Indian Journal of Animal Sciences **82** (2): 180–182, February 2012

Molecular investigation of glutathione peroxidase-1 (GPX-1) gene in Malvi cattle (*Bos indicus*) for draught capacity-- a comparative study

RAMESH JAGTAP[1] and SANJEEV SINGH[2]

Madhya Pradesh Pashu-Chikitsa Vigyan Vishwavidyalaya, Jabalpur, Madhya Pradesh 482 004 India

Received: 9 September 2010; Accepted: 14 October 2011

ABSTRACT

Glutathione peroxidase-1 (GPX-1) enzyme serves to detoxify peroxides by reacting with the GSH (reduced glutathione) which is responsible for the maintenance of the integrity of the essential bio-molecules. The study was conducted on 52 animals of Malvi breed of cattle (*Bos indicus*). The genomic DNA was isolated from the blood samples. Polymerase chain reaction was carried out to amplify 442 bp fragment (partial exon 2 and 3? UTR) of GPX-1 gene. Genetic polymorphism in the glutathione peroxidase-1(GPX-1) gene in Malvi breed of cattle was investigated by PCR-SSCP and direct sequencing. The study revealed the polymorphism in exon 2 region of GPX-1 gene in Indian cattle by PCR-SSCP and nucleotide sequencing. A and B alleles were distinguishable from each other due to point mutation at 189 position in 442 bp fragment. A allele had cytosine whereas B allele had thymine at this position. *Bos indicus* cattle showed 99.8% similarity with *Bos taurus* cattle. However, pig and mouse showed 88.2% and 76.0% similarity with the 442 bp fragment of GPX-1 gene of *Bos indicus* cattle, respectively. The genetic polymorphism identified in the present investigation would help in the identification of the draught capacity related alleles of the draught animals for their further genetic improvement.

Key words: Bos indicus, DNA sequencing, Draughtability, Genetic polymorphism, GPX-1, PCR-SSCP

A majority of Indian farmers possess small land holdings and largely depend on animals or manual power for different agriculture operations. Two-third of rural transportation and 70% of energy input in farm is contributed by draught animal power. Animal power is renewable natural resource that can assist in production conservation and in management of land and water. The programmes for improving draught animals have rather been meager, which resulted in enormous wastage of precious draught power resource. Therefore there is a need to generate simple and reproducible method for the assessment of draught capacity of animals which can be utilized for their genetic improvement. Strenuous aerobic exercise depletes antioxidants from the skeletal muscles, and sometimes also from other organs. Glutathione is an essential co-factor for antioxidant enzyme GPX-1 (glutathione peroxidase-1) which is encoded by GPX-1 gene. This GPX-1 enzyme serves to detoxify peroxides by reacting with GSH (reduced form) and thus contribute to the maintenance of integrity of essential bio-molecules like DNA, RNA and protein. The bullocks of Malvi breed are excellent draught animals, well adapted to the tropical conditions of their respective tracts. There is no scientific information available on draughtability and molecular marker studies related to exercise in Malvi breed of cattle. Handy *et al.* (2006) carried out the sequencing of this gene in *Bos taurus* species (accession no. NM_174076). In bovine GPX-1 gene is located on chromosome no. 22 and contains 2 exon and 1 intron (Mullenbach *et al.* 1988). Reports available in previous literature for the variability study in GPX-1 gene pertained to Homo sapiens (Hamanishi *et al*. 2004). No report is available about the genetic polymorphism study of GPX-1 gene in any breed of cattle. The strenuous exercise increases oxidative burden on body. GPX-1 gene is essentially involve in the antioxidant stress mechanism and contribute to reduce the oxidative stress. Furthermore, in humans, it was found that there is association between GPX-1 gene polymorphism (198 pro/leu) and the higher DNA damage, induced by oxidative stress (Miranda-Vilela *et al*. 2010). Therefore, the investigation was carried out to sequence the 442 bp fragment of GPX-1 gene (partial exon 2 and 3' UTR) of Indian cattle and compare it with the GPX-1 gene sequences of other species available at NCBI website.

