MAPPING OF QTL FOR POSTWEANING AVERAGE DAILY GAIN IN PIGS

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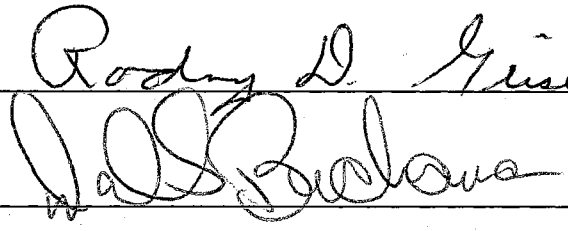

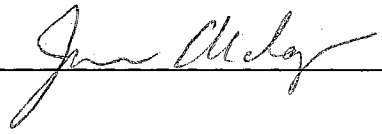

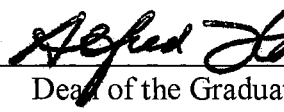

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ABBREVIATIONS

5-HT	Serotonin [5-hydroxytryptamine]
ACTH	Adenocorticotropin hormone
ADG	Average daily gain
AGRP	Agouti-related peptide
ARC	Arcuate nucleus
ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
β3AR	β3-adrenarlic receptor
BF	Backfat thickness
BLUP	Best linear unbiased prediction
BW	Body weight
BAT	Brown adipose tissue
BMI	Body mass index, (body weight)/(height)^2
CCK	Cholecystokinin
CCK-AR	Cholecystokinin type-A receptor
CCK-BR	Cholecystokinin type-B receptor
cM	Centi Morgan
CNS	Central nervous system
CRH	Corticotropin releasing hormone
CSF	Central spinal fluid
CV	Coefficient of variance
DD-PCR	PCR-base differential display
DMH	Dorsal medial hypothalamic nucleus
F	Fast growth
FCR	Feed conversion ratio
FE	Feed efficiency
FFA	Free fatty acid
FI	Feed intake
FISH	Fluorescent in situ hybridization
GH	Growth hormone
GLP-1	Glucagon-like peptide 1
GPCRs	G protein-coupled receptors
GR	Growth rate
i.c.v.	Intracerebroventricular
JAK	Janus kinase

LEP	Leptine (obese) gene
LEPR	Leptine (obese) receptor
LTFC	Lean tissue feed conversion
LOD	Logarism of odd
LTGR	Lean tissue growth rate
MAS	Maker assisted selection
MC1R	Melanocortin type 1 receptor
NC2R	Melanocortin type 2 receptor
MC3R	Melanocortin type 3 receptor
MC4R	Melanocortin type 4 receptor
MC5R	Melanocortin type 5 receptor
MCH	Melanin concentrating hormone
ME	Metabolizable energy
MSH	Melanocyte stimulating hormone
NPY	Neuropeptide Y
NPY Y5	Neuropeptide receptor Y5
NT	Neurotensin
OB	Obese (leptin) gene
OBR	Obese (Leptin) receptor gene
OX1R	Olexin type 1 receptor
OX2R	Olexin type 2 receptor
PCR	Polimerase chain reaction
POMC	Proopiomelanocortin
PPAR	Proxisome proliferate activated receptor
PVN	Paraventricular neuron
QTL	Quantitative trait loci
RFI	Residual feed intake
RFLP	Restriction fragment length polymorphism
STAT	Signal transducers and activators of transcription
STR	Simple tandem repeat
TRP	Triptophan
UCP1	Uncoupling protein 1
UCP2	Uncoupling protein 2
UCP3	Uncoupling protein 3
VMH	Ventromedial hypothalamus
WAT	White adipose tissue

I. Introduction

To be profitable, pork producers must achieve lower production costs and higher carcass values. Feed costs represents more than 60% of total pork production costs. Thus, efficient conversion of feed to weight gain is important for the profitable pork production. Efficiency of feed utilization can be expressed by the ratio of feed consumption and weight gain. Leanness is a primary factor determining carcass values so that gain of lean tissue is important as well. Growth is a process of accretion of body tissues that leads the animal to physical and sexual maturity.

Energy for maintenance of body functions is fundamental requirement and excessive energy can be converted to body growth. Growth rate and body composition can be manipulated by regulation of feed intake. Under the abundant feed supply, there would be variations in feed intake and accretion rate of lean and adipose tissue. Status of energy balance controls feeding behavior. Thus, exploitation of genetic effects on appetite regulation may be a way to improve feed efficiency and growth rate.

Significant improvement of economically important traits has been achieved in pigs. However, use of genomic data could boost the rate of genetic improvement of some traits. Advancements in molecular technology are the primary force for the localization of genes on chromosomes and elucidation of gene functions. Gene mapping has many applications to our life such as medicine and food production. In pig production, genetic markers can be utilized to improve pigs through selection, which is referred the marker assisted selection (MAS). Identifying genes controlling feeding behavior would provide us with the tools to manipulate growth rate and carcass traits. Since the first report of genetic markers for growth and fatness (Andersson et al., 1994), several genetic markers for growth traits have been reported (Yu et al., 1995a; Casas et al., 1997b; Knott et al., 1998; Wang et al., 1998; Marklund et al., 1999; Paszek et al., 1999). However, genome information for livestock species is still limited compared to the human and mouse. Development of a high resolution porcine genome map may permit greater genetic improvement of economically important traits such as lean growth rate and feed efficiency.

II. Review of literature

1. Genetic Aspects of Feed Intake and Growth

1.1 Genetic Correlation between Feed Intake and Growth

The reported genetic correlations between daily feed intake (FI) and daily body weight gain (GR) when pigs had *ad libitum* access to feed were in the range from 0.57 to 0.80. Cameron et al. (1988) reported estimates of 0.69 (Large White) and 0.73 (British Landrace) for pigs tested from 30 to 85 kg of body weight. Van Steenbergen et al. (1990) reported an estimate of 0.57 for boars tested from 25 to 105 kg of body weight, and Mrode and Kennedy (1993) reported 0.80 for boars tested from 30 to 90 kg of body weight. Von Felde et al. (1996) reported a genetic correlation of 0.62 between FI and GR for the pigs tested with a computerized feeding device which was capable to record the time of access to the feeder and volume of feed consumed by each pig between 100 and 170 days of age.

Many studies have been focused on improvement in feed conversion ratio (FCR) which is a ratio of FI to GR. Dickerson and Grime (1947) reported a heritability of .26 ($P < .05$) for FCR between 72 days of age and 102 kg of body weight in Duroc breed. Ellis and Smith (1979) reported that selection based on an index including FCR in addition to backfat (BF), average daily gain (ADG) under *ad libitum* feeding resulted in a significant reduction of feed intake. Sather and Fredeen (1978) reported improvement of FCR as a correlated response to index selection for ADG and BF. The pigs were selected with the index (I), $I=\Delta GR/\sigma_{GR} - \Delta BF/\sigma_{BF}$, where ΔG and ΔBF were deviations from the mean for GR and BF, respectively, and σ_{GR} and σ_{BF} were phenotypic standard deviations of GR and BF, respectively. After 9 generations of selection, GR was increased by .94 standard deviation, and BF was reduced by 1.51 standard deviation, whereas FI was reduced by 1.07 standard deviation.

Feed efficiency is the ratio of GR to FI. Therefore, the correlation between FI and feed efficiency (GR/FI) is not a relationship between two independent variables. The nature of this relationship has been discussed by Turner (1959) and Sutherland (1965). Both authors discussed the mathematical characteristics of the correlation between a ratio and its denominator, such as FI and GR/FI. The correlation between a ratio of two variables and either single variable can be predicted from the correlation between each of

the single variables and the coefficients of variation (CV). As the relationship between FI and FI/GR is dependent on the relative size of the CV for FI and GR, factors which change the CV will in turn change the phenotypic or genetic relationship between FI and GR. Drewry (1980) reported phenotypic correlations of 0.67 between FI and GR, 0.43 between FI and GR/FI, and -0.32 between GR and FI/GR. If, with restricted feeding, pigs are fed the same amounts of feed in a given period of time, variation in appetite is eliminated and a perfectly negative correlation between GR and FI/GR is expected. For instance, Smith et al. (1962) and Owen and Morton (1969) reported that there appears to be a progressive reduction in the genetic correlation between ADG and FCR from about -0.9 to -0.6 or less as one moves from a system of restricted feeding towards *ad libitum* access to feed. Cameron et al. (1988) reported genetic correlations between GR and FI/GR of –0.69 (*ad libitum*) and –1.03 (restricted).

Improvement of growth rate does not necessarily result in improvement of the overall value of the pig as a meat animal. Leanness is a primary determining factors of the total carcass value. Fowler et al. (1976) proposed to express the efficiency of growth based on lean tissue gain instead of total weight gain. One measurement of the efficiency is lean tissue growth rate (LTGR; lean/d). A second measurement is lean tissue feed conversion (LTFC; lean/feed). Selection index theory developed by Hazel (1943) is intended to maximize overall genetic gain of traits according to their economic values. Therefore, Fowler's index is called a "biological" index in order to distinguish it from Hazel's "economic" index. Fowler et al. (1976) theorized that higher LTGR can be achieved by increased lean growth without changes in food intake, while greater LTFC can be achieved by either increased lean growth rate, reduced food intake or both. Cameron and Curran (1994) reported the results of selection for LTGR or LTFC in Large White and British Landrace. Improvement of LTGR was due to increased growth rate with little change in daily food intake, but improvement of LTFC was due primarily to reduced daily food intake. These results were in agreement with the relationships between GR and FCR which were theorized by Fowler et al. (1976).

1.2 Genetic variation in Voluntary Feed Intake

Some genetic controls of voluntary food intake in the pig were indicated by differences between breeds. Bichard (1967) reported that Large White pigs had greater appetite than Landrace pigs in all growth phases. Cop and Buiting (1977) reported differences in voluntary feed intake and daily gain from 25 to 95 kg of body weight between six pure lines (Belgian Landrace, Dutch Landrace, Pietrain, Dutch Yorkshire, Duroc and Hampshire). Dutch Yorkshire gained the most and consumed the most feed while Pietrain pigs gained the least and consumed the least feed. Because Dutch Yorkshire pigs had low lean content and Pietrain pigs had low LTGR, both lines were similar in LTFC. The heritability estimates for food intake from 14 references summarized by Flock (1979) range from 0.12 to 0.59, with a median of 0.30. The heritability of food intake obtained by Flock (1979) was 0.32. Cameron et al. (1988) reported heritabilities of 0.17 (Large White) and 0.26 (British Landrace) for daily food intake. Mrode and Kennedy (1993) reported a heritability 0.45 for daily food intake. Von Felde et al. (1996) reported a heritability estimate of 0.22 for food intake between 40kg and 120kg of body weight.

Genetic variation in voluntary food intake is expected to increase with ad-libitum access to feed. Wyllie et al. (1979) analyzed data from Large White boars housed individually and given ad-libitum access to feed, and estimated that heritability was 0.23 for daily food intake. Smith and Fowler (1978) reported a heritability of 0.35 for daily food intake under semi-*ad libitum* (fed twice a day to appetite) feeding.

In term of energy utilization in pig production, our goal is to produce maximum lean tissue mass with minimum waste of ingested energy. Daily energy intake should meet the requirement for maintenance and production (Figure 1). Thorbek (1975) found that retention of 1 kcal in body protein required 2.09 kcal metabolizable energy (ME) whereas retention of 1 kcal in fat required 1.30 kcal ME in growing and finishing pigs. Energy retained per unit of energy intake was greater for genetically obese piglets than for genetically lean piglets (Cote and Wangsness, 1978). However, 1 kg lean tissue contains 740.8g water (Cole and Lawrie, 1975). Deposition of 1g of protein usually increases gain 2.0 to 4.0g, while a deposition of 1g of fat only increases gain 0.5 to 0.7g

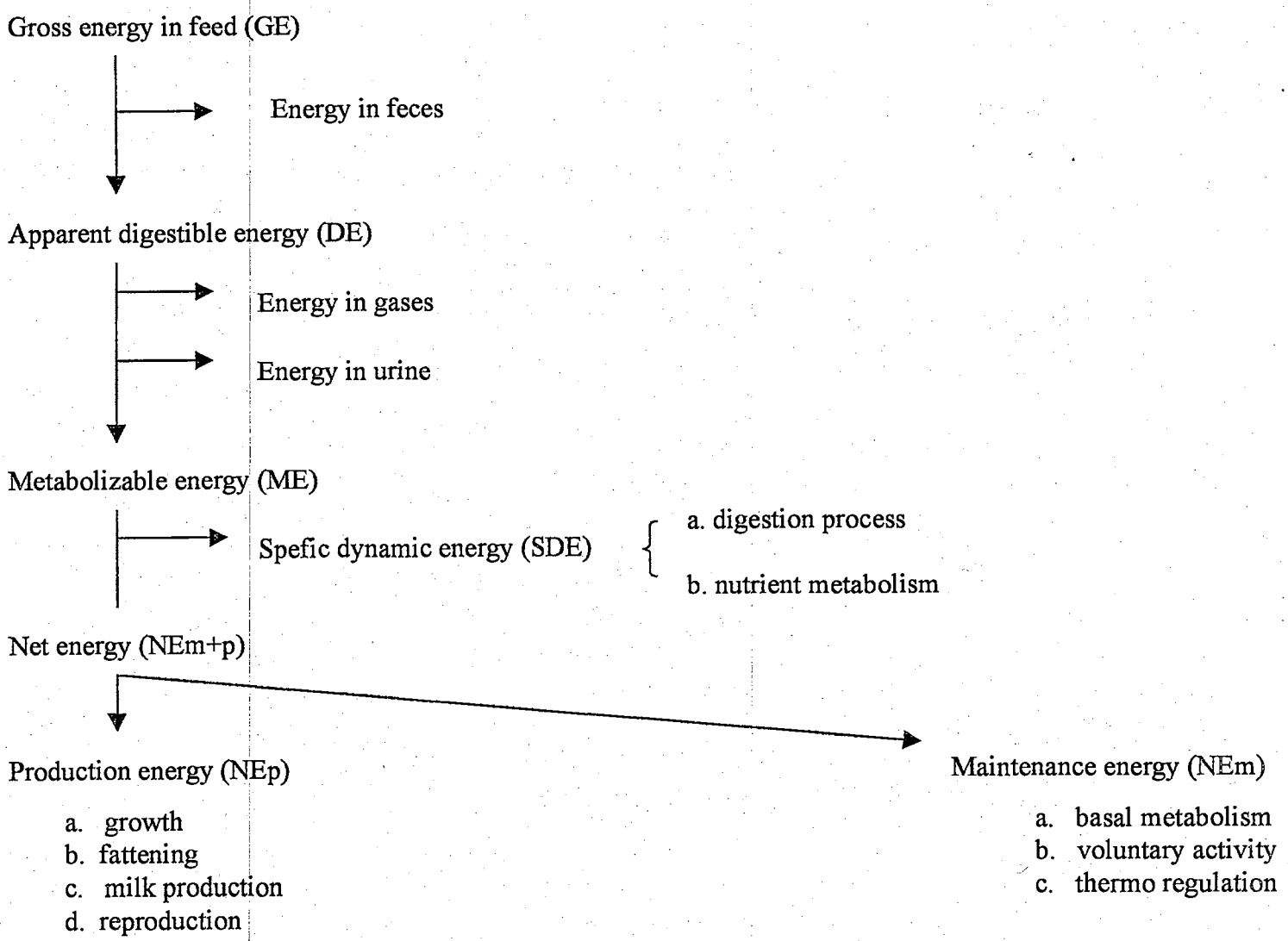

Fig 1. Principles of energy utilization in pigs. (Just, 1982)

(Just, 1982). Energy costs per unit gain of lean and fat tissue are similar (Webster, 1977). Thus, deposition of fat is expensive in terms of body weight gain and energy utilization.

Kanis (1988) found that adipose tissue growth rate had a high linear correlation with feed intake (r=0.85 to 0.95), indicating that a fixed proportion of the food was utilized to deposit adipose tissue. Predicted rates for daily protein retention increased from 20 kg to attain, at around 75 kg live weight, a maximum of 130 g (boar), 120 g (gilt) and 105 g (barrow). Between 45 and 110 kg live weight, rates of protein retention were maintained within 10 g of the maximum rate (Whittemore et al. 1988). Therefore, lean tissue growth potential is an important determinant factor of efficiency of energy use in pigs.

Theoretical estimates of maximal efficiencies of energy utilization for growth in the rat is in the range from 75 to 85% (Luiting, 1990). But observed efficiencies are often considerably below theoretical efficiencies. This variation in observed efficiencies may give us opportunities to identify animals that are capable of attaining maximum efficiencies of production. Variation in food intake may be a reflection of the energy requirement that is determined largely by body size. However, variation in maintenance requirements as predicted from metabolic body size is poorly associated with variation in food intake in pigs (Mrode and Kennedy, 1993), indicating that there may be variation in efficiency of utilization of food and/or maintenance requirements. Therefore, selection on a measure of the efficiency of energy utilization, other than FCR, may be more effective in terms of the correlated response in total or daily food intake.

Koch et al. (1963) proposed the concept of residual food intake (RFI). RFI is defined as the difference between the actual food intake and expected food requirement for growth and maintenance. In other words, animals showing equal production levels and body weights may differ considerably in feed intake, and therefore feed efficiency. Foster et al. (1983) investigated genetic variation in RFI of pigs. Feed intake was predicted based on maintenance requirement (metabolic body size or $BW^{.75}$), growth rate and backfat. Heritability of RFI was 0.30. Mrode and Kennedy (1993) predicted RFI based on 1) growth rate, 2) growth rate and backfat, or 3) lean growth rate. Heritabilities of RFI predicted in these three ways were 0.33, 0.30 and 0.38, respectively. Genetic and phenotypic correlations between growth and RFI were positive suggesting that fast

growing pigs had appetite greater than needs for growth. Cameron and Curran (1994) predicted feed requirement based on growth rate and backfat. The authors estimated a heritability of 0.16 to 0.17 for RFI. Feed intake and RFI was highly correlated, but growth rate and backfat were phenotypically independent from RFI. Similar studies were reported in the chicken (Luiting and Urff, 1991), in which estimated feed requirement was based on metabolic body size ($BW^{.75}$), daily egg production, and daily gain for eleven 4-wk periods (starting at 20 wk of age). The authors reported that the repeatability of RFI was 0.52 to 0.58, and the phenotypic correlation between periodic RFI and RFI accumulated over the whole testing period reached up to 0.8. Thus, RFI showed a considerable amount of consistent variation. Multiple regression analysis indicated that metabolic body weight was a significant component responsible for variation in RFI. Therefore, variation in energy required for maintenance is an important determinant of feed intake.

1.3 Genetic Variation in Energy Requirements

Energy balance is achieved by equal amounts of energy intake and energy expenditure. Energy equilibrium is rarely achieved from meal to meal. However, in the long-term, animals can maintain body weight despite considerable fluctuation in energy intake and energy expenditure. Therefore, when body weight is forced away from its normal level, animals undergo compensatory adjustments in voluntary food intake. Humans respond to under-nourishing or overfeeding by compensatory changes in energy expenditure (Leibel et al., 1995). Long-term increase in energy needs, such as lactation or cold exposure, lead to a compensatory hyperphagia. Lipid is ideally suited for long-term energy storage in mammals because lipid is calorie-dense. This is a fundamental physiological mechanism for all organisms to prepare the food depletion.

The fuels that are obtained through periodic eating are continuously released into the circulation by different energy stores. These include the gastrointestinal tracts, in particular the stomach, liver glycogen and the fat depots. Under *ad libitum* feeding, fuels absorbed from the gastrointestinal tract are the most important source of energy. If nutrients absorbed from the gastrointestinal tracts do not meet the energy requirements, glucose is released from glycogen, and fatty acids and glycerol are released from the fat

depots into blood circulation. Metabolizable energy exceeding maintenance requirements is available for production and growth (**Figure 1**).

Variation in maintenance requirements appears to be associated with body organs. Energy expenditures per unit of mass of liver and intestine are much higher than body energy expenditure per unit of mass (Reeds et al., 1993). Therefore, increases in the relative weights of these tissues increase average body energy expenditure. Selection for body composition will affect organ development during the growth period. During growth, the various organs of the body can be divided into two main groups, energy consuming and energy-supplying organs, according to their function in energy metabolism (Lilja, 1981). The energy-consuming organs are characterized by the requirement for energy for the deposition of protein and fats in tissues. The energy-supplying organs are responsible for making energy available for growth processes and are represented by the intestinal tract, the liver, the lungs, and the heart. These energy-supplying organs are characterized by their high oxygen requirement to supply their metabolic activity. Thus, animals characterized by a high growth rate ought to be characterized by a rapid early development of energy-supplying organs in relation to energy-consuming organs. Consequently, a rapid growth rate should also be accompanied by a high rate of oxygen consumption per gram of body weight gain. Growing pigs use about 30 to 35% of *ad libitum* gross feed energy consumption for maintenance purpose (Van Es and Buekholt, 1987). About 30% of total fasting heat production is attributed to the small intestine, liver and kidney (Pekas, 1991; Pekas and Wray, 1991). Therefore, variation in energy used for maintenance may play an important role in variation in gross energy efficiency.

It has been assumed that maintenance requirements vary only with body weight. However, some experiments show a considerable variation in maintenance requirements per unit of metabolic body weight (Verstegen et al., 1987). Farrell (1975) reported breed differences in the maintenance energy requirement in chickens. Also, Sabri et al. (1991) reported significant differences among the six lines in their energy requirements for maintenance and for egg production. Luiting et al. (1995) investigated genetic variation in maintenance requirements in growing pigs of Duroc and Landrace breeds. The pigs were fed to maintenance (zero growth) at 65 kg for 8 weeks and body composition was

predicted based on computerized tomography (CT) scan data. No significant body weight differences were observed between breeds. During maintenance feeding, fat utilization was significantly greater in Landrace pigs than in Duroc pigs. As a consequence, carcass fat:muscle ratio and energy content decreased more in Landrace than in Duroc pigs. This indicated that Landrace pigs had higher maintenance requirements than Duroc. In the growing period, growth rates in the lines were similar, 0.60 and 0.59 kg/d for Landrace and Duroc, respectively. But there was a tendency for daily feed consumption to be less in Landrace (1.51 kg/d) than Duroc (1.72 kg/d). The Landrace pigs had lower feed consumption during the growth period, but higher maintenance requirements per metabolic body weight. A possible explanation would be Landrace pigs were energy-efficient because they shifted accretion from fat to lean, which required less energy. Therefore, the Landrace pigs had lower maintenance requirements per metabolic body weight when growing, and were capable of changing body composition.

