SNP IDENTIFICATION IN THIOESTERASE DOMAIN OF FATTY ACID SYNTHASE GENE IN MURRAH BUFFALOES

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ABSTRACT

The aim of present study was to screen for the presence of genetic variability in thioesterase (TE) domain of the fatty acid synthase (FASN) gene in Murrah buffalo, an important milch breed of India. TE domain of FASN gene is responsible for termination of fatty acid chain during its de-novo synthesis which in turn affects the fatty acid composition and quality of milk fat. In this study, terminal exons 38 to 42, including TE domain were explored for the presence of SNP variations using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. An important SNP at g.18433A>G in exon-40 region of FASN gene was identified in Murrah buffaloes. Three types of genotypes, viz. AA, AG and GG were observed having 34%, 56% and 10% frequencies, respectively. The allele frequencies of A and G alleles were 0.62 and 0.38, respectively. The identified polymorphism was non-synonymous transition in FASN gene. The study will augment the information available and can be applied in future studies to determine the role of buffalo FASN gene as a candidate gene marker for a milk-fat content.

Key words: Murrah buffaloes, FASN, Genotypes, SNP, Thioesterase domain.

INTRODUCTION

Genetic or marker based selection followed with appropriate breeding methods for higher milk fat can facilitate genetic selection in different buffalo breed improvement programmes in India. Fatty acid synthase FASN is a multifunctional enzyme complex that regulates de novo biosynthesis of long chain fatty acids. This cytosolic enzyme catalyses palmitic acid synthesis from acetyl coenzyme-A and malonyl coenzyme-A in the presence of NADPH (Wakil et al., 1983). The FASN gene has been identified in rat, human, goose, chicken and cattle (Kameda and Goodridge 1991; Amy et al., 1992; Jayakumar et al., 1995; Chirala et al., 1997; Roy et al., 2006). Bovine fatty acid synthase gene (FASN) was mapped to chromosome 19 (BTA19) at q22 band (Roy et al., 2001) and is 19770 bp long and consists of 42 exons and 41 introns (Gen Bank Accession No. AF285607.2).

The studies on the bovine FASN gene structure have revealed occurrence of several single nucleotide polymorphisms (SNPs) linked to the fat content and fatty acids composition in milk (Roy et al., 2006). Based on QTL studies, Morris et al. (2007) identified FASN gene as a potential candidate gene for some milk production quality traits. Moreover, the fatty acid composition of milk is an important factor affecting human health (Mele et al., 2007; Kgwatallala et al., 2009). The thioesterase (TE) domain within the FASN complex regulates the termination of fatty acid synthesis. C14 acyl-ACP and C16acetyl-ACP are both substrates of the FASN (TE) domain. The hydrolysis rate of C14 acyl-ACP by FASN (TE) is slower than that of C16 acyl-ACP (Lin and Smith 1978; Pazirandeh et al., 1991). The TE domain has an important role in determining the product chain length of FASN. Therefore, the TE domain of the FASN gene can be a candidate gene for variance in fatty acid composition and might help to produce healthier livestock products regarding fatty acid composition (Zhang et al., 2008). The TE domain is the part between exons 39 to 41 of the FASN gene (Abe et al., 2009). SNPs affecting fatty acid composition, g.18663 (g – genomic sequence) T>C and g.17924 A>G, were identified in the TE domain (Zhang et al., 2008; Bhuian et al., 2009). Furthermore a Bos indicus associated SNP, g.18440 G>A is also reported in the same region (Bhuian et al., 2009). However, scanty literature is available regarding genetic variations in TE domain of the FASN gene among buffaloes. Keeping this in mind, an attempt was made to explore the polymorphism in FASN gene in Murrah buffaloes and to study its partial sequence comparison with other livestock species.

MATERIALS AND METHODS

Population studied and sample size: Random blood samples (approximately 8 to 10 mL) were collected from 196 buffaloes representative of the Murrah breed. Samples of Murrah buffaloes were collected from Livestock Farm of National Dairy Research Institute, Karnal (Haryana).

