A preliminary study of solute carrier family gene in adapted bovine breeds of Panama

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Abstract

SLC11A1, the solute carrier family gene formerly known as bovine natural resistance-associated macrophage protein (NRAMP), has the polymorphism of the associated microsatellites located in its 3′ untranslated region (UTR). SLC11A1 has been associated with susceptibility or resistance to several intracellular pathogens. In Panama, several genetic characterization studies have been performed on the Guaymí (GY) and Guabalá (GUA) Creole breeds. Given that these breeds adapted to the local environment over a long period, there is an extremely high probability that an examination of the SLC11A1 gene could reveal alleles of this gene that confer resistance to disease. Therefore, the aim of this study was to identify the associated microsatellites located in its 3′ UTR of the SLC11A1 gene in bovine breeds locally adapted to Panama. Four locally adapted bovine breeds were studied. In total, 35 of the amplified samples were sequenced, revealing new polymorphisms such as (GT)_{13}, (GT)_{6}, and (GT)_{4} in GT regions. The most common repeats among the evaluated populations were (GT)_{13}, (GT)_{6}, and (GT)_{4}, which were found in 34.3, 20.0 and 11.4% of samples, respectively. Moreover, the (GT)_{13} repeat, which is associated with natural resistance against brucellosis, was found in the GUA breed. In addition, based on the numbers of GT repeats found in the sequenced samples, the GUA breed exhibited the greatest number of polymorphisms among the examined breeds.

Introduction

SLC11A1, the solute carrier family gene previously known as bovine natural resistance-associated macrophage protein (NRAMP), has been mapped to chromosome 2q43-q44 (Feng et al., 1996) and has an approximate size of 16 kb (Céllier et al., 1994). This gene has a microsatellite polymorphism due to the variations in the number of GT repeats located in its 3′ untranslated region (UTR); as a result, this microsatellite exhibits polymorphic patterns known as N rapl.1 (Feng et al., 1996) and N rapl.2 (Horin et al., 1999). These patterns are produced not only by changes in the number of guanine and thymine (GT) repeats but also by variations in the number of 5′-adjacent G residues, which are generally caused by errors during DNA replication (Goldstein and Pollock, 1997). Prior to this study, recent reports have demonstrated that there are polymorphisms in a microsatellite of the SLC11A1 gene (Hasenauer et al., 2013). Research has indicated that the SLC11A1 gene is related to susceptibility or resistance to several intracellular pathogens. This gene encodes a divalent cation carrier located in the phagolysosome membranes of macrophages (Kerpola and Ames, 1992; Céllier et al., 1994). Studies have demonstrated that this gene plays an important role in innate immunity, promotes the elimination of bacteria by macrophages, and affects adaptive immunity in mice (Vidal et al., 1995). In cattle, this gene has been associated with resistance against infection by Brucella abortus (Feng et al., 1996; Adams and Templeton, 1998; Horin et al., 1996; Barthel et al., 2001). However, conflicting findings exist, such as the results reported by Kumar et al. (2005), and Paixao et al. (2007), who did not detect an association between 3′ UTR polymorphism in SLC11A1 and brucellosis resistance in examinations of crosses between Bos taurus and Hariana cattle in India. In Panama, genetic characterization studies of the Guaymí (GY) and Guabalá (GUA) Creole breeds have been performed (Villalobos Cortés et al., 2010). Given that these breeds have adapted to the Panamanian environment over a long period, it is likely that alleles of a microsatellite associated with the SLC11A1 gene that confer resistance to diseases can be found; such alleles would have great importance due to their potential use in genetic conservation and improvement programs (Figure 1).