Some studies found higher maintenance requirements per metabolic body weight in lean pigs than fatty pigs. For example, Sundstol et al. (1979) reported that fasted metabolism was significantly greater for lean (108 kcal/ $W^{.75}$ kg) than for fatty pigs (96 kcal/ $W^{.75}$ kg). Selection for reduced backfat and increased growth rate increased visceral organs such as heart, lungs, spleen, liver and kidney. Davey and Bereskin (1978) reported that fatty pigs had small kidney, heart and liver weights at 100 kg of body weigh. Furthermore, Koong (1983) reported lighter heart, spleen, pancreas and intestinal weights in fatty pigs than in lean pigs at 41 kg of body weight. Therefore, the lean pigs utilized more energy for maintenance. Pekas (1993) reported that when energy intake was restricted at a maintenance level after body weight reaches at 100 kg, it caused a reduction of viscera, such as small intestine, caecum, pancreas, liver, kidney and heart. When food intake was suppressed to attain maintenance, the requirements for digestion and absorption were greatly reduced and the digestive organs atrophied. Atrophic catabolism of digestive organs and liver mobilized nitrogenous substrates and these substrates were likely salvaged for the continued accretion of carcass lean tissue which was observed during terminal maintenance in his study. Reduction of viscera mass and simultaneous enlargement of the carcass account for the increased dressing proportion observed in his study.

Pigs weighing 50 kg live weight deposit about 290 g/day muscle and 170 g/day subcutaneous fat, whereas pigs near 105 kg deposit about 260 g/day muscle and 310 g/day subcutaneous fat (Just, 1984). Thus, differential lean gain was about +120g/day in 50 kg animals but –50g /day in 105kg animals. Whittemore et al. (1988) investigated the patterns in lean and fat deposition as a function of empty body weight. The ratio of lean to fat tissue accretion increased from a value near 3.0 at 10kg empty body weight to 1.1 at 50 kg, 0.65 at 105 kg, and 0.55 at 130 kg. Tissue deposition would be about 215 g/day lean and 190 g/day adipose tissue at 50 kg empty body weight and about 220 g/day lean and 345 g/day adipose tissue at 105 kg. This indicated that differential lean gain was about +25 g/day at 50 kg empty body weight but –125 g/day at 105 kg body weight.

Energy efficiency declines as animal grows. A large proportion of maintenance energy is consumed by organs and lean pigs tend to have higher maintenance requirement. However, lean pigs may utilize energy for production more efficiently than fatty pigs by accreting more lean than adipose tissue.

1.4 Genetic Aspects of Feed Intake and Growth – Mouse model

Laboratory animals can be a useful model for livestock breeding. The mouse has been used for genetic research because it is suitable to investigate consequences of long-term selection in a relatively short period. Thus, genetic parameters of growth traits in the mouse may be informative for predicting the selection responses in large animals.

Hanrahan et al. (1973) reported a realized heritability of 0.51 after 14 generations of selection for increased postweaning GR from 3 to 6 weeks of age. Bailey et al. (1988) observed that mice selected for 42-day body weight had greater growth rate and larger mature size than a control line. Thus, growth curve shape was changed by selection for growth. Also, selection for growth rate alters body composition and feed intake. A rapid-growth line increased lean tissue growth rate but also became obese and hyperphagic (Eisen et al., 1977; Eisen and Leatherwood, 1978).

Sutherland et al. (1970) reported a heritability of 0.20 for feed intake. The genetic correlation between FI and GR was 0.71, but between FI and FCR was only 0.36. The increased appetite did not completely convert to weight gain. Energy loss might due to increased energy expenditure. Moruppa (1990) reported that fasting heat production was

similar between mice selected for either high or low feed intake adjusted for body weight However, mice selected for increased appetite expend some of the excess energy intake by increasing physical activity.

Genetic correlations between FI and GR, and FI and FCR were similar to those reported in pigs. More importantly, potential genetic variation in energy efficiency was observed in mice.

2. Appetite Regulation

2.1 Feeding Behavior

Prolonged nutritional deprivation leads to increased food-seeking behavior and reduced energy expenditure. On the other hand, prolonged nutritional abundance leads to reduced food intake and increased energy expenditure (Keesey and Powley, 1986). In practice, however, availability of feed may vary from time to time and the diurnal rhythm affects feeding behavior. Thus, accurate adjustment of metabolic rates, which regulate energy balance, is critical for organisms to survive.

Feeding patterns are closely related with energy requirement for growth. In pigs, Auffray and Marcilloux (1980) reported that frequency of daily food intake decreased from a mean of 9.5 meals after weaning to about 3 at adulthood when growth was finished. Also, the animals preferred to eat in the daytime, and this diurnal character of food intake was accentuated gradually with age. Hyun et al. (1997) analyzed the automated feed intake records of group-housed pigs from 27 to 82 kg of body weight. The pigs visited the feeder most frequently between 0900 and 1100 and least frequently between 2000 and 0400. Correlations between feeding pattern and growth traits were low. Bigelow and Houpt (1988) reported feeding pattern in pigs from 10 to 130 kg of body weight. As the pigs grew, daily feed intake increased nearly threefold, while meal frequency fell from 14 to 7 per day. Consequently meal size and inter-meal interval increased. The increased meal size was attributed to the increased meal rate, not the increased time spent at the feeder.

Considering the feedback loop between energy balance and eating, it seems clear that nutrient availability or some measure of energy in metabolism affects feeding behavior. For example, intramuscular or intravenous administration of various fuels or

pharmacological manipulation of fuel utilization affects eating in human and animals ((Keesey and Powley, 1986). Glucose is used preferentially by most tissues and is almost the exclusive fuel of brain cell metabolism under normal conditions. Mayer (1953) postulated the existence of chemoreceptors that have a special affinity for glucose and all activated by the utilization of glucose. According to this "glucostatic theory", an increase or decrease in glucose utilization would serve as stimulus for satiety or hunger, respectively.

Louis and Le Magnen (1980) observed a 6 to 11% decline in blood glucose just before spontaneous meals in rats. Campfield et al. (1985) reported that intravenous glucose infusions blocked the premeal decline of blood glucose levels, and subsequently delayed the onset of the meal. Strubbe and Steffens (1977) observed that blood glucose levels increased in the rat during and after carbohydrate containing meals, suggesting that the premeal decline in blood glucose is a trigger to meal onset. In contrast to the initiation of feeding in response to low blood glucose level, attempts to induce satiety by intravenous administration of glucose reached different conclusions. Mayer (1953) and Tordoff and Friedman (1986) reported a marked suppression of food intake after intravenous administration of glucose. Furthermore, Walls and Koopmans (1989) reported that glucose and amino acids are more effective than fats in inhibiting food intake. However, Adair et al. (1968) reported no significant changes in food intake in the rat receiving continuous intravenous infusion of nutrients. In addition to these inconsistent results regarding the role of glucose as a satiety signal, the glucostatic theory fails to explain one common physiological condition, diabetic hyperphagia that is characterized as the increased food intake under a high blood glucose levels.

It has been known that body fat is a critical factor for survival during starvation and necessary for normal reproductive functions. Thus, body fat has been considered an important effector in regulation of food intake, energy expenditure, and nutrient partitioning. Kennedy (1953) proposed the "adipostat" theory, that adipocyte-derived mediators regulate body weight. This hypothesis suggests that the central nervous system and deviations sense the status of energy stores (body weight) in the intensity of a putative signal result in adjustments in food intake and energy expenditure. This theory was supported by a classical probiosis conducted by Hervey (1958) who connected blood

circulation of hypophysectomized obese mice to lean mice and observed the lean mice starving to death. This experiment suggested that obese animals produced high concentrations of appetite-suppressing factors.

2.2 Regulatory Mechanisms

The "set point" model implies the existence of four major components of an energy homeostasis system: afferent signals indicating the quantity of energy stores, efferent processes regulating energy storage and expenditure, efferent mechanisms controlling feeding behavior, and integration of those components in the central nervous system. The hypothalamus is an important part of the brain in regulation of appetite. Lesions of the ventromedial hypothalamus (VMH) increase food intake and lesions of the lateral hypothalamus reduce food intake (Keesey and Powley, 1986). Gastrointestinal hormones and peptides are secreted following ingestion of food. They mediate feed intake through central nervous system (CNS) (Figure 2). Hormones and peptides involved in regulatory mechanisms are classified anorectic or orexinergic peptides based on inhibitory or stimulatory effects on appetite, respectively.

2.2.1 Appetite-suppressing Factors

Cholecystokinin: Ingestion of food stimulates a neural mediator of the autonomic nervous system and secretion of gastrointestinal hormones. Among them, CCK is a well-characterized hormone, which is produced by endocrine cells of the intestinal mucosa, which are concentrated in the duodenum and proximal jejunum. Outside of the intestinal tract, CCK is synthesized by a subpopulation of pituitary cells (Rehfeld et al., 1984). CCK was discovered based on the ability of intestinal extracts to stimulate gallbladder contraction when infused (Ivy and Oldberg, 1928) and pancreatic enzyme secretion (Harper and Raper, 1943).

CCK is found in intestine, brain, and circulation with multiple molecular forms including 58, 39, 33, 25, 22, 18, 8, 7, 5, and 4 amino acids (Mutt and Jorpes, 1968; Muller et al., 1977; Kothary et al., 1983; Eberlein et al., 1987). Translated CCK mRNA consists of 114 amino acids (Gubler et al., 1994), but different cleavage patterns result in variation in length of CCK. The octapeptide of CCK (CCK-8) which consists of the

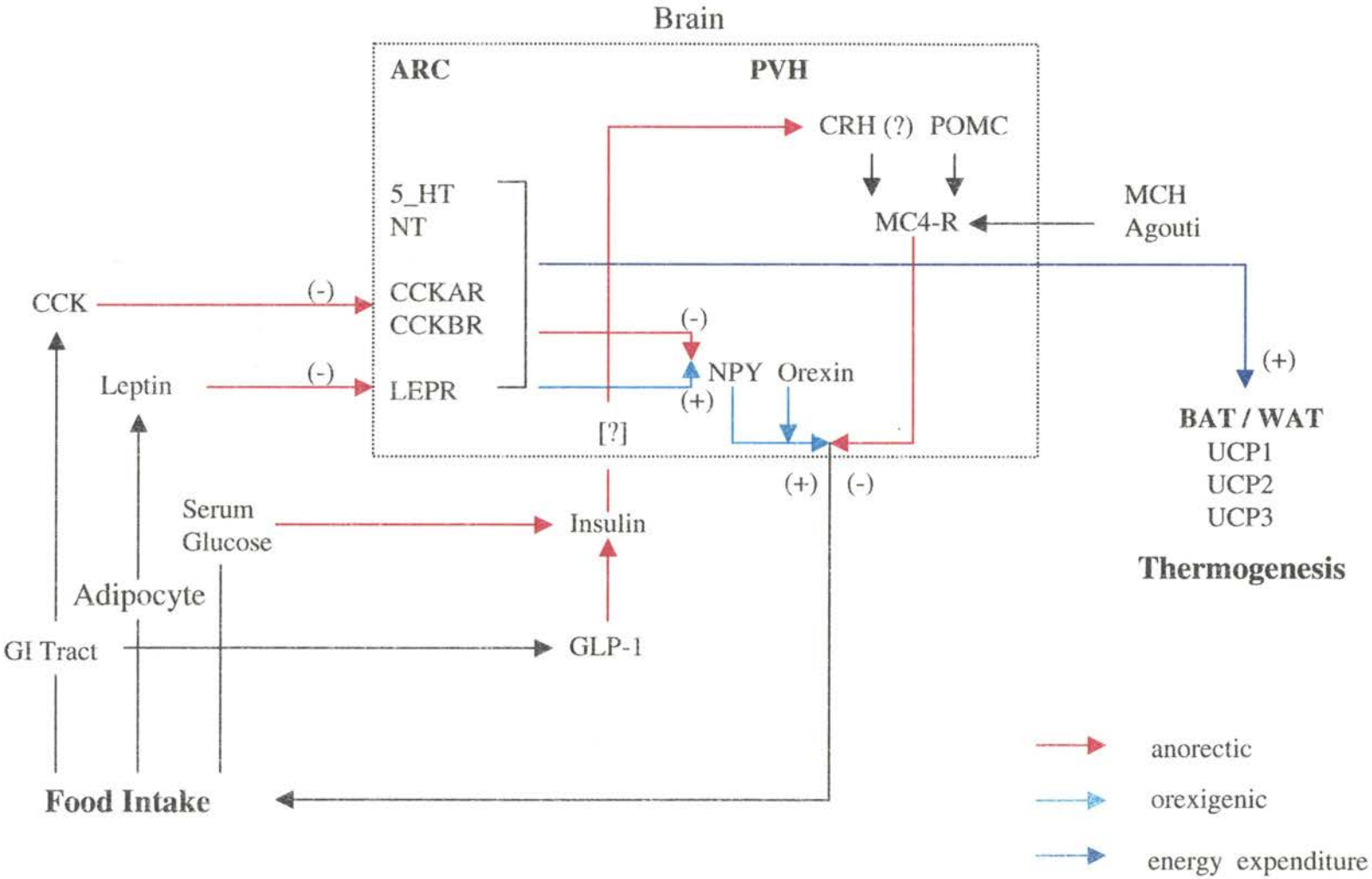

Figure 2 . Diagram of endocrine control of appetite.

carboxyl-terminal 8 amino acids of CCK is the most biologically potent small peptide of CCK. However, another study demonstrated that the most abundant molecular form of CCK in pig, dog, rat, and human was CCK-58 (Liddle, 1997).

CCK has been known for its inhibitory effect on gastric emptying (Moran and McHugh, 1982). Thus, CCK may have multiple target cells in producing satiety. Smith et al. (1985) demonstrated that satiety is mediated in the central nervous system via the vagus in rats. Also, Moran and McHugh (1982) reported peripheral actions of CCK on inhibition of gastric emptying. CCK acts with other neuropeptides and hormones in regulation of energy balance. In pigs, feed intake is suppressed by administration of CCK-8 peripherally (Pekas and Trout, 1990) or intracerebroventricularly (Baldwin and Sukhchai, 1996). However, a larger amount of peripheraly administration of CCK is required to suppress feed intake compared to central administration (Baldwin and Sukhchai, 1996). This indicates that satiety is regulated under the central effect. Pekas and Trout (1990) reported that pigs immunized against CCK-8 increased food intake by 8.2% and body weight gain by 10.6% compared to control animals.

Two types of CCK receptors, type A (CCK-AR) and type B (CCK-BR), mediate the action of CCK and gastrin (Jensen et al., 1989). CCK-AR mediate physiologic gallbladder contraction, pancreatic growth and enzyme secretion, delayed gastric emptying, relaxation of the sphincter of Oddi, and potentiation of insulin secretion (Jensen et al., 1989). Although CCK-AR are classified as peripheral receptors, they are present in the CNS and peripheral nervous system (vagus nerve) (Corp et al., 1993). CCK-BR are found predominantly in the CNS and gastrointestinal tracts (Corp et al., 1993).

Blocking CCK receptors can abolish the anorexic effects of CCK. Baldwin et al. (1994) and Baldwin and Sukhchai (1996) reported that intracerebroventricular (i.c.v.) administration of Devazepide, a CCK-AR antagonist, prior to i.c.v. injection of CCK abolished suppression of feeding in pigs. Reverse of the inhibitory effect of CCK by blocking CCK-AR has been reported in monkeys (Moran et al., 1993), mice (Hirose et al., 1993), and rats (Corwin et al., 1991; Brenner and Ritter, 1996; Hirose et al., 1993) indicating CCK-AR in the brain mediate satiety. CCK-AR deficient rats became hyperphagic and obese (Moran et al., 1998). In contrast, Dourish et al. (1989) reported

that both CCK-A and CCK-B receptor antagonists increased food intake, whereas CCK-B receptor antagonist was 100 times more potent than CCK-A receptor antagonist. Furthermore, Corp et al. (1997) reported that administration of selective antagonists for CCK-A and CCK-B into the lateral ventricle did not reverse the inhibitory effect of CCK-8. These inconsistent results could be due to 1) dose, 2) target organs, and 3) species. For example, the antagonists were injected subcutaneously (Corwin et al., 1991), intraperitonially (Brenner and Ritter, 1996), lateral ventricularly (Corp et al., 1997), and intraperitonially and intracerebroventricularly (Hirose et al., 1993). It remains to be determined whether endogenous CCK is acting peripherally by non-vagal mechanism, within the brain, or both peripherally and centrally.

CCK acts with other neuropeptides and hormones in regulation of energy balance. Administration of leptin and CCK (sub-threshold doses each inadequate to induce significant decrease in food intake by itself) in lean mice induced a quick suppression of food intake (Barrachina et al., 1997). CCK-A receptor antagonist but not CCK-B receptor antagonist blocked the leptin-CCK interaction (Wang et al., 1997a). Since CCK-AR are found in vagal fibers, CCK might initiate appetite suppression. Wang et al. (1997a) observed increased responsiveness of gastric vagal afferent to leptin by pretreatment with CCK. These findings suggest that CCK secretion at the paraventricular neuron (PVN) is part of the leptin-regulated pathway.

Leptin: Genetically obese mice have contributed a great deal of insight for the study of appetite/satiety regulation. The first recessive obesity mutation, the obese mutation (*ob*), was reported in 1950 (Ingalls et al., 1950). Characteristics of these obese mice were large body size, high fat content and large feed intake. Although inheritance patterns supported the hypothesis that a recessive allele of a major gene controlled fat deposition, the location of the gene and its functions in metabolic pathways were unknown for nearly 40 years. In 1994, Friedman and his colleagues isolated the gene and resulting peptide which is synthesized in adipocytes in response to elevated levels of energy storage (Zhang et al., 1994).

The predicted amino-acid sequence is 84% identical between human and mouse (Zhang et al., 1994). A nonsense mutation in codon 105 was identified in *ob/ob* mice that

causes severe obesity. Leptin (Greek: leptos, thin), the product of the *ob* gene, is a 16-kDa secreted protein which is primarily produced by white adipocytes (Halaas et al., 1995).

Pelleymounter et al. (1995) reported that intraperitoneal injection of leptin to *ob/ob* mice lowered food intake, body weight, body fat, and serum concentration of glucose and insulin. In addition, the authors reported that leptin enhanced metabolic rate, body temperature, and activity levels. Levin et al. (1996) demonstrated that leptin not only suppressed food intake but also increased energy expenditure. When the *ob/ob* mice were pair-fed with the leptin-infused *ob/ob* mice, the leptin-infused *ob/ob* mice reduced more fat than food-restricted *ob/ob* mice. Therefore, decreased food intake does not completely account for adiposity reduction, indicating that leptin increased energy expenditure as well as reduced food intake.

It is unclear whether the *ob* gene is expressed by brown adipose tissue (BAT), as well as white adipose tissue (WAT). Some reports supported from low to high expression of *ob* mRNA in BAT compared with WAT (Trayhurn et al., 1995; Murakami and Shima, 1995; Maffei et al., 1995a; Frederich et al., 1995; Ogawa et al., 1995; Moinat et al., 1995). Another study (Cinti et al., 1997) found BAT did not express leptin in lean mice. Status in energy balance regulates morphology of adipocytes and expression of uncoupling protein 1 (UCP1) that regulates non-shivering heat production (Morroni et al., 1995; Wolf and Phil., 1997; Ricquier, 1998). When mice are exposed to cold environment and the cell is stimulated, the morphology of adipocytes is BAT-type (multilocular, many large mitochondria, and high UCP1 expression), while when the cell is not stimulated, adipocytes remain in WAT-type (unilocular, small number of mitochondria, and low UCP1 expression). Cancello et al. (1998) reported that during the transformation of adipocytes from BAT-type to WAT-type, the UCP1 gene is suppressed and leptin gene expression is enhanced. Thus, the UCP1 and leptin genes appear to be reciprocally activated.

Administration of leptin to normal mice resulted in significant reduction in food intake, body weight, and body fat (Halaas et al., 1995). Leptin was injected intraperitoneally to *ob/ob* and normal mice. The *ob/ob* mice lost about 40% of body weight and food intake was significantly reduced compared to non-treatment *ob/ob* mice.

Leptin effectively reduced both food intake and body weight in *ob/ob* and diet-induced obesity mice, but not in diabetic (*db/db*) mice that develop obesity (Campfield, 1995). Mice with lesions of the hypothalamus, as well as mice mutant at the *db* locus, expressed higher level of leptin mRNA in adipose tissue than lean mice (Maffei et al., 1995a). Intraperitoneal injection of leptin into *db/db* mice increased plasma level of leptin but failed to reduce food intake and body weight (Halaas et al., 1995). Furthermore, obese humans and rodents are normal in their ability to produce leptin mRNA and its level is higher than in lean individuals (Considine et al., 1995a; Maffei et al., 1995b; Lonnqvist et al., 1995; Hamilton et al., 1995). These observations provided some indications of resistance against leptin in the *db* mouse.

Leptin receptor (*obr*) mRNA was detected in various tissues including hypothalamus (Tartaglia et ail., 1995; Lee et al., 1996; Ghilardi et al., 1996; Dyer et al., 1997). Lee et al. (1996) identified abnormal splicing of the *obr* gene among the *db/db* strain. In the *db/db* mouse, the mRNA for the long form of the receptor is abnormal and yields a receptor with a truncated intracellular domain that is unable to appropriately signal (Chen et al., 1996). At least five isoforms of the leptin receptor exist as the result of alternative splicing (Lee et al., 1996; Chen et al., 1996.). Major receptors are the long form of the receptor (OBRl) that contains a 302 amino acid intracellular domain and the short form of the receptor (OBRs) that contains 32-40 amino acid intracellular domain (Lee et al., 1996). These isoforms possess identical extracellular and transmembrane domains but differ in the intercellular domain. The long form of the receptor is expressed most highly in the hypothalamus (Tartaglia et al., 1995). Also, lung, kidney, liver, adipocytes, and pancreas β-cells express OBRl (Lollmann et al., 1997; Hoggard et al., 1997; Dyer et al., 1997). The short form of the receptor is distributed widely, including in skeletal muscle, and leptin has direct effects on skeletal muscle in vitro (Muoio et al., 1997). However, roles of the short form of the leptin receptor in energy balance are unknown.

