

# Effect of the polymorphism in 5' UTR region of pig prolactin gene on prolactin gene expression and reproduction performance in the female pig

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## Abstract

**OBJECTIVES:** The recent genetics and molecular biology progress seems to be a fascinating challenge for the interdisciplinary studies on the effects of genetic changes in gene structure that causes the modification of physiological functions of many important proteins including hormones. Pig prolactin is one of the interesting hormones for this study.

**AIM OF THE STUDY:** The aim of the study was to analyze the mutation in 5'UTR region of the pig prolactin (*PRL*) gene and to evaluate the effect of this polymorphism on changes in plasma prolactin concentration.

**RESULTS:** It was found that only two individual groups of animals differed by the genotype in examined *PRL* gene locus – homozygote C/C and heterozygote C/T. PRL plasma concentration was 38.4 ng/ml (for C/T animals) or 42.7 ng/ml (for C/C animals). Animals with C/C genotyped exhibited a tendency to elevate PRL concentration as compared to the C/T group ( $p < 0.07$ ).

**CONCLUSIONS:** This research combines the genetic, molecular and, *in vivo*, physiological study which allows focus on the possible relationship between the gene polymorphism and physiological status of animal.

## INTRODUCTION

Prolactin (PRL) is a protein hormone primarily synthesized and secreted by the anterior pituitary in response to many factors and also steroids such as estrogens. PRL is involved in many different endocrine activities and there are more than 300 separate actions of PRL reported in various verte-

brate species. A large percentage of these actions are in some way associated with reproduction either directly or indirectly (van Rens and van Lende 2002). Several studies showed that PRL not only considerably affects the ovaries and uterus but also plays an important role in growth and development of the fetus and is essential for reproductive performance of animals.

The role of PRL in the synthesis of milk proteins in mammals has been well characterized. After binding with its specific receptor, prolactin induces the transcription of genes such as  $\beta$ -casein and  $\beta$ -lacto albumin (Freeman et al. 2000). Moreover, PRL plays an important role in the maintenance of pregnancy in pigs by acting on *corpora lutea* cells and possibly initiates production of progesterone. During the estrous cycle in female pigs there are two fairly distinct peaks in plasma prolactin; one occurs four days before estrus and the other during the sexual receptivity phase (De Rensis 1999). In sows, extremely high concentrations of PRL have been detected on the day before farrowing (Dusza & Krzymowski 1981, van Landeghem et al. 1978). This increase is an essential prerequisite to normal lactation. After parturition, plasma PRL gradually decreases to the concentrations during lactation, which are still relatively elevated.

Based on its genetic, structural, binding and functional properties, PRL is estimated to belong to the prolactin/growth hormone/placental lactogen hormones family. In the human genome, a single gene which encodes *PRL* was found on chromosome 6. It is 10 kb in size and is composed of 5 exons and 4 introns. Transcription of the *PRL* gene is regulated by two independent promoter regions. The human prolactin cDNA is 914 nucleotides long and contains a 681-nucleotide open reading frame encoding the prolactin prohormone of 227 amino acids. In rats and mice, the pituitary prolactin consists of 197 amino acids with a molecular weight of ~ 23 kDa (Freeman et al. 2000). The sequence homology can vary from the striking 97% among primates to as low as 56% between primates and rodents. Comparative studies on human and pig genomes showed that there is conserved synteny between human chromosome 6 and pig chromosomes 1 and 7, but some gene locations are not well established. Swine *PRL* gene has been tentatively mapped to pig chromosome 7 using Southern RFLP analysis with a limited number of meioses (Vincent et al. 1998). The structure of prolactin gene is shown on Fig. 1.

The aim of our study was to analyze the mutation in 5'UTR region of the pig *PRL* gene and evaluate the possible effect of this polymorphism on changes of blood prolactin concentration in sows.

