# Quantitative Measurement of PIT1, GH, and PRL mRNA and Circulating Hormone Levels in Pig Families Segregating *PIT1* Genotypes

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## Summary and Implications

*PIT1* is a member of the POU-domain family gene and is a positive regulator for growth hormone (GH), prolactin (PRL), and thyrotroph-stimulating hormone β(TSHB) in several mammalian species. Previous studies in pigs have shown an association of *PIT1* polymorphisms with growth and carcass traits in which the primarily Chinese alleles were associated with heavier birth weight and greater backfat. To further investigate the role of *PIT1* in controlling pig growth traits, Chinese Meishan (MS) pigs segregating *PIT1* polymorphisms were used to study the differences of GH and PRL at both mRNA and circulating hormone levels. A total of 60 animals from nine litters was used to collect both pituitary and blood samples at day 1, 15, and 30 after birth. A novel procedure that involves standard curve quantification was used to quantify mRNA amount for GH, PRL, PIT1-α, and PIT1-β. Conventional hormone assays were done to measure the circulating amount of GH and PRL from the blood. Statistical analyses of *PIT1* genotypes, sex, and days on either mRNA (PIT1 alternative transcripts, GH, and PRL) or circulating hormone (GH and PRL) were done by using the least-squares procedure. This study investigates GH and PRL at both mRNA and circulating hormone levels in MS pigs segregating *PIT1* polymorphisms. Results from this study will provide useful information on the role of *PIT1* in controlling pig growth traits.

## Introduction

Hormones produced from the pituitary are important in controlling animal growth and reproduction. The control of expression of these hormones, however, is poorly understood in pigs. PIT-1 is a pituitary-specific regulator that controls gene expression of three important hormones: growth hormone (GH), prolactin (PRL), and thyroid stimulating hormone beta (TSH-β). PIT-1 mutations result in dwarfism in rodents and humans due to hypopituitarism and the absence of the above hormones. Thus, PIT-1 is a critical gene to understand for manipulating growth in the pig. We have investigated the different PIT-1 allelic forms for their association to economic traits and previously found that PIT-1 alleles are statistically associated with birth weight and a number of backfat thickness measurements (7).

To further investigate the biological function of *PIT1*, we have cloned the full length cDNA of pig *PIT1*, and found significant variability at pig *PIT1*, in specific allelic forms at the genetic level, as well as in alternative splicing mRNA forms at the metabolic level. The objectives of this study were to test association of *PIT1* genotypes with pituitary mRNA (PIT1-α, PIT1-β, GH, and PRL) and circulating hormone levels (GH and PRL) in pig families segregating *PIT1* genotypes.

## Materials and Methods

*Experimental animals and sample collections.* Animals were assigned to matings according to their *PIT1* genotype. Eight CD and one DD Meishan female pigs were mated to two CD Meishan males. Nine litters with a total of 92 piglets were delivered from September to November 1997 in the ISU Reproduction Laboratory at Ames. An average of 10ml of blood was collected from individual piglets at 0, 15 or 30 days of age. At the same time, pituitary tissues were obtained from randomly sacrificed animals and frozen in liquid nitrogen immediately. The day 0 samples were collected within 24–72 hours after birth.

*PIT1 genotyping.* Genomic DNA was isolated from tail tissue that was collected from all animals on the day of birth. A PCR-Msp I marker developed from the previously described Southern-RFLP marker (6) was used to genotype all newborn animals (T.P. Yu and C.K. Tuggle, unpublished data).

*RNA isolation and native/competitor template preparation.* Poly($A^+$) RNA was directly isolated from frozen pituitary tissues. 0.5–1 μg PCR amplified native/competitor template was used for in vitro transcriptions by using MAXIscript™ kit according to manufacture's protocol (Ambion, Austin, Tx). Concentration of the mRNA and in vitro transcribed RNA were determined by $OD_{260nm}$ absorption, then aliquoted and stored at –80°C until used.

*Primers used in quantitative competitive RT-PCR and subcloning.* Three sets of porcine-specific primers were designed based upon published nucleotide sequences for the GH and PRL genes and unpublished PIT1-α sequence (8). A highly conserved PIT1-β specific sequence from the human and mouse was used to design PIT1-β primers. All primers were designed to cross an intron for controlling

DNA contamination in the reverse transcriptase (RT) procedure.

*Plasmid construction and quantitative RT-PCR of targeted genes.* Eight engineered probes, including both native and competitor for pig PIT1-α, PIT1-β, GH, and PRL, were cloned from RT-PCR amplified fragments using Meishan pig pituitary mRNA. The cloned probes were sequenced and verified by comparison with the corresponding region of each gene. The PIT1-α, PIT1-β, GH and PRL mRNA transcripts in individual samples were quantified using a modified quantitative, competitive RT-PCR procedure (QC-RT-PCR; [5]).

*Quantification of plasma GH and PRL.* Blood was drawn from piglets at 1, 15, and 30 days of age and the plasma was stored at -20°C until being analyzed by using radioimmune assays (RIA). Both GH and PRL in blood plasma, in duplicate, are measured in a double antibody homologous RIA as described (1,2,4). For GH assay, USDA pGH-B1 was used at the reference preparation (5.4 IU/mg) and the assay sensitivity was 0.1 ng/tube; assay variances range from 6-15%. For PRL assay, USDA - pPRL-I1 (103.4IU/mg) was used as the reference preparation and the assay sensitivity was 0.3 ng/ml; assay variance range from 5–8%.