Signal transduction mechanisms for leptin receptors are similar to those for cytokine receptors, which signal via activation of Janus kinase (JAK) and the signal transducers and activators of transcription (STAT) (Baumann et al., 1996). Following ligand-receptor binding and receptor aggregation, phosphorylation events ultimately

result in activation of JAK. The JAK then phosphorylates specific receptor tyrosine residues that provide docking sites for members of the STAT family. Leptin signaling via the JAK-STAT pathway has been documented. In mice, Vaisse et al. (1996) reported that leptin activated STAT3 in the hypothalamus of wild type and *ob/ob* mice, presumably via leptin receptor. Bjorbaek et al. (1997) reported that in response to leptin, the long form, but not the short form of leptin receptor was capable of signaling the tyrosine phosphorylation that was enhanced by co-expression with JAK2. Thus, the OBRl mediates leptin-dependent activation of the STAT pathway and STAT-dependent transcriptional activation. Since STAT binding sites are missing in the cytoplasmic domain of the OBRs, they are not capable of such activity (Ghilardi et al., 1996). Based on these observations, STAT-dependent signaling seems to be an essential action in the avoidance of obesity.

In the human, body mass index [BMI (kg/m^2)] is positively correlated with serum leptin level (Considine et al., 1995b), and women have higher leptin levels than men even at the same BMI value (Montague et al., 1997). Rosenblum et al. (1996) reported that premenopausal women have higher serum leptin levels than postmenopausal women. Clement et al. (1998) reported obesity linked to a homozygous mutation in the leptin receptor gene. Individuals homozygous for the mutation had no pubertal development, low growth hormone and thyrotropin secretion, indicating a wide range of leptin effects on endocrine functions.

One possible explanation for obesity in humans would be that the brain lacks mechanisms for responding to leptin (leptin resistance). Serum leptin levels in obese human are high but leptin levels in central spinal fluid (CSF) do not follow the serum leptin levels (Caro et al., 1996), indicating that the blood-brain barrier might be critical in obesity. Many hormones and growth factors circulate in blood as forms of bound with binding proteins. Houseknecht et al. (1996) isolated three leptin-binding molecules in serum from rodents and two leptin-binding molecules in serum from humans. The majority of leptin in lean subjects circulated in the bound form. Central administration of leptin was more effective than peripheral administration of leptin on suppression of food intake. Thus, the bound form of leptin may be a biologically active form. However, the precise identity and function of binding proteins for leptin are currently unknown. Also,

Sinha et al. (1996) reported that the proportion of free leptin is positively correlated with obesity in human. However, only 10% of the bound leptin could attach to leptin receptors, thus the bound leptin in human is less significant in quantity. So far, the mechanism(s) of leptin resistance is not fully understood.

Serotonin (5-HT): Serotonin [5-hydoxytryptamine (5-HT)] is synthesized from the essential amino acid L-tryptophan (TRP), which is obtained naturally in the diet. In the brain, TRP is converted to 5-HT by the enzyme tryptophan hydroxylase. 5-HT is released from nerve endings that originate mainly from neurons in the midbrain nuclei and that terminate close to the arcuate nucleus (ARC)-PVN neurons (Guy et al., 1988). 5-HT receptors are abundant in the PVN, ARC and VMH (Leibowitz and Jhanwar, 1989). Serotonin is a potent anorectic agent and causes intense carbohydrate aversion (Leibowitz et al., 1990). Serotonin agonists such as fenfluramine, d-norfenfluramine, fluoxetine and sertraline reduced the rate of eating (Le Feuvre et al., 1991). These serotonin agonists had been widely used as a "diet pill" for humans until 1998 at which time a detrimental side effect on the heart was diagnosed. A specific role for medial hypothalamic serotonergic receptors in the control of feeding was supported by the finding that hypothalamic administration of general serotonin antagonists effectively and dose-dependently blocks the anorectic effect of serotonin in the PVN (Weiss et al., 1986). Later, Dryden et al. (1994) reported that the powerful central anorectic and thermogenic effects of serotonin might be mediated by decreased release of neuropeptide Y (NPY) from the ARC-PVN neurons. Therefore, serotonergic neurons may act in opposition to the NPY neurons and suppress feed intake. Furthermore, Dryden et al. (1995) demonstrated the inhibitory effect of serotonin on NPY mRNA expression in ARC using a serotonin antagonist. However, effectiveness of serotonin in suppressing NPY synthesis seems to be receptor dependent. Simensky (1996) reported that selective agonists to 5-HT1B, 5-HT2C or 5-HT2A receptors decreased food intake in rats but by different mechanisms. Stimulation of 5-HT1B receptors reduced meal size and 5-HT2C receptors decreased eating rate, whereas 5-HT2A receptors did not influence feeding behavior. These results indicate that serotonin regulates feeding behavior in multiple ways through its different receptors and possibly mediates expression of other neuropeptides besides NPY. Le Feuvre et al.

(1991) reported that fenfluramine reduces feed intake and increases energy expenditure in rats.

Neurotensin: Neurotensin (NT) was first isolated from bovine hypothalamus (Caraway and Leeman, 1973). Neurotensin is widely distributed in CNS and the gastrointestinal tract of a variety of species. In CNS, a high concentration of NT is found in the hypothalamus (Caraway and Leeman, 1976; Mangberg et al., 1981; Uhl and Snyder, 1977). Approximately 90% of the total body NT in rats is located outside the CNS, with the majority of this found in the small intestine (Caraway and Leeman, 1976). In humans, NT is found in the mucosa of the ileum and jejunum (Buchan et al., 1978; Helmstaedter et al., 1977; Polak et al., 1977) and in blood plasma (Blackburn and Bloom, 1979; Bloom et al., 1979; Mashford et al., 1978).

Plasma levels of NT change in response to food intake in humans. Plasma NT concentrations increase proportionally with an increase in meal size (Blackburn and Bloom, 1979; Bloom et al., 1979; Mashford et al., 1978). Peripheral administration of NT induces hyperglycemia, hyperglucagonemia, and hypoinsulinemia (Brown and Vale, 1976; Rosell et al., 1976; Ukai et al., 1977; Nemeroff et al., 1983). These findings suggest the possibility that NT may be involved with normal regulation of ingestive behavior. NT injected into the cerebral ventricular system decreases feeding in food-deprived rats (Levine et al., 1983; Luttinger et al., 1982). Microinjection of NT into VMH induced hypophagia, suggesting the VMH may be an important site for regulation of feeding in the rat. (Hawkins, 1986).

2.2.2 Appetite-stimulating Factors

Neuropeptide Y: Neuropeptide Y (NPY) is a 36-residue, single-chain peptide which is a member of the pancreatic polypeptide family of peptides. NPY was first discovered in pig brain (Tatemoto et al., 1982) and is now known to be one of the most abundant peptides in human brains. NPY levels are high in the hypothalamus, especially in ARC at the base of the third ventricle and in the PVN at the ventricle's apex (Chronwall et al., 1985). The PVN is a crucial integrating center for many neural pathways that influence energy homeostasis. The PVN is a site of termination of neurons that release many

neurotransmitters, including NPY, serotonin, noradrenaline and the opioid peptides, which affect feeding behavior and energy expenditure. NPY has potent appetite stimulating properties after i.c.v or intrahypothalamic administration (Stanley and Leibowitz, 1985). Injection of leptin into the third ventricle of the *ob/ob* mouse decreased the NPY mRNA levels (Stephens et al., 1995). On the other hand, injection of NPY into the brain of *ob/ob* mice resulted in the positive energy balance due to reduced thermogenesis with increased food intake (Billington et al., 1991). Expression of NPY in the dorsal medial hypothalamic nucleus (DMH) was detected in lethal yellow (A^y) or melanocortin 4-receptor (MC4R) knockout mice (Kesterson et al., 1997). A^y and MC4R knockout mice are obese because they can not inhibit NPY expression, so that the critical site for NPY expression in the brain is the DMH.

Five NPY receptor subtypes have been identified in rat and NPY Y5 receptor mediates the effect of NPY on food intake (Gerald et al., 1996; Schaffhauser et al., 1997). NPY Y5 is a 456-amino-acid protein and NPY Y5 mRNA is found primary in the CNS, including PVN of hypothalamus (Gerald et al., 1996).

Hypocretins (Orexin-A and Orexin-B): Sakurai et al. (1998) isolated two novel neuropeptides from mice, which were orexinergic. Examining peptides from different tissues to determine whether any of them activates orphan G protein-coupled receptors (GPCRs) led to the identification of these peptides, named orexin-A (3.5 kDa) and orexin-B (3.0 kDa). Prepro-orexin, which is a precursor of orexin-A and orexin-B, showed high homologies: the human and mouse were 83% and 95% identical to their rat counterparts, respectively. Prepro-orexin mRNA was expressed abundantly in lateral and posterior areas of the hypothalamus. Two types of orexin receptors, OX1R and OX2R, were located in the brain. OX1R is selective to orexin-A but OX2R act as receptor for both orexin-A and orexin-B.

Melanin Concentrating Hormone (MCH): Melanin-concentrating hormone (MCH) is a peptide originally isolated from fish pituitary (Kawauchi et al., 1983). MCH neurons project to a variety of areas throughout the brain, including the cortex, as well as the hindbrain (Skofitsch et al., 1985). Rat MCH is a 19 amino acid peptide that is expressed

in the lateral hypothalamus and the zona incerta (Nahon, 1994). MCH expression is upregulated in the hypothalamus of *ob/ob* mice, where it is further increased by fasting (Qu et al., 1996). MCH is a short-term appetite-stimulating peptide (Rossi et al., 1997). Orexigenic effects of MCH has been demonstrated by injection (i.c.v.) of MCH in the rat (Qu et al., 1996; Rossi et al., 1997). Qu et al. (1996) reported that expression of MCH was increased after fasting in both normal and obese mice. Injection of MCH into the lateral ventricles of rats increased food intake two- to threefold that of controls.

Glucagon-like Peptide 1(GLP-1): The gene encoding glucagon is expressed both in the intestine and in the pancreas (Mojsov et al., 1986; Novak et al., 1987). Glucagon-like peptide-1 amide (GLP-1) and glucagon-like peptide-2 are produced in the small intestine (Mojsov et al., 1986; Holst et al., 1987). GLP-1 stimulates insulin secretion, inhibits glucagon secretion, and enhances insulin sensitivity with great impact on glucose metabolism (Holst et al., 1987; Kreymann et al., 1987; Mojsov et al., 1987; Orskov et al., 1988). GLP-1 stimulates somatostatin secretion in rat pancreatic cells (d'Alessio et al., 1989). GLP-1 is able to inhibit gastric emptying rate in humans and rats (Wettergren et al., 1993; Gutniak et al., 1996; Tolessa et al., 1998). Although the roles of GLP-1 in appetite regulation are unclear, a study has shown GLP-1 does not bind to MC4R (Seeley et al., 1997).

2.2.3 Leptin-NPY System

Proopiomelanocortin (POMC): In normal mice, fasting lowered leptin levels and resulted in reduced POMC levels (Mizuno et al., 1998). The reduced POMC levels were also observed in *lep/lep* (leptin deficient) mice and *db/db* (leptin receptor defect) mice (Thorton et al., 1997; Mizuno et al., 1998). However, administration of leptin to *db/db* mice did not increase POMC levels because of the lack of leptin receptors (Schwartz et al., 1997). Therefore, Leptin signaling in the brain involves activation of the hypothalamic melanocortin system. POMC is thought to involved in regulation of feed intake because leptin receptors are on POMC neurons in the ARC that synthesize melanocortin (Cheung et al., 1997). However, injection of α-MSH antagonist without leptin had no effect on food intake and body weight (Satoh et al., 1998). Thus, leptin uses

the melanocortin pathway to inhibit feed intake and increase energy expenditure. These observations indicated that the hypothalamic melanocortin system plays a central role in both the satiety effect and sympathetic activation of leptin.

The agouti (A^y) mouse that has golden yellow coat color is a mouse model of obesity. Agouti mice display an age-onset (10-20 weeks) metabolic defect characterized by hyperphagia, increased adiposity, hyperglycemia, hyperinsulinemia and increased somatic growth. There are five known melanocortin receptors based on sequence homology that range from 35 to 60% homology between family members (Cone et al., 1996). Melanocortin-1 receptor (MC1R) is a G-protein coupled receptor and expressed primarily in the skin. MC1R regulate pigmentation in response to the melanocyte stimulating hormone (α-MSH), a potent agonist of MC1R (Cone et al., 1996). The coupling of α-MSH with MC1R produces black pigmentation. However, antagonism of the MC1R results in phaeomelanin (resulting in bleaching of the skin color). MC2R are expressed in the adrenal cortex and regulate corticoid synthesis in response to adrenocorticotropin hormone (ACTH) levels. The MC3R and MC4R are both expressed in CNS, and thought to play key roles in feeding behavior. The MC5R are expressed in exocrine glands throughout the body. MC5R may be involved in the control of lipid and pheromone production in these exocrine glands (Chen et al., 1997). Melanocyte stimulating hormones (MSH) are produced from pro-opiomelanocortin (POMC), a prohormone of 131 amino acids that is processed into three classes of hormones: MSH's (α, β, and γ), ACTH and endolphins (Lu et al., 1994). POMC is produced primarily in the pituitary, but also in the ARC of the hypothalamus.

The agouti gene encodes a protein that modifies the phenotype of coat color through MC1R in the skin. However, the agouti protein is a potent antagonist of the MC4R. A mutation in the promoter region of the agouti gene causes overexpression of the agouti gene (Bultman et al., 1992; Fan et al., 1997). This suggested that the characteristic phenotypes of the A^y mice arise from separable antagonist activities at the MC1R and MC4R: the yellow coat color from MC1R antagonism and the metabolic defects from the MC4R antagonism. An agouti-like molecule that is expressed in the normal brain has also been discovered (Ollmann et al., 1997), which is called the agouti-related protein (AGRP). Agouti protein and AGRP are antagonists of the MC3R and

MC4R. Chemical structure in the C-terminal in these proteins and α-MSH is very similar, and all have higher affinity for the MC4R than for MC3R (Tota et al., 1999). The MC4R knockout mouse had a normal coat color, but developed age-onset (12 to 20 weeks) obesity that resulted from hyperphagia (Huszar et al., 1997).

The MC4R are thought to be a key segment of the leptin-NPY regulatory mechanism for food intake. Agouti mice are highly resistance to leptin effects on food intake inhibition (Halaas et al., 1997). Also, long-form leptin receptors are found in the neurons of the ARC where POMC is synthesized (Cheung et al., 1997). POMC expression is elevated in response to rising leptin concentrations after feeding (Mizuno et al., 1998). α-MSH is an anorectic and i.c.v. administration of α-MSH suppressed feed intake in rodents (Tsuji and Bray, 1989). These observations suggest that activation of the leptin signaling pathway results in elevated POMC production. Consequently, the elevated α-MSH binds to MC4R and suppresses expression of NPY.

Treatment of rats with MC4R antagonist in increased *ad libitum* feed intake (Kask et al., 1998). Thus, melanocortic peptides exert a continuous activation of the MC4R, which prevents animals from overeating. The level of NPY mRNA is increased in the DMH of MC4R knockout mice (Kesterson et al., 1997) and NPY inhibits the release of α-MSH (Blasquez et al., 1995). These results suggest that NPY may be the downstream effector in this obesity syndrome. Both α-MSH and MCH bind to the receptor, MC4R. Therefore, they compete for MC4R and mediate feeding behavior in opposite directions. Tritos et al. (1998) demonstrated that i.c.v. administration of α-MSH abolished the orexinergic effect of MCH in the rat.

The data in rodent models support a role for MC4R in weight regulation. AGRP in humans has been identified (Suzuki et al., 1997). This protein is produced in the hypothalamus and has potent antagonist activity for both MC3R and MC4R (Ollmann et al., 1997). A frameshift mutation in human MC4R and association of that mutation and obesity has been reported (Vaisse et al., 1998; Yeo et al., 1998). Thus, AGRP, MC3R, and MC4R may play important roles in food intake regulation in humans.

2.2.4 Energy Homeostasis in Peripheral level

Adipose Tissue: When energy intake and energy expenditure are equal an energy balance is maintained. Energy equilibrium is of course not achieved from meal to meal. However, in the long-term animals are capable of maintaining body weight despite considerable fluctuations in energy intake and energy expenditure. Adipose tissue plays an important role under starvation, supplying glucose via free fatty acids. WAT and BAT play very different roles in mammalian thermogenesis.

WAT is the main long-term energy store in an animal and provides substrate in the form of fatty acids for utilization by other organs. In mammals, WAT is distributed in a number of locations throughout the body, both internal and subcutaneous. Up to 85% of WAT may consist of triacylglycerol, and WAT is usually described as having 'unilocular' arrangement of the lipid (i.e., there is a single fat droplet within each adipocyte) (Trayhurn, 1996).

The main function of BAT is to generate heat, either for thermoregulation or in relation to the regulation of energy balance. Relative to WAT, BAT is restricted in term of its anatomical localization and in that it has an extensive vascularization and is densely innervated by sympathetic nerves. BAT is described as having a 'multilocular' disposition of fat droplets (i.e. a number of individual lipid droplets within each adipocyte). There seems to be a continuum of adipose tissues which is interconvertible, rather than there being two quite distinct forms (Holloway, 1989). The traditional histological characteristics may not represent WAT and BAT (i.e., BAT appears unilocular in obese animals whereas WAT appear multilocular in fasting or during cold exposure (Trayhurn and Ashwell, 1987)). In addition to multiple lipid droplets, the major histrogical feature of active BAT is the presence of a large number of mitochondria.

Mitochondria use the energy contained in dietary sugars, fats, and other nutrients to drive the synthesis of adenosine triphosphate (ATP). This process depends on an electrochemical gradient set up across the inner of the two mitochondrial membranes when protons are pumped out of the interior chamber of the mitochondrion. The mitochondria of BAT oxidize fuel (particularly fatty acids) normally, passing electron through the respiratory chain to oxygen. This electron transfer is accompanied by proton pumping out of the matrix. Thus, proton gradient in the intermembrane space becomes

high and protons must reenter to the matrix. Usually protons reenter to matrix through the proton-specific channel and synthesize ATP. However, mitochondria of BAT have a unique protein in their inner membrane. Thus, an additional proton channel allows protons reenter to the matrix without ATP synthesis and dissipate heat (Nicholls and Locke, 1984). Because reentry of protons through this channel (protein) is not coupled with ATP synthesis, this protein is called the uncoupling protein (UCP). This protein, which is also called thermogenin, has a molecular mass of 32-33 kDa, and is a member of the family of mitochondrial carrier proteins which includes the ADP-ATP translocase and the phosphate carrier (Klingenberg, 1990).

UCP: Thermogenesis is initiated by the release of noradrenaline from the sympathetic nervous system so that noradrenaline is critical to the regulation of many of the processes that occur in BAT. BAT contains both α- and β-adrenergic receptors. Among them, β3-subtype was first identified in the tissue (Lafontan and Berlan, 1993).

Casteilla et al. (1987) reported changes in UCP levels in cow and sheep. In cattle, UCP is detected as early as 80 days before birth, is maintained at low levels until birth at which time it increases dramatically, then disappears in about 2 days. In sheep, changes in UCP levels between 2-day prior and 6 days after birth were similar to cattle. In humans, UCP expression reaches a peak at birth (Himms, 1995), but is very low among adults (Lean et al., 1986). In pigs, expression of UCP in prenatal stages is unknown, but the absence of UCP in pigs between 4 days and 8 weeks of age was reported (Trayhurn et al., 1989). Based on these studies, it is speculated that UCP expression at birth in pigs is increased and disappears in a short period. For these species, UCP seems not play a significant role after birth.

However, rodents increase expression of UCP when exposed to a cold environment (Foster and Frydman, 1979). Enerback et al. (1997) demonstrated that UCP knockout mice are intolerant to cold temperature. Obese mice can not utilize BAT because they fail to express UCP. Thus, the role of UCP in energy balance is investigated by using BAT deficient transgenic mice (Lowell et al., 1993). These BAT-deficient mice became obese without increased in food intake, indicating that UCP mediated energy balance. In pigs, significant reduction of body fat by injecting β-agonists has been

demonstrated. Treatment of pigs with β-agonists such as ractopamine has often resulted in improved growth and feed efficiency along with more deposition of protein and reduction of fat (Gu et al., 1991). The treatment was more effective in females than males and more effective in fatty animals (Gu et al., 1991). Recently, two novel proteins, which are structurally similar to the UCP, have been identified. Therefore, the original UCP is classified as uncoupling protein 1 (UCP1) and the others are classified as uncoupling protein 2 (UCP2) and uncoupling protein 3 (UCP3).

UCP2 has 59% amino acid homology to UCP1 (Fleury et al., 1997). UCP2 is widely expressed in adult human tissues and it is upregulated in white adipose tissue (Gimeno et al., 1997) and skeletal muscle (Aubert et al., 1997) in response to fat feeding. Unlike UCP1 and UCP3, UCP2 mRNA is expressed in the hypothalamus and it is not affected by cold exposure (Richard et al., 1998). The specifically localized expression of UCP2 mRNA suggests that this mRNA has a neuronal localization (Richard et al. 1998). The role played by UCP2 in the brain is unknown. UCP2 mRNA levels are increased in epididymal fat pads of mice upon high-fat feeding, suggesting that the flux of fatty acids entering adipose tissue may regulate UCP2 gene expression (Aubert et al., 1997). In mouse adipose cells, peroxisome proliferate activated receptors (PPARs) agonists such as carbacyclin and α-bromopalmite activate PPAR. However, norepinephrine does not enhance the expression of UCP2 gene. Therefore, a potential link between fatty acids metabolism and thermogenesis may exist in PPAR-expressing tissues (Aubert et al., 1997).

