Fluorescence in situ hybridization mapping of six loci containing genes involved in the dioxin metabolism of domestic bovids

Viviana Genualdo, Veronica Spalenza, Angela Perucatti, Alessandra Iannuzzi, Giulia Pia Di Meo, Annamaria Caputi-Jambrenghi, Gino Vonghia , Roberto Rasero, Carlo Nebbia, Paola Sacchi, Leopoldo Iannuzzi

Journal of Applied Genetics Vol. 52, Issue 2, pp 229-232, 2011, DOI: 10.1007/s13353-011-0043-3

Fluorescence in situ hybridization mapping of six loci containing genes involved in the dioxin metabolism of domestic bovids

Viviana Genualdo, Veronica Spalenza, Angela Perucatti, Alessandra Iannuzzi, Giulia Pia Di Meo, Annamaria Caputi-Jambrenghi, Gino Vonghia , Roberto Rasero, Carlo Nebbia, Paola Sacchi, Leopoldo Iannuzzi

Abstract Six loci containing genes involved in the dioxin metabolism (ARNT, AHR, CYP1A1, CYP1A2, CYP1B1 and AHRR) were assigned, for the first time, to cattle (Bos taurus, 2n = 60, BTA), river buffalo (Bubalus bubalis, 2n = 50, BBU), sheep (Ovis aries, 2n = 54, OAR) and goat (Capra hircus, 2n = 60, CHI) chromosomes by comparative FISH-mapping and R-banding using bovine BAC-clones. The following chromosome locations were found: ARNT to BTA3q21, BBU6q21, OAR1p21 and CHI3q21, AHR to BTA4q15, BBU8q15, OAR4q15 and CHI4q1 5; CY P1A 1 and CY P1A 2 to BTA 21q 17, BBU20q17, OAR18q17 and CHI21q17; CYP1B1 to BTA11q16, BBU12q22, OAR3p16 and CHI11q16, AHRR to BTA20q24, BBU19q24, OAR16q24 and CHI20q24. All loci were mapped at the same homoeologous chromosomes and chromosome bands of the four bovid species. Comparisons with corresponding human locations were also reported.

Dioxins are a large family of chlorinated compounds which includes polychlorodibenzodioxins (PCDDs), polychlorodibenzofurans (PCDFs) and dioxin-like polychlorobiphenyls (DL-PCBs). More than 200 congeners are known for both PCDD/PCDFs and DL-PCBs, although only 17 and 12 of them, respectively, are considered dangerous for human health and subjected to routine monitoring in dairy milk and other food commodities. For regulatory purposes, a toxic equivalent factor (TEF) of 1 has been assigned to the 2,3,7,8-tetrachlor- odibenzo-p-dioxin (TCDD), the toxicity of all other less toxic congeners being expressed as a fraction of 1. Dioxins are unintentionally produced in several industrial processes, as well as during illegal and legal waste incineration. Due to their high persistence, they are considered to be among the main causes of food chain and environmental pollution. Indeed, analyses performed to check dioxin levels in milk mass samples of bovids revealed higher values of dioxins than those legally permitted in several farms and this originated enormous economic damages to the farmers who were unable to sell milk or their derivative products for a long time (reviewed in Perucatti et al. 2006; Di Meo et al. 2010). Thus, the physical organization of genes involved in the dioxin metabolism can be of such interest to increase our knowledge on bovid genomes.

All dioxins bind to the cytosolic Aryl-hydrocabon Recep- tor (AHR) with subsequent migration in the cell nucleus where this complex binds the Aryl hydrocarbon Receptor Nuclear Translocator (ARNT). This heterodimer has the capacity to bind some specific DNA sites resulting in transcriptional activation or repression of several genes including cytochrome P450, family 1, subfamily A, poly-peptide 1 (CYP1A1), cytochrome P450, family 1, subfamily A, polypeptide 2 (CYP1A2) and cytochrome P450, family 1, subfamily B, polypeptide 1 (CYP1B1), amongst others (Nukaya et al. 2009). This is considered the key event in dioxin toxicity, which adversely affects immune, endocrine and reproductive systems (Mandal 2005).

In addition to AHR, also the Aryl-hydrocarbon Receptor Repressor (AHRR), regulated by AHR, acts as a transcrip- tional repressor of AHR function and, like the latter, can dimerize with the ARNT. The AHRR-ARNT complex can bind to AHR-responsive enhancer elements (AHREs) and the repression occurs through competition between AHR and AHRR for binding to AHREs as well as through additional mechanisms that do not involve competition for ARNT and are independent of AHRE binding by AHRR (for a review see Karchner et al. 2009).

