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[1] Departament of Animal Cytogenetics and Molecular Genetics,
National Institute of Animal Production, Krakowska 1, 32-083 Balice/Kraków,
e-mail: barbara.czech@izoo.krakow.pl
[2] Department of Pig Breeding and Production Technology, University of Life Sciences in Lublin,
Akademicka 13, 20-950 Lublin,
e-mail: marek.babicz@up.lublin.pl

BARBARA DANIELAK-CZECH[1], ANNA KOZUBSKA-SOBOCIŃSKA[1]
MAREK BABICZ[2]

Comparative cytogenetic mapping of the *HSPB1* locus in the genomes of *Bovidae*

Porównawcze cytogenetyczne mapowanie *locus HSPB1* w genomach bydłowatych

Summary. The HSPB1 protein, from the family of small heat shock proteins (sHsps), plays a functional role in the regulation of many intracellular processes and protection from environmental stress factors. Mutations of the *HSPB1* gene are the reason for neuronal cells dysfunction associated with myopathies, motor neuropathies and neurodegenerative disorders, including prion diseases. Precise, chromosomal localization of this gene may contribute to the identification of new QTL correlated with resistance/susceptibility to prion diseases in *Bovidae*. As a result of comparative mapping performed by FISH technique with species-specific and heterologous molecular probes the location of *HSPB1* gene was assigned to 25q22 cattle and goat genome region as well as sheep 24q22. Physical localization of the *HSPB1* gene in the genomes of the studied species assigned its attachment to the linkage and syntenic groups of genes, which is essential for the expectation of the genetic selection effects.

Key words: cattle, sheep, goats, chromosomes, FISH technique, *HSPB1* gene, small heat shock proteins, prion diseases

INTRODUCTION

Heat shock proteins (HSP), including small heat shock proteins (HSPB), are involved in a wide range of physiological cellular processes and are particularly known for their ability to help cells survive under stress conditions. HSPB, belonging to a family of molecular chaperons, contain a highly conserved sequence of 80–100 amino acids called the structural α-crystallin domain. This structural domain is responsible for many intra- and intermolecular interactions leading to the formation of dimmers, which are considered as the basic unit of these proteins [Wettstein *et al.* 2012, Arrigo 2013]. Majority of the HSPB, just

like HSPB1 (alternative name Hsp27), are expressed in all mammalian tissues (predominantly in astrocytes and primary neuronal cells as well as skeletal, smooth and cardiac muscles) and play functional role in preventing aggregation of improperly folder or partially denatured proteins [Acunzo *et al.* 2012]. Mutations of the *HSPB* genes are associated with significant neuronal cell dysfunction, which contributes to the development of deleterious myopathies, motor neuropathies or neurodegenerative disorders, including transmissible spongiform encephalopathies (TSEs) [Arrigo 2012, Boncoraglio *et al.* 2012].

Accumulating evidence demonstrates neuroprotective functions of endogenous expressed or stress-induced HSPB in infectious protein deposit diseases (with particular emphasis on prion diseases) in humans, rodents and domestic bovids [Tortosa *et al.* 2008, Vidal *et al.* 2009, Brownell *et al.* 2012, Arrigo 2013]. Nowadays research activity indicates that there are some *loci*, other than *PRNP* (prion protein locus), modulating resistance/susceptibility to BSE (bovine spongiform encephalopathy) in cattle and scrapie in sheep. Following this, several QTL associated with prion diseases incubation period have been identified recently on chromosomes of these species [Hernandez-Sanchez *et al.* 2002, Zhang *et al.* 2004, Moreno *et al.* 2008, 2010]. Additionally, some heat shock protein *loci* (among them *HSPB*) have been defined as a putative positional or functional candidate genes influencing polygenic response to prion diseases [Serrano *et al.* 2011, Bae *et al.* 2012]. For that reason, there is a strong impetus to study the potential new QTL associated with resistance/susceptibility to TSE in domestic bovids, basing on precise physical assignment of the *HSPB* genes in their genomes [Lewin *et al.* 2009, Hu *et al.* 2013]. In this context, FISH technique and comparative FISH–mapping (Zoo-FISH) seems to be a good tool to precisely localize these *loci* in specific chromosome regions, extending cytogenetic maps and improving *Bovidae* species genome assemblies needful for genetic analyses [De Lorenzi *et al.* 2010].

The aim of the presented study was comparative cytogenetic mapping of the *HSPB1* locus, selected because of its considerable association with neurodegenerative disorders in cattle, sheep and goats.

