels, switches the control of HNF4α to HNF1α. A reciprocal regulatory loop between HNF1α and HNF4α is then established which may lead to high concentrations of both HNF1α and HNF4α if left unchecked. This is regulated by COUP-TFII, which negatively regulates HNF4α and positively regulates HNF1α. Increased levels of HNF1α serves as its own negative control, which would also

prevent further activation of HNF4α. The efficient transactivation of HNF4α by HNF1α requires HNF6, which may provide an added level of control.

More recent studies have expanded the regulatory network of HNFs and established a link between the liver and pancreas. They show HNF involvement in general and HNF4α involvement in particular, in transcriptional regulatory loops that control the activity of human hepatic and islet cells (Smith et al. 2005; Odom et al. 2004; Gupta et al. 2005; Kulkarni and Kahn 2004).

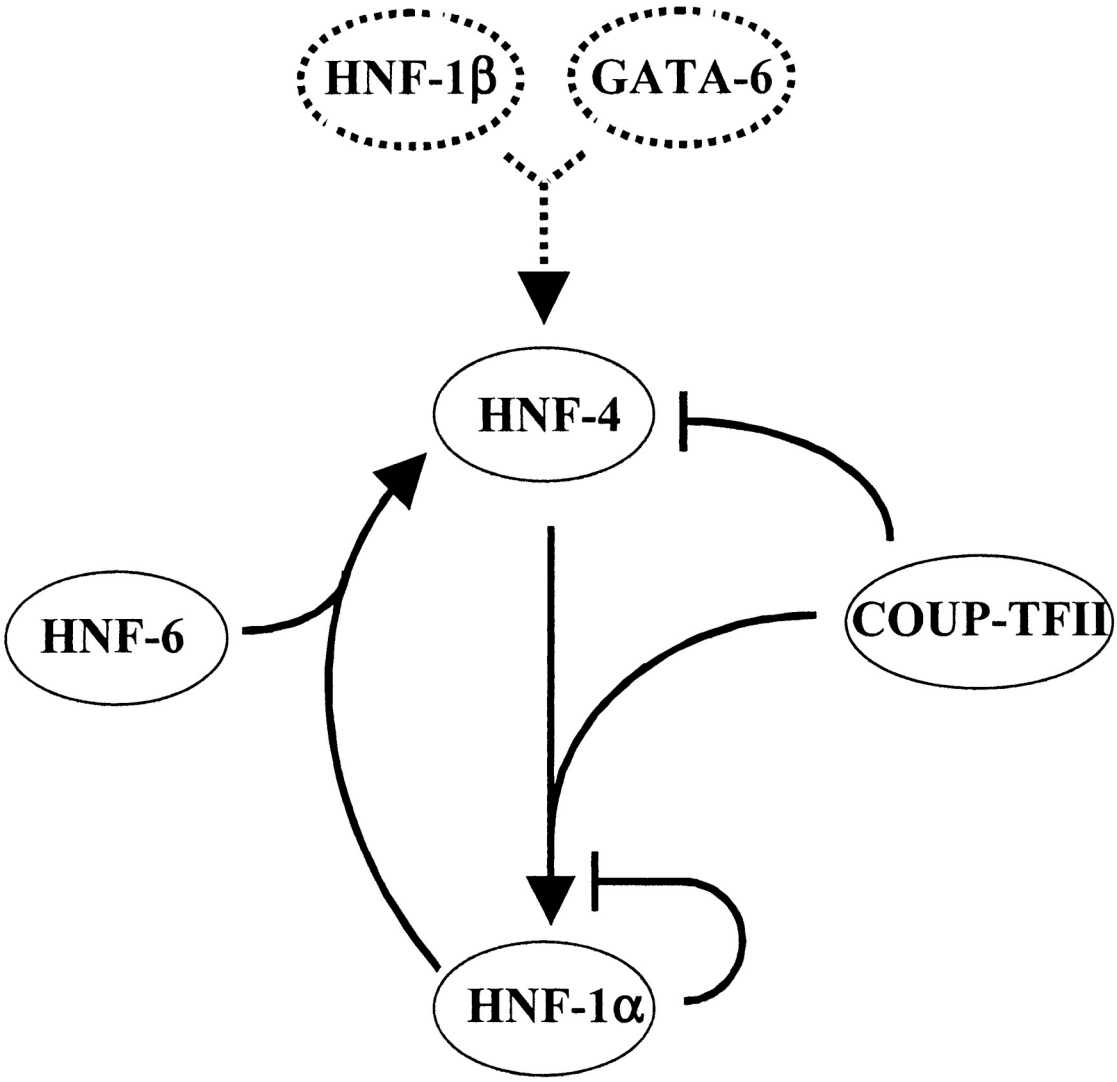

Fig 1.5 Proposed regulatory network involving liver enriched transcription factors
Multiple interdependent regulatory pathways control the expression of HNF1 and HNF4α in a stringent manner. Figure used with permission from the American Society for Microbiology (ASM). Hatzis and Talizndis 2001.

PGC1α coactivator

Coactivators serve as a link between physiologic stimuli to transcription factor activities (Finck and Kelly 2006). Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1α) is an example of these coactivators. PGC1α was identified through its interaction with Peroxisome proliferator-activated receptor gamma (PPARγ) in brown adipose tissue (Puigserver et al. 1998). PGC1α serves as a pleiotropic regulator of multiple pathways involved in cellular energy metabolism (Knutti and Kralli 2001; Puigserver and Spiegelman 2003). PGC1α has a variety of targets including PPARα (Vega, Huss, and Kelly 2000), HNF4α (Rhee et al. 2003), andFOXO1 (Puigserver et al. 2003). PGC1α is highly expressed in brown adipose tissue, heart, and slow-twitch skeletal muscle (Puigserver et al. 1998; Lin et al. 2002; Kressler et al. 2002). PGC1α expression is stimulated by stress, for example, by fasting in the liver (Rhee et al. 2003) and exercise in the skeletal muscle (Goto et al. 2000; Terada and Tabata 2004; Pilegaard, Saltin, and Neufer 2003).

The goal of this study

The goal of this study is to characterize the global changes that occur in the maternal liver during lactation. We will utilize genome-wide expression profiling in order to tease out molecular signatures of differentially up regulated and down regulated genes as a result of lactation and connect them with maternal adaptations that occur in order to meet the demands of the lactating mammary gland and the suckling pups. At the same time, we will focus on liver enriched transcription

factors, such as HNF4α, and the role they might play in lactation. Chapter 2 will focus on maternal adaptations that occur in order to meet the demands of the lactating breast and the suckling pups. We will be looking at maternal physical changes (i.e. whole body weight, whole liver mass), and metabolic changes (i.e. glucose levels, triglyceride levels) that occur during lactation. Chapter 3 is devoted to expression profiling of mRNA from the livers of virgin, non-lactating,and lactating mice. A close examination of the differential expression of genes during lactation should enable us to understand the role of the liver in lactation, and also find a link between the physical and/or metabolic changes that lactation brings and the possible health benefits to the mother.

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Chapter 2

Maternal Adaptations in Response to Lactation

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Introduction

The goal of pregnancy and lactation is the survival of reproductively viable offspring. Lactation includes the development of mammary tissue, synthesis and secretion of milk, and maintenance of milk production for the suckling young (Hartmann, Owens, and al. 1996; Lemay et al. 2007). Lactation is the most energetically demanding period in the life cycle of female mammals (Thompson and Nicoll 1986; Naya et al. 2008). The lactating mammal has developed maternal adaptations in order to meet the high energy demands of lactation. Maternal adaptations to lactation include overeating and phenotypic plasticity (Speakman and Krol 2005; Hammond and Kristan 2000). Phenotypic plasticity is an adaptive process (Agrawal 2001) that allows an organism to change its traits (i.e. organ morphology, body composition) in response to internal or external environmental changes, and these changes are reversible (Piersma and Drent 2003).

The liver plays an important role in lactation. The liver, along with the mammary gland and adipose tissues, is one of the three major sites of metabolism of fatty acids (Vernon 2005), which are a major component of milk. Lactation in turn has extensive effects on the liver (Drackley, Beaulieu, and Elliott 2001; Vernon et al. 2002). The high energy demands of lactation require the redirection of nutrients to the lactating mammary gland through the collaborative changes in the metabolic

activities of a variety of other tissues such as the liver and adipose tissue (Bauman and Currie 1980; Dunphy, Snell, and Clegg 1992).

Studies have shown that lactation lowers the incidence of metabolic syndrome among lactating mothers (Stuebe et al. 2005; Gunderson et al. 2007), and this health benefit may have long- term effects (Ram et al. 2008; Schwarz et al. 2009; Gunderson 2008). Metabolic Syndrome is a cluster of risk factors whose primary clinical outcome is cardiovascular disease (CVD) (Huang et al. 2009; Grundy et al. 2004). Metabolic syndrome includes hyperglycemia/insulin resistance, obesity, and dyslipidemia, (Huang et al. 2009; Grundy 2007; Zimmet, Alberti, and Shaw 2005), conditions that involve the liver (Xie et al. 2009; Kusminski et al. 2009). Nonalcoholic fatty liver disease (NAFLD), which involves the accumulation of fat in the liver, has also been associated with metabolic syndrome (Marchesini et al. 2003).

The liver enriched transcription factor Hepatocyte Nuclear Factor 4alpha (HNF4α) is vital to the development and functions of the liver (Chen et al. 1994; Hayhurst et al. 2001; Sladek and Seidel 2001). It regulates many essential genes related to transport and metabolism of nutrients such as amino acids, lipids, vitamins, and glucose. In addition, HNF4α has been associated with diseases such as diabetes, atherosclerosis, obesity, and metabolic syndrome (Yamagata et al. 1996; Sladek and Seidel 2001; Bagwell et al. 2005; Black et al. 2008; Weissglas-Volkov et al. 2006).

A healthy liver is important in lactation. It plays an important role in the detoxification of xenobiotics, and in glucose and lipid homeostasis. HNF4α plays an important role in adult liver functions such as lipid and glucose metabolism. We will be looking at maternal physiological and metabolic changes during lactation in mice. Examples of changes are maternal whole body weight, maternal whole liver weight, plasma glycerol concentration, plasma glucose concentration, hepatic triglyceride concentration, and plasma triglyceride concentration. Taken together, we may be able to paint a global picture of the liver during lactation, and thereby learn more about its function in lactation.

Materials and Methods

Animal care and treatment

Care and treatment of experimental animals was in accordance with guidelines from the University of Calfornia, Riverside, Institutional Animal Care and Use Committee (IACUC). Female, age-matched, virgin and timed pregnant C57Bl/6 mice were purchased from Charles River Laboratories (Charles River Laboratories, Wilmington, MA). All timed pregnant mice went through one successful round of reproduction (pregnancy, lactation, weaning) before mating for a second round. Timed pregnant mice were received at 15 days gestation at the UCR vivarium and were allowed to acclimate to their new environment for about 17 days. Virgin mice were never mated. At parturition, 12 dams were allowed to lactate for 10-12 days postpartum (lactating) and pups were adjusted to 6 pups/lactating dam at parturition. For the pregnant but non-lactating group (n=12), all pups were taken away at parturition. Each lactating dam (L) was housed separately with 6 suckling pups per cage. Non-lactating dams (NL) were also housed separately at parturition. Virgin mice (V) were housed 5 mice per cage. All mice were kept in a temperature-controlled room, exposed to 12 h light cycle, 12 h dark cycle, and fed a regular chow diet (Harlan Teklad, TD7012; Harlan Teklad, Madison, WI).

Fasting, whole body weights, and blood collection

In order to control for the effects that diet may have on lactation, each mouse (with suckling pups when applicable) was fasted for 18-21 h before sacrifice in the following manner. Each mouse (and their suckling pups when applicable) was transferred to a new cage without food. Virgin mice (5 per cage) were also transferred to a new cage. The bedding of each cage was changed to Sani-Chips (cat # 7090, Harlan Teklad, Madison, WI), non-edible wood chips that do not provide a source of nutrients as does standard bedding. All mice were allowed a bottle of water during the fast. At around 15 h post initiation of fast, (9:00 a.m. the next day) whole body weights were recorded for each mouse. Mice in their cages were then placed under a 60-watt heat lamp for 10 minutes. Each mouse was restrained, and their tails were nicked. Whole blood was then collected in two heparinized hematocrit tubes (Fisher Scientific, catalog # 21-176-6) per animal. Whole blood was subjected to centrifugation at 16,000 RCF for 10 min at 4^{o}C. Blood plasmas (supernatants) were collected, transferred to new Eppendorf tubes, and stored at -20^{o}C. After blood collection, mice were returned to their cages and allowed to recover before being sacrificed at around noon (18-21 h total fasting).

Tissue harvesting

Mice were euthanized by CO_2 exposure for 40 seconds. Whole body weights were recorded and tissues were harvested in a cold room (4^{o}C). The portal artery was cut and allowed to bleed before each liver was taken out and weighed. The largest liver

lobe (lobe 1) was harvested and stored for the following assays: two samples (10-30 mg) for RNA isolation, one sample (100 mg) for triglyceride (TG) assay, one sample (100 mg) for sectioning, the rest of lobe 1 (300 mg), for storage. The remaining liver (lobes two-six; 200 – 300 mg) was chopped up with a razor blade and stored in two aliquots for nuclear extraction. All liver samples were stored in screw cap cryovials (Nunc, cat # 12-565-170N, Thermo Fisher Scientific, Waltham, MA) in liquid nitrogen.

Plasma glycerol and plasma triglyceride quantification

Preparation of standards and sample plasmas

Plasma glycerol and plasma triglyceride concentrations were quantified using a triglyceride quantification assay kit (Cat# ETGA-200, BioAssay Systems, Hayward, Ca), as per the manufacturer's instructions. Briefly, assay standards (0 mmol/L, 0.3 mmol/L, 0.6 mmol/L, 1 mmol/L) were prepared as per the manufacturer's instructions. Heparinized plasmas (see Fasting, whole body weights, and blood collection section for whole blood collection details) of each animal (sample plasmas) were thawed on ice and diluted 5-fold in double distilled water.

Assay Procedure

Standards (10 μL) were added to their appropriate wells (standard wells) in singlicate, per manufacturer's instructions, in a 96-well plate (Costar assay plate 3595, Corning Scientific, Corning, NY). Each sample plasma (10 μL) was added to

the appropriate wells in singlicate (sample wells) and the same sample plasmas (10 μL) were added to separate wells (blank wells) in singlicate. The appropriate amount of working reagent (with lipase) and control reagent (no lipase) were prepared. Working reagent (100 μL) was added to each standard well and each sample well (all in singlicate), and 100 μL of control reagent was added to each blank well. The plate was tapped to mix all reagents. The plate was incubated at room temperature for 30 min. Standard, sample, and blank optical densities were determined by a plate reader (V Max kinetic microplate reader, Molecular Devices, Sunnyvale, CA), set at 570 nm.

Generating the standard curve

The standard curve was generated by subtracting the raw OD value of the zero standard from each raw OD value of all standards. These were plotted against known standard concentrations. The slope of the line was determined by linear regression.

Calculating plasma triglyceride concentrations

Plasma triglyceride concentrations of sample plasmas were determined by subtracting blank OD values of sample plasmas from their respective sample OD values. These were then divided by the slope, then multiplied by the dilution factor (in this case, 5). These values (in nmol/L) were multiplied by 88.5 (1 mmol/L TG equals 88.5 mg/dL) to get final values in mg/dL.

Calculating plasma glycerol concentrations

Plasma glycerol concentrations were calculated by subtracting the raw OD value of the zero standard from raw OD values of sample plasma blanks. These values were then divided by the slope of the standard curve and multiplied by the dilution factor (in this case, 5; unit: mmol/L).

Glucose Quantification

Preparation of standards and unknown samples

Plasmas were thawed and their fasting glucose levels were measured with a glucose assay kit per the manufacturer's instructions (cat # K606-100, BioVision, Mountain View, CA). A stock glucose standard was prepared by adding 10 μL of the Glucose Standard to 990 μL of Glucose Assay Buffer, for a final concentration of 1 nmol/μL. Zero nmol, 2 nmol, 4 nmol, 6 nmol, 8 nmol, and10 nmol standards were prepared by adding Glucose Assay buffer to 0 μL, 2, μL, 4 μL, 6 μL, 8 μL, and 10 μL of stock glucose standard in their corresponding eppendorf tubes. Each standard volume was adjusted to 50 μL per reaction, making enough for 2.5 reactions per standard. Unknown samples were diluted to 4 % in Glucose Assay Buffer (2 μL unknown sample in 48 μL Glucose Assay Buffer for 1 per reaction) in their respective Eppendorf tube, making enough for 2.5 reactions per unknown sample.

Glucose assay procedure

50 μL of all prepared standards and unknown samples were added to their assigned wells in a 96 well plate (Costar assay plate 3595, Corning Scientific, Corning, NY) in duplicate. Glucose Reaction Mix (Reaction mix) was prepared as per the manufacturer's instructions. Prepared Reaction mix (50 μL/well) was added to all standard and unknown sample wells, and mixed well by pipeting up and down. The plate was wrapped in foil and allowed to incubate at 37°C for 30 min. Standard and unknown optical densities were determined by a plate reader (V Max kinetic microplate reader, Molecular Devices, MDS Inc, Toronto, Canada), set at 570 nm.

Data Analysis

Data were collected and analyzed by using SoftMax Pro version 3.6 (Molecular Devices, MDS Inc, Toronto, Canada). The background was corrected by subtracting the raw value obtained from 0 nmol standard from all raw unknown sample values. Glucose concentrations of unknown samples were extrapolated from the standard curve generated.

Hepatic Triglyceride Quantification

Hepatic Triglyceride Isolation

Triglycerides (TG) were isolated from previously frozen liver chunks as per BioVision TG quantification kit instructions (cat # K622-100, BioVision, Mountain View, CA). Liver chunks were homogenized in the appropriate volume of 5%

Triton-X100 solution (5% Triton-X100 in water; 100 mg liver chunk in 1 mL 5% Triton-X100 solution). Homogenates were then transferred to 15 mL conical tubes and placed in an 80°C-100°C water bath for 2-5 minutes until cloudy. Heated homogenates were allowed to cool down to room temperature. Triglycerides were allowed to solubilize by heating cooled homogenates in an 80°C-100°C water bath for 2-5 minutes for a second time. Solubulized homogenates were spun at 400 x g in a table top clinical centrifuge (CL model, IEC). Supernatants (hepatic triglycerides) were transferred to Eppendorf tubes and stored at -20°C.

Preparation of standards and hepatic triglyceride samples

Hepatic triglycerides were quantified using a triglyceride quantification assay kit (Cat# ETGA-200, BioAssay Systems, Hayward, Ca), as per the manufacturer's instructions. Briefly: Assay standards (0 mmol/L, 0.3 mmol/L, 0.6 mmol/L, 1 mmol/L) were prepared (see Plasma glycerol and plasma triglyceride quantification section). Hepatic triglycerides samples (HTS) were thawed and diluted 10-fold in double distilled water.

Assay Procedure

Prepared standards (10 μL) were added to their assigned wells (standard wells) in singlicate, in a 96-well plate (Costar assay plate 3595, Corning Scientific, Corning, NY). HTS (10 μL) were added to both assigned wells (HTS wells) as well as to blank wells. The appropriate amount of working reagent (with lipase) and control reagent

(no lipase) were prepared as per the manufacturer's instructions. Working reagent (100 μL) was added to each standard well and each HTS well (all in singlicate), and 100 μL of control reagent was added to each HTS blank well. The plate was tapped to mix all reagents. The plate was incubated at room temperature for 30 min. Standard, HTS, and HTS blank optical densities were determined by a plate reader (V Max kinetic microplate reader, Molecular Devices, MDS Inc, Toronto, Canada), set at 570 nm.

Generating the standard curve

The standard curve was generated by subtracting the raw OD value of the 0 standard from each raw OD value of all standards. These were plotted against known standard concentrations. The slope of the line was determined by linear regression.

Calculating hepatic triglyceride concentrations

Hepatic triglyceride concentrations were determined by subtracting HTS blank OD values from their respective HTS OD values. These were then divided by the slope of the standard curve, then multiplied by the dilution factor (in this case, 10; unit mmol/L). The values (in mmol/L) were then multiplied by their respective total extract volumes (TEV) in liters and then normalized to their respective liver chunk weights in grams (normalized unit: mmol/g). Normalized values (mmol/g)

were then multiplied by the molecular weight of triglycerides (885 g/mmol) to get final units of mg/g.

Liver staining

Liver tissue chunks from liver lobe 1 per animal were placed in molds in (cat # 22-038-217, Fisher Scientific) containing OCT freezing medium (cat # 14-373-65 Andrew Scientific N.:4583, Fisher Scientific). Tissue samples in the molds were covered with OCT freezing tissue medium and placed on dry ice. These were then stored at -80^{o}C and sent to AML laboratories (AML laboratories, Rosedale,MD) on dry ice for Oil Red O staining. Whole mounts of Oil Red O-stained liver tissues were photographed at 200X. Oil Red-O stains fat red. All images were processed in an identical fashion. Red staining was enhanced by using the Auto Levels command in Adobe Photoshop CS, version 8.0. Images were further modified by using Powerpoint (Microsoft Office 2008) by adjusting their brightness levels (V image: -16%, NL image: -36%, L image: -22%) in the picture editing mode.

Preparation of maternal liver nuclear extracts

Crude mouse liver nuclear extracts (NE) were prepared using a combination of previously published methods (Costa et al. 1988; Sladek et al. 1990) with the following modifications. Frozen liver samples ear-marked for nuclear extract preparation were kept in liquid nitrogen until the time of extraction. Each frozen minced liver chunk was homogenized in 2mL Buffer A-T (10 mM Hepes, pH 7.8, 0.32 M sucrose, 0.3% Triton X-100, 25 mM KCl, 0.15 mM spermine, 0.5 mM spermidine, 1

mM EGTA, 1 mM EDTA, 1 mM DTT, 0.5 mM PMSF) containing protease and phosphatase inhibitors (P8340, P2850, P5726 cocktails, Sigma, St. Louis, MO) with an electric tissue grinder. The homogenate was passed through a 100 μm cell strainer (cat # 352360 BD Falcon, BD Biosciences, San Jose, CA) homogenized manually and centrifuged at 822 x g for 10 min at 4°C (Jouan CR4-12, Jouan, Winchester, VA). The supernatant was decanted and the pellet containing the nuclei was resuspended in Buffer A-T followed by two washes at 347 x g for 5 min at 4°C (Jouan CR4-12, Jouan, Winchester, VA) in Low Salt Buffer (as Buffer A-T except without sucrose or Triton X-100 but with 20% glycerol) before re-suspending in the same buffer and slowly adding High Salt Buffer (as Low Salt Buffer but with 0.5 M KCl) to a final salt concentration of 0.33 M KCl. The nuclei were extracted for 45-60 min with gentle agitation. The chromatin was pelleted and the supernatant (nuclear extract) was removed, aliquotted, snap-frozen on dry ice, and stored at -80°C. All steps were carried out at 4°C. Protein concentrations were determined by the BioRad Protein Assay (cat # 500-0201,Bio-Rad Laboratories, Hercules, CA).

Quantification of HNF4α protein by competitive ELISA

The HNF4α competitive ELISA (H4 ELISA) was developed and validated in our lab (Camba-Colón 2005), based on modifications of a procedure from Lomax et al (Lomax et al. 1998). The assay involves plate preparation, pre-incubation, competition, 2° AB, substrate, and plate reading.

Preparation of standard antigen, GST.HNF4α.LBDF/F (LBD/F)

GST.HNF4α.LBD/F (GST.LBD/F) was used as the standard antigen. Construction and expression of GST.LBD/F was in *E.coli* aspreviously described in Ruse et al (Ruse, Privalsky, and Sladek 2002). Expression of GST.LBD/F was verified by immunoblot (IB) analysis as described previously (Jiang et al. 1995; Maeda et al. 2002). GST.LBD/F was released from GST by cleavage with PreScission Protease (Amersham Biosciences, Piscataway, NJ) as per the manufacturer's protocol and verified by IB. The LBD/F concentration was determined using SDS-PAGE followed by transfer to Polyvinylidene fluoride (PVDF) transfer membrane (cat # ISEQ00010, Immobilon-PSQ membrane, Millipore, Billerica, MA) and staining with Coomassie Brilliant Blue. Different known quantities of bovine serum albumin (BSA) were loaded as standards. The NIH ImageJ program, a public domain Java image processing program, was used for quantification of the BSA and LBD/F bands.

Plate preparation

On day 1: 96-well non-treated, flat-bottomed, polystyrene microplates (Costar assay plate 3595, Corning Scientific, Corning, NY) were coated as follows. Day 1: HNF4α.LBD/F was diluted in 50mM carbonate buffer (16 mM Na_2CO_3, 34 mM $NaHCO_3$, pH 9.6) to a concentration of 4 mg/mL. 50 mL of 0.4 mg/mL HNF4α.LBD/F was added to each well, for a total of 20 ng per well. Three wells designated as non-specific binding (NSB) wells received 50 μL/well of NSB buffer (1% non-fat milk in 50 mM carbonate buffer) but no HNF4α.LBD/F. The plate was covered and

incubated overnight at 4°C on a tabletop shaker (Innova 4000, New Brunswick Scientific). On day 2: Plates were washed three times (1 min soak, followed by aspiration) with 200 μL/well phosphate buffered saline containing detergent (PBST: 0.02 M sodium phosphate, 0.15 M sodium chloride, pH 7.2, 0.05% Tween 20) to remove unbound HNF4α.LBD/F (antigen) and blocked by adding 50 μL/well of block solution (2% non-fat milk, PBST) to all wells. The plates were incubated for 45 min at 37°C on a platform shaker and then washed three times with PBST. The plate was aspirated, air dried, covered, and sealed in plastic and stored at 4°C overnight.

Preparation of 1° Antibody (α445-AP)

Also on day 2, a 1:500 dilution of affinity purified HNF4α primary antibody (Sladek et al. 1990) in PBST was prepared right before the preparation of standards, NSB sample, and unknowns. α445-AP is an affinity-purified antibody to HNF4α that reacts with the very C terminus of human, rat, and mouse HNF4α.

Preparation of standards, NSB sample, and unknowns

Day 2: Standards were prepared by serially diluting LBD/F in PBST. Standard concentrations were the following: 1600 ng/mL, 800 ng/mL, 400 ng/mL, 200 ng/mL, 100 ng/mL, 50 ng/mL, 25 ng/mL, 0 ng/mL. The unknowns were prepared by diluting maternal liver nuclear extracts 1:5 in PBST. The NSB sample consisted of PBST only. 100 μL of each standard, unknown, and NSB sample was transferred to

labeled Eppendorf tubes. 100 μL of α445-AP (diluted 1:500 in PBST from a starting concentration of 1.0 mg/mL) was added to prepared standards, NSB, and unknowns for a final volume of 200 μL per tube and nutated overnight at 4°C.

Competition

Day 3: 50 μL of each pre-incubated sample (NSB, standards, and unknowns) were added to wells in triplicate per sample. The plates were covered and sealed in plastic and incubated at room temperature on a nutator for three hours.

2° Antibody (GαR-AP)

Day 3 (cont): The samples were aspirated from the wells with micropipet tips. The wells were washed as described above for antigen coating. Goat anti-rabbit Alkaline phosphatase (GαR-AP) was diluted 1:2000 in PBST. 50 μL of GαR-AP was added to each well. The plates were covered and incubated for 1.5 h at room temperature with gentle shaking.

Substrate and color development

The substrate was prepared 15 min before the end of the 2° AB incubation by diluting 3 mL of 5x diethanolamine buffer (Bio-RAD, Hercules, CA) in 12 mL of deionized water in a 50 mL conical tube that was covered in aluminum foil and inverted several times. Three tablets of p-nitrophenylphosphate (PNP) (BIO-RAD, Hercules, CA) were completely dissolved in the diluted diethanolamine buffer by

shaking the conical tube vigorously. The plates were washed in PBST as previously described. 50 μL of the prepared substrate was added to each well. The plates were covered and wrapped in aluminum foil and then incubated at room temperature while shaking for 40 min to 1 h. The plates were read with a microplate reader (V Max kinetic microplate reader, Molecular Devices, MDS Inc, Toronto, Canada) at 450 nm.

Data Analysis

The program Softmax Pro version 3.6 (Molecular Devices, MDS Inc, Toronto, Canada) was used to initially gather and record the measurements of the samples (raw data). The raw data was then manually transcribed to Microsoft Excel and copied and pasted into Softmax Pro tables for further analysis. Softmax Pro generated standard curves from the raw data using the four parameter logistical curve fit (4-PL). Values for the unknowns were extrapolated by the program from the standard curve. Data from Softmax Pro was further analyzed using Microsoft Excel.

Quantitative Real Time Polymerase Chain Reaction (qPCR)

RNA Isolation

Liver chunks from each mouse (10-30 mg) ear-marked for RNA isolation were incubated overnight at 4^{o}C in 500 μL RNALater (cat # AM7023, Ambion, Austin, TX),

an RNA stablization solution. RNALater was removed from the previously incubated liver chunk the next day, and the remaining liver chunk was stored at -80^{o}C . RNA was then isolated from RNA-stabilized frozen liver chunks using the RNeasy mini kit (Qiagen, Valencia, CA). Briefly, each liver chunk was homogenized in complete cRLT buffer (1% beta mercaptoethanol in RLT buffer) in a 1.5 mL tube using a motor homogenizer until completely homogeneous. Homogenized lysates were centrifuged in room temperature at maximum speed (Eppendorf 5424,) for 2 min. to clear the lysate. The supernatant (cleared lysate) was transferred to a new 1.5 mL tube. An equal volume of 70% ethanol was added to the cleared lysate and immediately mixed by pipetting. Up to 700 μL cleared lysate in 70% ethanol was added to the spin column (spin columns come with collection tubes). Spin columns were then centrifuged at maximum speed for 15 sec at room temperature. The flow-through was discarded, and the remaining cleared lysate in 70% ethanol was added to the same spin column and centrifuged at maximum speed for 15 sec at room temperature, and the flow-through was discarded. The spin column was washed with Buffer RW1 by centrifuging at maximum speed for 15 sec at room temperature, and the flow-through discarded. The same column was washed this time with 500 μL of Buffer RPE by centrifuging at maximum speed for 15 sec at room temperature, then discarding the flow-through. The same tube was washed a second time with 500 μL of Buffer RPE, this time centrifuging at maximum speed for 2 min at room temperature to dry the spin column. Spin columns were then centrifuged at maximum speed for 1 min at room temperature, this time using fresh

collection tubes, to remove all ethanol. RNA was eluted by placing spin columns in fresh Eppendorf tubes and adding 50 μL RNAse free water then centrifuging at maximum speed for 1 min at room temperature. The flow-through (eluate) in the 1.5 mL tube is the RNA. A second elution was done by adding the eluate from the previous step to the collection tube and then centrifuged a final time at maximum speed for 1 min at room temperature. RNA concentration and quality were checked by nanodrop. RNA was stored at -80°C.

Digestion of genomic DNA

Digestion reactions were prepared for each RNA sample as per manufacturer's instructions (cat # 10 776 785 001Roche Applied Science, Penzberg, Germany): DNAse I mastermix (1x final concentration of 10x Incubation buffer, 0.5 units final concentration of DNase I recombinant RNase-free reagent for a total of 1.5 μL/reaction) was prepared for the appropriate number of reactions. RNA (final concentration of 0.3 mg/reaction) was added to 1.5 μL DNase I mastermix, then adjusted with RNase-free water for a total volume of 10 μL. Each reaction was incubated at room temperature for 20 min. DNase inactivation reagent (2 μL) (AM1906, Ambion, Austin, Tx) was added to each reaction and incubated at room temperature for 2 min. Reactions were then spun at 15000 RPM for 1.5 min at room temperature. Each supernatant (DNAse I treated RNAs) were transferred to new Eppendorf tubes and stored at -80°C.

cDNA preparation

cDNAs were prepared using the SuperScript™ III Synthesis System for RT-PCR (Invitrogen, Carlsbad, CA) as per the manufacturer's instructions. DNase I-treated RNAs were thawed on ice. The appropriate amount of primer mastermix (P-MM) (for one reaction: 1 μL 50 ng/μL random hexamers, 1 μL 10 mM dNTP mix) was prepared. DNase I-treated RNA (1 μg) was added to 2 μL of P-MM. Diethyl polycarbonate (DEPC)-treated water was added to each reaction tube for a total volume of 10 μL/reaction tube. Each reaction tube was then gently vortexed, then spun at 13,000 RPM at 4°C for 15 sec. All reaction tubes (RNA-P) were then incubated at 65°C for 5 min and placed on ice. The appropriate amount of cDNA synthesis mix master mix (cS-MM) was prepared (for one reaction: 10X RT buffer (2 μL), (4 μL) 25 mM $MgCl_2$, 0.1 M DTT (2 μL), RNaseoUTTM (1 μL, 40 U/mL), SuperScriptTM III reverse transcriptase (1 μL ,200 U/mL). cS-MM (10 μL) was added to each RNA-P mixture. All tubes were vortexed and spun 13,000 RPM at 4°C for 20 sec. All tubes were incubated in the PCR thermocycler with the following settings: step 1 at 25°C for 10 min, step 2 at 50°C for 50 min, step 3 at 85°C for 5 min, and step 4 at 4°C. All samples were then taken out of the thermocycler and placed on ice. Samples were then centrifuged at 13000 RPM at 4°C for 20s. Rnase H (1μL) was added to each sample. All samples were gently vortexed then centrifuged at 13,000 RPM at 4°C for 20 sec, then incubated at 27°C for 20 min. These are the cDNAs. All cDNAs were diluted 1:10 with DEPC-treated water. Standards for quantitative real time PCR (qPCR) were prepared by pooling all cDNA samples (180

μL total volume). Individual cDNAs and pooled cDNA were stored at -20°C for future use.

Quantitative real time PCR (qPCR)

Gene specific primers were validated before hand as previously described (Yuan et al. 2009). Briefly, primers were validated over four orders of magnitude and analyzed with the iQ5 Optical System Software. Primer pairs were deemed valid if an input log plot amount versus CT generated an efficiency of 100% ± 10% and a correlation coefficient of R^2=0950 ±0.05. Each condition (virgin, non-lactating, lactating) had three biological replicates. Each biological replicate was done in triplicate (technical replicate). The relative expression level of the genes was evaluated using the Pfaffl method (Pfaffl 2001), normalized to cyclophilipn A (*Ppia*) expression.

Statistical Analysis

Statistical analysis was performed using the Student's t test. Differences were considered significant at $p<0.05$. Uncertainty in our measurements was determined by calculating the standard deviation.

Results:

Trends in whole body weight in virgin, non-lactating, and lactating mice

In order to determine the effects of lactation on the maternal liver, we established 3 groups of mice: virgin (V), non-lactating (NL), and lactating (L). All 3 groups were age-matched. Virgin mice were never mated. Non-lactating and lactating mice had gone through one successful round of reproduction (pregnancy, lactation, weaning) then mated a second time. Mice were approximately 98 days old at second mating. Refer to the *Animal care and treatment* section of this chapter for details. All animals were fasted for 18-21 hours before sacrifice to normalize the amount of food they had eaten. Whole body weights of each animal were determined at different fasting time points (0 h, 15 h, 18-21 h) and plotted in Fig 2.1. At 0 h fasting, maternal whole body weights were slightly but significantly less in the non-lactating mothers compared to virgin mice (p_{NLvsV}=3.80E-2), but were much greater in the lactating mothers(p_{LvsV}=2.34E-08); the difference between non-lactating whole body weight and lactating whole body weight was also very significant (p_{LvsNL}=4.30E-09). The same trends were observed in both 15-h and 18-21-h fasting time points (Fig 2.1).

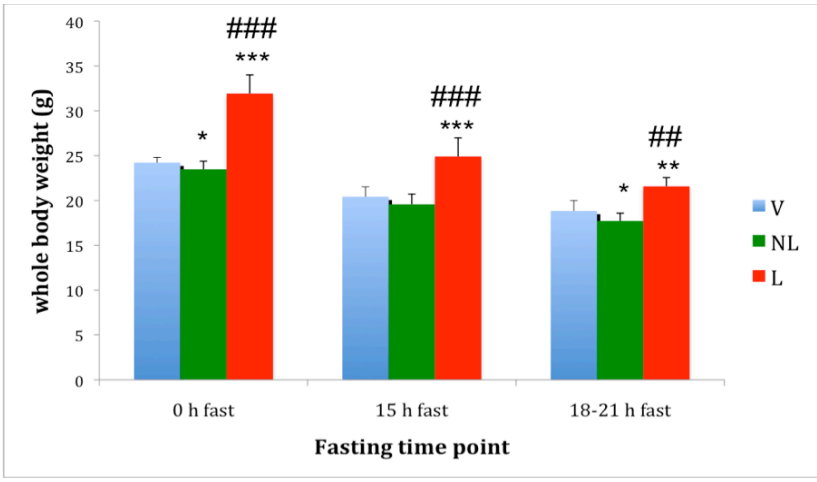


Fig 2.1: Lactation increases maternal whole body weight.
Age-matched virgin (V, blue bars, n_{0h}=5, n_{15h}=10, $n_{18\text{-}21h}$=9), non-lactating (NL, green bars, n_{0h}=11, n_{15h}=11, $n_{18\text{-}21h}$=9) and lactating (L, red bars, n_{0h}=11, n_{15h}=11, $n_{18\text{-}21h}$=5) mice were weighed at different fasting time points (0 h, 15 h, 18-21 h) on the day of sacrifice. Average whole body weights were subjected to the student T-test in order to determine whether differences in whole body weights for each group were significant: *=NL vs V or L vs V; #=L vs NL; * or #, p<5.00E-02; ** or ##, p< 5.00E-04; *** or ###, p< 5.00E-08.

Trends in percent of body weight lost (PWL)

Although maternal whole body weight increased with lactation, all mice lost weight during the 15-h fasting and 18-21-h fasting time points. In order to assess whether lactation affects weight loss, we calculated the percent of body weight lost (PWL) after the first 15h of fasting, and between 15 and 21h of fasting (Fig 2.2). During the first 15h of fasting, the percent of body weight lost (PWL) significantly increased with non-lactation ($PWL_{V0\text{-}15h}$= 12.90%, $PWL_{NL0\text{-}15h}$ = 16.67%, p_{NLvsV}=1.08E-02), and increased even more significantly with lactation (PWL_{L15h}=21.82%, p_{LvsV}= 7.06E-05). The difference in PWL between L and NL mice was also significant (p_{LvsNL}=2.05E-03). The same trend was observed at 15-21-h fasting although the percent changes were smaller. All mice continued to lose weight, with the most significant PWL occurring with lactation ($PWL_{L15\text{-}21h}$=10.23%,

$PWL_{NL15\text{-}21h}$=8.82%, $PWL_{V18\text{-}21h}$=7.73%, $PWL_{V15\text{-}21h}$=7.73%, p_{LvsV}= 3.16E-03, p_{LvsNL}=4.46E-02, p_{NLvsV}=4.27E-02).

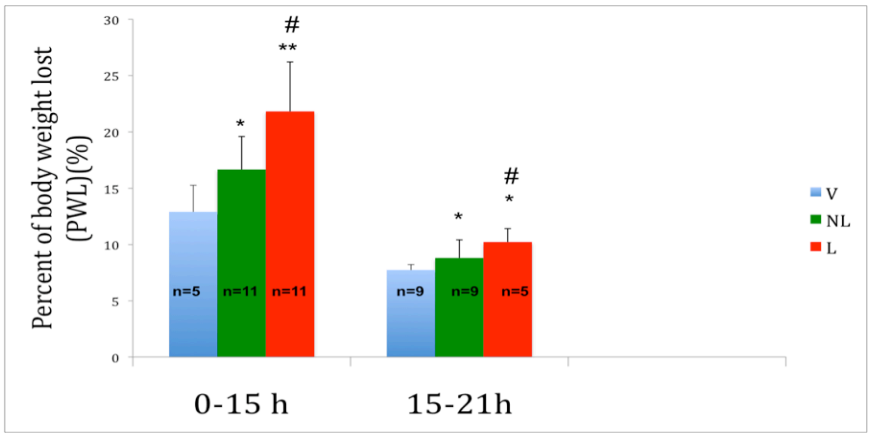


Fig 2.2: Percent of body weight lost (PWL) increases with lactation
Age-matched virgin (V, blue bars, $n_{0\text{-}15h}$=5, $n_{15\text{-}21h}$=4), non-lactating (NL, green bars, $n_{0\text{-}15h}$=11, $n_{15\text{-}21h}$=9) and lactating (L, red bars, $n_{0\text{-}15h}$=11, $n_{15\text{-}21h}$=5) mice were weighed at different fasting time points (0 h, 15 h, 18-21 h) on the day of sacrifice. Percent weight loss (PWL) for 15-h fasting time point for each animal was calculated by dividing the weight lost at 15h fasting time point (maternal whole body weight at 0-h fasting – maternal whole body weight at 15-h fasting) by maternal whole body weight at 0h fasting time point , all multiplied by 100%. PWL for fasting time point 15-21-h for each animal was calculated by dividing maternal whole body weight lost at 15-21h fasting time point (maternal whole body weight at 15-h fasting – maternal whole body weight at 18-21-h fasting) by maternal whole body weight at 15h fasting time point, all multiplied by 100%. Average PWL for animals per group were subjected to the student T-test in order to determine whether differences in PWL between groups were significant: *=NL vs V or L vs V; #=L vs NL; * or #, p<5.00E-02; ** or ##, p< 5.00E-04 .

Trends in rate of body weight lost (RWL)

In order to examine further the effect of lactation on weight loss, we looked at the trends in the rate of body weight lost among virgin, non-lactating, and lactating mice. We calculated the rate of body weight lost (RWL) at 0-15-h fasting and 15-21-h fasting (Fig 2.3). At 0-15h fasting, lactating mice had the highest rate of weight loss ($RWL_{V0\text{-}15h}$=0.208 g/h, $RWL_{NL0\text{-}15}$=0.260 g/h, $RWL_{L0\text{-}15h}$=0.468 g/h). The RWL for lactating mice was also significant for this time point ($p_{NLvsV0\text{-}15}$=1.61E-02, $p_{LvsV0\text{-}15h}$=4.34E-06, $p_{LvsNL0\text{-}15h}$=7.00E-06). The same trend is seen for 15-21h fasting

($RWL_{V15\text{-}21h}$=0.363 g/h, $RWL_{NL15\text{-}21h}$=0.38 g/h, $RWL_{L15\text{-}21h}$=0.54 g/h). The difference in RWL between non-lactating and virgin mice at 15-21h fasting was not significant (p_{NLvsV}=2.88E-01). Differences in RWL between lactating and virgin mice ($p_{LvsV15\text{-}21h}$=1.21E-04) and between lactating and non-lactating mice ($p_{LvsNL15\text{-}21h}$=2.55E-04) were significant.

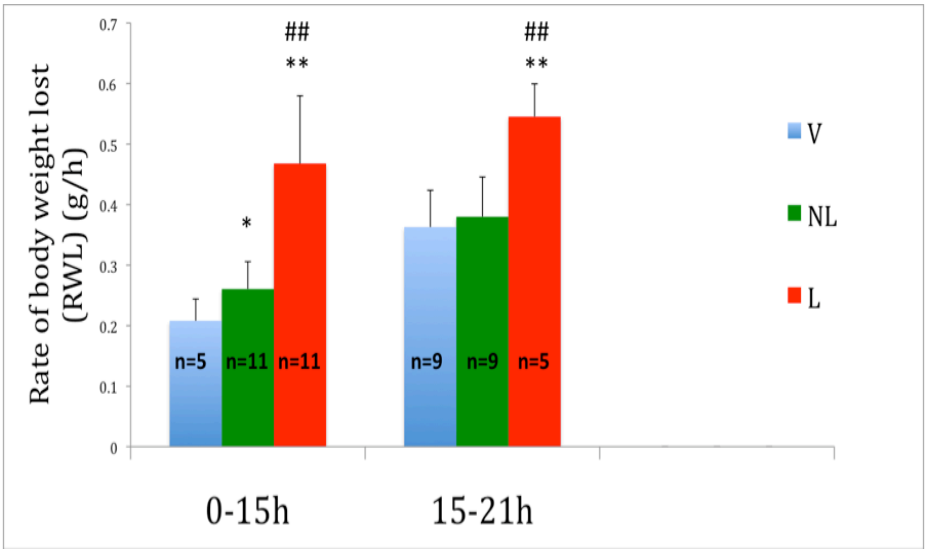


Fig 2.3: Rate of body weight lost (RWL) increases with lactation
Age-matched virgin (V, blue bars, $n_{0\text{-}15h}$=5, $n_{15\text{-}21h}$=9), non-lactating (NL, green bars, $n_{0\text{-}15h}$=11, $n_{15\text{-}21h}$=9) and lactating (L, red bars, $n_{0\text{-}15h}$=11, $n_{15\text{-}21h}$=5) mice were weighed at different fasting time points (0 h, 15 h, 18-21 h) on the day of sacrifice. Rate of body weight lost (RWL) at the 0-15h fasting time point was calculated by dividing the amount of weight lost (maternal whole body weight at 0 h fasting – maternal whole body weight at 15 h fasting) by 15 h. RWL for 15-21h time point was calculated by dividing the amount of whole body weight lost (maternal whole body weight at 15 h fasting – maternal whole body weight at 18-21 h fasting) by 4.5 h (the average number of hours between 15 h time point and 18-21 h time point). Average RWL for animals per group were subjected to the student T-test in order to determine the significance in the differences between groups: *=NL vs V or L vs V; #=L vs NL; * or #, p<5.00E-02; ** or ##, p< 5.00E-04.

Lactation affects plasma glycerol levels

Glycerol release is a marker of adipose tissue lipolysis (Judd et al. 1998). Previous animal and human *in vivo* studies have quantified the appearance of glycerol in the circulation as an index of fat mobilization (Issekutz, Shaw, and

Issekutz 1975; Bougneres et al. 1982; Beylot et al. 1987; Klein et al. 1986). In order to determine if the weight loss that occurred during lactation in our study was due to lipolysis of fat stores (adipocytes), we quantified plasma glycerol concentrations in virgin, non-lactating, and lactating mice (Fig 2.4). Plasma glycerol levels increased significantly with lactation compared to virgin (p_{LvsV}= 1.78E-03) and non-lactating levels (p_{LvsNL}= 5.52E-03). There is also a strong correlation between percent weight lost (PWL) at 15-h fasting and plasma glycerol concentrations of virgin, non-lactating, and lactating mice (Fig 2.5a, R^2=0.96635). This correlation holds true for the percent weight lost (PWL) at 18-21 h fasting time point as well (Fig 2.5b, R^2=0.964).

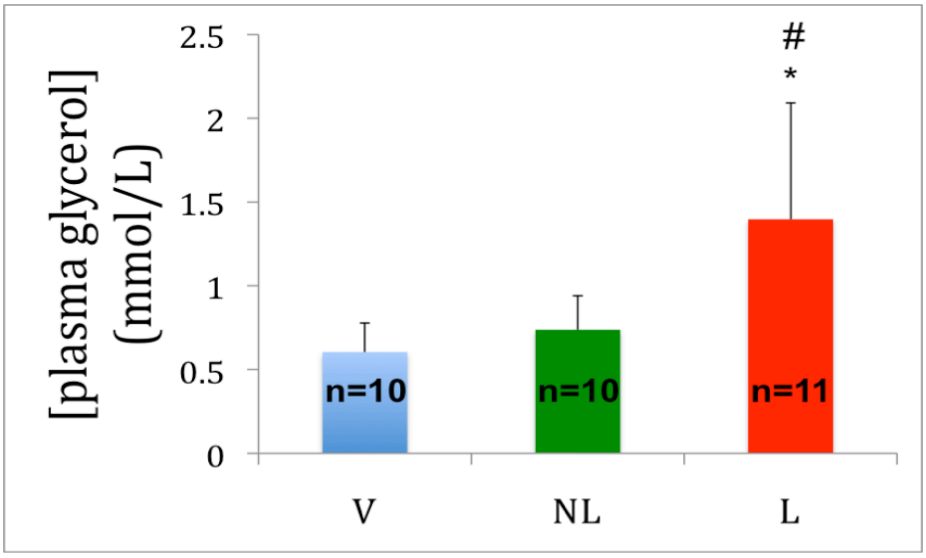
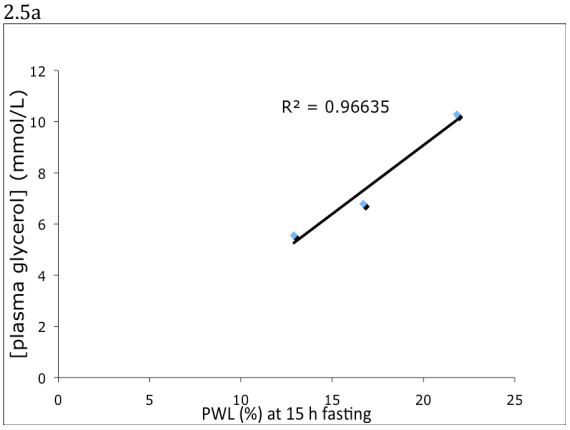


Fig 2.4: Plasma glycerol levels were significantly increased with lactation
Plasma glycerol levels of virgin (V, blue bar, n=10), non-lactating (NL, green bar, n=10), and lactating (L, red bar, n=11) quantified using a triglyceride assay kit (Cat# ETGA-200, BioAssay Systems, Hayward, Ca) per the manufacturer's instructions. Average plasma glycerol levels for each group were subjected to the student T-test in order to determine the significance in differences between groups. *=NL vs V or L vs V; #=L vs NL; * or # , p<5.00E-02.

