RESTRICTION FRAGMENT LENGTH POLYMORPHISM AT THE GROWTH HORMONE GENE IN SHEEP, GOATS AND CATTLE

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SUMMARY

Genomic DNA samples obtained from 53 rams and ewes of various breeds, from seven Saanen goats and seven Israeli Holstein dairy bulls were digested with BamHI, HindIII and EcoRI restriction enzymes and screened for restriction fragment length polymorphism (RFLP) at the growth hormone (GH) gene. Except for one DNA sample which showed a single hybridization band following digestion with BamHI or HindIII, all the sheep and goat DNA samples revealed two hybridization bands. On the other hand, only a single hybridization band was obtained following digestion of bovine DNA samples with BamHI or HindIII. Digestion of sheep or cattle genomic DNA samples with EcoRI revealed either one or two hybridization bands.
As there are no BamHI, HindII or EcoRI restriction sites within the ovine GH gene it is suggested that while at the bovine GH locus there is a single gene copy, two gene copies are present at the sheep and goat GH locus, and the RFLP at the ovine oGH locus is due to variation in copy number.

INTRODUCTION

Molecular variants giving rise to restriction fragment length polymorphism (RFLP) at the growth hormone (GH) gene or its flanking regions may cause variation in its activity, leading to alteration in growth or milk production in farm animals (Beckmann and Soller, 1983). While RFLP at the bovine GH gene has been described (Beckmann et al., 1986; Hallerman et al., 1987; Cowan et al., 1989; Theilmann et al., 1989), there is no or only limited information (Byrne et al., 1987) on RFLP at the GH gene in goats and sheep respectively. The aim of the present study was to investigate RFLP further at the GH gene in the Bovidae family, by examining DNA samples from several different breeds of sheep as well as from goats and cattle.

MATERIALS AND METHODS

Genomic DNA was isolated from semen or blood samples of Awassi (dairy breed), Assaf (dairy breed originated from East Friesian X Awassi cross), German Mutton Merino (mutton breed), Booroola- Merino (small body size wool breed) sheep; from blood samples of Saanen goats; and from frozen semen pellets of Israeli Holstein bulls.
DNA samples (10µg) were digested with EcoRI, BamHI or HindIII restriction enzymes. DNA fragments were separated by electrophoresis in agarose gel (0.8-1.2%) and transferred to Gene Screen plus membranes (NEN). Membranes were hybridized to bGH probe kindly provided by Rottman (Woychik et al., 1982).

RESULTS

The hybridization results with the GH probe of southern blots of EcoRI, BamHI or HindIII restriction digests of sheep, goat and cattle genomic DNA are presented in Table 1. Digestion with BamHI or HindIII restriction enzymes of genomic DNA samples obtained from 52 out of 53 sheep belonging to four breeds, and from seven Saanen goats, gave two hybridization bands. In one Awassi DNA sample, a single hybridization band was obtained following digestion with either BamHI or HindIII.
Digestion of sheep DNA samples with EcoRI gave one hybridization band of 12.2 kb in 49 of the 53 cases. Three samples showed two hybridization bands and in one DNA sample - the same one which gave the exceptional hybridizarion patterns following digestion with BamHI and HindIII - a single rare hybridization band of 6.7 kb was found.
In contrast to the situation in sheep and goats, digestion of bovine DNA with BamHI and HindIII revealed only one hybridization band. Following digestion with EcoRI, a single hybridization band was obtained in five of the seven bovine DNA samples tested, while two hybridization bands were found in the remaining two samples.

Table 1 Size (kb) and frequency of various growth hormone hybridization patterns obtained following digestion of sheep, goats and cattle genomic DNA with BamHI, EcoRI or HindIII.

Genotype	n	Restriction enzyme		
		BamHI	HindIII	EcoRI
Sheep		Fragment size (kb)		
Awassi	17	5.3+12.6	5.3+8.5	12.2
	1	5.3+12.6	5.3+8.5	6.7+12.2
	1	12.6	8.5	6.7
Assaf	18	5.3+12.6	5.3+8.5	12.2
	2	5.3+12.6	5.3+8.5	6.7+12.2
German Mutton Merino	10	5.3+12.6	5.3+8.5	12.2
Booroola Merino	4	5.3+12.6	5.3+8.5	12.2
Goat				
Saanen	7	5.3+12.6	5.3+8.5	N.D.
Cattle				
Israeli Holstein	5	12.6	23.0	4.3
	2	12.6	23.0	4.3+12.6

DISCUSSION

Digestion of bovine genomic DNA samples with BamHI or HindIII restriction enzymes revealed in the present study a single hybridization band using the GH as a probe. This finding is in agreement with results of other studies (Beckmann et al., 1986; Hallerman et al., 1987; Cowan et al., 1989; Theilmann et al., 1989) and supports the notion that the bGH gene is present as a single copy.
In contrast to the results with bovine DNA, two hybridization bands were obtained when genomic DNA samples obtained from various breeds of sheep and from goats were digested with BamHI or HindIII. High homology has been found between the ovine and the bovine GH genes (Byrne et al., 1987; Orian et al., 1988), and in neither gene was restriction sites of BamHI or HindIII present within the structural genes. Thus, the hypothesis is proposed that in sheep and in goats the GH gene is present in two copies.

RFLP at the bovine GH was observed in this study following digestion with EcoRI. Similar RFLP at the bGH locus was found also in other studies (Beckmann et al., 1986; Hallerman et al., 1987; Cowan et al., 1989; Theilmann et al., 1989) and was explained by the presence of insertion/deletion at the flanking region of the gene at the 3' region.
Using EcoRI, RFLP was observed also in this study at the ovine GH. In one DNA sample from Awassi sheep, a single hybridization band was obtained following digestion with BamHI, HindIII or EcoRI, indicating that in this sample only one GH copy is present. Thus, it is suggested that in sheep, RFLP at the GH locus can be due to variation in gene copy number.

Our finding of two GH copies in sheep and goat as opposed to one copy only in cattle, suggess that divergence of species within the Bovidae family involved alterations in the GH locus. Elucidation of the possible selective advantage of carrying one GH copy in cattle and two GH copies in sheep and goats may provide additional understanding of the involvement of the GH gene in control of growth and milk production in those related species.

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