DNA isolation and Primers used: Genomic DNA was isolated from aseptically collected venous blood using the
standard phenol/chloroform method with minor modifications (Sambrook and Russell, 2001). Quality check and quantification were done by nanodrop spectrophotometer and electrophoresis on 0.8% agarose gel. DNA concentration was determined and samples were diluted 10-40 times (approx. 50-80 ng/µl) with MiliQ water. For amplification of FASN gene (exon 38, 39, 40, 41 and exon 42) in Murrah buffalo the primers were designed from published cattle (Bos taurus) sequences available online in the Gen bank (www.ncbi.nlm.nih.gov) having accession no. AF285607.2 for FASN gene (Table 1). Designing of primers were done with help of primer 3.0 software (http://frodono.m.iit.edu/cgi-in/primer3/primer3www.cgi). The PCR-RFLP reaction conditions are shown in Table 2.

Statistical analysis: Genotype and allele frequencies of g. 18433 A>G polymorphisms in the TE domain were calculated by using the gene counting method as suggested by Falconer and Mackay (1996). A chi-square test was also performed to check Hardy-Weinberg equilibrium status in the studied population.

RESULTS AND DISCUSSION

The available genotypes of FASN exon 38 to 42 at position g.17980 G>T, g.18025 C>T, g.18433 A>G, g.18628 C>G and g.19056 C>T polymorphisms in the TE domain were determined by the PCR-RFLP method using the Rsal, PstI, MluCl, Mspl and DdeI restriction enzymes in Murrah buffaloes. The genotype and allele frequencies are shown in Table 3. Digestion of the 228bp PCR product of exon38 with Rsal revealed two fragments of 133 and 95bp and show the monomorphic band pattern. Digestion of the 696bp PCR product of exon39 with Pstl revealed three fragments of 296, 229 and 171bp in all the samples and was found to be monomorphic band pattern. Digestion of the 373bp PCR product of exon41 with Mspl revealed two fragments of 256 and 117bp and show the monomorphic band pattern, and digestion of the 453bp PCR product of exon42 with DdeI revealed three fragments of 135, 121 and 97bp and also show the monomorphic band pattern. Digestion of the 472bp PCR product of exon40 with MluCl revealed three fragments of 281, 191bp and uncut 472bp show the polymorphic band pattern and it is novel finding. RFLP test using MluCl restriction enzyme indicated that exon40 region of FASN gene is polymorphic, with the presence of three genotypes namely, AA, AG & GG, with two alleles in Murrah buffaloes. Frequency of AA, AG and GG genotypes were 34%, 56% and 10%, respectively. Out of the three GG was rare and AG being most common genotype. Allele frequencies of A and G alleles were 0.62 and 0.38, respectively. Moreover, the genotype frequencies of the exon40 polymorphisms in the TE domain was found to be in Hardy-Weinberg equilibrium.

Inferring that so far the studied population of Murrah buffaloes followed random mating and has not been subjected to selection with respect to TE domain of FASN gene for delete milk fat composition.

In recent years, genetic studies have focused on the manipulation of unsaturated fatty acid composition of livestock products which have healthier effects on human metabolism (Taniguchi et al., 2004; Mele et al., 2007; Schennink et al., 2009; Kgwatalala et al., 2009), Zhang et al. (2008) studied the association between g.17924A>G and g.18663 T>A polymorphisms in the FASN gene TE domain and beef fatty acid composition in Angus cattle. According to their results, g.17924 GG genotyped cattle had lower myristic acid (C14:0; P<0.0001), palmitic acid (C16:0; P<0.05), total SFA contents and higher oleic acid (C18:1; P<0.001) and total MUFA contents. The substitution from threonine to alanine due to this polymorphism may result in less C14:0 content and higher C:16:0 to C14:0 ratio in animals with the 17924 GG genotype (alanine) than in animals with the 17924 AA genotype (threonine) (Zhang et al., 2008). Bhuiyan et al. (2009) reported a significant association of the GG genotype of g.17924 A>G with C16:0 and C18:1 fatty acid content. GG genotype frequency was calculated as 13% in Angus cattle (Zhang et al., 2008) and 73% in Hanwoo cattle (Bhuiyan et al., 2009).

