Short Communication

DNA polymorphisms in the bovine thyroglobulin gene

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DNA polymorphisms that can be detected by restriction endonucleases are valuable in population and linkage studies. In this manuscript we have reported on the occurrence of DNA polymorphisms in a defined region of the bovine thyroglobulin gene. We have shown that an intron 9 PstI site and an exon 11 TaqI site are highly polymorphic, and occur only as the (+ +) or (– –) haplotypes at a frequency of 0.53 and 0.47 respectively in Afrikander cattle. The same sites have also been found to be polymorphic in the Belgian White-and-Blue breed. An additional TaqI polymorphism in the Belgian White-and-Blue breed is also described. The role of selective pressure on the polymorphic sites is discussed.

DNA-polimorfismes wat deur restriksie-endonukleases herken word is waardevol in bevolking- en koppelingstudies. In hierdie artikel word die voorkoms van DNA-polimorfismes in 'n spesifieke area van die bees-tiroglobuliengeen gerapporteer. 'n Intron-9-PstI- en 'n exon-11-TaqI-herkenningsvolgorde is polimorfies en kom slegs voor as die (+ +)- en (– –)-haplotipes met 'n frekwensie van 0.53 en 0.47 respektiewelik, in Afrikanerbeeste. Daar word verder aangetoon dat diesefde posisies ook in die Belgiese Wit-en-Blouras polimorfies is. 'n Addisionele polimorfiese TaqI-herkenningsvolgorde is ook in die Belgiese Wit-en-Blouras aangetoon. Die rol van selektiewe druk op hierdie polimorfismes word bespreek.

Keywords: Afrikander cattle, DNA, polymorphisms, thyroglobulin.

The establishment of linkage between specific genetic loci and hereditary traits or diseases is a powerful technique to plan breeding programmes to select for or against the linked traits. In addition, linkage analysis may lead to the identification and characterization of specific genes and mutations responsible for certain identifiable hereditary characteristics. DNA restriction fragment length polymorphisms (RFLPs) are very useful markers to map genetic diseases or traits to specific chromosomal regions by linkage analysis (Botstein *et al.*, 1980). In order to be informative, the polymorphisms should occur at a relatively high frequency in the population being studied. During an investigation into the molecular basis of hereditary goitre in cattle, DNA polymorphisms that affect recognition sites for the restriction endo-nucleases PstI and TaqI, were encountered in the thyroglobulin gene in the vicinity of the disease mutation (Ricketts *et al.*, 1985a; 1985b; 1987). The bovine thyroglobulin gene has recently been localized to chromosome 14, which makes knowledge of RFLPs and their frequencies in this gene particularly useful for linkage analysis studies (Threadgill *et al.*, 1990).

The DNA from thirty-two Afrikander cattle (the progeny of seven bulls from four different breeders) was screened by Southern blot (Vandenplas *et al.*, 1984) to determine the frequency of the TaqI and PstI polymorphic loci. A representative Southern blot (Figure 1A) shows that TaqI-digested DNA has fragments of ~1 600, ~1 500 and ~900 base-pairs (bp) hybridizing with the pbTg1.1 probe. All individuals have the 1 500 bp fragment which represents part of intron 8 and most of exon 9 (*cf.* Figure 3). However, the presence or absence of a TaqI site in exon 11 results in a hybridizing fragment of either ~900 bp or ~1 600 bp. Individuals heterozygous for the exon 11 TaqI site, have both the ~900 and the ~1 600 bp fragments (Figure 1A, lane 3). Figure 1B shows the same DNA samples cut with PstI and hybridized with the E1-8E probe. The polymorphic PstI site is within intron 9, 25 bases from the junction with exon 9 (Ricketts *et al.*, 1987). Individuals heterozygous for the PstI site, have two hybridizing bands (826 bp and ~1 000 bp). Of the 64 Afrikander cattle thyroglobulin alleles examined, 34 (53%) were found to be positive for the presence of both the polymorphic PstI site and the polymorphic TaqI site in exon 11. All seven individuals which were homozygous for the large TaqI fragment, were also homozygous for the large PstI fragment. Similarly, nine individuals were homozygous for the presence of PstI and TaqI sites in intron 9 and exon 11 respectively, and the remaining 16 were found to be heterozygous at both the TaqI and the PstI sites. The observed frequency shows that the polymorphisms are in Hardy–Weinberg equilibrium, with 50% of the animals being heterozygous. This suggests that there is no selective pressure operating with respect to these thyroglobulin alleles. The high frequency of these DNA polymorphisms makes them useful for linkage analysis studies. The presence of polymorphic sites was therefore investigated in an independent breed of cattle.

