

# INVESTIGATION OF POLYMORPHISMS AT GENES IN THE GROWTH HORMONE AXIS IN CATTLE AND PIGS

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## SUMMARY

Preliminary results from an ongoing investigation of restriction fragment length polymorphism (RFLP) at genes in the growth hormone axis are presented. Twelve heifers of Red Danish Cattle from two lines selected for high and low fat yield were screened for restriction fragment length polymorphisms around the growth hormone gene and the insulin-like growth factor-I gene. A polymorphism at the growth hormone gene consistent with an insertion/deletion of 0.9 Kb was found in the two lines. The frequency of each of the alleles was not different between lines. No RFLPs were found for the insulin-like growth factor-I gene. Screening of pigs from Landrace, Yorkshire, Duroc and Hampshire identified a RFLP consistent with a point mutation at the growth hormone gene with the DraI enzyme. Investigation of 8-19 unrelated pigs from each of the four breeds revealed significant differences in the frequencies of the two alleles between breeds.

## INTRODUCTION

The results of several livestock experiments indicate that hormones in the growth hormone axis play a key-role in the metabolic regulation and in the distribution of nutrients during lactation (Baumann et al., 1985). In mice a variant growth hormone gene haplotype was fixed within lines selected for growth rate (Salmon et al., 1988). It is therefore reasonable to presume, that genetic variation in production traits like milk yield and growth is partly caused by variation in the genetic constitution of the genes coding for hormones and receptors in the growth hormone axis.

Lines of Red Danish Cattle selected for high and low butterfat production (Christensen et al., 1985) and pigs from four breeds (Landrace, Yorkshire, Duroc and Hampshire) are presently examined for RFLP at genes in the growth hormone axis in our laboratory.

## MATERIALS AND METHODS

**Cattle:** In a selection experiment (Christensen et al., 1985) with Red Danish Cattle two lines have been established with a genetic difference for butterfat production of about 20%. Twentysix heifers from these lines have been subjected to physiological challenge tests (Løvendahl et al., 1988), and among these, seven animals from the high line and five from the low line with least possible relationship have been chosen for this investigation.

**Pigs:** Samples of blood from pigs at the Danish Slaughteries' test station Boegildgaard were obtained from four breeds, 19 Landrace, 17 Yorkshire, 16 Duroc and 8 Hampshire sows were selected for RFLP analysis. No grandparents were common to any of the animals. Three animals from each of the breeds were chosen for screening.

Probes: The probes used for hybridization are shown in Table 1. The cattle has been examined for RFLPs in the growth hormone gene and the insulin-like growth factor-I gene. The pigs have been examined for RFLPs in the growth hormone gene only.

**Table 1.** Probes used in the experiment

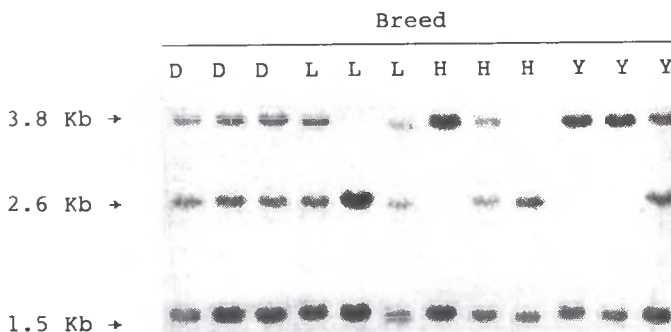
<u>Gene</u>	<u>Sequence</u>	<u>Designation</u>	<u>Reference</u>
Bovine growth hormone	cDNA	bGH	Miller (1980)
Porcine growth hormone	Genomic DNA	pGH	Vize & Wells (1987)
Porcine insulin-like growth factor-I	cDNA	pIGF-I	Tavakkol et al. (1988)

Laboratory methods: DNA was prepared from 10 ml whole blood according to a salt-precipitation procedure (Andersson, 1989). Seven µg DNA were digested with 35 units restriction enzyme and size separated by electrophoresis in 0.8% agarose gels. Southern transfer was made according to standard procedures. DNA probes were purified with "Gene-clean" and 200 ng labelled by random priming. Filters were hybridized ( $5 \times 10^5$  cpm/ml) over night at 60 °C. Filters were washed 2 x 15 min. at 60 °C in 0.7xSSC, 0.5% SDS with heterologous probes or 0.1xSSC, 0.5% SDS with homologous probes and exposed to Fuji HRG film at -80 °C 1-7 days.

