Identification of Polymorphisms in the Bovine Paraoxonase 1 Gene

1,2Juan Zhang, 1Shengguo Zhao, 1Zhaoming Lei, 1Xinrong Wang, 1Jianping Wu and 3Michael A. Brown
1Faculty of Animal Science and Technology, Gansu Agricultural University, Lanzhou, Gansu, People’s Republic of China
2Department of Animal Science, School of Agriculture, Ningxia University, Yinchuan, Ningxia, People’s Republic of China
3B&B Research and Development, LLC, El Reno, OK, USA and Faculty of Animal Science and Technology, Gansu Agricultural University, Lanzhou, Gansu, People’s Republic of China

Abstract: The Paraoxonase 1 gene (PON1) encodes paraoxonase which hydrolyzes organophosphate pesticides and nerve gases. In addition, PON1 has been shown to have significant antiatherosclerotic effects and is a major candidate gene associated with the reduction of atherosclerosis. Most of the literature reporting polymorphisms in PON1 has been in humans while less information about the polymorphism of PON1 gene has been reported in mammals, especially in bovine species. Polymorphisms in the PON1 gene were evaluated in Simmental x Chinese Yellow cattle (n = 32) and Longdong cattle (n = 37) using PCR-SSCP in exons 1-9 of the PON1 gene. Results demonstrated polymorphisms in the bovine PON1 gene at exons 4, 6, 8 and 9. DNA sequencing identified specific substitutions in exon 4, 6, 8 and 9 (g.338G>A, g.647C>T, g.896C>T and g.998T>C, respectively). Two genotypes were identified in exon 4 (GG and AG), exon 6 (TT and CT) and exon 9 (TT and CT) while 3 genotypes were identified in exon 8 (CC, CT and TT). The allelic frequency for the G allele was 92.08% and the A allele was 7.92% in exon 4. Allelic frequencies for the T and C alleles were 89.86 and 10.14%, respectively, in exon 6. In exon 8, frequencies for the C and T alleles were 94.93 and 5.07%, respectively. In exon 9, allelic frequencies for the T and C alleles were 88.41 and 11.59%, respectively.

Key words: Cattle, PON1 gene, exon, polymorphism, PCR-SSCP, China

INTRODUCTION

The paraoxonase family of genes consists of PON1, PON2 and PON3 genes. Paraoxonase genes are located in the p7 chromosome in humans, the 6th chromosome in mice and the 11th chromosome in bovine. The Paraoxonase 1 gene (PON1) contains nine exons and eight introns. The length of PON1 mRNA is 1205 bp, consisting of 355 amino acids and has a relative molecular mass of 43000-45000 (Zech et al., 1999; Martin-Burriel et al., 1997; Costa et al., 2005). The PON1 plays a role in insulin levels, insulin resistance and high-density lipoproteins in humans (Purnell et al., 2003; Watzinger et al., 2002) and has been shown to be a major candidate gene in lipometabolism and cardiovascular disease (Aviram et al., 2000; Ayub et al., 1999). Like Lipoprotein Lipase (LPL), PON1 plays a key role in the metabolism and physiology of mammalian growth (Harel et al., 2004). Recently, the PON1 crystalline structure has been verified. This demonstrates that the PON1 protein has catalytic action on several substrates with the basic function of hydrolysis of ring lactones (Khersonsky and Tawfik, 2005). The PON1 protein is synergistic with insulin, steatin, growth hormone, lipoprotein lipase and leptin (Curi et al., 2006a, b). The PON1 enzyme function in the movement of lipids across cell membranes and within the cells (Purnell et al., 2003). Little information is available in the literature for bovine PON1. Ji et al. (2008a, b, 2009a, b) studied the polymorphism of exon 6 of the PON1 gene using RFLP and analyzed associations between partial production traits and polymorphisms identified. Results showed that association of PON1/EcoR V genotypes with body weight, average daily gain, rib eye area and tenderness were evident (p<0.05). Variations in the PON1 gene could be important in resilience of cattle to organophosphates and impact phenotypic expression of metabolic and physiological growth processes in cattle. Consequently,
the objective of the study was to evaluate polymorphisms in all exons of PON1 in Simmental x Chinese Yellow cattle crosses and Longdong cattle to evaluate the potential genetic variability in this gene.

MATERIALS AND METHODS

Animals and DNA extraction: Blood samples were collected from 69 crossbred cattle from Pingliang and Kangle districts of Gansu Province representing different breed crosses: Simmental x Chinese Yellow cattle (Kangle, n = 32) and Longdong cattle (Pingliang, n = 37). Genomic DNA was extracted from the blood leukocytes using a phenol-chloroform extraction protocol followed by an ethanol precipitation step and the extraction was checked on 1% agarose gels in 1X TAE buffer.