2.5a



2.5b

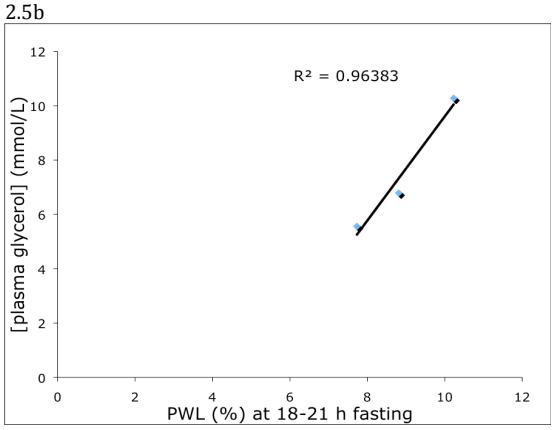


Fig 2.5 Strong correlation between percent of body weight lost (PWL) during fasting with increased plasma glycerol. Average PWL of age-matched virgin (n= 5), non-lactating (n=11) and lactating (n=11) mice were plotted against average plasma glycerol levels of virgin (n= 5), non-lactating (n=10), and lactating (n=11) mice. Linear regression was used to quantify the correlation between these factors. Panel 2.5a. Average PWL data at 15-h fasting time point are plotted against average plasma glycerol levels (refer to Fig 2.4 for details). Panel 2.5b. Average PWL data at 18-21 h fasting time point are plotted against average plasma glycerol levels. Please refer to Fig 2.2 and Fig 2.4 for PWL and plasma glycerol levels details.

Lactation affects maternal liver weight

In order to determine whether lactation had an effect on the maternal liver, whole livers of virgin (V), non-lactating (NL), and lactating (L) mice were weighed and recorded on the day of sacrifice after 21h of fasting. Average whole liver weights (WLW) were plotted in Fig 2.6a. Whole liver weights were normalized to maternal whole body weights in order to obtain the liver to body weight ratio (LBWR) (Fig 2.6b). Liver weights of virgin and non-lactating mice were equivalent (V_{WLW}=0.97g, NL_{WLW}=0.91g). Livers from lactating mice had a significantly greater mass than livers from virgin mice (L_{WLW}=1.5 g, L_V=0.97 g, p_{LVSV}= 7.98E-06) as well as from non-lactating mice (L_{WLW}=1.5 g, L_{NL}=0.91 g p_{LvsNL}= 1.41E-06). This was true

even when whole liver weights were normalized to maternal whole body weights (Fig 2.6b).

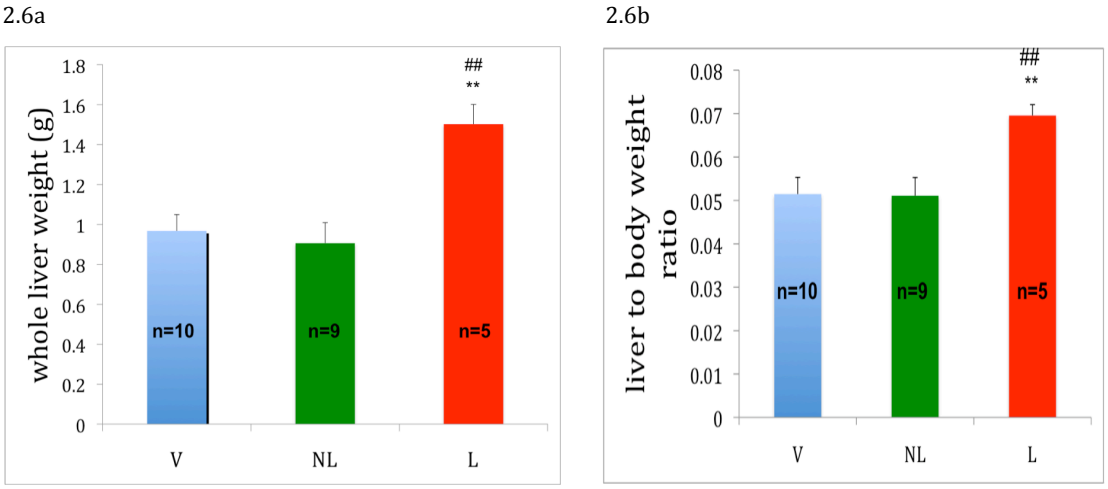


Fig 2.6: Lactation increases maternal whole liver weight
Panel 2.6a: Livers of age-matched virgin (V, blue bars, n=10), non-lactating (NL, green bars, n=9) and lactating (L, red bars, n=5) mice were harvested and weighed at sacrifice (18 to 21h fasting). Panel 2.6b: Maternal liver weights (as in panel 2.4a) were normalized to maternal whole body weights in order to obtain liver to body weight ratios of V, NL and L mice. Average liver weights and liver to body weight ratios were subjected to the student T-test in order to determine the significance of differences between groups of animals.

Trends in blood glucose levels

In order to assess whether lactation affects overall maternal health, we measured blood glucose levels of virgin, non-lactating and lactating mice (Fig 2.7). As the data in Fig 2.7 show, there was no discernable change in blood glucose levels for the three groups of mice since the differences in blood glucose levels were not statistically significant ($p_{NL\ vs\ V}$=0.14, $p_{L\ vs\ V}$=0.22, $p_{L\ vs\ NL}$=0.36).

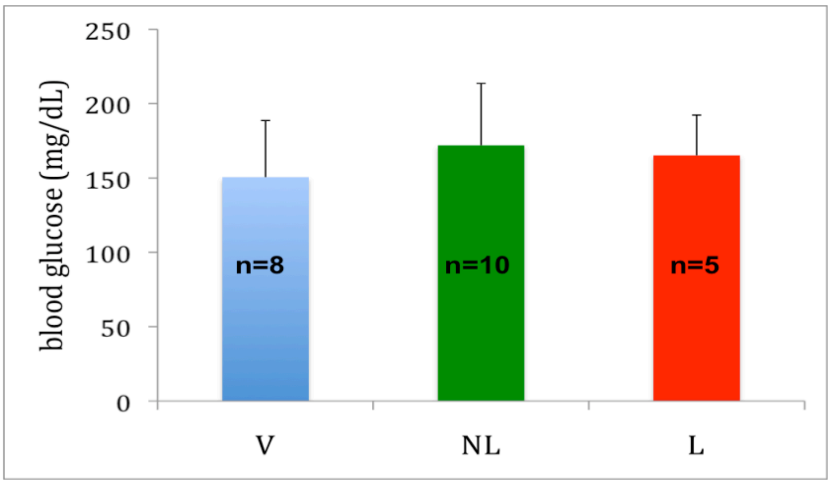


Fig 2.7 Blood glucose levels increase with non-lactation
Blood glucose levels of virgin (V, blue bar, n=8), non-lactating (NL, green bar, n=10), and lactating (L, red bar, n=5) were quantified using a glucose assay kit (Cat # K606-100, BioVision, Mountain View, Ca) per the manufacturer's instructions . Average blood glucose levels for each group were subjected to the student T-test in order to determine the significance in differences between groups. *=NL vs V or L vs V; #=L vs NL; * or # , p<5.00E-02; ** or ##, p< 5.00E-04;
*** or ###, p< 5.00E-08.

Trends in hepatic triglyceride levels

Hepatic fat accumulation (fatty liver) results in insulin resistance (Adams and Angulo 2005; Yki-Jarvinen and Westerbacka 2005). Fatty liver has also been associated with cardiovascular disease and type 2 diabetes (Targher et al. 2007; Edens, Kuipers, and Stolk 2009; Kotronen et al. 2008). For these reasons, we looked at hepatic triglyceride levels of virgin, non-lactating, and lactating mice (Fig 2.8). Hepatic triglyceride levels were significantly decreased with lactation (p_{LvsV}=9.07E-05, (p_{LvsNL}=7.65E-04). Since there was no significant difference in hepatic triglyceride levels between non-lactating and virgin mice, the drop in hepatic triglyceride levels must be due to lactation and not due to pregnancy and/or parturition. Liver Oil-Red-O staining confirmed the hepatic triglyceride

quantification data (Fig 2.9). Oil Red O is a lysochrome, a fat soluble dye used for staining neutral triglycerides on frozen sections. Overall, Red staining reflected hepatic fat content, although the lipid was present in different compartments in the virgin versus non-lactating group, in the sinusoids of the virgin animals and in lipid droplets in the non-lactating animals. Liver tissue from lactating mice were negative for Oil Red O staining.

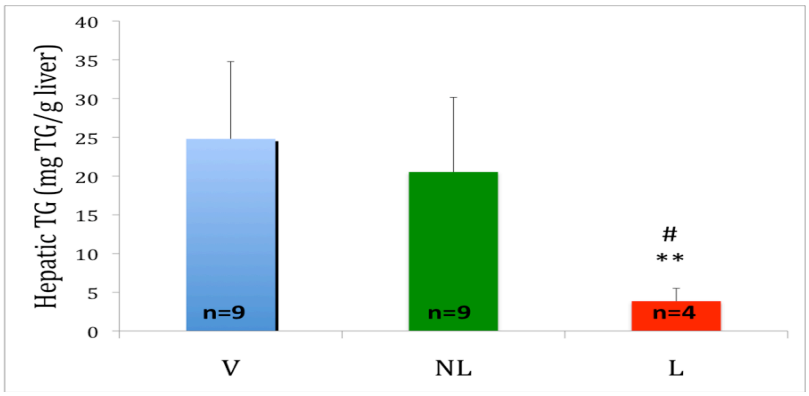


Fig 2.8: Hepatic triglyceride levels were significantly decreased with lactation
Triglycerides (TG) from livers of virgin (V, blue bar, n=9), non-lactating (NL, green bar, n=9), and lactating (L, red bar, n=4) mice Average TG levels for each group were subjected to the student T-test in order to determine the significance in differences between groups. *=NL vs V or L vs V; #=L vs NL; * or # , p<5.00E-02; ** or ##, p< 5.00E-04.

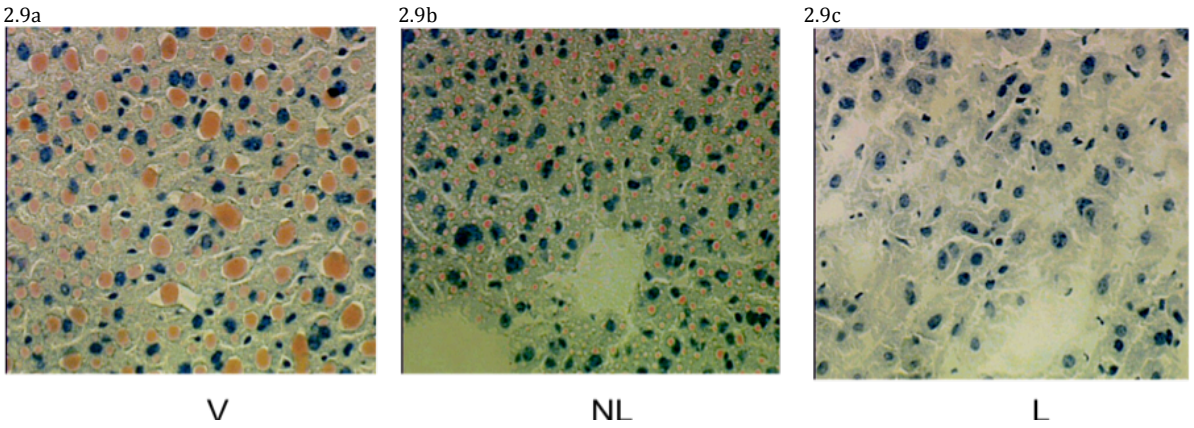


Fig 2.9 Lactation decreased hepatic fat content
Hepatic fat content of virgin (V n=2), non-lactating (NL, n=2), and lactating (L, n=2) were visualized by Oil Red O staining. Images shown are representative of two tissue samples per animal group. See Materials and Methods for further details.

Trends in plasma triglyceride levels

Plasma triglyceride levels are another important health parameter. Several studies have shown that fasting circulating triglyceride levels is a marker for metabolic syndrome (Hokanson and Austin 1996; Lahdenpera et al. 1996; Gaziano 1999; Kompoti et al. 2006). For this reason, we quantified plasma triglyceride levels of fasted virgin, non-lactating, and lactating mice (Fig 2.10). Plasma triglyceride levels significantly increased with lactation (p_{LvsV}=1.31E-05, p_{LvsNL}=1.04E-05). Plasma triglyceride levels of virgin mice were similar to circulating triglyceride levels of non-lactating mice (p_{NLvsL}=3.7E-01).

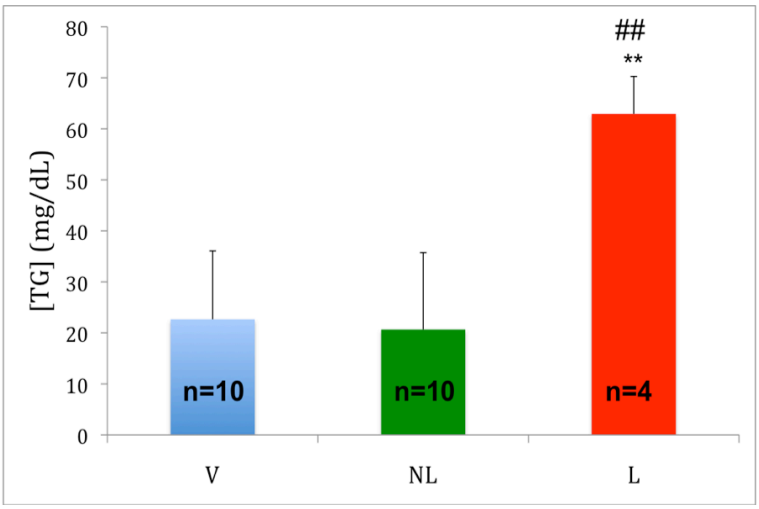


Fig 2.10 Plasma Triglyceride levels significantly increased with lactation
Plasma triglyceride (TG) levels of virgin (V, blue bar, n=10), non-lactating (NL, green bar, n=10), and lactating (L, red bar, n=4) mice were quantified from tail vein plasma. Average plasma TG levels for each group were subjected to the student T-test in order to determine the significance in differences between groups. *=NL vs V or L vs V; #=L vs NL; * or # , p<5.00E-02; ** or ##, p< 5.00E-04.

HNF4α protein increases postpartum

HNF4α plays a major role in adult liver functions including lipid metabolism. HNF4α has also been linked to diseases such as diabetes. We looked at HNF4α protein levels in order to determine whether lactation affects the expression of this liver enriched transcription factor. HNF4α protein was quantified in nuclear extracts prepared from V, NL, and L livers by competitive ELISA (H4 ELISA, Camba-Colón 2005) (Fig 2.11). HNF4α protein expression increased significantly with non-lactation ($HNF4\alpha_V$=1.21 ng/μg, $HNF4\alpha_{NL}$=3.15 ng/μg, p_{NLvsV}= 1.60E-03). HNF4α protein expression also increased significantly with lactation ($HNF4\alpha_L$=3.14 ng/μg, p_{LvsV}=1.53E-02). There was no significant difference in nuclear HNF4α protein concentration between non-lactation and lactation (p_{LvsNL}=4.92E-01) suggesting that the increase was the result of pregnancy and/or parturition rather than lactation.

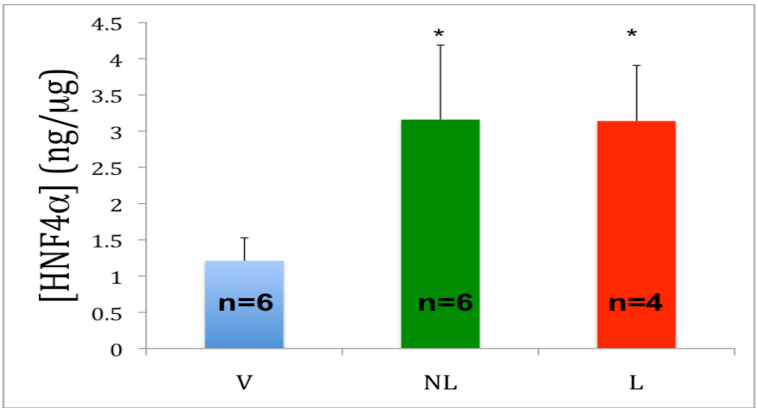


Figure 2.11: HNF4α protein expression increases postpartum
HNF4α protein was quantified from nuclear extracts (NE) prepared from virgin (V, blue bar, n=6), non-lactating (NL, green bar, n=6), and lactating (L, red bar, n=6) maternal livers by H4 ELISA (Camba-Colón, 2005). Representative H4 ELISA. Average HNF4α concentrations were subjected to the student T-test in order to determine the significance in differences between groups. *=NL vs V or L vs V; #=L vs NL; * or # , p<5.00E-02.

HNF4α gene expression increases postpartum

In order to determine whether lactation affects HNF4α expression at the transcription level, we quantified HNF4α mRNA by real time PCR (qPCR) (Fig 2.12). As seen in Fig 2.12, HNF4α mRNA expression increased significantly with both non-lactation ($HNF4\alpha_V$=1, $HNF4\alpha_{NL}$=1.86, p_{NLvV}=1.05E-03), and lactation ($HNF4\alpha_L$=, p_{LvsV}=6.78E-02) compared to virgin mice.

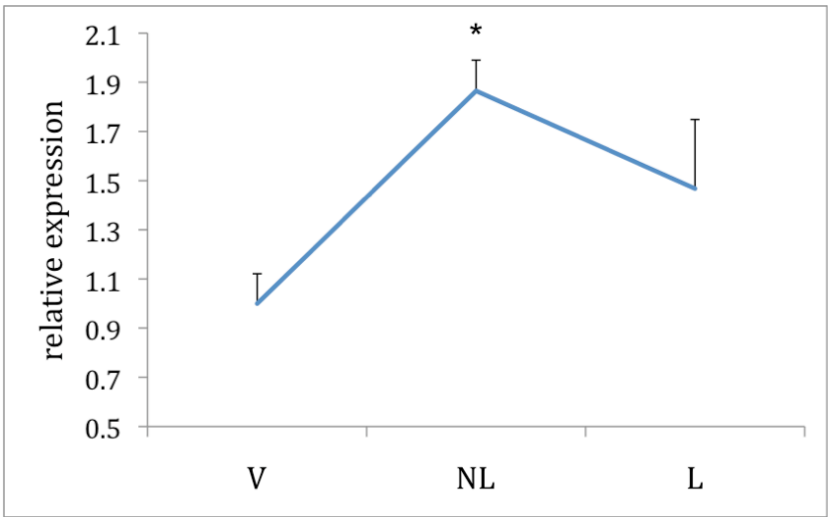


Fig 2.12: HNF4α mRNA expression increased postpartum
HNF4α mRNA expression was quantified from virgin (V, n=4), non-lactating (NL, n=4), and lactating (L, n=4) maternal livers by real time polymerase chain reaction (qPCR). Average HNF4α mRNA expression for each group were subjected to the student T-test in order to determine the significance in differences between groups. *=NL vs V or L vs V; #=L vs NL; * or # , p<5.00E-02; ** or ##, p< 5.00E-04; *** or ###, p< 5.00E-08.

Discussion

Lactation is the most energetically demanding period in the life cycle of female mammals (Thompson and Nicoll 1986; Naya et al. 2008). The glucose needs of the mammary gland are greater during lactation (Bauman and Currie 1980; Burnol et al. 1986). The lactating mammal has made several adaptations in order to meet this need. Increased food intake, for example, is a common characteristic of lactation in all mammals (Dunphy, Snell, and Clegg 1992; Speakman and Krol 2005) during this highly demanding period. As a response to the increased food intake, the lactating female's intestines, stomach, and liver increase in size (Naya et al. 2008; Barber et al. 1990). This is an example of phenotypic plasticity. Overeating, however, may not by itself be sufficient to meet the energy needs of the lactating mammary gland if food is limiting. Also occurring are changes in the metabolic activities of the mammary gland and other tissues such as adipose tissue and the liver (Vernon 2005; Bell and Bauman 1997). The net result is a change in the allotment of nutrients to the mammary gland, and also the amassing of stored energy in adipose tissue and the liver (McNamara 1997).

Effects of Lactation

Our results show a significant increase in whole body weight in lactating mice, compared to virgin and non-lactating mice (Fig 2.1). This could be explained by overeating in response to lactation. At first glance, the differences in whole body weights between virgin, non-lactating, and lactating mice may not point to lactation

as being beneficial to lactating females in terms of weight loss, because clearly, the lactating mouse is heavier than both virgin and non-lactating mice, and, non-lactating mice lost weight compared to virgin mice. Upon closer inspection, however, it seems that lactation is beneficial to the fasted, lactating mouse. Our experiment enabled us to monitor weight loss in our mice during a 24-hour period of lactation and fasting. Although the percent of body weight lost (PWL, Fig 2.2) significantly decreased in non-lactating mice at the 15-h fasting point, ($p_{NLvsV15h}$=1.08E-02), the increase of PWL in lactating mice was far more significant at this fasting time point ($p_{LvsV15h}$=7.06E-05, $p_{LvsNL15h}$=2.35E-03). The rate of weight lost (RWL, Fig 2.3) at the 15-h fasting time point was also greatest in lactating mice. This was also true of RWL at the 18-21 h fasting time point. The weight loss apparent with lactation can be explained by the mobilization of fat stores in order to meet the energy needs of the mammary gland. Animal studies have shown that the loss of fat stores (lipolysis) during lactation is common in mammals (Robinson 1986; Rauw et al. 2003; Hood, Oftedal, and Kunz 2006; Oftedal 2000). For example, lactating female rodents suckling many litters, gain body mass, probably from fat stores, during early lactation, and lose body mass later in lactation, in order to augment consumption when demands of the litter are highest (Hood, Oftedal, and Kunz 2006). Rauw et al (Rauw et al. 2003) in particular have shown that the absolute lipid mass and lipid percentage were higher in virgin females than in lactating female mice. This would explain the rapid weight lost during lactation in our experiment. Our data confirm findings of other studies that suggest the

mobilization of body stores during lactation (Rogowitz 1998; Hood, Oftedal, and Kunz 2006). Other studies have shown enhanced lipolysis in adipocytes in order to meet the energy demands of lactation (Vernon 1989; Aitchison, Clegg, and Vernon 1982; Sumner and McNamara 2007; McNamara 1997). A marker of increased lipolysis is the presence of glycerol in the circulation. Although we did not specifically measure the fat content of these animals, our data show a significant increase in glycerol plasma levels during lactation (Fig 2.4). In addition, there is a strong correlation between the percent of body weight lost during lactation and the increase in plasma glycerol levels (Fig 2.5). Taken together, this suggests that in order to meet the energy demands of lactation, the lactating mammal mobilizes the maternal lipid stores she accumulated during her pregnancy by increasing lipolysis of adipocytes. A benefit of this adaptation is the rapid and significant weight loss that occurs during lactation, as evidenced by the decrease in maternal whole body weights at different fasting time points for all groups of mice (Fig 2.2, Fig 2.3) (for comment a19).

We also observed evidence of phenotypic plasticity during lactation, as seen in the significant increase in whole liver weight (WLW_V=0.97 g, WLW_{NL}=0.91 g, WLW_L=1.50 g, p_{LvsV}=7.98E-06, p_{LvsNL}=1.41E-06) and liver to body weight ratio ($LBWR_V$=0.05, $LBWR_{NL}$=0.05, $LBWR_L$=0.07, p_{LvsV}=1.34E-07, p_{LvsNL}=1.44E-07) during lactation. The increase in liver size is in response to the increase in food demands in order to meet the energy demands of lactation (Naya et al. 2008). Our data

confirm the findings of other studies (Derting and Compton 2003). The increase in liver weight is most likely a consequence of hypertrophy (enlargement of an organ due to an increase in cell size), as opposed to hyperplasia (enlargement of an organ due to an increase in the number of cells) as Dunphy et al (Dunphy, Snell, and Clegg 1992) have demonstrated that hepatic proliferation does not occur during lactation. Rather, the increase in liver size is an indication of a metabolically active organ responding to the energy demands of lactation (Naya et al. 2008; McBride and Kelly 1990; Wang, Busk, and Overgaard 2001). Canas et al (Canas, Romero, and Baldwin 1982) report that there is a rise in metabolism in organs such as the liver during lactation, which is mostly due to an increase in organ weight with higher maintenance costs. Other contributors to consider in the liver weight increase would be liver growth factors such as Growth Hormone (GH) and Insulin-like Growth Factor 1 (IGF-1).

Lactation and Maternal Health

Lactation has been shown to bring about changes to the nursing mother that result in lowering the incidence of unhealthy conditions.(for comment pc21). In order to study the effects of lactation, we compared maternal adaptations in age-matched, fasted, virgin, non-lactating, and lactating mice. Although the non-lactating mice were not lactating during our experiment, they had a history of lactation (since they previously lactated).

In order to determine whether lactation affects the health of the lactating mother, we looked at several markers of overall health: plasma glucose levels (Fig 2.7), hepatic triglyceride levels (Fig 2.8, Fig 2.9), and plasma triglyceride levels (Fig 2.10). Our data did not show any significant differences between plasma glucose levels in virgin, non-lactating, and lactating mice. In contrast, hepatic triglyceride levels decreased dramatically and significantly with lactation (Fig 2.8, Fig 2.9). At first glance, the decrease in hepatic triglycerides indicates a positive effect of lactation on maternal health, since it suggests that lactation may decrease the incidence of fatty liver. In our system, however, both groups of postpartum mice (lactating and non-lactating) previously lactated. Our data therefore suggest that this "health benefit" is only apparent in currently lactating animals, and may not be a long term benefit after all since the non-lactating animals have higher hepatic triglyceride storage.

Plasma triglyceride levels, on the other hand, significantly increased with lactation (Fig 2.10). The increase in plasma triglyceride levels could be a result of increased adipocyte lipolysis. The increased plasma glycerol levels (Fig 2.4) during lactation indicates an increase in lipolysis (Judd et al. 1998). Previous studies have shown an increase of adipocyte lipolysis in order to provide for the demands of lactation (Vernon 1989; Aitchison, Clegg, and Vernon 1982; Sumner and McNamara 2007; McNamara 1997). An increase in lipolysis then leads to an increase of free fatty acids available for hepatic lipogenesis and the lipids are released in the circulation as very low density lipoproteins (VLDL, triglycerides) (Vernon 2005).

Studies have shown that there is an increase in hepatic lipogenesis during lactation (Grigor et al. 1982; Williamson et al. 1983; Casey et al. 2009). Thus, the high energy demands of lactation causes a mobilization of fat stores (increased lipolysis), the consequences of which include significant weight loss and an increase in the lipolytic marker, glycerol in the circulation. Lipolysis then increases free fatty acids, which serve as substrates for hepatic triglyceride synthesis, the product of which are triglycerides. The liver then packages the triglycerides (VLDL), which are secreted into the circulation. This would explain the low hepatic triglyceride levels during lactation, and also the high plasma triglyceride levels that occur at this time. The time point we have chosen is peak lactation, when the demand for lipids is presumably the greatest. The mice have also been constitutively lactating, up to the time of sacrifice. High plasma triglycerides may be a manifestation of this energy source being made available for the mammary gland.

Most of the changes we have observed are beneficial to the lactating mother: significant weight loss and no evidence of fatty liver (compared to non-lactating mice). Although these beneficial changes benefit the lactating mother, in mice, the benefits do not seem to be long-term. The previously lactating non-lactating mice had the opposite trends of less weight loss and increased hepatic storage. The elevated plasma triglycerides that accompany lactation are a reflection of the lactating mother's attempt to keep up with the energy demands of the mammary gland.

Although elevated triglyceride levels are considered an unhealthy marker, elevated triglyceride levels seem to be due to the increased hepatic lipogenesis and increased lipolysis going on in adipocytes. A close inspection of the liver tissue sections did not show evidence of inflammation (personal communication with Dr. Walker). Inflammation would accompany certain diseases such as atherosclerosis , although this would not be in the liver (Libby 2002). Our experiment, however, may suggest that in terms of plasma triglyceride levels, lactation may have a long-term benefit after all, since the previously lactating (and now non-lactating) mice had lower plasma triglyceride levels compared to the currently lactating mice, although the non-lactating plasma triglyceride levels are similar to the virgin animals.

The Liver, Lactation, and HNF4α

In order to understand the role of the maternal liver in lactation, we looked at the liver enriched transcription factor, HNF4α. A member of the nuclear receptor superfamily, HNF4α plays an important role in adult liver functions such as lipid metabolism and lipid homeostasis. It has also been linked to diseases such as obesity, atherosclerosis, and diabetes (Petrescu et al. 2002; Gupta and Kaestner 2004; Crestani et al. 2004). Given that changes in liver function are important in both pregnancy and lactation (Vernon 2005) and that HNF4α plays an important role in adult liver function, it is conceivable that HNF4α may also play a role in changes in response to lactation. For example, HNF4α regulates hepatic glucose metabolism and also the transport of cholesterol and triglycerides throughout the

body (Sladek and Seidel 2001; Bolotin, Schnabl, and Sladek 2009). The liver is also an important source of lipids during lactation (Vernon 2005) , and HNF4α controls hepatic lipid metabolism and transport (Xie et al. 2009; Rhee et al. 2006; Bolotin et al. 2009). We quantified HNF4α protein in nuclear extracts prepared from virgin, non-lactating, and lactating mouse livers by a competitive ELISA (H4 ELISA, Camba-Colón 2005). We also performed qPCR to examine the level of HNF4α mRNA. Our findings show a significant increase in HNF4α expression postpartum both at the level of protein and the mRNA (Fig 2.11, Fig 2.12). However, there was no significant difference in the levels of HNF4α between lactating and non-lactating mouse livers. Our qPCR result for HNF4α points to a trend of a decrease in HNF4α levels in lactating livers, which might lead to a decrease in HNF4α protein levels at a later time point after lactation. Our data show that a long term effect of lactation may be an increase in HNF4α expression, since this is the trend in our non-lactating mice. Further studies need to be done on pregnant, but never lactated mice in order to understand the role HNF4α may play in lactation.

Conclusions:

We have looked at physiological changes that occurred during peak lactation in mice in order to examine the role of the maternal liver during this important process. We saw an increase in maternal whole body weight and an increase in maternal whole liver weight during lactation. Although overeating accompanies lactation in mammals, the lactating animal needs to modulate its metabolism in order to meet the nutrient and energy needs of the mammary gland. In our experiment, we see rapid weight loss, exhibiting a significant change in maternal whole body weight of fasted lactating mice in a 24-hour period. This could be explained by the utilization of maternal stores, such as maternal lipids, in order to sustain lactation. The increase in maternal whole liver weight during lactation also reflects the increased metabolic activity of the liver during lactation. Other maternal adaptations in response to lactation include an increase in plasma glycerol, a decrease in the incidence amount of fat stored in the liver, and an increase in plasma triglyceride levels. These adaptations are a consequence of the increased mobilization of fat stores (adipocyte lipolysis) and hepatic lipogenesis that occur as a means to meet the increased metabolic demands of lactation. Finally, there is the trend of an increase in HNF4α gene expression postpartum which calls for more studies in pregnant but never lactated mice in order to investigate the possible role HNF4α may play in lactation.

There is a growing body of evidence that points to lactation as having beneficial effects against maternal metabolic syndrome. Although in our system, it would

seem that the benefits of lactation only apply in currently lactating mice. The maternal metabolic adaptations in response to lactation need to be looked at more closely. A closer examination of the lactating liver transcriptome will help us understand the role of the maternal liver during lactation, and, in the process, help us understand the maternal and metabolic changes (maternal adaptations) we observed in currently lactating mice, including a possible role of the liver enriched transcription HNF4α as well.

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Chapter 3:

Expression Profile Analysis of Maternal Liver Genes During Lactation

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Introduction

Pregnancy and lactation

The ultimate goal of reproduction (pregnancy and lactation) is the survival of reproductively viable offspring. This goal brings about many maternal adaptations during reproduction. For example, the acquisition of fat stores during pregnancy, as well as the use of such fat stores during lactation and mammary gland development (mammogenesis), milk synthesis and secretion (lactogenesis 1 and 2) all occur before, during, and after pregnancy. These adaptations continue well beyond gestation and continue postpartum with lactation. Hence, maternal adaptations during reproduction place high demands on maternal organs, the liver in particular.

The liver and metabolic syndrome

The liver acts as a detoxification center and plays an essential role in metabolism for both the mother and her offspring. The high physiologic and metabolic demands of reproduction can sometimes bring about diseases during pregnancy, such as gestational diabetes, acute fatty liver of pregnancy, and pre-eclampsia to name a few. Aspects of these diseases involve liver functions and diseases which manifest during pregnancy, may be harbingers of later susceptability to metabolic syndrome. Metabolic syndrome is a cluster of risk factors linked to diabetes and cardiovascular

disease (Ginsberg and Stalenhoef 2003; Alexander et al. 2003; Chan, Barrett, and Watts 2004). Studies have shown that lactation offers benefits the health of both mothers and their breast-fed offspring. For example, lactation ameliorates maternal diabetes (Stuebe *et al.* 2005) and lowers the incidence of maternal metabolic syndrome in menopausal women (Gunderson 2008; Ram et al. 2008; Schwarz et al. 2009).

Bioinformatics

We have laid the groundwork to understand the role of the maternal liver in lactation. Against this backdrop, broad strokes if you will, we begin the next step: adding details to our picture. We will use bioinformatics techniques to analyze microarray data (global gene expression profiling). Global gene expression profiling has been instrumental in increasing our understanding of the transcriptional basis of complex biological systems. It has allowed researchers to provide molecular signatures of diverse complex biological phenomena such as aging, reproduction, and growth and metabolism (Sharov *et al.* 2008; Helguera *et al.* 2009; Gleason *et al.* 2010). There is a growing body of evidence using gene expression profiling aimed at understanding the regulation of lactation, although most of the research has focused on bovine mammary gland (Lemay et al. 2007; Bionaz and Loor 2008; Ramanathan et al. 2008) and bovine liver (Loor *et al.* 2005; Wathes *et al.* 2007). Although there are some similarities between cows and humans (Elsik *et al.* 2009), the regulation of lactation is different. The mouse offers easy handling and quick

turnaround on processes such as lactation, which still make it an attractive and powerful experimental subject even though it also differs from the human in aspects of its reproductive physiology. To our knowledge, there are very few papers that examined molecular signatures of the mouse lactating liver, and none at the beginning of this project. Rudolph et al (Rudolph *et al.* 2007) used global expression profiling to examine lipid synthesis in the lactating mouse mammary gland and compared this to the lactating mouse liver, with an emphasis on the mammary gland. He et al. (He *et al.* 2007) used global expression profiling to examine the xenobiotic functions of the rat liver during pregnancy and lactation. In contrast, our study uses expression profiling of the liver to examine molecular signatures during peak lactation (day 10-12 postpartum) in the lactating mouse and compares those to livers from non-lactating (10-12 d postpartum) and age-matched virgin controls.

Goal of this study

The goal of this study is to examine the role of the liver in facilitating the additional demands of lactation. Transcription factors are vital in liver function, a close examination of liver-enriched transcription factor gene profiles is therefore warranted. Reproduction is regulated by hormones and growth factors, so we will look closely at hormone and growth factor receptor gene expression profiles. Since lipids are an important component of breast milk, we will be looking at genes involved in lipid production, lipid transport, and lipid secretion. Lipid homeostasis also plays an important role in diseases such as diabetes. Taken together, gene

expression profiles of these groups of genes may help us understand the role of the liver in lactation, and also the protective role that lactation plays against metabolic syndrome in the mother.

Materials and Methods

Animal care and treatment

Care and treatment of experimental animals was in accordance with guidelines from the University of Calfornia, Riverside, Institutional Animal Care and Use Committee (IACUC). Female, age-matched, virgin and timed pregnant C57Bl/6 mice were purchased from Charles River Laboratories (Charles River Laboratories, Wilmington, MA). All timed pregnant mice went through one successful round of reproduction (pregnancy, lactation, weaning) before mating for a second round. At parturition, 12 dams were allowed to lactate for 10-12 days postpartum (lactating) and pups were adjusted to 6 pups/lactating dam at parturition. For the pregnant but non-lactating group (n=12), all pups were taken away at parturition. Each lactating dam (L) was housed separately with 6 suckling pups per cage. Non-lactating dams (NL) were also housed separately at parturition. Virgin mice (V) were housed 5 mice per cage. All mice were kept in a temperature-controlled room, exposed to 12 h light cycle, 12 h dark cycle, and fed a regular chow diet (Harlan Teklad, TD7012; Harlan Teklad, Madison, WI).

Fasting, whole body weights, and blood collection

In order to control for the effects that diet may have on lactation, each mouse (with suckling pups when applicable) was fasted at 18-21 h before sacrifice in the following manner. Each mouse (and their suckling pups when applicable) was transferred to a new cage without food. Virgin mice (5 per cage) were also

transferred to a new cage. The bedding of each cage was changed to Sani-Chips (cat # 7090, Harlan Teklad, Madison, WI), non-edible wood chips that do not provide a source of nutrients as does standard bedding. All mice were allowed a bottle of water during the fast. At around 15 h post fasting, (9:00 a.m. the next day) whole body weights were recorded for each mouse. Mice in their cages were then placed under a 60-watt heat lamp for 10 minutes. Each mouse was restrained, and their tails were nicked. Whole blood was then collected in two heparanized hematocrit tubes (Fisher Scientific, catalog # 21-176-6) per animal. Whole bloods were spun (microcentrifuge specifics) for 10 min at 4^{o}C. Blood plasmas (supernatants) were collected, transferred to new Eppendorf tubes, and stored at -20^{o}C. After blood collection, mice were returned to their cages and allowed to recover before being sacrificed at around noon (18-21 h total fasting).

Tissue harvesting

Mice were euthanized by CO_2 exposure for 40 seconds. Whole body weights were recorded and tissues were harvested in a cold room (4^{o}C). The portal artery was cut and allowed to bleed before each liver was taken out and weighed. The largest liver lobe (lobe 1) was harvested and stored for the following assays: two samples (10-30 mg) for RNA isolation, one sample (100 mg) for triglyceride (TG) assay, one sample (100 mg) for sectioning, the rest of lobe 1 (300 mg), for storage. The remaining liver (lobes two-six; 200 – 300 mg) was chopped up with a razor blade and stored in two aliquots for nuclear extraction. All liver samples were stored in screw cap cryovials

(Nunc, cat # 12-565-170N, Thermo Fisher Scientific, Waltham, MA) in liquid nitrogen.

RNA Isolation

Liver chunks from each mouse (10-30 mg) ear-marked for RNA isolation were incubated overnight at 4°C in 500 μL RNALater (cat # AM7023, Ambion, Austin, TX), an RNA stablization solution. RNALater was pipeted out the next day, and the remaining liver chunk was stored at -80°C. RNA was then isolated from RNA-stabilized frozen liver chunks using the RNeasy mini kit (Qiagen, Valencia, CA). Briefly, each liver chunk was homogenized in complete cRLT buffer (1% beta mercaptoethanol in RLT buffer) in an 1.5 mL tube using a motor homogenizer until completely homogeneous. Homogenized lysates were centrifuged in room temperature at maximum speed (Eppendorf 5424,) for 2 min. to clear the lysate. The supernatant (cleared lysate) was transferred to a new 1.5 mL tube. An equal volume of 70% ethanol was added to the cleared lysate and immediately mixed by pipetting. Up to 700 μL cleared lysate in 70% ethanol was added to the spin column (spin columns come with collection tubes). Spin columns were then centrifuged at maximum speed for 15 sec at room temperature. The flow-through was discarded, and the remaining cleared lysate in 70% ethanol was added to the same spin column and centrifuged at maximum speed for 15 sec at room temperature, and the flow-through was discarded. The spin column was washed with Buffer RW1 by centrifuging at maximum speed for 15 sec at room temperature, and the flow-

through discarded. The same column was washed this time with 500 μL of Buffer RPE by centrifuging at maximum speed for 15 sec at room temperature, then discarding the flow-through. The same tube was washed a second time with 500 μL of Buffer RPE, this time centrifuging at maximum speed for 2 min at room temperature to dry the spin column. Spin columns were then centrifuged at maximum speed for 1 min at room temperature, this time using fresh collection tubes, to remove all ethanol. RNA was eluted by placing spin columns in fresh Eppendorf tubes and adding 50 μL RNAse free water then centrifuging at maximum speed for 1 min at room temperature. The flow-through (eluate) in the 1.5 mL tube is the RNA. A second elution was done by adding the eluate from the previous step to the collection tube and then centrifuged a final time at maximum speed for 1 min at room temperature. RNA concentration and quality were checked by nanodrop. RNA was stored at -80^{o}C.

Quantitative Real Time Polymerase Chain Reaction (qPCR)

Digestion of genomic DNA

Digestion reactions were prepared for each RNA sample as per the manufacturer's instructions (cat # 10 776 785 001, Roche Applied Science, Penzberg, Germany): DNAse I mastermix (1x final concentration of 10x Incubation buffer, 0.5 units final concentration of Dnase I recombinant RNase-free reagent for a total of 1.5 μL/reaction) was prepared for the appropriate number of reactions. RNA (final concentration of 0.3 mg/reaction) was added to 1.5 μL DNase I mastermix,

then adjusted with RNase-free water for a total volume of 10 μL. Each reaction was incubated at room temperature for 20 min. DNase inactivation reagent (2 μL) (AM1906, Ambion, Austin, Tx) was added to each reaction and incubated at room temperature for 2 min. Reactions were then spun at 15,000 RPM for 1.5 min at room temperature. Each supernatant (DNAse I-treated RNAs) were transferred to new 1.5-mL tubes and stored at -80°C.

cDNA preparation

cDNAs were prepared using the SuperScript™ III Synthesis System for RT-PCR (Invitrogen, Carlsbad, CA) as per the manufacturer's instructions. DNase I-treated RNAs were thawed on ice. The appropriate amount of primer mastermix (P-MM) (for one reaction: 1 μL 50 ng/μL random hexamers, 1 μL 10 mM dNTP mix) was prepared. DNase I-treated RNA (1 μg) was added to 2 μL of P-MM. Diethyl polycarbonate (DEPC)-treated water was added to each reaction tube for a total volume of 10 μL/reaction tube. Each reaction tube was then gently vortexed, then spun at 13,000 RPM at 4°C for 15 sec. All reaction tubes (RNA-P) were then incubated at 65°C for 5 min and placed on ice. The appropriate amount of cDNA synthesis mix master mix (cS-MM) was prepared (for one reaction: 10X RT buffer (2 μL), (4 μL) 25 mM $MgCl_2$, 0.1 M DTT (2 μL), RNaseoUTTM (1 μL, 40 U/mL), SuperScriptTM III reverse transcriptase (1 μL ,200 U/mL). cS-MM (10 μL) was added to each RNA-P mixture. All tubes were vortexed and spun 13,000 RPM at 4°C for 20 sec. All tubes were incubated in the PCR thermocycler with the following

settings: step 1 at 25°C for 10 min, step 2 at 50°C for 50 min, step 3 at 85°C for 5 min, and step 4 at 4°C. All samples were then taken out of the thermocycler and placed on ice. Samples were then centrifuged at 13000 RPM at 4°C for 20s. Rnase H (1μL) was added to each sample. All samples were gently vortexed then centrifuged at 13,000 RPM at 4°C for 20 sec, then incubated at 27°C for 20 min. These are the cDNAs. All cDNAs were diluted 1:10 with DEPC-treated water. Standards for quantitative real time PCR (qPCR) were prepared by pooling all cDNA samples (180 μL total volume). Individual cDNAs and pooled cDNA were stored at -20°C for future use.

Primer Design

Primers for mouse hepatocyte nuclear factor 4alpha (*HNF4α*), peroxisome proliferator –activated receptor gamma coactivator-1alpha (*Ppargc1a* or *Pgc1α*), cyclophilin A (*Ppia*), the long form of prolactin receptor (Prlr-L), and the 3 short forms of prolactin receptor (Prlr-S1, Prlr-S2, Prlr-S3) were designed by first going to Pubmed to identify the cDNA sequence of the genes (http://www.ncbi.nih.gov/entrez/query.fcgi). Exons were found by using the UCSC Genome database (http://genome.ucsc.edu). Specific primers for the gene in question were designed using the Primer3 program (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). This gave the melting temperature and product size of the primers. The specificity of the primers was checked by using the in silico PCR function in the UCSC Genome browser (http://www.genome.ucsc.edu).

Primers were further optimized by using the Scitools program on the IDT website (http://idtdna.com/Scitools/Scitools.aspx) to predict the hairpin structure, homodimerization ΔG and, heterodimerization ΔG. Primers were designed to go across two exons, have a product size <200 bp, homodimerization ΔG ≥ -10, and heterodimerization DG ≥ -10.

Table 1: Primers for genes used in real time PCR (qPCR). Primers for Hnf4a were modified from Briancon et al 2004. Primers for prolactin receptor isoforms (Prlr-L, Prlr-S1, Prlr-S2, Prlr-S3) were based on accession numbers from Fleenor et al 2006.

Gene	Accession number	product size (bp)	forward primer (5' to 3')	reverse primer (5' to 3')
Ppia	NC_000077	119	AGGGTGGTGACTTTACACGCCATA	ATTTGCCATGGACAAGATGCCAGG
Hnf4a	NM_008261	145	GAAAATGTGCAGGTGTTGACCA	AGCTCGAGGCTCCGTAGTGTTT
Ppargc1a	NM_008904	140	AATGCAGCGGTCTTAGCACT	ACGTCTTTGTGGCTTTTGCT
Prlr-L	BC005555	147	CCTGCATCTTTCCACCAGTT	AACTCCACCAGCAAGTCCTC
Prlr-S1	M22958	106	CAACTGTGTGGATCATTGTGG	GGTGGAAAGATGCAGGTCAT
Prlr-S2	M22959	136	CCCCTGACAAGGAAACATTC	GGGCCACTGGTTTTGTAGTC
Prlr-S3	M22957	122	TTAGCTCAGCCTCAGCCttc	CAGAAGCATGCAGAGGAACA

Quantitative real time PCR (qPCR)

Gene specific primers were validated before hand as previously described (Yuan *et al.* 2009). Briefly: Primers were validated over four orders of magnitude and analyzed with the iQ5 Optical System Software. Primer pairs were deemed valid if an input log plot amount versus CT generated an efficiency of 100% ± 10% and a correlation coefficient of R^2=0950 ±0.05. Each condition (virgin, non-lactating, lactating) had three biological replicates. Each biological replicate was done in triplicate (technical replicate). The relative expression level of the genes was

evaluated using the Pfaffl method (Pfaffl 2001), normalized to cyclophilipn A (*Ppia*) expression.

Microarray

Microarray study design

Three conditions were studied: virgin (V), non-lactating (NL), and lactating (L) as described in chapter 2. RNA samples isolated from mice were pooled and hybridized to Affymetrix MG_430A 2.0 chips as per the manufacturer's protocol in the Genomics Core at the UCR Institute for Integrated Genome Biology. Two microarray chips were used for each condition. Each virgin (V) array had 3 biological replicates/chip, for a total of 6 animals for this condition. Each non-lactating (NL) array also had 3 biological replicates/chip for, for a total of 6 animals for this condition. Each lactating (L) array had 2 biological replicates/chip for a total of 4 animals for this condition.

Microarray data analysis

Raw data from all 6 arrays were analyzed using GC Robust Multi-Array Average (GCRMA) background adjustment and quantile normalizaton on probe-level data sets with R software (http://www.bioconductor.org) by the lab's bioinformatics expert, Eugene Bolotin. Genes that were up regulated or down regulated at least 1.5-fold (L vs V) were looked at more closely. The gene sets were filtered further by eliminating genes whose non-log expression values were below 100.

Statistical Analysis

Statistical analysis was performed using the Student's t test. Differences were considered statistically significant at $p<0.05$. Uncertainty in our measurements was determined by calculalting the standard deviation.

Gene ontology (GO) analysis

Genes that were up regulated or down regulated at least 1.5 fold were further analyzed using the Database for Annotation, Visualization and Integrated Discovery (DAVID) (Huang da, Sherman, and Lempicki 2009; Dennis et al. 2003). Data are presented in a Functional Annotation Chart. The functional annotation chart lists annotation terms and their associated genes under study. Associated genes were subjected to Fisher Exact statistics to avoid over counting duplicated genes. This report includes percent genes and p value. Percent genes is calculated by dividing the genes identified for the arrays by the total number of genes in that category. P value is based on Fisher Exact statistics. The smaller the p value, the more overrepresented the gene is associated with the annotation term. Further analysis of annotated terms were done thru AmiGO (http://amigo.geneontology.org) (Blake and Harris 2008). Briefly, each annotated term in question (biological process) was uploaded to the AmiGO website and submitted under terms. This would list the term in question along with similar terms. The term is then viewed in a tree. The tree is similar to a family tree where the term in question is the child and is linked to

a parent term. A graphical view of the tree starts with the term in question (child), which is a part of the next term (parent) which is then a part of the next term (parent of the parent) and so on. The parent is a broader biological process, and the child is a specific example of the broad biological process. As is the case in biology, there is overlap within definitions of broad biological processes, and this results in linking a specific biological process to more than one broad biological process.

Results:

Experimental conditions

In order to study the role of the maternal liver in lactation, we studied age-matched mice that were virgin (V), non-lactating (NL), and lactating. Virgin mice were never mated. Non-lactating mice and lactating mice had two rounds of reproduction (complete pregnancy, complete lactation, weaning) before the current mating for this study. In order to normalize the mice and remove the confounding effects of diet, the mice were fasted.