#### RESULTS

In cattle a polymorphism was detected with BglII, EcoRV, PstI, PvuII, TaqI and XhoI. No RFLPs were observed with EcoRI and HindIII. The pIGF-I probe has been hybridized to the cattle DNA digested with BglII, EcoRI, EcoRV and HindIII without identification of any RFLPs. The frequencies of the deletion allele was  $p(D)=0.36$  in the high line and  $p(D)=0.1$  in the low line ( $P=0.065$ ).

The initial screening of three pigs per breed revealed no RFLPs using the pGH probe and the restriction enzymes BamHI, BglII, HindIII, PvuII and XbaI. Use of the DraI enzyme detected a RFLP for the porcine growth hormone gene (Fig. 1). The sequence of the pGH-gene contains a DraI site at position +216 and the polymorphic DraI site is situated about 2.6 Kb from this position.



**Fig. 1.** Southern blot of pig DNA digested with DraI and hybridized with pGH probe. L = Danish Landrace, Y = Yorkshire, D = Duroc, H = Hampshire. Fragment length has been estimated with a 1 Kb marker (Gibco).

The frequencies of the alleles with the extra DraI site are  $p(+\text{DraI})=0.74$ , 0.21, 0.44 and 0.56 in the breeds Landrace, Yorkshire, Duroc and Hampshire respectively (Table 2). The difference in the frequencies of the two alleles was significant between breeds.

**Table 2.** Number of animals, homozygotes (++) or (--) and heterozygotes (+-) and frequencies (%) for DraI polymorphic alleles at the growth hormone gene in four pig breeds.

	<u>Danish Landrace</u>	<u>Yorkshire</u>	<u>Duroc</u>	<u>Hampshire</u>
Number of animals	19	17	16	8
-Homozygotes (++)	10	1	2	2
-Heterozygotes (+-)	8	5	10	5
-Homozygotes (--)	1	11	4	1
p(+DraI) (2.6 Kb)	74	21	44	56
p(-DraI) (3.8 Kb)	26 <sup>a</sup> *)	79 <sup>b</sup>	56 <sup>c</sup>	44 <sup>c</sup>

\*) Frequencies with different superscripts are significantly different ( $p < 0.05$ )

#### DISCUSSION

Several authors have found RFLPs at the growth hormone gene in cattle (Beckmann et al., 1986; Hallerman et al., 1987; Hallerman et al., 1988; Cowan et al., 1989; Hilbert et al., 1989; Theilmann et al., 1989). Different polymorphisms have been reported. One is an insertion/deletion of approx. 0.9 Kb situated in the downstream direction of the coding region. Another is a point mutation changing a MspI restriction site within the coding region. The results from the two lines of Red Danish Cattle correspond to the insertion/deletion RFLP at the cattle growth hormone gene. The preliminary screening with the pIGF-I probe did not reveal any RFLPs, but certify the possibility of using non-species-specific probes for this gene. There are no other reports on RFLPs at the IGF-I gene in cattle.

The RFLP at the porcine growth hormone gene found with DraI enzyme in four breeds has not been reported earlier. The fragment lengths indicate that the polymorphic DraI site is situated outside the coding region. No other work reports RFLP analysis at the porcine growth hormone gene, but sequence analysis has been performed. Six different cDNA's coding for growth hormone have been cloned (Seeburg et al., 1983; Vize & Wells, 1987; O'Mahony et al., 1989) with eight different point mutations of which four causes aminoacid substitution in the leader peptide.

The possible biological significance of the polymorphisms will be further investigated. The results of the RFLP analysis in cattle will be related to results from physiological challenge tests and production figures. The results of the RFLP analysis in pigs will be related to plasma GH and IGF-I concentrations and to performance traits.

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