*Statistical analysis.* Both pituitary mRNA and plasma hormones were analyzed with the general linear model using the SAS program. The statistical model is as follows:

$Y_{ijkl} = u + DAY_i + LITTER_j + SEX_k + GENO_l + SEX_k * GENO_l + e_{ijkl}$

where

$Y_{ijkl}$ = trait measured on each of the ijklm$^{th}$ animal
u = population mean of the measurements
$DAY_i$ = fixed effect due to the i$^{th}$ day of measurement
$LITTER_j$ = fixed effect associated with the j$^{th}$ litter
$SEX_k$ = fixed effect due to the k$^{th}$ sex
$GENO_l$ = fixed effect associated with the l$^{th}$ *PIT1* genotype
$SEX_k * GENO_l$ = fixed effect due to interaction between the k$^{th}$ sex and l$^{th}$ *PIT1* genotype
$e_{ijkl}$ = random error effect of the ijklm$^{th}$ animal with expectation mean zero and variance.

## Results and Discussion

In this study, we have measured the level of circulating GH and PRL as well as the amount of pituitary mRNA for GH, PRL, PIT1-α, and PIT1-β in pig families segregating *PIT1* C/D genotypes. Pituitary mRNA concentrations were quantified by using the standard curve QC-RT-PCR method (5). A summarized result from the LS analysis is given in Table 1 which shows the LS mean and standard error of each measured trait under the currently applied model.

PIT1-α mRNA decreased dramatically from day 1 to day 30 ($P<0.01$). Although it is not significant, circulating GH concentration also decreased from day 1 to day 30 and this tendency is correlated with GH mRNA measurement as well. The decrease in both mRNA concentration and circulating GH agrees with a previous study (3), and is highly correlated with the mRNA expression pattern of both PIT1-α and PIT1-β. In addition, the pattern of pituitary GH and PIT1-β mRNA levels are highly similar, indicating a direct influence of PIT1-β mRNA on GH gene expression.

Significant sex by genotype interactions were obtained for both GH and PRL measurements ($P<0.05$). Genotype effect is significant on GH level ($P<0.1$) and both litter and day effects were found significantly associated with PRL plasma level. The genotype effect for GH approached significance ($0.1>P>0.05$), and an effect of genotype on PIT1-β mRNA and on PRL mRNA also was ($P = 0.2$). GH mRNA and plasma hormone levels both showed significant but different associations with the *PIT1* genotypes (Table 1). The CD animals expressed the highest amount of GH mRNA but had the lowest amount of GH in the plasma. This might suggest a post-transcriptional control of GH gene expression. Furthermore, the CC animals have lower plasma GH amount than the DD animals. In a different population, however, CC animals were found heavier from the birth to weaning (7). This observation implies a complicated regulation of GH in regulating early growth of pig.

Even though not indicated in Table 1, a genotype effect on PRL was obtained ($P= 0.21$) from analysis that suggests a possible association of *PIT1* genotype with PRL levels. The sex differences on PRL measurement were only observed on mRNA levels but not in the plasma hormone level. This study reports the first quantitative measurement of PRL mRNA and plasma hormone level in neonatal Meishan pigs, and the results suggest a possible association of PIT1 on PRL that should be further investigated.

This study investigated GH and PRL at both mRNA and circulating hormone levels in the MS pigs segregating the *PIT1* C/D polymorphism. Even though the sample size was limited, we observed a significant genotype effect on both GH mRNA and plasma levels (Table 1), which suggests an association of *PIT1* genotypes with GH gene expression. Our results also indicate the QC-RT-PCR is a sensitive and very reliable method for quantifying mRNA concentration in any tissue, especially for less abundant mRNA.

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**Table 1. The LS mean and standard error for each mRNA measurement.**

| | Genotypes | | | Sex | |
|---|---|---|---|---|---|
| | CC | CD | DD | F | M |
| | | Day | | | |
| | 1 | 15 | 30 | | |
| **mRNA (attomol/μg mRNA)*** | | | | | |
| **PIT1-α** | 1428±438 | 1682±235 | 1222±304 | 1443±290 | 1444±199 |
| | 2207±276[a] | 1261±283[a] | 863±285[a] | | |
| **PIT1-β** | 55±29[c] | 105±14[c] | 74±19[c] | 72±19 | 83±12 |
| | 92±18 | 74±18 | 67±17 | | |
| **GH** | 1.54±0.58[b] | 2.77±0.3[b] | 1.91±0.4[b] | 2.03±0.4 | 2.11±0.3 |
| | 2.59±0.4 | 2.0±0.4 | 1.63±0.4 | | |
| **PRL** | 1.23±13.5 | 18.5±6.7 | 30.6±8.8 | 8.13±9.0[c] | 25.4±5.8[c] |
| | 17.7±8.5 | 23.3±8.4 | 9.3±8.2 | | |
| **Plasma hormones (ng/ml)** | | | | | |
| **GH** | 8.52±3.8[b] | 6.74±1.7[b] | 13.3±2.05[b] | 11.2±2.5 | 7.73±1.7 |
| | 11.0±1.9 | 9.48±2.16 | 8.02±3.1 | | |
| **PRL** | 5.28±0.3 | 5.25±0.14 | 5.66±0.21 | 5.3±0.21 | 5.5±0.14 |
| | 5.76±0.16[a] | 5.43±0.18[a] | 5.0±0.25[a] | | |

Data obtained from statistical analysis under applied model (see Materials and Methods for details).
[a] P<0.05; [b] 0.1>P>0.05; [c] 0.1< P <0.2.
*GH and PRL mRNA are given in pmol/μg mRNA.