Verification of microarray data for selected genes by real time PCR (qPCR)

The use of microarrays along with real time PCR have been shown to be powerful tools to identify and validate differentially expressed genes in diverse applications that range from disease models to parturition (Zou et al. 2002; Hassan et al. 2006; Rajeevan et al. 2001). In this study, we validated the microarray expression values of the liver enriched transcription factor hepatocyte nuclear factor 4alpha (HNF4α) (*Hnf4a*) mRNA and the transcriptional coactivator peroxisome proliferator–activated receptor gamma coactivator-1alpha (Pgc1α) (*Ppargc1a*) from the livers of virgin (V), non-lactating (NL), and lactating (L) mice by quantifying their mRNA expression by real time PCR (Fig 3.1). Both genes play important roles in liver function and metabolism (Hayhurst et al. 2001; Rhee et al. 2006; Rhee et al. 2003). *Hnf4a* gene expression was higher in the livers of non-lactating mice (NL) compared to virgin mice (V). *Hnf4a* gene expression was also higher in the livers of lactating

mice (L) compared to virgin mice, but was lower than non-lactating mice. *Ppargc1a* (PGC1α) had a similar expression profile: the gene was up regulated with non-lactation (compared to the virgin state) and up regulated less with lactation.

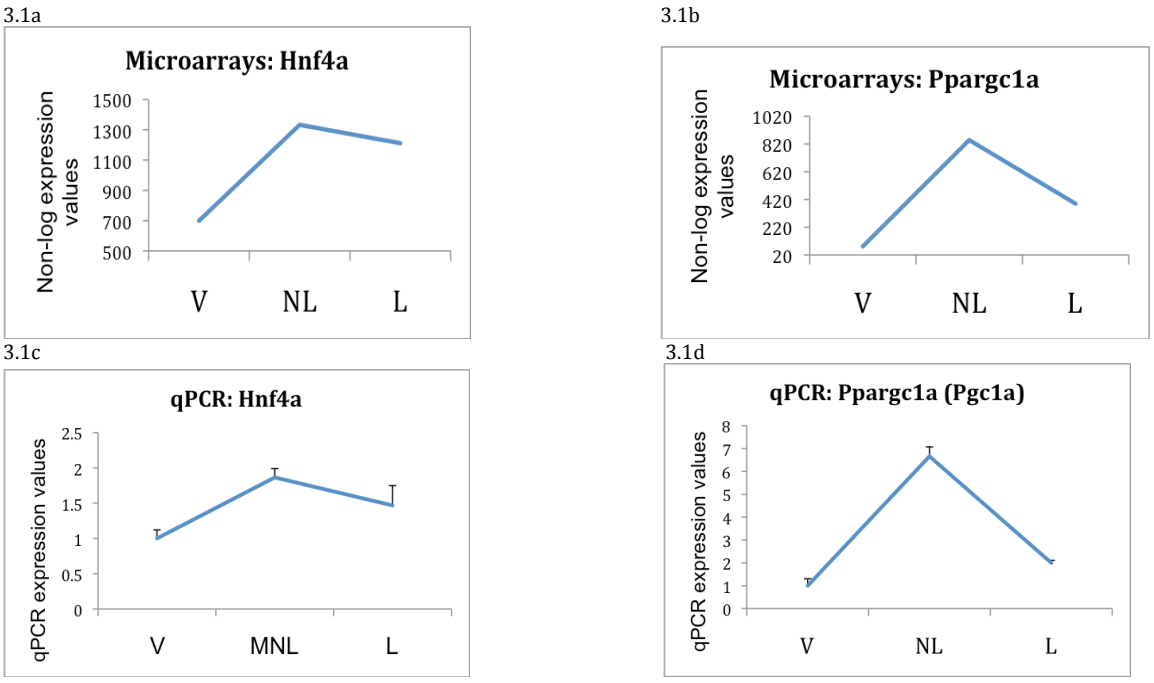


Fig 3.1 Verification of HNF4α (*Hnf4a*) and PGC1α (*Ppargc1a*) microarray data by quantitative real time PCR (qPCR)
Gene expression profiles ofHnf4a (Panel 3.1a) or Ppargc1a mRNA (Panel 3.1b) in livers of virgin (V, n=4), non-lactating (NL, n=4) and lactating (L, n=4) mice as determined by microarray analysis. mRNA of *Hnf4a* and *Ppargc1a* were quantified by real time PCR (qPCR) (Panel 3.1c and Panel 3.1d)(n_V=4, n_{NL}=4, n_L=4). For qPCR primers, refer to materials and methods and Table 1.

Prolactin is the major hormone responsible for lactation (Ostrom 1990; Svennersten-Sjaunja and Olsson 2005). The actions of prolactin are mediated by the prolactin receptor (PRLR). There are four forms of PRLR, three short forms and one long form. The long form is predominantly found in the mammary gland, while the short forms are predominant in the liver, although all forms are present in both

tissues (Jahn *et al.* 1997). The long form of prolactin receptor activates signaling pathways culiminating in milk protein gene transcription (Gouilleux et al. 1994; Lesueur et al. 1991; Das and Vonderhaar 1995). In order to study the differences in expression, if any, between the short forms and long form of Prlr, we quantified mRNA of the long form of prolactin receptor (Prlr-L) and the short forms of prolactin receptor (Prlr-S1, Prlr-S2, Prlr-S3) in the livers of virgin (V), non-lactating (NL), and lactating (L) mice. The molecular signature of prolactin receptor is as follows: no significant change in gene expression in the livers of non-lactating mice compared to virgin mice, but a noticeable increase in the livers of lactating mice. Real time PCR quantification of mRNA for the long and short isoforms of prolactin receptor showed a similar trend (Fig 3.2a-3.2d).

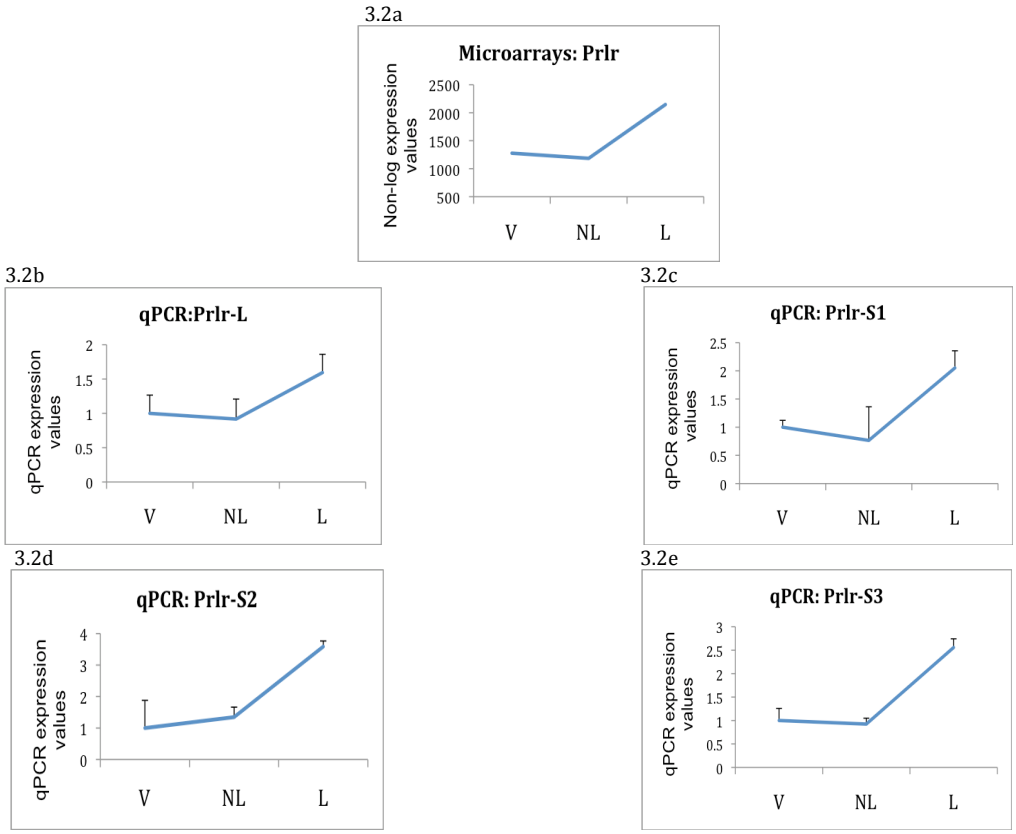


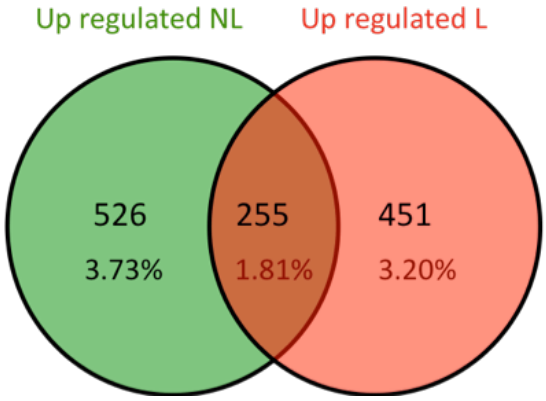
Fig 3.2 Verification of *Prlr* microarray data by quantitative real time PCR (qPCR)
The molecular signature of prolactin receptor (Prlr) in the livers of virgin (V, n=4), non-lactating (NL, n=4), and lactating (L,n=4) mice was determned by microarray analysis. mRNA of the long isoform of prolactin receptor (Prlr-L) and three short isoforms of prolactin receptor (Prlr-S1, Prlr-S2, Prlr-S3) in the livers of virgin (V, n=4), non-lactating (NL, n=4) and lactating (L, n=4) mice were quantified by real time PCR (qPCR).

Number of genes up and down regulated postpartum 1.5-fold or more

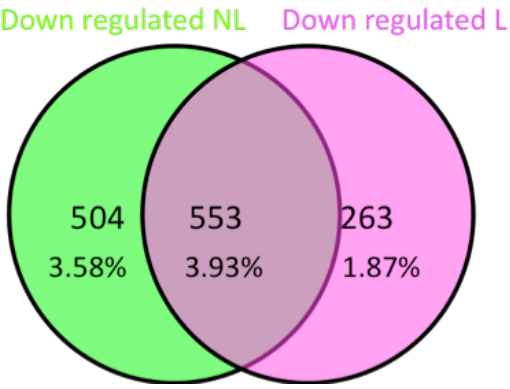
In order to obtain a global perspective of the liver during lactation, we examined genes that were up regulated or down regulated at least 1.5-fold in the livers of non-lactating (NL) and lactating (L) mice (Fig 3.3). Liver gene expression values of NL and L mice were compared to liver gene expression values of virgin (V) mice. There were 2552 genes (~ 18%) that were differentially up and down regulated after parturition (Fig 3.3). A total of 1232 genes were up regulated postpartum (~ 8.75%) while total of 1320 genes (~ 9.37%) were down regulated postpartum. (For

tables of genes up regulated or down regulated during lacatation or non-lactation, please see Appendix A). There were slightly more genes down regulated at least 1.5-fold postpartum (% genes $_{downreg}$ = 9.37, % genes$_{upreg}$=8.75, NL vs V or L vs V). A similar percentage of postpartum genes were up-regulated with non-lactation only or with lactation only (% genes$_{NLonly}$=3.73, % genes $_{Lonly}$=3.20%). In contrast, there was only a small percentage of genes up regulated under both postpartum conditions (% genes$_{common}$=1.81%). In comparison, the percentage of postpartum genes down regulated with non-lactation is greater that those with lactation only (%genes $_{NLonly}$=3.58, %genes$_{Lonly}$=1.87), while the percentage of common genes down regulated postpartum is similar to genes down regulated with non-lactation only (% genes$_{common}$=3.93). The large number of genes differentially up regulated or down regulated postpartum (with or without lactation) reflects the profound effect that pregnancy and parturition have on the maternal liver. Although our results indicate that a large number of genes that are differentially expressed in currently lactating livers (714, Fig 3.3), we can not discount the effects of past lactation, since our currently non-lactating mice lactated in the past. Although the physiological importance of the maternal liver in pregnancy and lactation has been recognized, few have documented this on the molecular level in a global scale. Ramanathan et al (Ramanathan *et al.* 2008) examined the mammary gland of highly fecund mouse strains during pregnancy and lactation. Casey et al (Casey *et al.* 2009) studied the homeorhetic response to lactation in late pregnant (day 20) and early lactation (day 1) (Bell 1995; Bauman 2000). Our study is unique in that we are studying the

maternal liver of virgin, non-lactating, and lactating mice, the latter during peak lactation. The non-lactating state allows us to examine long term effects of lactation because these mice lactated in the past. In this way, we hope to learn more about the role of the maternal liver in lactation, and any long term effects lactation may bring.



Total genes up regulated: 1232 (8.75%)



Total genes down regulated: 1323 (9.37%)

Fig 3.3: Genes up regulated and down regulated during lactation and non-lactation
Non-log values of relative gene expression of genes during lactation or non-lactation were compared to relative gene expression in virgins. Venn diagrams were created by copying and pasting a list of genes that were up regulated or down regulated at least 1.5-fold (compared to virgins) during lactation or non-lactation to the VENNY website (http://bioinfogp.cnb.csic.es/tools/venny/index.html, created by Juan Carlos Oliveros), an interactive tool for comparing lists by using Venn diagrams. NL = non-lactating, L = lactating.

Overrepresented Biological Processes when genes are differentially expressed at least 1.5-fold with lactation only

There are many biological processes that are affected during reproduction as a consequence of the up regulation and the down regulation of genes. In order to understand the role of the maternal liver in lactation, we used gene ontology to analyze biological processes that occur postpartum when genes are up regulated

(Fig 3.4) or down regulated (Fig 3.5) at least 1.5-fold (compared to the virgin state) with lactation.

The biological processes that are most overrepresented when genes are up regulated during lactation are metabolic processes (Fig 3.4). Some specific examples of metabolic processes include: hormone metabolic process (p value=1.99E-09), cholesterol metabolic process (p value=3.32E-08), and lipid metabolic process (p value=1.50E-04), catabolic process (p value=1.36E-02), and electron transport (p value=2.21E-02). Another overrepresented broad biological process when genes are up regulated during lactation is response to stress. Specific examples of response to stress include inflammatory response (p value=2.57E-03), innate immune response (p value=2.06E-02), and response to external stimulus (p value=2.87E-02). A third significant broad biological process is biological regulation. Specific examples of biological regulation include estrogen receptor signaling pathway (p value=1.70E-04), regulation of catalytic activity (p value=2.24E-04), regulation of a molecular function (p value=2.20E-02), regulation of MAP kinase activity (p value=2.53E-02), and regulation of body fluid levels (p value= 4.59E-02). Other overrepresented biological processes include cell cycle process (p value=3.26E-02), and blood coagulation (p value=1.29E-02).

The most enriched biological processes when genes are down regulated with lactation are also metabolic processes (p value= 8.88E-06), although the sub-categories tend to be different from the up regulated genes. For example, cofactor metabolic process (9.14E-03) and cell lipid metabolic process (p value=1.76E-02)

are significantly down but not up with lactation. Another biological process that is overrepresented when genes are down regulated during lactation is localization. Examples of localization include: macromolecule localization (p value = 1.09E-04), protein localization (p value=9.54E-04), and cell localization (p value= 1.65E-02). Other overrepresented biological processes include RNA transport (p value=1.20E-03), response to protein stimulus (p value=1.95E-03), and fatty acid oxidation (p value=4.20E-02).

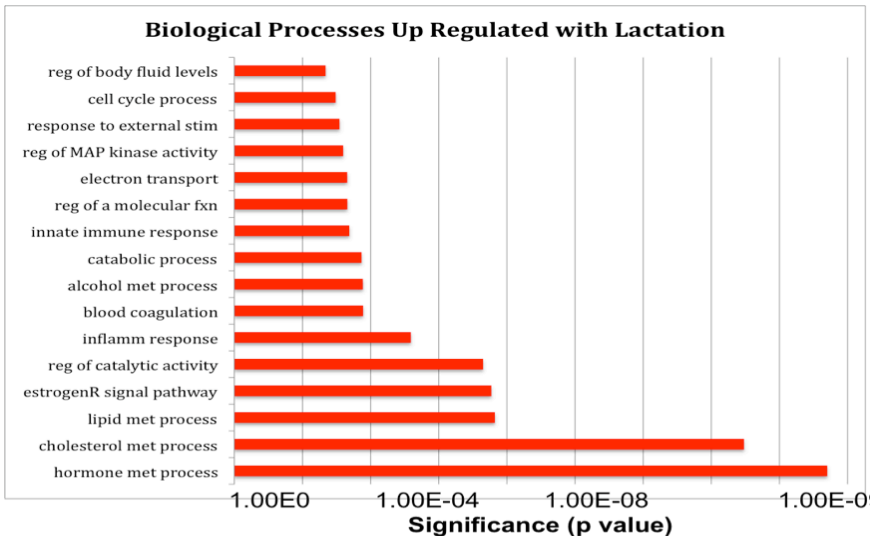


Fig 3.4 Overrepresented biological processes among genes up regulated with lactation only
Gene ontology analysis was used to evaluate the genes that are up regulated at least 1.5-fold in liver genes from lactating mice (compared to virgin mice). See Materials and Methods section for more details.

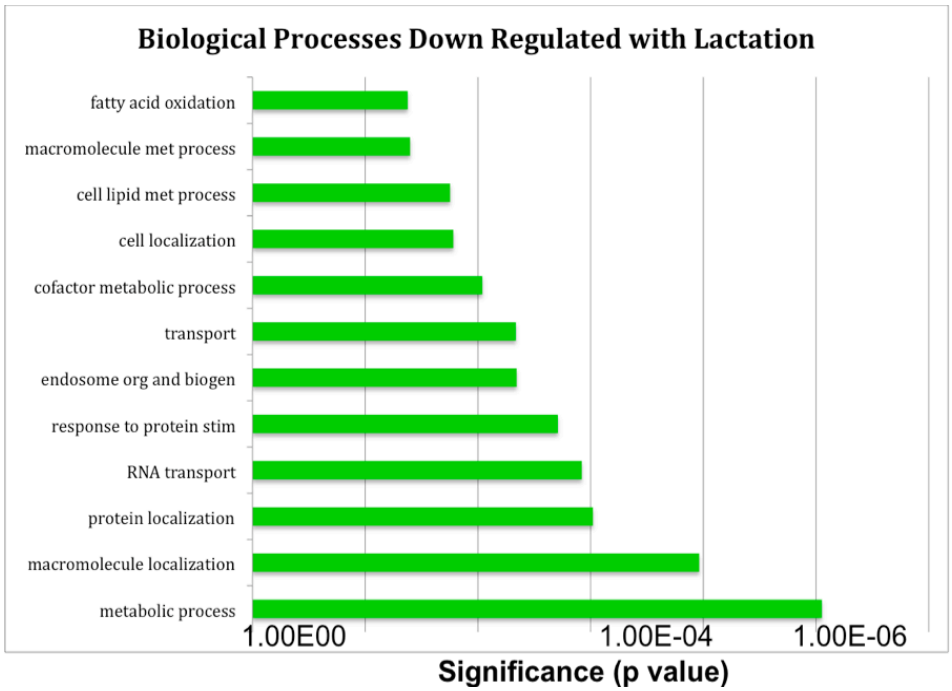


Fig 3.5 Overrepresented biological processes among genes down regulated with lactation only.
Gene ontology analysis was used to evaluate the genes that are down regulated at least 1.5-fold in liver genes from non-lactating mice (compared to virgin mice). See Materials and Methods for more details.

Overrepresented Biological Processes when genes are differentially expressed at least 1.5-fold without lactation (NL) only

We used Gene Ontology to analyze biological processes that are overrepresented when genes are up regulated (Fig 3.6) or down regulated (Fig 3.7) postpartum in non-lactating animals only. This serves as a control for the lactation analysis and provides further insight into the effect of pregnancy and parturition in the maternal liver without the confounding effect of lactation.

Again, metabolic processes were significantly overrepresented in genes that were up regulated in the non-lactating mice. Specific examples include: macromolecule metabolic process (p value=5.92E-17), gene expression (p value=4.89E-14), protein

metabolic process (p value=5.31E-07), RNA splicing (p value=8.63E-05), and DNA metabolic process (p value=2.96E-04). Additional biological processes that were overrepresented are cellular processes, localization, biological regulation, and developmental processes. Specific examples of cellular processes include: cellular component organization and biogenesis (p value=8.37E-07), chromatin modification (p value=3.40E-06), cellular process (p value=8.23E-06), organelle organization and biogenesis (p value=1.19E-04), and cell cycle cell (p value=6.07E-04). Specific examples of localization include: RNA transport (p value=2.14E-05), nuclear transport (p value=8.98E-04), and protein transport across a membrane (p value= 2.15E-03). Specific examples of biological regulation and developmental processes include the negative regulation of biological process (p value=2.76E-03) and cell development (p value=1.33E-02).

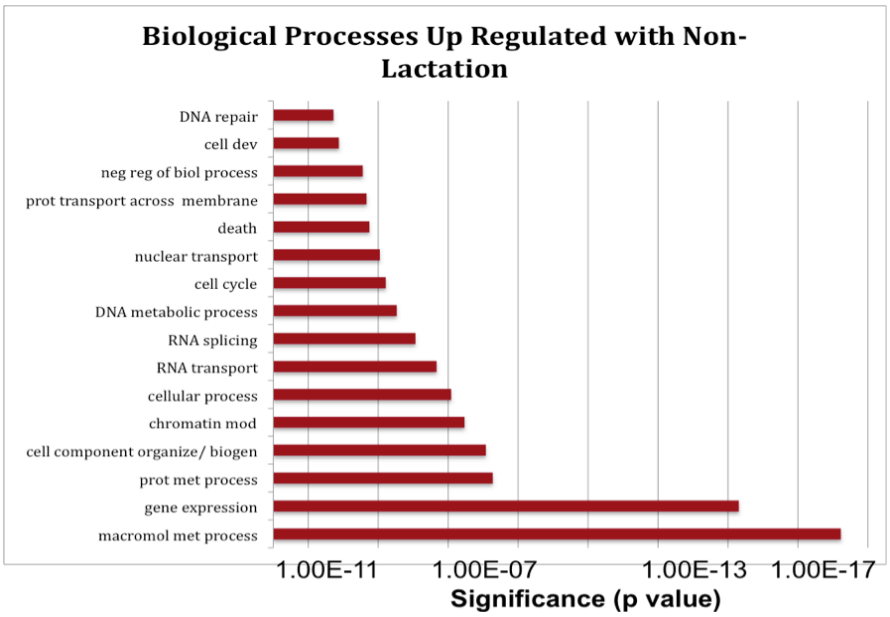


Fig 3.6 Overrepresented biological processes among genes up regulated with non-lactation.
Gene ontology analysis was used to evaluate the genes that are up regulated at least 1.5-fold in liver genes from non-lactating mice (compared to virgin mice). See Materials and Methods section for more details.

Finally, there were several biological processes that were significantly overrepresented in the genes down regulated postpartum without lactation (non-lactation) (Fig 3.7). The most significant category is immune system process/response to stress, including adaptive immune response (p value = 7.07E-04) and T cell proliferation (p value=8.11E-04).

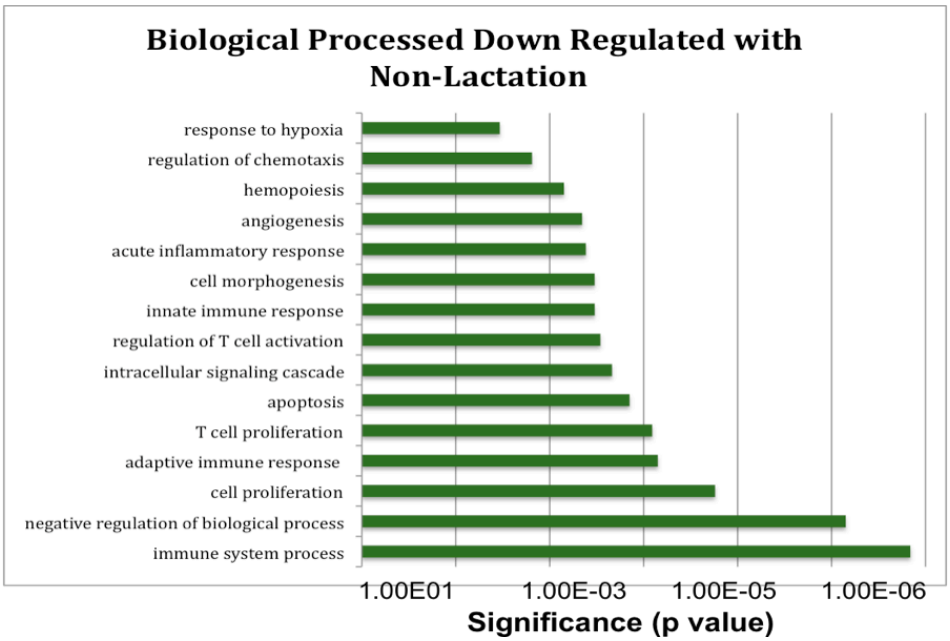


Fig 3.7 Overrepresented biological processes among genes down regulated with non-lactation.
Gene ontology analysis was used to evaluate the genes that are up regulated at least 1.5-fold in liver genes from non-lactating mice (compared to virgin mice). See Materials and Methods section for more details.

Gene expression profiles of transcription factors

In order to shed light on the mechanisms responsible for all the changes in gene expression during pregnancy and lactation, we looked at the gene expression profiles of liver enriched transcription factors and co-activators (Fig 3.8). Expression of these genes in maternal livers from non-lactating (NL) or lactating mothers were compared to those from livers of age-matched virgin females. Several

liver enriched transcription factor genes were up regulated postpartum. Hepatocyte nuclear factor 4alpha (*Hnf4a*), CCAAT/enhancer binding protein beta (*Ceppb*), retinoid X receptor beta (*Rxrb*), forkhead box P1 (*Foxp1*), forkhead box O1 (*Foxo1*), forkhead box A3 (*Foxa3*), and the co-activator peroxisome proliferative activated receptor gamma coactivator 1 alpha (*Ppargc1a* or *Pgc1a*) all had similar expression profiles: expression increased robustly postpartum but less so with lactation (Fig 3.8a-3.8g). This is consistent with the greater numbers of genes up regulated in the non-lactating samples (781 genes) compared to the lactating samples (706 genes). *Hnf4a (HNF4α)* gene expression increased 1.90-fold with non-lactation, and 1.73-fold with lactation (Fig 3.8a). This is in line with the postpartum increase in HNF4α protein expression that we observed in chapter 2 (Fig 2.11). *Cebpb (*C/EBP/β*)* gene expression increased 2.29-fold with non-lactation and 1.74-fold with lactation (Fig 3.8b). *Rxrb* gene expression increased 1.83-fold with non-lactation and 1.56-fold with lactation (Fig 3.8c). *Foxp1* gene expression increased 3.16-fold with non-lactation and 1.82-fold with lactation (Fig 3.8d). *Foxo1* gene expression increased 2.14-fold with non-lactation and 1.54-fold with lactation (Fig 3.8e). *Foxo3a* gene expression increased 2.36-fold with non-lactation and 2.21-fold with lactation (Fig 3.8f). Finally, the co-activator PGC1α (*Ppargc1a*) increased 10.37-fold with non-lactation and 4.76-fold with lactation (Fig 3.8g).

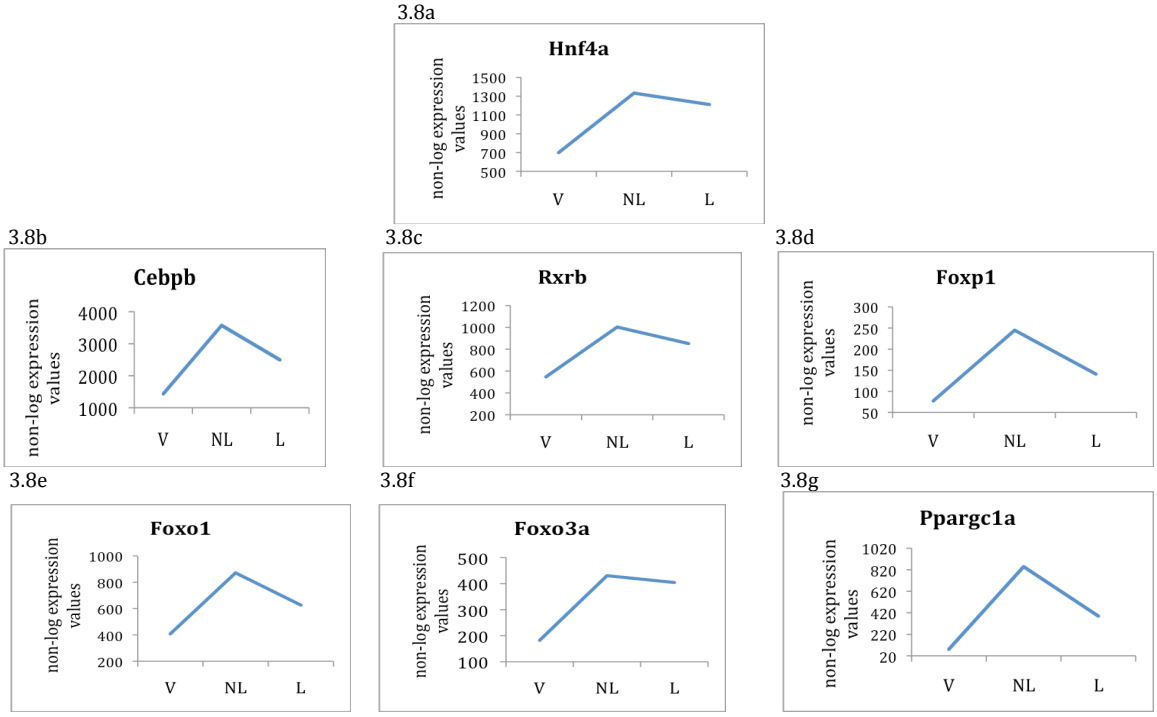


Fig 3.8 Molecular signatures of liver enriched transcription factor genes that are up regulated postpartum. Microarray data was analyzed using GC Robust Multi-Array Average (GCRMA) background adjustment and quantile normalizaton on probe-level data sets with R software (http://www.bioconductor.org). Genes that were differentially expressed at least 1.5-fold compared to virgin expression values were analyzed. From this group of genes, genes that had expression values >100 (non-log) were eliminated. Genes were normalized to GAPDH expression.

We also identified transcription factor genes that were were down regulated postpartum. Trascription factor 2 (*Tcf2*), also known as hepatocyte nuclear factor 1 beta (*Hnf1b*), peroxisome proliferator activated receptor gamma (*Pparg*), and one cut domain family member 1 Onecut1, also known as hepatocyte nuclear factor 6 beta (*Hnf6b*) gene expression decreased considerably with non-lactation, and increased slightly with lactation (Fig 3.9a-3.9c). *Hnf1b* gene expression decreased 2.15-fold with non-lactation 2.05-fold with lactation (Fig 3.9a). *Pparg* gene expression decreased 2.33-fold with non-lactation 2.23-fold with lactation (Fig 3.9b). *Hnf6b* gene expression decreased 2.45-fold with non-lactation and 2.06-fold

with lactation (Fig 3.6c). The decrease in expression of these transcriptional activators could be responsible for the down regulation of genes with lactation and with non-lactation (Fig 3.3).

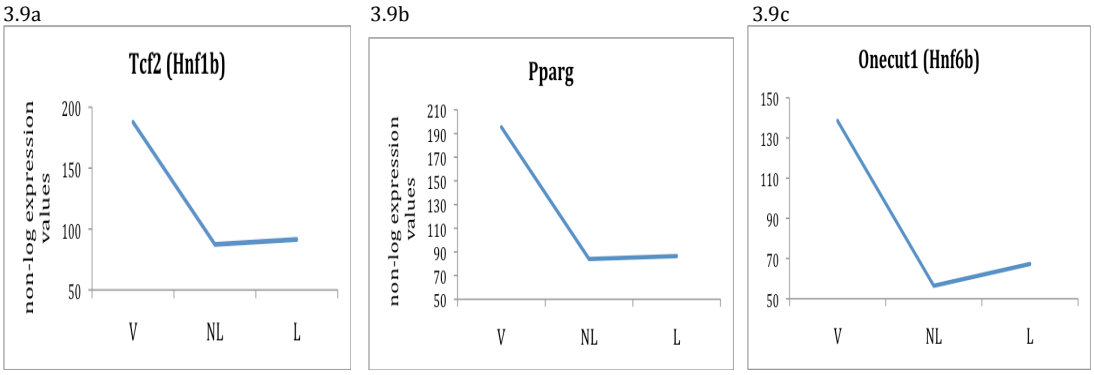


Fig 3.9: Molecular signatures of other liver enriched transcription factors were down regulated postpartum Gene expression profiles from the livers of virgin (V), non-lactating (NL), and lactating (L) mice of the indicated genes as described in Fig 3.8.

Expression profiles of hormone receptor genes

Hormones and their receptors are known to play key roles in lactation (Svennersten-Sjaunja and Olsson 2005). For this reason, we also looked at the postpartum molecular signatures of hormone receptors that were differentially expressed at least 1.5-fold with lactation (Fig 3.10a-3.10c). These hormone receptor genes included leptin receptor (*Lepr*), prolactin receptor (*Prlr*), and growth hormone receptor (*Ghr*). Leptin receptor gene expression increased 2.36-fold with non-lactation but less (1.86-fold) with lactation (Fig 3.10a). In contrast, the lactogenic hormone receptors *Prlr* and *Ghr* exhibited similar expression profiles: a slight decrease with non-lactation (1.09-fold for *Ghr*, and 1.07-fold for *Prlr*), and a

much larger increase with lactation (1.70-fold for *Ghr*, and 1.68-fold for *Prlr*) as one might expect (Fig 3.10b, Fig 3.10c).

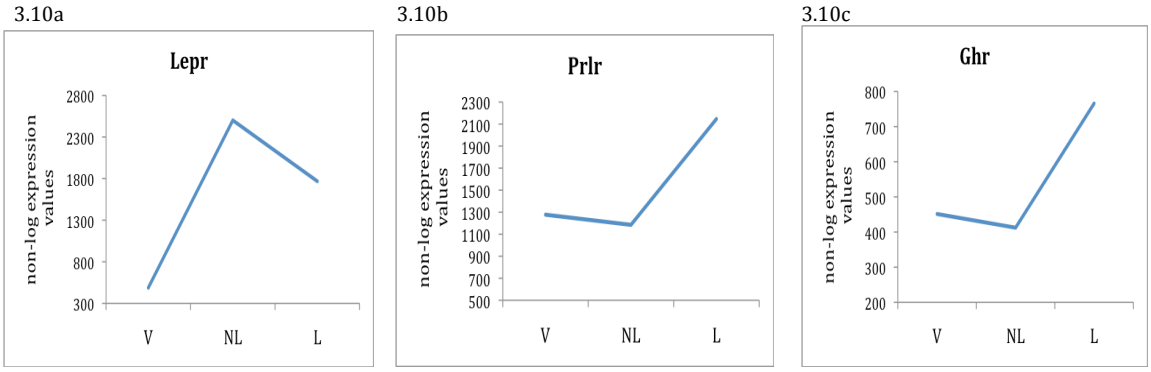


Fig 3.10 Postpartum molecular signatures of hormone receptor genes involved in lactation
Gene expression profiles from the livers of virgin (V), non-lactating (NL), and lactating (L) mice of the indicated genes as described in Fig 3.8.

Expression profiles of growth factors genes

Growth factors play an important role in lactation. For this reason, we examined the gene expression profiles of several growth factors and/or receptors that have been implicated in lactation in the maternal liver. Several growth factors were differentially up regulated postpartum (Fig 3.11a-3.11c). Insulin-like growth factor 1 (*Igf1*), epidermal growth factor receptor (*Egfr*), and insulin-like growth factor binding protein 1 (*Igfbp1*) were all up regulated postpartum in the non-lactating samples. *Igf1* and *Egfr* genes also had further up regulation under lactation. *Igf1* gene expression increased 1.18-fold with non-lactation and 1.69-fold with lactation (Fig 3.11a) while *Egfr* increased 1.75-fold with non-lactation and 2.23-fold with lactation (Fig 3.11b). *Igfbp1* exhibited a different expression profile: an up regulation with non-lactation (2.48-fold), but less of an up regulation with lactation (1.64-fold). In contrast, transforming growth factor alpha (*Tgfa*), transforming

growth factor beta induced (Tgfbi), and insulin-like growth factor binding protein 7 (*Igfbp7*) genes were significantly down regulated postpartum, but less so with lactation. *Tgfa* gene expression decreased 1.74-fold with non-lactation and 1.58-fold with lactation (Fig 3.12a). *Tgfbi* gene expression decreased 1.80-fold with non-lactation and 1.51-fold with lactation (Fig 3.12b). Finally, *Igfbp7* gene expression decreased 1.74-fold with non-lactation and 1.64-fold with lactation (Fig 3.12c).

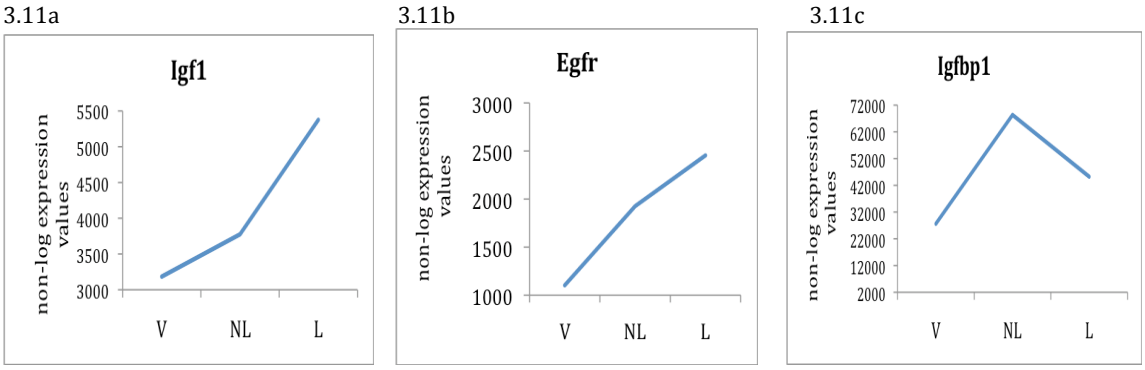


Fig 3.11: Growth factor/receptor genes are differentially up regulated postpartum
Gene expression profiles from the livers of virgin (V), non-lactating (NL), and lactating (L) mice of the indicated genes as described in Fig 3.8.

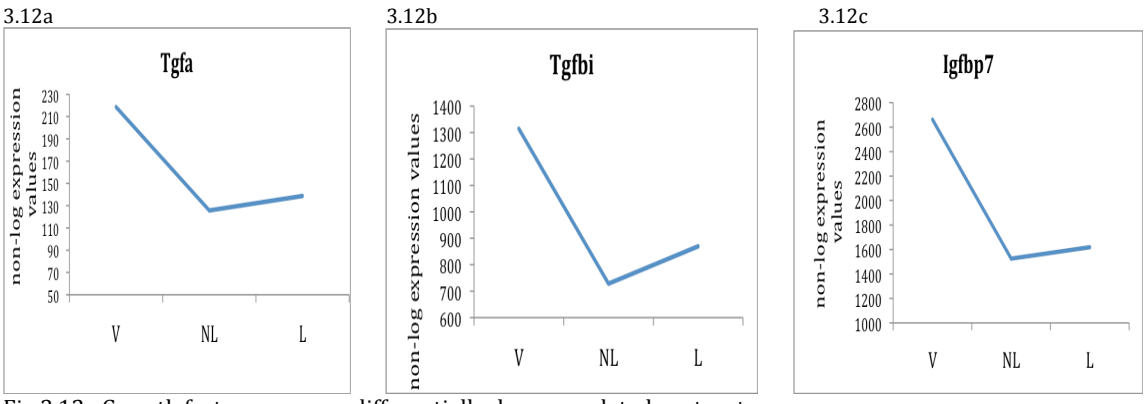


Fig 3.12: Growth factor genes are differentially down regulated postpartum
Gene expression profiles from the livers of virgin (V), non-lactating (NL), and lactating (L) mice of the indicated genes as described in Fig 3.8.

Expression profiles of pregnancy genes

A successful pregnancy contributes to a successful lactation, both of which are important in achieving the goal of reproduction. For this reason, we looked at gene expression profiles of genes that play a role in the maintenance of pregnancy (Bethin *et al.* 2003). Cluster of differentiation 14 (*Cd14*), Prostaglandin F receptor (*Ptgfr*), Prostaglandin-endoperoxide synthase 1 (*Ptgs1*) also known as Cytochrome C oxidase 1 (*Cox1*), LIM domain containing preferred translocation partner in lipoma (*LPP*) genes were all up regulated postpartum as expected. *Cd14* gene exhibited the following expression profile: a marked up regulation with non-lactation (11.97-fold), but less up regulation with lactation (10.30-fold) (Fig 3.13a). In contrast, *Ptgfr*, *Cox1*, and *Lpp* were all up regulated with non-lactation and further up regulated with lactation (Fig 3.13b-3.13d). *Ptgfr* gene had the highest expression fold-change of all 17,000 genes during non-lactation and lactation: 43.47-fold increase with non-lactation and 55.94-fold with lactation (Fig 3.10c). *Cox1* and *Lpp* genes had much more modest changes: *Cox1* increased 1.19-fold with non-lactation and 1.58-fold with lactation (Fig 3.13d), while *Lpp* increased 1.44-fold with non-lactation and 1.71-fold with lactation (Fig 3.13e).

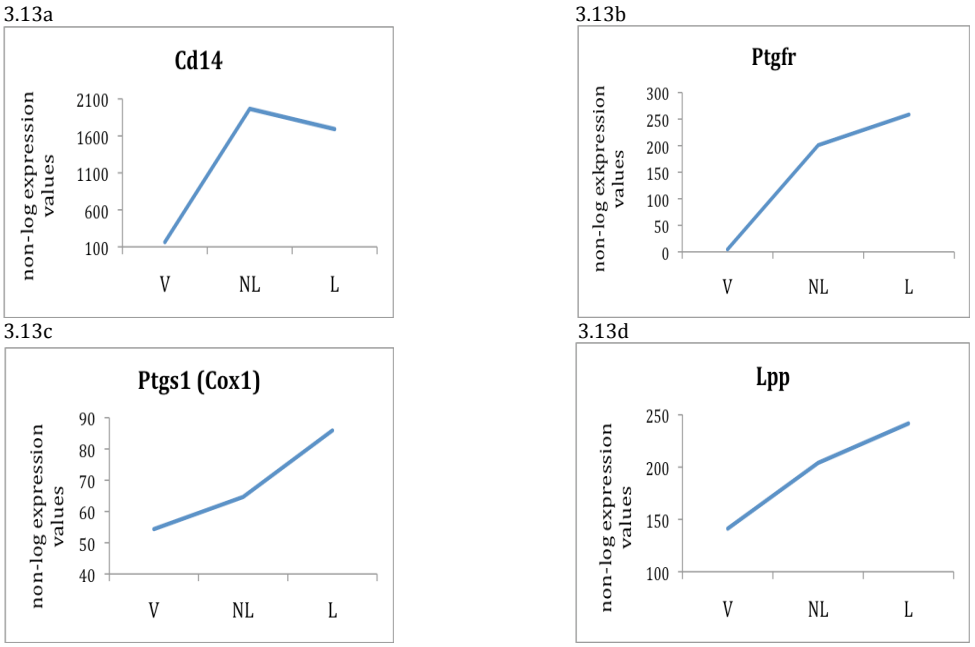


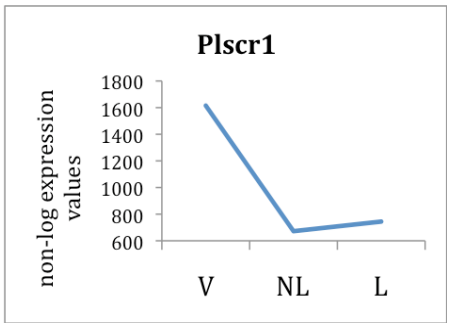
Fig 3.13: Pregnancy genes are up regulated postpartum
Gene expression profiles from the livers of virgin (V), non-lactating (NL), and lactating (L) mice of the indicated genes as described in Fig 3.8.

Gene expression profiles of other lactation genes

Genes involved in lactation have been characterized extensively in mammary gland studies (Lemay et al. 2007; Naylor et al. 2005; Rudolph et al. 2007; Anderson et al. 2007; Ramanathan et al. 2008). In contrast, relatively little is known about the expression of those genes associated with lactation in the maternal liver. Some lactation genes were down regulated postpartum (Fig 3.14), while others were up regulated postpartum (Fig 3.15). Phospholipid scramblase 1 (*Plscr1*) and Proviral integration site 1 (*Pim1*) genes share similar expression profiles: a large decrease in expression with non-lactation and slightly less of a decrease with lactation. *Plscr1* gene expression decreased 2.40-fold with non-lactation, and 2.17-fold with lactation

(Fig 3.14a). *Pim1* decreased 1.76-fold with non-lactation and 1.71-fold with lactation (Fig 3.14b).

3.14a



3.14b

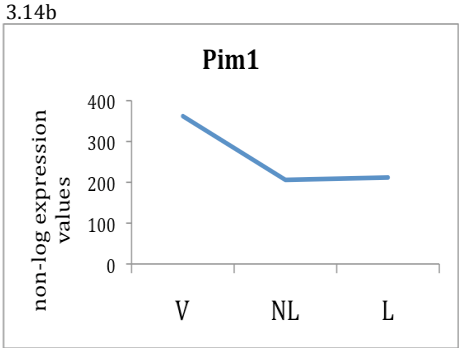


Fig 3.14 Lactation genes are down regulated postpartum in the maternal liver
Gene expression profiles from the livers of virgin (V), non-lactating (NL), and lactating (L) mice of the indicated genes as described in Fig 3.8.

Other genes that play a role in lactation were up regulated postpartum, just as they were in the mammary gland. These include Casein kinase 1 delta (*Csnk1d*), Suppressor of cytokine signaling 3 (*Socs3*), Phospholipase C epsilon 1 (*Plce1*), Signal transducer and activator of transcription 5B (*Stat5b*) (Fig 3.15a-d). *Csnk1d* gene had the following expression profile: up regulation with non-lactation (1.66-fold) and further up regulation with lactation (2.08-fold) (Fig 3.15a). *Socs3*, *Plce1*, and *Stat5b* genes exhibited similar expression profiles: an increase with non-lactation, and less of an increase with lactation. *Socs3* increased 3.26-fold with non-lactation, and 1.93-fold with lactation (Fig 3.15b). *Plce1* increased 1.81-fold with non-lactation, and 1.75-fold with lactation (Fig 3.15c). *Stat5b* increased 3.36-fold with non-lactation, and 3.15-fold with lactation (Fig 3.15d).

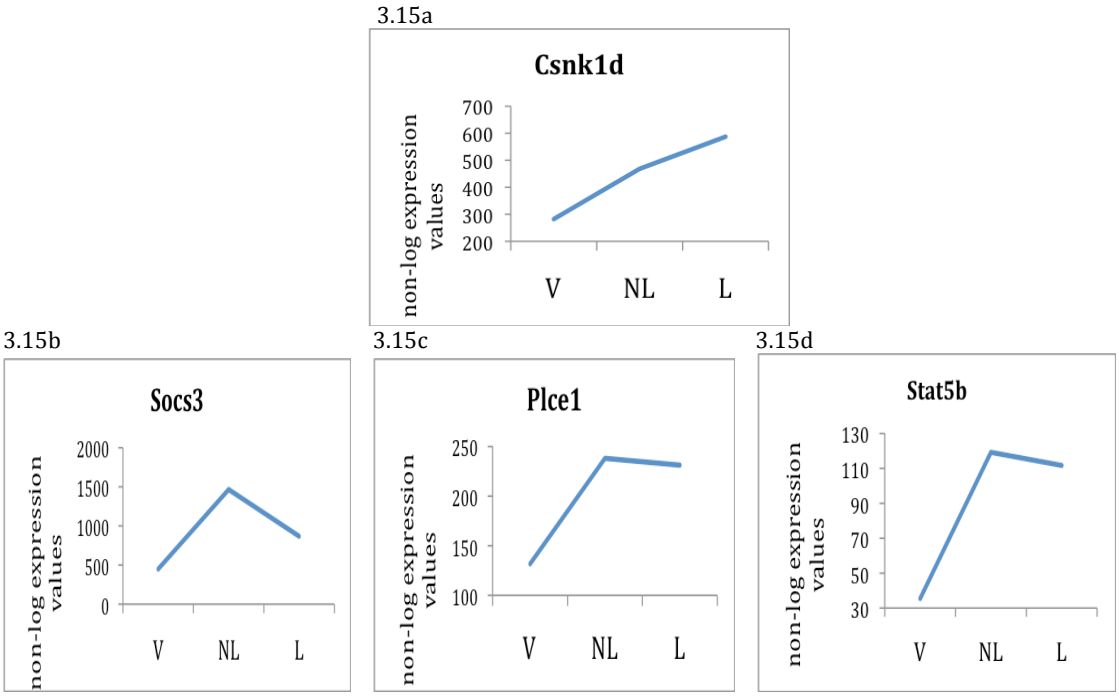


Fig 3.15: Lactation genes are up regulated postpartum in the maternal liver
Gene expression profiles from the livers of virgin (V), non-lactating (NL), and lactating (L) mice of the indicated genes as described in Fig 3.8.