The third member of the UCP family, UCP3, is 57% and 73% identical to UCP1 and UCP2, respectively in humans. Expression of UCP3 in skeletal muscle is highly specific and UCP3 is not involved in nonshivering thermogenesis (Boss et al., 1997). UCP3 expression is regulated by hormonal and dietary manipulations. Gong et al. (1997) reported that UCP3 levels were decreased three-fold in hypothyroid rats and increased six-fold in hyperthyroid rats. Thus, UCP3 is a strong candidate to explain the effects of thyroid hormone on themogenesis.

Nonshivering thermogenesis in the BAT is a major component of the energy expenditure to control body weight and metabolism in rodents. The UCP1 mRNA expression in the BAT is controlled mainly by the hypothalamus via the sympathetic

nervous system (Ricquier et al., 1985). β3-adrenergic receptors (β3-AR) were abundant in infant peri-renal brown adipose tissues, characterized by the presence of UCP1 mRNA. In adult adipose tissues, β3-AR mRNA levels were higher in deep deposits such as peri-renal and lower in subcutaneous. In these deposits, UCP1 mRNA levels paralleled those of β3-AR. However, isolated subcutaneous adipose cells enriched in white adipose tissues expressed β3-AR, but not UCP transcripts. β3-adrenergic mRNA was highly expressed in gallbladder, and to a much lower extent in colon, independently of UCP1 mRNA. Abdominal muscle, heart, liver, lung, kidney, thyroid, and lymphocytes did not express intrinsic β3-AR mRNA. These observations demonstrated that substantial amounts of brown adipose tissues exist throughout life in adipose deposits, which are generally classified as white adipose tissues. Therefore, β3-AR may be involved in the control of lipid metabolism, possibly from fat assimilation in the digestive tract, to triglyceride storage and mobilization in adipose tissues (Krief et al., 1993). Susulic et al. (1995) investigated the role of β3-AR using β3-AR knockout mice. β3-AR deficient mice had modestly increased fat stores, indicating that β3-ARs play a role in regulating energy balance. Treatment of normal mice with β3-AR agonist such as CL316243 increase serum free fatty acids levels (3.2-fold), insulin levels (140-fold), energy expenditure (2-fold), and reduced feed intake (45%). These effects were completely absent in β3-AR-knock-out mice. Thus, β3-AR involve mediation of energy expenditure and feeding behavior (Susulic et al., 1995).

However, regulation of energy balance via β3-AR seems to be tissue specific. Yoshitomi et al. (1998) reported that expression of UCPs in response to β3-AR agonist is different between BAT, WAT, and muscle: UCP1, UCP2, and UCP3 were elevated in BAT, UCP1 and UCP3 were elevated in WAT, but UCP2 and UCP3 were reduced in muscle. Administration of β3-AR significantly reduced circulating free fatty acids (FFA) compared to saline-injected mice. Thus, UCP2 and UCP3, which are not involved in nonshivering thermogenesis, may not be regulated via the β3-AR system. FFA is a substrate for glucose metabolism during negative energy balance. Samec et al. (1998) reported that UCP mRNA levels were elevated during starvation and reduced after feeding. Furthermore, Weigle et al. (1998) reported that administration of leptin to fasted

rats failed to reverse fasting-induced increase in UCP3 expression, and administration of glucocorticoid (anoretic agent) to fed rats failed to elevate UCP3 expression. These observations suggested that FFA is a potential mediator of the increase in muscle UCP3 expression that occurs under negative energy balance. A possible important role of UCP3 in energy expenditure has been supported in a human study. Schrauwen et al. (1999) reported that body mass index (BMI) was negatively correlated with expression levels of UCP3, and basal metabolic rate (adjusted for fat-free mass and fat mass) was positively correlated with expression of UCP3. Based on these observations, the search for factors determining circulating FFA levels may provide a better understanding of energy balance.

2.2.5 Synergetic Interaction Between Leptin and Other Hormones

Regulation of feeding behavior is a complex process. There have been many studies which examined regulatory functions of individual factors (e.g., hormones, neuropeptides, etc.) in feeding behavior. However, more studies are needed to focus on synergistic actions of the factors in energy balance. Leptin and NPY have been recognized as major players in appetite regulation as anorectic or orexinergic agents, respectively, but other factors mediate appetite independently or as part of the leptin-NPY system.

NPY: Leptin and NPY are antagonistic in their biological effects on feeding behavior: leptin is anorexic and NPY is orexinergic. Wang et al. (1997b) demonstrated that leptin treatment reduced NPY concentration in the ARC, PVN, DMH and NPY mRNA levels in the rat. On the other hand, NPY treatment induced hyperphagia and increased serum leptin levels. Erickson et al. (1996a) investigated the roles of NPY in leptin-induced feeding behavior. Feed intake in fasted NPY knockout ($NPY^{-/-}$ *OB/OB*) mice was compared with that of normal ($NPY^{+/+}$ *OB/OB*) mice. NPY knockout mice had normal feed intake and body weight, and feed intake was suppressed with exogenous leptin and body weight was reduced. In the second study, the role of NPY in absence of leptin was investigated using ob/ob mice by comparing obese ($NPY^{+/+}$ *ob/ob*), NPY-deficient and obese ($NPY^{-/-}$ *ob/ob*), and normal ($NPY^{+/+}$ *OB/OB*) mice. The $NPY^{-/-}$ *ob/ob*

mice were less obese than $NPY^{+/+}$ *ob/ob* mice because of reduced food intake and increased energy expenditure (Erickson et al., 1996b). Based on these two studies, NPY is not essential for feeding behavior or leptin actions (Study 1) but NPY is a central effector of leptin deficiency (Study 2). The results of Study 1 indicate that feeding is regulated by other factors.

Insulin: Insulin mediates serum glucose level that is under the control of many factors. The role of insulin in feeding behavior is complex. Wood et al. (1985) described that insulin level reflects peripheral metabolic status, thus acts as a sensor to the brain. For example, Vanderweele et al. (1989) mimicked the positive energy balance using administration of a low dosage of insulin chronically to rats, which suppressed feed intake. Saladin et al. (1995) reported that a single insulin injection in fasted rats increased leptin mRNA to levels of fed controls, indicating that insulin mediates leptin gene expression. Also, De Vos et al. (1995) reported glucocorticoids increased leptin gene expression in adipocytes followed by a concordant decrease in body weight gain and food intake. Furthermore, Kolaczynski et al. (1996) reported that insulin mediates *ob* gene expression and production of leptin in human adipocytes. This indicated that if insulin was absent, leptin production might be limited. On the other hand, leptin mediates insulin secretion into the blood stream. Seufert et al. (1999) reported that leptin reduces the transcriptional activity of the rat insulin I gene promoter and alters binding of distinct proteins including STAT5b complexes to upstream sequences within 5'-promoter region of the rat insulin I gene.

Corticotropin-releasing Hormone (CRH): CRH is synthesized in the hypothalamus, including the PVN, where leptin receptor is found. Arase et al. (1988) reported that injection of CRH into the third ventricle of rats reduces feed intake and increases sympathetic activity. Uehara et al. (1998) reported that the i.c.v. injection of leptin increased hypothalamic CRH in rats. Co-administration of leptin and CRH antagonist attenuated the anorectic effect of leptin, indicating that CRH may mediate the anorectic effect of leptin.

Growth Hormone (GH): Food deprivation suppressed GH secretion, but i.c.v. administration of leptin elevated GH level (Carro et al., 1997). Thus, leptin mediated GH secretion. Barb et al. (1998) reported that i.c.v. injection of leptin to prepubertal gilts reduced feed intake and stimulated growth hormone secretion. On the other hand, Karlsson et al. (1998) reported that injection of growth hormone subcutaneously for 9-months in the human male reduced body fat while serum leptin levels and body metabolic rate was not changed.

3. Molecular Approaches in Improvement of Growth Rate and Feed Efficiency

3.1 Molecular Aspect of Quantitative Traits

Traits whose distribution are continuous and fit to a bell-curve are referred to as quantitative traits. A quantitative trait is controlled by many genes so that genetic variation is the result of segregating allelic variants at numerous loci scattering through the genome. Putative loci responsible for genetic variation in quantitative traits are referred to quantitative trait loci (QTL).

Phenotype-based selection on some economically important traits such as growth rate is effective (Baird et al., 1952; Rahnefeld, 1973; Kuhlers and Jungst, 1990a; Kuhler and Jungst, 1990b; Woltmann et al., 1992). Selection response is a function of heritability. Heritability of growth traits in pigs is 30 to 50% (Baird et al., 1952; Rahnefeld, 1973; Kuhlers and Jungst, 1990a; Kuhler and Jungst, 1990b; Woltmann et al., 1992). However, improved accuracy of selection for economically important traits that are difficult to improve based on phenotypes may result in a greater genetic improvement. If we can identify QTL or linked markers affecting these traits we can utilize genotype information to predict on individual's genetic merit that can be used to assist selection. Selection with genetic marker information is called marker assisted selection (MAS). MAS has advantages in selection because 1) marker genotypes are independent from the environment, 2) animals can be tested for the genetic markers at any stage of life, and 3) markers for sex-limited traits can be tested in both sexes directly.

Simulation studies showed that addition of marker information to phenotypic data improves the rate of genetic gain compared to selection based on the best linear unbiased

prediction (BLUP) procedures (Zhang and Smith 1998). However, Gibson (1994) demonstrated that selection for a quantitative trait with single marker achieves less genetic gain than that with BLUP in a long term. Selection on a single marker increases the frequency of a favorable allele rapidly in expense of losing polygene effects which will not be recovered after fixation of the allele (Gibson, 1994). Thus, maximizing selection response using marker information (genotypic selection) and estimated breeding value (phenotypic selection) is essential challenge in the MAS schemes. Weights for the marker depend upon mainly heritability of traits and types of gene effect (additive or dominant). Dekkers (1998) developed a program that determines optimal index weights that maximize selection response in a long term.

3.2 Gene Map

QTL or linked markers need to be aligned on chromosomes. In order to localize them, genetic markers are used in physical and genetic maps. Location of the genes or genetic markers on the chromosome is described on either direct (physical) or linkage (genetic) maps.

On the genetic maps, location of the genes or QTL are described in distance (recombination rates) from genetic markers such as mapped genes (type I markers) or microsatellites (Type II markers). Recombination of genes occurs due to crossing of a pair of chromosomes during meiosis. Occurrence of recombination reflects the distances between loci (Morgan, 1928). However, recombination rates between multiple loci on the same chromosome are not in an independent manner. When genes are located with an order of A-B-C, rates of crossover between two distant genes, A-C, is not automatically a sum of crossovers in two subdivisions, A-B, and B-C. Genetic distances between A and C is not in additive function because of interference affecting recombination rates at two adjacent subdivisions. In order to adjust this nonadditive relationship between adjacent loci, the genetic distances are adjusted for interference (Kosambi, 1944). The genetic distance of 1 centi Morgan (cM) is estimated to be equivalent of one million base pairs (bp) of oligonucleotide in humans (Liu, 1998).

Botstein et al. (1980) developed the human gene map by using the restriction fragment length polymorphism (RFLP) technique. Because the RFLP provides biallelic

(for example, AA, BB, and BB) information for the markers, heterogenity of the markers in the population is crucial for RFLP to be informative. In contrast to Type I markers, Type II markers (microsatellites) are composed of tandem repeats of one to six oligonucleotides and abundant throughout genome (Litt and Luty, 1989). The number of the repeats in many microsattelites is highly variable in the population and informative (Archibald et al., 1995; Rohrer et al., 1996). In pigs, approximately 1500 type I and 1000 type II markers with an average spacing of 2 cM have been localized (Haley and Vasher, 1998). However, the densities of markers across regions of chromosomes have a large variation. The density of markers affects resolution of QTL and consequently the effectiveness of MAS.

On the physical maps, staining of chromosomes produces patterns of light and dark bands on chromosomes. This banding subdivides a chromosome into many regions and provides the basis for a physical map of the chromosomes. Location of genes on the chromosome can be determined by hybridization of labeled DNA probe to chromosome spreads. Fluorescent in situ hybridization (FISH) technique has been used widely for localization of genes (Rabin et al., 1985; Echard et al., 1986).

DNA sequence of genes in other species are used to identify novel genes in pigs (Hu et al., 1997). Genome data in other species, particularly humans and mice, are vital information for search of genes affecting traits in pigs.

3.3 Detection of Gene or QTL Affecting the Trait

Genes encoding hormones and enzymes involved in biological pathways may be responsible for variation of traits. Thus, genes that are identified based on their biological function are referred to as biological candidate genes. Biological candidate genes can be tested with relatively low cost and required less complicated family designs compared to more comprehensive approaches (Rothschild and Soller. 1997). However, the biological candidate gene approach has limitations in identifying genes that are truly affecting the trait. Our knowledge on biological significance of the each gene on bioligical processes is still limited. This limits the ability to choose the right candidate genes from among the many that are known to involve in the processes.

In contrast to the candidate gene approach, the whole genome scan approach is used to identify the regions of chromosomes affecting the trait. The whole genome scan procedure does not require prior knowledge of genes. Associations between markers and phenotype are used to determine the regions where genes affecting the trait are located. We assume that the region contains unknown number of genes (putative QTL) that affect the trait. Thus, recombination rate between the marker and a putative QTL affects power of detecting QTL. In outbred populations, allele type of the marker and the putative QTL become more dissociated; that is, linkage equiliblium is reduced between markers and due to recombination each generation. Under linkage equiliblium, it is difficult to evaluate associations between the marker and the trait, thus limiting the power of QTL detection. In contrast to outbred populations, a population produced by crossing two inbred lines retains a high degree of linkage between markers and putative QTL; that is, linkage disequiliblium. This disequiliblium between markers and QTL gives us a higher power to detect QTL based on marker information. Populations that are genetically diverted due to their origins or past selection are expected to possess diverted allele frequencies at many loci. Thus, family designs using two diverted breeds have been used for QTL studies in mammals.

Family data such as F_2 progeny of diverse lines are typed for a large number of genetic markers covering the entire genome. Association of each markers with phenotypic variation is tested, which results in a large number of independent point wise (nominal) tests across chromosomes. This testing procedure may produces many positive results in a genome-wide scan if a threshold level of 0.001 or 0.005 is used. A genome-wide significance level is the probability that one would encounter a deviation somewhere by chance in a whole genome scan. Thus, threshold levels for significance in the whole-genome scan approach are much higher than the pointwise (nominal) test (Lander and Kruglyak, 1995). Following detection of regions that have a significant effect on the trait, location of QTL can be estimated by the interval mapping procedures (Lander et al., 1987; Georges et al., 1995), which determines the highest likelihood of the location of QTL.

If QTL and markers are closely linked the accuracy of MAS with this marker is high. However, when QTL and markers are far apart linkage disequiliblium will be lost

quickly in each generation. In the latter case, new linked markers are required. To develop the markers, the region containing QTL needs to be cloned (positional cloning) and sequenced. However, the number of DNA base pairs composing the region is so large that it will require huge efforts to sequence the whole region. In livestock species, a low density of markers limits successful positional cloning of QTL (Andersson et al., 1998).

There are many regions on chromosome on which genomic organization is highly conserved across species (Johansson et al., 1995; Rohrer et al., 1997; Rohrer, 1999). For example, many genes found on porcine chromosome 8 are also found on human chromosome 4 and mouse chromosome 5. More genes have been mapped in humans and mice than pigs. Thus, location of novel genes in pigs can be predicted based on comparative genome data of humans, mice or other species. This approach is proceeded by 1) detection of the region containing QTL (position), 2) investigation of synteny with other species (comparative map), and 3) selection of candidate genes in the region for sequencing (cloning). Thus, this comprehensive approach is referred to comparative positional candidate cloning. For example, the *KIT* gene, in which mutations causes pigmentation disorders resulting in the dominant white coat colors in pigs was discovered in the region identified by a scan followed by comparative mapping of mouse and human (Johansson et al., 1996).

Differences in expression of genes affecting the trait may cause variation of performance. Thus, our interest is to know which genes have crucial effects on performance. To detect differences in gene expression, PCR-based differential display (PCR-DD) and DNA microarray (DNA Chip) techniques are available. PCR-DD is used to identify genes that are expressed differently between samples. mRNA are screened during cDNA synthesis by using many arbitrary primers that contain different sets of oligonucleotides next to poly(T) repeats. Then, cDNA are amplified with other arbitrary primers in PCR procedures. Synthesis of cDNA and PCR produce many primer specific fragments (Liang and Pardee, 1992). Fragments are labeled with radio active or fluorescent and separated by electrophoresis. Identity of the differentially expressed genes is determined by comparing their sequences to the known genes.

The DNA Chip technique can hybridize the sample to a large number of oligonucleotide or DNA that are immobilized on arrays of a glass slide (Schena et al.,

1995). Furthermore, two samples, each sample is dyed with unique fluorescent color, can be analyzed simultaneously (Shalon et al., 1996). With DNA Chip technique, single test can detect multiple genes that may regulate the trait (Schna et al., 1998). Thus, DNA Chip technique can be used to identify potential genes that may be responsible for differences in performance.

3.4 Genetic markers for growth rate and body composition in pigs

Clamp et al. (1992) reported significant association of enzyme alleles (chromosome 6) with ADG. A total of 186 progeny were produced by a Duroc boar that was homozygous for haptoglobin (HP), glucosephosphate isomerase (GPI), Phosphogluconate dehydrogenase (PGD), and esterase D (ESD). Pigs that inherited the GPI A allele from the sire had a 22 g higher ADG ($p < .02$). Yu et al. (1995) reported significant effects of PIT1 gene (chromosome 13), which was a regulatory factor of growth hormone and prolactin, on growth performance in progeny of Chinese-American breed crosses. Pigs that were PIT1 CC genotype had 120 g heavier at birth and 4 mm fatter (average backfat of first rib, last rib, and last lumber) at market weight (90 kg) than PIT1 DD genotype pigs. Casas et al. (1997a) reported significant association of IGF-I locus (chromosome 5) with ADG ($P < .01$; .032 kg/d) and backfat ($P < .05$; .22 cm) but no association of growth hormone (chromosome 12) with ADG nor backfat was detected. Jiang and Gibson (1999) reported significant association between one of four polymorphisms at leptin locus (chromosome18) and fatness at 100 kg of body weight in one replicate group of Landrace population.

The whole genome scan procedure with various family designs identified QTLs affecting the growth rate, appetite, and body composition (Andersson et al. 1994; Cassas et al., 1997b; Knott et al., 1998; Marklund et al., 1999). Andersson et al. (1994) reported QTL for GR between birth to 70 kg on chromosome 4, and GR between birth to 30 kg on chromosome 13 in F_2 progeny of the European Wild boar and the Large White. Rohrer and Keele (1998) reported QTLs for BF at the 10th rib on chromosome 1, 7, and X in Meishan-Large White population. Cassas et al. (1997b) reported QTLs for postweaning GR on chromosome 3 and 8 in half-sib progeny of the divergent selection lines. Marklund et al. (1999) reported QTL for GR and fatness on chromosome 4 in progeny of

European wild hog and large White/Landrace backcross. Wang et al. (1998) reported QTL for ADG on chromosome 4 and QTL for BF (10th-rib, last-rib, and average) on chromosome 7 in Chinese-American populations. Paszek et al. (1999) reported detection of QTL for postweaning GR on chromosome 1 in Meishan-Yorkshire population.

QTL or genetic markers associating with GR or fatness have been detected on many chromosomes. Differences in allelic frequency at marker loci between breeds, particularly Chinese and European/American breeds, may be a source of variation between studies.

4. Summary

Selection for growth rate increases feed intake and tends to cause more accretion of fat than protein. Improvement of lean growth efficiency can be achieved by either increased lean growth without changes in feed intake or reduced feed intake. One third of gross feed energy is used to maintain skeletal and organ tissues. Many organs are important for skeletal muscle growth as energy-supplies and size of organs are flexible to adjust to various status of energy balance. Metabolic traits including maintenance requirement may be exploited as a possible source of genetic variation.

Consumption of energy is an essential action for organisms to survive. Energy balance is mediated by food intake and energy expenditure. Initiation of feeding and induction of satiety determine the amount of food intake, which is the energy source for maintenance, production and reproduction. Central and peripheral mechanisms, which sense energy balance and mediate food intake and energy expenditure, regulate feeding behavior. Ingestion of food induces various physiological reactions including gastrointestinal distention, elevated serum macronutrient levels and enlarged adipocytes. Hormones or neuropeptides that may be involved in appetite regulatory mechanisms are summarized in Figure 2. Appetite is suppressed by positive energy status. CCK inhibits gastric emptying, stimulates gallbladder, and inhibits feeding through the central nervous system. Elevated serum glucose stimulates insulin secretion from the pancreas and stimulates *ob* gene expression in adipocytes. Also, triacylglyceride level in adipocytes is elevated, which stimulates *ob* gene expression. Leptin is released from adpocytes into the circulatory system and binds to leptin receptors at the hypothalamus and inhibits NPY

mRNA expression and increasing energy expenditure. Serotonin binds its receptor at the arcuate nucleus and projects into the paraventicular nucleus, suppressing NPY mRNA expression and possibly increases energy expenditure. Other anorexinergic factors such as GPL-1 and neurotensin are believed to inhibit NPY gene expression. The study with NPY-knockout mice demonstrated the presence of other factors that stimulate feeding. Although MCH and orexin stimulate feed intake, the feeding-stimulatory mechanism by these two neuropeptides has not yet been elucidated.

The hypothalamic melanocortin system is one of the key steps for leptin to have actions such as reduced NPY mRNA expression, increased OPMC, increased UCP1 mRNA expression, and suppression of food intake. The studies on MC4R in mice suggest the need for future studies of effects of M4R on appetite regulation in swine. MCH binds to MC4R and increases food intake in mice. Also, obesity in yellow obese agouti mice is caused by unique binding affinity of the agouti protein to MC1R and MC4R. A study using NPY-knockout mice demonstrated that the leptin-NPY mechanism is not a whole picture of appetite regulation, and appetite is under the other regulatory pathways as well.

