Short Communication

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MITOCHONDRIAL CYTOCHROME B GENE BASED PHYLOGENY OF LOHI AND THALLI SHEEP BREEDS OF PAKISTAN

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ABSTRACT

Small ruminants (sheep and goat) are major contributors to the livestock sector of Pakistan with 95.7 million heads raised mainly for meat, milk and wool. The 34 sheep breeds spread throughout the country manifest precious genetic diversity in Pakistan. The genetic structure of two sheep breeds (Lohi and Thalli) was explored using complete mitochondrial Cytochrome b gene sequences (1140 bp). The sequence analysis revealed four Lohi and five Thalli haplotypes. There were more transitions than transversions in these sequences. The phylogenetic analysis of Lohi and Thalli breeds with previously reported sheep breeds from different countries indicated two distinct groups. Pakistani and Indian sheep breeds clustered together while Turkish breeds clustered with European sheep breeds. This study was found informative for establishing relationships between breeds from different parts of the world. This study may facilitate the future researchers and breeders for better understanding the genetic interactions and breed differentiation for devising future breeding and conservation strategies to preserve the rich animal genetic reservoir of the country.

Key word: Sheep genetic diversity, Mitochondrial Cytochrome b gene, Haplotypes, Phylogenetic analysis, Pakistan

INTRODUCTION

Sheep (29.1 million) and goat (66.6 million) in Pakistan have valuable contributions to the economy of the country (Economic Survey, 2013-14). The 34 breeds of sheep are raised throughout the country, in almost all climatic and geographical regions of Pakistan, forming an important component of Animal Genetic Resource and playing its significant role in economic income of poor farmers. (Anonymous 2010-11). Pakistan is the tenth largest sheep producing country in the world (Khan *et al.,* 2008). In spite of great sheep gene resources, there were very less works available in Pakistan about the genetic architecture of different sheep breeds.

To evaluate the genetic structure of sheep breeds of Pakistan, initially the two sheep breeds (Lohi and Thalli) were selected from the Punjab province for their genetic evaluation on the basis of mitochondrial Cytochrome b gene. Mitochondrial DNA (mtDNA) is an important tool for phylogenetic studies and has been widely used to study genetic differentiation, genetic complexity, evolutionary relationships and origins of many domestic animals (Wilson *et al*. 1985; Carmela *et al.,* 2000) including cattle (Loftus *et al.,* 1994), buffalo (Babar *et al.,* 2011a), sheep (Loehr *et al.,* 2006) and goats (Joshi *et al.,* 2004; Babar *et al.,* 2011b). The present study was conducted to access the genetic structure and phylogenetic relationships between them and with other sheep breeds of Pakistan and the world.

MATERIALS AND METHODS

Collection of Blood Samples and DNA Isolation: True representative animals of Lohi and Thalli sheep breeds were selected from Livestock Experiment Station, Bahadurnagar (Okara) and Angora Goat Farm, Rukh Khairewala, (Layyah). Ten mL blood sample was collected aseptically from 25 unrelated individuals of both breeds into ethylenediamine tetra-acetic acid (EDTA) added tubes and transported to laboratory in ice. DNA was extracted from whole blood using the method as described by Hussain *et al*., 2013. All DNA samples were brought to the final concentration of 50 ng/µL and stored at -80 C°.

Amplification of Mitochondrial Cytochrome b gene: Specific primers were designed from flanking regions of cytochrome b gene (Table 1) using software Primer3 (http://frodo.wi.mit.edu/) (Steve and Skaletsky, 2000) from complete mitochondrial genome of *Ovis aries* (AF010406) available on NCBI (http:// www. ncbi. nlm. nih.gov). Bio-Rad thermocycler was used for primer optimization and PCR amplification of all samples for Cytochrome b gene. A volume of 25 µL was used as reaction mixture for PCR using conditions: 95 C° for 4 minutes as initial denaturation, 94 C° for 30 seconds of denaturation, 59 C° for 30 seconds for annealing, 72 C° for 30 seconds for extension and 72 C° for 10 minutes for final extension. PCR products were indentified using 1.2 % agarose gel and positive samples were purified by

ethanol for DNA sequencing using ABI PRISM Genetic Analyzer 3130 xl. (Sanger *et al*., 1977)

Sequence Alignment and Analysis: Cytochrome b gene sequences were visualized using Chromas software (Tatusova *et al.,* 1999) aligned with the help of BLAST software available on NCBI. Single Nucleotide Polymorphisms (SNPs) were detected from aligned sequences and consensus sequences, and haplotypes were construted. For phylogenetic analysis *MEGA* 5 software (Tamura *et al*. 2011) was used.