Lipogenic genes are up regulated with lactation

Fatty acids, as components of triglycerides in particular, are an important component of milk (Vernon 2005). In order to understand the role the liver plays in fatty acid metabolism during lactation, we looked at the gene expression profiles of lipogenic genes. Sterol regulatory element binding factor 1 (SREBP1c) is a master regulator of fatty acid or cholesterol synthesis (Sun et al. 2007; Brown et al. 2002). The SREBF1c gene (*Srebf1)* gene decreased slightly with non-lactation (1.11-fold) but increased with lactation (1.9-fold) (Fig 3.16a). Stearoyl-Coenzyme A desaturase 1 (*Scd1*) and thyroid hormone responsive SPOT14 homolog (*Thrsp*) genes exhibited similar expression profiles: a decrease with non-lactation and an increase with

lactation. *Scd1* gene expression increased 1.71-fold with non-lactation and 5.27-fold with lactation (Fig 3.16b). *Thrsp* gene expression increased 1.69-fold with non-lactation and 4.96-fold with lactation (Fig 3.16c). Lpin1 (*Lpin1*) gene expression, on the other hand, increased significantly with non-lactation (3.76-fold) but less so with lactation (2.82-fold) (Fig 3.16d). All told, these lipogenic genes are up regulated postpartum, ¾ of which are further increased with lactation. This suggests that the liver is synthesizing more lipids during lactation.

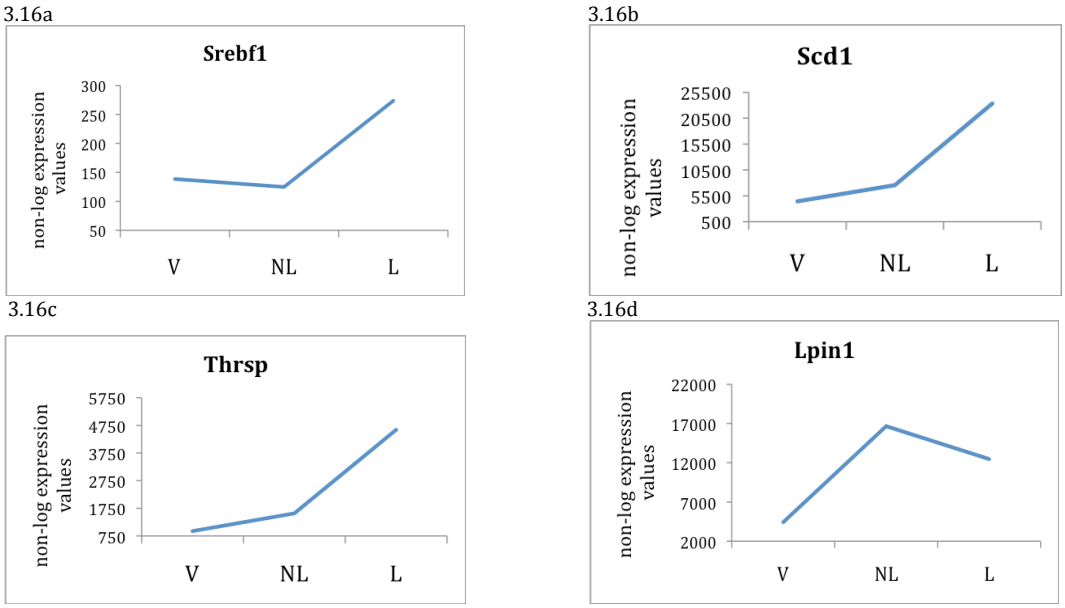


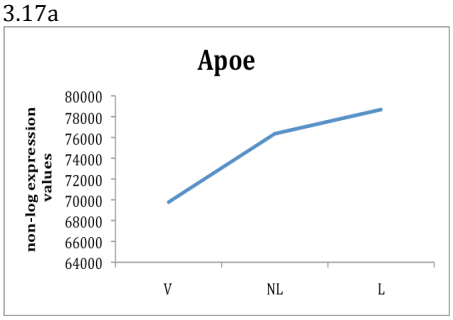
Fig 3.16 Lipogenic genes are up regulated postpartum.
Gene expression profiles from the livers of virgin (V), non-lactating (NL), and lactating (L) mice of the indicated genes as described in Fig 3.8.

Expression profiles of genes involved in triglyceride secretion

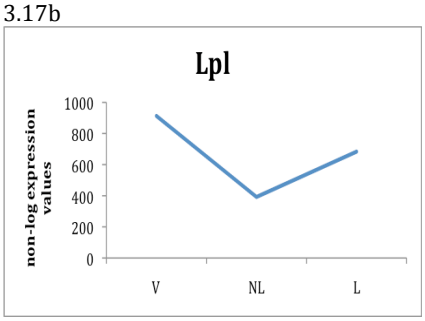
Our current study has shown that one of the consequences of lactation is an elevation of plasma triglycerides (Fig 2.10). For this reason, we looked at the expression profiles of genes involved in triglyceride secretion. Genes that play a

role in triglyceride secretion include apolipoprotein E (*Apoe*), lipoprotein lipase (*Lpl*), and very low density lipoprotein receptor (*Vldlr*). Lpl is the key enzyme in triglyceride hydrolysis in triglyceride-rich lipoproteins such as very low density lipoproteins (VLDL). During lipolysis, lipoproteins are broken down and enriched with the apolipoprotein Apo E, whose remnants are taken up by receptors that recognize Apo E. An example of such a receptor is VLDLR. VLDRL therefore binds triglyceride-rich lipoproteins via Apo E. Transgenic studies have shown that increased *Apoe* expression results in increased triglyceride secretion and that in the liver, increased *Apoe* exoression results in the mobilization of hepatic triglycerides. In our study, *Apoe* is up regulated postpartum with non-lactation (1-fold) and up regulated further with lactation (1.13-fold) (Fig 3.17a). *Lpl*, on the other hand, is down regulated with non-lactation (1.74-fold) but down regulated less with lactation (1.34-fold); the net fold change between lactation and non-lactation is a 2.33-fold increase (Fig 3.17b). *Vldlr* has a similar expression profile. Although *Vldlr* is down regulated with non-lactation, it is up regulated with lactation; the net fold change between lactation and non-lactation is 2.27 (Fig 3.17c). These results indicate that there is a net increase in genes involved in triglyceride transport in the lactating liver.

3.17a



3.17b



3.17c

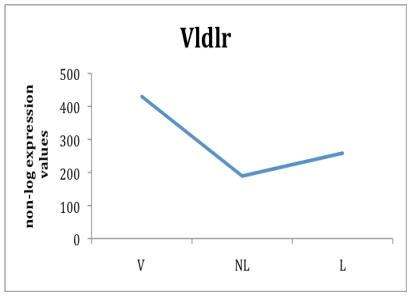


Fig 3.17 Triglyceride secretion genes are up regulated with lactation
Gene expression profiles from the livers of virgin (V), non-lactating (NL), and lactating (L) mice of the indicated genes as described in Fig 3.8.

Expression profile genes involved in fatty acid transport

Since fatty acid tranport genes play an important role in the trafficking of fatty acids (Furuhashi and Hotamisligil 2008), we looked at the expression profiles of several genes involved in fatty acid transport (Fig 3.18a-3.18d). Liver fatty acid binding protein (*Fabp1*), intestinal fatty acid binding protein (*Fabp2*), Solute carrier family 27 member 4, also known as fatty acid transfer protein 4 (*Slc27a4*), and fatty acid translocase/Cd36 (*Cd36*) all share the same expression profile: a decrease with non-lactation and a further decrease with lactation. *Fabp1* gene expression decreased 1.29-fold with non-lactation and 1.56-fold with lactation (3.18a). *Fabp2* gene expression decreased 1.67-fold with non-lactation and 2.46-fold with lactation (Fig 3.18b). *Slc27a4* gene expression decreased 1.07-fold with non-lactation and a

1.47-fold with lactation (Fig 3.18c). Finally, *Cd36* gene expression decreased 1.39-fold with non-lactation and 2.52-fold with lactation (Fig 3.18d). All told, there is a significant decrease in the expression of genes involved in moving fatty acid into the maternal liver during lactation.

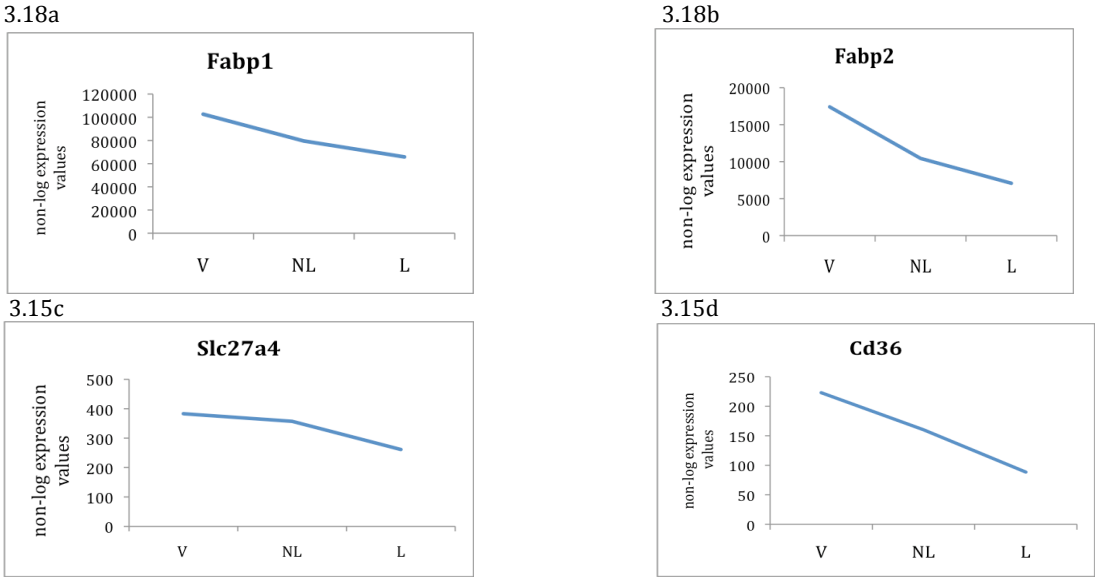


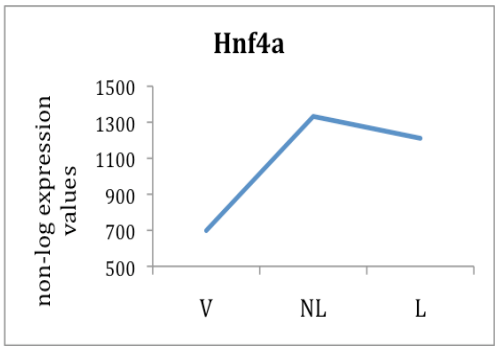
Fig 3.18 Fatty acid transport genes are down regulated postpartum
Gene expression profiles from the livers of virgin (V), non-lactating (NL), and lactating (L) mice of the indicated genes as described in Fig 3.8.

Expression profiles of HNF4α and some of its targets

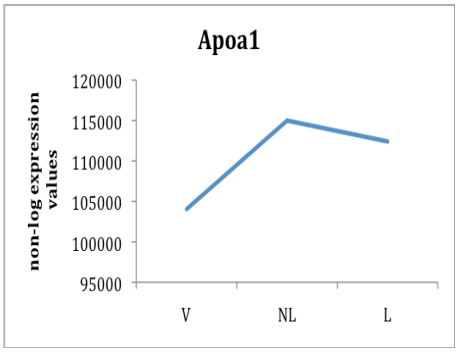
HNF4α is a master regulator of hepatic lipid metabolism (Hayhurst et al. 2001; Bolotin, Schnabl, and Sladek 2009). The postpartum molecular signature of the HNF4α gene (*Hnf4a*) is an induction during non-lactation, and less induction with lactation (Fig 3.8). In order to understand the role of HNF4α in the maternal liver during lactation, we looked at the expression profiles of some of its target genes. Apolipoprotein A1 (*Apoa1*), apolipoprotein B (*Apob*), and phosphoenolpyruvate

carboxykinase 1 (*Pck1/Pepck)* genes all had similar expression profiles: increased expression with non-lactation and less of an increase with lactation. *Apoa1* gene expression increased 1.12-fold with non-lactation and 1.10-fold with lactation. *Apob* gene expression increased 1.56-fold with non-lactation and 1.3-fold with lactation. *Pck1* gene expression increased 1.24-fold with non-lactation and 1.17-fold with lactation.

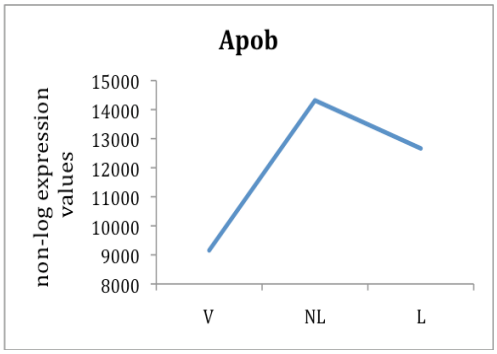
3.19a



3.19b



3.19c



3.19d

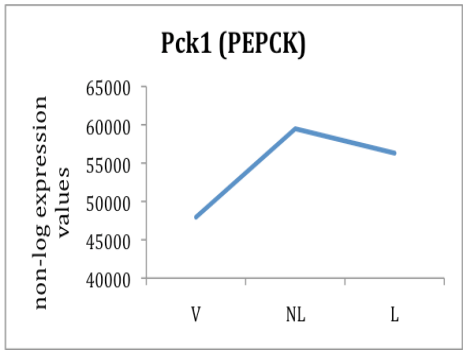


Fig 3.19 HNF4α and its targets have similar postpartum molecular signatures.
Gene expression profiles from the livers of virgin (V), non-lactating (NL), and lactating (L) mice of the indicated genes as described in Fig 3.8.

Discussion

Establishing our microarray system

The goal of this study is to understand the role the liver plays in lactation. We have employed gene expression profile analysis (microarray) in order to give us a global perspective of the maternal liver during lactation. In order to have confidence in our results, we verified the postpartum molecular signatures of select genes with quantitative real time PCR (qPCR). We chose to verify the postpartum molecular signatures of HNF4α and its co-activator PGC1α (*Ppargc1a*), since they have been shown previously to play roles in liver metabolism (Rhee *et al.* 2006). We also chose to verify the molecular signature of the prolactin receptor (*Prlr*) because it mediates the effects of prolactin, a hormone that is essential for lactation, at least in the mammary gland (Svennersten-Sjaunja and Olsson 2005). And indeed, the postpartum molecular signatures of the liver enriched transcription factor HNF4α, and its co-activator PGC1α, were validated by qPCR (Fig 3.1a-3.1d). The same is true for the long and short isoforms of prolactin receptor (Fig 3.2a-3.2e). As hypothesized, prolactin receptor expression was increased during lactation. In addition, our data show a few of the genes involved in lactation that are up regulated in the mammary gland during lactation are also up regulated in the liver with lactation.

These results validate key aspects our microarray data.

Overall Effects in Gene Expression During Lactation

Lactation elicits many maternal adaptations as a means to meet the demands of the highly activated mammary gland and the suckling pups. For this reason, we looked at the number and percentage of genes that were differentially expressed postpartum with or without lactation (Fig 3.3). The percentage of genes that were up regulated with non-lactation was similar to that of genes that were up regulated with lactation. Although the animals in this study were not pregnant at the time of sacrifice, the postpartum non-lactating state represents the post-pregnant condition because the lactating group (L) and the non-lactating group (NL) had both undergone pregnancy, the only difference is the occurrence or non-occurrence of lactation. Since pregnancy and lactation are closely tied, then the similarity in the percentage of up regulated genes with or without lactation is as expected. There were more genes differentially down regulated (9.37%, Fig 3.3) compared to genes that were differentially up regulated (8.75%, Fig 3.3) postpartum. The percentage of genes that were differentially up regulated with lactation was nearly double that of genes that were differentially down regulated with lactation. This would reflect a highly active liver during lactation, perhaps in order to meet the many demands that lactation imposes on the nursing mother.

We next used Gene Ontology analysis to examine the biological processes that were overrepresented postpartum with or without lactation. The diversity in significant biological processes among genes that are either are up regulated or

down regulated with lactation or with non-lactation demonstrate the complexity of lactation, which could result in some of the maternal adaptations we noted in Chapter 2 as consequences of lactation. Some of the most notable examples are biological processes that involve lipid metabolism and the immune system (Fig 3.4 – 3.7)

The most overrepresented biological process with lactation and with non-lactation is metabolic process (Fig 3.4 and Fig 3.6). The over representation of lipid, cholesterol, and hormone metabolism among genes up regulated with lactation reflects an increase in the metabolic activity of the liver, especially in regard to high energy substrates such as lipids that are required to meet the needs of the mammary gland, and ultimately, the suckling pups.

Immune Response During Lactation

It is of interest to note that biological processes that involve the immune system (i.e. inflammatory response, innate immune response) are highly represented in the lactating state among genes that are differentially up regulated (Fig 3.4), while biological processes that involve the immune system (e.g. adaptive immune response, T cell proliferation, regulation of T cell activation, innate immune response) are over represented among genes that are differentially down regulated in the non-lactating state (Fig 3.7).

There are many hormones involved in lactation, and these hormones may also play a role in the immune system during reproduction. For example, the molecular

signature of growth hormone receptor (*Ghr*) (slight decrease with non-lacation and a much larger increase with lactation, Fig 3.10c) in our study may provide an explanation for the difference in immune response processed with lactation and non-lactation. In addition to promoting growth, growth hormone has a positive influence on the immune system (Kelly *et al.* 1991). Several studies have shown that growth hormone stimulates T cell and B cell proliferation and immunoglobulin synthesis (Morrhaye *et al.* 2009). The increase in *Ghr* gene expression during lactation may be an explanation of the increased immune response during lactation and could explain why genes involved in T cell proliferation are not down regulated as they are in the non-lactating condition. In contrast, in the non-lactating state, genes involved in the immune response tend to be down regulated (Fig 3.7). Examples of these are innate immune response and acute inflammatory response. Lower immune response during pregnancy is a way to provide a means for the maternal immune system to tolerate the fetus (Tincani *et al.* 2009). For example, it has been suggested that thrombotic/inflammatory processes often accompany recurrent pregnancy loss (Kwak-Kim, Yang, and Gilman-Sachs 2009). Human breast milk, on the other hand, contains leukocytes and other factors that have antimicrobial effects (Jackson and Nazar 2006; Goldman 1993), suggesting a stimulated immune system. Studies have shown that the adaptive immune system of the nursing mother can provide passive protection to the suckling newborn by passing on antibodies through the mother's milk (Wilson and Butcher 2004; Holmgren et al. 1976; Stoliar et al. 1976). In contrast, lower immune response

during non-lactation that continued from the pregnant state that serves to protect the trophoblast from maternal immunity, and hence, maintain pregnancy.

Weight Loss During Lactation

A noticeable consequence of lactation in our study was significant weight loss in fasted,currently lactating animals (Fig 2.2, 2.3). This could be due to the mobilization of fat stores, or enhanced lipolysis of adipose tissue, in order to meet the high energy demands of lactation (Vernon 1989; McNamara 1997; Sumner and McNamara 2007) . The significant increase in plasma glycerol, a marker of adipose tissue lipolysis (Judd *et al.* 1998) during lactation (Fig 2.4) and the strong correlation between percent weight lost and high glycerol levels during lactation (Fig 2.5) supports the idea that the rapid weight loss that accompanies lactation is due to the mobilization of fat stores in the body. Growth hormone is lipolytic and may play a role in the rapid weight loss we observed. Reduced growth hormone secretion is associated with obesity (Makimura *et al.* 2009). Growth hormone been shown to promote lipid mobilization (Savastano et al. 2009; Rhoads et al. 2004). The molecular signature of the growth hormone receptor gene (*Ghr*) in our study is as follows: down regulation with non-lactation and an up regulation with lactation. This would suggest an elevation of growth hormone action with lactation, which would result in an increase in lipolysis (mobilization of fat stores), and could explain the rapid weight loss that accompanies lactation.

Increased Liver Mass with Lactation

Another consequence of lactation is an increase in liver mass (Fig 2.6a, 2.6b). The increased liver mass may be the result of increased food intake during lactation (Speakman and Krol 2005; Naya et al. 2008), a parameter that was not measured in our study. The increase in liver weight may also be due to hypertrophy and not hyperplasia. Dunphy et al (Dunphy, Snell, and Clegg 1992), for example, demonstrated that hepatic proliferation does not occur during lactation. In this case, one would expect to see elevated levels of hormones that promote growth, such as growth hormone and prolactin. Our data show similar postpartum molecular signatures for the receptors of these hormones, growth hormone receptor (*Ghr*) and prolactin receptor (*Prlr*): a decrease in gene expression with non-lactation and a dramatic increase in gene expression with lactation (Fig 3.10b, Fig 3.10c). Transgenic mice overexpressing growth hormone develop hepatocellularmegaly (Quafe et al 1989; Wolf et al 1993). Prolactin, on the other hand, has also been shown to increase cell number in liver regeneration in rats (Olazabal *et al.* 2009), but it likely has many more functions which contribute to hypertrophic responses. In addition, transforming growth factor alpha (*Tgfa*), a factor that has been shown to promote hepatocyte proliferation (Sato *et al.* 2006) is down regulated postpartum (Fig 3.12b). In addition antigen identified by monoclonal antibody Ki-67 (*Mki67*), a marker of cellular proliferation, is down regulated with non-lactation and further down regulated with lactation (data not shown). Taken together, our data suggest that the increase in liver mass during

lactation is a consequence of the up regulation of growth promoting hormone genes (growth hormone receptor and prolactin receptor), which promote hypertrophy, and not due to cell proliferation (down regulation of *Tgfa and Mki67*).

Lipid Metabolism During Lactation

Genes involved in lipid metabolic processes were significantly overrepresented among genes are up regulated during lactation. Lipids such as fatty acids are important components of milk, a majority of which is in the form of triglycerides (Anderson *et al.* 2007). The liver, the mammary gland, and adipocytes are the main sources of lipids during lactation (Vernon et al. 2002; Barber et al. 1997). During times of stress, very high rates of lipolysis can occur, resulting in the availability of free fatty acids (FFA) in the blood. The liver then takes up the FFA and esterifies them to triglycerides which are then secreted back into the circulation as very low density lipoproteins (VLDL) (Vernon *et al.* 2002; Vernon 2005). *In vivo* and microarray data from our current study support this scenario. First, there is the rapid weight loss that accompanies lactation, when the animal is fasting, which suggests an increase in lipolysis, which would then increase free fatty acids available to the liver (Fig .2, 2.3). Second, several genes that are involved in the synthesis of triglycerides are up regulated postpartum: *Srebf1, Scd1, Thrsp*, and *Lpin1* (Fig 3.16a-d). This would suggest an increase in triglyceride synthesis in the maternal liver of lactating mice. Third, plasma triglyceride levels are elevated with lactation (Fig 2.7). In addition, genes involved in triglyceride secretion are up regulated with lactation

(Fig 3.17). Taken together, the increase in plasma triglyceride levels and the stimulation of genes involved in triglyceride secretion suggests that the liver synthesizes triglycerides which are then secreted into the blood. Hepatic triglyceride levels are reduced with lactation (Fig 2.8). The lipogenic genes *Srebf1, Scd1,* and *Lpin1* have been shown to play important roles in triglyceride synthesis (Sun et al. 2007; Miyazaki and Ntambi 2003; Finck and Kelly 2006). It is of interest to note that although *Lpin1* is up regulated with non-lactation, the degree of up regulation is less with lactation. Finck et al (Finck and Kelly 2006) have shown that stimulation of *Lpin1* in the liver suppressed hepatic lipogenesis and TG secretion, and increased hepatic TG storage. This could explain the decrease in hepatic TG storage and the increase in TG secretion that we observed during lactation when compared to non-lactation. In addition, Im et al (Im *et al.* 2009) show that *Srebf1* plays a role in regulating the partitioning of lipids in hepatocytes. The liver has the ability to either oxidize the fatty acids it derives from adipose tissue or synthesize these same fatty acids into triglycerides. A deficiency in *Srebf1* leads to an unbalanced partitioning of fatty acids, favoring fatty acid oxidation resulting in an excess amount of ketone bodies and less triglyceride for storage. Consistent with this model is a decrease in the expression of fatty acid oxidation genes with lactation.

Taken together, our data implies an increase in growth hormone with lactation and this results in an increase of adipose tissue lipolysis, which leads to increased free fatty acids in the circulation. The liver is then able to utilize the free fatty acids

to synthesize triglycerides. A decrease in *Lpin1* gene expression that accompanies lactation up regulates lipogenic genes, including *Srebf1*. The decrease in *Lpin1* expression also results in an up regulation of genes involved in triglyceride secretion. As a result, there is a decrease in hepatic storage and an increase in plasma triglyceride levels with lactation, exactly what we observed. In addition, the increase in *Srebf1* expression pushes the liver to favor triglycide production, and not fatty acid oxidation.

Regulatory Factors in Lactation

A growing body of evidence suggests that the metabolic changes that occur as a consequence of lactation result in better health for the mother (Stuebe *et al.* 2005; Schwarz *et al.* 2009). A closer examination of microarray data from our current study may give us clues on how lactation brings about a healthier maternal profile. Several liver enriched transcription factors, including C/EBPβ, PGC1α, and HNF4α, share similar postpartum molecular signatures: an up regulation of gene expression with non-lactation and less up regulation with lactation (Fig 3.8). Several studies suggest that a decrease in *Cebpb* expression with lactation may lessen the incidence of diabetes. Dhahbi et al (Dhahbi *et al.* 2003) showed that C/EBP b levels are increased in Streptozotocin induced diabetes (SID). PGC1α is a co-activator of several transcription factors that regulates lipid and metabolic pathways in the liver (Lin, Handschin, and Spiegelman 2005; Spiegelman and Heinrich 2004). Type 1 and Type 2 diabetes models have shown an increase in hepatic *Pgc1a* expression

and activity (Herzig *et al.* 2001; Rhee *et al.* 2003). Therefore, a decrease in the up regulation of *Pgc1a* suggests that lactation lowers the incidence of diabetes in nursing mothers . The liver enriched transcription factor hepatocyte nuclear factor 4alpha (HNF4α) has been linked to diseases such as diabetes and atherosclerosis. Its postpartum molecular signature is as follows: an up regulation with non-lactation and less of an up regulation with lactation. Some of its targets, including PEPCK (*Pck1*) and Apolipoprotein B (Apob) have similar postpartum molecular signatures (Fig 3.19). An increase in PEPCK results in an increase in gluconeogenesis (Rosella *et al.* 1993). An increase in gluconeogenesis is associated with diabetes (Magnusson et al. 1992; Consoli et al. 1989). Apo B is the major protein component of low density lipoproteins (LDL) and is involved in the transport of lipids and cholesterol (Mahley *et al.* 1984). High levels of LDL in the circulation are associated with diabetes and heart disease (Therond 2009). Our data would suggest that the lower levels of HNF4α (*Hnf4a*) and its targets (*Pck1* and *Apob*) during lactation may lead to less of an incidence of diabetes and heart disease. However, HNF4α is known to directly regulate hundreds of genes in hepatocytes (Bolotin et al. 2009), the majority of which are involved in metabolism and homeostasis. Therefore, it is likely to play a very complex role in the maternal liver.

Not all transcription factors are up regulated postpartum. PPARγ (*Pparg*) is down regulated with lactation (Fig 3.9b). A study by Schadinger et al (Schadinger *et al.* 2005) shows that an up regulation of *Pparg2* induces lipid accumulation in hepatocytes, and it does so by stimulating pathways regulating de novo lipid

synthesis, and this ultimately results in hepatic steatosis (fatty liver). This would suggest that the down regulation of *Pparg* during lactation may lower the incidence of accumulating triglycerides in the liver. Suppressor of cytokine signaling 3 (*Socs3*), a gene that plays a role in lactation in the mammary gland (Le Provost *et al.* 2005), is up regulated with non-lactation and up regulated less with lactation (Fig 3.15b). Ukeki et al (Ueki, Kadowaki, and Kahn 2005) have shown that *Socs3* overexpression in liver causes insulin resistance and hepatic steatosis. Howard et al (Howard *et al.* 2004), on the other hand, showed that *Socs3* knock out mice are protected from diet induced obesity. Our data would therefore suggest that, the differential expression of these genes may promote weight loss and lessen hepatic triglyceride storage in the lactating mouse.

Conclusions

Our data show that there are many genes that are differentially up regulated and down regulated postpartum. This would reflect the complexity of pregnancy, parturition, and lactation, and the profound effects they have on the maternal liver. The differential up regulation and down regulation of genes during lactation brings about maternal adaptations to satisfy the energetic needs of the lactating mammary gland and suckling pups. Examples include, hepatic hypertrophy (increase in liver weight), increased immune response, and increased circulating triglyceride levels. Other consequences of the differential gene expression during lactation include rapid weight loss upon fasting and decreased hepatic triglyceride storage, which may be due to The liver, therefore, is highly active metabolically in currently lactating mice. It also plays a role in supplying the triglycerides that the lactating mammary gland needs by perhaps utilizing the free fatty acids and synthesizing these into triglycerides, which it then secretes into the circulation. It is interesting to note that maternal physiological and metabolic changes such as rapid weight loss upon fasting and decreased hepatic triglyceride storage may suggest improved maternal health during lactation. In our study, these trends are only apparent in currently lactating animals. Although human studies point to lactation as providing long term beneficial effects for the lactating mother, in our mouse study, the beneficial trends that we observe may not extend to long term effects because the non-lactating mice exhibit different trends from the lactating mice. Studies involving mice in the post-lactating state (weaning) and mice that have gone

through pregnant but never lactated may help us tease out the role of the maternal liver in lactation.

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Chapter 4

Conclusions

Chapter 4

Conclusions:

The Big Picture

Lactation is a complex process that requires various hormones whose presence or absence result in the orchestration of different metabolic activities of various maternal organs in order to meet the energy demands of the lactating mammary gland (Dunphy, Snell, and Clegg 1992; Vernon 2005). The liver is a highly active organ that plays an important role in this process. We used *in vivo* experimentation and bioinformatics in order to get a global picture of the liver in an attempt to further understand the role it plays in lactation. We chose to examine peak lactation (lactation day 10-12) because we thought we would see the most dramatic effects at this time. We also chose to compare the livers of lactating mice to virgin and non-lactating mice. Out of a total of ~ 14000 gene probe sets, 2552 genes were up regulated (~ 18%) postpartum (with lactation and without lactation) (Fig 3.3). This is a tremendous percentage and shows pregnancy and parturition have a profound effect on the liver. Lactation by itself also has significant effects on the maternal liver, since 714 genes or ~ 5% are differentially up regulated or down regulated. Taken together, pregnancy, parturition, and lactation greatly affect the maternal liver.

Gene Ontology

Gene Ontology analysis sheds some light on the consequences of the differential regulation of genes with or without lactation, by identifying the biological processes that are overrepresented when genes are up regulated or down regulated. The most overrepresented biological processes that occur when genes are differentially up regulated in lactating mice are different from the most overrepresented biological processes that occur when genes are differentially up regulated in non-lactating mice (Fig 4.1). For example, the biological processes that accompany lactation when genes are up regulated include hormone metabolism and cholesterol metabolism. The same can be said for the most overrepresented biological processes when genes are differentially down regulated in lactating mice or in non-lactating mice (Fig 4.1). Examples include macromolecular metabolism and DNA metabolism. Significantly enriched biological processes when genes are down regulated with lactation include metabolic processes, macromolecular localization and protein localization. The diversity in these biological processes emphasize the complexity of pregnancy and lactation.

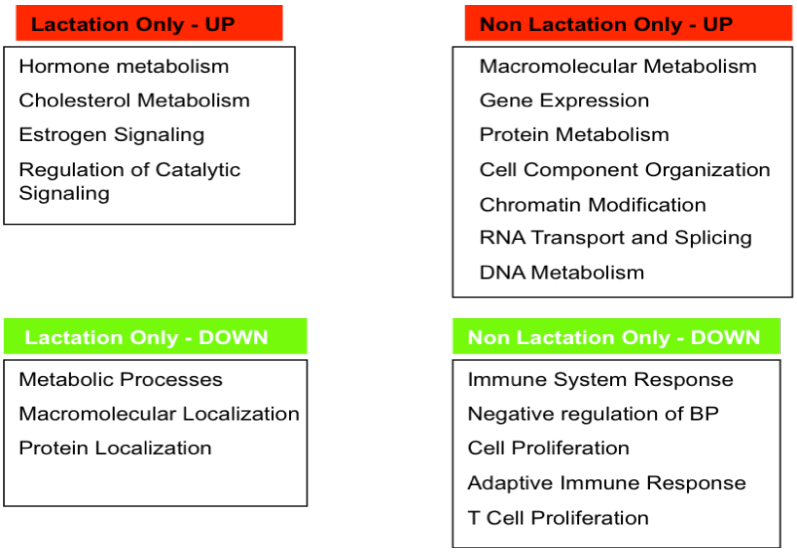

Fig 4.1
Shown are the most over represented Biological Processes (<1.0E-3) in each of the indicated categories from the expression profiling array results. All samples are compared to virgin controls. See Fig 3.3 for details.

In vivo data and gene expression profiling data

Chapter 2 documents the many maternal adaptations that occur in order to meet the many demands of lactation. These include the capacity for rapid and significant weight loss (Fig 2.2, Fig 2.3), an increase in plasma glycerol levels (Fig 2.4), an increase in maternal liver weight (Fig 2.6), an increase in plasma triglyceride levels (Fig 2.8), and a decrease in hepatic triglyceride levels (Fig 2.9). In order to examine these maternal adaptations further, we employed gene expression profiling analysis in Chapter 3. Gene expression profiling data reveal differential up regulation or down regulation of different categories of genes. Notable categories include transcription factors (Fig 3.8, Fig 3.9), hormone receptors (Fig 3.10), genes involved in lipogenesis (fig 3.16), and genes involved in triglyceride secretion (Fig 3.17). These categories contribute to the complex processes that occur during lactation which result in the many maternal adaptations that we observed. For example,

rapid weight loss upon fasting during lactation may come as a result of an increase in growth hormone. The increase in growth hormone receptor gene expression during lactation suggests an increased sensitivity to circulating growth hormone. Growth hormone has been shown to increase the mobilization of fat stores (lipolysis) (Savastano et al. 2009; Rhoads et al. 2004). The increase in lipolysis also leads to an increase in free fatty acids in the blood. The up regulation *Scd1* and *Srebf1,* for example, results in the partitioning of fatty acids in the liver towards triglyceride synthesis (as opposed to fatty acid oxidation), and results in an increase of triglyceride secretion (Ntambi and Miyazaki 2003; Im et al. 2009). Added evidence is supported by the up regulation of *Lpl, Apoe*, and *Vldlr* during lactation, genes involved in triglyceride secretion. The increase in triglyceride secretion leads to elevated plasma triglyceride levels, that provide the triglycerides that the mammary gland needs in order to make milk to sustain the suckling pups. The secretion of triglycerides leads to a decrease in hepatic triglyceride storage. This in consistent with our *in vivo* result of decreased hepatic triglyceride levels in livers of lactating mice (Fig 2.8), the latter further corroborated with histological staining (Fig 2.9). Taken together, this would suggest that the liver is highly active during lactation. It also provides evidence that the liver plays an important role in synthesizing and providing triglycerides to the lactating mammary gland.

Lactation and its effects on maternal health

A growing body of evidence from human studies suggests that lactation brings about metabolic changes that contribute to a unique maternal profile that promotes the prevention of metabolic diseases. For example, gluconeogensis is associated with diabetes (Magnusson et al. 1992). HNF4α is required in PGC1α mediated gluconeogenesis during fasting (Rhee et al. 2003). Our data show HNF4α and PGC1α sharing the same postpartum molecular signature: an increase in gene expression with non-lactation, and less of an increase in gene expression with non-lactation. In addition, PEPCK (*Pck1*) gene expression has a similar postpartum molecular signature. Taken together, our microarray data suggests that there is less gluconeogenesis with lactation. Although a decrease in gluconeogenesis might suggest lower potential risk for developing diabetes, there is no apparent hyperglycemia in our mice, since glucose levels were similar for all three conditions. What is apparent in our mouse model is the weight loss upon fasting that comes with current lactation, which could protect the currently lactating mouse against obesity, while the mouse is lactating.

Lactation, metabolic syndrome, and long term effects

Recent studies suggest that the maternal health benefits gained from lactation may have long term effects. For example, Gunderson et al (Gunderson 2009) (gunderson et al 2009) show that women who nursed their babies for a short amount of time significantly lowered their risk for developing metabolic syndrome

many years later. What's more, the risk for developing metabolic syndrome later in life is lowered the longer a woman breastfeeds. This study verifies other studies (Ram et al. 2008; Pirkola et al. 2009; Davis and Olson 2009) that point to the long term effects of lactation on maternal health. This sounds very promising. In order to further this promising outcome of lactation, we looked at weaned mice. These mice had at least 2 successful rounds of reproduction, having gone through a full term pregnancy, parturition, lactation, weaning (removal of suckling pups after 21 days of lactation), and were sacrificed at least 21 days post weaning. We looked at circulating triglyceride levels and hepatic triglyceride levels of weaned mice (Fig 4.2, Fig 4.3)

In contrast to the increase in plasma triglyceride levels that we saw in lactating mice, the plasma triglyceride levels of weaned mice significantly decreased (compared to lactating mice), or went back to virgin levels (Fig 4.2). A similar trend is apparent with hepatic triglyceride levels. Recall that hepatic trigyceride levels decreased with lactation. Hepatic triglyceride levels of weaned mice significantly increased, compared to lactating mice (Fig 4.3). Hepatic triglyceride levels of weaned mice were not significantly different from that of virgin and non-lactating mice. These data suggest that the increase in plasma triglycrides and decrease in hepatic triglyceride storage are transient.

Our results would suggest that the increase in plasma triglycerides that accompanied lactation is a transient phenomenon. Perhaps this was a way for the lactating mother to provide energy to the lactating mammary gland. In our system,

however, the lactating mice were also fasting. This is a confounding factor. Perhaps the fasting aspect increased the need for lipolysis (mobilization of fat stores), and, in the process, increased the need to secrete more triglycerides into the circulation for the mammary gland. Recall too that the time point we chose was peak lactation, where the maternal mouse was continuously lactating, the pups were continuously suckling, and so there was a dramatic need for triglycerides, an energy source. The same can be said for hepatic triglyceride levels. During lactation, there is decreased lipogenesis in adipocytes, and increased lipogenesis in the liver (Vernon 2005). It has been shown that lipogenesis in adipocytes increases when pups are taken away from the lactating mother (Vernon 1989). Perhaps in this weaned condition, the liver still synthesizes triglycerides but lessens its secretion.

In our model, the trends we saw such as increased triglyceride secretion and decreased hepatic triglyceride storage are transient. Although our model examines long term effects of lactation through the non-lactating condition, it would be of interest to examine the long term effects of pregnancy alone on the maternal liver. This study would require mice that have gone through pregnancy but have never lactated. This would enhance our understanding of the role of the liver in reproduction.

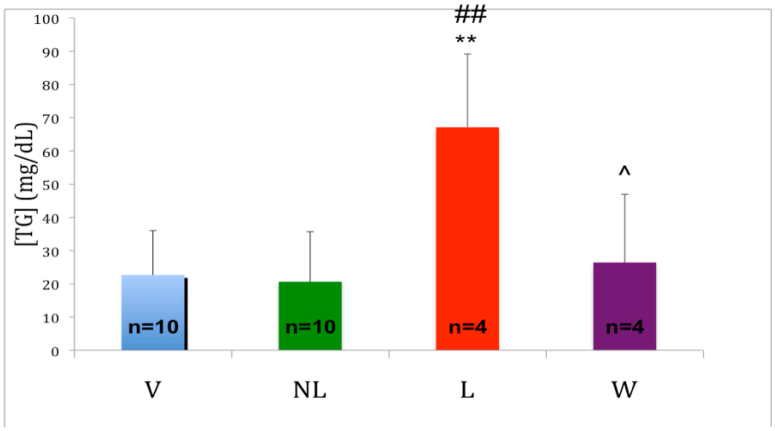

Fig 4.2: Postpartum plasma triglyceride levels (TG) of weaning mice significantly decrease (compared to levels of lactating mice).
Plasma triglyceride (TG) levels of virgin (V, blue bar, n=10), non-lactating (NL, green bar, n=10), and lactating (L, red bar, n=4). Materials and methods section in chapter 2 for details. *=NL vs V or L vs V; #=L vs NL; ^=WvsL; * or # or ^ , p<5.00E-02; ** or ## or ^^, p< 5.00E-04.

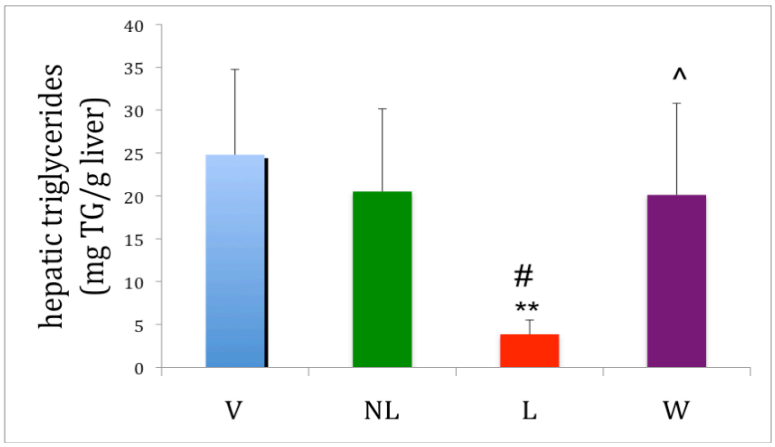

Fig 4.3: Hepatic triglyceride levels return to virgin and non-lactating levels with weaning.
Triglycerides (TG) from livers of virgin (V, blue bar, n=9), non-lactating (NL, green bar, n=9), and lactating (L, red bar, n=4). See materials and methods section in chapter 2 for details. *=NL vs V or L vs V; #=L vs NL; ^=W vs L; * or # or ^ , p<5.00E-02; ** or ## or ^^, p< 5.00E-04.

The Role of the Liver in Lactation

Our results show a highly active maternal liver during lactation. The sheer number of genes that are induced in the liver of lactating mice can attest to this. In addition, there is an increase in liver mass. An increase in liver mass has been associated with an increase in liver metabolism (Grigor et al. 1982; Reynolds et al.

2004). The up regulation of certain genes, such as growth hormone receptor (*Ghr*) and prolactin receptor (*Prlr*), mediators of hormones that play important roles in lactation, may contribute to an increase in liver mass. Our data does not exhibit cell proliferation since *Tgfa*, a factor that promotes hepatocyte proliferation, and Ki-67 (Mki67), a marker of cell proliferation is down regulated during lactation.

The liver is an important player in lipid metabolism (Grigor et al. 1982; Hayhurst et al. 2001; Bolotin, Schnabl, and Sladek 2009; Sladek and Seidel 2001). Lipid metabolism is a highly overrepresented biological process in lactation. Triglycerides, in particular, provide the energy needed by the lactating mammary gland . Our microarray data shows an increase in lipogenic genes (*Scd1* and *Srebf1*) that prefer triglyceride synthesis over fatty acid oxidation. Genes that are involved in triglyceride secretion (*Apoe, Vldl, Lpl*) are stimulated in the liver of lactating mice. Together, this results in an increase of triglyceride synthesis and triglyceride secretion in the liver, the consequences of which are increased circulating triglycerides and decreased hepatic triglyceride storage. Our data also show evidence of a decrease in gluconeogenesis, despite the fasting state of the lactating mouse, as suggested by the decrease in the up regulation of *Hnfa, Pgc1a*, and PEPCK (*Pck1)*, all important players in gluconeogenesis.

In summary, during lactation, we see a highly metabolically active liver, as evidenced by hepatic hypertrophy. Our data also suggest that the liver supplies the major source of energy to the lactating mammary gland. Some consequences of lactation include rapid weight loss upon fasting, a decrease in gluconeogenesis, an

increase in plasma triglycerides, and a decrease in hepatic triglyceride storage, but these trends are transient. The benefits to the mother brought on by lactation are only beneficial during lactation in the mouse. Further studies involving mice that have gone through pregnancy but have never lactated will enhance our understanding on the role the liver plays in lactation.

