Pakistan J. Zool., vol. 48(6), pp. 1993-1995, 2016.

Short Communication

Comparative Genetic Diversity of *CYP11b1*, *OLR1* and *SCD* Gene in Bovid and non-Bovid Species

Maryam Javed,[1,*] Masroor Ellahi Babar,[2] Asif Nadeem,[1] Mohammad Imran,[1] Tahir Yaqub,[3] Tanveer Hussain,[2] Akhtar Ali,[1] Nadia Mukhtar[3] and Abu Saeed Hashmi[1]

[1]*Institute of Biochemistry & Biotechnology, University of Veterinary & Animal Sciences, Lahore, Pakistan, 54000*

[2]*Virtual University, Lahore, Pakistan, 54000*

[3]*Department of Microbiology, University of Veterinary & Animal Sciences, Lahore*

ABSTRACT

Present study was planned to probe the rate of molecular evolution of three genes (CYP11b1, OLR1 and SCD) involved in milk fat contents. The DNA sequencing phylogenetic analysis and construction of Neighbor-Joining trees for these genes showed buffaloes sharing cluster with other members of bovines as cattle, sheep, goat etc. So it was concluded that along with similarities with respect to morphologic and genetic characters, buffaloes and some other bovines are evolutionarily less divergent with respect to selected candidate genes.

Article Information

Received 3 April 2015
Revised 11 February 2016
Accepted 9 April 2016
Available online 20 October 2016

Authors' Contributions

MJ conceived the project, perform dry and wet lab. experiments and wrote the article. AN and AA helped in laboratory experimentation. TH, AA were involved in research sampling. MI, TY, NM and ASH analyzed the data. MEB supervised the work.

Key words

Buffalo, Milk production OLRI, SCD, CYP11b1, Phylogenetic analysis

Variation in genetic make-up of dairy animals leads to variation in milk fat content even in the animals of same breed (Pasha and Hayat, 2012). In many regions of the world, techniques have been practiced in molecular genetics in combination with conventional animal breeding to improve animal breeding programmes, ensuing higher fat content due to better genetics (Afzal, 2010). Buffalos are raised in many regions of the world especially Asian countries including Pakistan (Baber *et al*., 2009). Improvement of animals via genetics has been proven effective (Bilal and Sajid, 2005). Many of the genes controlling production traits that are genetically informative in one specie or breed have been found less revealing in others and vice versa (Han *et al*., 2012; Hussain *et al*., 2006, Jiang *et al*., 2010). Some of the confirmed markers (as in DGAT-1, Leptin, Prolactin) could not be validated in buffaloes. Although buffalo-cattle homology is more than 80% but still specie differences are there. Therefore it is needed to find the regions of high similarity in cattle-buffalo and other bovines to identify common genetic markers which can be useful across species.

Keeping in view this negation, present study was planned to approximate the rate of evolution of three genes (CYP11b1, SCD and OLR1) in buffaloes. These genes have been found to be involved in controlling fat content of the milk. Many of previous reports provide information about the association of CYP11b1 gene with milk quality traits especially fat content (Khan *et al*., 2012). Oxidized low density lipoprotein receptor 1 (OLR1) is a type-II membrane surface protein that belongs to C-type Lectin family (Khatib *et al.*, 2006). This receptor acts as the major cell surface receptor for oxidized low density lipoprotein (Ox-LDL) (Kataoka *et al*., 2000). SCD is a multifunctional complex enzyme important in the cellular biosynthesis of fatty acids (Ntambi and Miyazaki, 2004).

Materials and methods

Fifty blood samples of true representatives of Nili-Ravi buffalo were collected from different areas of Punjab province of Pakistan, and processed for genomic DNA extraction by opting method described by Maryam *et al.* (2012). Standard sequences of other species were retrieved from DNA data base NCBI (www.ncbi.nlm.nih.gov).

Primers designed for coding regions of three candidate genes (CYP11b1, OLR1 and SCD) reported by Javed *et al.* (2013a,b,c) were used for PCR amplification. The amplicons were purified and sequenced by Sanger's chain termination method.

Multiple sequence alignment was performed for all sequences under investigation. Then phylogenetic analysis was performed by using software MEGA6 (www.megasoftware.net). Neighbor-Joining method of phylogeny was used to construct evolutionary trees for three genes under investigation.

* Corresponding author: maryam.javed@uvas.edu.pk
0030-9923/2016/0006-1993 $ 8.00/0

Results and discussion

The sequences all the three candidate genes were aligned and compared in MEGA6. Phylogenetic trees were constructed for CYP11b1, OLR1 and SCD gene sequences (Figs. 1, 2 and 3). In-silico evolutionary analysis of these genes illustrated their high sequence similarity among different bovid species and common or shared ancestry leading towards comparatively less species divergence than many other species (except bovid). These genes were analyzed independent of each other and similar results were found in all the trees.

