SHORT COMMUNICATION

The polymorphism in MUC1 gene in Nelore cattle

F. R. P. de Souza¹, D. B. Dentillo¹, J. Meola¹, F. H. Biase¹, M. V. Andréa², P. A. Vozzi¹, R. B. Löbo¹, L. R. Martelli³

¹ Departamento de Genética, Faculdade de Medicina de Ribeirão Preto, Ribeirão Preto, SP, Brazil
² Departamento de Zootecnia, Centro de Ciências Agrárias e Ambientais, Universidade Federal da Bahia, Cruz das Almas, BA, Brazil

Introduction

MUC1 is a transmembrane glycoprotein mucin exclusively expressed on the apical surfaces of the uterine epithelial tissue with predicted functions in protection and cell–cell adhesion. These properties are closely related with the repetitive region [variable number of tandem repeats (VNTR)] of the extracellular domain and with the O-glycosylation in their serine and threonine residues. This study describes a polymerase chain reaction (PCR) protocol to analyze MUC1 bovine genetic polymorphism and demonstrates the existence of a VNTR within the sites for O-glycosylation. Oligonucleotide primers based on the Bos taurus mucin (MUC1) gene sequence GenBank AF399757 were used to amplify five VNTR MUC1 alleles from a study group of 56 pure Nelore bovines. The number of repeats varied between 10 and 24, being more prevalent than the alleles with less number of repeats. The DNA sequence analysis revealed two repeats and one of them presented 100% homology with the bovine consensus sequence already reported. The second repeat showed codons that translate to serine and proline amino acids, which are conserved in the MUC1 of several species. This study is the first description of allelic variation and the VNTR structure in the Nelore breed MUC1 gene, and we suggest that this genetic variability can be tested for association with variation in reproductive traits in Nelore cattle.
and serine residues serve as sites for the O-glycosyla-
tion responsible for both positive and negative ade-
hesive properties of the molecule. The proline residues
are associated with this role, giving the molecule an
extended form and raising it well above the surface
of the cell membrane (Patton et al. 1995; Gendler
2001; Dekker et al. 2002).

In the eight species studied, the PTS-conserved consensus amino acid sequence included one serine and
two proline residues highly conserved. The remain-
ing sequence presents high content of these residues
with a significant degree of conservation (Pallensen
et al. 2001; Rasero et al. 2002; Sacchi et al. 2004). The
lack of perfect conservation between species is caused
by the position of these residues varying within the
repeat, as the VNTR varies in length. In bovines and
caprines the repeats can show 80–100% of homology
with the derived PTS consensus repeat. In Italian Frie-
sian and Piedmontese breeds all repeats in the VNTR
share on average 93% DNA sequence homology with the consensus sequence. In mouse MUC1, this homol-
ogy between repeats is down to 75% on average
(Rasero et al. 2002; Sacchi et al. 2004).

DNA polymorphism in the VNTR of dairy cattle and caprines has been used for association studies
with improved selection schemes in mind. In taurus bovine subspecies, four alleles for the VNTR region
have been described with size ranging from 778 base-pairs (bp) to approximately 1600 bp (Rasero
et al. 2002; Sacchi et al. 2004).

The role of the MUC1 protein in the uterine envi-
ronment suggests it may be an informative marker
for variation in fertility traits. The aim of this study
was thus to develop a polymerase chain reaction
(PCR) protocol for analysing polymorphism of the MUC1 VNTR in the Brazilian Nelore breed for future
association studies of traits such as early puberty,
and the success rate of embryo implantation
obtained with in vitro fertilization techniques (IVF).

Materials and Methods

Sample collection

The study material comprised DNA derived from 56
pure Nelore breed fertile cows selected in the Pro-
gram for Genetic Improvement of the Nelore Breed
(PMGRN) at the FMRP – University of Sao Paulo,
Brazil. The sample was characterized by low values
of relationship coefficient and inbreeding. We found
a similar effective number of founders as reported by
Vozzi et al. (2006) on the breed indicating that the
sample was representative of the Brazilian Nelore
cattle.

DNA extraction

Molecular genetic analysis was performed on ge-
nomic DNA obtained by extraction from peripheral
blood lymphocytes. DNA was extracted by precipita-
tion in NaCl using standard techniques (Olerup &

MUC1 polymorphism analysis

The forward and reverse primer sequences (5’-CGC
AGA ACT ACG CCA GTT TCC-3′ and 5’-AGA GCG
GGT GGT CAT GGA TG-3′) were based on the bovine
sequence GenBank AF399757 and adapted from the
primer sequences which flank the repetitive VNTR
region of the bovine MUC1 published by Rasero et al.
(2002). About 100 ng of genomic DNA was mixed
with 15 pmol of each primer in a total volume of
25 μl containing 200 μM of each dNTP, 0.75 mM
MgSO4, 0.5 U of Platinum Pfx Taq DNA polymerase,
Platinum Pfx Amplification Buffer and PCR Enhancer
Solution (Invitrogen, Carlsbad, CA, USA), both in
the concentration of 1X. PCR was performed on a What-
man Biometra TGradiente Thermocycler at the fol-
lowing conditions: a denaturation step at 95°C for
5 min, 35 cycles of 95°C for 40 s, 58°C for 40 s, 68°C
for 1 min and 30 s, and a final extension step of 68°C
for 5 min. The amplified fragments were checked in a
1.5% agarose gel electrophoresis prepared with 0.5X
Tris–borate–ethylenediaminetetraacetic acid (EDTA)
buffer. The gel was photographed and allele lengths
were estimated by the software Kodak Digital Science
1D Image Analysis Software with the Marker 1 kb
Plus DNA Ladder molecular weight standard.