The selected genotypes obtained through RFLP results were sequenced an on sequence comparison (Fig.1) it was revealed that the g.18433 G>A polymorphism in the TE domain leads to non-synonymous transition as observed in the third base of codon in FASN gene. The protein translation of exon40 (Fig.2) reveals a change of L (Bos taurus) to P Murrah buffaloes. However, both Leucine and Proline are non-polar neutral amino acid. In Murrah this change in amino acid may affect the quality and quantity of milk fat. However, detailed association studies needs to be carried out to confirm such association. On analysis of variations in adjacent sites to g.18433 it is confirmed that buffalo germplasm is quite different from Bos taurus at many locations but within buffalo samples it is polymorphic at this particular site (Fig.1). Phylogenetic analysis of sequence characterized for exon40 in Murrah was compared with other reported sequences (NCBI Accession No. AF285607.2, NW_005395032, NW_005785022.1, XM_012186804.1, AC_006105.3, NW_005785022.1) in different livestock species (Fig.3) which revealed that Murrah is closer to Bubalus, taurus cattle and Yak species which lies in an adjacent cluster. Other livestock species forms different clusters. Therefore, it is suggested that A allele of g.18433 G>A is a Murrah associated allele.

It is concluded that genetic variation is present in the coding region of TE domain in FASN gene with guanine to adenine transition in well-known Murrah buffalo is being reported first time. Results suggest that
exon40 region of FASN gene in Murrah buffaloes is polymorphic and sufficient genetic variability is present in this gene in contrast to other well-known candidate gene for fat percentage like DGAT gene and its alleles are reported fixed in Murrah buffaloes. The genotypic frequencies of the exon40 polymorphisms in the TE domain were found to be in Hardy-Weinberg equilibrium. It infers that there is no selection of animal for FASN gene in Murrah herd. This G to A transition can be used further as SNP marker, which could be helpful to breeders for conducting future association studies and detailed analysis of other SNPs localized within this gene, could also possibly allow for indicating quantitative trait loci for milk fat and its metabolism, and can be used in marker assisted selection of river buffaloes.

Table 1. Primer sequences used for amplifying various exon regions of FASN gene

<table>
<thead>
<tr>
<th>Gene / Region</th>
<th>Primer sequence</th>
<th>Product length</th>
<th>A. °C</th>
<th>GC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EXON 38</td>
<td>F- 3’GACCTTGACCGCTCAACT-5’ R- 3’GGGCACACGCTAGGTTTAG-5’</td>
<td>228 bp</td>
<td>60</td>
<td>55</td>
</tr>
<tr>
<td>EXON 39</td>
<td>F- 3’AGAGCTGACGGACTCCACAC-5’ R- 3’CTGCATGAAGAAGCACATGG-5’</td>
<td>696 bp</td>
<td>58</td>
<td>60</td>
</tr>
<tr>
<td>EXON 40</td>
<td>F- 3’CTGCACACCTTCGTAGTG-5’ R- 3’CACGTTGCCGTTGTAAGTG-5’</td>
<td>472 bp</td>
<td>60</td>
<td>50</td>
</tr>
<tr>
<td>EXON 41</td>
<td>F- 3’CGCTCAGCTGCTGCTCTAC-5’ R- 3’GCTGTAATAAATACTAAGGGATGGA-5’</td>
<td>373 bp</td>
<td>60</td>
<td>50</td>
</tr>
<tr>
<td>EXON 42</td>
<td>F- 3’GCCTGCGGCGGCGCTCAACTCTC-5’ R- 3’CCCCCATGGCGACGCGAATAAAT-5’</td>
<td>453 bp</td>
<td>61</td>
<td>54.5</td>
</tr>
</tbody>
</table>

A. is annealing temperature

Table 2. PCR-RFLP reaction conditions used for digesting the different exons of FASN gene.