In addition, from an animal production perspective, there are other interesting breeds, such as Brahman (BRH) and Senepol (SEN), and crosses, such as Brown Swiss and/or Holstein × BRH, that might produce cattle with alleles of the SLC11A1 gene that are associated with resistance and susceptibility to diseases. Therefore, the aim of this study was to identify and sequence a microsatellite of the SLC11A1 gene in certain locally adapted bovine breeds in Panama.
15 seconds and incubated at 98°C for 2 min. After the second incubation, samples were stored at -20°C for future use. The theoretical DNA yield reported by the manufacturer was from 20 to 140 ng/µL for each 10-hair sample. The amplification of the 3’ UTR of the SLC11A1 gene was performed using the aforementioned PCR machine. The SLC11A1 oligonucleotides of reference used were FW (5’-GGAATGAGTGGGCAAGTCCGC-3’) and RV (5’-CCTTCCAGAACTCCTCTCGC-3’), which were reported by Vásquez-Flores et al. (2006), were used in the PCR amplification; these fragments were designed by Vásquez-Flores et al. (2006) to amplify a 223-bp fragment corresponding to the microsatellite 311-22 (GenBank: U12862.1). Primers (0.5 uM) were included in a 25-µL reaction mixture that contained 70 to 100 ng of DNA, 0.2 mM of each dNTP, 1× PCR buffer, 1.5 mM MgCl2 and 1 U of Taq DNA Polymerase. The amplification program consisted of an initial denaturation of 3 minutes at 94°C; 30 cycles of 45 seconds at 94°C, 30 seconds at 55°C and 90 seconds at 72°C; and a final extension step of 10 minutes at 72°C.

The PCR products were verified using electrophoresis in 2% agarose gels, stained with SYBR Safe® (Invitrogen, Waltham, MA, USA) and visualized in a UV® Biodoc-it™ imaging system. In addition, analysis was performed using a Qiagen® capillary electrophoresis fragment analyzer (Qiagen, Valencia, CA, USA); this technique is similar to agarose gel electrophoresis, but faster and less contaminating than the previous one. The presence of a band of 224 bp indicated that the sampled animal possessed an amplicon of the associated microsatellites located in its 3’ UTR of the SLC11A1. Qiagen® QX DNA Size Marker 25-500 bp (50 µL) v2.0 was used as an internal marker. Sequencing was accomplished by puri-

| GT repeats | n  | %   |
|-----------|----|-----|
| GT(11)    | 12 | 34.3|
| GT(6)     | 7  | 20.0|
| GT(4)     | 4  | 11.4|
| GT(13)    | 3  | 8.6 |
| GT(5)     | 2  | 5.7 |
| GT(9)     | 2  | 5.7 |
| GT(3)     | 1  | 2.9 |
| GT(7)     | 1  | 2.9 |
| GT(8)     | 1  | 2.9 |
| GT(10)    | 1  | 2.9 |
| GT(14)    | 1  | 2.9 |

GT, guanine and thymine.

Table 2. Distribution of guanine and thymine repeats per breed.

| Breed             | GT repeats         |
|-------------------|--------------------|
| Brahman           | 5, 6, 9, 11        |
| Guabala           | 3, 4, 7, 9, 11, 13, 14 |
| Guaymi            | 5, 6, 11           |
| Brown swiss×Zebu  | 4, 6, 10, 11       |
| Senepol           | 4, 6, 8, 11        |

GT, guanine and thymine.

Figure 1. The Guaymi Creole (A) and Guabalá Creole (B) bovine breeds, which have adapted to the Panamanian environment.

Figure 2. Capillary electrophoresis of 224-bp polymerase chain reaction fragments corresponding to the 3’ untranslated region of the SLC11A1 gene of Guaymi sample population.
fying the amplified SLC11A1 fragments with ExoSAP-IT and sequencing these fragments with BigDye Terminator v3.1. Finally, the runs were edited and aligned using Sequencer 5.0 (GeneCodes) and compared with blastn of Basic Local Alignment Tool (BLAST) sequences.

Results and discussion

The amplification of a fragment of 224 bp in all five examined populations (GY, GUA, SEN, BRH and Brown Swiss × BRH) was achieved using the 2% agarose gel electrophoresis (data not shown) and capillary electrophoresis techniques (Figure 2).