## MATERIALS AND METHODS

### Blood samples for molecular analysis

The blood samples were collected from 100 sows belonging to the commercial line 990. The animals were maintained at a test station at the National Research Institute of Animal Production in Pawlowice. All test animals were fed the same commercial-based ration during the feeding period and raised in the same common environment and were adapted to experiments by the frequent presence of veterinarians and frequent imitations of injections to avoid stress as much as possible. Blood samples of all sows were drawn into 10 ml tubes containing potassium ethylenediaminetetraacetic acid (EDTA) as an anticoagulant and stored at  $-78^{\circ}\text{C}$  until DNA isolation.

### Record and analysis of reproductive performance

Several reproductive performance traits were recorded for all parities (from 1–6) for investigated sows. Litter traits included: total number of born piglets (TNB), number of piglets born alive (NBA), number of piglets at 21 days of age (NP21), number of weaned piglets (NWP), litter weight at 21 days of age (LW21), litter weight at weaning (LWW) and farrowing interval (FI).

### Polymorphisms in 5' UTR of the *PRL* gene

Using the Primer3 software available from the website (<http://www.genome.wi.mit.edu/cgi-bin/primer/primer3>) all pairs of primers shown in the Table 1 were designed on the basis of the bovine prolactin promoter gene's sequence (GeneBank X01452). Three different polymerase chain reactions using three different primers par were carried out. Three overlapping fragments (A, B and C) covered about 400 nucleotides of the promoter of the *PRL* gene and 87 nucleotides of exon 1 were obtained (Fig.2). In these three fragments the multi-temperature SSCP analysis was performed, two of them were sequenced and deposited in the GenBank (Korwin-Kossakowska et al. 2003, 2005) Only fragment C of size 162 bp appeared to show a different MSSCP (*Multi Temperature Single-Strand Conformation Polymorphism*) pattern that means it is mutation located in this region. The samples of differing MSSCP conformers were sequenced and the restriction analysis of

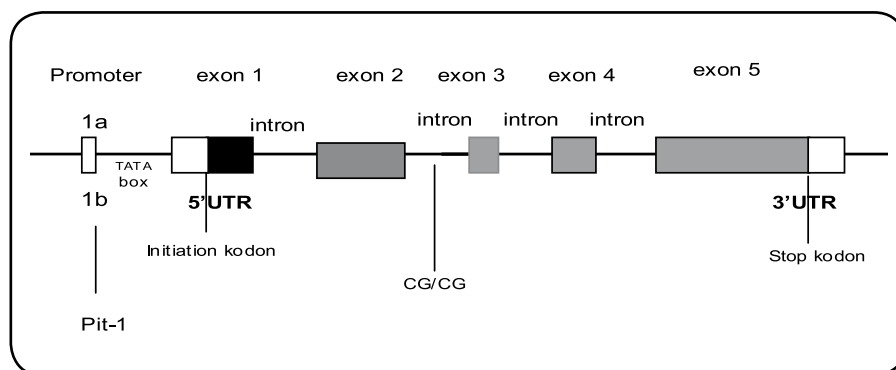


Fig. 1: Structure of the pig *PRL* gene

the DNA was performed using the AnnHyb software (<http://www.bioinformatics.org/annhyb>).

The C449T transition was identified in the 5'UTR (Untranslated Region) region of the gene. The restriction analysis allowed the choice of *HphI* restriction enzyme to recognize the novel SNP (*Single nucleotide polymorphism*) (Korwin-Kossakowska et al. 2006). The PRL/C499T polymorphism in all hundred animals was analyzed by the PCR-RFLP method. As the results of the digestion by *HphI* enzyme in the studied population of hundred sows, two different alleles was obtained: allele C characterized by the fragments -111 base pairs, 32 base pairs and 19 base pairs length also allele T -111 base pairs, 16 base pairs, 16 base pairs and 19 base pairs fragments length.

#### Assays of blood plasma concentrations of PRL

Twenty-two sows were chosen from the group of hundred animals for the plasma concentration analysis. There were ten days after parturition and characterized by different PRL/C499T genotypes.

