© Indian Academy of Sciences

RESEARCH NOTE

Comparative FISH mapping of *BMPR1B*, *BMP15* and *GDF9* fecundity genes on cattle, river buffalo, sheep and goat chromosomes

A. FARHADI[1,2], V. GENUALDO[2], A. PERUCATTI[2], S. H. HAFEZIAN[1], G. RAHIMI-MIANJI[1], L. DE LORENZI[3], P. PARMA[3], L. IANNUZZI[2] and A. IANNUZZI[2]*

[1]*Laboratory for Molecular Genetics and Animal Biotechnology, Department of Animal Science, Faculty of Animal Science and Fisheries, Sari Agricultural Sciences and Natural Resources University, P.O. Box 578, Sari, Iran*
[2]*National Research Council (CNR), Institute for Animal Production System in Mediterranean Environment, Laboratory of Animal Cytogenetic and Gene Mapping, Via Argine. 1085, 80147 Napoli, Italy*
[3]*Department of Agricultural and Environmental Sciences, University of Milan, Via Celoria 2, 20133 Milan, Italy*

[Farhadi A., Genualdo V., Perucatti A., Hafezian S. H., Rahimi-Mianji G., De Lorenzi L., Parma P., Iannuzzi L. and Iannuzzi A. 2013 Comparative FISH mapping of *BMPR1B*, *BMP15* and *GDF9* fecundity genes on cattle, river buffalo, sheep and goat chromosomes. *J. Genet.* **92**, 595–597]

Introduction

One of the main goal in livestock genomic projects is the preparation of extensive genomic maps that identify loci affecting economically important traits. For this reason, the aim of this study was to compare several physical maps using bovine artificial chromosomes (BAC) clones containing genes related to fecundity genes. Fluorescent *in situ* hybridization (FISH) was performed on three gene sequences: growth differentiation factor 9 (*GDF9*), bone morphogenetic protein 15 (*BMP15*) and its type I receptor (*BMPR1B*). They were studied on cattle (BTA), river buffalo (BBU), sheep (OAR) and goat (CHI) for the first time. *GDF9* was assigned to BTA7q22.3, BBU9q24, OAR5q22.3 and CHI7q22.3; *BMP15* was assigned to BTAXq31, BBUXq36, OAR/CHIXq24; *BMPR1B* was assigned to BTA/OAR/CHI6q15 and BBU7q21. All loci were mapped on homologous chromosome and chromosome bands, underling the high degree of chromosome homologies among bovids and extending the cytogenetic maps of these four economically important species.

Cytogenetic maps are useful tools for various applications in animal cytogenetics for several reasons: precise physical position on single chromosome bands of both type I and type II loci, especially using FISH mapping on R-banded chromosome preparations (Di Meo *et al.* 2007); use of molecular markers to confirm chromosomes involved in chromosome abnormalities; anchoring linkage and RH maps; genome sequence contigs to specific chromosomal regions (Goldammer *et al.* 2009). Cattle (*Bos taurus*, $2n = 60$, BTA), river buffalo (*Bubalus bubalis*, $2n = 50$, BBU), sheep (*Ovis aries*, $2n = 54$, OAR) and goats (*Capra hircus*, $2n = 60$, CHI) are very close to each other from the evolutionary point of view and are also the four major domestic bovid species of great economic importance. Although the location of a lot of genes in these species was identified by linkage and RH mapping, only a small percentage of those loci were physically assigned to the corresponding bands of specific chromosomal location (Di Meo *et al.* 2007). So far, several studies on the physical gene mapping using FISH methodology were reported for cattle, river buffalo, sheep, goat and other livestock (Iannuzzi *et al.* 2003). In the present study, three major fecundity genes (*BMPR-1B, BMP15* and *GDF9*) were comparatively FISH mapped on cattle, sheep, goat and river buffalo R-banded chromosomes for first time, extending the cytogenetic maps of these species. The role of these genes as either a major gene that influences the prolificacy or a molecular genetic marker was reported in different studies. Sheep carrying the *Booroola* gene have a mutation in *BMPR-IB* expressed in the ovary; in fact, this autosomal gene has an additive effect on ovulation rate and incomplete dominance effect on litter size (Mulsant *et al.* 2001). On the other hand, six mutations were described in sex-linked *BMP15* gene affecting prolificacy in sheep (Monteagudo *et al.* 2009). There is also a mutation in the autosomal *GDF9* gene that causes increased ovulation rates in heterozygous ewes (Hanrahan *et al.* 2004).

Peripheral blood samples from cattle (Agerolese breed), sheep (Laticauda breed), goat (Cilentana breed) and river

*For correspondence. E-mail: alessandra.iannuzzi@ispaam.cnr.it.
A. Farhadi and V. Genualdo contributed equally to this work.

Keywords. FISH mapping; fecundity genes; cattle; river buffalo; sheep; goat.

Table 1. BAC-probes, indentified DNA sequences of FISH-mapped genes in cattle (BTA), river buffalo (BBU), sheep (OAR), goat (CHI) chromosomes (ISCNDB2000, Di Berardino *et al*. 2001 and CSKBB 1994), comparison with human (HSA) chromosomes (HGNC-genenames.org).