The DNA from nine individuals of the White-and-Blue breed from Belgium was analysed for TaqI and PstI RFLPs in the same region of the thyroglobulin gene. The results are presented in Figure 2. Three of the nine animals were found to be heterozygous for both the exon 11 TaqI site and the PstI site (Figure 2, lanes 2, 4 and 6). However, a fragment of about 1 300 bp (instead of ~900 bp seen in Afrikander cattle) is present in the Belgian White-and-Blue breed. This indicates that an additional TaqI polymorphism is present in the Belgian White-and-Blue breed, possibly in intron 9. The PstI and TaqI polymorphic sites were found to be present in 17% of the DNA samples from the White-and-Blue breed analysed. This finding shows that this region of the bovine thyroglobulin gene is also polymorphic in breeds other than the Afrikander.

A restriction map which illustrates the polymorphic sites and DNA fragments in the Afrikander cattle is presented in Figure 3. In a previous wider search for RFLPs in the Belgian White-and-Blue breed, six polymorphic sites have been

identified, but their precise location is not known (Georges *et al.*, 1987). The polymorphic sites described herein were not detected in the earlier study.

In Afrikander cattle, all animals homozygous at the intron 9 PstI site were also homozygous for the exon 11 TaqI site. This strongly suggests that individual alleles are either positive (+ +) or negative (– –) for both these restriction sites. The absence of intermediate haplotypes [(+ –) or (– +)] could have been the result of some genetic bottleneck, such as the South African War (1899—1902) or rinderpest epidemic (1896).

Analysis of the specific polymorphic sites has shown that two sites are in introns but are not associated with splice junctions. The exon 11 TaqI site is in a region of the thyroglobulin gene where four possible point mutations in the published sequence (at nucleotides 2630, 2704, 2724 and 2790) could have resulted in a TaqI site (Mercken *et al.*, 1985), but none of these would have altered the encoded amino acid. There is therefore no indication that selective pressure is operating on any of the polymorphisms described in this manuscript, and it is expected that these sites will be polymorphic in many bovine populations. The demonstration of the same polymorphisms in the Belgian White-and-Blue breed supports this conclusion.