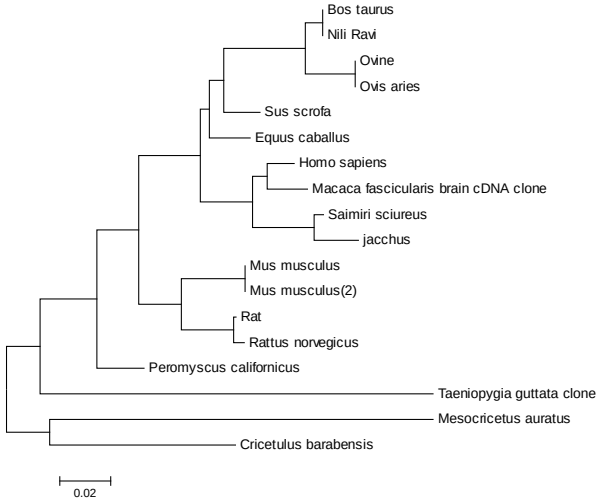

Fig. 1. Neighbor Joining Tree for CYP11b1 gene (*Steroid 11-beta-hydroxylase*).

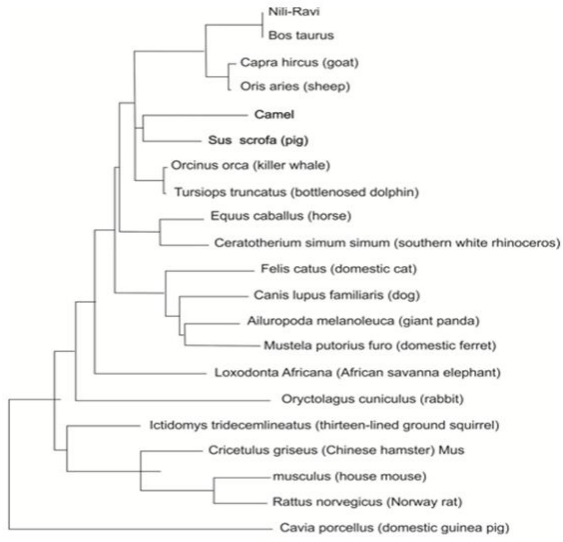

Fig. 2. Neighbor-joining tree for OLR1 (oxidized low density lipoprotein (lectin-like) receptor 1).

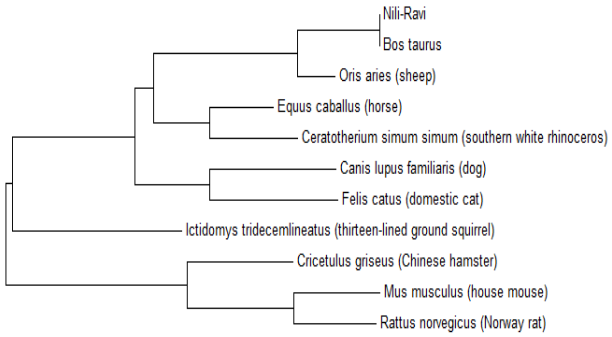

Fig. 3. Neighbor-joining tree for SCD (stearoyl-CoA desaturase).

Along with evolutionary trees, evolutionary divergence between sequences of each gene was also estimated. These results have been mentioned in Supplementary Tables I, II and III. Number of base substitutions per site from between sequences have been shown. Analyses were conducted using the Maximum Composite Likelihood model (Tamura *et al.*, 2004). All positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted in MEGA6.

Present study investigated the genetic homology of river buffalo with other bovid and non-bovid species. In CYP11b1, Same results were reported previously by Aditi *et al*. (2013) for some other genes controlling milk quality. The gene tree and species tree analysis revealed a clear homology between members of Bovidae which cluster together. Vijh *et al.* (2008) also reported phylogenetic relatedness of buffalo with other members of bovidae family. This study illustrated that genes involved in production traits have high sequence homology in members of bovid group, so common markers for improved milk production can be identified. Another study was carried out to reveal haplotype and phylogenetic analysis of OLR1 gene in Jaffarabadi and Surti by Shabir *et al.* (2011). They identified intra-breed variations that could be use as probable markers for fat content in milk. Goldammer and co-workers (2007) also reported the evolutionary conserved regions in buffalo chromosome number-7. Kumar *et al*. (2007) also reported mitochondrial DNA analyses of Indian water buffalo and illustrated distinct genetic origin of river and swamp buffalo than other members of family Bovidae. Wani *et al.* (2014) reported similar results for Murrah buffalo after sequencing and phylogenetic analysis of CXCR2 gene controlling genetic resistance against mastitis. He also concluded that only single set of markers can be used for selection of genetically superior animals of all members of bovidae.