Primer and PCR amplification: The PCR primers (Table 1) were designed from the bovine PON1 gene nucleotide sequence of DNA in GenBank (accession number NC_007302.5, EU289337)(Ji et al., 2009a, b) using the primer 3 program (http://frodo.wi.mit.edu/). The PCR reaction was performed in a volume of 25 µL using genomic DNA (approximately 50-100 ng), 1 µL, 10 pmol of each primer, 1 µL; Taq DNA polymerase (5 U µL⁻¹), 0.5 µL; dNTP (2.5 mM L⁻¹), 2.0 µL; 10X PCR Buffer (Mg²⁺ free), 2.5 and 17 µL ddH₂O. The PCR cycling conditions were 94°C for 3 min for the first cycle and 94°C for 30 sec for the remainder of the cycles for denaturation, 60-63.5°C (Table 1) for 30 sec for annealing and 72°C for 30 sec for polymerization; a total of 35 cycles. The final extension was at 72°C for 10 min. The amplification result was verified on 1% agarose gel (ABI 2720, USA).

Table 1: Primer sequences used in study

<table>
<thead>
<tr>
<th>Primers</th>
<th>Sequence 5'-3'</th>
<th>Size (bp)</th>
<th>Annealing temperature°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>PONI-EX1F</td>
<td>AAGCAAGTCCCGCGAGTCC</td>
<td>365</td>
<td>63.0</td>
</tr>
<tr>
<td>PONI-EX1R</td>
<td>ACACTCTCTCTCCTCTGAT</td>
<td>323</td>
<td>60.6</td>
</tr>
<tr>
<td>PONI-EX2F</td>
<td>GAGCCCAAAGTACAGTAAAAGA</td>
<td>327</td>
<td>63.5</td>
</tr>
<tr>
<td>PONI-EX2R</td>
<td>AAAATCTCTCTGCTTAGTAG</td>
<td>345</td>
<td>60.0</td>
</tr>
<tr>
<td>PONI-EX3F</td>
<td>GGAGATGATGAGAGCAAG</td>
<td>385</td>
<td>62.0</td>
</tr>
<tr>
<td>PONI-EX3R</td>
<td>GAATGGGCTACACGGTTG</td>
<td>387</td>
<td>63.0</td>
</tr>
</tbody>
</table>

was screened using SSCP. The 3 µL of PCR product was mixed with 7 µL of denaturation buffer (58% deionized formamide, 10 mM EDTA pH8.0, 0.025% bromophenol blue and 0.025% xylene-cyanol). The samples were denatured at 98°C for 10 min. Samples were cooled on ice for at least 5 min and loaded onto 10% polyacrylamide gels (39.1 acrylamide:bisacrylamide). For exon 4 and exon 9, electrophoresis was carried out at 19°C and 190 V for 10 h in 1X TBE buffer; 12% polyacrylamide gels (39.1 acrylamide:bisacrylamide). For exon 6 and exon 8, electrophoresis was carried out at 19°C and 180 V for 10 h in 1X TBE buffer; gels were silver-stained (Byun et al., 2009).

Sequencing and sequence analysis: The PCR products, two genotypes in exon 4, 6 and 9 and three genotypes in exon 8 were identified by DNA sequencing. Several inserts from positive clones for each animal were confirmed by PCR using the primers mentioned above. The SSCP patterns were determined in each positive clone and clones corresponding to different PCR-SSCP band patterns were chosen for DNA sequencing. Each distinct SSCP pattern that corresponded to different clones and individuals was sequenced (Sheng Gong Company, China). The sequence alignments, translations and comparisons were carried out with BioEdit 7.0 Software (Ibis Biosciences, Carlsbad, CA, USA).

RESULTS AND DISCUSSION

The PCR amplification products of exons 4, 6, 8 and 9 of the PON1 gene tested using SSCP and results are shown in Fig. 1-4.

Two genotypes were identified in exons 4 (GG, AG), 6 (TT and CT) and 9 (TT and CT) and 3 genotypes were identified in exon 8 (CC, CT and TT) using PCR-SSCP. Sequence alignment diagrams with the published cattle sequence (GenBank Accession EU289337) are given in Fig. 5-8. DNA sequencing identified specific substitutions.