Recent discovery of UCP subfamilies indicates unique roles of each UCP in thermogenesis. UCP1 in BAT and WAT provides heat in newborn animals including humans, and non-shivering thermogenesis in rodents. UCP2 is found in many tissues including the gastrointestinal tract but their function in thermogenesis is not clear. On the other hand, UCP3 is expressed abundantly and preferentially in the skeletal muscle and BAT. Because adult humans and pigs have little BAT, skeletal muscle is the tissue where UCP3 may have important roles in energy balance. Many hormones and receptors are involved in the central and peripheral regulation of energy homeostasis. Thus, investigation of genetic variation in these peptides is justified in order to improve energy efficiency and body composition.

Analysis of phenotypic variation in quantitative traits at the molecular level has become more feasible due to advancement in molecular biology. Many major genes and QTLs for the performance traits in pigs have been reported, and some of them have been implemented in commercial operations. However, success of the biological candidate gene approach is still limited because of poor understanding of biological mechanisms in quantitative traits. Unlike the biological candidate gene approach, the whole genome-scan

approach does not require any prior knowledge of candidate genes. However, linkage disequiliblium between genetic markers and putative QTL affects the power in detection of QTL. Also, informativeness of genetic markers is crucial for detection of QTL. Microsatellite markers are multi-allelic and highly informative.

Whole-genome scans can identify chromosomal regions that are associated with variation in performance. DNA sequencing of detected regions can identify nucleotide variants that may be associated with variation in the trait. However, this positional cloning approach requires large efforts to sequence the entire regions in livestock species. In humans and laboratory animal species, DNA sequencing and localization of genes are in progress. These species and pig share much synteny in their genomes. Thus, genes that locate in the synteny regions in other species may be candidate genes for pigs.

MAS may improve the rate of genetic improvement. However, polygene effects remain as a major cause of variation of quantitative traits until the entire genome is known. Overemphasis on few genetic markers could be detrimental for the long-term genetic gain.

III. Mapping of genes that may associate with growth, appetite, and feed efficiency in pigs

1. Assignment of the porcine obese (leptin) gene to Chromosome 18 by linkage analysis of a new PCR-based polymorphism

S. Sasaki, A.C. Clutter and D. Pomp.

Mamm. Genome 1996. 7: 471-472

***Species*:** Pig

Locus name: Obese (leptin)

Locus symbo: *ob* (Lep)

Map position: *ob* maps to porcine Chromosome (Chr) 18. Sex average recombination frequencies of *ob* with microsatellites S0062 and S0120 were 0.22 (LOD = 4.41) and 0.24 (LOD = 3.56), respectively.

Method of mapping: Individuals from three-generation reference families of European Wild boar x Large White and Meishan x Large White pigs (Archibald et al., 1995) were genotyped for *ob* alleles to determine linkage relationships between the *ob* gene and previously mapped loci. A total of 88 progeny from eight full-sib families were genotyped (Table 1).

Table 1. Number of pigs typed for leptin gene locus by generation

Generation	Genotype			Total
	AA	BB	AB	
Grandsire	10	6	0	16
Granddam	6	10	0	16
Parent	0	2	14	16
Offspring	15	31	42	88

Molecular reagents: Sequence of ob cDNA has beed reported for the mouse (Zhang et al., 1994), human (Considine et al., 1995a), and rat (Funahashi et al., 1995). Exon/intron

organization of the mouse *ob* gene was obtained from GenBank (Accession Number: U22421). Primers to amplify the porcine *ob* gene were designed on the basis of homology between mouse, human, and rat to flank an expected intron of 1730 bp (Figure 3). Primer sequences were 5'-primer-5'GTCACCAGGATCAATGACAT3'; and 3'-primer-5'AGCCCAGGAATGAAGTCCAA3'.

PCR (25 µl final volume) was performed with 50 ng genomic DNA, 0.75 mM $MgCl_2$, 100 µM each dNTP, 0.5 µM each primer, 0.4 units *Taq* polymerase and its supplied reaction buffer. Thermal cycling began with an initial cycle of 95°C for 2 min, 60°C for 1 min, and 72°C for 2 min, followed by 34 cycles of 94°C for 1 min, 60°C for 1 min, 72°C for 1 min, with a final extension at 72°C for 10 min. The reaction resulted in a single product of ~2200 bp. Terminal-end sequencing was used to verify that the product included the expected exon regions of the *ob* gene.

Allele detection: Digestion of the product with the restriction enzyme *Aci*I without reaction buffer revealed a polymorphism with a corresponding single pair of segregating alleles (Figure 4).

Previously identified homologs: Mouse *ob* maps to the proximal end of Chr 6 (Zhang et al., 1994) and human OBS to Chr 7q32 (Geffroy, 1995).

Discussion: Genetically obese (*ob/ob*) mice exhibit hyperphasia, hyperglycemia, and severe obesity (Bray and York, 1979). Isolation and sequencing of mouse *ob* and its human homolog (Zhang et al., 1994) have allowed studies to determine the mechanisms of gene action. Mutations in mouse *ob* resulted in either the absence of *ob* mRNA or a premature stop codon and an incomplete protein (Zhang et al., 1994), but the same mutations were not observed in human subjects (Considine et al., 1995a). Treatment with leptin, the protein encoded by the ob gene reduced feed intake and body fat in both *ob/ob* and wild-type mice (Pelleymounter et al., 1995; Halaas et al., 1995). The *ob* gene may be an interesting candidate for studies of genetic variation in appetite, body composition and obesity in the pig.

The only previously reported Type I marker mapped to porcine Chr 18 (IGFBP3) is conserved on human Chr 7 (Archibald et al., 1995). Thus, assigment of *ob* to Chr 18 broadens the homology between porcine 18 and human 7 (Figure 5), and establishes homology between porcine 18 and mouse 6.

rat	CTATGTTCAAGCTGTGCCTATCCACAAA
mouse	CTATGTTCAAGCTGTGCCTATCCAGAAA
human	CTATGTCCAAGCTGTGCCCATCCAAAAA
rat	GTCCAGGATGACACCAAAACCCTCATCA
mouse	GTCCAGGATGACACCAAAACCCTCATCA
human	GTCCAAGATGACACCAAAACCCTCATCA
rat	AGACCATTGTCACCAGGATCAATGACAT
mouse	AGACCATTGTCACCAGGATCAATGACAT
human	AGACAATTGTCACC AGGATCAATGACAT
rat	TTCACACACG .... CAGT
mouse	TTCACACACG .... Intron .... CAGT
human	TTCACACACG .... CAGT
rat	CGGTATCCGCCAGGCAGAGGGTCACCGG
mouse	CGGTATCCGCCAGGCAGAGGGTCACCGG
human	CGGTATCCGCCAGGCAGAGGGTCACCGG
rat	TTTGGACTTCATTCATTCCCGGGCTTCAC
mouse	TTTGGACTTCATTCATTCCCGGGCTTCAC
human	TTTGGACTTCATTCATTCCCGGGCTTCAC
rat	CCCTCCGCCAGGCAGAGGGTCACCGGT
mouse	CCCTCCGCCAGGCAGAGGGTCACCGGT
human	CCCTCCGCCAGGCAGAGGGTCACCGGT

Figure 3 . Nusleotide sequence of the rat, mouse, and human leptin cDNA. Conservative nucleotide sequence (underlined) were utilized to design the forward and reverse primersflanking exon 2 and exon 3 are underlined.

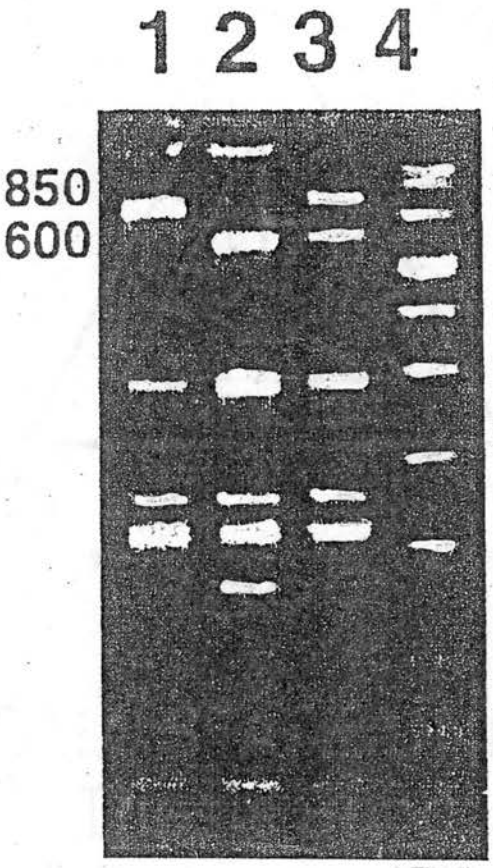

Figure 4. *Aci*I restriction length polymorphism in porcine ob PCR product of ~2200 bp. Lane 1 through 3 are from individuals of AA (~850 bp), BB (~600 bp), and AB (~850 and 600 bp) genotypes, respectively. Lane 4 is Boehringer Mannheim (Indianapolis, IN) DNA molecular weight marker VIII.

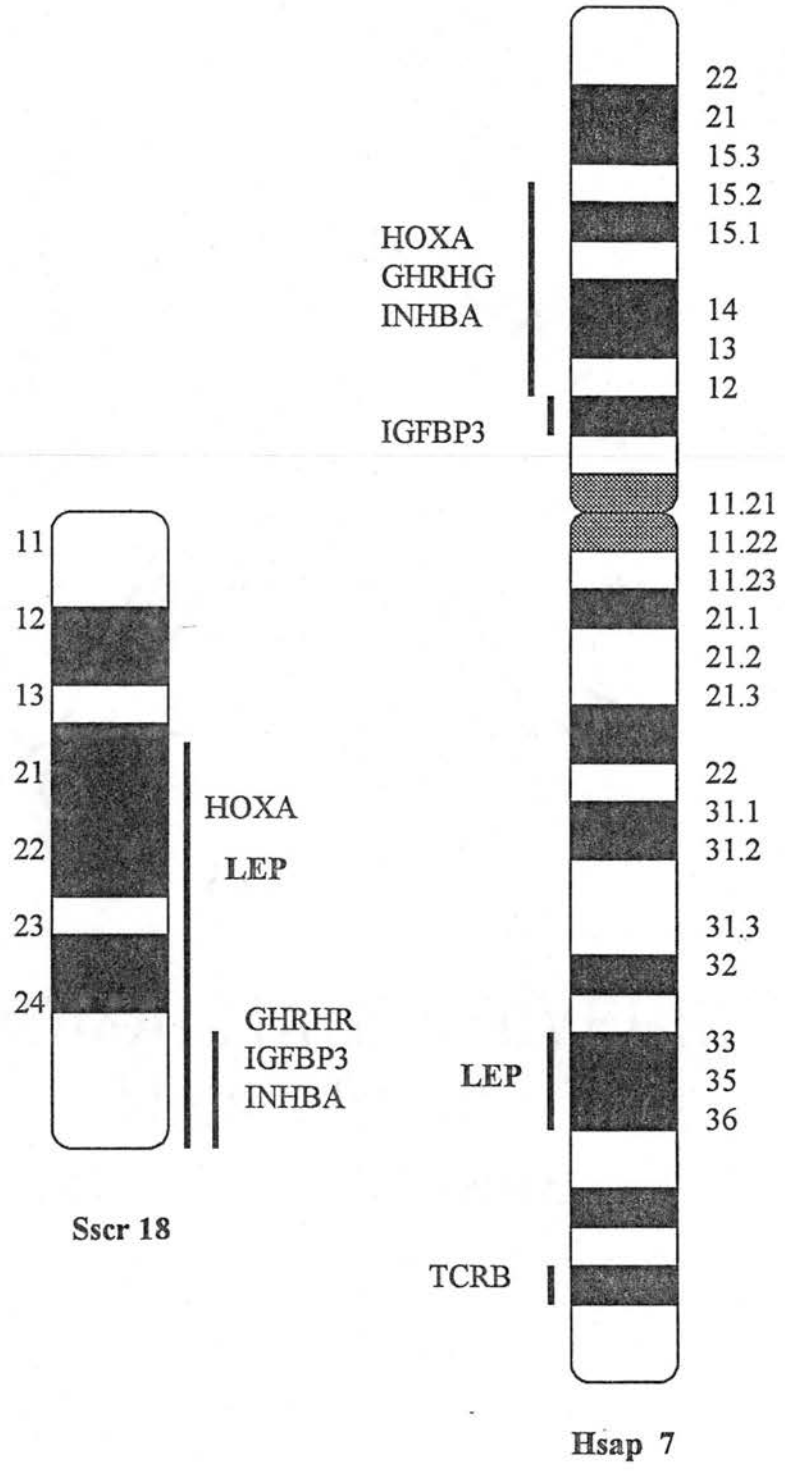

Figure 5. Genomic homology between pig chromosome 18 (Sccr 18) and human chromosome 7 (Hsap 7).

2. Polymerase chain reaction-based polymorphisms in the porcine cholecystokinin (CCK) gene and assignment to chromosome 13.

S. Sasaki, A.C Clutter, and D. Pomp.

Anim. Genet. 1996 27: 369-370

Abstract:

Polymorphisms were identified in the porcine cholecystokinin (CCK) gene by digestion of products from polymerase chain reaction (PCR) with the restrictipn enzyme *Dpn*II. Individuals from the European pig gene mapping project (PiGMap) consortium reference families (eight full-sib families, 91 total progeny) were genotyped to determine linkage relationships between the CCK gene and previously mapped loci. Linkage analysis revealed that the CCK gene is located on porcine chromosome 13.

Introduction: Cholecystokinin (CCK) is a heterogeneous peptide hormone that exists in molecular forms of varying length, created by post-translational modification of a single gene product (Deschenes et al., 1985). In its active forms, CCK stimulates pancreatic enzyme secretion and gall bladder contraction, and is a putative satiety mechanism in a variety of species including the pig (Reidelberger 1994). The objectives of the present study were to identify a polymorphism in the porcine CCK gene, and determine its chromosomal location.

Molecular reagents: Takahashi et al. (1985) and Vitale et al. (1991) reported the exon/intron organization of the human and mouse CCK genes, respectively. Porcine cDNA sequence (Gubler et al. 1994) corresponding to portions of human and mouse exons 1 and 2 was used to design primers to amplify a region of porcine CCK flanking an expected intron of ~4-6 kb (Figure 6). Primer sequences were; 5'-primer, 5'-CTGGCCAGATACATCCAGCA-3'; and 3'-primer, 5'-ATCCATCCAGCCCATGTAGT-3'.

Polymerase chain reaction (PCR) (25 µl final volume) was performed using 50 ng genomic DNA, 1 mM $Mg(Oac)_2$, 200 µM each dNTP, 0.35 µM each primer, 0.4 units rTth DNA polymerase-XL (Perkin-Elmer) and its supplied buffer. A hot start was used

mouse TGCTCCGACCGGACAGGGAGCCCCGAGCG
pig TGCAAAAGGTAGACGGCGAGTCCCGAGCG
human CGCAGAGAACGGATGGCGAGTCCCGAGCG

mouse CGCCTGGGCGCACTGCTAGCGCGATACAT
pig CACCTGGGCGCGCTGCTGGCCAGATACAT
human CACCTGGGCGCGCCCCTGGCAAGATACAT

mouse CCAGCAGG .... CTCCTTCT
pig CCAGCAGG ..... Intron .... CACCTTCT
human CCAGCAGG .... CTCCTTCT

mouse GGCCGCATGTCCGTTCTTAAGAACCTGCA
pig GGCCGAGTATCTATGATTAAGAATCTGCA
human GGACGAATGTCCATCGTTAAGAACCTGCA

mouse GAGCCTGGACCCCAGCCATAGAATAAGTG
pig GAGCCTGGACCCCAGCCACAGAATAAGTG
human GAACCTGGACCCCAGCCACAGGATAAGTG

mouse ACCGGGACTACATGGGCTGGATGGATTTT
pig ACCGGGACTACATGGGCTGGATGGATTTT
human ACCGGGACTACATGGGCTGGATGGATTTT

Figure 6. Nucleotide sequence of the mouse, pig, and human cholecystokinin DNA. Nucleotide sequence of porcine CCK (underlined) were utilized to design the forward and reverse primers flanking exon 2 and exon 3 are underlined.

(polymerase added at 80°C) and thermal cycling began with an initial cycle of 95°C for 1 min, 60°C for 1 min and 72°C for 5 min, followed by 34 cycles of 94°C for 30 s, 60°C for 1 min and 72°C for 5 min. The last 19 cycles included additional, cumulative extension periods of 15 s per cycle. The reaction resulted in a single product of ~3.57 kb. Terminal end-sequencing was used to verify that the product included the expected exon regions of the porcine CCK gene.

Allele detection: Digestion of the product with the restriction enzyme *Dpn*II revealed two independently identifiable polymorphisms (CCK-1 and CCK-2) with corresponding pairs of segregating allels (A/B and C/D, respectively). The allele pairs are depicted separately in Figure 7, and examples of the genotypes observed are shown in Figure 8.

Individuals from three-generational reference families of European Wild Boar x Large White and Meishan x Large White pigs (Archibald et al., 1995) were genotyped for CCK-1 and CCK-2 alleles. A total of 91 progeny from eight full-sib families were genotyped (Table 2). Frequencies in the parental generation were 0.5 and 0.5 for alleles A and B, respectively, and 0.875 and 0.125 for allele C and D, respectively. Haplotype frequencies in the parental generation were 0.4375, 0.0625, 0.4375 and 0.0625 for AC, AD, BC and BD, respectively. There were no recombinations observed between CCK-1 and CCK-2 in the present population.

Table 2. Number of pigs typed for CCK (CCK-1 and -2) gene loci by generation.

Generation	Haplotype									Total
	AA CC	AA DD	AA CD	BB CC	BB DD	BB CD	AB CC	AB DD	AB CD	
Grandsire	9	0	0	4	0	0	0	2	0	15
Granddam	4	0	0	4	2	0	0	0	4	14
Parent	0	0	2	2	0	10	0	0	2	16
Offspring	18	0	5	23	0	3	31	2	9	91

Assignment of loci: Genotypes were used to determine linkage relationships between the CCK gene and previously mapped loci. CCK-1 was segregating in seven of the eight families, but CCK-2 in only two. The relatively greater informativeness of CCK-1 compared with CCK-2 was reflected in the LOD scores associated with linkage of each

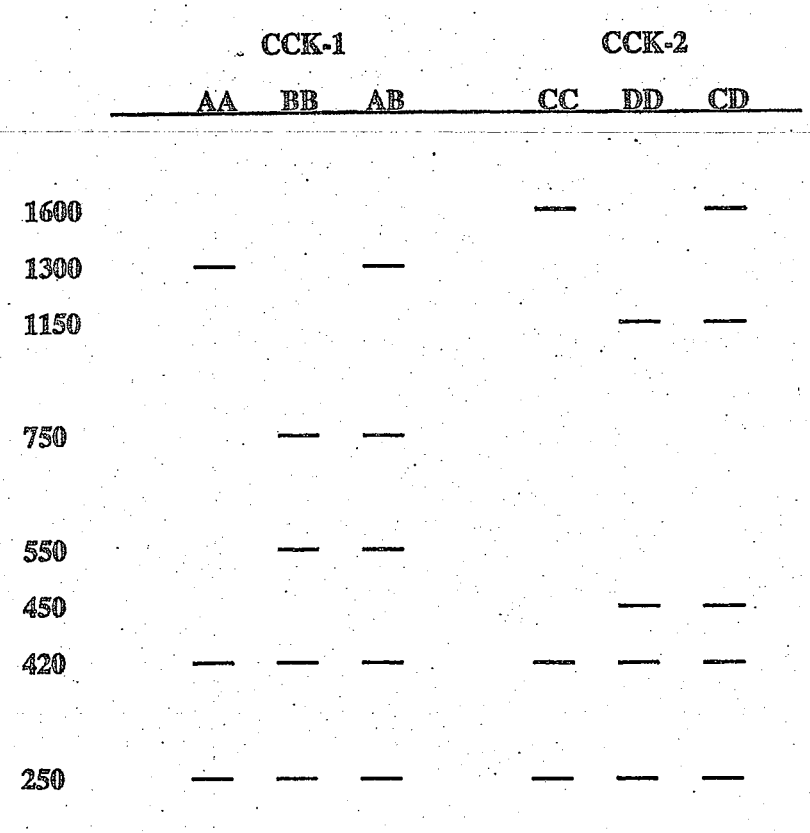

Figure 7. Separate schematic depiction of polymorphisms (CCK-1 and CCK-2) produced by digestion with the restriction enzyme *Dpn*II. For CCK-1, the A allele yielded a fragment of ~1300 bp; the B allele resulted in corresponding fragment of ~750 and 550 bp. For CCK-2, allele C resulted in a fragment of ~1600 bp; the alternative allele yielded fragments of ~1150 and 450 bp. Monomorphic bands were observed at ~420 and 250 bp.

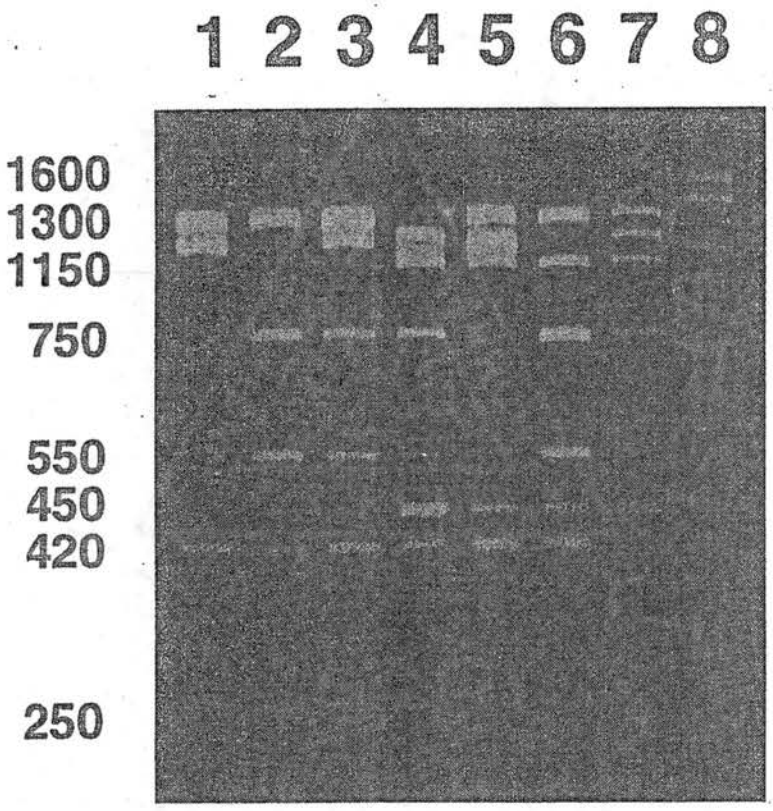

Figure 8. *Dpn*II restriction length polymorphisms in porcine CCK PCR product of ~3570 bp. Lanes 1-7 are observed genotypes for two sets of segregating alleles (see Fig. 7 for separate depiction of genotypes): AACC (~1600. 1300, 420 and 250 bp), BBCC (~1600, 750, 550, 420 and 250 bp), ABCC (~1600, 1300, 750, 550, 420 and 250 bp), ABDD (~1300, 1150, 750, 550, 450, 420, 250 bp), AACD (~1600, 1300, 1150, 450, 420 and 240 bp), BBCD (~1600, 1150, 550, 450, 420 and 250 bp), ABCD (~1600, 1300, 1150, 750, 550, 450, 420 and 250 bp). Lane 8 is Boehringer Mannheim (Indianapolis, IN) DNA molecular-weight marker VI.

marker with itself (27.39 and 12.34, respectively). Significant linkage was revealed between CCK-1 and two previously mapped loci on porcine chromosome 13. CCK-1 showed no recombination with the type I marker ITIH (LOD = 7.53), and the sex average recombination frequency with microsatellite S0288 was 0.02 (LOD = 6.96).