Secretion of the hormone was induced by TRH S.C. injection (*Thyrotropin-releasing hormone*). The dose of 0.20 µg TRH for one kilo of body mass was applied. For this analysis, sows in the same lactation stage (10

**Table 1.** Primer sequences and amplified fragments of the porcine PRL gene

	Symbol of fragments	Length of the PCR fragments (bp)	Access Number
1	A	238	AY341908
2	B	187	-
3	C	162	AY905690

days after farrowing) had blood samples taken from a vein into 5 ml tubes three times: before injection of the TRH dose ("time 1"), 30 min. ("time 2") and 60 min. ("time 3") after TRH injection in the same concentration and content. The level of PRL in blood plasma was examined by RIA method, and the results represent 66 observations (22 sows, the three different experimental points during three times).

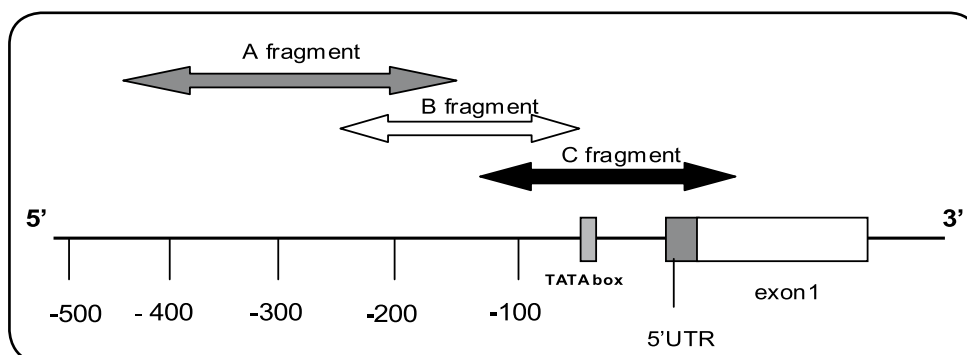
#### Statistical analysis

The relationships between PRL/C499T genotype and data of the PRL hormone level in blood of experimental sows were evaluated. In the second stage, the relationships between PRL/C499T genotype and data of the reproduction of individual sows were also evaluated.

**Table 2.** Reproductive performance traits of the sows bearing the PRL/C499T genotype. Results are expressed in last squares means (LSM) and standard errors (SE).

Analyzed traits	Number of observations	PRL Genotype			
		C/C		C/T	
		LSM	SE	LSM	SE
TNB(n)	107	9,80	0,47	9,89	0,32
NBA(n)		8,83	0,45	9,27	0,30
NP21(n)		8,49	0,48	8,91	0,32
NWP(n)		8,57	0,50	8,65	0,34
LW21(kg)		45,58	2,35	47,02	1,58
LWW(kg)		60,26	3,61	58,76	2,42
FI(days)		156,52	2,91	153,74	1,92

TNB- total number born piglets, NBA - number of piglets born alive, NP21- number of piglets at 21 days of age, NWP- number of weaned piglets, LW21 - litter weight at 21 days of age (kg) LWW - litter weight at weaning (kg) and FI- farrowing interval .



**Fig.2:** The overlapping fragments A, B, C covered about 400 nucleotides of the PRL promoter region and part of the exon 1.

**Table 3.** Prolactin blood levels in the sows bearing the *PRL/C499T* genotype after the TRH stimulation (in ng/ml) in the 3 groups after the TRH injection (0.20 µg/kg of body weight). Results are expressed in last squares means (LSM) and standard errors (SE).

Time*	<i>PRL</i> Genotype			
	C/C		C/T	
	LSM	SE	LSM	SE
Time 1 (ng/ml)	24,05	6,52	27,84	4,93
Time 2 (ng/ml)	51,42	6,50	49,35	4,88
Time 3 (ng/ml)	52,61	6,49	37,88	4,91
Average from all three times	42,69	3,76	38,36	2,84

\*time after the injection of the TRH dose, "time 1" - before injection of the TRH (control); "time 2"-30 min. after TRH injection "time 3"-60 min after TRH injection

Data were analyzed using the GLM procedure within the SAS software according to two different statistical models. In the first model the significance of relations were determined between individual *PRL/C499T* genotypes (C/C and C/T) and plasma prolactin concentration measured in three different times ("time 1", "time 2", "time 3"). The model for plasma prolactin concentration included the fixed effect of *PRL* genotypes of a sow, time of blood sampling and interaction between *PRL* genotype and time of taking the samples. In the second model, the significance of relations was determined between individual *PRL* genotypes of sow and values recorded for traits characterizing the reproductive performance of sows. The model for TNB, NBA, NP21, NWP, LW21, LWW and FI included the fixed effect of *PRL* genotype of the tested sows and number of parity.