In this study we assign for the first time these six loci to specific chromosome regions of cattle (Bos taurus, 2n = 60, BTA), river buffalo (Bubalus bubalis, 2n = 50, BBU), sheep (Ovis aries, 2n = 54, OAR) and goat (Capra hircus, 2n = 60, CHI) by using bovine BAC-clones containing specific DNA-fragments and the FISH-technique on R-banded chromosome preparations.

Peripheral blood samples from five cattle, six river buffaloes, four sheep and four goat were cultured and treated for late BrdU-incorporation to obtain R-banded chromosome preparations as previously reported (Iannuzzi and Di Berardino 2008).
One BAC clone for each gene was identified by PCR screening of the 4-genome equivalent INRA bovine BAC library (CRB-Gadie, Ressources Biologiques en Génomique –INRA, Jouy-en-Josas, France) (Eggen et al. 2001) and the CHORI-240 Bovine BAC library (Children's Hospital Oakland Research Institute, Oakland, California, USA). Details of primers and BAC clones containing ARNT, AHR, CYP1A1, CYP1A2, CYP1B1 and AHRR are reported in Table 1. The identity of the PCR products was verified by sequencing and DNA probes were obtained from each of the six BAC clones. However, later we found that both BAC clones identified with CYP1A1 and CYP1A2 primers contained both genes.
Biotin incorporation, FITC-signal detection, RBPI- banding, microscope chromosome observation and image processing were as reported earlier (Iannuzzi and Di Berardino 2008). At least 30 metaphases were studied for each probe and species. Chromosome identification and banding followed the most recent standard nomenclatures. The official gene symbol followed human gene nomencla- ture HGNC (http://www.genenames.org/).
Representative FISH-mapping of ARNT, AHR, CYP1A1, CYP1A2, CYP1B1 and AHRR in the four bovid species is shown in Fig. 1. The precise localizations of these six loci are reported in Table 1. With the exception of CYP1A1 and CYP1A2, all loci map in different chromosomes. Frequency of FITC signals (double or single signals in both or single chromosomes or chromatids) varies from 45% in both cattle and river buffalo with CYP1A1 to 23% in sheep with AHR. All six loci map in homologous chromosomes and chromosome bands of the four species, as expected given the high degree of autosome homologies among bovids (reviewed in Iannuzzi et al. 2009), although a simple translocation event differentiated "bovinae" chromosomes 9 and 14 from homologous chromosomes of "caprinae" (and remaining bovid subfamilies) (reviewed in Iannuzzi et al. 2009). Corresponding human locations HGNC (http:// www.genenames.org/) of the six genes are in agreement with comparative mapping data between bovids and humans (Table 1).
This study adds further information to the previous cytogenetic maps of the domestic bovids (BOVMAP (http://dga.jouy.inra.fr/cgi-bin/lgbc/main.pl?BASE=); Goldammer et al. 2009; Schibler et al. 2009; Di Meo et al. 2008) and assigns, for the first time, six loci containing genes which are strongly involved in the metabolism of a variety of xenobiotics, including polycyclic aromatic hydrocarbons (PAHs) and halogenated aromatic hydro- carbons, in chromosomes of cattle, sheep, river buffalo and goat.
Although assembled genome sequences are available for several species, especially for cattle, and chromosome gene location can be predicted based on available comparative maps of Bovids, FISH-mapping, especially applied on R- banded chromosome preparations, is still useful to construct precise genomic maps. Indeed, in a recent study using FISH, De Lorenzi et al. (2010) demonstrated the existence of errors in gene location in the Btau_4.0 assembly being DFNA5 and CHCHD6 genes located on BTA4 and BTA22, respectively, instead of BTA10 and BTA3, as erroneously displayed by Btau_4.0.

Acknowledgments
We are extremely grateful to Domenico Incarnato, CNR-ISPAAM, for his excellent technical assistance. The study was supported in part by the Italian Ministry of University and Research "PRIN-2008 - Contaminazione da diossine e composti diossino-simili: ricerca di biomarcatori in popolazioni bovine e bufaline esposte" and in part by the "Fondazione Cassa di Risparmio di Puglia", project "Monitoraggio ambientale di diversi areali zootecnici pugliesi mediante traccianti genetici".

