GENETIC POLYMORPHISM ANALYSIS OF MONOACYL GLYCEROL TRANSFERASE2 (MOGAT2) GENE IN MURRAH BUFFALO (Bubalus bubalis)

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ABSTRACT

The polymerase chain reaction single-strand conformation polymorphism (PCR-SSCP) was identified within the Murrah buffalo MOGAT2 gene. The result exhibited three SSCP patterns in the MOGAT2 gene with variable frequency in samples studied, indicating that Murrah buffaloes have genetic variability at that locus. This identified SSCP was confirmed by DNA sequencing which revealed one single nucleotide polymorphism (SNP) viz, c.193T>C within 245 bp fragment of the MOGAT2 gene spanning exon 5 of the Murrah buffalo. Identified SNP (c.193T>C) was used to genotype the 106 samples of Murrah buffalo in which frequencies for C and T variants were found to be 0.25 and 0.75, respectively. The frequency of genotypes within the breed group was in accordance with Hardy-Weinberg proportions. Murrah buffalo MOGAT2 allelic variant sequence was 95% pairwise similar with cattle sequence and comparison of the two revealed eleven computational SNPs. The statistical analysis using general linear model procedure (SYSTAT) for association study indicated that Murrah Buffalo MOGAT2 c.193T>C SNP genotypes did not differ significantly (P>0.01) from Murrah buffalo milk production traits.

Keywords: MOGAT2, SSCP, DNA sequencing, association study, Murrah buffalo

INTRODUCTION

India is rich in buffalo genetic resources having 97 million animals in 2003 (Annual Report, 2006) which accounted for 59.5% to total world buffalo population. Buffalo milk contributes 55.6% to the country’s total milk production. The Murrah buffalo is the most important dairy breed with superior genetic potential for milk production. However, their inherent potential for growth and production has not been exploited due to inadequate information about their genetic architecture. The river buffalo, along with domestic cattle, belongs to the subfamily Bovinae, and these species have been shown to be closely related, sharing homology in chromosome banding and gene mapping (Di Meo et al., 2005) and have been cytogenetically characterized in detail. The first generation whole genome RH map of the river buffalo was reported based on comparison to domestic cattle (Amaral et al., 2008). Therefore these preliminary maps, based on cattle-derived markers, demonstrated that the bovine genome is a useful source of markers for the buffalo genome mapping, allowing rapid
and efficient transfer of information from cattle to buffalo.

The publication of the entire genome sequences of several livestock species will allow easy identification of genetic markers in buffaloes, which will aid buffalo breeding and genetic improvement. The comparative genomics and genome analysis biotechniques have opened new possibilities for evaluation of the buffalo genome. The PCR-SSCP analysis (Orita et al., 1989) is a technique based on the principle that single-stranded DNA molecules form specific sequence-based secondary structures under non-denaturing conditions. The association of genetic polymorphisms with milk production traits and composition (Ganai et al., 2008) has stimulated interest in identifying the genetic markers influencing production traits which will be used in marker assisted selection (MAS) to improve productivity of farm animals.

In the marker assisted selection of dairy animals some genes are proposed as potential candidates associated with dairy performance traits. Fat is one of the major constituents of milk. Triglycerides are the major energy storage molecules in eukaryotes, and their final and presumably rate-limiting step of synthesis is catalyzed by a diacylglycerol acyltransferase (DGAT). A few years back, DGAT1 was the first identified gene encoding a protein with DGAT activity in which a mutation has been shown to be significantly associated with variation in milk fat percentage in cattle (Grisart et al., 2002; Gautier et al., 2007). DGAT-like activity has also been shown in other enzymes encoded by other genes and led to the detection of diacylglycerol transferase2 (DGAT2), monoacyl glycerol transferases1 (MOGAT1) and monoacyl glycerol transferases2 (MOGAT2), which are members of the same family (Winter et al., 2003). The members of this gene family show similarity in their nucleotide sequences and arose from same ancestral gene by duplication. However, this family has not yet been fully characterized in any single mammalian species (Winter et al., 2003).

As the MOGAT2 gene is in the family related with QTL influencing milk production traits and its functional role similarity in triglyceride synthesis indicates that it might be a useful candidate to reveal genetic polymorphisms. As the Murrah buffalo is very important dairy breed of buffalo contributing the lions share in country’s milk production, it is necessary to screen candidate genes implicated for milk production, viz, the MOGAT2 in Murrah buffalo genome. Therefore the present study was undertaken to detect genetic variation in the MOGAT2 gene using PCR-SSCP followed by DNA sequencing and any find association with milk production traits.