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Appendix A

Table 2: Genes up regulated at least 1.5-fold in lactating mice

Table 3: Genes down regulated at ltaeast 1.5-fold in lactating mice

Table 4: Genes up regulated at least 1.5-fold in non-lactating mice

Table 5: Genes down regulated at least 1.5-fold in non-lactation mice

A 1.5 cut-off was chosen because this is biologically relevant

Table 2: Genes up regulated at least 1.5-fold in lactating mice. Abbreviations:
affyID: annotated files from Affymetrix that identifies probeset IDs for genes on its chips.
fcVvsL: the fold change of genes in lactating mice compared to virgin mice, in non-log form.

affyid	Gene Symbol	fcVvsL
1420349_at	*Ptgfr*	55.94479534
1453924_a_at	*Ptgfr*	50.18992891
1415965_at	*Scd1*	13.32077958
1449565_at	*Cyp2g1*	11.22030498
1417268_at	*Cd14*	10.29870309
1420447_at	*Sult1e1*	8.654675869
1420379_at	*Slco1a1*	8.603027877
1421681_at	*Nrg4*	8.086997542
1439380_x_at	*Gtl2*	7.901134069
1452183_a_at	*Gtl2*	7.463535783
1428306_at	*Ddit4*	7.335056041
1449844_at	*Slco1a1*	7.230866655
1451548_at	*Upp2*	7.04807296
1434496_at	*Plk3*	6.261145056
1416927_at	*Trp53inp1*	6.198418842
1426850_a_at	*Map2k6*	5.980429523
1417273_at	*Pdk4*	5.639607976
1416258_at	*Tk1*	5.473164919
1418028_at	*Dct*	5.433977043
1424969_s_at	*Upp2*	5.364860117
1422973_a_at	*Thrsp*	5.336276966
1415964_at	*Scd1*	5.270366481
1460256_at	*Car3*	5.266677378
1451452_a_at	*Rgs16*	5.24662071
1424737_at	*Thrsp*	4.963602635
1419209_at	*Cxcl1*	4.904110018
1431214_at	*LOC100041156 /// LOC100041932*	4.788928727
1460336_at	*Ppargc1a*	4.759141003
1451612_at	*Mt1*	4.638461628
1421041_s_at	*Gsta1 /// Gsta2 /// LOC100042295*	4.635625249
1423905_at	*Pvr*	4.500879799
1417168_a_at	*Usp2*	4.499985514
1426452_a_at	*Rab30*	4.497499645
1424401_at	*LOC100047937*	4.438219136
1427425_at	*9130208E07Rik*	4.38127263
1449890_at	*Ugt2b37*	4.33714473
1427537_at	*Eppk1 /// LOC626152*	4.335375059
1460258_at	*Lect1*	4.263272932
1424273_at	*Cyp2c70*	4.260006036
1448364_at	*Ccng2*	4.243825738

1450611_at	*Orm3*	4.230069989
1421092_at	*Serpina12*	4.222228528
1450703_at	*Slc7a2*	4.099542014
1416505_at	*Nr4a1*	3.994851061
1433711_s_at	*LOC100047324 /// Sesn1*	3.964219624
1416250_at	*Btg2*	3.901244053
1423556_at	*Akr1b7*	3.892690414
1436767_at	*Luc7l2*	3.890325707
1426037_a_at	*Rgs16*	3.86996157
1451787_at	*Cyp2b10*	3.832170672
1455457_at	*Cyp2c54*	3.792071225
1421040_a_at	*Gsta2*	3.739166294
1433675_at	*Snord22*	3.699870895
1425875_a_at	*Lepr*	3.622784346
1436766_at	*Luc7l2*	3.605839083
1426444_at	*Rhbdd2*	3.598677262
1433674_a_at	*Snord22*	3.594839812
1454699_at	*LOC100047324 /// Sesn1*	3.578955155
1437751_at	*Ppargc1a*	3.572237784
1418600_at	*Klf1*	3.561286864
1416926_at	*Trp53inp1*	3.555467919
1417169_at	*Usp2*	3.543900504
1436952_at	*2310051E17Rik /// Klf9*	3.533944979
1418697_at	*Inmt*	3.533134052
1420438_at	*Orm2*	3.51854887
1455683_a_at	*Tbc1d8*	3.5150528
1448538_a_at	*D4Wsu53e*	3.485567207
1426064_at	*Cyp3a44*	3.473216469
1439244_a_at	*Tnrc6a*	3.458864393
1431722_a_at	*Afmid*	3.404126544
1436898_at	*LOC100045887 /// Sfpq*	3.400278107
1423100_at	*Fos*	3.39104584
1419069_at	*Rabgef1*	3.373036161
1425751_at	*BC014805*	3.368746388
1423196_at	*Nedd1*	3.367007623
1426936_at	*BC005512 /// EG641366 /// LOC629242*	3.357817094
1451793_at	*Klhl24*	3.320974435
1428529_at	*2810026P18Rik*	3.304607721
1436643_x_at	*Hamp2*	3.290651231
1427152_at	*Qser1*	3.251814952
1434099_at	*Ppargc1a*	3.231096011
1419430_at	*Cyp26a1*	3.22083668
1415988_at	*Hdlbp*	3.19988096

1422257_s_at	*Cyp2b10*	3.179400598
1442025_a_at	*AI467657*	3.176286019
1452426_x_at	---	3.163834303
1424607_a_at	*BC003993 /// LOC100038980 /// LOC100039088 /// LOC100039101 /// LOC100039204 /// LOC100039404 /// LOC100040136 /// LOC100040620 /// LOC100040646 /// LOC100040656 /// LOC100040790 /// LOC100040936 /// LOC100041238 /// LOC100041416 /// LOC100042151 /// LOC1*	3.149202284
1422102_a_at	*Stat5b*	3.149122012
1449434_at	*Car3*	3.142957003
1455128_x_at	*Tnrc6a*	3.134573647
1434390_at	*Hnrpu*	3.122125147
1450184_s_at	*Tef*	3.103193879
1449486_at	*Ces1*	3.085145933
1451486_at	*Slc46a3*	3.078955769
1423891_at	*Gstt3*	3.057698612
1434282_at	*Ibtk*	3.051151898
1427377_x_at	*Hsd3b3*	3.036375133
1456395_at	*Ppargc1a*	3.033987889
1425649_at	*Slc39a14*	3.010414733
1418206_at	*Sdf2l1*	3.005633507
1448997_at	*Pscd1*	2.99754425
1427371_at	*Abca8a*	2.994139574
1425645_s_at	*Cyp2b10*	2.988951099
1432004_a_at	*Dnm2*	2.981023879
1424609_a_at	*BC003993 /// LOC100038980 /// LOC100039088 /// LOC100039101 /// LOC100039204 /// LOC100039404 /// LOC100040136 /// LOC100040620 /// LOC100040646 /// LOC100040656 /// LOC100040790 /// LOC100040936 /// LOC100041238 /// LOC100041416 ///*	2.9791619

	LOC100042151 /// LOC1	
1435628_x_at	*BC005512 /// EG641366 /// LOC629242*	2.973615897
1427229_at	*Hmgcr*	2.967121932
1418301_at	*Irf6*	2.96541455
1460232_s_at	*Hsd3b2 /// Hsd3b3 /// Hsd3b6*	2.937814718
1416191_at	*Sec61a1*	2.934755527
1438931_s_at	*LOC100047324 /// Sesn1*	2.925105738
1425792_a_at	*Rorc*	2.902381767
1419572_a_at	*Abcd4*	2.896012797
1426721_s_at	*Tiparp*	2.860634744
1419283_s_at	*Tns1*	2.854351415
1416222_at	*Nsdhl*	2.854316468
1452160_at	*Tiparp*	2.844017439
1455265_a_at	*Rgs16*	2.834094966
1418288_at	*Lpin1*	2.818112259
1448950_at	*Il1r1*	2.785887436
1450505_a_at	*1810015C04Rik*	2.780735373
1425281_a_at	*Tsc22d3*	2.774639915
1425752_at	*BC014805*	2.769000932
1426516_a_at	*Lpin1*	2.766391553
1451530_at	*Egfr*	2.740583287
1416996_at	*Tbc1d8*	2.73451754
1422975_at	*Mme*	2.68879158
1416237_at	*Mpzl2*	2.687943178
1417602_at	*Per2*	2.677323055
AFFX-PyruCarbMur/L09192_5_at	*Pcx*	2.670883891
1451738_at	*Ogt*	2.669187189
1425644_at	*Lepr*	2.668995473
1439060_s_at	*Wipi1*	2.647688362
1439399_a_at	*Snord22*	2.644788454
1419559_at	*Cyp4f14*	2.643240131
1434100_x_at	*Ppargc1a*	2.636851989
1417065_at	*Egr1*	2.628259568
1421063_s_at	*LOC100044139 /// Snrpn /// Snurf*	2.625637023
1438686_at	*Eif4g1*	2.622525347
1451964_at	*Mia2*	2.620483412
1451160_s_at	*Pvr*	2.604272556
1448898_at	*Ccl9*	2.602455946
1449901_a_at	*Map3k6*	2.599061737
1430332_a_at	*Gusb*	2.588194943
1416488_at	*Ccng2*	2.575184384

1421987_at	*Papss2*	2.567272793
1436549_a_at	*Hnrpa1*	2.565237171
1452602_a_at	*1700001C19Rik*	2.55974095
1417936_at	*Ccl9*	2.556774534
1449615_s_at	*Hdlbp*	2.553538733
1432543_a_at	*Klf13*	2.548307149
1449326_x_at	*Saa2*	2.545610638
1424934_at	*Ugt2b1*	2.507011127
1449001_at	*Ivd*	2.505659253
1448241_at	*Gm2a*	2.490821263
1416190_a_at	*Sec61a1*	2.487177716
1419134_at	*Rhbg*	2.486411101
1452708_a_at	*Luc7l*	2.482826455
1452831_s_at	*Ppat*	2.479266003
1417001_a_at	*D4Wsu53e*	2.460596576
1455100_at	*Akr1d1*	2.460385236
1426184_a_at	*Pdcd6ip*	2.459452358
1423959_at	*Ropn1l*	2.45737043
1448529_at	*Thbd*	2.451265712
1416188_at	*Gm2a*	2.435780455
1424245_at	*Ces2 /// LOC667754*	2.425282963
1425483_at	*LOC100044677 /// Tox*	2.416661549
1437218_at	*Fn1*	2.412054466
1416959_at	*Nr1d2*	2.408990883
1453740_a_at	*Ccnl2*	2.406167476
1448978_at	*Ngef*	2.405901667
1420772_a_at	*Tsc22d3*	2.403872507
1446147_at	*Rbm39*	2.392684677
1425771_at	*Akr1d1*	2.388317089
1448272_at	*Btg2*	2.383656946
1418138_at	*Sult1d1*	2.366375036
1416216_at	*Reps1*	2.359095978
1438264_a_at	*Tpp2*	2.357934764
1421218_at	*Bche*	2.354203569
1415997_at	*Txnip*	2.353014739
1429144_at	*Prei4*	2.352935272
1417707_at	*B230342M21Rik*	2.350614093
1429758_at	*15-Sep*	2.348435088
1424457_at	*Apbb3*	2.329307936
1449773_s_at	*Gadd45b*	2.322148559
1448021_at	---	2.3171933
1418250_at	*Arl4d /// LOC100038842 /// LOC100044157*	2.315052344
1418007_at	*1810007M14Rik*	2.312252665
1452406_x_at	*Erdr1*	2.310061255

1430575_a_at	*Tpp2*	2.306383329
1419068_at	*Rabgef1*	2.301934922
1416062_at	*Tbc1d15*	2.300872823
1416501_at	*Pdpk1*	2.2936607
1419459_a_at	*2610529C04Rik*	2.285359994
1425686_at	*Cflar*	2.279915662
1456424_s_at	*Pltp*	2.278249785
1427037_at	*Eif4g1*	2.272717362
1425705_a_at	*Ero1lb*	2.27098213
1452125_at	*Thrap3*	2.267399766
1427546_at	*Abca8b*	2.266545701
1424942_a_at	*Myc*	2.265268359
1437667_a_at	*Bach2*	2.258496477
1452418_at	*1200016E24Rik /// LOC100039378 /// LOC100039464 /// LOC100040148 /// LOC100041150 /// LOC100041274 /// LOC100042075 /// LOC100042387 /// LOC100043154 /// LOC100043406 /// LOC100043650 /// LOC100043680 /// LOC100047599 /// LOC100048290*	2.251023088
1419089_at	*Timp3*	2.250306537
1419687_at	*Macrod1*	2.249006613
1425344_at	*Narf*	2.246571613
1433758_at	*Nisch*	2.24340782
1423418_at	*Fdps*	2.233684752
1448792_a_at	*Cyp2f2*	2.233419464
1452113_a_at	*Rab23*	2.23330441
1460420_a_at	*Egfr*	2.225768543
1423867_at	*Serpina3k*	2.224686034
1433508_at	*Klf6*	2.221146354
1434832_at	*Foxo3a*	2.216562354
1448607_at	*Pbef1*	2.212581403
1432344_a_at	*Aplp2*	2.201578273
1427089_at	*Ccnt2*	2.198708367
1424917_a_at	*Wipi1*	2.196297998
1448231_at	*Fkbp5*	2.194594813
1424412_at	*Ogfrl1*	2.191963971
1460684_at	*Tm7sf2*	2.190872467
1436506_a_at	*Snhg6*	2.189389439
1425745_a_at	*Tacc2*	2.188034408

1452416_at	*Il6ra*	2.187978736
1435169_at	*A930001N09Rik*	2.186731813
1425918_at	*Egln3*	2.183930262
1452187_at	*Rbm5*	2.183241993
AFFX-r2-Bs-lys-M_at	---	2.17767883
1452638_s_at	*Dnm1l*	2.17651559
AFFX-PyruCarbMur/L09192_MA_at	*Pcx*	2.176482776
1427472_a_at	*C8b*	2.174745707
1434278_at	*Mtm1*	2.173242902
1451788_at	*F11*	2.169749966
1425201_a_at	*Hyi*	2.166018918
1422230_s_at	*Cyp2a4 /// Cyp2a5 /// LOC100047711*	2.153167493
1418645_at	*Hal*	2.149553633
1433733_a_at	*Cry1*	2.143454617
1451577_at	*Zbtb20*	2.13534142
1421212_at	*Abcc6*	2.131613339
1420990_at	*Chd1*	2.128950484
1420973_at	*Arid5b /// LOC100044968*	2.128802207
1416661_at	*Eif3s10*	2.12235735
1455961_at	---	2.122294304
1449525_at	*Fmo3*	2.119689435
1419874_x_at	*Zbtb16*	2.118888182
1417033_at	*Ube2g2*	2.107007062
1428543_at	*Ppat*	2.105424005
1450899_at	*Nedd1*	2.099172784
1425036_a_at	*Tnrc6a*	2.094255294
1417813_at	*Ikbke*	2.085108658
1423622_a_at	*Ccnl1*	2.084526746
1419659_s_at	*Chic2*	2.083539779
1452837_at	*Lpin2*	2.083439957
1422479_at	*Acss2*	2.081467026
1418889_a_at	*Csnk1d*	2.080329962
1432436_a_at	*Ak3*	2.0739023
1419994_s_at	*D10Ertd641e*	2.07303112
1424576_s_at	*Cyp2c44*	2.072647162
1418300_a_at	*Mknk2*	2.069555329
1430271_x_at	*Josd3 /// LOC666781*	2.06898621
1421989_s_at	*Papss2*	2.068572445
1439167_at	*Pecr*	2.065550469
1424484_at	*Mobkl1b*	2.064782805
1416067_at	*Ifrd1*	2.060038072
1460498_a_at	*Dnajc5*	2.049380561
1418322_at	*Crem*	2.048349512

1437985_a_at	*2310061I04Rik*	2.047494153
1427011_a_at	*Lancl1*	2.043522423
1417254_at	*Spata5*	2.043354211
1418238_at	*Ivd*	2.042650736
1428468_at	*3110043O21Rik*	2.037876175
1416130_at	*Prnp*	2.03748216
1419677_at	*Masp1*	2.032716903
1450715_at	*Cyp1a2*	2.031117695
1443696_s_at	*Habp2*	2.028906067
1451628_a_at	*Ank3*	2.026978628
1427559_a_at	*Atf2 /// LOC100047997*	2.024120256
1425516_at	*Ogt*	2.022068819
1427134_at	*Sfrs12*	2.019670976
1434831_a_at	*Foxo3a*	2.018049826
1423198_a_at	*Smek2*	2.015582723
1418653_at	*Cyp2c50*	2.015533846
1438736_at	*Thoc2*	2.014402362
1422478_a_at	*Acss2*	2.013203574
1431721_a_at	*Proz*	2.010054784
1452720_a_at	*Fip1l1*	2.008997004
1452124_at	*Ank3*	2.005878438
1429369_at	*Tnpo3*	2.005718379
1422341_s_at	*Lypla3*	2.005029305
1419067_a_at	*Rabgef1*	2.002702578
1425576_at	*Ahcyl1*	2.001603624
1438714_at	*Zfp207*	2.001331661
1450971_at	*Gadd45b*	1.997195927
1415996_at	*Txnip*	1.996133314
1454106_a_at	*Cxxc1*	1.995194947
1427189_at	*Arih1*	1.995144628
1420622_a_at	*Hspa8*	1.99333459
1452754_at	*Creld2*	1.990090541
1448948_at	*Rag1ap1*	1.989916733
1428908_at	*Rbm25*	1.989479415
1417629_at	*Prodh*	1.989188735
1435626_a_at	*Herpud1*	1.988177467
1449374_at	*Pipox*	1.986576119
1435091_at	*Zfp568*	1.985522478
1455252_at	*Tsc1*	1.984833143
1426727_s_at	*LOC100039405 /// LOC100044621 /// LOC677319 /// Ppp1r10*	1.983087542
1425246_at	*0610008F07Rik*	1.982802204
1449558_at	*F8*	1.98230026
1426690_a_at	*Srebf1*	1.975554054

1427885_at	*Pold4*	1.973533629
1423832_at	*Prkag2*	1.969225573
1427305_at	*Piga*	1.968376844
1449405_at	*Tns1*	1.967801053
1431680_a_at	*Ptprk*	1.966829978
1448136_at	*Enpp2*	1.964854719
1419319_at	*Saa4*	1.964446266
1416022_at	*Fabp5*	1.96328124
1427932_s_at	*1200003I10Rik /// 1200015M12Rik /// 1200016E24Rik /// A130040M12Rik /// E430024C06Rik /// LOC100039464*	1.961596305
1450084_s_at	*Ivns1abp*	1.961395439
1438443_at	*Zbtb20*	1.958378279
1450006_at	*EG627557 /// Ncoa4*	1.953688542
1448420_a_at	*Fbxl12*	1.951530275
1422270_a_at	*Il6ra*	1.948693657
1422842_at	*Xrn2*	1.945992228
1428225_s_at	*Hnrpdl*	1.943774547
1426911_at	*Dsc2*	1.942846491
1430205_a_at	*Cdc37l1*	1.941084849
1422904_at	*Fmo2*	1.941027232
1418940_at	*Sult1b1*	1.940362501
1427983_at	*Suhw3*	1.936357367
1419191_at	*Hipk3 /// LOC100048439*	1.93525911
1426787_at	*Sfi1*	1.933851341
1455899_x_at	*Socs3*	1.933124024
1451927_a_at	*Mapk14*	1.9318833
1427474_s_at	*Gstm3*	1.931363507
1448973_at	*Sult1d1*	1.929703109
1450398_at	*Kcnk5*	1.928970764
1446148_x_at	*Rbm39*	1.925541448
1422850_at	*Pabpn1*	1.924379763
1426975_at	*Os9*	1.920650775
1439251_at	*LOC100042616*	1.919775464
1460290_at	*Lpin2*	1.917565721
1431274_a_at	*Hspa9*	1.916937221
1449248_at	*Clcn2*	1.91660743
1416236_a_at	*Mpzl2*	1.916357861
1426837_at	*Metap1*	1.916136023
1451212_at	*Ccdc21*	1.916101849
1418488_s_at	*Ripk4*	1.914248496
1425329_a_at	*Cyb5r3*	1.913634502
1437863_at	*Bche*	1.912836901

1425718_a_at	*Ivns1abp*	1.910430691
1422959_s_at	*Zfp313*	1.909342888
1449966_s_at	*Cab39l*	1.90728926
1421184_a_at	*Higd1c /// LOC554292 /// Mettl7a /// Ubie*	1.905234445
1416255_at	*Gja4*	1.904964994
1434393_at	*Usp34*	1.904923177
1451735_at	*Arfrp1*	1.90371901
1438397_a_at	*Rbm39*	1.901064609
1435666_at	*Mast3*	1.901056523
1423048_a_at	*Tollip*	1.900025285
1419523_at	*Cyp3a13*	1.897795814
1421912_at	*Slc23a1*	1.897445523
1424211_at	*Slc25a33*	1.895412291
1434743_x_at	*Rusc1*	1.894388216
1418470_at	*Yes1*	1.890045268
1434842_s_at	*Upf3b*	1.889129807
1455141_at	*Tnrc6a*	1.888034504
1417967_at	*Mms19l*	1.886569247
1427711_a_at	*Ceacam1*	1.883919214
1416442_at	*Ier2*	1.882989832
1417785_at	*Pla1a*	1.882976591
1422624_at	*Rev1*	1.882414529
1417496_at	*Cp*	1.881841768
1426271_at	*Smc5*	1.880412323
1425834_a_at	*Gpam*	1.880218899
1420357_s_at	*LOC100044050 /// LOC100044051 /// LOC100044094 /// LOC100044314 /// Xlr3a /// Xlr3b /// Xlr3c*	1.878321628
AFFX-18SRNAMur/X00686_3_at	---	1.877964972
1417811_at	*Slc24a6*	1.876375288
1426502_s_at	*Gpt1*	1.875451343
1423117_at	*Pum1*	1.874467473
1427797_s_at	*Ctse*	1.874377544
1422553_at	*Pten*	1.873980642
1460695_a_at	*2010111I01Rik*	1.872183162
1424400_a_at	*Aldh1l1 /// LOC100047937*	1.867668375
1421156_a_at	*Dsc2*	1.866079726
1417904_at	*Dclre1a*	1.865914217
1450934_at	*Eif4a2*	1.864650163
1452885_at	*Sfrs2ip*	1.864476667
1448256_at	*Gosr1*	1.861491551

1460180_at	*Hexb*	1.860943832
1416774_at	*Wee1*	1.860390628
1428844_a_at	*Bclaf1*	1.860137369
1423648_at	*Pdia6*	1.858337705
1436936_s_at	*Xist*	1.85742114
1418898_at	*Lin7c*	1.857236059
1425022_at	*Usp3*	1.854840228
1424932_at	*Egfr*	1.852392615
1425508_s_at	*Arfrp1*	1.851374466
1426780_at	*D14Ertd436e*	1.851037807
1428012_at	*C8a*	1.850613805
1449043_at	*Naga*	1.849072221
1417702_a_at	*Hnmt*	1.848103198
1448271_a_at	*Ddx21*	1.84617482
1419349_a_at	*Cyp2d9*	1.844282131
1453604_a_at	*Hbs1l*	1.843291254
1436443_a_at	*Kdelc1*	1.840462096
1416660_at	*Eif3s10*	1.838806023
1435635_at	*Pcmtd1*	1.83868142
1423266_at	*2810405K02Rik*	1.8377486
1423078_a_at	*Sc4mol*	1.836595449
1448456_at	*Cln8*	1.835023994
1432195_s_at	*Ccnl2*	1.831661337
1418586_at	*Adcy9*	1.830977671
1427024_at	*Slain2*	1.829318766
1450392_at	*Abca1*	1.829091497
1424570_at	*Ddx46 /// LOC100046698*	1.826971593
1424544_at	*Nrbp2*	1.826201718
1455991_at	*Ccbl2*	1.82617971
1449118_at	*Dbt*	1.826051033
1448478_at	*Med20*	1.82596973
1449334_at	*Timp3*	1.825669339
1421422_at	*5033411D12Rik*	1.825198485
1419173_at	*Acy1*	1.823127871
1448185_at	*Herpud1*	1.822170845
AFFX-PheX-M_at	---	1.820595368
1422017_s_at	*4833439L19Rik*	1.820231552
1421141_a_at	*Foxp1*	1.818584488
1427136_s_at	*Sfrs12*	1.81854473
1450744_at	*Ell2*	1.816558495
1452096_s_at	*D230025D16Rik*	1.814529039
1416662_at	*Sardh*	1.810752248
1422100_at	*Cyp7a1*	1.810751216
1419075_s_at	*Saa1*	1.80900141

1421829_at	*Ak3l1 /// LOC100047616 /// LOC635960*	1.808386499
1424022_at	*Osgin1*	1.807057202
1415899_at	*Junb*	1.80634931
1418050_at	*Gpld1*	1.80540565
1450665_at	*Gabpa*	1.804797174
1418366_at	*Hist1h2ad /// Hist1h2an /// Hist2h2aa1 /// Hist2h2aa2 /// Hist2h2ac*	1.801555314
1448193_at	*5730403B10Rik*	1.801238987
1424744_at	*Sds*	1.798372591
1421976_at	*Mmp19*	1.797878477
1450434_s_at	*Pcyt1a*	1.795137309
1423425_at	*1300012G16Rik*	1.794124448
1421907_at	*Med1*	1.793539892
1448038_at	*1810021B22Rik*	1.792249859
1421163_a_at	*Nfia*	1.791271389
1420966_at	*Slc25a15*	1.789759288
1460425_at	*1700001C19Rik*	1.787690133
1451715_at	*Mafb*	1.786804121
1427035_at	*Slc39a14*	1.785508433
1434155_a_at	*2310061I04Rik*	1.784592445
1429159_at	*Itih5*	1.783778459
1418979_at	*Akr1c14*	1.783742322
1452730_at	*Rps4y2*	1.783542242
1448233_at	*Prnp*	1.782076408
1433757_a_at	*Nisch*	1.78130496
1418435_at	*Mkrn1*	1.781244189
1421365_at	*Fst*	1.780938381
1454929_s_at	*Safb*	1.780886918
1419321_at	*F7*	1.777764671
1421889_a_at	*Aplp2*	1.777759459
1419163_s_at	*Dnajc3a*	1.776981284
1451260_at	*Aldh1b1*	1.775677634
1428021_at	*Mccc2*	1.77562731
1431606_a_at	*Angel2*	1.774028644
1451093_at	*Polr2e*	1.773046988
1424657_at	*Taok1*	1.772888743
1425837_a_at	*Ccrn4l /// LOC100047134*	1.771930401
1419506_at	*Ggps1*	1.771569688
1460164_at	*LOC100046080 /// Spin1*	1.771268983
1418490_at	*Sdsl*	1.771108847
1416467_at	*Ddx3x /// LOC100045923*	1.770801068
1425723_at	*Nr1i2*	1.770197193

1454967_at	---	1.769789209
1427319_at	*A230046K03Rik*	1.769499767
AFFX-r2-Bs-phe-M_at	---	1.768476644
1451019_at	*Ctsf*	1.767425025
1419024_at	*Ptp4a1*	1.76481911
1427345_a_at	*Sult1a1*	1.761952317
1448426_at	*Sardh*	1.761839203
1451439_at	*BC027231*	1.759369465
1455892_x_at	---	1.758130985
1419040_at	*Cyp2d22*	1.757488544
1426521_at	*D230025D16Rik*	1.75600965
1452398_at	*Plce1*	1.754990346
1418562_at	*Sf3b1*	1.753900104
1422990_at	*Met*	1.752740617
1417502_at	*Tspan7*	1.751658901
1424947_at	*Dync1li1*	1.751331085
1429003_at	*Snw1*	1.750863595
1450699_at	*LOC100044204 /// Selenbp1*	1.750528367
1450161_at	*Ikbkg*	1.749880328
1425484_at	*LOC100044677 /// Tox*	1.74894592
1459992_x_at	*Cln8*	1.748089968
1448265_x_at	*Mpzl2*	1.747068724
1418768_at	*LOC100046998 /// Opa1*	1.745979375
1417852_x_at	*Clca1*	1.745595913
1449373_at	*Dnajc3a*	1.743055791
1421622_a_at	*Rapgef4*	1.742860538
1422751_at	*Tle1*	1.742024359
1438040_a_at	*Hsp90b1*	1.741989937
1437100_x_at	*Pim3*	1.741978672
1418492_at	*Grem2*	1.738853888
1419039_at	*Cyp2d22*	1.73827297
1449382_at	*Slc6a12*	1.737787939
1427844_a_at	*Cebpb*	1.737485662
1430976_a_at	*Mrpl9*	1.736702537
1450447_at	*Hnf4a*	1.733838735
1435357_at	*D4Wsu53e*	1.733304421
1438115_a_at	*Slc9a3r1*	1.731433175
1417963_at	*Pltp*	1.727713382
1422491_a_at	*Bnip2*	1.727467074
1418640_at	*Sirt1*	1.72731476
1449375_at	*Ces6*	1.726599142
1427127_x_at	*Hspa1b*	1.726249078
AFFX-r2-Bs-phe-5_at	---	1.726207103
AFFX-PheX-5_at	---	1.725671257

1450039_at	*Usp9x*	1.725028668
1450061_at	*Enc1*	1.719674707
1453124_at	*Tnpo3*	1.718681701
1455696_a_at	*Prpf4b*	1.717182088
1427074_at	*Pcmtd2*	1.716855549
1427137_at	*Ces5*	1.71637645
1454928_at	*Safb*	1.715799759
1424326_at	*Lemd2*	1.715345031
1453128_at	*Lyve1*	1.714791096
1421411_at	*Pstpip2*	1.713854622
1437235_x_at	*Lpp*	1.712053864
1449620_s_at	*D16Wsu65e*	1.711801345
1434899_s_at	*Tnrc6a*	1.710554076
1452079_s_at	*Dcun1d1 /// LOC100046841 /// Tes3-ps*	1.70988522
1453724_a_at	*Serpinf1*	1.709071165
1449854_at	*Nr0b2*	1.707290714
1437314_a_at	*Trmt1*	1.707281031
1422845_at	*Canx*	1.70723288
1419950_s_at	*Tnpo3*	1.706730784
1424683_at	*1810015C04Rik*	1.706699568
1416051_at	*C2*	1.704800695
1459894_at	*Iqgap2*	1.70353052
1436298_x_at	*Paics*	1.703223594
1417580_s_at	*LOC100044204 /// Selenbp1*	1.703165353
1452286_at	*Slain2*	1.701899574
1421074_at	*Cyp7b1*	1.701637646
1456340_at	*2610205E22Rik*	1.700895168
1415955_x_at	*Prm1*	1.700010923
1428935_at	*Canx*	1.699658384
1451871_a_at	*Ghr*	1.696745215
1451678_at	*Narf*	1.696402542
1427798_x_at	---	1.695133249
1453474_at	*1300007F04Rik*	1.695087319
1426986_at	*2810485I05Rik*	1.694030128
1423565_at	*Paics*	1.693833697
1438000_x_at	*Zfp622*	1.690738047
1453287_at	*5730557B15Rik*	1.690696321
1421415_s_at	*Gcnt2*	1.6892375
1422866_at	*Col13a1*	1.689084619
1420727_a_at	*Tmlhe*	1.688985857
1419519_at	*Igf1*	1.688462408
1432499_a_at	*Ube4b*	1.687782568

1422477_at	*Cables1*	1.687733873
1416125_at	*Fkbp5*	1.687584745
1450970_at	*Got1*	1.686467562
1426951_at	*Crim1*	1.683888727
1427174_at	*LOC100039000 /// LOC100039123 /// LOC100043403 /// LOC100043915 /// LOC329575 /// LOC626832 /// OTTMUSG00000016327 /// RP23-232H13.5 /// RP23-330D3.3 /// RP23-376N23.4 /// RP23-442I10.2 /// RP24-562O19.2*	1.683831812
1427531_a_at	*Slc22a18*	1.681843873
1423251_at	*Luc7l2*	1.681236933
1450226_at	*Prlr*	1.680659823
1426628_at	*Tmem34*	1.679893883
1426722_at	*Slc38a2*	1.679224884
1428988_at	*Abcc3*	1.678106788
1419867_a_at	*Ankhd1*	1.677178173
1428112_at	*Armet*	1.677087096
AFFX-LysX-5_at	---	1.677086409
1427253_s_at	*Suz12*	1.677034944
1422793_at	*Pafah1b2*	1.675545755
1434697_at	*1110001P04Rik*	1.675073908
1415795_at	*LOC100046080 /// Spin1*	1.67480063
1419100_at	*Serpina3n*	1.674605071
1417190_at	*Pbef1*	1.674185724
1450788_at	*Saa1*	1.673856206
1426829_at	*Uimc1*	1.673847153
1426876_at	*4732466D17Rik*	1.673708704
1416795_at	*Cryl1*	1.673651228
1451152_a_at	*Atp1b1*	1.673423776
1418430_at	*Kif5b*	1.673053214
1425656_a_at	*Baiap2*	1.67290511
1436162_at	*C730048C13Rik*	1.670331749
1434714_at	*Ero1lb*	1.669560124
1449092_at	*LOC100045551 /// Ppm1d*	1.669138556
1427104_at	*Zfp612*	1.668887061
1428255_at	*Luc7l*	1.664228907
1448854_s_at	*Mug-ps1 /// Mug1 /// Mug2 /// Mug4*	1.662776287
1449888_at	*Epas1 ///*	1.662287141

	LOC100048537	
1448276_at	*Tspan4*	1.661975839
1418367_x_at	*Hist1h2ad /// Hist1h2an /// Hist2h2aa1 /// Hist2h2aa2 /// Hist2h2ac*	1.661801413
1449308_at	*C6*	1.661667675
1450424_a_at	*Il18bp*	1.661146028
1436316_at	*9430029L20Rik*	1.661067187
1452162_at	*Wdr48*	1.660762233
1449945_at	*Ppargc1b*	1.658408994
1448455_at	*Cln8*	1.657097942
1450915_at	*Ap3b1*	1.6569493
1425030_at	*Zfp622*	1.655959483
1451140_s_at	*Prkag2*	1.655188376
1455475_at	*3110057O12Rik*	1.654366488
1435222_at	*Foxp1*	1.654220064
1420641_a_at	*Sqrdl*	1.653698052
1426942_at	*Aim1*	1.653634162
1424367_a_at	*Homer2*	1.653574515
1426062_a_at	*Casp7*	1.65215282
1448385_at	*Slc15a4*	1.650350539
1416833_at	*Keg1*	1.649918686
1439036_a_at	*Atp1b1*	1.649249237
1431345_a_at	*Taf1b*	1.649237291
1424748_at	*Galnt11*	1.648842197
1424252_at	*Hnrpdl*	1.648440995
1420013_s_at	*Lss*	1.648293739
1429339_a_at	*Acad10*	1.646507856
1423437_at	*Gsta3*	1.645814068
1438138_a_at	*Pex6*	1.642766557
1425853_s_at	*Prlr*	1.642529988
1451415_at	*1810011O10Rik*	1.640702615
1423633_at	*6530403A03Rik*	1.63854407
1451355_at	*Asah3l*	1.638238414
1420971_at	*Ubr1*	1.637983481
1418918_at	*Igfbp1*	1.637809742
1436510_a_at	*Lrrfip2*	1.637606526
1416963_at	*Ubac1*	1.637590832
1434437_x_at	*Rrm2*	1.637368116
1419183_at	*Papd4*	1.637261539
1421679_a_at	*Cdkn1a*	1.637146794
1426243_at	*Cth*	1.636714023
1427945_at	*Dpyd*	1.636370819
1449338_at	*D10Ertd641e*	1.63428391
1417792_at	*Zfml*	1.633090622

1425319_s_at	*6530403A03Rik*	1.632807895
1416942_at	*Arts1*	1.632755504
1448769_at	*Slc35b1*	1.631841253
1456377_x_at	*Limd2 /// LOC632329*	1.629430077
1421973_at	*Gfra1*	1.626787357
1423890_x_at	*Atp1b1*	1.626672068
1428083_at	*2310043N10Rik*	1.625838668
1416493_at	*Ddost*	1.625734794
1422185_a_at	*Cyb5r3*	1.625109053
1451557_at	*Tat*	1.62462492
1422123_s_at	*Ceacam1 /// Ceacam2*	1.623817443
1438116_x_at	*Slc9a3r1*	1.623732444
1438980_x_at	*4732466D17Rik*	1.623224311
1421894_a_at	*Tpp2*	1.622853824
1449972_s_at	*BC018101*	1.622120545
1455372_at	*Cpeb3*	1.621741224
1451234_at	*BC021381*	1.621579083
1427255_s_at	*Zfp445*	1.620886582
1422551_at	*Zkscan3*	1.618681286
1426726_at	*LOC667766 /// LOC677319 /// Ppp1r10*	1.618337243
1425507_at	*Arfrp1*	1.618204045
1418453_a_at	*Atp1b1*	1.617051458
1434151_at	*Mettl7a*	1.616882987
1449269_at	*F5*	1.616667336
1422660_at	*LOC100043257*	1.616003919
1449029_at	*Mknk2*	1.615979422
1430692_a_at	*Sel1l*	1.615240262
1437801_at	*EG627352 /// LOC433598 /// LOC433955 /// LOC626309 /// Morf4l1*	1.613544816
1415830_at	*Orc5l*	1.613418312
1452429_s_at	*Abcf1*	1.612108524
1425023_at	*Usp3*	1.612051666
1416451_s_at	*Taf8*	1.610356458
1423142_a_at	*Gtpbp4*	1.610278831
1419251_at	*Eps15*	1.610188439
1453988_a_at	*Ide*	1.609903447
1426463_at	*Gphn*	1.609085553
AFFX-LysX-M_at	---	1.608440106
1418652_at	*Cxcl9*	1.604689552
1432099_a_at	*Prodh2*	1.604209417
1449816_at	*Sult5a1*	1.603554101
1418960_at	*Phf20l1*	1.601509462
1449292_at	*Rb1cc1*	1.599676177

1421075_s_at	*Cyp7b1*	1.599660701
1451494_at	*LOC100044766 /// Wac*	1.598544751
1422015_a_at	*Abcb8*	1.598199381
1427254_at	*Zfp445*	1.597827564
1426762_s_at	*Aof2 /// LOC100046934*	1.597822481
1451496_at	*Mtss1*	1.594996039
1419030_at	*Ero1l*	1.5948672
1418431_at	*Kif5b*	1.594411194
1452333_at	*Smarca2*	1.594319338
1452364_at	*Suz12*	1.594085355
1448181_at	*Klf15*	1.593822043
1450378_at	*Tapbp*	1.593444242
1417969_at	*Fbxo31*	1.592231412
1430020_x_at	*Hnrpa1*	1.591030424
1423233_at	*Cebpd*	1.59042426
1422568_at	*Ndel1*	1.590103578
1416205_at	*Glb1*	1.590042399
1416354_at	*Rbmx*	1.589918545
1452929_at	*Clip1*	1.589234153
1421324_a_at	*Akt2 /// LOC100048123*	1.589159177
1450841_at	*Stt3a*	1.588979251
1450038_s_at	*Usp9x*	1.58849471
1450901_a_at	*Smek2*	1.588452204
1419393_at	*Abcg5*	1.587495754
1439381_x_at	*Marveld1*	1.587290327
1418712_at	*Cdc42ep5*	1.585930665
1424861_at	*D930016D06Rik*	1.585030531
1419811_at	*D16Wsu65e*	1.584992548
1434987_at	*Aldh2*	1.584851221
1434272_at	*Cpeb2*	1.584665533
1421977_at	*Mmp19*	1.583454992
1424738_at	*4932432K03Rik*	1.583106817
1419528_at	*C730007P19Rik*	1.582937893
1418245_a_at	*Rbm9*	1.582615612
1427464_s_at	*Hspa5*	1.58252575
1428853_at	*Ptch1*	1.582064458
1429979_a_at	*1810073N04Rik*	1.582027531
1427929_a_at	*Pdxk*	1.581395016
1452836_at	*Lpin2*	1.581317799
1448694_at	*Jun*	1.581228024
1421013_at	*Pitpnb*	1.581204308
1421097_at	*Endog*	1.580807514
1428129_at	*Lman1*	1.580705632
1423564_a_at	*Paics*	1.580465875
1427459_at	*Cpn2*	1.577797173

1439119_a_at	*BC010304*	1.577445427
1448629_at	*Hps4*	1.577212541
1448348_at	*Caprin1*	1.576892409
1417513_at	*Evi5*	1.576386049
1452381_at	*Creb3l2*	1.576029538
1426459_s_at	*AW549877*	1.575599615
1430019_a_at	*EG434858 /// EG665646 /// Hnrpa1 /// LOC100043855 /// LOC100044632 /// LOC654467*	1.574517648
1451075_s_at	*Ctdsp2*	1.574334977
1434988_x_at	*Aldh2*	1.573982044
1450644_at	*Zfp36l1*	1.573445722
1448754_at	*LOC100045055 /// Rbp1*	1.573408029
1420922_at	*Usp9x*	1.572485608
1423738_at	*Oxa1l*	1.571249218
1451109_a_at	*Nedd4*	1.570300447
1452532_x_at	*Ceacam1*	1.570078617
1418673_at	*Snai2*	1.568820891
1452753_at	*Foxk2*	1.568358361
1423667_at	*Mat2a*	1.56826119
1425682_a_at	*Tprkb*	1.568075618
1424041_s_at	*C1s /// LOC100044326*	1.567882916
1421839_at	*Abca1*	1.567850849
1420908_at	*Cd2ap*	1.566698662
1451658_a_at	*Polr3c*	1.566557811
1419274_at	*C80913*	1.565394436
1418605_at	*Nr2c1*	1.565381463
1455305_x_at	*Hnrpa1*	1.565257539
1423143_at	*Gtpbp4*	1.565106569
1419423_at	*Stab2*	1.564720434
1455446_x_at	*Acadsb*	1.564527217
1422072_a_at	*Gstm6*	1.563458107
1418862_at	*Echdc3*	1.562126057
1439433_a_at	*Slc35a2*	1.561545015
1449321_x_at	*LOC100046187 /// LOC667951 /// Serpina1a /// Serpina1b /// Serpina1c /// Serpina1d /// Serpina1e*	1.560440824
1424862_s_at	*Mib2*	1.56042576
1426124_a_at	*Clk1*	1.559709174
1420623_x_at	*Hspa8*	1.559398489
1419553_a_at	*Rabggtb*	1.558892604
1416990_at	*Rxrb*	1.558564824

1451723_at	*Cnot6l*	1.557934556
1452268_at	*2810485I05Rik*	1.557830194
1452236_at	*Abcf1*	1.555894788
1427188_at	*Arih1*	1.553659543
1455958_s_at	*Pptc7*	1.553530224
1455002_at	*Ptp4a1*	1.553189971
1452218_at	*Ccdc117*	1.553089629
1451072_a_at	*Rnf4*	1.551565369
1451759_at	*Masp2*	1.55137259
1427631_x_at	*Mup3*	1.550100835
1449419_at	*Dock8*	1.549797247
1417409_at	*Jun*	1.549719728
1423095_s_at	*Crbn*	1.548396693
1424486_a_at	*Txnrd1*	1.548306135
1424672_at	*Dmxl1*	1.546460603
1418375_at	*Mbd6*	1.546237965
1426263_at	*Cadm4*	1.54602968
1427411_s_at	*Dleu2*	1.546023309
1434391_at	*Hnrpu*	1.545887059
1429410_at	*Eny2*	1.545759517
1428394_at	*Lrrc8a /// Phyhd1*	1.545674522
1451458_at	*Tmem2*	1.545647092
1423481_at	*Riok2*	1.545418951
1427561_a_at	*Afm*	1.545415793
1416601_a_at	*Rcan1*	1.544797751
1420088_at	*Nfkbia*	1.544732194
1451251_at	*Appbp2*	1.544441077
1450982_at	*Slc9a3r1*	1.544267426
1424452_at	*Sltm*	1.543201145
1422286_a_at	*Tgif1*	1.543197725
1425862_a_at	*Pik3c2a*	1.541935903
1427369_at	*Nlrp6*	1.541602583
1452158_at	*Eprs /// LOC633677*	1.541133788
1428087_at	*Dnm1l*	1.540659001
1450201_at	*Proz*	1.539245374
1428848_a_at	*Macf1*	1.539134482
1437772_s_at	*Fuca1*	1.538651889
1425893_a_at	*Fhit*	1.53848503
1454735_at	*LOC100047199 /// Odf2*	1.537909766
1421305_x_at	*Rabep1*	1.537207648
1416983_s_at	*Foxo1*	1.536006545
1416691_at	*Gtpbp2*	1.535899137
1452318_a_at	*Hspa1b*	1.534614743
1455789_x_at	*Hspa8*	1.534255857
1454899_at	*Lpp*	1.534029465

1427663_a_at	*Clk4*	1.533858531
1427826_a_at	*Slco1b2*	1.531792915
1448490_at	*Adck4*	1.530906153
1417706_at	*Naglu*	1.530673723
1425547_a_at	*Klc4*	1.529828917
1418017_at	*Pum2*	1.529052816
1450351_a_at	*Clip1*	1.528769325
1426008_a_at	*Slc7a2*	1.528407455
1437521_s_at	*Ammecr1l*	1.527699554
1417651_at	*Cyp2c29*	1.527599595
1434542_at	*Gpt2*	1.527355472
1450396_at	*Stag2*	1.527174899
1451296_x_at	*LOC100048654 /// Pabpc4*	1.526902324
1422468_at	*Ppt1*	1.526739833
1418896_a_at	*Rpn2*	1.526646559
1448927_at	*Kcnn2*	1.52635429
1455388_at	*Pcmtd1*	1.525707855
1451621_at	*5830417C01Rik*	1.524931676
1426365_at	*2810403A07Rik*	1.524927413
1448568_a_at	*Slc20a1*	1.52441823
1448545_at	*Sdc2*	1.52351269
1456615_a_at	*Bptf*	1.522822473
1448923_at	*Prkra*	1.522139884
1436362_x_at	*2700079J08Rik /// Ccrn4l /// LOC100040359 /// LOC100041308 /// LOC100042078 /// LOC100042092 /// LOC100043548 /// LOC100043775 /// LOC100044145*	1.522097419
1426078_a_at	*Gpr108*	1.522088923
1449335_at	*Timp3*	1.521763403
1427197_at	*Atr*	1.521261318
1421957_a_at	*Pcyt1a*	1.521022175
1436308_at	*Zfp292*	1.520722339
1427097_at	*Wwp1*	1.519115531
1418146_a_at	*LOC635075 /// Rbl2*	1.518717566
1427946_s_at	*Dpyd*	1.518271468
1415828_a_at	*D3Ucla1*	1.517789435
1427410_at	*Dleu2*	1.517524774
1451989_a_at	*Mapre2*	1.517237532
1419176_at	*Vps37a*	1.517174276
1432416_a_at	*EG668347 /// LOC100046628 /// Npm1*	1.517023684

1456080_a_at	*Serinc3*	1.517019421
1449262_s_at	*Lin7c*	1.516063892
1418427_at	*Kif5b*	1.516040468
1421064_at	*Mpp5*	1.515412015
1448761_a_at	*Copg2*	1.515233859
1434888_a_at	*Matr3*	1.515111701
1422512_a_at	*Ogfr*	1.51467509
1423371_at	*Pole4*	1.51388872
1424485_at	*Angptl3*	1.513215186
1420965_a_at	*Enc1*	1.512969801
1430289_a_at	*Wdr77*	1.512039772
1422621_at	*Ranbp2*	1.511655163
1426361_at	*Zc3h11a*	1.511634886
1427231_at	*Robo1*	1.510532444
1421940_at	*LOC100045442 /// Stag1*	1.509526081
1434510_at	*Papss2*	1.509056036
1448915_at	*Zfp524*	1.508609992
1429318_a_at	*LOC100046895 /// Qk*	1.508250927
1425577_at	*Zmym5*	1.506440938
1448810_at	*Gne*	1.505920183
1429655_at	*Nudcd1*	1.505611499
1426667_a_at	*Unc84a*	1.505491466
1422968_at	*Ihpk1*	1.504897069
1451218_at	*Edem1*	1.504688424
1456341_a_at	*2310051E17Rik /// Klf9*	1.503786963
1449862_a_at	*Pi4k2b*	1.502843623
1423194_at	*Arhgap5*	1.502130455
1426950_at	*Parp16*	1.501801323
1420630_at	*8430419L09Rik*	1.501601436
1454905_at	*Ibtk*	1.501338673
1438377_x_at	*Slc13a3*	1.500224346
1423441_at	*Tfb2m*	1.500123716
1423432_at	*Phip*	1.499801254
1426117_a_at	*Slc19a2*	1.49900764

Table 3: Genes down regulated at least 1.5-fold in lactating mice.
Abbreviations: affyID: annotated files from Affymetrix that identifies probeset IDs for genes on its chips. fcVvsL: the fold change of genes in lactating mice compared to virgin mice, in non-log form.

affyid	Gene Symbol	fcVvsL
1416055_at	*1810008N23Rik /// Amy2 /// Amy2-1 /// Amy2-2 /// LOC100043684 /// LOC100043686 /// LOC100043688*	527.2090714
1435012_x_at	*Ela3 /// LOC242711 /// LOC638198 /// LOC638418*	398.1844811
1437326_x_at	*Ela3 /// LOC638418*	369.4113804
1448281_a_at	*RP23-395H4.4*	331.700733
1433431_at	*Pnlip*	298.3060846
1422434_a_at	*2210010C04Rik*	296.4476479
1431763_a_at	*Ctrl*	281.6921475
1415954_at	*1810049H19Rik /// Prss1 /// Try10 /// Try4*	275.4665842
1448220_at	*Ctrb1*	251.4445019
1433459_x_at	*Prss2*	239.9084972
1428062_at	*Cpa1*	206.356074
1428102_at	*Cpb1*	180.0922418
1415883_a_at	*Ela3*	171.8811441
1435611_x_at	*Ela3*	139.8153501
1415805_at	*Clps*	113.1102818
1428359_s_at	*1810010M01Rik*	89.31957441
1438612_a_at	*Clps*	88.82847877
1422435_at	*2210010C04Rik*	81.76767188
1415884_at	*Ela3*	78.94854171
1416523_at	*Rnase1*	77.71395072
1415777_at	*Pnliprp1*	59.51504078
1417257_at	*Cel*	40.59883807
1415905_at	*Reg1*	38.32051292
1422916_at	*Fgf21*	31.96545626
1422144_at	*Inhbe*	28.07855705
1416666_at	*Serpine2*	19.58607536
1434089_at	*Synpo*	19.12889939
1428358_at	*1810010M01Rik*	17.37579616
1416930_at	*Ly6d*	16.75786261
1417682_a_at	*Prss2*	16.38875446
1435507_x_at	*Prss2*	16.05069803
1424528_at	*Cgref1*	15.50971984

1417812_a_at	*Lamb3*	13.1778114
1418209_a_at	*Pfn2*	11.34169547
1424529_s_at	*Cgref1*	11.05039651
1452260_at	*Cidec*	10.26852523
1418126_at	*Ccl5*	10.23475034
1433573_x_at	*Prss2*	10.21022585
1418665_at	*Impa2*	10.10136298
1421262_at	*Lipg*	8.388886056
1421430_at	*Rad51l1*	7.592310585
1425409_at	*Chrna2*	7.13807985
1449299_at	*Lrp5*	6.853171322
1448700_at	*G0s2*	6.784395326
1427660_x_at	*Cr1 /// ENSMUSG00000076577 /// Igk-C /// Igk-V21-4 /// Igk-V28 /// Igk-V8-16 /// LOC100046552 /// LOC100046793 /// LOC100047628*	6.739546659
1426959_at	*Bdh1*	6.599399651
1434137_x_at	*1810010M01Rik*	6.455117571
1452417_x_at	*2010205A11Rik /// Cr1 /// ENSMUSG00000076577 /// Igk-C /// Igk-V21-4 /// Igk-V28 /// Igk-V8-16 /// LOC100046552 /// LOC100046793 /// LOC100047628*	6.402506913
1425300_at	*Dak*	6.2417876
1417896_at	*Tjp3*	6.046398096
1424123_at	*Flvcr2*	5.068199888
1427455_x_at	*Cr1 /// ENSMUSG00000076577 /// Igk-C /// Igk-V21-4 /// Igk-V28 /// Igk-V8-16 /// LOC100046552 /// LOC100046793 /// LOC100047628*	4.885411824
1418210_at	*Pfn2*	4.852737029
1423141_at	*Lipa*	4.8363833
1423693_at	*Ela1*	4.751124282
1452336_at	*BC027382*	4.704638252
1448029_at	*Tbx3*	4.653199272
1450391_a_at	*Mgll*	4.453615871
1419394_s_at	*S100a8*	4.43732933
1427002_s_at	*Arsg*	4.251245588
1427347_s_at	*Tubb2a*	4.214088067

1422001_at	*Inhbc*	4.210992962
1424118_a_at	*Spc25*	4.143104061
1427008_at	*Rnf43*	4.083347062
1422925_s_at	*Acot3*	3.906153758
1423389_at	*Smad7*	3.892015015
1448698_at	*Ccnd1*	3.877268704
1449365_at	*Edg8*	3.856768371
1425964_x_at	*Hspb1*	3.829023002
1418181_at	*Ptp4a3*	3.819030835
1418468_at	*Anxa11 /// LOC100039484 /// LOC100039503*	3.7621731
1452277_at	*Arsg*	3.749378626
1451046_at	*LOC100047651 /// Zfpm1*	3.699230912
1425355_at	*BC018371 /// EG433604*	3.694236208
1422943_a_at	*Hspb1*	3.676174357
1423854_a_at	*Rasl11b*	3.583399024
1451361_a_at	*Pnpla7*	3.565871365
1423140_at	*Lipa*	3.546480016
1448756_at	*S100a9*	3.456049522
1426464_at	*Nr1d1*	3.420493585
1433471_at	*Tcf7*	3.354918305
1452257_at	*Bdh1*	3.33352003
1429126_at	*2600001M11Rik*	3.326232619
1460212_at	*Gnat1*	3.326081258
1448249_at	*Gpd1*	3.303356784
1418991_at	*Bak1*	3.296165331
1425927_a_at	*Atf5*	3.263303064
1427099_at	*Maz*	3.243034053
1452463_x_at	*ENSMUSG00000076577*	3.234850834
1416846_a_at	*Pdzrn3*	3.232255613
1448945_at	*Pllp*	3.216771548
1424626_at	*2010003K11Rik*	3.198653472
1417420_at	*Ccnd1*	3.190804502
1449443_at	*Decr1*	3.18011507
1448729_a_at	*5-Sep*	3.177009287
1427981_a_at	*Csad*	3.171208502
1453836_a_at	*Mgll*	3.10949332
1449072_a_at	*N6amt2*	3.100450319
1418369_at	*Prim1*	3.097324271
1427329_a_at	*Igh-6*	3.086481095
1418446_at	*LOC100045628 /// Slc16a2*	3.083812336
1449125_at	*Tnfaip8l1*	3.064612298
1418133_at	*Bcl3*	3.061454124