BAC FISH probe	Identified DNA sequence within BAC and locus symbol (HGNC)	Gene name	Cytogenetic localization on RBPI-bands				
			BTA	BBU	OAR	CHI	HAS
BtINRA-152G11	*BMPR1B*	Bone morphogenetic protein receptor 1 B	6q15	7q21	6q15	6q15	4q23-q24
BtINRA-748C10 + BtINRA-320H10	*BMP15*	Bone morphogenetic protein 15	Xq31	Xq36	Xq24	Xq24	Xp11.2
BtINRA-544F11	*GDF9*	Growth differentiation factor 9	7q22.3	9q24	5q22.3	7q22.3	5q31.1

buffalo were cultured and treated for late BrdU and Hoechst 33258 incorporation according to Iannuzzi and Di Berardino (2008). The bovine BAC clones overlapping studied genes (table 1) were screened by database searching and ordered from INRA bovine BAC library (Biological Resources Centre dedicated to livestock genomics (CRB), INRA, Jouy-en Josas, France). Extraction of DNA was done using CHORI (Children's Hospital Oakland Research Institute, Oakland, USA) recommended protocol. DNA was labelled with biotin using nick-translation kit (Roche, Monza, Italy). Slides were then treated for FISH with BAC clones overnight in presence of bovine COT-l DNA and sonicated salmon sperm allocated in a moist chamber. After detection steps with FITC-avidin and anti-avidin antibodies, slides were stained with propidium iodide (PI), and mounted with 1:1 ratio of Antifade/PI. Both RBPI-banded metaphases (R-banding by late incorporation of BrdU and propidium iodide staining) and fluorescent FITC signals were separately captured by a CCD-camera (Photometrics cool SNAP, Nikon, Cusago Milano, Italy) and processed by superimposing FITC signals on RBPI-banded preparations. Chromosome identification and banding followed the standard karyotypes for cattle,

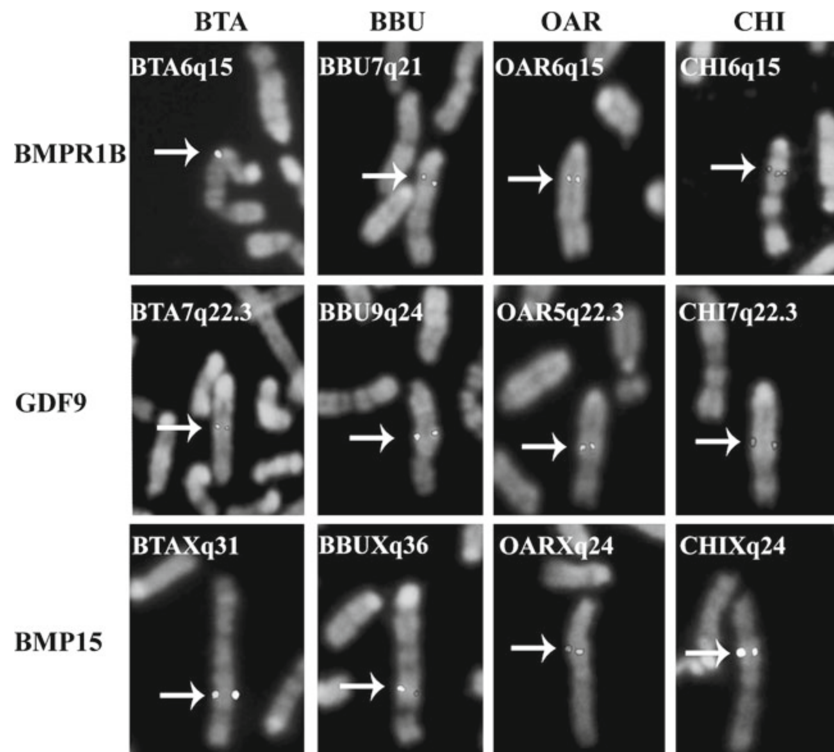

Figure 1. Representative FISH results on cattle (BTA), river buffalo (BBU), sheep (OAR) and goat (CHI) chromosomes, using bovine BAC clones containing genes related to fecundity (*BMPR1B*, *GDF9* and *BMP15*). FITC signals were superimposed on RBPI-banding (R-banding using early BrdU-incorporation and propidium iodide staining).

sheep and goat (ISCNDB2000, Di Berardino *et al.* 2001) and river buffalo (CSKBB 1994).

Three major genes (*BMPR1B, BMP15* and *GDF9*), affecting ovulation rate and prolificacy, were physically FISH mapped on cattle, river buffalo, sheep and goat metaphase chromosomes (figure 1). The frequency of FITC signals (chromosomes with single or double-spot in one or both chromatids) varied from 35% (*BMPR1B*) in sheep to 80% (*BMP15*) in cattle. Loci FISH mapped with locus name, symbol, clone identification and chromosome localization are reported in table 1. *BMPR1B* maps on BTA/OAR/CHI6q15 and BBU 7q21; *BMP15* maps on BTAXq31, BBU Xq36 and OAR/CHIXq24; *GDF9* was assigned to BTA7q22.3, BBU9q24, OAR5q22.3 and CHI7q22.3. The three loci were located in homologous chromosomes and chromosome bands of the four species extending the cytogenetic maps in these three species chromosomes. Further, no BAC covering *BMP15* gene are available, so we chose two BAC covering genomic regions before and after the gene and used as a mixing probe.