## RESULTS AND DISCUSSION

So far, there is a little knowledge about pig *PRL* gene structure (Fig. 1) as well as expression of this gene in different areas of the reproductive tract. One of the most interesting areas in the *PRL* gene is the 5' UTR. The 5'UTR fragment may contain sequences that regulate translation efficiency (for example, that promote the initiation of translation) or mRNA stability and may play an important regulatory role in the posttranscriptional *PRL* processes.

### Polymorphism in 5' UTR of the *PRL* gene

The *PRL/C499T* polymorphism found by Korwin-Kossakowska et al (2006) was analyzed in the population of hundred sows. Only two groups of animals which differed by the genotype in *PRL* locus – homozygote C/C and heterozygote C/T were distinguished within this population.

### The relationship between gene polymorphism and reproduction performance

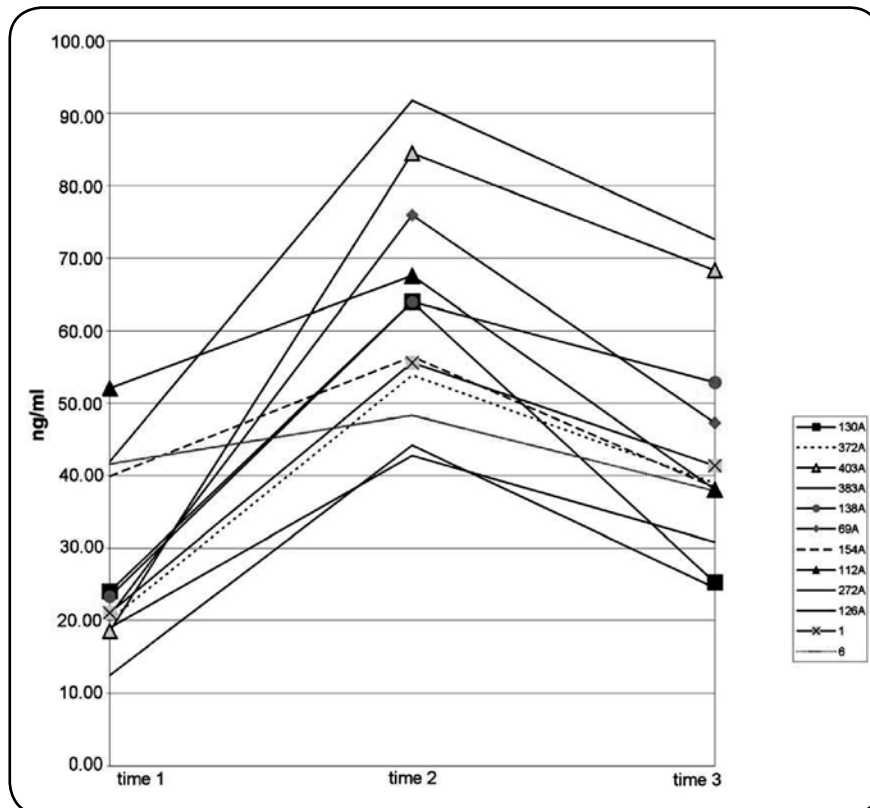
A total number of 107 litters were obtained from twenty two sows chosen for the prolactin plasma concentration analysis. Seven reproductive traits were noted: four connected with the litter size, two characterized litter weight and interval between consecutive farrowing. There were no statistical significant effects of the *PRL/C499T* genotype on the examined reproductive traits. Until now only Korwin-Kossakowska et al. (2003) found a relationship of this gene with reproduction traits. The two polymorphic sites in the *PRL* gene reported by Vincent et al. 1998 (both recognized as RFLP-*Bst*UI) and then identified in research performed by Korwin-Kossakowska et al (2003), were located in intron 2. In the cited paper six possible genotypes were observed in all tested sows, nevertheless the numbers of animals in some of these groups were very low.