1449459_s_at	*Asb13*	3.059944991
1426785_s_at	*Mgll*	3.036153985
AFFX-r2-Bs-thr-5_s_at	---	3.00971431
1435275_at	*Cox6b2*	3.007661166
1424790_at	*Slc25a42*	2.991901733
1434071_a_at	*Pelo*	2.952170255
1434560_at	*Wdtc1*	2.94427658
1451124_at	*Sod1*	2.927136722
1449498_at	*Marco*	2.873952925
1423726_at	*Vat1*	2.858196041
1438654_x_at	*Mmd2*	2.854352217
1456125_a_at	*Dynll1 /// EG627788*	2.833263844
1425519_a_at	*Cd74*	2.8234555
1419761_a_at	*Gabpb1*	2.810672313
1416028_a_at	*Hn1*	2.807489948
1419365_at	*Pex11a*	2.804456548
1417568_at	*Ncald*	2.788819887
1417851_at	*Cxcl13*	2.760360888
1422537_a_at	*Id2*	2.754473012
AFFX-ThrX-5_at	---	2.743226622
1416032_at	*Tmem109*	2.735700906
1426242_at	*Polr2a*	2.690769867
1452472_at	*Rtp3*	2.681206096
1421424_a_at	*Anpep*	2.681173897
1418701_at	*Comt*	2.673171257
1416318_at	*Serpinb1a*	2.658735291
1419403_at	*BC017612*	2.6470018
1456691_s_at	*LOC100044230 /// Srd5a2l*	2.638209607
1451453_at	*Dapk2*	2.635847049
1417231_at	*Cldn2*	2.63195396
1427357_at	*Cda*	2.629749415
1418863_at	*Gata4*	2.628933537
1415865_s_at	*Bpgm*	2.623962948
1417654_at	*Sdc4*	2.622154356
1424383_at	*Tmem51*	2.613214336
1422474_at	*Pde4b*	2.610146293
1434599_a_at	*Tjp2*	2.602543664
1450843_a_at	*Serpinh1*	2.588749458
1418142_at	*Kcnj8*	2.579027906
1417219_s_at	*LOC100042319 /// LOC100045828 /// Tmsb10*	2.568607759
1455071_at	*Zbtb7b*	2.568413634

1417434_at	*Gpd2*	2.565419982
1419031_at	*Fads2*	2.563046479
1421057_at	*Dnase1l3*	2.561627723
1417008_at	*Crat*	2.559780426
1418884_x_at	*LOC100045728 /// LOC636070 /// LOC676756 /// Tuba1a*	2.544427468
1448986_x_at	*Dnase2a*	2.537812113
1425239_at	*Setd4*	2.533301332
1434329_s_at	*Adipor2*	2.525393698
1422771_at	*Smad6*	2.52210317
1450884_at	*Cd36*	2.521600674
1418739_at	*Sgk2*	2.511397681
1416246_a_at	*Coro1a*	2.502464205
1423596_at	*Nek6*	2.488640028
1423574_s_at	*Srd5a2l*	2.480635444
1417399_at	*Gas6*	2.47537136
1429033_at	*Gcc1*	2.462153304
1418438_at	*Fabp2*	2.461959804
1415840_at	*Elovl5*	2.461632357
1416204_at	*Gpd1*	2.454504726
1422432_at	*Dbi*	2.454025005
1416762_at	*S100a10*	2.448689625
1437733_at	*Eif4ebp2*	2.448595133
1424048_a_at	*Cyb5r1*	2.448453824
1423152_at	*Vapb*	2.447840976
1426733_at	*Itpk1*	2.446436843
1437211_x_at	*Elovl5*	2.444211502
1453317_a_at	*Khdrbs3*	2.441387003
1425343_at	*Hdhd3*	2.431325664
1456642_x_at	*S100a10*	2.428638608
1438422_at	*Lrrc20*	2.428277479
1448605_at	*Rhoc*	2.426074674
1434138_at	*Prune*	2.42459216
1417419_at	*Ccnd1*	2.404523357
1426418_at	*Atoh8*	2.401384901
1422473_at	*Pde4b*	2.392672153
1460316_at	*Acsl1*	2.391918399
AFFX-ThrX-M_at	---	2.374606855
1455639_at	*Slc25a39*	2.370733672
1423642_at	*Tubb2c*	2.358285896
1418164_at	*Stx2*	2.351313523
1422493_at	*Cpox*	2.349337689
1416151_at	*Sfrs3*	2.347027096

1448171_at	*Siah2*	2.342950532
1425764_a_at	*Bcat2*	2.342467813
1427079_at	*Mapre3*	2.339253933
1436902_x_at	*LOC100043712 /// LOC100047613 /// Tmsb10*	2.338456103
1452501_at	*Cyp2c38*	2.328567809
1417431_a_at	*Sphk2*	2.328227888
1427042_at	*Mal2*	2.323904526
1416946_a_at	*Acaa1a /// Acaa1b*	2.323812379
1418518_at	*Furin*	2.31611365
1434653_at	*Ptk2b*	2.315683891
1418649_at	*Egln3*	2.315589856
1423632_at	*Gpr146*	2.314837566
1426432_a_at	*Slc4a4*	2.311318802
1451079_at	*Adpgk*	2.310834389
1448019_at	*2900006A08Rik*	2.304864837
1434920_a_at	*Evl /// LOC100047333*	2.303943469
1416985_at	*Sirpa*	2.297554248
1451857_a_at	*Notum*	2.296317883
1418746_at	*Pnkd*	2.295689839
1420879_a_at	*Ywhab*	2.294676104
1450872_s_at	*Lipa*	2.289377063
1417839_at	*Cldn5*	2.289154591
1417339_a_at	*Dynll1 /// EG627788*	2.287628754
1428789_at	*Ralgps2*	2.282147265
1448382_at	*Ehhadh*	2.281398263
1416029_at	*Klf10*	2.269309439
1437723_s_at	*Derl1*	2.265615699
1428357_at	*2610019F03Rik*	2.265520727
1448651_at	*Nudt5*	2.262579247
1418925_at	*Celsr1*	2.259993444
1451321_a_at	*Rbm43*	2.259684147
1420715_a_at	*Pparg*	2.25932215
1448318_at	*Adfp*	2.25511857
1423387_at	*Psmd9*	2.251523585
1455894_at	*Ptrh2*	2.249414871
1450014_at	*Cldn1*	2.249161229
1427039_at	*Epn1*	2.248340784
1417604_at	*Camk1*	2.246838183
1417843_s_at	*Eps8l2*	2.244825201
1422997_s_at	*Acot1 /// Acot2 /// LOC100044830*	2.24224703
1415802_at	*Slc16a1*	2.23935494
1448138_at	*Ppp2r4*	2.238372449

1420679_a_at	*Aig1*	2.238361565
1435394_s_at	*Rhoc*	2.238017542
1437651_a_at	*Dtnb*	2.237924023
1427480_at	*Leap2*	2.232830102
1417819_at	*Tor1b*	2.230603432
1417991_at	*Dio1*	2.228854897
1431056_a_at	*LOC669888 /// Lpl*	2.222556868
1450903_at	*Rad23b*	2.221597981
1424239_at	*2310066E14Rik*	2.215304661
1421344_a_at	*Jub*	2.213953617
1448392_at	*LOC100046740*	2.20443315
1428954_at	*Slc9a3r2*	2.203240768
1427243_at	*Rell1*	2.197454467
1460173_at	*Lasp1*	2.196116051
1419518_at	*Tuba8*	2.194506623
AFFX-r2-Bs-thr-M_s_at	---	2.192266833
1416178_a_at	*Plekhb1*	2.192065431
1448682_at	*Dynll1*	2.191961358
1415853_at	*Def8*	2.191652856
1435493_at	*Dsp*	2.187618831
1449846_at	*Ear2 /// Ear3*	2.185243798
1417141_at	*Igtp*	2.184292576
1454993_a_at	*Sfrs3*	2.183518515
1449349_at	*Nudt1*	2.181508071
1416947_s_at	*Acaa1a /// Acaa1b*	2.175928701
1435834_at	*LOC100040608 /// LOC100046423*	2.175465938
1417703_at	*Pvrl2*	2.173068275
1453181_x_at	*Plscr1*	2.170051191
1426253_at	*4933428G09Rik*	2.16441406
1418188_a_at	*Malat1*	2.163780796
1426857_a_at	*Hsdl2*	2.161292901
1422703_at	*Gyk*	2.160249686
1456315_a_at	*Ptpla*	2.159363216
1436736_x_at	*D0H4S114*	2.158415918
1415864_at	*Bpgm*	2.153690647
1425108_a_at	*BC004728*	2.150401057
1422472_at	*Pex13*	2.145707193
1428905_at	*Rraga*	2.140783512
1448859_at	*Cxcl13*	2.137822104
1419401_at	*Asb13*	2.135852877
1418982_at	*Cebpa*	2.131211174
1427661_a_at	*Tssc4*	2.130107854
1426645_at	*Hsp90aa1*	2.12890682

1450826_a_at	*Saa3*	2.128107125
1437497_a_at	*Hsp90aa1*	2.126372717
1452743_at	*Pole3*	2.124159088
1449195_s_at	*Cxcl16*	2.123311138
1418128_at	*Adcy6*	2.120785033
1422704_at	*Gyk*	2.120504211
1433408_a_at	*Mcm10*	2.118829734
1416331_a_at	*Nfe2l1*	2.114896095
1425753_a_at	*Ung*	2.113519345
1422076_at	*Acot4*	2.112432278
1448944_at	*Nrp1*	2.111762074
1417311_at	*Crip2*	2.10803174
1450395_at	*Slc22a5*	2.105136439
1425004_s_at	*Mocs1*	2.104342682
1448170_at	*Siah2*	2.101705094
1453578_at	*Pter*	2.100476115
1417941_at	*Nanp*	2.096554478
1419964_s_at	*Hdgf*	2.093943363
1450685_at	*Arpp19*	2.093717481
1423846_x_at	*EG434428 /// LOC100041240 /// LOC100042266 /// LOC100045127 /// LOC100046947 /// Tuba1b*	2.092060253
1426856_at	*Hsdl2*	2.08949672
1452227_at	*2310045A20Rik*	2.089463513
1417365_a_at	*Calm1*	2.087187543
1436838_x_at	*Cotl1*	2.084578797
1435193_at	*A230050P20Rik*	2.084501494
1460205_at	*Dcakd*	2.076286266
1415776_at	*Aldh3a2*	2.070867955
1435494_s_at	*Dsp*	2.070259876
1417866_at	*Tnfaip1*	2.069665475
1423765_at	*Athl1*	2.06578734
1417605_s_at	*Camk1*	2.065647337
1435176_a_at	*Id2*	2.063009461
1427228_at	*Palld*	2.062930601
1433670_at	*Emp2*	2.05889773
1421447_at	*LOC100048479 /// Onecut1*	2.058816746
1451644_a_at	*0610037M15Rik /// H2-Q6 /// LOC436493*	2.054995812
1451687_a_at	*Tcf2*	2.052414009
1420632_a_at	*Bscl2*	2.0479691
1424564_at	*2410001C21Rik*	2.044463772

1415974_at	*Map2k2*	2.044358361
1426875_s_at	*Srxn1*	2.040148485
1424684_at	*Rab5c*	2.038563931
1450779_at	*Fabp7*	2.034275553
1415812_at	*Gsn*	2.03424413
1417744_a_at	*Ralb*	2.029663642
1424995_at	*6230410P16Rik*	2.029248652
1434109_at	*Sh3bgrl2*	2.024364052
1439393_x_at	*Ppp2r4*	2.022037351
1456733_x_at	*Serpinh1*	2.020717921
1450966_at	*Crot*	2.018599147
1433991_x_at	*Dbi*	2.017187776
1426607_at	*EG633640*	2.015934195
1420842_at	*Ptprf*	2.013769612
1426513_at	*Rbm28*	2.013472186
1437277_x_at	*Tgm2*	2.013290494
1424132_at	*Hras1*	2.012417675
1416156_at	*Vcl*	2.010494824
1424351_at	*Wfdc2*	2.009786075
1418213_at	*Krt23*	2.005391794
1460569_x_at	*Cldn3*	2.004684556
1452100_at	*Dullard /// LOC100048221*	2.004198385
1431833_a_at	*Hmgcs2*	2.003361427
1428635_at	*Comtd1*	2.002487706
1436783_x_at	*Ywhab*	2.001748136
1418015_at	*Pum2*	2.000932247
1417449_at	*Acot8*	2.000429743
1425439_a_at	*Slc41a3*	2.000317315
1427183_at	*Efemp1*	1.999024474
1416152_a_at	*Sfrs3*	1.996670325
1416101_a_at	*Hist1h1c*	1.996270729
1426873_s_at	*Jup*	1.994782388
1417632_at	*Atp6v0a1*	1.989928992
1424191_a_at	*Tmem41a*	1.988742486
1422807_at	*Arf5 /// LOC100046958*	1.982829729
1449442_at	*Pex11a*	1.981794839
1438058_s_at	*Ptov1*	1.981022622
1448893_at	*Ncor2*	1.980320892
1449694_s_at	*Commd5*	1.976908776
1451341_s_at	*LOC620966 /// Tmem189*	1.97666218
1460341_at	*Plekhb2*	1.97496132
1433558_at	*Dab2ip*	1.974881356
1417900_a_at	*Vldlr*	1.973421842

1417172_at	*Ube2l6*	1.973026407
1450648_s_at	*H2-Ab1*	1.960699132
1423557_at	*Ifngr2*	1.960054786
1416980_at	*Mettl7b*	1.959690769
1437932_a_at	*Cldn1*	1.957715708
1424895_at	*Gpsm2*	1.957281555
1437185_s_at	*LOC100042319 /// LOC100043712 /// LOC100045828 /// LOC100047613 /// LOC100048142 /// Tmsb10*	1.954695835
1449362_a_at	*LOC100048805 /// Mink1*	1.950633907
1455470_x_at	*Lasp1*	1.945585135
1434481_at	*4121402D02Rik*	1.941685643
1434512_x_at	*EG632248 /// Sfrs3*	1.940946743
1448985_at	*Dusp22*	1.935229863
1417232_at	*Cldn2*	1.934087324
1450622_at	*Bcar1*	1.933141028
1455976_x_at	*Dbi*	1.93100171
1425608_at	*Dusp3*	1.929809354
1437345_a_at	*Bscl2*	1.928104114
1422799_at	*Bat2*	1.926217157
1418890_a_at	*Rab3d*	1.926164849
1417473_a_at	*LOC634682 /// LOC667114 /// Ppcs*	1.923694856
1460174_at	*Dexi*	1.923317489
1448942_at	*Gng11*	1.922060482
1449660_s_at	*Coro1c*	1.92018232
1438093_x_at	*Dbi*	1.918144201
1419737_a_at	*Ldha*	1.916842958
1417655_a_at	*Ars2*	1.915414412
1416271_at	*Perp*	1.915022663
1455900_x_at	*Tgm2*	1.913805182
1448232_x_at	*EG626534 /// Tuba1c*	1.91342069
1423571_at	*Edg1*	1.913035702
1417500_a_at	*Tgm2*	1.911161718
1448848_at	*Tor1b*	1.910942314
1417793_at	*Iigp2*	1.910065775
1439368_a_at	*Slc9a3r2*	1.907006354
1425850_a_at	*Nek6*	1.906531568
1428367_at	*Ndst1*	1.903032504
1419091_a_at	*Anxa2*	1.899972612
1460218_at	*Cd52*	1.89923967
1416960_at	*B3gat3*	1.898778887
1417440_at	*Arid1a /// LOC675933*	1.895838615

1417038_at	*10-Sep*	1.895375362
1419270_a_at	*Dut*	1.893029202
1424411_at	*Tmem189*	1.889640229
1424780_a_at	*Reep3*	1.889212957
1449065_at	*Acot1 /// LOC100044830*	1.887854873
1417376_a_at	*Cadm1*	1.886226129
1421917_at	*Pdgfra*	1.884183757
1424133_at	*Tmem98*	1.882788828
1460362_at	*2410001C21Rik*	1.882567039
1424451_at	*Acaa1b*	1.881079089
1455441_at	*Map3k7*	1.880308948
1424291_at	*Nup93*	1.880039115
1424552_at	*Casp8*	1.879345683
1416435_at	*Ltbr*	1.877689816
1417027_at	*Trim2*	1.876698933
1438633_x_at	*Lasp1*	1.875716957
1417593_at	*Tusc2*	1.874648146
1439264_x_at	*Lasp1*	1.874463493
1438855_x_at	*Tnfaip2*	1.872100709
1416772_at	*Cpt2*	1.870533401
1418442_at	*Xpo1*	1.869557283
1418118_at	*Slc22a1*	1.869512118
1452210_at	*Dna2l*	1.869251415
1424182_at	*Acat1*	1.868898531
1452882_at	*Pgrmc2*	1.867298132
1424835_at	*Gstm4*	1.866021771
1431900_a_at	*Foxa3*	1.865829618
1424530_at	*Sec14l2*	1.862804262
1452359_at	*Rell1*	1.861836534
1452170_at	*2010209O12Rik*	1.86094644
1418084_at	*Nrp1*	1.860671811
1424033_at	*Sfrs7*	1.856945194
1426765_at	*Commd7 /// LOC631742 /// LOC674161*	1.856708593
1449093_at	*Ctf1*	1.855339208
1454636_at	*Cbx5*	1.854362623
1449460_at	*Asb13*	1.854325289
1452919_a_at	*1700012G19Rik*	1.854007923
1435964_a_at	*Taok3*	1.854006503
1435458_at	*Pim1*	1.853884229
1415932_x_at	*Atp9a*	1.851800265
1425921_a_at	*1810055G02Rik*	1.850994726
1424923_at	*Serpina3g*	1.850641259
1417728_at	*Mbd3*	1.850512302
1429527_a_at	*LOC433328 ///*	1.848538881

	LOC677340 /// Plscr1	
1415728_at	*Pabpn1*	1.846862875
1418486_at	*Vnn1*	1.8468366
1422718_at	*Ap3s2*	1.845842253
1428095_a_at	*Tmem24*	1.845561927
1433428_x_at	*Tgm2*	1.844756383
1428063_at	*Ankrd46*	1.844325428
1428189_at	*5730494M16Rik*	1.84343774
1449491_at	*Card10*	1.843216522
1424456_at	*Pvrl2*	1.84297801
1422508_at	*Atp6v1a*	1.842508592
1416589_at	*LOC100046740 /// Sparc*	1.841693061
1433901_at	*Caprin1*	1.839104965
1448106_at	*Necap1*	1.837834642
1448422_at	*Tmed4*	1.837160374
1417112_at	*Arl2bp*	1.83705587
1425844_a_at	*Rngtt*	1.835777413
1436994_a_at	*Hist1h1c*	1.83544989
1419054_a_at	*Ptpn21*	1.835172751
1427604_a_at	*Atp9a*	1.831547126
1418321_at	*Dci*	1.829175326
1434651_a_at	*Cldn3*	1.828334908
1427963_s_at	*Rdh9*	1.827765327
1422996_at	*Acot2*	1.822980396
1436981_a_at	*Ywhaz*	1.82285474
1460004_x_at	*Stx6*	1.822760272
1449027_at	*Rhou*	1.821898771
1435652_a_at	*Gnai2 /// LOC100045883*	1.8181512
1424425_a_at	*Mtap*	1.815821624
1424651_at	*BC021611*	1.812510445
1436946_s_at	*Gng5 /// LOC100041120 /// LOC100041703 /// LOC100043507 /// LOC100044719 /// LOC100045733 /// LOC100045948 /// LOC100047170 /// LOC100048366 /// LOC100048410*	1.808075622
1422637_at	*Rassf5*	1.808060291
1426897_at	*Rcc2*	1.807381103
1423267_s_at	*Itga5*	1.806989885
1416444_at	*Elovl2*	1.806656201
1451036_at	*LOC100048562 /// Spg21*	1.803875847
1438902_a_at	*Hsp90aa1*	1.803552146

1417769_at	*Psmc6*	1.803419111
1421009_at	*Rsad2*	1.800812144
1434879_at	*Cdc34 /// LOC100046898*	1.800785629
1460740_at	*LOC100046457*	1.799042056
1448559_at	*Flot1*	1.798789905
1448592_at	*Crtap*	1.798778913
1450643_s_at	*Acsl1*	1.795679107
1451313_a_at	*1110067D22Rik*	1.793913792
1455733_at	*Taok3*	1.791429736
1439451_x_at	*Gpr172b*	1.790974907
1454753_at	*Rnpepl1*	1.78969838
1417378_at	*Cadm1*	1.78962134
1435454_a_at	*BC006779*	1.789341762
1451037_at	*Ptpn9*	1.786231638
1449055_x_at	*Pcbp4*	1.783517102
1434836_at	*Nfatc2ip*	1.783083733
1449010_at	*Hspa4l*	1.783038458
1419648_at	*Myo1c*	1.782827725
1450018_s_at	*Slc25a30*	1.782478882
1424380_at	*Vps37b*	1.782109896
1415925_a_at	*Nup62*	1.781834339
1416609_at	*BC004004*	1.781258971
1451154_a_at	*Cugbp2*	1.780212559
1417671_at	*Scly*	1.779955754
1435417_at	*AI464131*	1.7793408
1418520_at	*LOC100038890 /// Tgoln1*	1.776283519
1450027_at	*Sdc3*	1.775240004
1448722_s_at	*Ppcs*	1.774708037
1417025_at	*H2-Eb1*	1.773988962
1423282_at	*Pitpna*	1.773097337
1431008_at	*0610037M15Rik /// H2-Q6*	1.772220205
1418359_at	*Wbscr27*	1.770355009
1449377_at	*Nicn1*	1.769375664
1426706_s_at	*Xylb*	1.768987367
1452298_a_at	*Myo5b*	1.76766776
1454698_at	*Ptplad1*	1.766285612
1418014_a_at	*B4galt1*	1.76368456
1451058_at	*Mcts2*	1.763349268
1427943_at	*Acyp2*	1.763316515
1424040_at	*Mtap7d1*	1.761394313
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1435981_at	---	1.75872123
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1417377_at	*Cadm1*	1.758215163
1417272_at	*9130005N14Rik*	1.757270099
1437331_a_at	*Arf3*	1.755830178
1438670_at	*Ptpn1*	1.754593979
1455422_x_at	*5-Sep*	1.752617994
1448180_a_at	*Hn1*	1.748852369
1417374_at	*Tuba4a*	1.748679544
1420541_at	*Rdh16*	1.747495394
1449403_at	*Pde9a*	1.746535502
1416924_at	*Bri3*	1.744193708
1425028_a_at	*Tpm2*	1.743227467
1449718_s_at	*4930453N24Rik*	1.742933525
1417688_at	*BC004044*	1.742716579
1448239_at	*Hmox1*	1.74225102
1422780_at	*Pxmp4*	1.742039434
1438091_a_at	*H2afz /// LOC626950*	1.740304285
1454870_x_at	*Gpr172b*	1.740165111
1416321_s_at	*Prelp*	1.739104038
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1435448_at	*Bcl2l11*	1.737105422
1450044_at	*Fzd7*	1.736830765
1435376_at	*Ddhd2*	1.736550424
1422124_a_at	*Ptprc*	1.736317547
1460178_at	*Lonp2*	1.734561052
1452201_at	*2310047B19Rik*	1.734263208
1448300_at	*Mgst3*	1.733536729
1419103_a_at	*Abhd6*	1.732862849
1433512_at	*Fli1*	1.731493236
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1419573_a_at	*Lgals1*	1.7311367
1426982_at	*Flywch1*	1.729824662
1455556_at	*Notch2*	1.72958906
1439005_x_at	*Ywhaz*	1.7295608
1448546_at	*Rassf3*	1.7290374
1424463_at	*2210010L05Rik*	1.725568997
1417761_at	*Apoa4*	1.725320417
1435243_at	*Zfp746*	1.725298534
1434472_at	*Dusp3*	1.725220632
1422147_a_at	*Pla2g6*	1.724461927
1448648_at	*9130005N14Rik*	1.722757235
1423045_at	*Ncbp2*	1.720033378
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1422077_at	*Acot4*	1.709723049
1435872_at	*Pim1*	1.708527645
1424358_at	*Ube2e2*	1.708363251
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1419115_at	*Alg14*	1.706458828
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1435527_at	*Nfic*	1.663280663
1434465_x_at	*Vldlr*	1.662756765
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1416150_a_at	*Sfrs3*	1.662405444
1416540_at	*Hgs*	1.662183029
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1454725_at	*Tra2a*	1.661592297
1433493_at	*5730472N09Rik*	1.661245138
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1436737_a_at	*Sorbs1*	1.656528581
1432848_a_at	*1200004M23Rik*	1.655166292
1415677_at	*Dhrs1*	1.655063011

1422457_s_at	*Sumo3*	1.654472116
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1425778_at	*Indol1*	1.652537249
1456011_x_at	*Acaa1a*	1.652163409
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1437423_a_at	*Sra1*	1.651155444
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1423584_at	*Igfbp7*	1.644580776
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1448668_a_at	*Irak1*	1.63489652
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1416017_at	*Copg*	1.633995246
1417879_at	*Nenf*	1.633686164
1416548_at	*Slc35b4*	1.630755889
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1425610_s_at	*Galnt2*	1.62378174
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1449009_at	*LOC100039796 /// Tgtp*	1.623162204
1422730_at	*Limd1*	1.622495271
1419568_at	*Mapk1*	1.620986345
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1428580_at	*Blvra*	1.620447932
1416268_at	*Ets2*	1.619864076
1438769_a_at	*Thyn1*	1.619467932
1460346_at	*Arsa*	1.619415937
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1418011_a_at	*Sh3glb1*	1.616057525
1452182_at	*Galnt2*	1.615280737
1429582_at	*Btbd14a*	1.615189982
1418258_s_at	*Dynll2*	1.61454138
1452062_at	*Prpsap2*	1.614392048
1418117_at	*Ndufs4*	1.613170438
1422499_at	*Lima1*	1.611482417
1424329_a_at	*Prrg2*	1.61115298
1427003_at	*Ppp2r5c*	1.610460807
1426435_at	*Tmem135*	1.610388598
1436339_at	*1810058I24Rik*	1.609410589
1417267_s_at	*Fkbp11*	1.60797995
1428265_at	*Ppp2r1b*	1.607082987
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1439964_at	*Tmem170*	1.60647493
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1417569_at	*Ncald*	1.605660939
1417292_at	*Ifi47*	1.605576051
1426473_at	*Dnajc9*	1.605467979
1438164_x_at	*Flot2*	1.605380495
1437908_a_at	*Ergic1*	1.605215389
1418603_at	*Avpr1a*	1.604654421
1419256_at	*Spnb2*	1.604574529
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1433658_x_at	*Pcbp4*	1.602792866
1448718_at	*2400001E08Rik*	1.601687254
1426756_at	*Galnt2*	1.600132204
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1417084_at	*Eif4ebp2*	1.598285765
1424611_x_at	*Trub2*	1.597940104
1422593_at	*Ap3s1*	1.597485834
1449054_a_at	*Pcbp4*	1.597474943
1426804_at	*Smarca4*	1.595759381
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1426965_at	*Rap2a*	1.595201328
1421267_a_at	*Cited2*	1.594580688
1434642_at	*Hsd17b11*	1.593991375
1417544_a_at	*Flot2*	1.593784117
1420834_at	*Vamp2*	1.593736384
1424370_s_at	*Psmf1*	1.591230502
1418058_at	*Eltd1*	1.591184498
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1424877_a_at	*Alad /// LOC100046072*	1.584148128
1422492_at	*Cpox*	1.583548624
1416556_at	*Tspan31*	1.582671403
1426693_x_at	*Cox15*	1.582188404
1427082_at	*4632417N05Rik*	1.582125219
1422819_at	*Mrpl36*	1.580885556
1417535_at	*Fbxo25*	1.580785199

1425824_a_at	*Pcsk4*	1.580436273
1433514_at	*Etnk1*	1.579863227
1425603_at	*Tmem176a*	1.57974531
1434020_at	*Pdap1*	1.579493272
1434886_at	*Opa3*	1.578405487
1436504_x_at	*Apoa4*	1.578101222
1423566_a_at	*Hsp110*	1.577388027
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1421943_at	*Tgfa*	1.575624887
1438644_x_at	*Commd9*	1.575316479
1420829_a_at	*Ywhaq*	1.571987859
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1426988_at	*Klhdc5*	1.571497051
1428172_at	*Prpf39*	1.570573267
1416389_a_at	*Rcbtb2*	1.570138847
1451896_a_at	*Cherp*	1.569754079
1434827_at	*Thoc6*	1.569305156
1438156_x_at	*Cpt1a*	1.569258658
1452146_a_at	*Cox15*	1.569071048
1451918_a_at	*Loh12cr1*	1.568668134
1424904_at	*1300010F03Rik*	1.566971673
1425616_a_at	*Ccdc23*	1.566703236
1436959_x_at	*Nelf*	1.566366537
1427005_at	*Plk2*	1.565142428
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1451721_a_at	*H2-Ab1*	1.563368264
1419061_at	*Rhod*	1.563342896
1449818_at	*Abcb4*	1.562929275
1425986_a_at	*Dcun1d1*	1.56261584
1421858_at	*Adam17*	1.562464476
1438365_x_at	*Laptm4b*	1.562273607
1416458_at	*Arf2*	1.561754313
1419455_at	*Il10rb*	1.561714164
1419275_at	*Dazap1*	1.560140039
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1450919_at	*Mpp1*	1.559497806
1423680_at	*Fads1*	1.559138356
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1417369_at	*Hsd17b4*	1.556348278
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1425351_at	*Srxn1*	1.556322918
1453198_at	*LOC100043468 /// Zfp422-rs1*	1.55607228
1428465_at	*Tmem147*	1.555269221
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1424293_s_at	*Tmem55a*	1.554140856
1451197_s_at	*Gatad2a /// LOC100047029*	1.554069578
1426987_at	*5430417L22Rik*	1.552774564
1439241_x_at	*LOC100044230 /// Srd5a2l*	1.552375827
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1424082_at	*Tbc1d13*	1.550287799
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1433582_at	*1190002N15Rik /// LOC100044725*	1.549371908
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1423971_at	*Thoc3*	1.547429844
1417271_a_at	*Eng*	1.547340407
1438963_s_at	*Tfpt*	1.546191291
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1433803_at	*Jak1*	1.542321191
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1433666_s_at	*Vps41*	1.540207058
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1421910_at	*Tcf20*	1.539053053
1418893_at	*Pbx2*	1.538470351
1431469_a_at	*Cxxc5*	1.5377319
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1450629_at	*Lima1*	1.537105125
1449641_at	*Adk*	1.536871546
1455206_at	*C130006E23*	1.536746738
1423883_at	*Acsl1*	1.536168015
1452272_a_at	*Gfer*	1.536093506
1421534_at	*Dfna5h*	1.53588836
1426902_at	*Coq6*	1.535808259
1416016_at	*Tap1*	1.53409957
1424908_at	*Mtfmt*	1.533215744
1419751_x_at	*AB056442*	1.532339042
1438656_x_at	*Timm17b*	1.532227028
1417466_at	*Rgs5*	1.531291669
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1424147_at	*Ahsa1*	1.530182772
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1452354_at	*2810459M11Rik*	1.527721545
1424602_s_at	*LOC100044170 /// Xrcc4*	1.527454662
1416395_at	*Guk1*	1.527401631
1424497_at	*1110032O16Rik*	1.52692164
1460633_at	*Prpf19*	1.526463155
1435270_x_at	*N6amt2*	1.525628041
1448463_at	*4933434E20Rik*	1.5255125
1425596_at	*AI317395*	1.525296302
1424720_at	*Mgat4b*	1.52515146
1450097_s_at	*LOC100048021*	1.524867236
1417508_at	*Rnf19*	1.524804655
1425676_a_at	*Elovl1*	1.524492518
1460318_at	*Csrp3*	1.524467729
1449317_at	*Cflar /// LOC100040853 /// LOC100046787*	1.523651039

1423547_at	*Lyzs*	1.523597713
1423087_a_at	*1110002E23Rik /// LOC100047839*	1.52357717
1428494_a_at	*Polr2i*	1.522043434
1460348_at	*Mad2l2*	1.521991749
1425983_x_at	*Hipk2*	1.521236853
1437419_at	*Bmp2k*	1.520858202
1420841_at	*Ptprf*	1.520848254
1424585_at	*Ranbp10*	1.520846639
1424697_at	*Dtwd1*	1.520565961
1448969_at	*Ftsj2*	1.520470893
1424108_at	*Glo1*	1.52026312
1433597_at	*9430010O03Rik*	1.520239835
1421654_a_at	*Lmna*	1.519988401
1425120_x_at	*1810023F06Rik*	1.519455072
1460590_s_at	*LOC100039786 /// LOC664895 /// LOC676123 /// Ywhaq*	1.51887073
1415760_s_at	*Atox1*	1.518146486
1415687_a_at	*Psap*	1.517337606
1459890_s_at	*1110008P14Rik*	1.517315837
1448822_at	*LOC100048803 /// Psmb6*	1.516335951
1423447_at	*Clpx*	1.516328163
1422917_at	*Epha1*	1.515614669
1416400_at	*Pycrl*	1.515152688
1452173_at	*Hadha*	1.514445802
1427245_at	*Arfgap1*	1.513421841
1433486_at	*Clcn3*	1.513298499
1427201_at	*Mustn1*	1.513297149
1426373_at	*Ski*	1.513130031
1416632_at	*LOC630951 /// LOC677317 /// Mod1*	1.513035071
1451168_a_at	*Arhgdia*	1.512988819
1424062_at	*Ube2d1*	1.512535157
1448123_s_at	*Tgfbi*	1.511367065
1416907_at	*Tsn*	1.511298052
1451303_at	*BC002230*	1.511282168
1428554_a_at	*1810035L17Rik*	1.511200472
1417585_at	*Nup210*	1.510280551
AFFX-MURINE_b1_at	---	1.509562871
1436012_s_at	*Scrn2*	1.509198171
1436342_a_at	*D19Ertd721e*	1.508933085
1448539_a_at	*Acy3*	1.508764364
1416320_at	*Sec22a*	1.50842826

1428160_at	*Ndufab1*	1.50736955
1429005_at	*Mfhas1*	1.507105104
1460639_a_at	*Atox1*	1.506873158
1454862_at	*Phldb2*	1.506776112
1428257_s_at	*Dynlrb1*	1.506504979
1420984_at	*Pctp*	1.506204682
1418496_at	*Foxa1 /// LOC100047556*	1.506202927
1425532_a_at	*Bin1*	1.505697378
1428708_x_at	*Ptms*	1.505469735
1424726_at	*Tmem150*	1.504809201
1417412_at	*F8a*	1.504772991
1433655_at	*Rnf141*	1.504011931
1427957_at	*9530008L14Rik*	1.503627166
1428657_at	*Rreb1*	1.503401656
1422155_at	*Hist2h3c2*	1.50248001
1422484_at	*Cycs*	1.502461125
1420172_at	*---*	1.502395893
1451680_at	*Srxn1*	1.502194229
1434276_x_at	*BC004004*	1.502111541
1433706_a_at	*Ptplad1*	1.501770898
1427060_at	*Mapk3*	1.501015973
1434148_at	*Tcf4*	1.500966703
1421847_at	*Wsb2*	1.500874164
1417772_at	*Grhpr*	1.500624533
1416810_at	*Mea1*	1.500556408
1451563_at	*Emr4*	1.500296389
1452307_at	*Cables2*	1.499562079
1425993_a_at	*Hsp110*	1.499363873
1437226_x_at	*Marcksl1*	1.499362084
1431591_s_at	*Isg15 /// LOC100038882 /// LOC100044225 /// LOC677168*	1.498151105
1417068_a_at	*Ptpn1*	1.497962152

Table 4: Genes up regulated at least 1.5-fold in non-lactating mice. Abbreviations: affyID: annotated files from Affymetrix that identifies probeset IDs for genes on its chips. fcVvsNL: the fold change of genes in non-lactating mice compared to virgin mice, in non-log form.

affyid	Gene Symbol	fcVvsNL
1420349_at	*Ptgfr*	43.47535413
1421912_at	*Slc23a1*	30.66288222
1453924_a_at	*Ptgfr*	30.66288222
1428306_at	*Ddit4*	15.18551709
1449862_a_at	*Pi4k2b*	15.18551709
1427001_s_at	*Hnf4a*	12.85202734
1453752_at	*Rpl17*	11.9673676
1416010_a_at	*Ehd1*	10.36519371
1423556_at	*Akr1b7*	10.36270468
1422102_a_at	*Stat5b*	9.957268376
1433570_s_at	*Mak10*	8.674516703
1423191_at	*Fnbp4*	8.495354535
1416756_at	*Dnajb1*	6.734997848
1415703_at	*Huwe1*	6.499333885
1424996_at	*Cflar*	6.498825681
1452627_at	*LOC100048528 /// Senp6*	5.93107577
1449615_s_at	*Hdlbp*	5.903115016
1437610_x_at	*Rps8*	5.84025782
1418275_a_at	*Elf2*	5.708973627
1423838_s_at	*2400003C14Rik*	5.691487383
1420497_a_at	*Cebpz*	5.635713435
1428129_at	*Lman1*	5.58247585
1416868_at	*Cdkn2c*	5.306991144
1455991_at	*Ccbl2*	5.282349852
1436763_a_at	*2310051E17Rik /// Klf9*	5.205893241
1449565_at	*Cyp2g1*	5.123748848
1448715_x_at	*Ccrn4l /// Cog6 /// ENSMUSG00000073624 /// LOC100043821 /// Sgip1*	4.942761241
1420033_s_at	*Ppp2r2d*	4.870162582
1428502_at	*Actr6*	4.708245584
1455540_at	*Cps1*	4.674885051
1448639_a_at	*Spata5*	4.670733092
1416189_a_at	*Sec61a1*	4.641470474
1421955_a_at	*Nedd4*	4.561716258
1416940_at	*Ppif*	4.544865665
1437845_x_at	*Pofut2*	4.506625676
1421075_s_at	*Cyp7b1*	4.454265157
1448324_at	*LOC100040661 ///*	4.213043695

	LOC100044797 /// Rnps1	
1426426_at	*Rbm13*	4.206111544
1449732_at	*Zscan21*	4.203282365
1416570_s_at	*Gfm1*	4.196544694
1425518_at	*Rapgef4*	4.132219891
1418834_at	*Cno*	4.126754964
1417663_a_at	*Ndrg3*	4.054467426
1460356_at	*Esam1*	3.992743609
1452220_at	*Dock1*	3.989811023
1415708_at	*Tug1*	3.988912209
1424723_s_at	*Cstf3*	3.921073956
1416755_at	*Dnajb1*	3.911839242
1426408_at	*Cugbp1*	3.903224984
1415965_at	*Scd1*	3.899652575
1422491_a_at	*Bnip2*	3.786196995
1426011_a_at	*Ggnbp2*	3.764879854
1448900_at	*D16H22S680E*	3.74196715
1450104_at	*Adam10*	3.653433229
1426117_a_at	*Slc19a2*	3.653041582
1449151_at	*Pctk3*	3.63188893
1418512_at	*Stk3*	3.594437082
1427255_s_at	*Zfp445*	3.570154142
1417166_at	*Psip1*	3.531948195
1449800_x_at	*Phf7*	3.52978842
1460338_a_at	*Crlf3*	3.523003038
1415916_a_at	*Mthfd1*	3.516453253
1426380_at	*Eif4b*	3.504205842
1448990_a_at	*Myo1b*	3.502594593
1425837_a_at	*Ccrn4l /// LOC100047134*	3.497109112
1434256_s_at	*Cds2*	3.494640555
1431359_a_at	*1110007C09Rik*	3.494200915
1418586_at	*Adcy9*	3.444362199
1419704_at	*Cyp3a41a /// LOC100041375*	3.415090765
1453729_a_at	*Rpl37*	3.413739837
1437210_a_at	*Brd2 /// LOC100044024*	3.396314775
1452997_at	*6820431F20Rik*	3.394712201
1424570_at	*Ddx46 /// LOC100046698*	3.360679474
1433757_a_at	*Nisch*	3.346559665
1423371_at	*Pole4*	3.303336587
1452462_a_at	*Banp*	3.296263095
1459546_s_at	*Enpp1*	3.280738084
1449107_at	*LOC100048122 /// Nudt4*	3.278589303

1422793_at	*Pafah1b2*	3.27545811
1418168_at	*Zcchc14*	3.263750651
1455826_a_at	*Bace1*	3.262867877
1438625_s_at	*Pctk1*	3.224102157
1416060_at	*Tbc1d15*	3.214830953
1422636_at	*Dmtf1*	3.186464935
1451536_at	*Mtfr1*	3.176418694
1431804_a_at	*Sp3*	3.159080628
1435524_at	*2010109N14Rik*	3.107632589
1426254_at	*Tm2d1*	3.103099654
1451980_at	*Casd1 /// LOC100045658*	3.07910581
1418144_a_at	*Pip5k1a*	3.064885823
1428362_at	*Eif4g2*	3.04021745
1451496_at	*Mtss1*	3.015581711
1449200_at	*Nup155*	3.005719468
1448657_a_at	*Dnajb10*	2.999712796
1436991_x_at	*Gsn*	2.994434402
1432499_a_at	*Ube4b*	2.970714999
1449932_at	*Csnk1d*	2.965348474
1450392_at	*Abca1*	2.965109374
1424280_at	*Mospd1*	2.952385988
1435881_at	*Pcbp2*	2.948863835
1426394_at	*Eif3j /// LOC100042807 /// LOC100044332*	2.948718858
1433482_a_at	*Fubp1*	2.947664868
1418007_at	*1810007M14Rik*	2.941257436
1427297_at	*Mrpl9*	2.930507864
1424656_s_at	*Usp19*	2.930104634
1416600_a_at	*Rcan1*	2.912186431
1433924_at	*Peg3*	2.904276639
1448793_a_at	*Sdc4*	2.895198745
1442025_a_at	*AI467657*	2.874241721
1420961_a_at	*Ivns1abp*	2.863202806
1423782_at	*Mobkl1b*	2.858064285
1419650_at	*Zfr*	2.850069015
1436834_x_at	*Mdh1*	2.835914103
1416501_at	*Pdpk1*	2.824680897
1451577_at	*Zbtb20*	2.821674255
1416182_at	*Apba3*	2.81346871
1419835_s_at	*Plec1*	2.81298187
1429246_a_at	*Anxa6*	2.795214267
1426381_at	*Pprc1*	2.79504055
1449405_at	*Tns1*	2.795008515
1454754_a_at	*Aamp*	2.794540469

1426689_s_at	*Sdha*	2.786132136
1421972_s_at	*Hcfc1*	2.778701894
1424116_x_at	*Ppp5c*	2.765892604
1450084_s_at	*Ivns1abp*	2.762703209
1450489_at	*Sall1*	2.749614594
1448014_s_at	*LOC100039616 /// LOC100048006 /// Usp24*	2.739942829
1421987_at	*Papss2*	2.738957658
1437027_x_at	*LOC100040661 /// LOC100044797 /// Rnps1*	2.722994722
1416215_at	*Gosr1*	2.715163028
1454955_at	*Ipo7*	2.714460434
1426936_at	*BC005512 /// EG641366 /// LOC629242*	2.712383573
1422842_at	*Xrn2*	2.711983592
1455958_s_at	*Pptc7*	2.702671171
1428810_at	*2700097O09Rik*	2.670754602
1417875_at	*Ddx50*	2.666804181
AFFX-PyruCarbMur/L09192_MB_at	*Pcx*	2.658199375
1426700_a_at	*Usp52*	2.656206255
1424247_at	*Erc1*	2.650290432
1426331_a_at	*Frmd4b*	2.607926138
1417629_at	*Prodh*	2.602145808
1456352_a_at	*Sf3b2*	2.594752342
1425466_at	*Senp2*	2.590772481
1427127_x_at	*Hspa1b*	2.590248325
1426792_s_at	*Rusc2*	2.583797543
1428226_at	*Ngdn*	2.578812886
1448175_at	*Ehd1*	2.576007387
1437377_a_at	*Polrmt*	2.570542802
1439271_x_at	*Ik*	2.567597796
1449620_s_at	*D16Wsu65e*	2.564998695
1460335_at	*Lysmd3*	2.556474717
1431213_a_at	*LOC100041156 /// LOC100041932*	2.555240985
1450655_at	*Pten*	2.546824746
1427058_at	*Eif4a1*	2.528904964
1429473_at	*1200003I07Rik*	2.526080346
1433850_at	*LOC627908 /// LOC673151 /// Ppp4r2*	2.525227659
1428280_at	*Fip1l1*	2.517019836
1434542_at	*Gpt2*	2.516093997
1438397_a_at	*Rbm39*	2.514988747
1422490_at	*Bnip2*	2.511530836

1448456_at	*Cln8*	2.506430195
1427971_at	*Cdc73*	2.504367259
1416140_a_at	*Dhx30*	2.497561644
1423163_at	*Bat4*	2.486679051
1427248_at	*Whsc2*	2.474834876
1423659_a_at	*Tbc1d17*	2.471444246
1426831_at	*Ahcyl1*	2.468546649
1427074_at	*Pcmtd2*	2.463035596
1427460_at	*LOC100046932 /// Taf4a*	2.462933205
1452753_at	*Foxk2*	2.462406894
1423430_at	*Mybbp1a*	2.45832132
1451452_a_at	*Rgs16*	2.452748167
1437626_at	*Zfp36l2*	2.448818269
1433871_at	*R3hdm1*	2.447410141
1422953_at	*Fpr-rs2*	2.444839175
1426635_at	*Acbd3*	2.439472492
1427546_at	*Abca8b*	2.42997918
1418901_at	*Cebpb*	2.42538159
1460241_a_at	*St3gal5*	2.424589856
1449851_at	*Per1*	2.414149622
1422959_s_at	*Zfp313*	2.414061169
1424607_a_at	*BC003993 /// LOC100038980 /// LOC100039088 /// LOC100039101 /// LOC100039204 /// LOC100039404 /// LOC100040136 /// LOC100040620 /// LOC100040646 /// LOC100040656 /// LOC100040790 /// LOC100040936 /// LOC100041238 /// LOC100041416 /// LOC100042151 /// LOC1*	2.41277919
1460646_at	*Csnk2a2*	2.399617277
1423144_at	*Pik3ca*	2.396573226
1450980_at	*Gtpbp3*	2.394952642
1415869_a_at	*Trim28*	2.386986433
1435626_a_at	*Herpud1*	2.374980416
1450628_at	*Slc2a8*	2.372490218
1422017_s_at	*4833439L19Rik*	2.366299135
1419030_at	*Ero1l*	2.360421522
1452372_at	*Bsdc1*	2.357837841
1451251_at	*Appbp2*	2.354055686
1428529_at	*2810026P18Rik*	2.345782983

1423432_at	*Phip*	2.345688862
1451977_at	*Dyrk1a*	2.344493962
1417090_at	*Rcn1*	2.336276283
1418237_s_at	*Col18a1*	2.33618306
1454631_at	*Gtf2a1*	2.319524186
1460705_at	*Rps6kb1*	2.314580577
1416354_at	*Rbmx*	2.312363595
1456433_at	*Rcbtb1*	2.307097256
1452708_a_at	*Luc7l*	2.299103358
1423961_at	*LOC100045629*	2.296793837
1426290_at	*Dimt1*	2.293504277
1426516_a_at	*Lpin1*	2.29042638
1449335_at	*Timp3*	2.282299599
1415715_at	*Tmem129*	2.273253556
1431805_a_at	*Rhpn2*	2.269503432
1418896_a_at	*Rpn2*	2.266364113
1460691_at	*Zfp598*	2.266112524
1448568_a_at	*Slc20a1*	2.260080734
1433443_a_at	*Hmgcs1 /// LOC100040592*	2.248626409
1434035_at	*Dnajb6 /// LOC100039994 /// LOC100048324*	2.246601441
1453015_at	*4933407C03Rik*	2.242050126
AFFX-PheX-5_at	---	2.241794104
1417737_at	*Mrps31*	2.238552959
1423978_at	*Sbk1*	2.236289943
1452186_at	*Rbm5*	2.235761618
1448135_at	*Atf4*	2.22941127
AFFX-PyruCarbMur/L09192_MA_at	*Pcx*	2.221149705
1437751_at	*Ppargc1a*	2.21498663
1417254_at	*Spata5*	2.212129268
1420481_at	*Cnnm3*	2.211072966
1422862_at	*Pdlim5*	2.205636189
1418655_at	*B4galnt1*	2.202747266
1452276_at	*Smarcad1*	2.194899132
1428267_at	*Dhx40*	2.189906136
1418127_a_at	*Aifm1*	2.188309919
1419281_a_at	*Zfp259*	2.183765121
1427132_at	*Sbf2*	2.179792864
1420651_at	*Ate1*	2.177488888
1451188_at	*LOC100045629 /// Wdr26*	2.176705907
1423773_at	*Gpbp1*	2.176565695
1420640_at	*Jmy*	2.174410469

