DETECTION OF A DELETION IN THE LAST INTRON OF BOVINE FACTOR XI

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SUMMARY

A sequence variation of approximately 20-base pairs has been detected near the middle of the last intron of bovine Factor XI. The variation is associated with the presence or absence of a TaqI restriction site and polymorphisms with several other restriction enzymes. It appears to be inherited in a codominant manner and was found in Holstein-Friesian, Limousin, and Hereford cattle in Australia.

INTRODUCTION

Factor XI is a serine protease that is a component of the intrinsic blood coagulation cascade. A deficiency of Factor XI, inherited as an autosomal recessive trait, leads to a bleeding disorder that can be lethal in cattle (Gentry and Black, 1980). Bovine Factor XI has been mapped to chromosome 17 (Zhang et al., 1992). The last three exons (13, 14 and 15) for bovine Factor XI were isolated from cDNA libraries and have been sequenced (DeLeeuw, 1993). The present study was designed to characterize the last intron of bovine Factor XI and led to the discovery of the deletion.

MATERIALS AND METHODS

Polymerase chain reaction was employed to amplify the last two exons and the intervening intron of bovine Factor XI from leukocyte or fibroblast DNA. The primers used were based on the nucleotide sequences at the 5′ end of exon 14 and untranslated sequence 3′ to the stop codon in exon 15. Stringent annealing conditions, predicted from the primer sequences, were used. The amplified segments were electrophoresed on 2% agarose, with ethidium bromide detection and on 6% acrylamide with silver stain detection.

RESULTS AND DISCUSSION

Upon electrophoresis on agarose, the amplified segment was approximately 920 bp in length and its authenticity was supported by BanII cleavage of a 170 bp fragment, as expected from the sequence for exon 15. Therefore, the product was considered to consist of 128 bp from exon 14, 188 bp from exon 15 and the untranslated 3′ end and the intron of about 600 bp. The analogous intron of human Factor XI has about 700 bp (Asakai et al., 1987).

This amplified segment exhibited polymorphisms with respect to a variety of restriction enzymes. TaqI was expected to cleave

approximately 70bp from the 3' end of the amplified product. However, with some animals Taq1 digestion resulted in the presence of 2 bands approximately 450 and 400bp in length, revealing a Taq 1 site near the middle of the intron. With product from other animals only one band, approximately 820-850bp was evident following Taq1 digestion. Three bands were evident following digestion of the product from others with the bands being approximately 400, 450 and 825-850bp in length. For several other enzymes, polymorphism were evident that involved presence or absence of doublets for one of the bands produced. With each enzyme the doublets, irrespective of length of the bands appeared to vary by approximately 20bp.

Acrylamide electrophoresis revealed that the PCR product from some animals was a doublet. Other subjects had either the longer or the shorter of the two bands comprising the doublet. TaqI cleaved only the longer of the bands comprising the doublet. XmnI, BstYI, AvaI, AvaII, and BanII sites were found in the intron of all animals. Polymorphisms, that consisted of 20bp variations, were evident with each of the enzymes. The polymorphisms were variations, consistent within enzymes, in the length of either the longer or the shorter band created by cleavage. These doublets were only evident if the uncut product was also a doublet.

The inheritance of the deletion was examined in offspring of parents of known genotype. Matings between subjects with the doublet (heterozygous) produced 2 offspring with only the shorter of the uncut doublet bands (homozygous short), 11 heterozygotes and 4 where the product was cleaved to 450 and 400bp pieces (homozygous cut). Matings between heterozygotes and homozygous cut individuals produced 3 heterozygotes and 7 homozygous cut. These results are consistent with a two-allele, single locus. Heterozygotes have been detected in Holstein-Friesian, Limousin and Hereford cattle, indicating that the deletion is widely distributed. The observations are consistent with either a deletion or an insertion near the center of intron 14 that includes a Taq1 restriction site. The existence of this deletion/insertion may be of value in defining the bovine genome, especially since it seems to be widely distributed.

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