Discussion: Results of the present study confirm those of Rettenberger et al. (1996), which placed the CCK gene on porcine chromosome 13 using somatic cell hybridization, and provided a more precise location of the CCK locus. The CCK gene maps to chromosome 3 in the human (Takahashi et al. 1986). The type I marker linked most closely to CCK-1 in the present study (ITIH) is conserved on human chromosome 3 (Archibald et al. 1995), as is a second type I marker apparently linked to CCK-1 (TF, recombination frequency = 0.19, LOD = 3.29). Therefore, assignment of CCK to porcine chromosome 13 is consistent with available comparative information between the pig and human (Figure 9).

3. The *Cholecystokinin Type-A Receptor (CCKAR)* Gene Maps to Porcine Chromosome 8

S.Sasaki, A.C. Clutter, and D. Pomp

J. Anim. Sci. 1998. 76:1983-1984

Species: *Sus scrofa.*

Locus Name: *Cholecycstokinin Type-A Receptor.*

Locus Symbol: *CCKAR.*

Map Position: *CCKAR* maps to porcine chromosome 8. Sex average recombination frequencies of *CCKAR* with microsatellites S0086 and S0017 were .19 (LOD = 4.40) and .18 (LOD = 3.14), respectively.

Method of Mapping: Pigs from three-generational European Wild boars x Large White and Meishan x Large White reference families (Archibald et al., 1995) were genotyped for *CCKAR* alleles to determine linkage relationships between *CCKAR* and previously mapped loci. A total of 91 progeny from eight full-sib families were

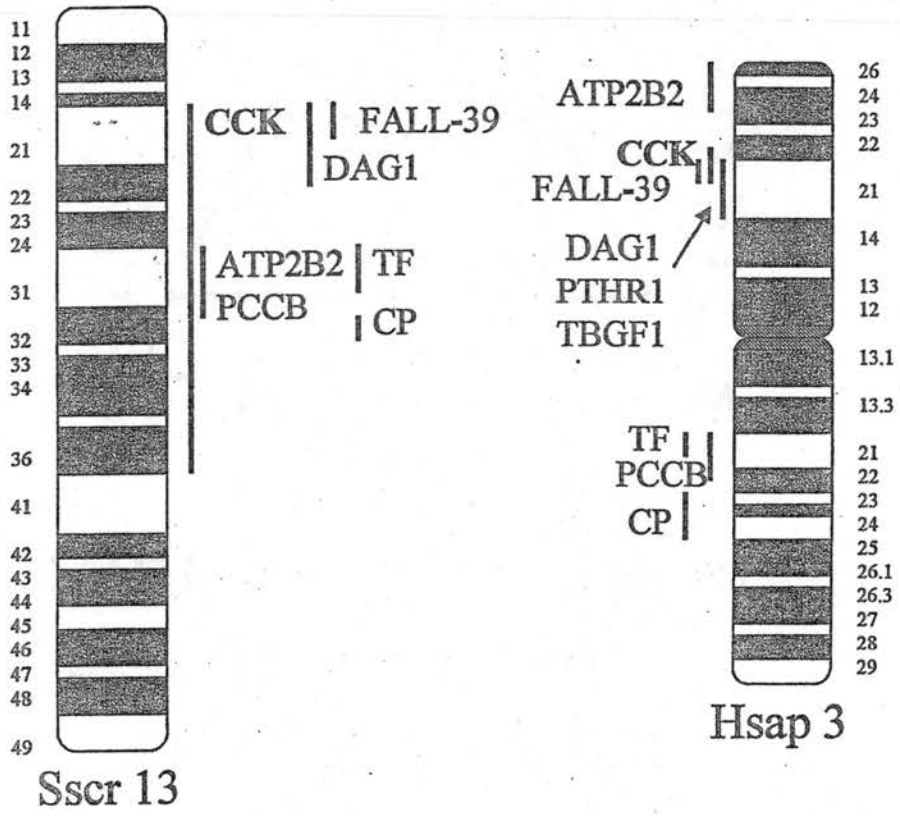

Figure 9. Genomic homology between pig chromosome 13 (Sccr13) and human chromosome 3 (Hsap3).

genotyped. Alleles of the *CCKAR* marker were segregating in four of the eight families (Table 3). The marker was informative in families from both combinations of divergent grandparental breeds (Wild Boar x Large White and Meishan x Large White). No departure from Mendelian inheritance of *CCKAR* alleles was observed in the present population.

Table 3. Number of pigs typed for CCKAR gene locus by generation

Generation	Genotype			Total
	AA	BB	AB	
Grandsire	2	12	2	16
Granddam	1	11	4	16
Parent	0	11	5	16
Offspring	9	65	17	91

Molecular Reagents: Genomic organization of rat *CCKAR* was reported by Takatashi al. (1995). Sequence of cDNA for CCKAR has been reported for guinea pig (de Weerth et al., 1993a) and human (de Weerth et al., 1993b). Primers designed to amplify porcine *CCKAR* were based on homology between rat, guinea pig, and human (Figure 10) and were expected to flank an intron of ~700 bp. The expected total product size was ~900 bp. Primer sequences were 5'-primer; 5'TGAATGGGAGCAACATCACT3; 3'-primer: 5'CATCCTCTTGTTTCGAATCAGC3'.

PCR (25 µL final volume) was performed with 50 ng of genomic DNA 100 *µM* each of dNTP, .1 *µM* of each primer, .875 units of Taq polymerase, and PCR reaction buffer (Tris-HCL, 10 *mM*; $MgCl_2$, 1.5 *mM*; KCL, 50 *mM*; pH 8.3). Thermal cycling began with an initial cycle of 95°C for 2 min, 55°C for 1 min, and 72°C for 2 min followed by 34 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min, concluding with a final extension of 72°C for 9 min. The reaction resulted in a single product of ~900 bp. Terminal-end sequencing was used to verify that the amplified product displayed 80% homology with targeted regions of exon 1 and 2 in rats (52/65 and 57/71, respectively; Takata et al., 1995).

rat ATGGACGTGGTCGACAGCCTTCTTATGAAT
guinea pig ATGGACGTGGTAGACAGCCTTTTTGTGAAT
human ATGGATGTGGTTG ACAGCCTTCTTGTGAAT

rat GGGAGCAACATCACTCCCCCCTGTGAACTC
guinea pig GGAAGCAACATCACTTCTGCCTGCGAGCTC
human GGAAGCAACATCACTCCTCCCTGTGAACTC

rat GGACTGGAAAATGAGACGCTTTTCTGCTTG
guinea pig GGCTTTGAAAATGAGACACTTTTCTGCTTG
human GGGCTCGAAAATGAGACGCTTTTCTGCTTG

rat GATCAACCTCAACCTTCAAAAG.....intron..
guinea pig GATCGGCCCCGGCCTTCCAAAG.....intron..
human GATCAGCCCCGTCCTTCCAAAG ..... intron..

rat .....AGTGGCAGTCTGCACTGCAGATTCTCC
guinea pig ... ..AGTGGCAGCCGGCGGTGCAGATTCTCT
human .....AGTGGCAGCCAGCGGTGCAGATTCTCC

rat TGTACTCCATCATATTCCTTCTCAGTGTGCT
guinea pig TGTATTCCTTGATATTCCTGCTCAGCGTGCT
human TGTACTCCTTGATATTCCTGCTCAGCGTGCT

rat GGGGAACACGCTGGTTATAACGGTGCTGAT
guinea pig GGGAAACACGCTGGTAATCACGGTGCTGAT
human GGGAAACACCCTGGTCATCACCGTGCTGAT

rat TCGAAACAAGAGGATGCGGACGGTCACCAA
guinea pig TCGGAACAAGAGGATGCGGACGGTCACCAA
human TCGAAACAAGAGGATGCGGACGGTCACCAA

Figure 10 . Nucleotide sequence of the rat, mouse, and human cholecystokinin type-A receptor DNA. Conservative nucleotide sequence (underlined) were utilized to design the forward and reverse primers flanking exon 21 and exon 2 are underlined.

Allele Detection: A polymotphism with a corresponding set of segregating alleles was revealed by digestion of the PCR product with the restriction enzyme Fnu4HI (Figure 11).

Previously Identified Homologs: The *CCKAR* gene maps to chromosome 4 in humans (de Weerth et al., 1993b) (Figure. 12) and chromosome 5 in mice (Samuelson et al., 1995).

Discussion: The peptide cholecystokinin was first identified for its ability to stimilate gall bladder contractions and secretion of pancreatic enzymes (Mutt and Jorpes, 1968), but it has since been implicated in numerous studies as a mediator of satiety (see review by Crawley and Corwin, 1994). Two types of cholecystokinin receptors (type-A and type-B) have been identified and specific antagonists to each developed. Although both receptor types are widely distributed, type-B receptors are predominantly located in the brain and type-A receptor in the periphery. Systemic administration of an antagonist for type-A receptor resulted in a 50% increase in feed intake of pigs (Ebenezer et al., 1990), suggesting an important role of type-A receptor in the satiety action of cholecystokinin. The present report of a marker for porcine *CCKAR*, along with previously reported markers for porcine CCK (Clutter et al., 1996) and CCKBR (Hu et al., 1997), provides the opportunity to investigate the importance of these loci as contributors to genetic variation in appetite, growth, and body composition in pigs.

The assignment of this marker to porcine chromosome 8 results in the addition of *CCKAR* to a rather large syntenic block conserved on human chromosome 4 (Figure 12), of which a subset on the proximal end is conserved on mouse chromosome 5 (Johansson et al., 1996). Platelet-derived growth factor receptor alpha (PDGFRA), a gene at the proximal border of this block, is distal to *CCKAR* in humans (4q11-q12 and 4q15.2-p15.1, respectively; Genome Data Base, 1998). Although *CCKAR* did not display linkage to Type I loci in the present analysis, previous reports of the relative distance between PDGFRA and S0086 (3 cM; Ellegren et al., 1993) suggested that *CCKAR* may extend this syntenic group conserved in pigs, humans, and mice.

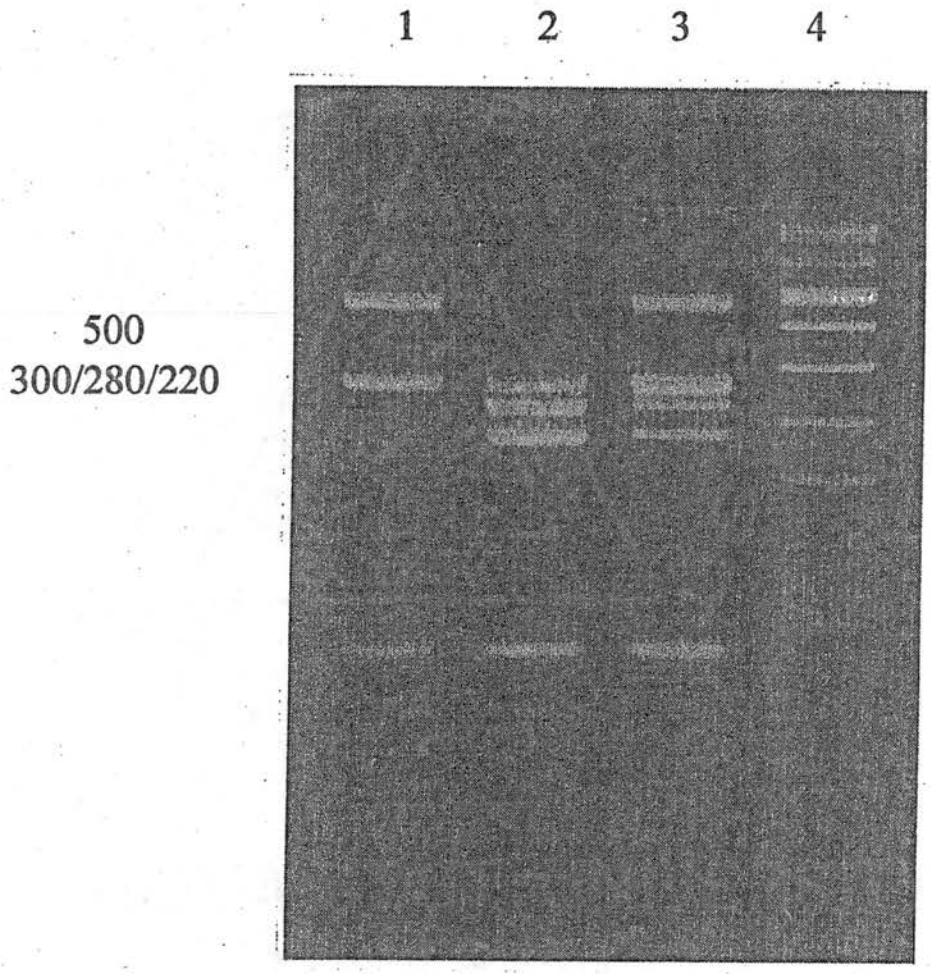

Figure 11. *Fnu*4HI restriction length polymorphisms in porcine CCKAR PCR product of ~900 bp. Lanes 1 through 3 are from individuals of AA (~500 and 300 bp), BB (~300, 280, and 220 bp), and AB (~500, 300, 280, and 220 bp) genotypes, respectively. A monomorphic fragment of ~100 bp was also observed. Lane 4 is Boehringer Mannheim (Indianapolis, IN) DNA molecular weight marker VIII.

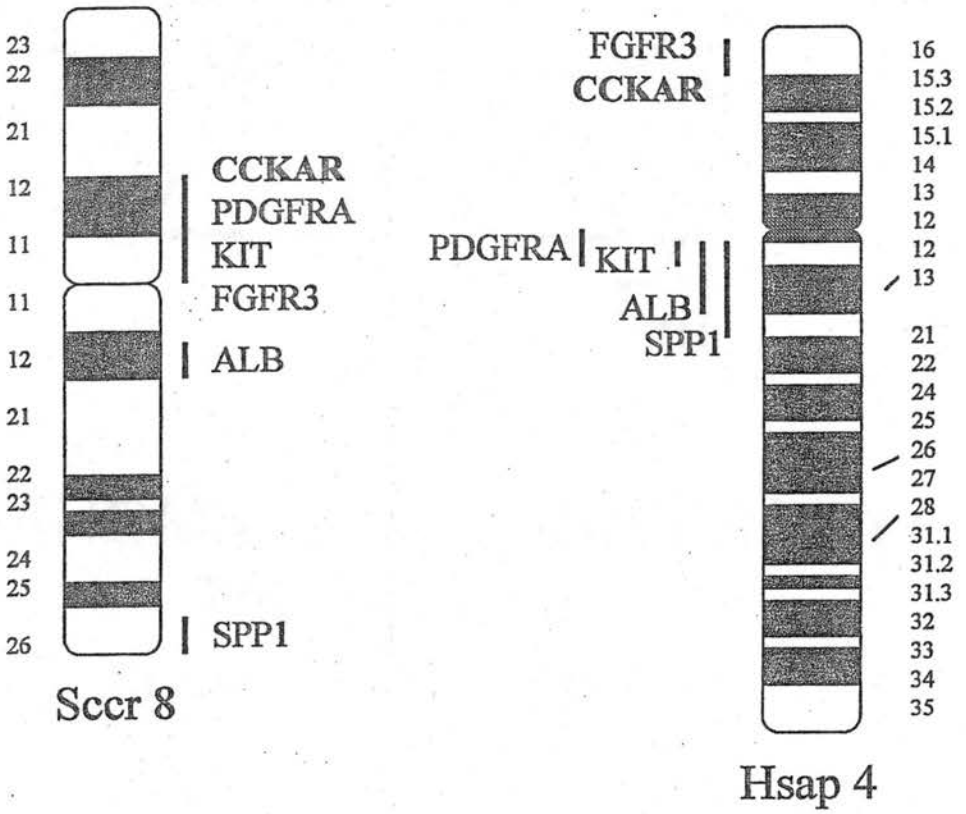

Figure 12. Genomic homology between pig chromosome 8 (Sccr8) and human chromosome 4 (Hsap4).

IV. Greater resolution of a QTL on porcine chromosome 3 for postweaning average daily gain.

Sasaki, S., A.C. Clutter, B.W. Kirkpatrick, E. Casas, A. Prill-Adams and S.G. Price.
Plant and Animal Genome 1999. VII, P342.

Abstract

Ten generations of divergent selection on postweaning average daily gain (ADG) resulted in 40 % better postweaning ADG in the fast growth line (F) compared to the slow-growth line (S). Detection of quantitative trait loci (QTL) for postweaning ADG was attempted with a whole-genome screen approach using half-sib progeny of F_1 (F x S) sires. Two F_1 boars produced a total of 339 progeny (29 litters). A suggestive QTL affecting postweaning ADG had been detected on chromosome 3 in the initial scan of one of the sire families. To increase the resolution of the QTL map, four new microsatellite markers in the region were used for analysis. Regression analysis indicated significant association of microsatellite markers, SW72, SW833, and S0206 with postweaning ADG in the same sire family. Effects associated with these markers on postweaning ADG were 0.03 to 0.04 kg/d ($P<.01$). Interval mapping suggested QTL between SW833 and S0206 (LOD 2.56).

Introduction

Approximately 1,500 markers including 500 functional (Type I) markers have been mapped in pigs (Haley and Visscher, 1998). Some genetic markers have been utilized in marker assisted selection (MAS) in pig production. The ryanodine recptor (halothane) gene (Fujii et al., 1991) that is associated with malignant hyperthermia is also associated with the increased carcass yield but unfavorable meat quality (Leach et al., 1996). Other genetic markers include the KIT gene (Johansson et al., 1996) for white coat color, and MC1R gene (Kijas et al., 1998) for skin pigmentation. With a DNA test, the unfavorable allele can be eradicated from a population. Gene markers for coat colors can be used to produce white coat color market hogs that packers prefer for processing

reason. Breed-specific allele can be introduced to existing population by marker assisted introgression..

Estimates of heritabilities for growth rate in pigs range from .03 to .49 (Clutter and Brascamp, 1998). Significant genetic improvement in growth traits has been achieved by selection based on phenotypes. However, MAS can enhance genetic gain when marker information is added to BLUP (Meuwissen and Goddard, 1997; Zhang and Smith, 1992). Following the initial detection of putative QTL for growth and fatness in pigs (Andersson et al., 1994), more QTL for growth rate and fatness have been reported (Andersson et al., 1998; Casas et al., 1997a; Casas et al., 1997b; Rohrer and Keele, 1998; Paszek et al., 1999). At this moment, genome maps of pigs contain a relatively small number of markers that are spaced unevenly. Therefore, development of genome maps with high density of markers can increase resolution of QTL or linked markers on chromosomes.

Casas et al. (1997b) detected QTL affecting postweaning average daily gain (ADG) in half-sib progeny of F_1 sires which were products of two divergent lines selected 10 generations for either fast or slow ADG (Clutter et al., 1998b). To identify genomic regions that are associated with ADG, the authors typed selected samples (one pig with highest ADG and one pig with lowest ADG per litter) for 46 (sire: 96-5) and 65 (sire: 98-3) informative markers. Allele frequency in selected groups were tested by chi-square test. All nominally significant markers were used for typing family members of pigs used in the selective typing. Association of marker allele with ADG was examined by regression analysis. The selective genotyping and regression analysis indicated significant effects on ADG associated with markers on chromosome 1, 2, 3, 8, and 12 in at least one of the sire families. To determine the location of QTL affecting postweaning ADG, interval mapping was performed using the ANIMAP program, maximum likelihood program designed for half-sib family analyses. Interval mapping indicated that several chromosomal regions containing putative QTL, of which the most significant putative QTL was located on chromosome 3 and accounted for 5% of the phenotypic variation in ADG. However, the distance between two flanking markers for this QTL was larger than 20 cM.

The objective of the present study was to localize QTL affecting ADG by using additional marker information in the same region on chromosome 3 where QTL for ADG had been detected in the previous study.

Material and Methods

Animals

Base population: In 1976, 25 gilts and four boars for each of the Landrace and Spotted breeds were introduced to an existing herd of the Duroc and Yorkshire. Gilts were produced from a four breed diallel mating system involving the Duroc, Yorkshire, Spotted and Landrace breeds. Boars were randomly mated to at least one dam of each breed. The sires selected from each breed were chosen on the basis of the following index: $I = 100 + 60 (G - G) - 75(F - F) - 70(B - B)$, where G is average daily gain, F is feed efficiency (kg feed consumed/kg gain), and B is backfat thickness. Replacement gilts were selected from within the herd on the basis of a similar index and assigned to a sire at random.

In 1979, 12 Hampshire boars were purchased for Spring and Fall breeding seasons and mated to three- and four-breed cross gilts consisting of Duroc, Yorkshire, Landrace and Spotted breeds. Litters sired by the Hampshire boars were born in the Spring and Fall of 1980.