1460645_at	*Chordc1*	2.172953163
1448763_at	*Atad1*	2.170953387
1428766_at	*Rnmtl1*	2.170617256
1455204_at	*Pitpnc1*	2.170133864
1435369_at	*Fastkd5*	2.161237382
1438506_s_at	*Abi1*	2.159741281
1456054_a_at	*Pum1*	2.159502896
1455076_a_at	*4933424B01Rik*	2.151580855
1416659_at	*Eif3s10*	2.149038187
1424843_a_at	*Gas5*	2.147478963
1419039_at	*Cyp2d22*	2.143874187
1418332_a_at	*Agtpbp1*	2.142333036
1433631_at	*Eif5 /// LOC100047658*	2.137350801
1423849_a_at	*Clk3*	2.135951738
1415787_at	*Ganab*	2.133745426
1416493_at	*Ddost*	2.133546034
1426829_at	*Uimc1*	2.132232429
1439119_a_at	*BC010304*	2.132203305
1416162_at	*Rad21*	2.126541595
1450915_at	*Ap3b1*	2.124773293
1426008_a_at	*Slc7a2*	2.121005519
1450724_at	*Drctnnb1a*	2.120119768
1452189_at	*Wdr82*	2.115056323
1418333_at	*Mtf1*	2.111053979
1421140_a_at	*Foxp1*	2.110506444
1433511_at	*Gtf2a1*	2.107724481
1450136_at	*Cd38*	2.10702722
1428845_at	*Bclaf1*	2.106997908
1428853_at	*Ptch1*	2.10419089
1425508_s_at	*Arfrp1*	2.100507052
1420519_a_at	*Eral1*	2.097355269
1421894_a_at	*Tpp2*	2.093791422
1417087_at	*Glg1*	2.093146776
1417869_s_at	*Ctsz*	2.087294577
1437586_at	*Cnot4*	2.084133384
1416926_at	*Trp53inp1*	2.08250967
1417371_at	*Peli1*	2.079815852
1422569_at	*Yy1*	2.07542894
1424403_a_at	*Rufy3*	2.073930326
1428448_a_at	*Gtf3c2*	2.072780584
1438386_x_at	*Mat2a*	2.071091257
1426555_at	*Scpep1*	2.064092623
1448038_at	*1810021B22Rik*	2.062631218
1420850_at	*Crnkl1*	2.060897085
1416662_at	*Sardh*	2.058366638

1416190_a_at	*Sec61a1*	2.05723677
1451663_a_at	*Trim3*	2.054786278
1434586_a_at	*Ptdss2*	2.054643697
1427310_at	*Bptf*	2.047308284
1431832_x_at	*Exosc10*	2.046548429
1427631_x_at	*Mup3*	2.046484608
1448915_at	*Zfp524*	2.045733949
1435030_at	*Upf2*	2.044969575
1433866_x_at	*Prdx1*	2.037905937
1435362_at	*Foxj3*	2.033633725
1435018_at	*5930434B04Rik*	2.032887005
1428087_at	*Dnm1l*	2.028632395
1426942_at	*Aim1*	2.022814769
1427305_at	*Piga*	2.020099562
1420478_at	*Nap1l1*	2.017540009
1437308_s_at	*F2r*	2.016979155
1421415_s_at	*Gcnt2*	2.015833919
1423105_a_at	*Yeats4*	2.014764695
1415771_at	*Ncl*	2.009265169
1460711_at	*4930461P20Rik*	2.00871637
1450845_a_at	*Bzw1*	2.008377278
1416963_at	*Ubac1*	2.005915306
1416944_a_at	*Tlk2*	2.005333134
1424913_at	*2310044G17Rik*	2.005033649
1450378_at	*Tapbp*	2.001916399
1423040_at	*Bzw1*	2.001185139
1437985_a_at	*2310061I04Rik*	1.997656313
1434612_s_at	*Sbno1*	1.997332817
1425194_a_at	*6330577E15Rik /// LOC100048533*	1.995565796
1454606_at	*4933426M11Rik*	1.988841092
1431951_a_at	*Usp16*	1.985936485
1455372_at	*Cpeb3*	1.98496593
1459894_at	*Iqgap2*	1.983735125
1428871_at	*LOC100047441*	1.979151427
1451639_at	*Cebpg*	1.976886158
1438233_at	*6030443O07Rik*	1.975003381
1449618_s_at	*2900092E17Rik*	1.974534512
1451004_at	*Acvr2a*	1.972041337
1460258_at	*Lect1*	1.971497432
1452787_a_at	*Prmt1*	1.968693195
1428073_a_at	*Nup88*	1.96532579
1427052_at	*Acacb /// LOC100047358*	1.962494056
1424422_s_at	*Flad1 /// Lenep*	1.959570166

1427844_a_at	*Cebpb*	1.957066874
1449773_s_at	*Gadd45b*	1.954498401
1427273_at	*Rnf214*	1.954186194
1422264_s_at	*2310051E17Rik /// Klf9*	1.953212381
1415911_at	*Impact*	1.948661884
1426954_at	*Trim33*	1.945224968
1424917_a_at	*Wipi1*	1.945045244
1451649_a_at	*Wdr75*	1.940549616
1426260_a_at	*Ugt1a1 /// Ugt1a10 /// Ugt1a2 /// Ugt1a5 /// Ugt1a6a /// Ugt1a6b /// Ugt1a7c /// Ugt1a9*	1.939437113
1429979_a_at	*1810073N04Rik*	1.935317323
1420956_at	*Apc /// LOC100048863*	1.935306032
1433717_at	*D19Wsu162e*	1.930005368
1435742_at	*Smek1*	1.924069621
1423663_at	*Flcn*	1.922987937
1426761_at	*Aof2 /// LOC100046934*	1.922829384
1460279_a_at	*Gtf2i*	1.920595695
1456040_at	*Sf3b2*	1.919023484
1418605_at	*Nr2c1*	1.916433016
1424802_a_at	*3300001P08Rik*	1.915609149
1418911_s_at	*Acsl4*	1.914742543
1450950_at	*Smc3*	1.910763596
1429410_at	*Eny2*	1.910577957
1425150_at	*C730036D15Rik*	1.907848645
1424672_at	*Dmxl1*	1.906841944
1419883_s_at	*Atp6v1b2*	1.90580718
1450674_at	*Cdk5*	1.90480593
1448480_at	*Nip7*	1.901271659
1423722_at	*Tmem49*	1.898374952
1424005_at	*B230219D22Rik*	1.891167915
1448124_at	*Gusb*	1.888889179
1421889_a_at	*Aplp2*	1.887255718
1455899_x_at	*Socs3*	1.886608919
1451736_a_at	*Map2k7*	1.88656146
1454789_x_at	*Prpf6*	1.886208451
1451237_s_at	*Rbm7*	1.885498492
1424083_at	*Rod1*	1.88493797
1425516_at	*Ogt*	1.883001348
1452168_x_at	*Gspt1*	1.88219308
1438278_a_at	*BC003993*	1.880269396
1428081_at	*Klhl21*	1.878954271
1435137_s_at	*1200015M12Rik /// 1200016E24Rik /// A130040M12Rik ///*	1.877464151

	E430024C06Rik	
1423106_at	*Ube2b*	1.872594828
1452116_s_at	*Atf2*	1.872071359
1415867_at	*Cct4*	1.871371561
1421797_a_at	*Snx12*	1.869671566
1453099_at	*LOC100046483*	1.869261176
1449029_at	*Mknk2*	1.86914942
1433784_at	*9030612M13Rik*	1.86798339
1416982_at	*Foxo1*	1.867803999
1436308_at	*Zfp292*	1.863471648
1418034_at	*Mrps9*	1.862002303
1455908_a_at	*Scpep1*	1.859651849
1448892_at	*Dock7*	1.856945921
1423867_at	*Serpina3k*	1.856438578
1452153_at	*Fbxo18*	1.856437818
1418288_at	*Lpin1*	1.856435978
1448801_a_at	*Timm44*	1.855766024
1456088_at	*Birc4*	1.855203541
1424711_at	*Tmem2*	1.855141779
1448620_at	*Fcgr3*	1.854452928
1448272_at	*Btg2*	1.853424323
1417126_a_at	*Rpl22l1*	1.853275521
1451388_a_at	*Atp11b*	1.852214854
1451640_a_at	*Rsrc2*	1.851753588
1419100_at	*Serpina3n*	1.851439761
1423675_at	*Usp1*	1.849825541
1425547_a_at	*Klc4*	1.843014722
1430289_a_at	*Wdr77*	1.842071556
1451612_at	*Mt1*	1.841580583
1452035_at	*Col4a1*	1.839743886
1436801_x_at	*Cdc42ep4*	1.839193402
1433816_at	*Mcart1*	1.836715646
1455479_a_at	*Ube2d3*	1.835576473
1437843_s_at	*Nupl1*	1.834728655
1439185_x_at	*D430028G21Rik*	1.830973907
1456716_s_at	*3110002H16Rik*	1.82829281
1437061_at	*Mbd1*	1.827098167
1431304_a_at	*Tmem183a*	1.82444424
1424873_at	*Rnf2*	1.82214085
1435055_a_at	*EG545878 /// Tom1*	1.821878472
1427457_a_at	*Bmp1*	1.82090701
1426911_at	*Dsc2*	1.820516081
1418138_at	*Sult1d1*	1.817795365
1429318_a_at	*LOC100046895 /// Qk*	1.817726617
1422844_a_at	*Wdr77*	1.816119107

1434468_at	*Otud4*	1.815986835
1419753_at	*Nfx1*	1.815300115
1416071_at	*Ddx18*	1.815185137
1434659_at	*5830411G16Rik*	1.81435194
1416216_at	*Reps1*	1.813273742
1426671_a_at	*Rbm39*	1.812356606
1433656_a_at	*Gnl3*	1.812109386
1452406_x_at	*Erdr1*	1.81203102
1424928_at	*2210018M11Rik*	1.810195209
1452096_s_at	*D230025D16Rik*	1.808159509
1448989_a_at	*Myo1b*	1.807810405
1418295_s_at	*Dgat1*	1.807282535
1422528_a_at	*Zfp36l1*	1.805407199
1449515_at	*Zfp292*	1.804658686
1425381_a_at	*Trfr2*	1.802680773
1453169_a_at	*Gtf2h1*	1.802029309
1428288_at	*2310051E17Rik*	1.800860971
1423462_at	*Map3k7ip2*	1.799012691
1427885_at	*Pold4*	1.798688228
1427188_at	*Arih1*	1.798499522
1436950_at	*Crkl*	1.798380865
1434554_at	*Trim37*	1.797443501
1417775_at	*Rpo1-4*	1.793046577
1426184_a_at	*Pdcd6ip*	1.792658482
1428255_at	*Luc7l*	1.791009366
1449648_s_at	*Rpo1-1*	1.789166633
1460325_at	*Pum1*	1.788045485
1449354_at	*Zrsr1*	1.787482562
1453013_at	*Zfp740*	1.786524183
1425161_a_at	*Trabd*	1.783594593
1428907_at	*Rbm25*	1.783405696
1427075_s_at	*Pcmtd2*	1.781686544
1450942_at	*Ccdc16*	1.781205951
1450420_at	*LOC100045442 /// Stag1*	1.779214382
1418322_at	*Crem*	1.778355807
1426874_at	*Edc4*	1.77818752
1449119_at	*Arih2*	1.777909089
1425576_at	*Ahcyl1*	1.777792305
1438169_a_at	*Frmd4b*	1.776874254
1454643_at	*Ubap2l*	1.776846678
1417001_a_at	*D4Wsu53e*	1.776255804
1417973_at	*Itih1*	1.774996744
1426491_at	*Herc2*	1.773886391
1416127_a_at	*Dnpep*	1.77386862
1418860_a_at	*Letmd1*	1.772205252

1417065_at	*Egr1*	1.770862884
1451087_at	*Wdr36*	1.770277074
1434714_at	*Ero1lb*	1.769368335
1439214_a_at	*Api5*	1.769211755
1417496_at	*Cp*	1.76846118
1420345_at	*Cldn14*	1.766776505
1455171_at	*Suv420h1*	1.766510957
1425553_s_at	*Hip1r*	1.766222217
1427797_s_at	*Ctse*	1.765477587
1455550_x_at	*Serinc3*	1.764339468
1454136_a_at	*4921524J17Rik*	1.763817469
1427236_a_at	*Mll5*	1.761922673
1416794_at	*Arl6ip2*	1.761802056
1429227_x_at	*Nap1l1*	1.759448591
1417315_at	*Gripap1*	1.758475278
1417743_at	*Clk2*	1.758139889
1426698_a_at	*Hnrpm*	1.757213981
1420408_a_at	*Abcc9*	1.757065029
1435946_at	*Sepsecs*	1.75703393
1449555_a_at	*Fetub*	1.753901443
1449374_at	*Pipox*	1.75316719
1423100_at	*Fos*	1.752183968
1419637_s_at	*4833420G17Rik*	1.752137798
1427490_at	*Abcb7*	1.750537777
1416129_at	*Errfi1*	1.750022914
1425280_at	*Leng1*	1.747661248
1453307_a_at	*Anapc5*	1.747024246
1420628_at	*LOC100045958*	1.746915899
1427073_at	*Lace1*	1.74670129
1423322_at	*Lin7c*	1.746447665
1417209_at	*Sertad2*	1.745757178
1460394_a_at	*Inppl1*	1.745739883
1427199_at	*Fryl*	1.744943567
1418553_at	*Arhgef18*	1.744870546
1448398_s_at	*Rpl22*	1.744495025
1423894_a_at	*Dalrd3*	1.743178806
1427174_at	*LOC100039000 /// LOC100039123 /// LOC100043403 /// LOC100043915 /// LOC329575 /// LOC626832 /// OTTMUSG00000016327 /// RP23-232H13.5 /// RP23-330D3.3 /// RP23-376N23.4 /// RP23-*	1.743038603

	442I10.2 /// RP24-562O19.2	
1448667_x_at	*Tob2*	1.742215568
1450103_a_at	*Pscd2*	1.740952126
1456726_x_at	*Qars*	1.740683632
1418861_at	*Pias4*	1.733173783
1418444_a_at	*Mir16*	1.73297889
1427032_at	*Herc4*	1.731641884
1419181_at	*Zfp326*	1.730544822
1460695_a_at	*2010111I01Rik*	1.730175943
1417852_x_at	*Clca1*	1.729325307
1416690_at	*Gtpbp2*	1.727934843
1416881_at	*LOC632101 /// Mcl1*	1.727905972
1427266_at	*Pbrm1*	1.727723969
1419047_at	*Pcnx*	1.726391205
1451218_at	*Edem1*	1.726065283
1419519_at	*Igf1*	1.724730771
1452070_at	*Dedd2*	1.724730771
1424038_a_at	*2310044H10Rik*	1.723189963
1437235_x_at	*Lpp*	1.721506758
1427358_a_at	*Dapk1*	1.721498563
1422477_at	*Cables1*	1.718528394
1428193_at	*Usp9x*	1.71572716
1423991_at	*Nol14*	1.715584166
1418300_a_at	*Mknk2*	1.714602885
1426419_at	*Rbm26*	1.713721768
1426124_a_at	*Clk1*	1.713387015
1416780_at	*Pfkm*	1.712333574
1416923_a_at	*Bnip3l*	1.711699722
1426879_at	*1190005F20Rik*	1.710724053
1416011_x_at	*Ehd1*	1.71062642
1424943_at	*Cyp4a31 /// LOC100044218*	1.710308768
1421233_at	*Pknox1*	1.709942042
1455036_s_at	*LOC675851 /// Ndufc2*	1.709803843
1437032_x_at	*Rbm14*	1.707305003
1424744_at	*Sds*	1.706672579
1424937_at	*2310076L09Rik*	1.704763317
1453722_s_at	*LOC100048559 /// Sfrs1*	1.704718954
1423425_at	*1300012G16Rik*	1.703342096
1435965_at	*Cnot3*	1.702363009
1419384_at	*Pick1*	1.702272277
1435174_at	*LOC100045795 /// Rsbn1*	1.701951008
1421681_at	*Nrg4*	1.700614545
1448133_at	*Nmd3*	1.698982201

1416880_at	*Mcl1*	1.697701198
1419069_at	*Rabgef1*	1.697322856
1421197_a_at	*Acin1*	1.695211606
1428468_at	*3110043O21Rik*	1.695101014
1423566_a_at	*Hsp110*	1.694792269
1424420_at	*Ccpg1*	1.694038896
1416739_a_at	*Brap*	1.693702434
1448426_at	*Sardh*	1.693233351
1421156_a_at	*Dsc2*	1.692778308
1438790_x_at	*Tmem41b*	1.692635096
1455030_at	*Ptprj*	1.692504332
AFFX-r2-Bs-lys-M_at	---	1.6920545
1450611_at	*Orm3*	1.689591993
1426536_at	*Narg2*	1.689230849
1424483_at	*Mobkl1b*	1.688580365
1424642_at	*Thoc1*	1.687824304
1428674_at	*Prpf38b*	1.687298775
1418152_at	*Nsbp1*	1.686949701
1452171_at	*Grwd1*	1.686548927
1426118_a_at	*Tomm40*	1.686313149
1434942_at	*Esf1*	1.685488773
1437461_s_at	*Rnpc3*	1.684436145
1452155_a_at	*Ddx17*	1.683920981
1423821_at	*Tmem168*	1.682720402
1419659_s_at	*Chic2*	1.679852452
1416272_at	*Map2k1ip1*	1.67692627
1427988_s_at	*Safb2*	1.676910827
1438975_x_at	*Zdhhc14*	1.676258966
1452024_a_at	*Ldb1*	1.675884551
1436956_at	---	1.67526931
1450038_s_at	*Usp9x*	1.675028593
1450072_at	*Ash1l*	1.674174581
1426810_at	*Jmjd1a*	1.673786942
1426780_at	*D14Ertd436e*	1.673301258
1452009_at	*1810054D07Rik*	1.672534198
1426416_a_at	*Yipf4*	1.671734645
1422123_s_at	*Ceacam1 /// Ceacam2*	1.670323115
1421143_at	*Diap1*	1.669908879
1423641_s_at	*Cnot7*	1.66918082
1416061_at	*Tbc1d15*	1.667904701
1418388_s_at	*4930548G07Rik*	1.667627687
1424275_s_at	*LOC100046003 /// Trim41*	1.666487852
1428194_at	*Usp9x*	1.66505424
1449410_a_at	*Gas5*	1.663754517

1417454_at	*Cul4b*	1.662724388
1427983_at	*Suhw3*	1.6626244
1421840_at	*Abca1*	1.661868734
1451242_a_at	*Ppp5c*	1.660934905
1431606_a_at	*Angel2*	1.660118883
1424007_at	*Gdf10*	1.659425673
1427670_a_at	*Tcf12*	1.657965173
1418458_at	*Anapc7*	1.65580495
1416442_at	*Ier2*	1.65487231
1421184_a_at	*Higd1c /// LOC554292 /// Mettl7a /// Ubie*	1.654024533
1426046_a_at	*Rabggta*	1.652625605
1418596_at	*Fgfr4*	1.651762824
1417765_a_at	*Amy1*	1.651682789
1434100_x_at	*Ppargc1a*	1.650307604
1452661_at	*Tfrc*	1.650302573
1417012_at	*Sdc2*	1.649495208
1426985_s_at	*2810485I05Rik*	1.647818342
1426377_at	*Zfp281*	1.647759049
1436909_at	*Slc25a44*	1.647631641
1417622_at	*LOC100047237 /// Slc12a2*	1.646953451
1418301_at	*Irf6*	1.646364551
1449444_a_at	*LOC100048499 /// Mfap1a /// Mfap1b*	1.645928466
1452986_at	*Hgd*	1.645597549
1430533_a_at	*Ctnnb1*	1.64460776
1452221_a_at	*Cxxc1*	1.64429846
1426556_at	*Suhw4*	1.644219008
1434103_at	*Slc35e1*	1.642643785
1434004_at	*Dhps*	1.641664277
1450732_a_at	*Bicd2*	1.641393345
1438917_x_at	*Nup62*	1.641054246
1423839_a_at	*Btf3 /// LOC100041823 /// LOC100046341*	1.640025711
1433711_s_at	*LOC100047324 /// Sesn1*	1.639543628
1419067_a_at	*Rabgef1*	1.638752673
1452835_a_at	*Polrmt*	1.638520353
1421989_s_at	*Papss2*	1.63836198
1452330_a_at	*Mxra8*	1.63827821
1421743_a_at	*Pcbp2*	1.637804845
1448978_at	*Ngef*	1.637499055
1448828_at	*Smc6*	1.636877504
1422648_at	*Slc7a2*	1.636164284
1420990_at	*Chd1*	1.635408065

AFFX-LysX-5_at	---	1.635056113
1416461_at	*Caprin1*	1.634568675
1424776_a_at	*Slc25a28*	1.63396188
1419508_at	*Ripk1*	1.633018334
1432646_a_at	*2900097C17Rik*	1.632323494
1451376_at	*5730596K20Rik*	1.631459832
1455128_x_at	*Tnrc6a*	1.631075157
1434005_at	*Rbms1*	1.631005109
1426378_at	*Eif4b*	1.630776875
1417406_at	*Sertad1*	1.630708082
1447977_x_at	*LOC626832*	1.629642769
1424112_at	*Igf2r*	1.628324375
1455475_at	*3110057O12Rik*	1.628118702
1423884_at	*Cirh1a*	1.627514032
1456213_x_at	*Qars*	1.626039408
1417831_at	*Smc1a*	1.624112205
1417169_at	*Usp2*	1.623256834
1436315_at	*Myst3*	1.623142288
1451621_at	*5830417C01Rik*	1.622625875
1416811_s_at	*Ctla2a /// Ctla2b*	1.622297514
1452784_at	*Itgav*	1.620686365
1433804_at	*Jak1*	1.619485291
1451295_a_at	*Chd4*	1.619429797
1434514_at	*Rbm15*	1.618818381
1448385_at	*Slc15a4*	1.618701456
1424023_at	*Atpbd3*	1.618577914
1450184_s_at	*Tef*	1.617761972
1415988_at	*Hdlbp*	1.617346627
1426830_a_at	*Ahcyl1*	1.617340994
1434999_at	*Suv420h1*	1.61670064
1416660_at	*Eif3s10*	1.616388358
1421225_a_at	*Slc4a4*	1.61552477
1452429_s_at	*Abcf1*	1.614472715
1428511_at	*Phkg2*	1.613663827
1424442_a_at	*Pja2*	1.611190897
1451044_at	*Sip1*	1.611076077
1451628_a_at	*Ank3*	1.610687348
1417033_at	*Ube2g2*	1.610500936
1437991_x_at	*Rusc1*	1.6101595
1426602_at	*Araf*	1.609797496
1416142_at	*Rps6*	1.608990196
1425652_s_at	*LOC100044395 /// Rbpms*	1.608555985
1428133_at	*Snip1*	1.607401275
1432419_a_at	*2700078K21Rik*	1.607371926

1424472_at	*Nol6*	1.605833227
1417167_at	*Exosc5*	1.605741725
1416172_at	*Pes1*	1.604864778
1450690_at	*Ranbp2*	1.604783785
1451081_a_at	*Tcf25*	1.603651651
1417273_at	*Pdk4*	1.603456191
1416819_at	*Cdc37*	1.603388967
1450656_at	*Gna13*	1.603197651
1460213_at	*Golga4*	1.602418004
1424261_at	*Zfp672*	1.599527628
1417130_s_at	*Angptl4*	1.599508756
1418600_at	*Klf1*	1.599507905
1427997_at	*1110007M04Rik*	1.597612256
1449897_a_at	*Mtcp1*	1.597392156
1418932_at	*LOC100046232 /// Nfil3*	1.59694935
1416030_a_at	*Mcm7*	1.596903621
1423565_at	*Paics*	1.596302837
1417762_a_at	*Rpl8*	1.59623064
1434773_a_at	*Slc2a1*	1.594011219
1424390_at	*Nupl1*	1.593850228
1423338_at	*Ccdc16*	1.593583831
1416921_x_at	*Aldoa*	1.59350704
1423445_at	*Rock1*	1.593349081
1448013_at	*LOC100039616 /// LOC100048006 /// Usp24*	1.592768008
1452286_at	*Slain2*	1.590965546
1417279_at	*Itpr1*	1.59088168
1451152_a_at	*Atp1b1*	1.590652253
1416210_at	*Imp3*	1.589585685
1432416_a_at	*EG668347 /// LOC100046628 /// Npm1*	1.588968194
1415982_at	*Herpud2*	1.588608873
1428931_a_at	*Parp6*	1.588601138
1437801_at	*EG627352 /// LOC433598 /// LOC433955 /// LOC626309 /// Morf4l1*	1.588559269
1418279_a_at	*Akap1*	1.587428851
1439441_x_at	*Lats2*	1.586015254
1452124_at	*Ank3*	1.585904446
1433580_at	*Nup54*	1.585840929
1433576_at	*Mat2a*	1.58579726
1422972_s_at	*Gcn5l2*	1.584913908
1434831_a_at	*Foxo3a*	1.58327422
1428091_at	*Klhl7*	1.582739053

1425027_s_at	*Sft2d2*	1.581798728
1434113_a_at	*Rexo4*	1.580915782
1449084_s_at	*Sh3d19*	1.580622243
1418470_at	*Yes1*	1.580009948
1436362_x_at	*2700079J08Rik /// Ccrn4l /// LOC100040359 /// LOC100041308 /// LOC100042078 /// LOC100042092 /// LOC100043548 /// LOC100043775 /// LOC100044145*	1.579043918
1449109_at	*Socs2*	1.578789705
1426300_at	*Alcam*	1.575223542
1429002_at	*Snw1*	1.574879483
1430147_a_at	*Josd3*	1.574598897
1416826_a_at	*Med20*	1.574007811
1432488_a_at	*Sf3a3*	1.573737411
1423982_at	*Fusip1*	1.573295569
1439122_at	*Ddx6*	1.573202433
1423048_a_at	*Tollip*	1.57291972
1456399_at	*---*	1.572843627
1452202_at	*Pde2a*	1.572818626
1424951_at	*Baiap2l1*	1.572545012
1424205_at	*Smarca5*	1.571796853
1422570_at	*Yy1*	1.571742968
1438155_x_at	*Pigo*	1.571723091
1451019_at	*Ctsf*	1.571702966
1423459_at	*Cops2*	1.571560905
1420870_at	*Mllt10*	1.571263977
1450199_a_at	*Stab1*	1.57083556
1436510_a_at	*Lrrfip2*	1.570677814
1418427_at	*Kif5b*	1.569777747
1428289_at	*2310051E17Rik /// Klf9*	1.568984648
1432094_a_at	*Ccdc132*	1.568527065
1433510_x_at	*LOC100043483 /// LOC100043718 /// LOC100048459 /// LOC100048508 /// LOC622534*	1.566932427
1419321_at	*F7*	1.566847559
1425723_at	*Nr1i2*	1.566762075
1419687_at	*Macrod1*	1.566467066
1450873_at	*Gtpbp4*	1.566288728
1418436_at	*Stx7*	1.565132426
1452187_at	*Rbm5*	1.563877052

1448183_a_at	*Hif1a*	1.563613067
1423648_at	*Pdia6*	1.563549827
1448934_at	*Ndufa10*	1.562031611
1452381_at	*Creb3l2*	1.561843373
1422904_at	*Fmo2*	1.561283978
1459992_x_at	*Cln8*	1.561211724
1415688_at	*Ube2g1*	1.560784132
1454635_at	*Fbxl3 /// LOC100044862*	1.560329499
1427425_at	*9130208E07Rik*	1.560021459
1420509_at	*Srfbp1*	1.55871534
1419572_a_at	*Abcd4*	1.557053109
1418121_at	*Vrk3*	1.55656
1424748_at	*Galnt11*	1.556529371
1431284_a_at	*Spns1*	1.554980557
1418507_s_at	*Socs2*	1.553918138
1423740_a_at	*Rbm10*	1.552618815
1416953_at	*Ctgf*	1.552512193
1423233_at	*Cebpd*	1.552502077
1428092_at	*Cdc5l*	1.552168624
1419364_a_at	*EG621699 /// EG624124 /// LOC634044 /// Rps7*	1.551827554
1437614_x_at	*Zdhhc14*	1.55096773
1439244_a_at	*Tnrc6a*	1.550353516
1460398_at	*Phf8*	1.550121277
1431921_a_at	*LOC100045442 /// Stag1*	1.548688415
AFFX-LysX-M_at	---	1.548233423
1451599_at	*Sesn2*	1.547319922
1421849_at	*Stag2*	1.545857746
1425514_at	*Pik3r1*	1.545160159
1417871_at	*Hsd17b7*	1.545061614
1428904_at	*Ammecr1l*	1.545047519
1437758_a_at	*Slc4a1ap*	1.54496051
1438736_at	*Thoc2*	1.543626191
1449890_at	*Ugt2b37*	1.543571288
1426663_s_at	*Slc45a3*	1.543147181
1456243_x_at	*Mcl1*	1.541951719
1452629_at	*Safb2*	1.541747989
1428169_at	*Atg16l1*	1.541738883
1423269_a_at	*Nedd4l*	1.54169206
1448271_a_at	*Ddx21*	1.541386532
1425644_at	*Lepr*	1.539695523
1449337_at	*Tdo2*	1.538688084
1415966_a_at	*Ndufv1*	1.538486909
1438478_a_at	*Ppp3ca*	1.53818457
1451093_at	*Polr2e*	1.53808306

1426257_a_at	*Sars*	1.537831873
1424932_at	*Egfr*	1.537437404
1448454_at	*Sfrs6*	1.537344981
1426559_at	*LOC100047496 /// Sbno1*	1.537156978
1425981_a_at	*LOC635075 /// Rbl2*	1.536544719
1419816_s_at	*Errfi1*	1.535628887
1432099_a_at	*Prodh2*	1.535564794
1418453_a_at	*Atp1b1*	1.534630125
1424161_at	*Ddx27*	1.533817991
1419950_s_at	*Tnpo3*	1.533200524
1450901_a_at	*Smek2*	1.532798213
1428862_at	*Ttc17*	1.532629193
1423667_at	*Mat2a*	1.532442047
1423645_a_at	*Ddx5*	1.531161298
1451528_at	*Tmem82*	1.531056946
1449445_x_at	*Mfap1a*	1.530833261
1426383_at	*Cry2*	1.530478364
1451936_a_at	*Txnrd2*	1.530427419
1451092_a_at	*Rangap1*	1.527191437
1421950_at	*Pfdn2*	1.526742551
1426301_at	*Alcam*	1.526488874
1455563_at	*Ddx49*	1.526241054
1424650_at	*Pdia5*	1.526239903
1423713_at	*Abcb8*	1.525754849
1418998_at	*Kmo*	1.524683751
1426355_a_at	*6330578E17Rik*	1.524402682
1423196_at	*Nedd1*	1.524266373
1416661_at	*Eif3s10*	1.522196401
1423336_at	*Orc4l*	1.521638154
1450778_a_at	*Rnuxa*	1.521018389
1448455_at	*Cln8*	1.520714564
1456516_x_at	*LOC640502 /// Uap1*	1.520699691
1418503_at	*Hspa9*	1.520625333
1424250_a_at	*Arhgef3*	1.520537839
1427888_a_at	*Spna2*	1.519846571
1449106_at	*Gpx3*	1.519154211
1418028_at	*Dct*	1.518840715
1450970_at	*Got1*	1.518815622
1435630_s_at	*Acat2*	1.518748362
1438714_at	*Zfp207*	1.518523356
1448389_at	*Wdr5*	1.518337979
1430777_a_at	*Golph3*	1.517696693
1419549_at	*Arg1*	1.517247471
1433588_at	*D6Wsu116e*	1.516500712

1455242_at	*Foxp1*	1.514579618
1416722_at	*Hmg20a*	1.514415347
1426950_at	*Parp16*	1.514278615
1429491_s_at	*Rif1*	1.514068431
1424942_a_at	*Myc*	1.51204532
1436936_s_at	*Xist*	1.511999585
1417422_at	*Gnmt*	1.510566701
1460544_at	*Mak10*	1.509970537
1419565_a_at	*Zfx*	1.509286017
1452464_a_at	*Metapl1*	1.508709772
1429897_a_at	*D16Ertd472e*	1.50822605
1426521_at	*D230025D16Rik*	1.508062638
1421836_at	*Mtap7*	1.507991851
1423532_at	*Rnf44*	1.506766091
1452438_s_at	*LOC100046932 /// Taf4a*	1.506604525
1417740_at	*Cdc37l1*	1.505534824
1417707_at	*B230342M21Rik*	1.505504289
1450095_a_at	*Acyp1*	1.504961884
1416994_at	*Ttc1*	1.502579323
1415689_s_at	*Zkscan3*	1.502235068
1418401_a_at	*Dusp16*	1.502214436
1427406_at	*Trip11*	1.501993058
1449121_at	*Fusip1*	1.501477656
1452061_s_at	*Strbp*	1.500867461
1423201_at	*Ncor1*	1.500571067
1437354_at	*C230091D08Rik*	1.500447692
1450479_x_at	*Ptpn12*	1.50028987
1427254_at	*Zfp445*	1.500003007
1416078_s_at	*Raf1*	1.499302912
1451190_a_at	*Sbk1*	1.49896603
1434799_x_at	*Aldoa*	1.497989671

Table 5 : Genes down regulated at least 1.5-fold in non- lactating mice Abbreviations: affyID: annotated files from Affymetrix that identifies Probeset IDs for genes on its chips. fcVvsNL: the fold change of genes in non- lactating mice compared to virgin mice, in non-log form.

affyid	Gene Symbol	fcVvsNL
1416055_at	*1810008N23Rik /// Amy2 /// Amy2-1 /// Amy2-2 /// LOC100043684 /// LOC100043686 /// LOC100043688*	434.958683
1435012_x_at	*Ela3 /// LOC242711 /// LOC638198 /// LOC638418*	356.6884872
1448281_a_at	*RP23-395H4.4*	326.0138579
1437326_x_at	*Ela3 /// LOC638418*	311.565369
1433431_at	*Pnlip*	295.1078795
1431763_a_at	*Ctrl*	276.2077642
1448220_at	*Ctrb1*	251.5824223
1433459_x_at	*Prss2*	235.1797488
1415954_at	*1810049H19Rik /// Prss1 /// Try10 /// Try4*	235.1293775
1428062_at	*Cpa1*	205.8258283
1422434_a_at	*2210010C04Rik*	181.7922939
1428102_at	*Cpb1*	178.8417494
1415883_a_at	*Ela3*	171.1465103
1435611_x_at	*Ela3*	132.9130062
1415805_at	*Clps*	112.618415
1428359_s_at	*1810010M01Rik*	89.08473323
1438612_a_at	*Clps*	87.68984143
1422435_at	*2210010C04Rik*	81.92824284
1415884_at	*Ela3*	78.05723823
1416523_at	*Rnase1*	77.43278806
1415777_at	*Pnliprp1*	59.46039841
1417257_at	*Cel*	40.99879527
1415905_at	*Reg1*	38.08741211
1428358_at	*1810010M01Rik*	17.28772742
1417682_a_at	*Prss2*	17.07960013
1416930_at	*Ly6d*	16.54611194
1416666_at	*Serpine2*	15.86097652
1421868_a_at	*Pnlip*	15.73974144
1435507_x_at	*Prss2*	15.71515581
1434089_at	*Synpo*	15.35166015
1434803_a_at	*Sycn*	12.88110326
1422144_at	*Inhbe*	12.86967578
1424528_at	*Cgref1*	12.23170186
1417812_a_at	*Lamb3*	11.65306945

1448107_x_at	*Klk1*	10.27423681
1433573_x_at	*Prss2*	10.19894359
1449452_a_at	*Gp2*	10.14059924
1418126_at	*Ccl5*	10.06876153
1424529_s_at	*Cgref1*	9.648921746
1436845_at	*Axin2*	9.608269039
1421998_at	*Tor3a*	9.156833563
1434137_x_at	*1810010M01Rik*	8.814243778
1418665_at	*Impa2*	8.661749731
1422916_at	*Fgf21*	8.018080791
1452260_at	*Cidec*	7.728273063
1418648_at	*Egln3*	7.692904561
1415837_at	*Klk1*	7.09715282
1448698_at	*Ccnd1*	6.752201515
1418287_a_at	*Dmbt1*	6.572674493
1448756_at	*S100a9*	6.562682503
1424140_at	*Gale*	6.379929326
1423693_at	*Ela1*	6.346914993
1417419_at	*Ccnd1*	6.06123671
1417420_at	*Ccnd1*	6.005113594
1426959_at	*Bdh1*	5.999645942
1448700_at	*G0s2*	5.963824751
1450188_s_at	*Lipg*	5.957666572
1424534_at	*Mmd2*	5.784510289
1423467_at	*Ms4a4b*	5.548852972
1427008_at	*Rnf43*	5.485965899
1421262_at	*Lipg*	5.443032182
AFFX-r2-Bs-thr-5_s_at	---	5.372454414
1418396_at	*Gpsm3*	5.226302204
1451228_a_at	*Sycn*	5.151648053
1452336_at	*BC027382*	5.105657693
1417896_at	*Tjp3*	5.03734778
1448029_at	*Tbx3*	5.009129637
1449299_at	*Lrp5*	4.848559677
1427356_at	*2310031A18Rik /// LOC100047808*	4.848498684
1418091_at	*Tcfcp2l1*	4.540729085
1438165_x_at	*Vat1*	4.456193862
1451095_at	*Asns*	4.443365163
1423141_at	*Lipa*	4.431275421
1418133_at	*Bcl3*	4.267564346
AFFX-ThrX-5_at	---	4.236869752
1425409_at	*Chrna2*	4.199741316
1416318_at	*Serpinb1a*	4.193653486

1418209_a_at	*Pfn2*	4.112971816
1431056_a_at	*LOC669888 /// Lpl*	4.095685341
1424123_at	*Flvcr2*	4.093138814
1449365_at	*Edg8*	4.037222067
1418181_at	*Ptp4a3*	3.962288264
1424383_at	*Tmem51*	3.899533833
1417839_at	*Cldn5*	3.888858104
1424118_a_at	*Spc25*	3.888479011
1418210_at	*Pfn2*	3.850726449
1460674_at	*Paqr7*	3.790690837
1418468_at	*Anxa11 /// LOC100039484 /// LOC100039503*	3.757481823
1425300_at	*Dak*	3.722740428
1423140_at	*Lipa*	3.684820573
1433966_x_at	*Asns*	3.684500681
1425343_at	*Hdhd3*	3.678941629
1418701_at	*Comt*	3.664164724
1435495_at	*Adora1*	3.653471548
1423854_a_at	*Rasl11b*	3.645509818
1452417_x_at	*2010205A11Rik /// Cr1 /// ENSMUSG00000076577 /// Igk-C /// Igk-V21-4 /// Igk-V28 /// Igk-V8-16 /// LOC100046552 /// LOC100046793 /// LOC100047628*	3.643144921
1419394_s_at	*S100a8*	3.63693563
1437932_a_at	*Cldn1*	3.5366832
1423596_at	*Nek6*	3.533235195
1449629_s_at	*Snrpd3*	3.528805045
1450035_a_at	*Prpf40a*	3.452483011
1425355_at	*BC018371 /// EG433604*	3.418460731
1434449_at	*Aqp4*	3.403624835
1434920_a_at	*Evl /// LOC100047333*	3.388549186
1423389_at	*Smad7*	3.36671688
1451566_at	*Zfp810*	3.352848461
1448631_a_at	*Hipk2*	3.344177268
1423726_at	*Vat1*	3.342312993
1417092_at	*Pthr1*	3.326172607
1450391_a_at	*Mgll*	3.313435544
1436833_x_at	*Ttll1*	3.303006434
1427476_a_at	*Trim32*	3.298538623
1450014_at	*Cldn1*	3.284897798
1427347_s_at	*Tubb2a*	3.284450491
1424882_a_at	*Nt5dc2*	3.279676356
1427455_x_at	*Cr1 ///*	3.267073384

	ENSMUSG00000076577 /// Igk-C /// Igk-V21-4 /// Igk-V28 /// Igk-V8-16 /// LOC100046552 /// LOC100046793 /// LOC100047628	
1416160_at	*Nr2f2*	3.254818299
1436736_x_at	*D0H4S114*	3.230314932
1421052_a_at	*LOC671878 /// Sms*	3.228760493
1427099_at	*Maz*	3.220141912
1460218_at	*Cd52*	3.21977552
1427351_s_at	*Igh-6*	3.191491946
1452431_s_at	*H2-Aa*	3.166502873
1451827_a_at	*Nox4*	3.158089141
1449146_at	*Notch4*	3.152603198
1451046_at	*LOC100047651 /// Zfpm1*	3.146356407
1418863_at	*Gata4*	3.127468315
1434175_s_at	*2210010N04Rik*	3.118483005
AFFX-ThrX-M_at	---	3.107658891
1426025_s_at	*Laptm5*	3.105825569
1422537_a_at	*Id2*	3.101950312
1425519_a_at	*Cd74*	3.088583936
1456388_at	*LOC100045280*	3.060129006
1419573_a_at	*Lgals1*	3.040533612
1460173_at	*Lasp1*	3.037814305
1425059_at	*Prmt6*	3.03425691
1421424_a_at	*Anpep*	3.031759833
1450241_a_at	*Evi2a*	3.017543648
1418587_at	*Traf3*	3.016807614
1449479_at	*Cyp2b13*	3.014744448
1418991_at	*Bak1*	2.996658507
1452257_at	*Bdh1*	2.961309648
1448182_a_at	*Cd24a*	2.959124001
1417399_at	*Gas6*	2.94646587
1427327_at	*Pilra*	2.938294441
1428319_at	*Pdlim7*	2.926653054
1448930_at	*3010026O09Rik*	2.925698323
1427480_at	*Leap2*	2.922606875
1452463_x_at	*ENSMUSG00000076577*	2.919737259
1450027_at	*Sdc3*	2.915777255
1449125_at	*Tnfaip8l1*	2.899993237
1433575_at	*Sox4*	2.8961333
1425764_a_at	*Bcat2*	2.88939197
1426464_at	*Nr1d1*	2.888699042
1454623_at	*Cpa2*	2.888088839

1418213_at	*Krt23*	2.871231965
1427660_x_at	*Cr1 /// ENSMUSG00000076577 /// Igk-C /// Igk-V21-4 /// Igk-V28 /// Igk-V8-16 /// LOC100046552 /// LOC100046793 /// LOC100047628*	2.869530239
1448777_at	*Mcm2*	2.866417327
1416550_at	*Slc35b4*	2.865509099
1423836_at	*Zfp503*	2.856544663
1449072_a_at	*N6amt2*	2.85471127
1421057_at	*Dnase1l3*	2.853495348
1422001_at	*Inhbc*	2.825445683
1425850_a_at	*Nek6*	2.825220374
1421149_a_at	*Atn1*	2.822082913
1455470_x_at	*Lasp1*	2.80545638
1433471_at	*Tcf7*	2.800233911
1419161_a_at	*Nox4*	2.794113316
1416032_at	*Tmem109*	2.788773391
1416246_a_at	*Coro1a*	2.782786155
1419483_at	*C3ar1*	2.768017152
1448249_at	*Gpd1*	2.758481183
1431694_a_at	*Ctnnbip1*	2.75608695
1427838_at	*Tubb2a*	2.751467371
1426893_at	*C230093N12Rik*	2.746954899
1425222_x_at	*D630002G06Rik*	2.739818758
1450928_at	*LOC100045546*	2.738083909
1450268_at	*Fign*	2.735948244
1431591_s_at	*Isg15 /// LOC100038882 /// LOC100044225 /// LOC677168*	2.716737906
1417231_at	*Cldn2*	2.714655689
1421344_a_at	*Jub*	2.711241143
1424998_at	*Emr4*	2.710477605
1421009_at	*Rsad2*	2.694385902
1450839_at	*D0H4S114*	2.692571341
1449164_at	*Cd68*	2.681703685
1433691_at	*Ppp1r3c*	2.669946809
1453836_a_at	*Mgll*	2.667888763
1425028_a_at	*Tpm2*	2.658395675
AFFX-r2-Bs-thr-M_s_at	---	2.645165025
1434560_at	*Wdtc1*	2.638349397
1451124_at	*Sod1*	2.638095561
1421326_at	*Csf2rb*	2.628694224

1415898_at	*Mgst1*	2.626853743
1452277_at	*Arsg*	2.625720249
1417807_at	*2700038N03Rik*	2.625505438
1426997_at	*LOC100047427 /// Thra*	2.621778234
1449492_a_at	*Lect2*	2.620380725
1417232_at	*Cldn2*	2.610357697
1435792_at	*Csprs /// EG665317 /// EG665338 /// LOC100040158 /// LOC100040213*	2.595712059
1449846_at	*Ear2 /// Ear3*	2.5956405
1426501_a_at	*T2bp*	2.581642794
1438634_x_at	*Lasp1*	2.572831793
1460345_at	*2610208M17Rik*	2.572167542
1416431_at	*Tubb6*	2.568467907
1435417_at	*AI464131*	2.565819849
1418204_s_at	*Aif1*	2.564463915
1425243_at	*Cd207*	2.561210622
1460212_at	*Gnat1*	2.560080034
1418123_at	*Unc119*	2.545292839
1424524_at	*1200002N14Rik*	2.535851523
1436902_x_at	*LOC100043712 /// LOC100047613 /// Tmsb10*	2.535649521
1448945_at	*Pllp*	2.525406466
1429126_at	*2600001M11Rik*	2.52218336
1416985_at	*Sirpa*	2.52075249
1421008_at	*Rsad2*	2.519591441
1452227_at	*2310045A20Rik*	2.514597268
1451453_at	*Dapk2*	2.504724039
1425548_a_at	*Lst1*	2.50464591
1417654_at	*Sdc4*	2.498014152
1417877_at	*2310005P05Rik*	2.489274025
1423571_at	*Edg1*	2.487302978
1452501_at	*Cyp2c38*	2.480832062
1419366_at	*Zmat5*	2.478874547
1420699_at	*Clec7a*	2.475657547
1415865_s_at	*Bpgm*	2.473191715
1416796_at	*LOC100044475 /// Nck2*	2.471133687
1451115_at	*Pias3*	2.468591201
1416935_at	*Trpv2*	2.467202558
1423603_at	*LOC100047651 /// Zfpm1*	2.466229044
1456739_x_at	*Armcx2*	2.458143214
1421447_at	*LOC100048479 /// Onecut1*	2.454993793
1422771_at	*Smad6*	2.444571459

1435981_at	---	2.444191317
1449403_at	*Pde9a*	2.442727002
1422925_s_at	*Acot3*	2.44108233
1423632_at	*Gpr146*	2.438497936
1418446_at	*LOC100045628 /// Slc16a2*	2.436132121
1418711_at	*Pdgfa*	2.43572031
1418188_a_at	*Malat1*	2.435223614
1417292_at	*Ifi47*	2.43317629
1424895_at	*Gpsm2*	2.429192671
1417568_at	*Ncald*	2.424022082
1429859_a_at	*Arl2bp*	2.413389604
1451407_at	*Igsf5*	2.411343715
1450648_s_at	*H2-Ab1*	2.409122166
1419476_at	*Adamdec1*	2.408612793
1417174_at	*1810021J13Rik*	2.408007799
1416762_at	*S100a10*	2.407168143
1453181_x_at	*Plscr1*	2.403139014
1427357_at	*Cda*	2.402304029
1418782_at	*Rxrg*	2.400734561
1422982_at	*Ar*	2.398138467
1429527_a_at	*LOC433328 /// LOC677340 /// Plscr1*	2.396376459
1452666_a_at	*Tmcc2*	2.391724629
1438633_x_at	*Lasp1*	2.390168118
1436838_x_at	*Cotl1*	2.390134709
1455269_a_at	*Coro1a*	2.389572775
1417462_at	*Cap1*	2.389132936
1425004_s_at	*Mocs1*	2.379530993
1449259_at	*Rab3d*	2.37688532
1449498_at	*Marco*	2.375822181
1420842_at	*Ptprf*	2.375130519
1426242_at	*Polr2a*	2.374675957
1426785_s_at	*Mgll*	2.374101563
1418142_at	*Kcnj8*	2.364549282
1427002_s_at	*Arsg*	2.358376059
1417744_a_at	*Ralb*	2.353976524
1417141_at	*Igtp*	2.352320258
1427385_s_at	*Actn1*	2.351129937
1417391_a_at	*Il16*	2.348897076
1417461_at	*Cap1*	2.339694723
1454078_a_at	*Gal3st1*	2.339688163
1448894_at	*Akr1b8*	2.335169638
1417605_s_at	*Camk1*	2.331523379
1448475_at	*Olfml3*	2.331300614

1417703_at	*Pvrl2*	2.329115145
1415904_at	*Lpl*	2.327558362
1418649_at	*Egln3*	2.327205131
1425631_at	*Ppp1r3c*	2.326284468
1420715_a_at	*Pparg*	2.325270147
1448374_at	*Med28*	2.32054823
1426278_at	*Ifi27*	2.315294628
1419123_a_at	*Pdgfc*	2.313747978
1450919_at	*Mpp1*	2.310823676
1451635_at	*C730048C13Rik /// D630002G06Rik*	2.310764347
1450872_s_at	*Lipa*	2.309725216
1436993_x_at	*Pfn2*	2.307289606
1424004_x_at	*4930444A02Rik*	2.299292085
1434465_x_at	*Vldlr*	2.271630972
1450783_at	*Ifit1*	2.270556164
1448180_a_at	*Hn1*	2.269694363
1416206_at	*Sipa1*	2.269688455
1430798_x_at	*Mrpl15*	2.268330714
1416114_at	*Sparcl1*	2.264559263
1423586_at	*Axl*	2.258313342
1435176_a_at	*Id2*	2.253079449
1430127_a_at	*Ccnd2*	2.247764253
1431302_a_at	*Nudt7*	2.246074016
1448539_a_at	*Acy3*	2.245292252
1437208_at	*11-Sep*	2.2435564
1455961_at	---	2.240566707
1416630_at	*Id3*	2.239648816
1460713_at	*BC048355*	2.238032213
1451361_a_at	*Pnpla7*	2.237926788
1455281_at	*Wdr33*	2.233063293
1417027_at	*Trim2*	2.231560712
1456642_x_at	*S100a10*	2.227201537
1424794_at	*Rnf186*	2.226510545
1448729_a_at	*5-Sep*	2.222972759
1418164_at	*Stx2*	2.221628776
1418739_at	*Sgk2*	2.219185974
1448392_at	*LOC100046740*	2.218961173
1420928_at	*St6gal1*	2.217889616
1449124_at	*Rgl1*	2.217803295
1418927_a_at	*Habp4*	2.212924763
1418535_at	*Rgl1*	2.212555083
1455071_at	*Zbtb7b*	2.212265289
1452201_at	*2310047B19Rik*	2.211818718
1448232_x_at	*EG626534 /// Tuba1c*	2.20969501