Present address : [1]Ph.D. Scholar (docrameshjagtap @gmail.com), Department of Animal Biotechnology, Madras Veterinary College, Vepery, Chennai 600 007 India.

[2]Senior Scientist (drsanjeevivri@yahoo.com), NBAGR, Karnal, Haryana 132 001 India.

MATERIALS AND METHODS

The genomic DNA was isolated from 52 animals of Malvi breed of Cattle (*Bos indicus*) by phenol - chloroform extraction method (Sambrook and Russell 2000). Quantification and quality checking of DNA was carried out by spectrophotometer.

PCR-SSCP analysis: A 442 bp fragment of GPX-1 gene was amplified by PCR using primer pair P1 and P2.

P1: GAAAAGTGCGAGGTGAATGG

P2: GCTGTGGTCTGGGAAAGG

PCR-SSCP was carried out and the products were resolved on 12% PAGE with silver staining (Bassam *et al.* 1991). Gene and genotypic frequencies were calculated by gene counting method (Falconer and Mackey 1996).

Nucleotide sequencing: The nucleotide sequencing of the identified alleles was carried out by using an automated DNA sequencer (ABI prism). The nucleotide sequences were compared with the other available sequences of livestock species. The NCBI gene bank accession number of the sequences of various species considered were NM_174076 (*Bos taurus*), AF532927 (*Sus scrofa*), BC 000742 (*Homo sapiens*) and BC086649 (*Mus musculus*).

RESULTS AND DISCUSSION

Genotypic frequencies for 442 bp exon 2 fragment of GPX-1 gene in Malvi breed of cattle for different SSCP patterns are given in Table 1. Two alleles A and B were identified in the Malvi breed of cattle. The gene frequencies of A and B alleles were 0.749 and 0.249, respectively.

The A and B alleles were sequenced and 442 bp sequences of A and B allele of GPX-1 gene have been submitted to NCBI GenBank (National Center for Biotechnology Information, USA). The NCBI, GenBank accession numbers allotted for the A and B alleles are FJ467558 and FJ467559, respectively.

Table 1. Genotype frequency for 442 bp fragment of GPx-1 gene in Malvi bullocks

SSCP pattern	Genotype	Genotypic frequency
1	AA	0.53846 (n,28)
2	AB	0.423077 (n,22)
3	BB	0.0385(n,2)

Further, the sequence analysis revealed that A and B alleles were distinguishable from each other due to point-mutation at 189 position. A allele had cytosine whereas B allele had thymine at this position. This variation being a silent mutation did not alter the reading frame of the GPX-1 protein.

The comparative study of the A and B allele sequences of 442 bp fragment of GPX-1 gene of Malvi breed of cattle was carried out with other available NCBI sequences of the cattle, pig, human and mouse GPX-1 gene by DNASTAR software (DNASTAR Inc., USA). The pair distances between the species were observed (Fig.1) and phylogenetic tree (Fig.2) was also constructed to show the relatedness between the species with respect to GPX-1 gene fragment. The phylogenetic tree showed that the observed 442 bp sequences of A and B alleles of GPX-1 gene of *Bos indicus* cattle and GPX-1 gene of *Bos taurus* cattle fall in single monophyletic group. The pig is most closely related species to the observed A and B allele sequences of *Bos indicus* cattle next to *Bos taurus* cattle. The species next to pig is human being (*Homo sapiens*). The mouse was found to be most distantly related to the observed *Bos indicus* sequences as compared to other species. Furthermore, the similarity and divergence study as shown in Fig.1 revealed that the *Bos indicus* cattle showed

Percent Identity

	1	2	3	4	5	6		
1		99.8	100.0	88.2	79.4	76.7	1	A allele
2	0.2		99.8	88.0	79.2	76.5	2	B allele
3	0.0	0.2		88.2	79.4	76.7	3	*Bos taurus*
4	12.0	12.3	12.0		81.4	78.5	4	*Sus scrofa*
5	21.0	21.3	21.0	19.2		76.0	5	*Homo sapiens*
6	24.0	24.3	24.0	24.5	27.7		6	*Mus musculus*
	1	2	3	4	5	6		