In 1980, 25 (13 high indexing and 12 low indexing) Duroc boars were purchased and mated randomly to Hampshire-sired gilts produced from the previous year's matings. Litters sired by Duroc boars were born in Spring and Fall of 1981. Any gilt that farrowed was grouped into one of two breeding of dam categories, depending on her sire's classification, as either a high or low indexing boar.

Selection criterion: Selection was initiated in 1981 to evaluate phenotypic and physiological responses to divergent selection for postweaning ADG. Fast-growth line (F) and slow-growth line (S) were defined based on ADG from 9 wk of age through 100 kg. Selection of F males in the base generation was for fast ADG among boars sired by high-indexing Duroc boars and out of females sired by high-indexing Hampshire boars. F females were selected for fast ADG from among all gilts sired by high-indexing Duroc

boars. Conversely, S males in the base generation were selected for slow ADG among boars sired by low-indexing Duroc boars and out of females sired by low-indexing Hampshire boars. S females were selected for slow ADG from among all gilts sired by low-indexing Duroc boars.

Selection response: In subsequent generations, within-line, mass selection of boars and gilts for fast or slow ADG was practiced in F and S, respectively. The divergent selection lines were represented in each of two farrowing groups. The spring group farrowed during mid-March through April and the fall group farrowed during mid-September through October. Each line was maintained with approximately 48 females and 8 males per farrowing group. Annual replacement rate of boars and gilts was 100% and thus generation interval was 1 yr.

These selection lines were used for studies investigating physiological differences in growth and feed intake. Clutter et al. (1995) reported gilts from F had greater plasma concentrations of IGF-1 and lesser amounts of IGFBP than those from S. Clutter et al. (1998b) reported pigs from F had less plasma concentration of CCK per unit of feed consumed than those from S. Also, pigs from F were less sensitive to the CCK than those from S.

Selection responses in ADG, FI, and BF after 10 generation of selection for ADG (Clutter et al., 1998b) are shown in Table 4. ADG was 46 to 48% greater in the F line, FI was 36% greater in the F line, and BF was 9 to 17% greater in the F line than those in the S line.

Table 4. Least square means of postweaning ADG, daily feed intake, and backfat after 10 generations of selection and differences between line.

Trait	Sex	F line	S line	Diff. (F - S)
Postweaning ADG	Barrow	0.98 (.01)	0.67 (.01)	.31 (46%)
	Gilt	0.92 (.01)	0.62 (.01)	.30 (48%)
Daily Feed Intake	Barrow & Gilt	2.83 (.03)	2.08 (.04)	.75 (36%)
Backfat	Barrow	30.99 (.050)	28.3 (.071)	2.62 (9%)
	Gilt	31.03 (.032)	26.63 (.047)	4.40 (17%)

Clutter et al.(1998)

Resource family: Two F1 boars were produced by crossing the F and S lines. These boars were mated to 30 dams from an unrelated herd of unselected crossbred animals with mostly Yorkshire and Landrace inheritance. A total of 160 and 159 offspring with ADG data were produced by sire 96-5 and 98-3, respectively. Pigs were weaned at 28 d of age and slaughtered at an average weight of 110 kg. Postweaning ADG was calculated as the difference between live weight at slaughter and weaning divided by days on test.

Typing of animals

DNA Extraction: DNA was extracted from all parent animals and their progeny as described by Casas et al. (1997b). Briefly, blood was collected from all dams and either semen or blood was collected from sires, from which DNA was extracted using a phenol/chloroform extraction method followed by ethanol precipitation (Strauss, 1991). Ear tissue from offspring was frozen, and DNA was extracted using a salt procedure followed by ethanol precipitation (Miller et al., 1988). Working solutions of extracted DNA were adjusted to 50 ng/μL of concentration. These DNA samples were provided to Oklahoma State University in 1996.

Chemical reagents: Primers for microsatellite markers were synthesized by Operon Technologies (Alameda, CA) and kindly provided by the US Swine Genome Coordinator. All PCR reagents were purchased from Promega (Madison, WI). Chemicals for polyacrylamide gel electrophoresis were purchased from Bio-Rad Laboratories (Hercules, CA).

Microsatellite markers: Six new markers on chromosome 3, SW072, SW833, S0100, S0174, S0206 and SWR1637, at the region where QTL for postweaning ADG had been reported by Casas et al. (1997b) were chosen. These markers located between the markers SW2429 and SW251 (Figure 13). Sequences of primers and allelic patterns for markers are listed in Table 5. Genotype data for S0100 locus was not utilized because genotypes of parents and progeny did not fit mendelian inheritance patterns. SWR1637 was not informative (homozygous) in both sires and did not used in this study.

Table 5. Nucleotide sequence of primers, number of alleles, and fragment size for microsatellite markers.

Marker	Primers	No. Alleles	Fragment size
SW72	F: 5'-ATCAGAACAGTGCGCCGT-3' R: 5'-TTTGAAAATGGGGTGTTTCC-3'	5	101 103 109 111 113
SW833	F: 5'-CTGACTGTTTTGCTGCAGTG-3' R: 5'-TCCACTGAGGTCTCTCACTCTC-3'	5	171 77 179 181 183
S0100	F: 5'-CCTCTAGGAAGCTGTGTA-3' R: 5'-AGCCATGACAGGAACGCCAGTAG-3'	5	165 173 175 177 179
S0174	F: 5'-AATCACCATGCACCCTAGG-3' R: 5'-GACATTCAGCCTGAGACT-3'	5	104 112 113 115 123
S0206	F: 5'-TGGGTGTGGTCAACAACCAA-3' R: 5'-ACGTGCCTGCCTCTACCATC-3'	5	175 191 195 199 201

F: Forward
R: Reverse

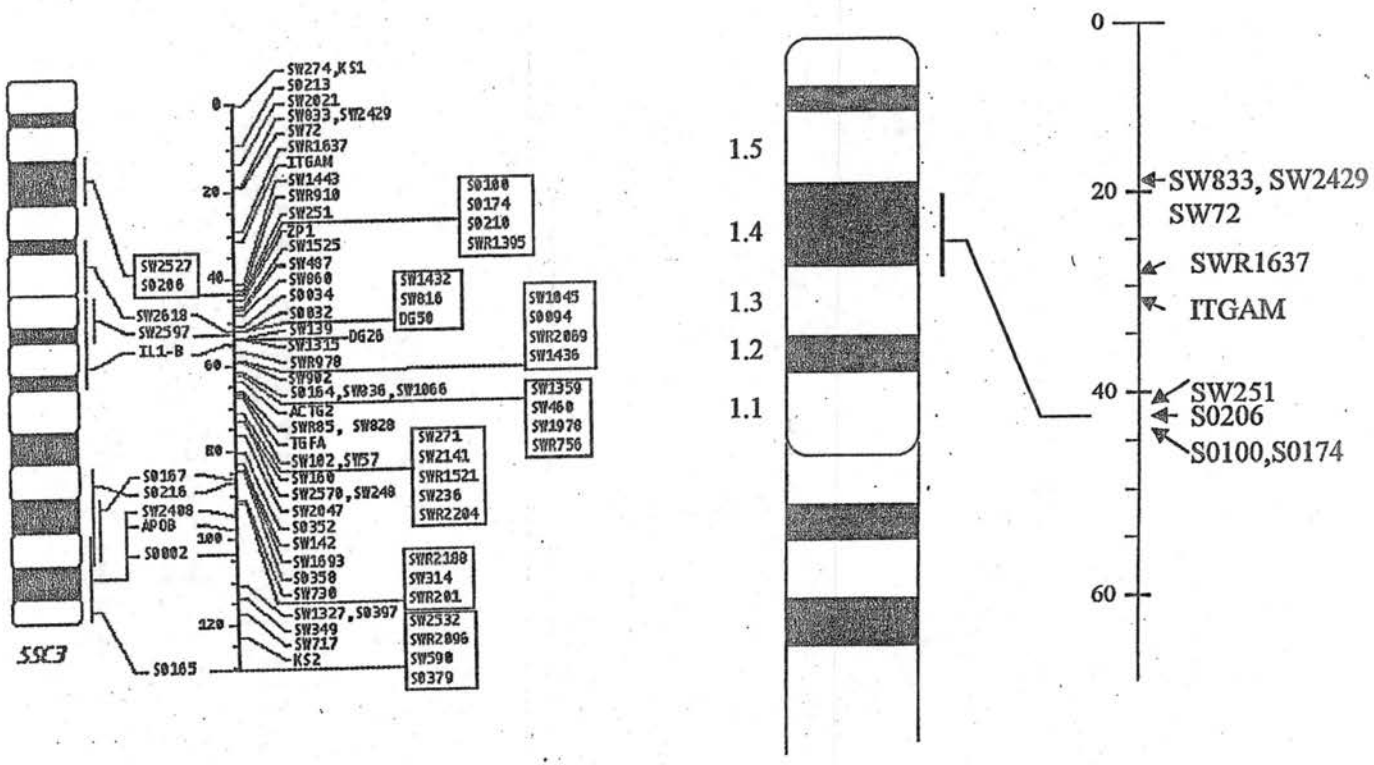

Figure 13. Ideogram of microsatellite markers on pig chromosome 3 utilized in the study

Genotyping of animals: Microsatellite markers were amplified using PCR. PCR reactions with 50 ng DNA were carried out with buffer (50 mM KCl, 10 mM Tris-HCl, and 0.1% Triton® X-100), 150 μM $MgCl_2$, 200 μM each dNTP and respective primers and Taq polymerase. Concentrations of primers and Taq polymerase for SW072, SW833, S0100 and S0206 were; 0.2 μM and 0.625 U, 0.5 μM and 0.875 U, 0.2 μM and 0.625 U, and 0.5 μM and 0.875 U, respectively. All PCR were carried out in a thermocycler PTC-100 (JB Research. Watertown, MA). The initial cycle consisted of 95°C for 2 min, respective annealing temperatures for 1 min and 72°C for 2 min. Annealing temperatures for the markers were; 55°C (S0100 and S0174), 58°C (SW072 and S0206), 60°C (SW833), and 65°C (SWR1637). After the initial thermal cycle, additional thermal cycles (94°C for 1 min, annealing for 1 min and 72°C for 1 min) were carried out 27 times (S0174, SW833 and S0206), 30 times (SW072) or 35 times (S0100). The markers Sw72, SW833, and S0100 were separated by electrophoresis with on 8% acrylamide gel (19:1 acrylamide:bis-acrylamide) by the following conditions: 200V for 30 min (SW72), 60mA for 4.5h (SW833), 48mA for 8h (S0100), 48 mA for 4h or 200V for 30 min (S0100 and SWR1637). The maeker S0174 was separated by electrophoresis with on 12% (19:1) acrylamide gel by 200V for 30 min. The gel was then stained with ethidium bromide and exposed to the UV light.

Statistical Analysis

Regression analysis: Regression analysis was performed for each marker to test association of paternal inheritance with postweaning ADG. Paternal marker inheritance was established by examining offspring and paternal genotypes. For each marker, one sire allele was designated as "1" and the alternative allele as "2". When the dam and sire were both heterozygotes and the offspring was heterozygous at the marker loci, origin of marker allele could not be determined. In order to utilize the information from the offspring with ambiguous paternal inheritance, probability of inheriting allele 1 from the sire was expressed as a coefficient (Dentine and Cowan, 1990). When paternal inheritance was precisely determined, the coefficient was either 1 or 0. If offspring was ambiguous because both parents were heterozygous, the coefficient was 0.5. These

coefficients were used as regressors in the statistical model. Regression analysis was performed for each sire group using the GLM procedure of SAS (1988). The statistical model used for the regression analysis was:

$Y_{ijk} = \mu + L_i + S_j + \beta_1 X_1 + \beta_2 X_2 + e_{ijk}$

Where Y_{ijk} = ADG of k th individual, j th sex, and i th litter

μ = population mean

L_i = effect of i th litter

S_j = effect of j th sex (j = 1, 2)

β_1 = regression coefficient of ADG on weaning weight

X_1 = weaning weight used as covariate

β_2 = regression on probability of inheriting marker allele 1 from sire

X_2 = probability of inheriting marker allele 1 from sire

e_{ijk} = random error

Nominal significant associations of markers on chromosome 3 with postweaning ADG had been already confirmed in the previous study (Casas et al., 1997b). In the present study, the markers were located in the same region on chromosome 3 where nominal significant associations of markers and postweaning ADG had previously been detected already. Therefore, nominal significant associations of new markers with postweaning ADG was expected.

Interval mapping analysis: A maximum likelihood procedure, the ANIMAP linkage analysis programs (Georges et al., 1995) were used to test marker-QTL linkage and construct the most probable location of QTL. These programs were designed to perform linkage studies in half-sib pedigrees and were capable of 1) generating LOD score tables between pairs of markers, 2) performing multi-point linkage analysis (maximum likelihood recombination rates between adjacent markers are determined for all or a subset of marker orders) and 3) generating LOD score between a QTL whose position can be varied with respective to a set of markers whose relative positions are held fixed.

In the first step, pairwise linkage analysis was performed between all pairs of markers. In this analysis, likelihoods of the pedigree data were computed for a range of

fixed recombination rates (θs). LOD scores were computed as $\log_{10}$ (likelihood of pedigree data for $\theta \neq 0.5$ / likelihood of pedigree data for $\theta = 0.5$).

In the second step, different linkage groups were sequentially tested for the presence of a linked QTL affecting ADG. Residuals from a statistical analysis that included the effects of litter, sex, and weaning weight as a covariate for ADG were used as data in this analysis. When the program generates location scores or performs interval mapping, the position of the postulated QTL is changed with respect to the markers composing the linkage group held in fixed positions. Recombination rates between markers were estimated and Kosambi map distance were calculated (Ott, 1992).

The experimental-wise threshold values for linkage results from genome-wide QTL analysis were calculated based on the formulas developed by Lander and Kruglyak (1995) with values of 18 for chromosome number and 21.5 for autosomal genome length in M. LOD threshold values were 3.29 for a significant linkage, and 1.98 for suggestive linkage. With a LOD score greater than 1.98, probability of detecting a marker-QTL linkage in every genome scan by chance is 0.0024, whereas, with a LOD score greater than 3.29, probability of detecting a marker-QTL linkage in every genome scan by chance is 0.0001.

Results

Marker Typing: Microsatellite marker S0174 produced polymorphic PCR products. However, segregation pattern did not fit to Mendelian inheritance (Figure 14). Therefore, marker information from S0174 was excluded from data analysis. Both sires were homozygous for SWR1637, thus no further genotyping was performed. The differential simple tandem repeats amplified by microsatellite markers SW72, SW833, S0100, and S0206 are shown in Figures 15, 16, 17, and 18, respectively. The number of litters and half-sib progeny by sire are listed in Table 5. Sire 96-5 but not his progeny could not be typed for SW833 due to unknown technical difficulties and consequently no data for SW833 in 96-5 sire family was utilized. Sire 98-3 was homozygous for S0100 and no data for S0100 in 98-3 sire family was utilized (Table 6).

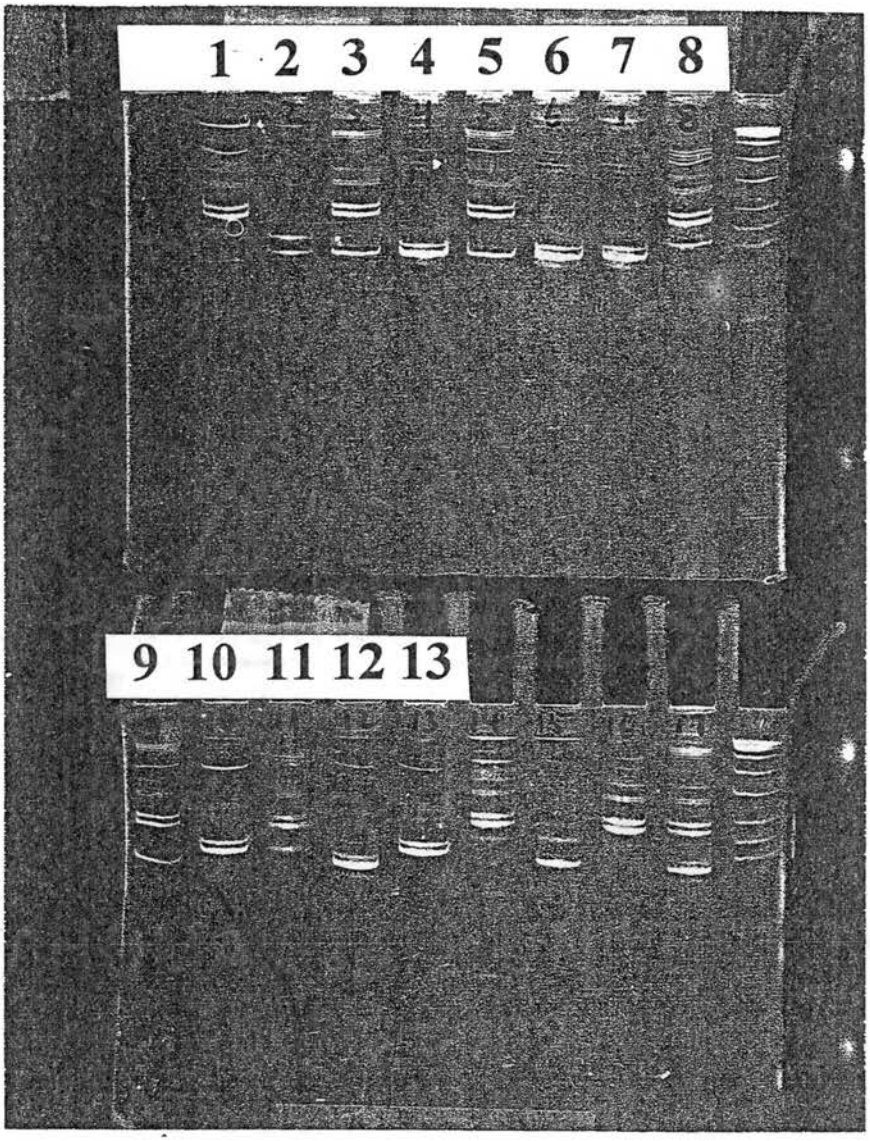

Fig 14. Differential simple tandem repeats by microsatellite marker S0174. PCR products are electrophoresed at 203 V for 30 min on 12% polyacrylamide gel. Lane 1 and 2 are sire and dam, respectively. Lane 3 - 13 are their progeny. Inheritance patterns did not fit the Mendelian inheritance and marker information were not informative.

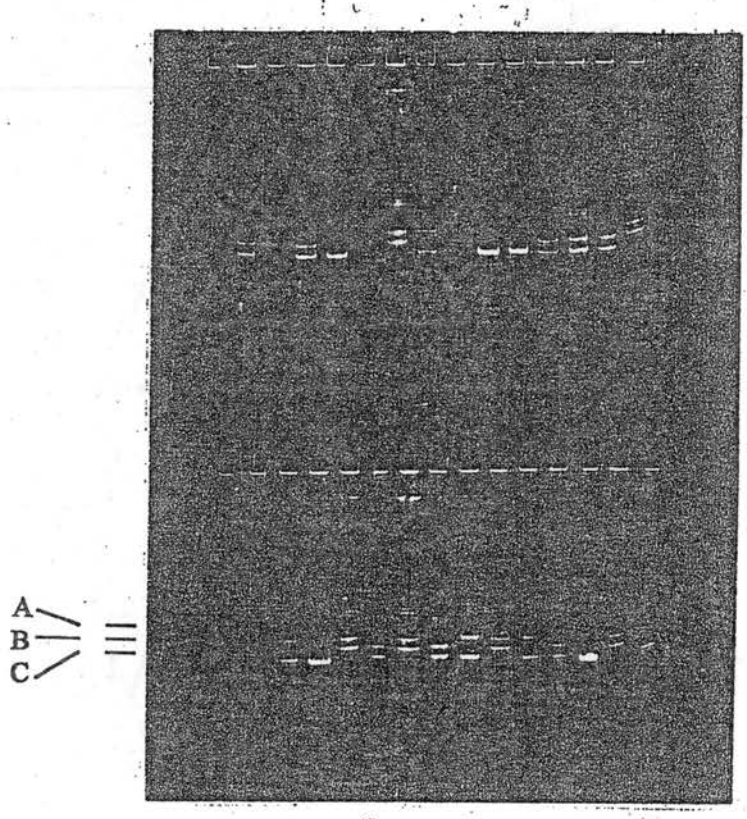

Fig 15. Differential simple tandem repeats by microsatellite marker SW72. PCR products are electrophoresed at 206 V for 30 min on 8% polyacrylamide gel.

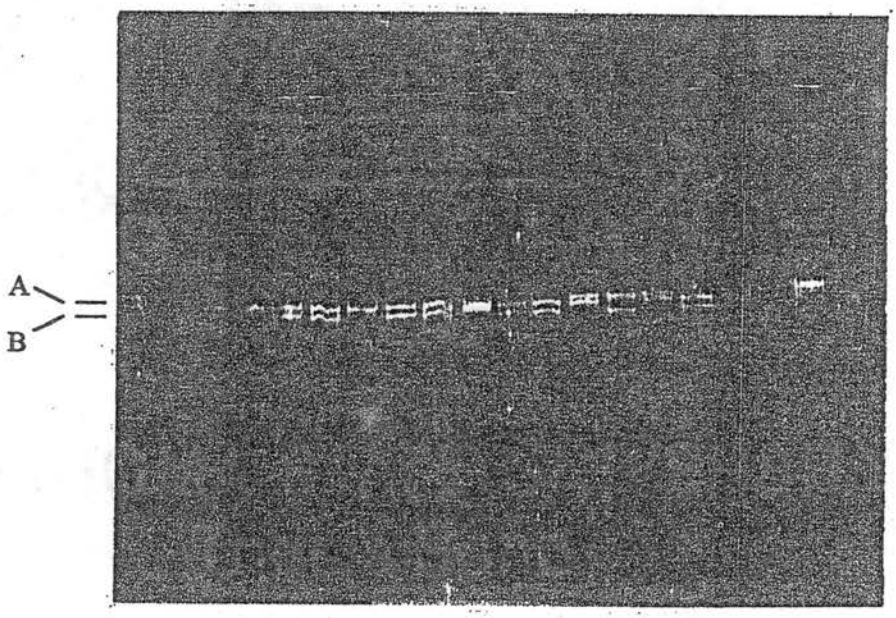

Fig 16. Differential simple tandem repeats by microsatellite marker SW833. PCR products are electrophoresed at 60 mA for 2.5 hrs on 8% polyacrylamide gel.