1416782_s_at	*Praf2*	2.209322108
1449093_at	*Ctf1*	2.208757337
1460174_at	*Dexi*	2.208499834
1417172_at	*Ube2l6*	2.20548877
1415864_at	*Bpgm*	2.204252831
1434599_a_at	*Tjp2*	2.203443615
1421425_a_at	*Rcan2*	2.199066702
1424968_at	*2210023G05Rik*	2.196868569
1435193_at	*A230050P20Rik*	2.196489938
1424868_at	*Glyat*	2.194501649
1418260_at	*Hunk /// LOC630567*	2.190421266
1424835_at	*Gstm4*	2.187852698
1437733_at	*Eif4ebp2*	2.187759114
1417025_at	*H2-Eb1*	2.186754357
1450966_at	*Crot*	2.185944986
1419748_at	*Abcd2*	2.184303322
1417991_at	*Dio1*	2.183982814
1416882_at	*Rgs10*	2.183440727
1450622_at	*Bcar1*	2.183284469
1456371_a_at	*Shroom1*	2.180057192
1454903_at	*Ngfr*	2.17219013
1450629_at	*Lima1*	2.17201631
1449459_s_at	*Asb13*	2.171074409
1417884_at	*Slc16a6*	2.170945355
1425601_a_at	*Rtkn*	2.169058107
1417604_at	*Camk1*	2.168928336
1424754_at	*Ms4a7*	2.168287065
1416368_at	*Gsta4*	2.159167849
1416589_at	*LOC100046740 /// Sparc*	2.156168415
1433558_at	*Dab2ip*	2.154290894
1427895_at	*2310004N24Rik*	2.153934611
1437651_a_at	*Dtnb*	2.151264592
1419403_at	*BC017612*	2.150735786
1418580_at	*Rtp4*	2.149968663
1451687_a_at	*Tcf2*	2.148619918
1417434_at	*Gpd2*	2.148236526
1440230_at	*Tsku*	2.141835167
1433408_a_at	*Mcm10*	2.141018987
1422637_at	*Rassf5*	2.136051574
1450958_at	*Tm4sf1*	2.135584823
1424339_at	*Oasl1*	2.134961413
1416590_a_at	*Rab34*	2.134905508
1452020_a_at	*Siva1*	2.129336096
1449362_a_at	*LOC100048805 /// Mink1*	2.127177018
1451721_a_at	*H2-Ab1*	2.126896939

1417786_a_at	*Rgs19*	2.125847188
1417793_at	*Iigp2*	2.125591136
1449195_s_at	*Cxcl16*	2.122749191
1416204_at	*Gpd1*	2.122423283
1451367_at	*Cops6*	2.122359117
1417219_s_at	*LOC100042319 /// LOC100045828 /// Tmsb10*	2.120072622
1448876_at	*Evc*	2.119966932
1421430_at	*Rad51l1*	2.118421631
1417112_at	*Arl2bp*	2.117645207
1422061_at	*Akr1c20*	2.116145612
1422718_at	*Ap3s2*	2.115668332
1435290_x_at	*H2-Aa*	2.112366087
1438855_x_at	*Tnfaip2*	2.109593254
1418982_at	*Cebpa*	2.10569181
1417633_at	*Sod3*	2.104775036
1438480_a_at	*Thyn1*	2.104460219
1435652_a_at	*Gnai2 /// LOC100045883*	2.104390601
1424012_at	*Ttc30a1*	2.104079819
1425927_a_at	*Atf5*	2.102273771
1424169_at	*LOC100039683 /// LOC100045937 /// LOC100047518 /// Tax1bp3*	2.101266172
1417867_at	*Cfd*	2.100704386
1455422_x_at	*5-Sep*	2.098632529
1439264_x_at	*Lasp1*	2.097452417
1451857_a_at	*Notum*	2.096761188
1422072_a_at	*Gstm6*	2.09189803
1418356_at	*Mpst*	2.088777267
1450696_at	*Psmb9*	2.088174968
1417671_at	*Scly*	2.084979111
1460423_x_at	*Cr1*	2.082543077
1424763_at	*1700027N10Rik*	2.080605958
1427005_at	*Plk2*	2.077986761
1428789_at	*Ralgps2*	2.076970839
1426894_s_at	*C230093N12Rik*	2.07681005
1438769_a_at	*Thyn1*	2.068609556
1456691_s_at	*LOC100044230 /// Srd5a2l*	2.067611614
1422432_at	*Dbi*	2.063938071
1428057_a_at	*Ahnak*	2.063242521
1416435_at	*Ltbr*	2.062341419
1416560_at	*Slc13a3*	2.060884
1427331_at	*Adora1*	2.053820268
1438422_at	*Lrrc20*	2.048229474

1460569_x_at	*Cldn3*	2.048081261
1460205_at	*Dcakd*	2.047979462
1415938_at	*Spink3*	2.047593175
1455232_at	*Cml2*	2.046129544
1437185_s_at	*LOC100042319 /// LOC100043712 /// LOC100045828 /// LOC100047613 /// LOC100048142 /// Tmsb10*	2.045863271
1451313_a_at	*1110067D22Rik*	2.044006333
1451562_at	*BC006662*	2.041830929
1426440_at	*Dhrs7*	2.040859858
1425191_at	*Ocel1*	2.040406034
1415802_at	*Slc16a1*	2.03557023
1423574_s_at	*Srd5a2l*	2.035304653
1416406_at	*Pea15a*	2.030747874
1417152_at	*Btbd14a*	2.025568833
1424239_at	*2310066E14Rik*	2.019538817
1425477_x_at	*H2-Ab1*	2.019402691
1421358_at	*H2-M3*	2.016771376
1451563_at	*Emr4*	2.014973633
1417084_at	*Eif4ebp2*	2.013044145
1422807_at	*Arf5 /// LOC100046958*	2.011996698
1425596_at	*AI317395*	2.010973439
1436339_at	*1810058I24Rik*	2.008738248
1420722_at	*Elovl3*	2.008463641
1448942_at	*Gng11*	2.007496304
1417900_a_at	*Vldlr*	2.005131983
1448506_at	*Serpina6*	2.003164776
1437211_x_at	*Elovl5*	2.001081277
1451675_a_at	*Alas2*	1.999926848
1438654_x_at	*Mmd2*	1.999297689
1422411_s_at	*Ear1 /// Ear12 /// Ear2 /// Ear3*	1.999093158
1425407_s_at	*Clec4a2 /// Clec4b1*	1.998441867
1452485_at	*Phospho1*	1.998213982
1455575_at	*Eif4ebp2*	1.997215927
1416332_at	*Cirbp*	1.996168512
1448754_at	*LOC100045055 /// Rbp1*	1.995797696
1435494_s_at	*Dsp*	1.994377674
1423642_at	*Tubb2c*	1.993863606
1422656_at	*Rasl2-9*	1.993281417
1424923_at	*Serpina3g*	1.992705709
1417819_at	*Tor1b*	1.98819623
1417569_at	*Ncald*	1.986941289

1418652_at	*Cxcl9*	1.985435231
1418317_at	*Lhx2*	1.983428255
1439388_s_at	*Bcar1*	1.98252361
1428580_at	*Blvra*	1.982072689
1449694_s_at	*Commd5*	1.979169302
1417640_at	*Cd79b*	1.978384908
1415840_at	*Elovl5*	1.977648431
1451321_a_at	*Rbm43*	1.977409259
1452085_at	*Gatad1*	1.976797383
1425616_a_at	*Ccdc23*	1.975720849
1448893_at	*Ncor2*	1.975404182
1424790_at	*Slc25a42*	1.974510621
1460316_at	*Acsl1*	1.97357767
1430780_a_at	*Pmm1*	1.973443886
1448119_at	*Bpgm*	1.972789192
1449519_at	*Gadd45a*	1.972581056
1418746_at	*Pnkd*	1.97110273
1450731_s_at	*Tnfrsf21*	1.970254541
1426253_at	*4933428G09Rik*	1.969821869
1451860_a_at	*Trim30*	1.966278238
1425824_a_at	*Pcsk4*	1.965328675
1449226_at	*Hic1*	1.961971268
1433775_at	*C77080*	1.958781309
1452599_s_at	*AI413582*	1.957438752
1450685_at	*Arpp19*	1.955271658
1454862_at	*Phldb2*	1.955167564
1415871_at	*Tgfbi*	1.954553427
1423468_at	*Steap3*	1.954364073
1452743_at	*Pole3*	1.953603159
1431506_s_at	*EG665989 /// LOC433064 /// LOC629952 /// LOC666411 /// Ppih*	1.94890432
1427039_at	*Epn1*	1.948881783
1427865_at	---	1.948244836
1448300_at	*Mgst3*	1.947659084
1452354_at	*2810459M11Rik*	1.945004681
1448943_at	*Nrp1*	1.944207936
1418825_at	*Irgm*	1.944014443
1427228_at	*Palld*	1.943307926
1434736_at	*Hlf*	1.943182233
1455439_a_at	*Lgals1*	1.939888485
1416591_at	*Rab34*	1.939752531
1416896_at	*Rps6ka1*	1.9347481
1417879_at	*Nenf*	1.934050589
1448315_a_at	*Pycr2*	1.930160281

1421534_at	*Dfna5h*	1.926816228
1456733_x_at	*Serpinh1*	1.925864648
1418890_a_at	*Rab3d*	1.923044712
1455685_at	*Mical2*	1.922658133
1417366_s_at	*Calm1*	1.922492752
1456309_x_at	*Lasp1*	1.91971304
1416656_at	*Clic1*	1.916014863
1419547_at	*Fahd1*	1.915237229
1426323_x_at	*Siva1*	1.914709188
1417267_s_at	*Fkbp11*	1.913957062
1424291_at	*Nup93*	1.913245193
1433725_at	*Acvr1b*	1.912774096
1460361_at	*5033414D02Rik*	1.909654273
1435394_s_at	*Rhoc*	1.908464177
1456393_at	*2310002J21Rik*	1.908123468
1416946_a_at	*Acaa1a /// Acaa1b*	1.908051066
1422703_at	*Gyk*	1.907910126
1424048_a_at	*Cyb5r1*	1.90788796
1451419_at	*Spsb4*	1.907845724
1422499_at	*Lima1*	1.906888276
1418014_a_at	*B4galt1*	1.906886056
1449660_s_at	*Coro1c*	1.901694022
1443695_at	*Habp2*	1.897848771
1456315_a_at	*Ptpla*	1.897508023
1449027_at	*Rhou*	1.896950112
1451036_at	*LOC100048562 /// Spg21*	1.896331619
1417374_at	*Tuba4a*	1.89430893
1434329_s_at	*Adipor2*	1.89207227
1449254_at	*Spp1*	1.89207136
1424425_a_at	*Mtap*	1.891535187
1437165_a_at	*Pcolce*	1.890328122
1416103_at	*Ywhaz*	1.890222461
1451058_at	*Mcts2*	1.888941212
1438403_s_at	---	1.885819426
1460251_at	*Fas*	1.883866334
1427042_at	*Mal2*	1.882955143
1417941_at	*Nanp*	1.882545574
1460348_at	*Mad2l2*	1.881255386
1422978_at	*Cybb*	1.88116775
1434944_at	*Dmpk*	1.881081254
1420679_a_at	*Aig1*	1.880100866
1434261_at	*Sipa1l2*	1.878405232
1450826_a_at	*Saa3*	1.877985511
1435275_at	*Cox6b2*	1.876995416
1448749_at	*Plek*	1.875589681

1417593_at	*Tusc2*	1.875118657
1426733_at	*Itpk1*	1.874303043
1427183_at	*Efemp1*	1.874065145
1449443_at	*Decr1*	1.872459108
1422472_at	*Pex13*	1.872164419
1422527_at	*H2-DMa*	1.871744114
1416846_a_at	*Pdzrn3*	1.871649453
1423846_x_at	*EG434428 /// LOC100041240 /// LOC100042266 /// LOC100045127 /// LOC100046947 /// Tuba1b*	1.870269127
1434651_a_at	*Cldn3*	1.869348169
1448757_at	*Pml*	1.866895312
1435143_at	*---*	1.866019361
1425050_at	*Isoc1*	1.865469832
1435493_at	*Dsp*	1.865267136
1439368_a_at	*Slc9a3r2*	1.865067567
1435335_a_at	*Gnptab*	1.86456973
1449183_at	*Comt*	1.864526517
1427243_at	*Rell1*	1.863524487
1419042_at	*Iigp1*	1.862448713
1427422_at	*EG624219*	1.861365924
1433661_at	*Nlrx1*	1.860384243
1456700_x_at	*Marcks*	1.857222277
1417500_a_at	*Tgm2*	1.857147304
1417714_x_at	*Hba-a1 /// Hba-a2*	1.856687881
1422948_s_at	*Hist1h4a /// Hist1h4b /// Hist1h4c /// Hist1h4h /// Hist1h4i /// Hist1h4j /// Hist1h4k /// Hist1h4m /// LOC100041230*	1.855425164
1426332_a_at	*Cldn3*	1.855172287
1425276_at	*Fbrs*	1.855053708
1418084_at	*Nrp1*	1.854936852
1437327_x_at	*Enoph1*	1.853075636
1448979_at	*Muted*	1.852145903
1453317_a_at	*Khdrbs3*	1.851552029
1450843_a_at	*Serpinh1*	1.851442628
1450903_at	*Rad23b*	1.849529842
1449310_at	*Ptger2*	1.849454971
1423630_at	*Cygb*	1.849299417
1421109_at	*Cml2*	1.849217165
1448328_at	*Sh3bp2*	1.846980669
1420936_s_at	*Cpsf2*	1.845342289
1452298_a_at	*Myo5b*	1.845148333

1448137_at	*Aldh7a1*	1.841783694
1416947_s_at	*Acaa1a /// Acaa1b*	1.841558821
1456003_a_at	*Slc1a4*	1.841288125
1418449_at	*Lad1*	1.840693011
1419054_a_at	*Ptpn21*	1.839722757
1439393_x_at	*Ppp2r4*	1.838976095
1436236_x_at	*Cotl1*	1.837687193
1449474_a_at	*Nelf*	1.836038243
1434653_at	*Ptk2b*	1.835937223
1450842_a_at	*Cenpa*	1.834878857
1437345_a_at	*Bscl2*	1.833601093
1436320_at	---	1.832804269
1438731_at	*Sgsh*	1.83118675
1437537_at	*Casp9*	1.83051842
1424785_at	*Angptl6*	1.829800506
1423607_at	*Lum*	1.82899562
1417688_at	*BC004044*	1.828828461
1435259_s_at	*Tmem141*	1.828083531
1422962_a_at	*Psmb8*	1.827820523
1455556_at	*Notch2*	1.827196541
1422076_at	*Acot4*	1.826416954
1449002_at	*Phlda3*	1.825640263
1450700_at	*Cdc42ep3*	1.825289239
1454974_at	*LOC672215 /// Ntn1*	1.824892617
1416101_a_at	*Hist1h1c*	1.823753495
1436058_at	*Rsad2*	1.823642016
1425452_s_at	*AW125753 /// Ptprj*	1.823044458
1418386_at	*N6amt2*	1.821286357
1451315_at	*Tmem101*	1.820858976
1420427_a_at	*Dhx32*	1.820431529
1427329_a_at	*Igh-6*	1.819214669
1417888_at	*Trim13*	1.818557899
1428012_at	*C8a*	1.818368632
1415776_at	*Aldh3a2*	1.817417936
1419115_at	*Alg14*	1.815143576
1435129_at	---	1.814786585
1436670_x_at	*1700019G17Rik*	1.814185446
1450044_at	*Fzd7*	1.813756207
1422155_at	*Hist2h3c2*	1.81373567
1433666_s_at	*Vps41*	1.810899425
1424877_a_at	*Alad /// LOC100046072*	1.809167044
1435559_at	*Myo6*	1.808534809
1425826_a_at	*Sorbs1*	1.808073079
1418369_at	*Prim1*	1.807877934
1449718_s_at	*4930453N24Rik*	1.807873709

1432282_a_at	*2010305C02Rik*	1.805510024
1427883_a_at	*Col3a1*	1.805450231
1421108_at	*Cml2*	1.805380985
1448123_s_at	*Tgfbi*	1.80455987
1448861_at	*Traf5*	1.803746747
1422875_at	*Cd84*	1.802873471
1455505_at	*Gatad2a*	1.802413465
1422124_a_at	*Ptprc*	1.801952395
1418240_at	*Gbp2*	1.801057155
1417544_a_at	*Flot2*	1.800062657
1449460_at	*Asb13*	1.799249404
1417010_at	*Zfp238*	1.799113034
1416980_at	*Mettl7b*	1.797719285
1417843_s_at	*Eps8l2*	1.797502789
1451341_s_at	*LOC620966 /// Tmem189*	1.796889427
1452170_at	*2010209O12Rik*	1.796484235
1419401_at	*Asb13*	1.795934183
1423061_at	*Arvcf*	1.79555789
1434138_at	*Prune*	1.793168099
1448138_at	*Ppp2r4*	1.791062888
1423348_at	*Fzd8*	1.791000074
1434600_at	*Tjp2*	1.788253754
1426883_at	*Slc25a45*	1.787312597
1448648_at	*9130005N14Rik*	1.786191227
1420632_a_at	*Bscl2*	1.78520974
1448944_at	*Nrp1*	1.783856051
1417458_s_at	*Cks2 /// LOC100039474 /// LOC100044750 /// LOC100044764*	1.782684953
1427079_at	*Mapre3*	1.782633091
1424995_at	*6230410P16Rik*	1.780928631
1419742_at	*1700037H04Rik*	1.780854292
1419765_at	*Cul2*	1.779465776
1424369_at	*Psmf1*	1.779406801
1448985_at	*Dusp22*	1.779376056
1439465_x_at	*Agbl5*	1.779020318
1417700_at	*Rab38*	1.778970666
1425603_at	*Tmem176a*	1.778948564
1416271_at	*Perp*	1.77800095
1449491_at	*Card10*	1.776662678
1451701_x_at	*Cldn3*	1.775861433
1419365_at	*Pex11a*	1.775234902
1448592_at	*Crtap*	1.774709174
1450454_at	*Tor3a*	1.77469173
1419455_at	*Il10rb*	1.773658705

1425108_a_at	*BC004728*	1.773656287
1418328_at	*Cpt1b*	1.77321577
1421098_at	*AI586015 /// LOC100047840*	1.772226372
1428955_x_at	*Slc9a3r2*	1.772194228
1422474_at	*Pde4b*	1.771903334
1436981_a_at	*Ywhaz*	1.771294722
1448171_at	*Siah2*	1.770946247
1417272_at	*9130005N14Rik*	1.770606978
1448498_at	*Rps6ka4*	1.768575961
1416156_at	*Vcl*	1.768163607
1451346_at	*Mtap*	1.766797176
1422782_s_at	*Tlr3*	1.766608384
1417365_a_at	*Calm1*	1.766451512
1419270_a_at	*Dut*	1.766179347
1424380_at	*Vps37b*	1.765890522
1454268_a_at	*Cyba*	1.765453945
1434109_at	*Sh3bgrl2*	1.761511694
1428534_at	*2310073E15Rik*	1.760648569
AFFX-ThrX-3_at	---	1.760478029
1434836_at	*Nfatc2ip*	1.760241151
1435872_at	*Pim1*	1.75845936
1424404_at	*0610040J01Rik*	1.756100957
1427364_a_at	*Odc1*	1.755195128
1426768_at	*1700060H10Rik*	1.754919918
1456530_x_at	*Elovl1*	1.753042233
1427110_at	*Raver1*	1.752092447
1423627_at	*Nqo1*	1.750914579
1424303_at	*Depdc7*	1.75056309
1427927_at	*Hscb*	1.748897552
1417038_at	*10-Sep*	1.747470947
1428357_at	*2610019F03Rik*	1.747197441
1433860_at	*6030458C11Rik*	1.746993311
1424832_at	*4732429D16Rik*	1.74688299
1418809_at	*Pira2*	1.746195906
1423584_at	*Igfbp7*	1.74492617
1422493_at	*Cpox*	1.743740741
1416199_at	*Kifc3*	1.743636418
1420438_at	*Orm2*	1.741766102
1436737_a_at	*Sorbs1*	1.739315961
1424132_at	*Hras1*	1.739256602
1421943_at	*Tgfa*	1.738894135
1421993_a_at	*Tmem134*	1.738698386
1422508_at	*Atp6v1a*	1.738182733

1424726_at	*Tmem150*	1.73783814
1428189_at	*5730494M16Rik*	1.737215222
1449349_at	*Nudt1*	1.737215151
1434735_at	*Hlf*	1.736619261
1420653_at	*Tgfb1*	1.735981635
1416750_at	*Oprs1*	1.734754344
1419353_at	*Dpm1*	1.733827836
1456125_a_at	*Dynll1 /// EG627788*	1.73378171
1435458_at	*Pim1*	1.733705284
1427003_at	*Ppp2r5c*	1.733307804
1419509_a_at	*Nagk*	1.732353624
1427954_at	*BC048403*	1.731551079
1426873_s_at	*Jup*	1.731360298
1426897_at	*Rcc2*	1.731300916
1450017_at	*Ccng1*	1.730930642
1424829_at	*A830007P12Rik*	1.73055757
1421402_at	*Mta3*	1.729498361
1416897_at	*Parp9*	1.728811707
1437277_x_at	*Tgm2*	1.726363951
1431704_a_at	*Ralgps2*	1.72588058
1415973_at	*Marcks*	1.725870038
1450947_at	*2610528J11Rik*	1.725373648
1420172_at	---	1.724516905
1428788_at	*1700012G19Rik*	1.724491597
1426988_at	*Klhdc5*	1.723704129
1417384_at	*Entpd5*	1.72369056
1417379_at	*Iqgap1*	1.722561315
1423267_s_at	*Itga5*	1.721281261
1417431_a_at	*Sphk2*	1.721048548
1422704_at	*Gyk*	1.720845817
1455065_x_at	*EG667410 /// Gnpda1*	1.720151716
1418088_a_at	*Stx8*	1.719579499
1451091_at	*Txndc5*	1.719288939
1450010_at	*Hsd17b12*	1.719218527
1452472_at	*Rtp3*	1.719094808
1419045_at	*Slc25a23*	1.718204337
1418015_at	*Pum2*	1.717881121
1450803_at	*Ntf3*	1.716129966
1452062_at	*Prpsap2*	1.71561893
1439005_x_at	*Ywhaz*	1.714566333
1418508_a_at	*Grb2*	1.71414527
1452768_at	*Tex261*	1.71327581
1422687_at	*Nras*	1.713257913
1431701_a_at	*Pdzk1*	1.713038861
1424530_at	*Sec14l2*	1.713035691

1455106_a_at	*Ckb*	1.712216395
1424631_a_at	*Ighg*	1.711986684
1428823_at	*Hddc2*	1.709730953
1433582_at	*1190002N15Rik /// LOC100044725*	1.709529291
1451369_at	*Commd5*	1.70881424
1438676_at	*Mpa2l*	1.708552172
1416184_s_at	*Hmga1*	1.707606164
1456250_x_at	*Tgfbi*	1.707312606
1449852_a_at	*Ehd4*	1.705878653
1418518_at	*Furin*	1.70512236
1456169_at	*EG226654*	1.704999553
1419648_at	*Myo1c*	1.704708902
1427060_at	*Mapk3*	1.704654127
1417377_at	*Cadm1*	1.704040206
1419737_a_at	*Ldha*	1.70358432
1425778_at	*Indol1*	1.703518525
1418884_x_at	*LOC100045728 /// LOC636070 /// LOC676756 /// Tuba1a*	1.703158623
1449009_at	*LOC100039796 /// Tgtp*	1.702696278
1452598_at	*Gins1*	1.702502877
1426004_a_at	*Tgm2*	1.700168773
1448904_at	*D6Wsu176e*	1.700071819
1418962_at	*Necap2*	1.699869495
1454373_x_at	*Ubc*	1.699808267
1422910_s_at	*Smc6*	1.699680653
1417135_at	*Srpk2*	1.698943476
1421237_at	*Tmpo*	1.698773438
1451841_a_at	*Ncor2*	1.698337165
1424981_at	*Nln*	1.698234724
1422597_at	*Mmp15*	1.69784533
1448848_at	*Tor1b*	1.697432041
1448436_a_at	*Irf1*	1.696875706
1429033_at	*Gcc1*	1.696803097
1423557_at	*Ifngr2*	1.696130604
1427082_at	*4632417N05Rik*	1.694513512
1438602_s_at	*Masp1*	1.693637183
1448382_at	*Ehhadh*	1.693168991
1420626_at	*Gtf3c6*	1.692343279
1425985_s_at	*Masp1*	1.691801625
1415677_at	*Dhrs1*	1.690950256
1419097_a_at	*Stom*	1.690873479
1420901_a_at	*Hk1*	1.690765638
1449907_at	*Bcmo1*	1.689704912

1418359_at	*Wbscr27*	1.688156834
1421654_a_at	*Lmna*	1.687834657
1419209_at	*Cxcl1*	1.687737821
1422748_at	*Zeb2*	1.687342733
1422815_at	*C9*	1.686776037
1448318_at	*Adfp*	1.686415729
1451969_s_at	*Parp3*	1.686114216
1422070_at	*Adh4*	1.685485629
1424450_at	*Gprc5c /// LOC100044349*	1.685382386
1451060_at	*Gpr146*	1.685281239
1423169_at	*Taf7*	1.682463403
1416389_a_at	*Rcbtb2*	1.681680425
1438312_s_at	*Ltbp3*	1.681143054
1436994_a_at	*Hist1h1c*	1.680619758
1424614_at	*Frag1*	1.679561355
1448651_at	*Nudt5*	1.678843772
1451641_at	*Dbr1*	1.677464229
1415906_at	*LOC100047211 /// Tmsb4x*	1.67702591
1421183_at	*Tex12*	1.676348683
1424411_at	*Tmem189*	1.676082267
1416924_at	*Bri3*	1.674918917
1428082_at	*Acsl5*	1.674111017
1420879_a_at	*Ywhab*	1.673670531
1417828_at	*Aqp8*	1.67316508
1436158_at	*Eif4ebp2*	1.672654771
1418891_a_at	*Rab3d*	1.672440053
1420843_at	*Ptprf*	1.672380263
1424191_a_at	*Tmem41a*	1.672173499
1420567_at	*Prkcn*	1.671867701
1460231_at	*Irf5*	1.671461911
1451362_at	*Rab7l1*	1.671049609
1448559_at	*Flot1*	1.671034456
1427103_at	*Plekhq1*	1.670158132
1438910_a_at	*Stom*	1.669892525
1429170_a_at	*Mtf1*	1.669750385
1421116_a_at	*Rtn4*	1.669646631
1421144_at	*Rpgrip1*	1.668791461
1418438_at	*Fabp2*	1.667912364
1434512_x_at	*EG632248 /// Sfrs3*	1.666881661
1455959_s_at	*Gclc*	1.666388122
1422684_a_at	*Exoc4*	1.665331902
1416257_at	*Capn2*	1.665263814
1424003_at	*4930444A02Rik*	1.665216371
1424300_at	*Gemin6*	1.664834493
1426692_at	*Ccdc97*	1.664350115

1424927_at	*Glipr1*	1.663955817
1422473_at	*Pde4b*	1.663682447
1450519_a_at	*Prkaca*	1.663488385
1424266_s_at	*AU018778*	1.662008836
1427423_at	*2810410C14Rik*	1.660394862
1428494_a_at	*Polr2i*	1.658723845
1417632_at	*Atp6v0a1*	1.658454664
1425120_x_at	*1810023F06Rik*	1.65818565
1448605_at	*Rhoc*	1.658090098
1417728_at	*Mbd3*	1.657999104
1425944_a_at	*Rad51l3*	1.657845189
1424667_a_at	*Cutl1*	1.657749608
1416028_a_at	*Hn1*	1.656940937
1428442_at	*BC029722*	1.656637751
1418445_at	*LOC100045628 /// Slc16a2*	1.656428627
1423957_at	*Isg20l1*	1.656347899
1438164_x_at	*Flot2*	1.655510329
1451896_a_at	*Cherp*	1.654738358
1451307_at	*Mrpl14*	1.6542727
1455798_at	*Galk2*	1.653746677
1452457_a_at	*Prkab1*	1.653742107
1435527_at	*Nfic*	1.653519187
1428707_at	*Ptms*	1.653322614
1426530_a_at	*Klhl5*	1.653038015
1416458_at	*Arf2*	1.652813445
1450410_a_at	*4930570C03Rik*	1.65279273
1460179_at	*Dnaja1*	1.652686056
1417188_s_at	*Hip2*	1.652408538
1449303_at	*Sesn3*	1.650871348
1416178_a_at	*Plekhb1*	1.650570626
1426432_a_at	*Slc4a4*	1.650002296
1448390_a_at	*Dhrs3*	1.647979288
1455900_x_at	*Tgm2*	1.647972323
1453578_at	*Pter*	1.647623005
1448960_at	*Cxxc5*	1.647299644
1418294_at	*Epb4.1l4b*	1.64721742
1417204_at	*Kdelr2*	1.646990995
1425753_a_at	*Ung*	1.646974398
1448823_at	*Cxcl12*	1.646418592
1431003_a_at	*2610002J02Rik*	1.646378296
1423765_at	*Athl1*	1.645556901
1417408_at	*F3*	1.644613128
1448422_at	*Tmed4*	1.644270597
1451601_a_at	*Spns2*	1.643401228

1434087_at	*Mthfr*	1.643276892
1419091_a_at	*Anxa2*	1.642298479
1448678_at	*3110048E14Rik*	1.642167297
1436038_a_at	*Pigp*	1.642026792
AFFX-r2-Bs-thr-3_s_at	---	1.641809029
1421214_at	*Cmah*	1.641744324
1418174_at	*Dbp*	1.641723048
1416128_at	*EG626534 /// Tuba1c*	1.641505578
1418804_at	*Sucnr1*	1.641321041
1415853_at	*Def8*	1.641204044
1422730_at	*Limd1*	1.640242702
1436232_a_at	*Gabpb1*	1.640226232
1424626_at	*2010003K11Rik*	1.640009071
1448186_at	*Pnliprp2*	1.639885135
1424293_s_at	*Tmem55a*	1.639272402
1419114_at	*Alg14*	1.637561212
1448463_at	*4933434E20Rik*	1.637363757
1452359_at	*Rell1*	1.637060018
1416320_at	*Sec22a*	1.637059519
1439398_x_at	*Nelf*	1.637054171
1448450_at	*Ak2 /// LOC100047005*	1.636777619
1451426_at	*Dhx58*	1.63601019
1452649_at	*Rtn4*	1.635557172
1450011_at	*Hsd17b12*	1.635097611
1419745_at	*4933428G20Rik*	1.635057859
1424040_at	*Mtap7d1*	1.63393998
1453343_s_at	*Vrk2*	1.63363044
1433991_x_at	*Dbi*	1.632847403
1416888_at	*Fadd*	1.63245479
1435788_at	*2900086B20Rik*	1.632411973
1432848_a_at	*1200004M23Rik*	1.632349123
1460565_at	*Slc41a1*	1.631683338
1417070_at	*Cyp4v3*	1.630942653
1418073_at	*Acot9*	1.630783416
1429566_a_at	*Hipk2*	1.630756162
1417535_at	*Fbxo25*	1.62916107
1452235_at	*Man1b1*	1.628695692
1448752_at	*Car2*	1.62769168
1450779_at	*Fabp7*	1.627176563
1418377_a_at	*Siva1*	1.626908633
1427513_at	*BC024137*	1.626273695
1450967_at	*Ptplad2*	1.625546295
1425058_at	*Zfp472*	1.624607595
1452280_at	*Farp1 /// LOC100045542*	1.624225978

1417331_a_at	*Arl6*	1.623961011
1431800_at	*1300010F03Rik*	1.621946593
1416527_at	*Rab32*	1.621187543
1438761_a_at	*EG666231 /// EG668343 /// LOC546355 /// LOC627245 /// LOC632337 /// LOC677259 /// Odc1*	1.620769717
1428708_x_at	*Ptms*	1.620114049
1450792_at	*Tyrobp*	1.620045268
1423721_at	*Tpm1*	1.619925716
1424351_at	*Wfdc2*	1.619865361
1422768_at	*Syncrip*	1.618870586
1438067_at	*Nf1*	1.61824397
1418450_at	*Islr*	1.617150789
1422498_at	*Mageh1*	1.615029719
1448699_at	*Mrpl54*	1.613423949
1433670_at	*Emp2*	1.613357958
1448412_a_at	*Tsc22d4*	1.613305609
1417866_at	*Tnfaip1*	1.612684897
1417294_at	*Akr7a5*	1.612624421
1439266_a_at	*Polr3k*	1.612348006
1451363_a_at	*Dennd2d*	1.612346947
1428063_at	*Ankrd46*	1.612333573
1425053_at	*Isoc1*	1.611871282
1435834_at	*LOC100040608 /// LOC100046423*	1.611612905
1422701_at	*Zap70*	1.611556898
1429270_a_at	*Syce2*	1.611524484
1418906_at	*Nubp1*	1.611138743
1418187_at	*Ramp2*	1.610736029
1416840_at	*Mid1ip1*	1.608745244
1422692_at	*Sub1*	1.608339138
1421050_at	*Vps25*	1.607890309
1423217_a_at	*2510049I19Rik*	1.607823026
1417449_at	*Acot8*	1.605823384
1450259_a_at	*Stat5a*	1.605652857
1417184_s_at	*Hbb-b1 /// Hbb-b2*	1.604679211
1420603_s_at	*Raet1a /// Raet1b /// Raet1c /// Raet1d /// Raet1e*	1.604529097
1417040_a_at	*Bok*	1.604077261
1428367_at	*Ndst1*	1.60371826
1417373_a_at	*Tuba4a*	1.603655217
1423816_at	*Cxx1b*	1.603645
1448170_at	*Siah2*	1.602385315

1454993_a_at	*Sfrs3*	1.602369319
1426812_a_at	*9130404D14Rik*	1.601795685
1428160_at	*Ndufab1*	1.601546833
1424564_at	*2410001C21Rik*	1.601371876
1451079_at	*Adpgk*	1.600499021
1424929_a_at	*Trim26*	1.599379143
1434930_at	*Tpcn1*	1.598454649
1452828_at	*Fbxo21*	1.598110645
1427168_a_at	*Col14a1*	1.596933651
1427604_a_at	*Atp9a*	1.596421935
1451644_a_at	*0610037M15Rik /// H2-Q6 /// LOC436493*	1.59638192
1448718_at	*2400001E08Rik*	1.59565843
1417104_at	*Emp3*	1.593945003
1423976_at	*4930453N24Rik*	1.593871766
1434879_at	*Cdc34 /// LOC100046898*	1.593556631
1460220_a_at	*Csf1*	1.592811784
1418248_at	*Gla*	1.592735415
1448682_at	*Dynll1*	1.592672961
1423049_a_at	*Tpm1*	1.59238654
1428714_at	*Pgrmc2*	1.592270744
1448969_at	*Ftsj2*	1.591914448
1460411_s_at	*AW548124 /// LOC100048505*	1.591627342
1450715_at	*Cyp1a2*	1.591420119
1418766_s_at	*Timd2*	1.591031585
1422516_a_at	*Fibp*	1.590885764
1434330_at	*Tbcel*	1.589817249
1433515_s_at	*Etnk1*	1.589091563
1416700_at	*Rnd3*	1.588322976
1448618_at	*Mvp*	1.588292941
1416614_at	*Eid1*	1.587290866
1421089_a_at	*2610028A01Rik*	1.587020624
1418623_at	*Rab2*	1.58691661
1420827_a_at	*Ccng1*	1.586891249
1457265_at	*Sfrs17b*	1.586065841
1450981_at	*Cnn2*	1.585900457
1423729_a_at	*Rnf181*	1.585464283
1452074_at	*Tmem135*	1.585456572
1449317_at	*Cflar /// LOC100040853 /// LOC100046787*	1.585438277
1416009_at	*Tspan3*	1.584881828
1431008_at	*0610037M15Rik /// H2-Q6*	1.584730269
1418737_at	*Nudt2*	1.584512243
1424108_at	*Glo1*	1.584505592
1428257_s_at	*Dynlrb1*	1.583951552

1420507_a_at	*3110031B13Rik*	1.583862204
1450252_at	*Onecut1*	1.58351122
1424497_at	*1110032O16Rik*	1.583164932
1448940_at	*Trim21*	1.583098121
1420636_a_at	*Dusp12*	1.582904832
1425385_a_at	*LOC100047788*	1.582688219
1432136_s_at	*Zdhhc4*	1.582618688
1428905_at	*Rraga*	1.582184012
1428954_at	*Slc9a3r2*	1.581414826
1451224_at	*Scamp5*	1.580808196
1455976_x_at	*Dbi*	1.580268222
1422753_a_at	*Polr3k*	1.579798836
1421871_at	*Sh3bgrl*	1.578812603
1448748_at	*Plek*	1.578808998
1418661_at	*Abhd2*	1.578646444
1448617_at	*Cd53*	1.578309585
1460376_a_at	*Cox15*	1.577848173
1451219_at	*Ormdl1*	1.577649218
1451110_at	*Egln1*	1.577545594
1423375_at	*1700023B02Rik*	1.577142139
1451918_a_at	*Loh12cr1*	1.576854409
1454753_at	*Rnpepl1*	1.576649991
1460228_at	*LOC100047980*	1.575584772
1449193_at	*Cd5l*	1.575398708
1425844_a_at	*Rngtt*	1.575370951
1419630_a_at	*Trim11*	1.575338323
1424451_at	*Acaa1b*	1.575324449
1437723_s_at	*Derl1*	1.575225036
1419467_at	*Clec14a*	1.575144278
1452664_a_at	*Tm7sf3*	1.574889905
1452757_s_at	*Hba-a1 /// Hba-a2*	1.574717087
1416508_at	*Med28*	1.574075034
1420132_s_at	*Pttg1ip*	1.573890703
1431226_a_at	*Fndc4*	1.57376381
1452055_at	*Ctdsp1 /// LOC100047249*	1.573699833
1415974_at	*Map2k2*	1.573032919
1460644_at	*Bckdk*	1.572998455
1423986_a_at	*Scotin*	1.572541547
1418078_at	*Psme3*	1.572019392
1423547_at	*Lyzs*	1.571919598
1425599_a_at	*Gatad1*	1.571771535
1450406_a_at	*St3gal3*	1.571610301
1419493_a_at	*Tpd52*	1.571532846
1439451_x_at	*Gpr172b*	1.570545515
1421221_at	*Bcdo2*	1.570228728

1416564_at	*Sox7*	1.569767555
1434020_at	*Pdap1*	1.569594561
1420643_at	*Lfng*	1.569496117
1452919_a_at	*1700012G19Rik*	1.569472359
1425567_a_at	*Anxa5*	1.568550491
1451651_at	*LOC100044885 /// Vsig4*	1.568469434
1451393_at	*Pex26*	1.568308636
1420612_s_at	*Ptp4a2*	1.568200291
1422593_at	*Ap3s1*	1.568173582
1451803_a_at	*Vegfb*	1.567960744
1426765_at	*Commd7 /// LOC631742 /// LOC674161*	1.567817666
1425297_at	*BC012278*	1.567595835
1416046_a_at	*Fuca2*	1.567547347
1434291_a_at	*Serf1*	1.567097492
1438670_at	*Ptpn1*	1.567087533
1434071_a_at	*Pelo*	1.566547568
1450425_a_at	*2700062C07Rik*	1.5664008
1415732_at	*Bat5*	1.56503999
1417211_a_at	*1110032A03Rik /// LOC100048251*	1.564931242
1417611_at	*Tmem37*	1.564818876
1437463_x_at	*Tgfbi*	1.564644908
1429884_at	*Srgap2*	1.564554767
1449353_at	*Zmat3*	1.564397754
1452100_at	*Dullard /// LOC100048221*	1.563541707
1436050_x_at	*Hes6*	1.562908196
1455719_at	*Tubb5*	1.56215549
1419714_at	*Cd274*	1.562125336
1421899_a_at	*Mr1*	1.561340575
1416692_at	*Coil*	1.561340085
1417177_at	*Galk1*	1.561216018
1452622_a_at	*Tradd*	1.561107242
1452021_a_at	*Hes6*	1.560724603
1449455_at	*Hck*	1.560527685
1425983_x_at	*Hipk2*	1.560170002
1417472_at	*Myh9*	1.559859646
1448494_at	*Gas1*	1.558347356
1448416_at	*Mgp*	1.557745561
1432176_a_at	*Eng*	1.557094388
1452327_at	*Iqsec1*	1.55696688
1460341_at	*Plekhb2*	1.556404922
1416444_at	*Elovl2*	1.556071158
1431469_a_at	*Cxxc5*	1.555589566
1417457_at	*Cks2 /// LOC100039474 /// LOC100044764*	1.555172944

1426499_at	*Sh3glb2*	1.554826339
1417205_at	*Kdelr2*	1.554706353
1426454_at	*Arhgdib*	1.554686969
1439383_x_at	*Ppp2r4*	1.554368229
1437670_x_at	*Cd151*	1.554148364
1456377_x_at	*Limd2 /// LOC632329*	1.553776664
1423271_at	*Gjb2*	1.553719591
1450672_a_at	*Trex1*	1.553561746
1419627_s_at	*Clec4n*	1.553012855
1425052_at	---	1.552441188
1417810_a_at	*Kcnb1 /// Pacsin2*	1.551669973
1460739_at	*Zmiz2*	1.551574941
1419761_a_at	*Gabpb1*	1.550942613
1433563_s_at	*Derl1*	1.549835274
1420459_at	*Ripply3*	1.5493303
1427137_at	*Ces5*	1.549234244
1417376_a_at	*Cadm1*	1.548944436
1438058_s_at	*Ptov1*	1.548806626
1455940_x_at	*Wdr6*	1.548607373
1451435_at	*Cutl1*	1.548298342
1421259_at	*Pklr*	1.548011269
1419747_at	*Asgr2*	1.547862811
1422917_at	*Epha1*	1.547706661
1451755_a_at	*Apobec1*	1.547210258
1448852_at	*Rgn*	1.547088443
1435525_at	*Kctd17*	1.546985446
1449131_s_at	*Cd1d1*	1.546727651
1427048_at	*Smo*	1.546665589
1460178_at	*Lonp2*	1.545776243
1452294_at	*Pcdh1*	1.54571593
1451207_at	*Cbara1*	1.544825518
1435170_at	*Tsr2*	1.544726184
1455639_at	*Slc25a39*	1.542833577
1450855_at	*Arvcf*	1.541654616
1415950_a_at	*Pebp1*	1.54080983
1419031_at	*Fads2*	1.540439913
1419964_s_at	*Hdgf*	1.539815992
1455045_at	*Srr*	1.539804412
1428406_s_at	*Hcfc1r1*	1.539572704
1451457_at	*Sc5d*	1.539423413
1434923_at	*Cox19*	1.539396371
1419614_at	*Pla2g12b*	1.539069406
1417378_at	*Cadm1*	1.539024878
1417085_at	*Akr1c6*	1.538944942
1423680_at	*Fads1*	1.538876558

1427261_at	*Wwc1*	1.538749316
1424068_at	*Tcta*	1.538150464
1452166_a_at	*Krt10*	1.53780045
1422826_at	*Igfals*	1.537699711
1424133_at	*Tmem98*	1.537383264
1416909_at	*Pigyl*	1.536713191
1426799_at	*Rab8b*	1.536460063
1424370_s_at	*Psmf1*	1.53622042
1460344_at	*2310033F14Rik*	1.535681766
1416608_a_at	*BC004004*	1.535494418
1449059_a_at	*Oxct1*	1.535397011
1426880_at	*Etl4*	1.534761004
1451777_at	*BC013672*	1.53367394
1423282_at	*Pitpna*	1.533645592
1423573_at	*LOC100044230 /// Srd5a2l*	1.533002484
1416152_a_at	*Sfrs3*	1.532543697
1424093_x_at	*Cd151*	1.532388758
1460362_at	*2410001C21Rik*	1.531766729
1434881_s_at	*Kctd12*	1.531735682
1455008_at	*LOC100048021*	1.531595419
1436996_x_at	*Lyz*	1.530984886
1452063_at	*2410081M15Rik*	1.530885974
1426875_s_at	*Srxn1*	1.53053521
1418258_s_at	*Dynll2*	1.530401459
1426746_at	*1810026J23Rik*	1.529948932
1452312_at	*2810002D19Rik*	1.529917599
1433595_at	*LOC100047674*	1.529490452
1426306_a_at	*LOC100046560 /// Maged2*	1.529464927
1424590_at	*Ddx19b*	1.529069239
1434417_at	*Solh*	1.529060943
1429514_at	*Ppap2b*	1.52825209
1416433_at	*Rpa2*	1.527636951
1419833_s_at	*Centd3*	1.527529274
1428552_at	*2610001J05Rik*	1.527501887
1451899_a_at	*Gtf2ird1*	1.527400488
1448722_s_at	*Ppcs*	1.527190395
1423909_at	*Tmem176a*	1.526590936
1418496_at	*Foxa1 /// LOC100047556*	1.525983295
1417820_at	*Tor1b*	1.525427935
1415855_at	*Kitl*	1.525337533
1426435_at	*Tmem135*	1.525049348
1433655_at	*Rnf141*	1.524917477
1424648_at	*Rabl4*	1.524693082
1453470_a_at	*Gna13*	1.524526449

1416810_at	*Mea1*	1.524167597
1424552_at	*Casp8*	1.523992212
1451352_s_at	*Mta3*	1.523792141
1452882_at	*Pgrmc2*	1.522753198
1426965_at	*Rap2a*	1.522595501
1420329_at	*4930455C21Rik*	1.521588166
1428635_at	*Comtd1*	1.521002329
1416549_at	*Slc35b4*	1.520514318
1430125_s_at	*Pqlc1*	1.520372031
1433690_at	*2210016L21Rik*	1.520207985
1424277_at	*LOC100044098 /// LOC100048005 /// Uchl5ip*	1.520156233
1435764_a_at	*Gemin7*	1.51995476
1434856_at	*Ankrd44*	1.519654734
1449377_at	*Nicn1*	1.519247684
1450016_at	*Ccng1*	1.519010017
1438093_x_at	*Dbi*	1.51856484
1420984_at	*Pctp*	1.518004701
1431241_at	*LOC100046321*	1.517537919
1417251_at	*LOC100046410 /// Palmd*	1.517532031
1416155_at	*Hmgb3*	1.517304064
1454805_at	*LOC100043168 /// LOC100047730 /// Wtap*	1.517129654
1455530_at	*Ighv14-2*	1.516880139
1449536_at	*Kcnn1*	1.516849451
1438211_s_at	*Dbp*	1.516655656
1448346_at	*Cfl1*	1.51616631
1424358_at	*Ube2e2*	1.515211826
1422752_at	*Polr3k*	1.51495614
1451455_at	*Thnsl2*	1.51461555
1425211_at	*2010003O18Rik*	1.514547828
1451371_at	*Mrap*	1.514547709
1434273_at	*BC034069*	1.514392644
1416773_at	*Wee1*	1.513874528
1424163_at	*Rmnd5b*	1.513467285
1437341_x_at	*Cnp*	1.513208804
1416151_at	*Sfrs3*	1.5131443
1426362_at	*Tmem144*	1.511927001
1435270_x_at	*N6amt2*	1.511774882
1438133_a_at	*Cyr61*	1.511585707
1430274_a_at	*Stard3nl*	1.511296564
1434642_at	*Hsd17b11*	1.510939151
1415961_at	*Itm2c*	1.510533559
1450097_s_at	*LOC100048021*	1.509819509
1427934_at	*Lyrm2*	1.509643143

1455894_at	*Ptrh2*	1.509358743
1417648_s_at	*Snx5*	1.508148664
1424720_at	*Mgat4b*	1.508093559
1425491_at	*Bmpr1a*	1.507307515
1424863_a_at	*Hipk2*	1.506778362
1424576_s_at	*Cyp2c44*	1.506650738
1435454_a_at	*BC006779*	1.506509651
1460424_at	*Tmem160*	1.506396883
1460724_at	*Ap2a1*	1.505720314
1419518_at	*Tuba8*	1.505487738
1421846_at	*Wsb2*	1.505460198
1448765_at	*Fyn*	1.505362669
1420650_at	*Zfhx3*	1.504445162
1448797_at	*Elk3*	1.503874203
1451760_s_at	*2010001E11Rik /// AI317395*	1.503711127
1415693_at	*Derl1*	1.503647535
1416016_at	*Tap1*	1.503625959
1424394_at	*Selm*	1.503497301
1425493_at	*Bmpr1a*	1.502940973
1449139_at	*2310008M10Rik*	1.502840685
1456011_x_at	*Acaa1a*	1.502787104
1417077_at	*Bcap29*	1.502696402
1418340_at	*Fcer1g*	1.502422373
1450484_a_at	*Tyki*	1.502040656
1418923_at	*Slc17a3*	1.501738697
1433428_x_at	*Tgm2*	1.501558379
1448967_at	*Nipsnap3a*	1.500247852
1433685_a_at	*6430706D22Rik*	1.500001854
1426524_at	*Gnpda2*	1.499893329
1453016_at	*2900042B11Rik*	1.499502445