The size of the obtained fragment corresponds to the 3' UTR region of the SLC11A1 gene. In particular, the findings were similar to the results reported by Vásquez-Flores et al. (2006), who observed banding patterns between 224 bp in an evaluation of 16 bovine breeds (10 B. Taurus and 6 B. indicus). Pinedo et al. (2009) reported bands ranging from 273 to 289 bp in a study of the associations between SLC11A1 and paratuberculosis resistance. Kadarmideen et al. (2011) detected banding patterns between 211 and 217 bp for African zebu. Lower weights were observed by González et al. (2006) in Colombia, who reported banding patterns between 175 and 189 bp for eight bovine breeds (Holstein, BRH and six Colombian Creole breeds). Similarly, Hasenauer et al. (2013) found banding patterns between 175 and 189 bp in populations of B. taurus, B. indicus, and crosses between these two species. The banding patterns found and reported by other authors exhibit variability with respect to their sizes in base pairs and their corresponding (GT)n repeated sequences; under this point of view, it is better to use GT repeats. Trujillo et al. (2006) reported that band sizes of 265, 209, 211 and 213 bp correspond to (GT)10, (GT)11, (GT)12 and (GT)16, respectively. In contrast, Paixao et al. (2006) reported that band sizes of 175, 177 and 179 bp correspond to (GT)13, (GT)14 and (GT)15, respectively. The different primers used in various studies, which could produce fragments of different sizes after amplification, might cause the aforementioned differences. Therefore, we sequenced all of our amplicons to obtain greater precision regarding the size of (GT)n.

Capillary electrophoresis exhibited similar resolution with gel electrophoresis with respect to identifying 224-bp banding patterns. Therefore, capillary electrophoresis could not be used to identify the polymorphisms of the alleles corresponding to microsatellites. This is because the Qiagen® capillary electrophoresis fragment analyzer give a resolution of 3-5 bp for fragments; thus, the sequencing of 35 fragments from sampled individuals of the examined breeds was performed.

The sequenced fragments are similar to Bos indicus breed, Holstien Fresian S X Sahiwal solute carrier family member 1 (SLC11A1) gene, 3' UTR-Sequence ID: gi|88862033|gb|IQ048024.11 and Bos taurus solute carrier family 11 (proton-coupled divalent metal ion transporters), member 1 (SLC11A1), mRNA. NM_174652.

Table 1 indicates the frequencies of tandem repeats observed in this study. As indicated in this table, the least and greatest number of repeats detected were GT(3) and GT(14), respectively. The most frequently occurring repeat was GT(11), which was found in 34.3% of samples, and the least frequently occurring repeats were GT(3), GT(7), GT(9), GT(10) and GT(14), which were each found in 2.9% of samples.

Table 2 indicates the distribution of markers per breed. This table indicates that the breed with the lowest repeat diversity was GY, which only exhibited the GT(5), GT(6) and GT(11) markers, and the breed with the greatest marker diversity was GUA, which exhibited the GT(3), GT(4), GT(7), GT(9), GT(11) GT(13) and GT(14) markers. GT(11) was a common marker across all examined breeds. The GT(3), GT(8), and GT(10) were exclusively present in GUA, SEN and SXC samples, respectively. The current investigation is a preliminary study; thus, to follow up on the findings presented here, a larger study should be conducted to determine which alleles are associated with resistance and susceptibility to certain diseases in the breeds examined in this study.

Conclusions

Fragments corresponding to the 3' UTR region of the SLC11A1 gene were amplified and sequenced in locally adapted breeds. The Guabala breed appears to possess the highest allelic diversity among the breeds examined in this study. This study will augment the information available about new GT's repetitions and will be useful in further studies to determine the role of the SLC11A1 gene in diseases resistance/susceptibility and for selection of diseases resistant animals like Guabala and Guaymi breeds. More population of each breeds need to be analyzed and sequenced by more different markers to gain more precision in the knowledge of this gene.

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