DNA sequencing and VNTR fragment analysis

Polymerase chain reaction purification derived from
two independent Nelore homozygotes (no. 28 and no.
41 in Figure 1b) for the same allele (1036 bp) was
performed with GFX PCR DNA and Gel Band Puri-
ication Kit (Amersham Biosciences, Pittsburgh, PA,
USA). For quantification, the samples were applied in
a 2% agarose gel with Low DNA Mass Ladder
(Invitrogen, Carlsbad, CA, USA). The sequencing was
performed in the MegaBACE™ 1000 Analysis System
(Amersham Biosciences) with DYEnamic ET Dye
Terminator Cycle Sequencing Kit and Thermo Sequenase™ II DNA Polymerase and the sequence
information was entered into GenBank as DQ644555
on 12 April 2006. The sequences were analysed by
the Sequence Analyzer software using the Base Caller
Cimarron 3.12 (Amersham Biosciences, Pittsburgh,
PA, USA). Both purification and sequencing reactions were performed by the DNA sequencing and PCR products purification services of Human Genome Research Center (CEGH).

Chromas software (http://www.technelysium.com.au) was used to compare the homology with other sequences in GenBank. The Tandem Repeat Finder Program (http://tandem.bu.edu/trf/trf.html) was used to identify and count the number of repetitions of the sequence (Benson 1999). The estimation of the number of repeats in other alleles was performed utilizing the following formula:

$$ETR = \frac{(NCR)(NR)}{L1036 \text{ bp}}$$

where ETR is the size (bp) of the non-repeat sequence, NCR the bp number in the consensus repeat, NR the number of repeats in the 1036 sequence and $L1036$ bp the bp length of the allele 1036.

$$NTR(X) = \frac{FNS(X) - ETR}{60}$$

where $X$ is the allele, NTR the number of repeats and FNS the allele length.

**Results**

Five alleles were identified in the VNTR MUC1 locus and they were initially named according to the size, based on estimates provided by the Image Analysis Software (Figure 2). The 1036-bp allele was the most prevalent, followed by the 1323- and 1175-bp alleles. The 1526- and 1894-bp alleles were the least frequent (Table 1).

$$ETR = \frac{(NCR)(NR)}{L1036 \text{ bp}}$$

where ETR is the size (bp) of the non-repeat sequence, NCR the bp number in the consensus repeat, NR the number of repeats in the 1036 sequence and $L1036$ bp the bp length of the allele 1036.

$$NTR(X) = \frac{FNS(X) - ETR}{60}$$

where $X$ is the allele, NTR the number of repeats and FNS the allele length.
sequence of the sample no. 41, comprised four consensus repeats, and six imperfect repeats. One imperfect repeat showed conserved codons for proline and serine residues. The number of repeats in the other alleles was estimated to be 12, 15, 18 and 24. With the exception of the alleles with 12 and 18 repeats, the other alleles were described in Bos taurus taurus by Rasero et al. (2002) (Figure 1c,d).

Discussion

Mucin genes are characterized by the unusual presence of intragenic repeats within the transcript. Most genes with variable number of tandem repeats (VNTR) in the coding region are surface proteins involved in cell–cell interactions. The quantitative alterations in the cell-surface phenotypes are manifested as differential adhesiveness and different levels of flocculation in cells (Verstrepen et al. 2005). The wide variability in the functional efficiency of these molecules arises from length variation of the functional VNTR, caused by misalignment of tandem repeats and unequal crossing-over at meiosis.

In the reproductive tract of rodents, pigs and ruminants, the glycoprotein MUC1 is repressed by progesterone control at the time of embryo implantation. During the same period, in humans and rabbits, progesterone stimulates uterine MUC1 expression, and its repression is limited to the site of embryo implantation. These studies suggest that variables in the VNTR region of the molecule can influence the interaction between the embryo and uterine luminal cells by ‘steric hindrance’ or different patterns of O-glycosylation (Aplin et al. 2001).

For the function involving differential cellular adhesiveness, the MUC1 VNTR polymorphisms have been associated with genetic susceptibility to increased risk for metastasis in gastric carcinoma and for infertility (Carvalho et al. 1997; Horne et al. 2001; Goulart et al. 2004). In goats and bovines, MUC1 has been associated with yield, health and reproductive traits (Hens et al. 1995; Patton et al. 1995; Sacchi et al. 2004). The present study shows five new allelic variations in the Nelore cattle in which the alleles with 12 and 18 repeats have not been previously described in Bos taurus Piedmontese and Italian Friesian cattle (Rasero et al. 2002). The higher frequency of the alleles with minor sizes presented in this study is in contrast to the report of Sacchi et al. (2004) who found the prevalence of alleles with moderate sizes in five cattle breeds.

The consensus sequence described by Spicer et al. (1995), differs from the sequence described by Pallensen et al. (2001) and from the findings we present. This variation may reflect genetic differences among breeds or exemplify the relative instability of the VNTR region in these populations. Despite the variation in the extent of homology for some repeats, the repeats typically maintain proline, threonine and serine residues, suggesting that the major selective pressure for MUC1 protein is to maintain the potential O-glycosylation sites.

We conclude that the high variability of MUC1 polymorphism in Brazilian Nelore cattle, the VNTR structure, and the presence of codons that translate for serine and threonine residues in the VNTR consensus sequence provide new information for finding association between the polymorphisms at the gene and the variation in fertility traits in cattle. In addition to these traits, the polymorphism may be associated with other economically important characteristics relevant to these breeds such as yield and overall health.

Acknowledgements

This work was supported by CAPES, ANCP and PRONEX/CNPq. We thank Dr Jeremy A. Squire from University of Toronto for suggestions and text revision.

References