**MATERIALS AND METHODS**

The study group included 106 Murrah buffaloes with milk production records from the Institute herd. Blood samples (10 ml) were collected by jugular veinipuncture using vacuum tubes containing acid citrate dextrose solution (ACD) as an anticoagulant. Genomic DNA was isolated from blood using the phenol chloroform extraction protocol (Clamp et al., 1993) with some modifications. The integrity of the DNA was assessed following electrophoresis in a 0.8% agarose gel with ethidium bromide staining. In addition, the OD ratio 260/280 nm was measured to check for protein contamination and to calculate the DNA concentration. All stock DNA samples were kept at -80°C for longer storage, and the
The PCR primers were designed for exon V of the MOGAT2 gene on the basis of cattle gene sequence covering nucleotide substitution using PRIMER3 software (http://www-genome.wi.mit.edu). The polymerase chain reaction (PCR) was carried out on about 100 ng of genomic DNA in a 25 μL reaction volume. The reaction mixture consisted of 2.5 μL of 10x PCR assay buffer containing 1.5 mM MgCl2, 200 μM each of dNTPs, 0.75 unit Taq DNA polymerase and 10 pmole of each primer (Integrated DNA Technologies, Inc). The primers (Forward primer: 5’-TTT GGT CTT ATG CCC TAC CG-3'; Reverse primer: 5’-GGA CAG GGT GAT CTT TTG GA-3’) were used for amplification of exon V of the Buffalo MOGAT2 gene. Amplification was carried out in a Biometra thermal cycler using PCR cycling conditions as (95°C for 5 minutes) and 34 cycles of 45 seconds at 95°C, 65°C and 72°C consecutively, followed by a five minute final extension at 72°C. The PCR amplification was verified by electrophoresis of the PCR products with loading dye on 2% (w/v) agarose gel in 0.5 x TBE buffer using a 100 bp ladder as marker for confirmation of the length of the PCR products. The amplified products (5 μL) were detected on 2% agarose gel using 1 μL of loading dye as a stop dye, electrophoresed and visualized using UV light.

The MOGAT2 gene PCR products were resolved by SSCP analysis using PCR product (5 ul), acrylamide concentration (18%), presence glycerol (10%), voltage (200 volts), running time (24 hours) and temperature (15°C). PCR products were diluted in denaturing solution (95% formamide, 10 mM NaOH, 0.05% xylene cyanol and 0.05% bromophenol blue, 20 mM EDTA) and heat denatured at 95°C for ten minutes. The PCR products were resolved on a non-denaturing 18% acrylamide: bis-acrylamide (49: l) gel for SSCP analysis. Gels were silver-stained (Sambrook and Russell, 2001) and photographed using a digital camera for SSCP pattern analysis.

The PCR products representing different SSCP patterns were directly got sequenced. The nucleotide sequence analysis was carried out using Geneious software. The DNA sequence polymorphism observed was used to genotype Murrah buffalo population. The frequency of polymorphic allele variant, genotypes and their accordance with Hardy-Weinberg law was assessed by POPGENE 1.31 software (http://www.ualberta.ca/~fyeh). The association between polymorphic allelic variants of the MOGAT2 gene and milk production traits was analyzed using GLM procedure (SYSTAT). The following model was used,

\[ Y_{ijkl} = \mu + g_i + s_i + p_j + h_k + e_{ijkl} \]

- \( Y_{ij} \): observation on jth animal ith genotype
- \( \mu \): population mean
- \( g_i \): effect of ith genotype (i=1, 2)
- \( s_i \): effect of i season
- \( p_j \): effect of j parity
- \( h_k \): effect of k year,
- \( e_{ijkl} \): random error

RESULTS AND DISCUSSIONS

The SSCP analysis of amplified gene fragments of exon 5 of the MOGAT2 gene resulted in three different patterns viz, A, B and C (Figure1) with the following frequencies in Murrah buffaloes (A = 0.49, B = 0.36, C = 0.15). This study has revealed the polymorphic nature of the 3’ UTR region of exon 5 of the buffalo MOGAT2 gene.

The direct DNA sequencing and nucleotide
sequence analysis of MOGAT2 amplified PCR products (Table 1) representing different SSCP patterns (A, B and C), revealed one SNP (T-C substitution) in exon 5 at the 193rd nucleotide position (denoted as c.193 T>C) within the MOGAT2 gene sequence of the Murrah buffalo (Figure 2). These MOGAT2 allelic variant nucleotide sequences were analyzed and submitted in NCBI GenBank with accession No. (EU239373, EU239374). The nature of mutation was T-C transversion between SSCP pattern A and B confirming them as variants (A and B). The SSCP patterns B and C had identical nucleotide sequences; therefore only two variants (A and B) were confirmed. The PCR products representing SSCP pattern B were homozygote: TT in position 193 while SSCP patterns A and B were heterozygote: TC in position 193.