Fig. 1: PON1 gene amplification products in SSCP at exon 4
in exon 4, 6, 8 and 9 (g.338G>A, g.647C>T, g.896C>T and g.998T>C, respectively) but without apparent alteration of amino acid (BioEdit7.0, Ibis Biosciences, Carlsbad, CA, USA). In this study, the frequencies of the two alleles in the 69 cattle studied are shown in Table 2. Allele G was the most frequent allele with a frequency of 92.03%, followed by allele A at 7.97% in exon 4. Allele T was the most frequent allele in exon 6 with a frequency of 89.86% followed by allele C at 10.14%. In exon 8, allele C was the most frequent allele with a frequency of 94.93% followed by allele T at 5.07%. In exon 9, allele T was the most frequent allele with a frequency of 88.41%, followed by allele C at 11.59% in exon 4 genotypic frequencies were 84.06% and 15.94% for GG and AG, respectively.

Fig. 2: PON1 gene amplification products in SSCP at exon 6

Fig. 3: PON1 gene amplification products in SSCP at exon 9

Fig. 4: PON1 gene amplification products in SSCP at exon 8

Fig. 5: Sequence alignment of the bovine exon 4 of PON1 alleles together with the published cattle sequence (EU289337). Dots represent nucleotides identical to the top sequence. Nucleotides in exon 4 are shown in a solid box (Sequenced by up primer).

Fig. 6: Sequence alignment of the bovine exon 6 of PON1 alleles together with the published cattle sequence (EU289337). Dots represent nucleotides identical to the top sequence. Nucleotides in exon 4 are shown in a solid box (Sequenced by up primer).
Fig. 7: Sequence alignment of the bovine exon 8 of PON1 alleles together with the published cattle sequence (EU289337). Dots represent nucleotides identical to the top sequence. Nucleotides in exon 4 are shown in a solid box (Sequenced by up primer).

Fig. 8: Sequence alignment of the bovine exon 9 of PON1 alleles together with the published cattle sequence (EU289337). Dots represent nucleotides identical to the top sequence. Nucleotides in exon 4 are shown in a solid box (Sequenced by up primer).

Table 2: Allelic and genotypic frequencies of bovine PON1

<table>
<thead>
<tr>
<th>Breed population</th>
<th>Gene</th>
<th>n</th>
<th>GC</th>
<th>AG</th>
<th>GG</th>
<th>A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crossed cattle</td>
<td>PON1-EX8</td>
<td>32</td>
<td>0.8750</td>
<td>0.1250</td>
<td>0.0375</td>
<td>0.0625</td>
</tr>
<tr>
<td>Crossed cattle</td>
<td>PON1-EX9</td>
<td>37</td>
<td>0.8378</td>
<td>0.1622</td>
<td>0.0989</td>
<td>0.0811</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>69</td>
<td>0.8406</td>
<td>0.1594</td>
<td>0.0923</td>
<td>0.0797</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Breed population</th>
<th>Gene</th>
<th>n</th>
<th>TT</th>
<th>CT</th>
<th>TT</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crossed cattle</td>
<td>PON1-EX6</td>
<td>32</td>
<td>0.8125</td>
<td>0.1875</td>
<td>0.0964</td>
<td>0.0936</td>
</tr>
<tr>
<td>Crossed cattle</td>
<td>PON1-EX7</td>
<td>37</td>
<td>0.7838</td>
<td>0.2162</td>
<td>0.0891</td>
<td>0.1081</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>69</td>
<td>0.7971</td>
<td>0.2029</td>
<td>0.0896</td>
<td>0.1014</td>
</tr>
</tbody>
</table>

Genotypic frequencies in exon 6 were 79.71% and 20.29% for TT and CT, respectively. In exon 8, the genotypic frequency for CC was 91.30% and 7.25% for CT and 1.45% for TT. Genotypic frequencies in exon 9 were 76.81% for TT and 23.19% for CT. Researchers concluded that there are two alleles (G and A; T and C; C and T and T, C, respectively for exon 4, 6, 8 and 9) within both groups (Simmental x Chinese Yellow cattle; Longdong cattle) and G, T, C and T have the greater frequency at exons 4, 6, 8 and 9, respectively. By sequencing, researchers found polymorphisms in both breed-types, suggesting the potential for phenotypic differences resulting from gene expression. In this research, the genetic variation was detected using 69 cattle. While a greater sample size would have probably been more definitive, the current numbers were sufficient to determine that genetic differences occurred compared to the published PON1 sequence. Further research is warranted to determine more precise estimates of allelic and genotype frequencies in Chinese cattle and crosses and to determine if these polymorphisms influence production performance.

CONCLUSION

Results from this study suggest that unique polymorphisms were identified in the bovine PON1 gene.
and further study is needed to determine if they might have an impact on phenotypic expression of growth and production traits in beef cattle.

ACKNOWLEDGEMENTS

The research was supported by the Agricultural Biotechnology Research and Application of Gansu (GNSW-2010-4, GNSW-2011-27) and Transforming Feed with Straw of the Agriculture and Animal Husbandry of Gansu (2130106).

REFERENCES