<table>
<thead>
<tr>
<th>Region</th>
<th>Exon 38</th>
<th>Exon 39</th>
<th>Exon 40</th>
<th>Exon 41</th>
<th>Exon 42</th>
</tr>
</thead>
<tbody>
<tr>
<td>Template DNA Conc. ng/µl</td>
<td>150</td>
<td>200</td>
<td>150</td>
<td>180</td>
<td>100</td>
</tr>
<tr>
<td>Restriction Enzyme</td>
<td>Rsal</td>
<td>PstI</td>
<td>MluCl</td>
<td>MspI</td>
<td>Ddel</td>
</tr>
<tr>
<td>Restriction Site</td>
<td>GTAC</td>
<td>CTGCA</td>
<td>AATT</td>
<td>CGG</td>
<td>TNAG</td>
</tr>
<tr>
<td>Buffer used</td>
<td>5 µl (10x)</td>
<td>5 µl (10x)</td>
<td>5 µl (10x)</td>
<td>5 µl (10x)</td>
<td>5 µl (10x)</td>
</tr>
<tr>
<td>Enzyme Concentration</td>
<td>3 IU</td>
<td>6 IU</td>
<td>4 IU</td>
<td>7 IU</td>
<td>3 IU</td>
</tr>
<tr>
<td>Time for digestion</td>
<td>8 to 12 hr. at 37°C</td>
<td>8 to 12 hr. at 37°C</td>
<td>8 to 12 hr. at 37°C</td>
<td>8 to 12 hr. at 37°C</td>
<td>8 to 12 hr. at 37°C</td>
</tr>
<tr>
<td>Temp. &amp; Time of Denaturation</td>
<td>80°C for 15 min.</td>
<td>80°C for 15 min.</td>
<td>65°C for 20 min.</td>
<td>65°C for 20 min.</td>
<td>80°C for 15 min.</td>
</tr>
</tbody>
</table>

Table 3. Genotype and allele frequencies of different exons in FASN gene.

<table>
<thead>
<tr>
<th>Position</th>
<th>Region</th>
<th>Genotype</th>
<th>Genotype frequency</th>
<th>Allele</th>
<th>Allele frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>g 17980 G&gt;T (Rsal)</td>
<td>Exon38</td>
<td>GG</td>
<td>1.00</td>
<td>G</td>
<td>1.00</td>
</tr>
<tr>
<td>g 18025 C&gt;T (PstI)</td>
<td>Exon39</td>
<td>CC</td>
<td>1.00</td>
<td>C</td>
<td>1.00</td>
</tr>
<tr>
<td>g 18632 A&gt;G (MluCl)</td>
<td>Exon40</td>
<td>AA</td>
<td>0.34</td>
<td>A</td>
<td>0.62</td>
</tr>
<tr>
<td>g 19056 C&gt;T (Ddel)</td>
<td>Exon42</td>
<td>CC</td>
<td>1.00</td>
<td>C</td>
<td>1.00</td>
</tr>
</tbody>
</table>
Figure 1. Nucleotide sequence alignment of different genotypes of exon-40 region in FASN gene and SNP at g.18433A>G position among different Bubalus bubalis (Murrah buffalo) and their comparison with Bos Taurus (Accession no. AF285607.2)


Figure 2. Comparison of different protein sequence (single letter symbol of proteins) of exon 40 region of TE domain of FASN gene

Figure 3. Phylogenetic diagram of TE domain of FASN gene based on NJ method using ClustalW2-Phylogeny program.

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REFERENCES