During the last 15 years, FISH techniques have been used in domestic animals research mainly to identify chromosomal rearrangements, gene mapping, comparative mapping, and evolutionary chromosome studies. The localization of *BMPR1B* on BTA/OAR6, *BMP15* on OARX and *GDF9* on OAR5, using FISH analysis, was in agreement with those previously reported with linkage mapping analysis (Kim *et al.* 2003). Their localization on homologous chromosomes and chromosome bands in cattle, sheep, goat and river buffalo (figure 1; table 1) confirmed the high conservation of autosomal chromosomes among the bovid species and extended the cytogenetic maps of the four economically important domestic species. Remembering that De Lorenzi *et al.* (2010) reported the wrong position of *DFNA5* and *CHCHD6* genes in cattle genome assembly using FISH mapping analysis, it is important to verify the data of cattle and other animal genomes assemblies using physical localization of genes.

Acknowledgements

The authors are very grateful to Domenico Incarnato, CNR-ISPAAM, Naples for excellent technical assistance. The study has been supported by PSR, Misura 214, e2 of Campania Region, Project 'Razze Autoctone a Rischio di Estinzione della Regione Campania – RARECa', CISIA-VARIGEAV project and Iran National Science Foundation (INSF).

References

CSKBB 1994 Standard karyotype of the river buffalo (*Bubalus bubalis* L., 2*n* = 50). Report of the committee for the standardization of banded karyotype of the river buffalo (Iannuzzi L., coordinator). *Cytogenet. Cell Genet.* **67**, 102–113.

De Lorenzi L., Molteni L. and Parma P. 2010 FISH mapping in cattle (*Bos taurus* L.) is not yet out of fashion. *J. Appl. Genet.* **51**, 497–499.

Di Berardino D., Di Meo G. P., Gallagher D. S., Haves H. and Iannuzzi L. 2001 International system for chromosome nomenclature of domestic bovids ISCNDB 2000. *Cytogenet. Cell Genet.* **92**, 283–299.

Di Meo G. P., Perucatti A., Floriot S., Hayes H., Schibler L., Rullo R. *et al.* 2007 An advanced sheep (*Ovis aries*, 2*n* = 54) cytogenetic map and assignment of 88 new autosomal loci by fluorescence in situ hybridization and R-banding. *Anim. Genet.* **38**, 233–240.

Goldammer T., Di Meo G. P., Lühken G., Drögemüller C., Wu C. H., Kijas J. *et al.* 2009 Molecular cytogenetics and gene mapping in sheep (*Ovis aries*, 2*n* = 54). *Cytogenet. Genome Res.* **126**, 63–76.

Hanrahan P. J., Gregan S. M., Mulsant P., Mullen M., Davis G. H., Powell R. and Galloway S. M. 2004 Mutation in the genes for oocyte-drived growth factors GDF9 and BMP15 are associated with both increased ovulation rate and sterility in Cambridge and belclare sheep (*Ovis aries*). *Biol. Reprod.* **70**, 900–909.

Iannuzzi L. and Di Berardino D. 2008 Tools of the trade: diagnostics and research in domestic animal cytogenetics. *J. Appl. Genet.* **49**, 357–366.

Iannuzzi L., Perucatti A., Di Meo G. P., Schibler L., Incarnato D. and Cribiu E. P. 2003 Chromosomal localization of sixty autosomal loci in sheep (*Ovis aries*, 2*n* = 54) by fluorescence in situ hybridization and R-banding. *Cytogenet. Genome Res.* **103**, 135–138.

Kim J. G., Smith T. P. L., Snelling W. M., Vallet J. L. and Christenson R. K. 2003 Linkage mapping of the bovine bone morphogenetic protein receptor-1B (BMPR1B) to chromosome 6. *Anim. Genet.* **34**, 302–318.

Monteagudo L. V., Ponz R., Teresa Tejedor M., Lavina A. and Sierra I. 2009 A 17 bp deletion in the Bone Morphogenetic Protein 15 (BMP15) gene is associated to increased prolificacy in the Rasa Aragonesa sheep breed. *Anim. Reprod. Sci.* **110**, 139–146.

Mulsant P., Lecerf F., Fabre S., Schibler L., Monget P., Lanneluc I., *et al.* 2001 Mutation in bone morphogenetic protein receptor-IB is associated with increased ovulation rate in Booroola Merino ewes. *Proc. Natl. Acad. Sci. USA* **98**, 5104–5109.

Received 19 March 2013, in revised form 18 July 2013; accepted 24 July 2013
Published on the Web: 6 December 2013