References

De Lorenzi L, Molteni L, Parma P (2010) FISH mapping in cattle (Bos taurus L.) is not yet out of fashion. J Appl Genet 51(4):497–499

Di Meo GP, Perucatti A, Floriot S, Hayes H, Schibler L, Incarnato D,
Di Berardino D, Williams J, Cribiu E, Eggen A, Iannuzzi L (2008) An extended river buffalo (Bubalus bubalus, 2n = 50) cytogenetic map: assignment of 68 autosomal loci by FISH- mapping and R-banding and comparison with human chromo- somes. Chromosome Res 16:827–837
Di Meo GP, Perucatti A, Genualdo V, Caputi-Jambrenghi A, Rasero R, Nebbia C, Iannuzzi L (2010) Chromosome fragility in dairy cows exposed to dioxins and dioxin-like PCBs. Mutagenesis 26 (2):269–272
Eggen A, Gautier M, Billaut A, Petit E, Hayes H, Laurent P, Urban C, Pfister-Genskow M, Eilertsen K, Bishop MD (2001) Construc- tion and characterization of a bovine BAC library with four genome-equivalent coverage. Genet Sel Evol 33(5):543–548
Goldammer T, Di Meo GP, Lühken G, Drögemüller C, Wu CH, Kijas J, Dalrymple BP, Nicholas FW, Maddox JF, Iannuzzi L, Cockett NE (2009) Molecular cytogenetics and gene mapping in sheep (Ovis aries, 2n = 54). Cytogenet Genome Res 126:63–76
Iannuzzi L, Di Berardino D (2008) Tool of the trade: diagnostic and research in domestic animal cytogenetics. J Appl Genet 49:357–366
Iannuzzi L, King WA, Di Berardino D (2009) Chromosome evolution in domestic bovids as revealed by chromosome banding and FISH-Mapping techniques. Cytogenet Genome Res 126:49–62
Karchner SI, Jenny MJ, Tarrant AM, Evans BR, Kang HJ, Bae I, Sherr DH, Hahn ME (2009) The active form of human aryl hydrocarbon receptor (AHR) repressor lacks exon 8, and its Pro185 and Ala185 variants repress both AHR and hypoxia- inducible factor. Mol Cell Biol 29(13):3465–3477
Mandal PK (2005) Dioxin: a review of its environmental effects and its aryl hydrocarbon receptor biology. J Comp Physiol B 175:221–230
Nukaya M, Moran S, Bradfield CA (2009) The role of the dioxin- responsive element cluster between the Cyp1a1 and Cyp1a2 loci in aryl hydrocarbon receptor biology. PNAS 106(12):4923–4928
Perucatti A, Di Meo GP, Albarella S, Ciotola F, Incarnato D, Caputi Jambrenghi A, Peretti V, Vonghia G, Iannuzzi L (2006) Increased frequencies of both chromosome abnormalities and SCEs in two sheep flocks exposed to high dioxin levels during pasturage. Mutagenesis 21:67–75
Schibler L, Di Meo GP, Cribiu EP, Iannuzzi L (2009) Molecular cytogenetics and comparative mapping in goats (Capra hircus, 2n = 60). Cytogenet Genome Res 126:77–85.

Identified genes in BACs	Gene name	Source	Name of FISH probe and accession no.	Gene specific primers	Cytogenetic localization				
					BTA	BBU	OAR	CHI	HSA
ARNT	Aryl hydrocarbon receptor nuclear translocator	CRB-Gadie[b] (INRA)	BI 1098H07	1F: 5′- ACA GCT AAT TTA TCC CTG G -3′ 2R: 5′- TAG CTG GTT AGT CCA TCT C -3′	3q13	6q15	1p13	3q13	1q21
AHR	Aryl hydrocarbon receptor	CHORI[a]	CH240-131P6 Reference 49560	1F: 5′- GGT GAA GAT GAC TTC AGA G -3′ 2R: 5′- AAG ACG TAC TGC TGG AGG -3′	4q15	8q15	4q15	4q15	7p15
CYP1A1	Cytochrome P450, family 1, subfamily A, polypeptide 1	CRB-Gadie[b] (INRA)	BI 0438A06	1F: 5′- CGA GAA TGC CAA TAT CCA GC -3′ 2R: 5′- AGG ATA AAG GCC TCC AAA TAG -3′	21q17	20q17	18q17	21q17	15q24.1
CYP1A2	Cytochrome P450, family 1, subfamily A, polypeptide 2	CRB-Gadie[b] (INRA)	BI 0961H04	1F: 5′- AGC TTC ACC TTG GTC ACT G -3′ 2R: 5′- GAC TGA GCA TCT CCT TAC TG -3′ 2R: 5′- CTGGAGCACACTG CATCCTA -3′	21q17	20q17	18q17	21q17	15q24.
CYP1B1	Cytochrome P450, family 1, subfamily B, polypeptide 1	CRB-Gadie[b] (INRA)	BI 0084H11	3F: 5′- TCC TCG AAT TGC TCA GCC AC -3′ 4R: 5′- GAT GAA AGC GTC CAT CAT GTC -3′	11q16	12q22	3p16	11q16	2p22.2
AHRR	Aryl hydrocarbon receptor repressor	CRB-Gadie[b] (INRA)	BBAA plate 97.C 01 (INRA)	1F: 5′– GATTTTGCACACC TGGCTTT -3′	20q24	19q24	16q24	20q24	5p15.33