The polymorphic Murrah buffalo MOGAT2 allelic variant sequence (EU239373) was compared with Bos taurus reference sequence (AJ534379) using alignment tool (Geneious Software) which revealed eleven computational mutations. The Murrah buffalo MOGAT2 variant sequence (EU239373) was 95% pairwise similar with, the cattle sequence (AJ534379). A neighbor-joining tree (Figure 3) was constructed based on comparison of the Murrah buffalo MOGAT2 consensus sequence (EU239373) and consensus GenBank sequences of cattle (AC149756, NM_0010011154 and AJ534379) and buffaloes (EF208205, EU239373) at the same MOGAT2 locus (Geneious Software). The phylogenetic tree based on partial consensus sequence agreed with taxonomic relationship of cattle and buffaloes.

The association analysis was carried out between MOGAT2 c.193T>C SNP and milk production traits to find any relationship between them. The ANOVA results indicated non-significant (P>0.01) effects of different Murrah buffalo MOGAT2 genotypes: c.193T>C TT and c.193T>C TC on 305 days milk yield, fat percentage as well as SNF percentage. The effect of non-genetic factors, viz. season, parity and year of calving on milk yield were found to be significant (p<0.01). The least squares mean values of the milk production traits studied in Murrah buffaloes differing in their MOGAT2 c.193T>C genotypes are given in Table 1.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>n</th>
<th>Milk Yield±SE</th>
<th>FAT*±SE</th>
<th>SNF*±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.193T&gt;C TT</td>
<td>52</td>
<td>1775.81±241.06</td>
<td>0.297±0.005</td>
<td>0.315±0.001</td>
</tr>
<tr>
<td>c.193T&gt;C TC</td>
<td>54</td>
<td>1678.21±264.85</td>
<td>0.297±0.005</td>
<td>0.314±0.001</td>
</tr>
</tbody>
</table>

Where,
* are scale-transformed values
superscript NS are means not differing significantly at p≤0.05.
Figure 1. MOGAT2 PCR-SSCP genotype patterns resolved on 18% PAGE and visualized by silver staining in Murrah buffalo.

Figure 2. Multiple sequence alignment using CLUSTAL W (1.83) indicating T-C substitution at 193\textsuperscript{rd} position.

Figure 3. Neighbor-Joining Tree based on Murrah buffalo MOGAT2 variant A consensus sequence (EU239373) and related consensus sequences of cattle and buffaloes (Geneious Software).
sub-region of the dairy animal genome. In view of the above, the MOGAT2 gene was screened for polymorphism in Murrah buffalo using SSCP followed by sequencing and association study.

In the present study, one SNP has been identified within the population at the MOGAT2 gene locus of the Murrah buffalo. The identified SNP genotypes at the MOGAT2 gene locus did not differ significantly from Murrah buffalo milk production traits. However in their bovine MOGAT2 gene polymorphism study, Winter et al. (2003) found 15 SNPs outside exons and two silent exon SNPs (ID 358 and 363) and reported non-significant association of allele frequencies with breeding values for milk fat content in analyzed dairy breeds.

CONCLUSION

The present study revealed that PCR-SSCP followed by DNA sequencing is an effective molecular biological technique to detect DNA sequence variation at candidate gene loci in buffaloes. The identified SSCP within the MOGAT2 gene after DNA sequencing revealed one SNP (c.193T>C) in exon 5 of the Murrah buffalo. However, the statistical analysis revealed non-significant effect of the observed polymorphism genotypes on Murrah buffalo milk production traits. The possible reasons for non-significant effect might be the small size of the sample, absence of some genotypes, high standard error and uneven distribution of data.

The studies concerning associations between DGAT gene polymorphism and production traits of riverine buffaloes are, however, fairly scarce. Information regarding the actual physiological role of DGAT2 and the closely related MOGAT genes in vivo is only just becoming available. In view of this, it is necessary to screen all the regions of these genes in buffalo genome and to continue association studies as DGAT2/ MOGAT genes have genetic and functional similarity to DGAT1 influencing milk production traits in genetically related Bos taurus. The identified DNA polymorphism after validation study will open up possibilities for buffalo breeding and improvement in gene assisted selection.

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