Also Babicz et al. (2008) while searching for a putative molecular marker for reproductive performances in the Polish Pulawska breed found a novel mutation of the InDel type identified in exon 5 encoding the 3'UTR *PRL* region in which the 11-bp deletion/insertion at position 212-222 was found. The results presented by Babicz et al. (2008) may also serve as a starting point for further studies concerning the effect of the *PRL* gene on reproduction performance of pigs.

We attempted to correlate the identified gene polymorphism with the prolactin synthesis and storage rate by measuring plasma PRL after the TRH stimulation of its release by RIA method.

### Blood plasma concentrations of prolactin

It is now established that in sows mean prolactin concentrations in the blood plasma ranged from 4.4 to 13.0 ng/ml during pregnancy up to the 2<sup>nd</sup> day pre partum. PRL concentration increased to 20.3 ng/ml two days before and then to 103.4 ng/ml one day before the start of farrowing. During farrowing, peak values ranged from 124.2 to 147.3 ng/ml (Dusza & Krzymowska 1981; Van Landeghem 1978). Although plasma prolactin after parturition was shown to gradually decrease, it still remained at a relatively elevated level (Dusza & Krzymowska 1981; Van Landeghem et al.1978) which was directly associated with the lactation process (Bever 1978; Stevenson et al. 1981). Indeed, in the early post partum period active secretion of PRL was observed with the resulting hormone blood concentration ranging widely between 12,0 and 89,6 ng/ml (De Rensis et al. 1991). Since high individual variability in plasma prolactin concentration which was observed in their study was not dependent on either seasonal changes or differences in litter size, it was suggested to be due to genetic differences between animals. Similarly, Bever et al. (1978) did not observe a correlation between the plasma prolactin concentration and the number of piglets nursing.



**Fig.3:** Prolactin hormone concentration (ng/ml) in the blood plasma in 12 from 22 of the investigated sows at three different times 1, 2 and 3.

"time 1" - before injection of the TRH

"time 2" -30 min. after TRH injection

"time 3" - 60 min after TRH injection

During mid-lactation, plasma prolactin concentrations remain high (Bevers et al 1978, Van Landeghem et al 1978, Dusza & Krzymowski 1981) and then starts to decline between day 20 and day 30 post-partum (Stevenson et al. 1981).

In the present study, plasma PRL concentration in sows with genotype C/C and genotype C/T was estimated. The mean PRL plasma concentration in animals ten days after parturition was 38.4 ng/ml in C/T animals and 42.7 ng/ml in C/C animals (Tab.3). Among all 22 investigated sows the range of prolactin concentration within "time 1" was between 12.3 to 52.1 ng/ml, within "time 2" between 18.5 to 91.8 ng/ml and within "time 3" the obtained values varied from 16.8 to 81.9 ng/ml.

#### The relationship between different genotype groups and PRL blood level

There were no significant differences in blood prolactin concentration between the two investigated genotype groups of sows (Table 3; Fig. 3). However, it should be mentioned that the increase in prolactin blood level observed between C/C and C/T animals in "time 3" (60 minutes after injection of the TRH ) was close to the significant value ( $p < 0.07$ ).

## CONCLUSIONS

Despite a lack of clear relationship between *PRL/C499T* polymorphism in 5' UTR of the *PRL* gene and plasma prolactin concentration, gene polymorphism's impact on the reproductive performance cannot be excluded. Such potential influence may occur via modulation of *PRL* gene transcript stability which consequently may affect the reproductive process. Further investigations of *PRL/C499T* polymorphism in 5' UTR based on a large number of animals will certainly reach a decisive conclusion in this respect. In the future, we will focus on the polymorphism(s) in coding regions of *PRL* gene as well as on their possible influence on the prolactin mRNA level in the anterior pituitary and other tissues.

## ACKNOWLEDGEMENT

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