[a] Children's Hospital Oakland Research Institute, USA

[b] Ressources Biologiques en Génomique – INRA, Jouy-en-Josas

Table 1 Identified genes, BAC-clones used, specific primers and chromosome localization of ARNT, AHR, CYP1A1, CYP1A2, CYP1B1 and AHRR in cattle (BTA), river buffalo (BBU), sheep (OAR) and goat (CHI). Comparison with human (HSA) locations are also reported on the basis of the HGNC (http://www.genenames.org/)

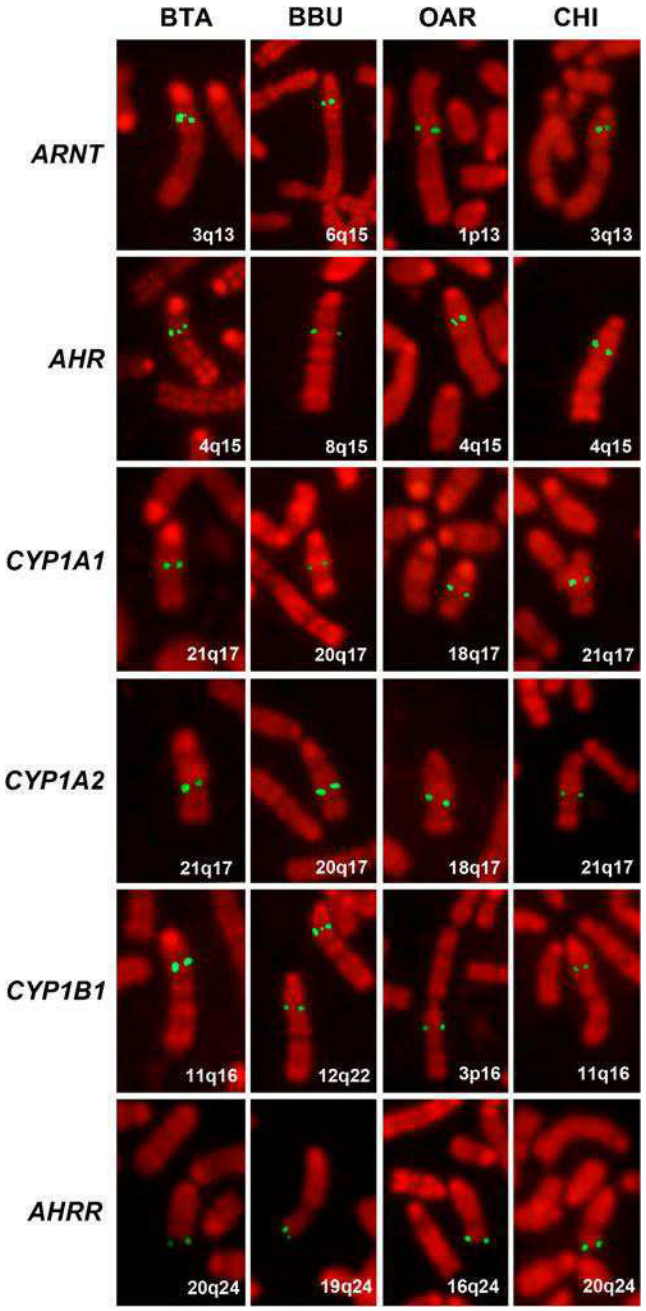

Fig. 1 Details of chromosome preparations of cattle (BTA), river buffalo (BBU), sheep (OAR) and goat (CHI) treated for simultaneous visualization of RBPI-banding (R-banding by using late BrdU incorporation and propidium iodide staining) and FITC signals after FISH-mapping with bovine BAC clones containing ARNT, AHR, CYP1A1, CYP1A2, CYP1B1 and AHR