Divergence

Fig 1. Nucleotide sequence similarity and divergence between various species on the basis of 442 bp fragment of GPX-1 gene

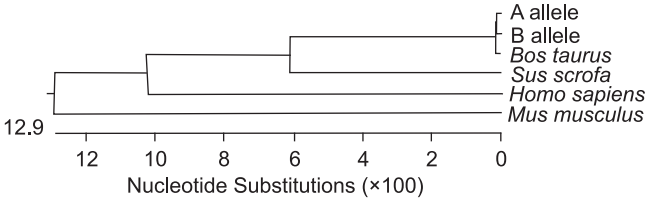

Fig 2. Phylogenetic tree based on 442 bp sequence of GPX-1 gene

99.8% similarity with *Bos taurus* cattle. However, the pig, human and mouse showed 88.2%, 81.4% and 76.0% similarity with the 442 bp fragment of GPX-1 gene of *Bos indicus* cattle, respectively.

GPX-1 gene is involved in the antioxidant stress mechanisms. Studies revealed that reduced glutathione (GSH) which is a cofactor of GPX-1 enzyme is depleted by exercise (Sen 1999). Moreover, it has been observed that natural selection plays an important role in the patterning of the genome of an organism. In positive Darwinian selection, some alleles are favoured over the other because of their survival or reproductive advantages. The evolution of this gene fragment in cattle from other livestock species might be due to its selective advantages in cattle for the draught purpose. Finally, it may be concluded that the 442 bp fragment of GPX-1 gene is polymorphic at nucleotide level in Malvi cattle.

ACKNOWLEDGEMENTS

The authors thank to the Director Biotechnology Center, Director Research Services, JNVV, Jabalpur, Madhya Pradesh and In-charge of Cattle Breeding Farm on Malvi cattle, Malwa region of Madhya Pradesh for providing the necessary facilities.

REFERENCES

Bassam B J, Caetano-Anolies G and Greshoff P M. 1991. Fast and sensitive silver-staining of DNA in polyacrylamide gels. *Analytical Biochemistry* **196**: 80–83.

Falconer D S and Mackay T F C.1996. *Introduction to Quantitative* Genetics. 4th edn., Addison wesely Longman Ltd. Essere, England, 1–2.

Hamanishi T H, Furuta H, Kato A, Doi M, Tamai H, Shimomura S, Sakagashira M, Nishi H, Sasaki T, Sanke and Nanjo K. 2004. Functional variants in the glutathione peroxidase-1 (GPx-1) gene are associated with increased intima-media thickness of carotid arteries and risk of macro vascular diseases in Japanese type 2 diabetic patients. *Diabetes* **53**: 2455–60.

Handy D E, Hang G, Seolaro J, Metes N, Razaq N, Yang Y and Loscalzo J. 2006. Aminoglycosides decrease glutathione peroxidase-1 activity by interfering with selenocysteine incorporation. *Journal of Biological Chemistry* **281**(6): 3382–88.

Miranda-Vilelal A L, Penha C Z A, Akimoto1 A K, Graciana S L, Carlos A G, Cesar K G and Maria N. 2010. Gene polymorphisms against DNA damage induced by hydrogen peroxide in leukocytes of healthy humans through comet assay: a quasi-experimental study. *Environmental Health* **9**: 21.

Mullenbach G T, Tabrizi A, Irvine B D, Bell G I, Tainer J A and Hallewell R A. 1988. Selenocysteine's mechanism of incorporation and evolution revealed in c-DNAs of three glutathione peroxidases. *Protein Engineering* **2**(3): 239–46.

Sambrook J and Russel D W. 2000. *Molecular Cloning: A Laboratory Manual*. 3rd edn. Cold Spring Harbor Laboratory Press, New York, USA.

Sen C K. 1999. Glutathione homeostasis in response to exercise training and nutritional supplements. *Molecular and Cellular Biochemistry* **196**: 31–42.