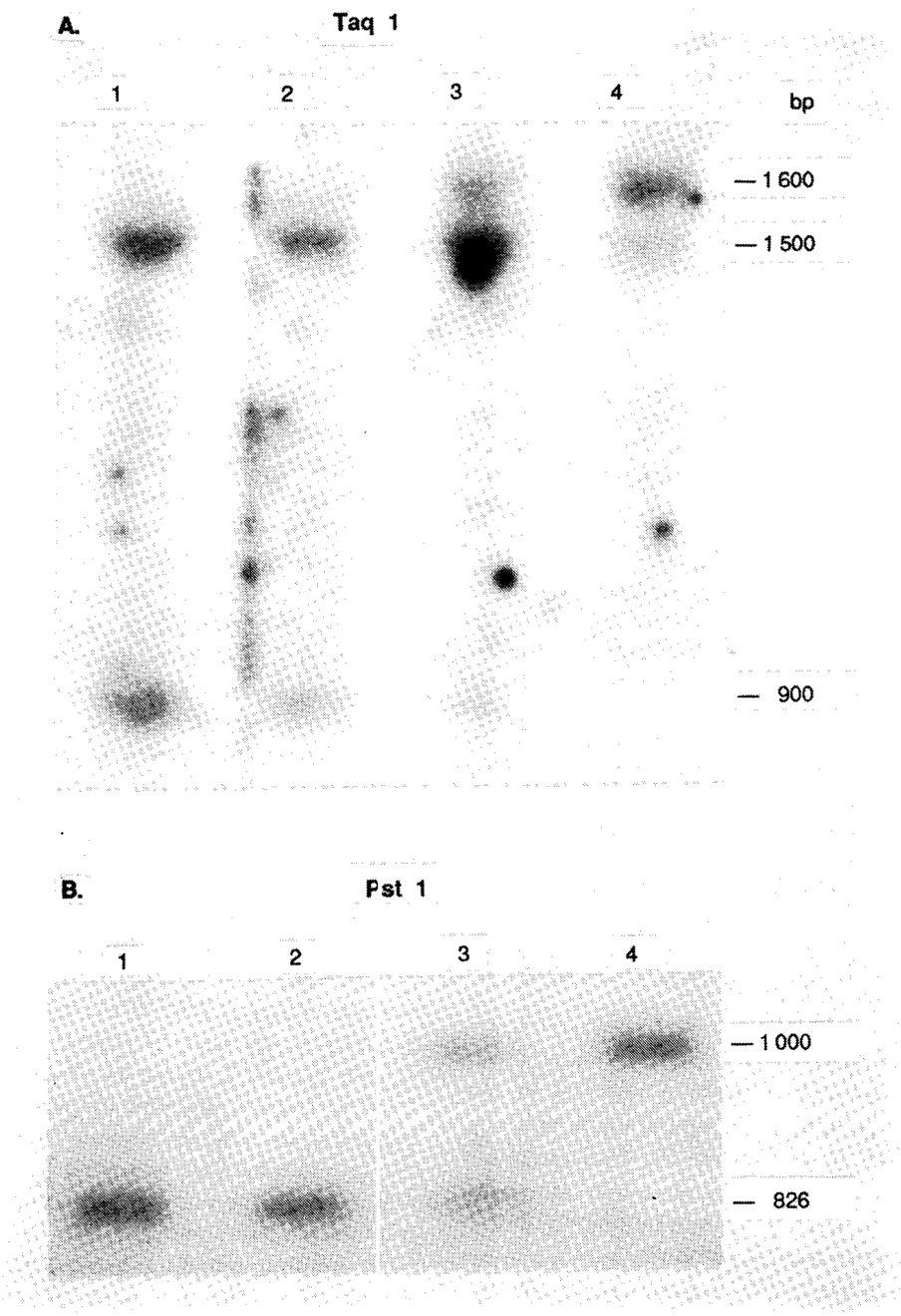

Figure 1 Southern blot analysis of four representative Afrikander cattle DNA samples. The DNA samples were cut with TaqI and probed with pbTg1.1 (A), or cut with PstI and probed with E148E (B). Lanes 1 and 2: DNA from animals homozygous for the presence of both the exon 11 TaqI site and the intron 9 PstI site; lane 3: DNA from an animal heterozygous for the exon 11 TaqI and intron 9 PstI sites; lane 4: DNA from an animal homozygous for the absence of the exon 11 TaqI and intron 9 PstI sites. The length of the DNA fragments (in base-pairs) is indicated on the right.