Conclusion

This study provides information about relationship of the riverine buffalo of Pakistan, covering three genes involved in milk producing abilities. Genetic relatedness of these genes among different bovine species provides their equal usefulness and worth across species which can be a baseline in our objectives of finding common genetic markers for improved milk quality that would be equally valid across species and would save many of the research inputs.

Conflict of interest

There is no conflict of interest for the contents presented in this paper.

Supplementary Tables I, II and III is available at website (http://zsp.com.pk/pdf48/QPJZ-0147-2015-F%20SUPPLEMENTARY%20TABLES.pdf)

References

Aditi, S., Kanwar, S.S., Tantia, M.S. and Vijh, R.K., 2011. *Int. J. Livest. Prod.*, **2:** 172-181.

Afzal, M., 2010. *Pakistan Vet. J*., **30**: 187-190.

Baber, M.E., Hussain, T., Nadeem, A., Jabeen, R. and Javed, M., 2009. *Pakistan J. Zool.*, **9**(Suppl.ser.): 361- 366.

Bilal, M.Q. and Sajid, M.S., 2005. *The Nation.* May. 29: 2005.

Han, X., Lee, F.L., Zhang, L. and Guo, M.R., 2012. *Funct. Fds. Hlth. Dis*., **2**: 86-106.

Goldammer, T., Weikard, R., Miziara, M.N., Brunner, R.M., Agarwala, R., Schäffer, A.A., Womack, J.E. and Amaral, M.E.J., 2007. *Cytogen. Genom. Res*., **119**: 235–241.

Hussain, Z., Javed K., Hussain, S.M.I. and Kiyani, G.S., 2006. *J. Anim. Pl. Sci*. **16**: 15-19.

Jiang, L., Liu, J., Sun, D., Ma, P., Ding, X., Yu, Y. and Zhang, Q., 2010. *PloS One*, **5**: e13661.

Kataoka, H., Kume, N., Miyamoto, S., Minami, M., Murase, T., Sawamura, T., Masaki, T., Hashimoto, N. and Kita, T., 2000. *J. biol. Chem*., **275**: 6573-6579.

Khan, S., M.S. Qureshi, N., Chand, A., Sultan, Rafulah, I. Khan, Ihsanulah, A.J. Tanwer S.M. Sohail M. Husain, A. Akhtar., D. Khan., 2012. *Sarhad J. Agric*., **28**: 469-476.

Khatib, H., Leonard, S.D., Schutzkus, V., Luo, W. and Chang, Y.M., 2006. *J. Dairy Sci*., **89**: 1753–1760.

Kumar, S., Nagarajan, M., Sandhu, J.S., Kumar, N., Behl, V. and Nishanth, G., 2007. *Anim. Genet*., **38**: 227–232.

Maryam, J., Babar, M.E., Nadeem, A. and Hussain, T., 2012. *Mol. biol. Rep*., **39**: 4565–4570.

Javed, M., Babar, M.E., Nadeem, A., Yaqub, T. and Hussain T., 2013a. *Buffalo Bull*., **32**: 701-705.

Javed, M., Nadeem A., Babar, M.E. and Manzoor, S., 2013b. *Buffalo Bull*., **32**: 697-700.

Javed, M., Nadeem, A. and Babar, ME., 2013c. *Buffalo Bull*., **32**: 710-713.

Ntambi, J.M. and Miyazaki, M., 2004. *Progr. Lipid Res*., **43**: 91–104.

Pasha, T.N. and Hayat, Z., 2012. *J. Anim. Pl. Sci. Suppl*., **3**: 250-256.

Shabir, N., Jawale, C.V., Bhong, C.D., Naikoo, M., Rank, D.N. and Josh, C.G., 2011. *Vet. World*, **4:** 396-398.

Tamura, K., Nei, M. and Kumar, S., 2004. *Proc. natl. Acad. Sci. (USA)*, **101:** 11030-11035.

Vijh, R.K., Tantia, M.S., Mishra, B. and Bharani-Kumar S.T., 2008. *J. Anim. Sci*., **86:** 1495-1502.

Wani, S.A., Sangwan, M.L., Dar, M.A., Kumar, A., Rafee, M.A. and Baro, D., 2014. *Vet. World*, **7:** 342-346.