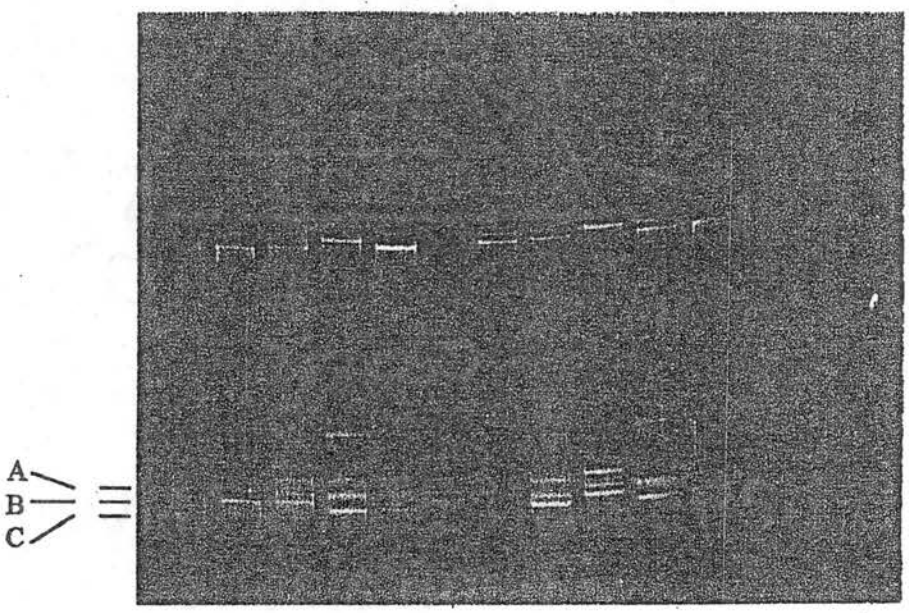

Figure 17. Differential simple tandem repeats by microsatellite marker S0100. PCR products are electrophoresed at 48mA for 8.5 hrs on 8% polyacrylamide gel.

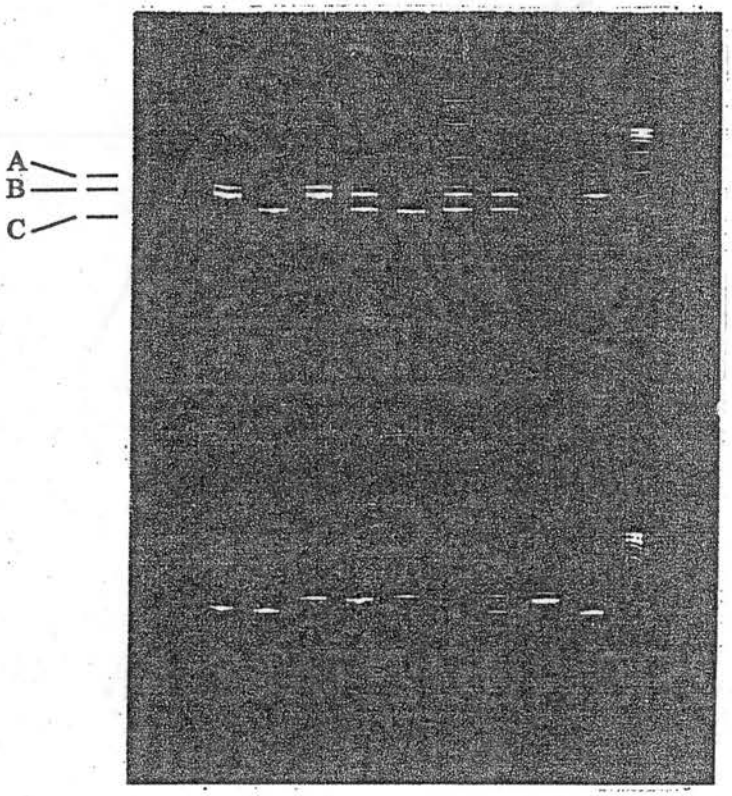

Figure 18. Differential simple tandem repeats by microsatellite marker S0206. PCR products are electrophoresed at 197V for 30 min on 6% polyacrylamide gel.

Table 6. Total number of families, total number of progeny, number of litters typed, and number of progeny typed by sire family.

Sire	Total No. of Families	Total No. of Progeny	Number of litters and progeny typed by marker			
			SW72	SW833	S0100	S0206
96-5	15	160	11 (156)	0 (0)[a]	15 (152)	15 (157)
98-3	15	159	15 (147)	15 (144)	0 (0)[b]	15 (143)

Regression analysis: Nominal significant association (P = .003) between SW251 on chromosome 3 and postweaning ADG was obtained in the family of sire 98-3 in the initial study. In this study, nominal significant associations between markers, SW72, SW833, and S0206, and postweaning ADG was obtained in the family of sire 98-3. The results of the regression analysis of these markers are shown in Table 7. For the family of sire 98-3, regression of ADG on inheritance of SW072 and S0206 alleles were 0.03 to 0.04 kg/d (all P < .01).

Table 7. Regression of postweaning ADG on probability of inheriting designated marker allele from sire.

	96-5		98-3	
Marker	P value[c]	b value[d]	P value[c]	b value[d]
SW72	.56	.02	.007	.03
SW833[a]	N/A	N/A	.0001	.04
S0100[b]	.93	0	N/A	N/A
S0206	.17	.02	.002	.04

a Sire 96-5 was not typed.
b Sire 98-3 was homozygous.
c Probability of rejecting the hypothesis that the regression is equal to zero, when in fact it is true.
d Regression of phenotype on probability of receiving designated sire allele.

Interval mapping analysis: With the addition of these three markers, a total of seven microsatellite markers on chromosome 3 were determined to be associated with ADG in

the family of 98-3. Results of the updated interval analysis for these seven markers are shown in Figure 19. Map order and linkage distances in this analysis were generally consistent with the previous map generated from this population (Casas et al., 1997b) and the USDA porcine map of chromosome 3 in that the three new markers were located within the region bracketed by SW2429 and S0151. SW833 and SW72 are placed at the nearly equal distance from SW2429 and SW251 in this study, while they are placed adjacent to SW2429 in the USDA map. The threshold for the genome-wide significance level (1.98) was used for the evidence suggestive of linkage to a QTL. The location of QTL is in the region bracketed by SW833 and S0206 QTL, where LOD score is 2.56. The location of putative QTL is consistent with the previous result that found a putative QTL between SW2429 and S0151 (Casas et al., 1997b).

Discussion

This study was conducted to develop a map of greater resolution for a putative QTL for ADG on chromosome 3. The putative region was based on the results of an initial QTL analysis reported by Casas et al. (1997b). Due to technical problems and lack of heterozygosity, number of marker information was reduced. The presence of a potential QTL affecting postweaning ADG was detected previously in the sire family of 98-3. The order of markers used in the early (Casas et al., 1997b) and present study was SW2429-SW72-SW833-S0206-S0151-SW251-SW1066 (Figure 19). Suggested location of the putative QTL detected in the family of 98-3 was between SW833 and S0206. The predicted location of QTL for postweaning ADG was between SW2429 and S0151 in the early study (Casas et al., 1997b). The drawing of LOD scores (Figure 19) had three peaks that were exceeded a LOD score for the suggestive association between putative QTL and postweaning ADG. This might indicate the presence of more than one QTL affecting postwening ADG in this region or simply lack of microsatellites between the present markers. However, no other informative markers in this region were available. Therefore, no further mapping of the QTL in this region for this resource families was possible.

Andersson et al. (1994) reported detection of QTL affecting growth from birth to 70 kg and average backfat on chromosome 4 in F_2 progeny of the European Wild boar

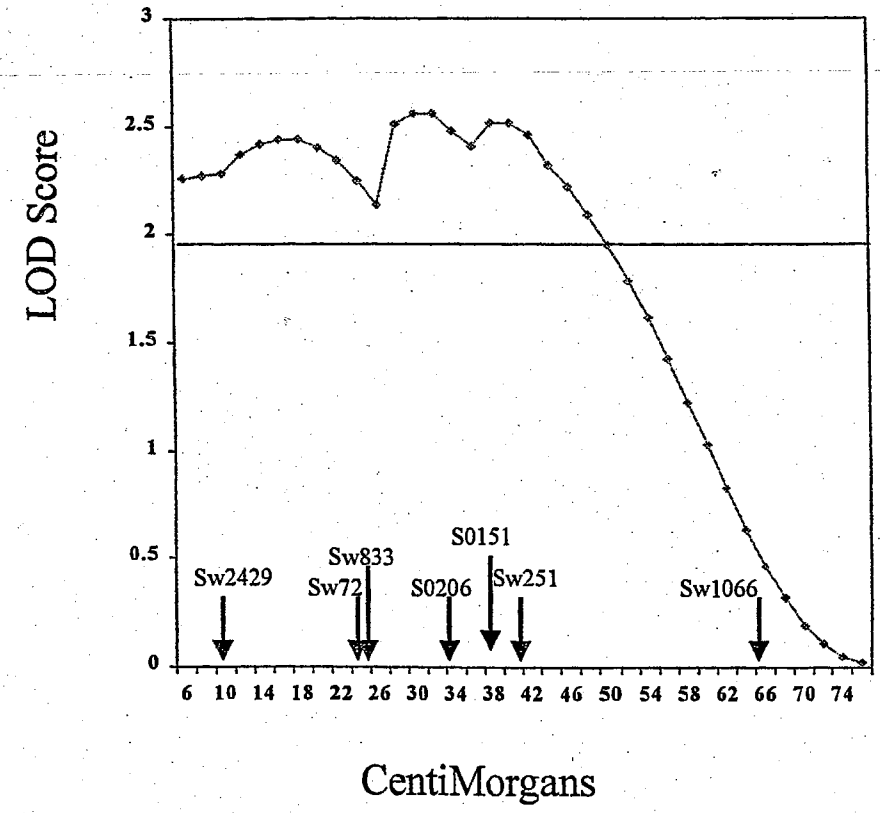

Figure 19. Maximum likelihood estimate of QTL position on pig chromosome 3.

and the Large White. Wang et al. (1998) detected a significant association of QTL on chromosome 7 with fatness but detected only a suggestive QTL affecting ADG on chromosome 4 in Chinese x American resource families. In the initial study, the whole genome scan was performed with 75 informative markers in two sires (Casas et al., 1997b). No significant nominal association between markers on chromosome 4 and postweaning ADG was detected from the selective genotyping. Therefore, QTL affecting growth on chromosome 4 (Anderoson et al., 1994: Marklund et al., 1999) might not be segregating in the families we studied.

A QTL affecting postweaning ADG was detected on chromosome 1 in Meishan x Yorkshire resource families (Paszek et al., 1999). This QTL was mapped between SW377 and SW1301 in telomere of chromosome 1 and accounted for 26% of the F2 phenotypic variance. In the early study (Casas et al., 1997b) of the present population, a marker SW974 on chromosome 1 was nominally significant after the selective genotyping. The marker SW974 is approximately 20 cM away from SW373. Therefore, investigation of this region in our resource families with new markers would be interesting.

Chromosomal regions that may contain potential QTL for growth can be investigated based on the positional candidate gene approach. A large portion of the p-arm on pig chromosome 3 is homologous to human chromosomes 16 and 7 (Figure 20). Protamin protein 1 (PRM1; Rettenberger et al., 1994), protamin 2 (PRM2; Engel et al., 1992), and transition protein 2 (TNP2; Rettenberger et al., 1994; Johansson et al., 1995) correspond to the p-arm of human chromosome 16. Somatostatin receptor 5 gene (SSTR5) on human chromosome 16p13.3 is a member of somatostatin receptor family and mediates inhibition of insulin secretion (Bell et al., 1995). Another gene of interest is smooth muscle myosin heavy chain 11 (MYH11) on human chromosome 16p13.13-p13.12. In chicken, myosin heavy chain is strongly expressed in developing atria from a very early stage to the adult stage. This gene is also expressed in the ventricle, somite (the precursor to skeletal muscle) and skeletal muscle during embryonic stages but not in adults (Oana et al., 1998). Moreover, the number of myofibers expressing IGF-I and IGF-II coincides with the increased expression of developmental myosin heavy chain during wound healing in mice (Keller et al., 1999).

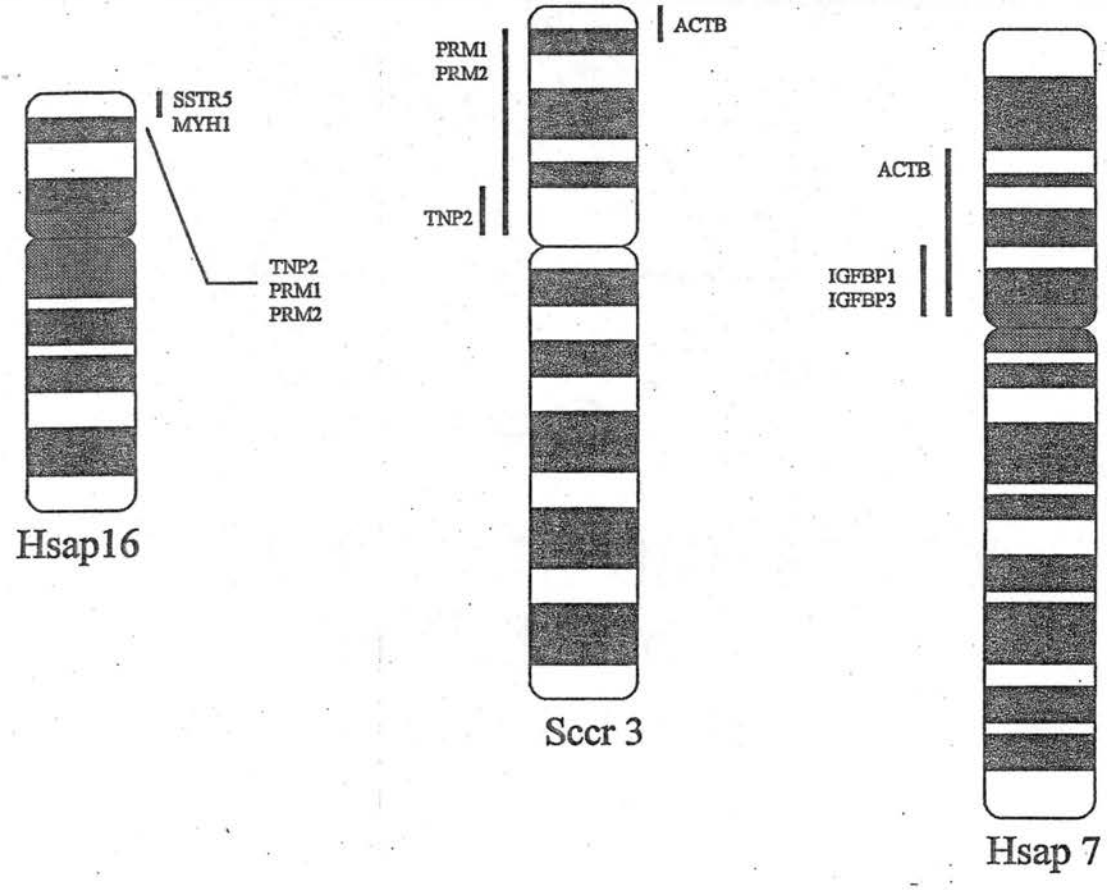

Figure 20. Genomic homology between pig chromosome 3 (Sccr3) and human chromosome 16 (Hsap16) and 7 (Hsap7).

Our ability to choose right genes that have large effects on growth rate, feed intake, or feed efficiency is still limited at this time. However, research efforts have identified few genes that may have significant effects on growth rate. Information of hormones or neuropeptides that have been investigated for their effects on these traits in other species can be useful. Genes encoding those hormones or neuropeptides can be considered as candidate genes. Many studies in humans, rodents, and other livestock species indicate CCK, CCKAR, and Leptin have important roles in feed intake and energy utilization. We developed markers for these genes and can be tested their associations with growth rate, appetite, or feed efficiency.

Possible associations of GH or IGFs with growth performance in pigs were reported. Norton et al. (1989) investigated concentration of GH in S and F. The authors reported gilts from F had higher mean GH concentrations than the gilts from S. However, a later study in the same population found no difference in mean GH concentrations between F and S. In the same study, the authors found gilts from F had higher concentrations of IGF-1 than gilts from S, whereas F had less concentrations of IGFBP2 than S.

Intramuscular injection of porcine somatotropin increases IGF-I levels and enhances anabolic performances in pig. Elevated IGF-I was followed by the increased IGFBP3 and the decreased IGFBP2 concentrations (Coleman et al., 1991). Thus, concomitant concentration of IGFBP2 and IGFBP3 may be associated with activity of IGF-I. However, at least one study demonstrated no significant correlation between plasma GH and IGF-I, IGF-II, or IGFBP in pigs selected for growth rate (Owen et al., 1991).

Although there seem to be no direct effects of GH on energy efficiency GH seem to play important roles through IGF-1 and IGFBP in energy balance. Prepubertal surge of IGF-1 was detected in rats (Handelsman et al., 1987; Hiney et al., 1996). In gilts, surge of IGF-II triggers sexual maturity (Buonomo and Klindt, 1993). A threshold level of body fat is required to reach puberty in pigs (Kirkwood and Aherne, 1985; Beltranena et al., 1991). Therefore, change in body composition coincides with the change in IGF levels. Potential roles of IGF in control of body composition was suggested. Buonomo and Klindt, (1993) reported the rise in IGF-II concentration between weeks 12 and 22 was

greater in amplitude and had a longer duration in the obese line pigs overall. This result may be relevant to the finding of a higher IGF-II levels during fetal growth in lean line than that in obese line pigs (Hausman et al., 1991). Another study indicated IGF-I and IGF-II might interact to increase backfat in pigs (Lamberson et al, 1995; Lamberson et al, 1996).

V. Summary

Quantitative approaches have demonstrated their effectiveness in improvement of growth and feed efficiency. Feed intake, growth, and body composition are associated with each other through energy balance. Feed intake and growth are positively associated. Estimated heritability of feed intake ranges from 0.12 to 0.59. Fast growing pigs consume more feed and tend to deposit excess energy in body fat. Body weight increases in a nonlinear fashion and accretion of fat exceeds accretion of lean mass in the late growth phase. Because approximately 75% of lean tissue is water, energy cost to accrete one unit of body weight with fat is expensive compared to lean tissue.

Lean content of the carcass is an important economic trait. Genetic variation in energy efficiency must be exploited to improve growth performance. Selection for growth rate with restricted feeding (no variation in FI) or residual feed intake were practiced to evaluate energy efficiency. Some studies demonstrated that energy requirements for maintenance might vary between lean pigs and fat pigs. Approximately 30% of metabolizeable energy are required to maintain the organs. Lean pigs tend to have a larger proportion of body weight in organs. A study indicated that lean pigs are capable of utilizing energy efficiently by shifting toward accretion of lean which is more energy efficient than accretion of fat. One study in mice indicated a hyperactive behavior in lean mice attributed to the less accretion of fat compared to obese mice.

Regulatory mechanisms of feeding behavior evolved from the glucostatic theory to the adipostat theory. Mendelian inheritance of obesity was known in a strain of mouse in 1953. Presence of a circulating chemical compound that controls feeding behavior in mice was demonstrated by using probiosis in 1958. Molecular characteristics of the obese gene and its product, leptin, were determined in 1994. Some hormones or neuropeptides associating with energy hompstasis have been identified. Along with discovery of novel neuropeptides or hormones, associations between them are gradually elucidated. An energy homeostasis is maintained by the following neural networking; 1) reporting quantity of energy stores, 2) balancing energy storage and expenditure, 3) directing eat or stop eating , and 4) integration of those components in the CNS.

The Leptin-NPY system is the one of regulatory mechanisms for energy homeostasis that is supported by balance of leptin (anorectic) and NPY (orexinergic).

However, many hormones and neuropeptides involve in the leptin-NPY system or novel systems. The characteristics and putative roles of many hormones and neuropeptides were reviewed. Among those, malanocortin receptors seem to have interesting roles in feed intake. More novel hormones or neuropeptides will be identified in the future.

Molecular dissection of quantitative traits can identify causes of variation and may make selection of pigs more effective. For feed intake, CCK is a hormone that suppresses feed intake in many species including pigs. A mutation in the *ob* gene causes hyperphagia and obesity in rodents. We amplified the porcine Lep, CCK, and CCKAR genes and detected polymorphisms in them. Using linkage analysis, we mapped Lep, CCK, and CCKAR to porcine chromosome 18, 13, and 8, respectively. Other peptides such as Lepr, CCKBR, YNPY, NPY_5, MCH, serotonin, and orexins are also candidate genes for appetite in pigs.

Many QTLs affecting growth rate have been detected in different resource families. Power of QTL detection is affected by design of resource families. Linkage disequilibrium generated by within-family or intercross between divergent populations have been used in many QTL studies. Data from F_2 progeny maximizes detection of QTL because deviation of gene effect in the population is maximum, and all recombination phases are informative.

In the present study, postweaning ADG of half-sib progeny produced by two F_1 sires of the F and S lines were analyzed. Thus, the effects of paternally inherited alleles on ADG was evaluated by regressing probabilities of inheriting a paternal marker allele on ADG. A previous study had detected QTL for postweaning ADG on chromosome 3 (LOD = 2.9). Genotype information for four additional microsatellite markers in the same region were added to the previously collected marker information. The interval mapping analysis indicated a putative QTL between SW833 and SW251 on chromosome 3 (LOD = 2.56).

QTL are hypothetical genes based on statistical inference, thus specific biological roles of the QTL are unknown. However, variance explained by the QTL can be evaluated and utilized in a MAS program. The region where QTL have been mapped may be examined for presence of known or novel genes. For the discovery of novel genes in pigs, candidate genes identified with comparative genome information may be the most

effective approach. Based on homology between pig chromosome 3 and human chromosome 7 and 16, SSTE5, and IGFBP1 were identified as positional candidate genes for ADG.

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