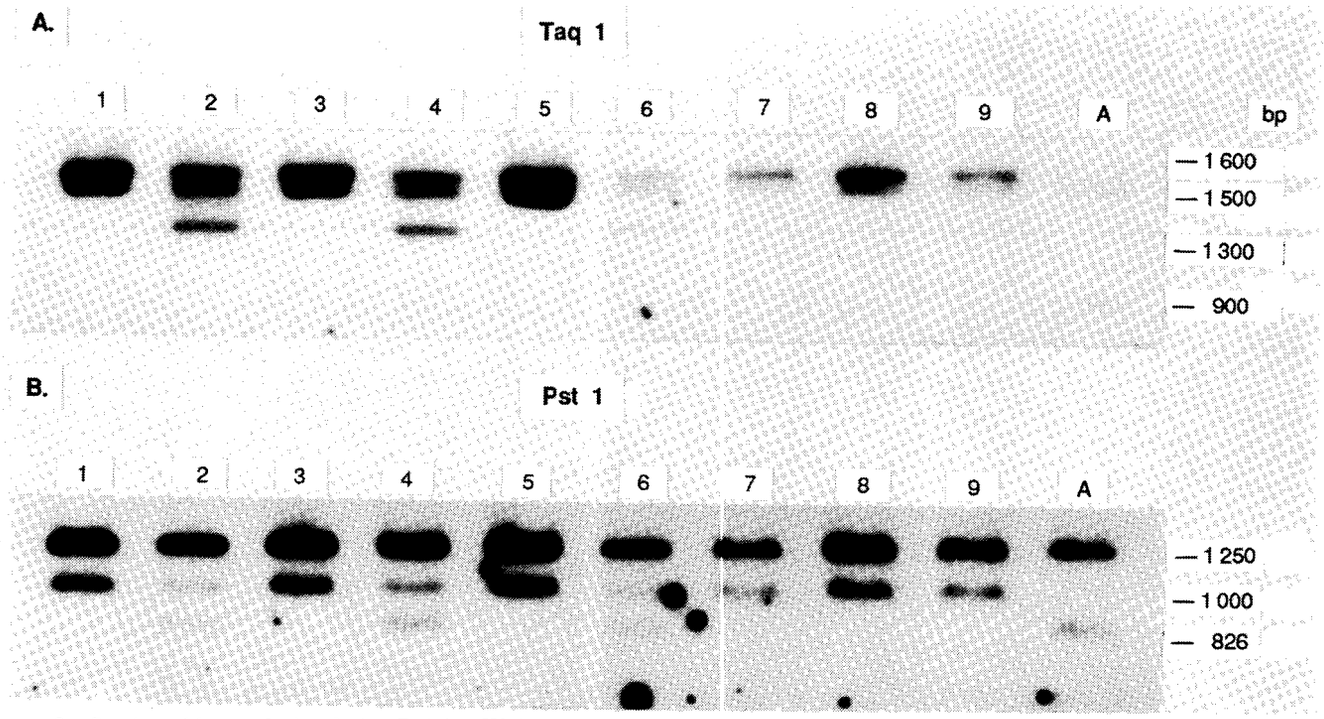

Figure 2 Southern blot analysis of nine Belgian White-and-Blue DNAs (lanes 1—9) and 1 Afrikander DNA (lane A). The DNA was cut with TaqI (A) or PstI (B). Both blots were probed with pbTg1.1. The length of the DNA fragments (in base-pairs) is indicated on the right.

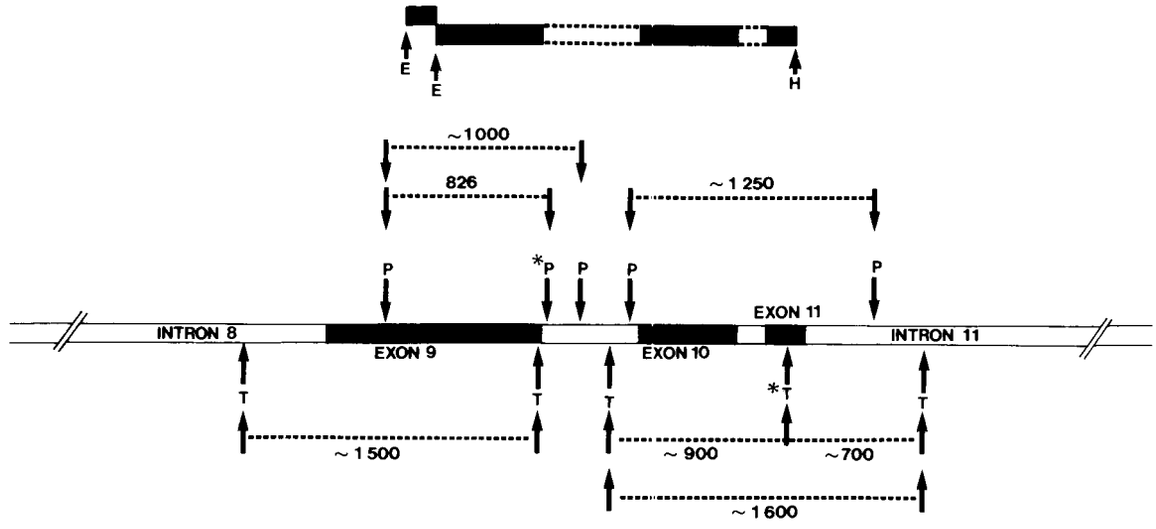

Figure 3 Restriction map of a section of the thyroglobulin gene from intron 8 to intron 11. The top bar represents the cDNA probes (E148E and pbTg1.1) in relation to the gene, which spans the centre of the figure. The fragments obtained by digesting with either TaqI ('T', below the gene) or PstI ('P', above the gene) are shown with their sizes in base-pairs. The polymorphic sites are indicated with an asterisk.

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