RESULTS AND DISCUSSION

The analysis of complete Cytochrome b sequences (1140 bp) in selected animals revealed a total of 9 different haplotypes (5 in Thalli and 4 in Lohi breed). A/T contents were rich in all the haplotypes. More transitions were found than transversions in the haplotypes. The phylogenetic tree of Lohi and Thalli haplotypes were constructed using UPGMA method with reported sheep sequences from India (Deccani FJ218118, Tibetan AY879582, Bannur FJ218019, Garole FJ218040), Turkey (Tuj DQ851960, Sakiz DQ852006, Karayaka DQ097408), Scotland (Finn Dorset DQ320090), Finland (Grey Finn sheep AY879520), Italy (Musimon FR873151), Germany (Merino land sheep NC001941) with goat (*Capra hircus*) and cattle (*Bos indicus*) as out groups (Fig. 1).

The UPGMA phylogenetic tree indicated two distinct groups. All Pakistani haplotypes were clustered together with Indian sheep breeds. In the second group, Turkish and European countries sheep sequences clustered together. The tree indicated the close relationships between Lohi and Thalli sheep breeds of Pakistan, further these breeds were found closer to Indian sheep breeds as well referring to the possibility of some common domestication history in the past. The Turkish sheep breeds clustered with European sheep breeds also strengthened the concept of sheep breeds transferred from Turkey to Europe (Fig. 1).

This work resulted in useful information about the genetic relationships of selected Pakistani sheep breeds with sheep breeds from different regions of the world. This data may be used for further phylogenetic and evolutionary studies in future. The findings of present study may help the researchers and animal breeders to better understand genetic relationships between indigenous and exotic sheep breeds, and help to design effective breeding policies in the country. The present information also contributed to the evolutionary data of the modern sheep breeds. The data obtained in this study is significant as very little information is available about Pakistani sheep phylogenetics and origin.

Table 1: Sheep Mitochondrial Cytochorme b gene Primers

Primers	5'-3' Sequence	Product length (bp)
Cyto b –I	Forward: CATGGAATCTAACCATGACCAA	676
	Reverse: CTCTTCCTCCACGAAACAGG	
Cyto b –II	Forward: CGATTTTTCGCCTTTCACTT	677
	Reverse: GAAGGAGAACAACCAACCTCC	

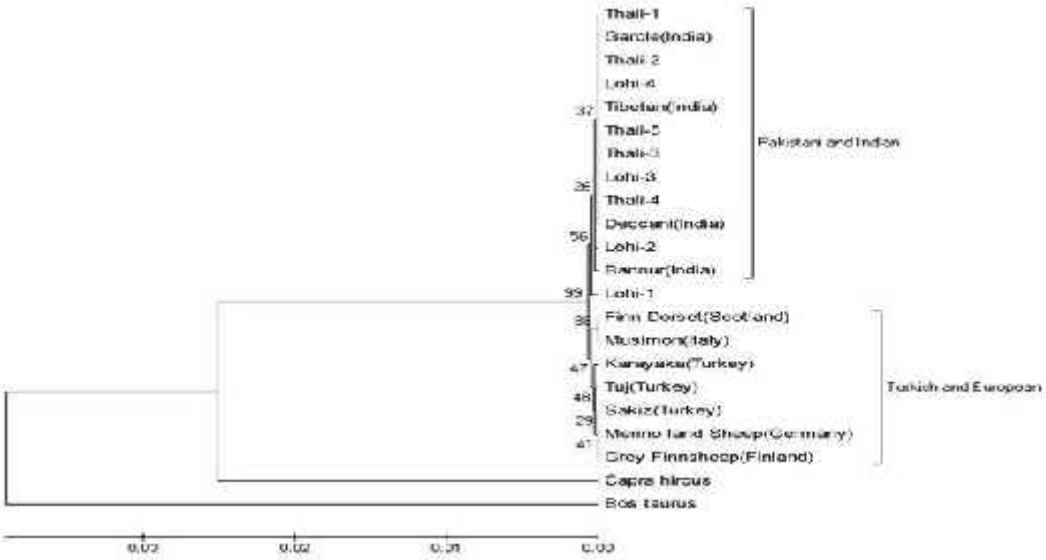

Fig. 1. Phylogenetic tree (UPGMA method with bootstrap value 1000) of Pakistani haplotypes with reported sequences from India, Turkey, Scotland, Finland, Italy and Germany using MEGA5 software.

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REFERENCES

Anonymous, (2013-14). Pakistan Economic Survey. Government of Pakistan, Finance division, Economic Advisor's Wing Islamabad.

Babar, M.E., T. Hussain, M. Imran, M. Nagarajan and S. Kumar (2011a). Mitochondrial DNA diversity patterns in Pakistani buffalo. Anim. Genetics. 43(3):315-7.

Babar, M. E., T. Hussain, H. Sadia, M. Shaheen, A. Nadeem, A. Ali, A. Wajid and S. A. Shah, (2011b). Molecular characterization, evaluation and conservation of indigenous goat breeds of Pakistan. Proceedings of RBI 8th Global Conference on the Conservation of Animal Genetic Resources Tekirdag, Turkey, 4-8 October: 43-50.

Carmela, G., A. Reyes, G. Pesole and C. Saccone (2000). Lineage-Specific Evolutionary Rate in Mammalian mtDNA. Mol. Biol. Evol. 17 (7):1022-1031.

Hussain T, M. E. Babar, H. Sadia, M. Shaheen, A. Nadeem, A. Ali, A. Wajid and S. A.Shah. (2013). Microsatellite markers based genetic diversity analysis in Damani and Nachi goat breeds of Pakistan. Pakistan Vet. J. 33(4): 520-522

Joshi, M. B., P. K. Rout, A. K. Mandal, C. Tyler-Smith and L. Singh (2004). Phylogeography and origin of Indian domestic goats. Mol. Biol. Evol. 21(3): 454-462.

Khan M.S., M.A. Khan, S. Mahmood (2008). Genetic resources and diversity in Pakistani goats. Int J Agric Biol.10(2): 227-231.

Loehr, J., K. Worley, A. Grapputo, J. Carey, A. Veitch and D.W. Coltman (2006). Evidence for cryptic glacial refugia from North American mountain sheep mitochondrial DNA. J. Evol. Biol. 19(2): 419-430.

Loftus R. T., D. E. MacHugh, D. G. Bradley, P. M. Sharp and P. Cunningham (1994). Evidence for two independent domestications of cattle. Proc. Natl Acad. Sci. USA. 91(7):2757-2761.

Sardina, M. T., M. Ballester, J. Marmi, R. Finocchiaro, J. B. C. H. M. van Kaam, B. Portolano and J. M. Folch (2006). Phylogenetic analysis of Sicilian goats reveals a new mtDNA lineage. Anim. Genetics. 37(4): 376-378.

Steve, R., and H. J. Skaletsky, (2000). Primer 3 on the www for general users and for biologist programmers. In: Krawetz S, Misener S (eds) Bioinformatics Methods and Protocols: Methods in Molecular Biology. Humana Press, Totowa,NJ.132: 365-386.

Tamura K., D. Peterson, N. Peterson, G. Stecher, M. Nei and S. Kumar (2011). MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution. 28(10):2731-9.

Wilson, A. C., R. L. Cann, S. M. Carr, M. George, U.B. Gyllensten, K. M. Helm-Bychowski, R. J. Higuchi, S. R. Palumbi, E. M. Prager, R. D. Sage and M. Stoneking (1985). Mitochondrial DNA and two perspectives on evolutionary genetics. Biology J. Linnean Society. 26(4): 375-400.