MATERIAL AND METHODS

The late-replicating banded chromosome preparations for fluorescence *in situ* hybridization (FISH) detection were obtained from synchronized cattle, sheep and goat lymphocyte cultures, treated 6 h before harvesting with 10 μg/ml BrdU and 20 μg/ml H33258 (Sigma) and counterstained by DAPI (following the protocol described by Iannuzzi and Di Berardino 2008). Chromosome identification followed the standard cattle, sheep and goat karyotypes and ideograms, according to the international chromosome nomenclature for domestic bovids ISCANDB 2000 [Di Berardino *et al.* 2001].

The bovine BAC clone CH240-362H14 containing *HSPB1* gene was screened by database searching (http://bacpac.chori.org/libraries.php) and obtained from the CHORI-240 Bovine BAC Library (BACPAC Resources) (http://www.chori.org/bacpac/ bovine240.htm) The presence of the studied gene in the selected clone was confirmed by PCR using gene-specific primers (table 1). The BAC DNA was extracted according to the alkaline lysis miniprep protocol (Qiagen), labelled with biotin-16-dUTP by standard nick translation kit (Roche), and used as probe in the FISH experiments on bovid chro-

mosomes. The probe with an excess of bovine competitor DNA were denatured for 5 min at 75°C, preannealed for 15 min at 37°C, and applied onto chromosome preparations, denatured previously in formamide for 1 min at 70°C. Hybridizations were carried out overnight (up to three days in cross-species experiments) at 37°C. After detection step with the use of FITC-avidin (fluorescein isothiocyanate-avidin) (Vector Laboratories) and anti-avidin antibodies (Sigma), slides were counterstained with DAPI (4,6-diamidino-2-phenylindole) solution (0.24 μg/ml) in Antifade (Vector Laboratories) to obtain DAPI-banded chromosomes (with patterns corresponding to the Q bands).

The cross-species *in situ* hybridization experiments (Zoo-FISH) with the human commercial probe (Vysis LSI ELN 7q11.23 Spectrum Orange) overlapping *HSPB1* locus were performed according to the manufacturer's protocol.

Slides with DAPI-banded chromosomes and FISH-signals were analyzed in Axio Imager.D2 (Zeiss) fluorescence microscope equipped with Axio Vision computer-assisted image analysis system.

RESULTS

The FISH experiments allowed for the successful assignment of bovine BAC clone, harboring the small heat shock protein gene – *HSPB1,* to the cattle: BTA25q22, sheep: OAR24q22 and goat: CHI25q22 chromosome regions (fig. 1 and fig. 2). Frequency of FITC signals (double or single spots on both or single chromosomes or chromatids) varied from 73% in cattle and goats to 35% in sheep. Cross-species *in situ* hybridizations with human probe, specific for HSA7q11.23 genome region involving *HSPB1* locus, completely confirmed localization in the same chromosome bands of the studied bovids.

DISCUSSION

In this study we present FISH-based mapping of the *HSPB1* locus on homologous chromosomes and chromosome bands (25q22/24q22) of the three species, as expected given the high degree of autosome homologies among bovids, which are very close to each other from the evolutionary point of view [Iannuzzi *et al.* 2009]. This physical locations, extending the cytogenetic maps of cattle/goat 25 and sheep 24 autosome, are in agreement with their corresponding human location in the proximal q11.23 segment of chromosome 7 (HGNC) (http://www.genenames.org), based on the comparative painting, radiation hybrid or marker mapping data between bovids and humans [Chowdhary *et al.* 1996, Everts-van der Wind *et al.* 2004, Darlymple *et al.* 2007, Schibler *et al.* 2009]. Moreover, these chromosomal locations of the studied gene are in accordance with results of our earlier provisional comparative mapping of the *HSPB loci* in domestic bovids [Danielak-Czech *et al.* 2014a, 2014b]. Besides, cross-species *in situ* hybridizations carried out in three *Bovidae* species with the use of bovine and human probes confirmed conserved nature of the linkage groups containing *HSPB1* gene and homology of HSA7q11.23 and BTA25q22/OAR24q22/CHI15q22 chromosome regions, which can be a basis for evolutionary investigations. On the whole, the study performed may help to elucidate the role of *HSPB1* gene in the development of neurodegenerative disorders in *Bovidae* and other livestock species.

Table 1. PCR protocol verifying presence of the *HSPB1* gene in BAC clone
Tabela 1. Protokół reakcji PCR weryfikującej obecność genu *HSPB1* w klonie bakteryjnym

Gene Gen	BAC clone Klon BAC	GenBank accession number Numer akcesyjny GenBank	PCR			
			primers (5'-3' sequences) startery (sekwencje 5'-3')	Ta (°C)	product size (bp) długość produktu (pz)	gene fragment fragment genu
HSPB1	CH240-134C10	AB605262	ccgagatcaccattcccgtc ggctatagtggtaagggcag	58	87	exon 2

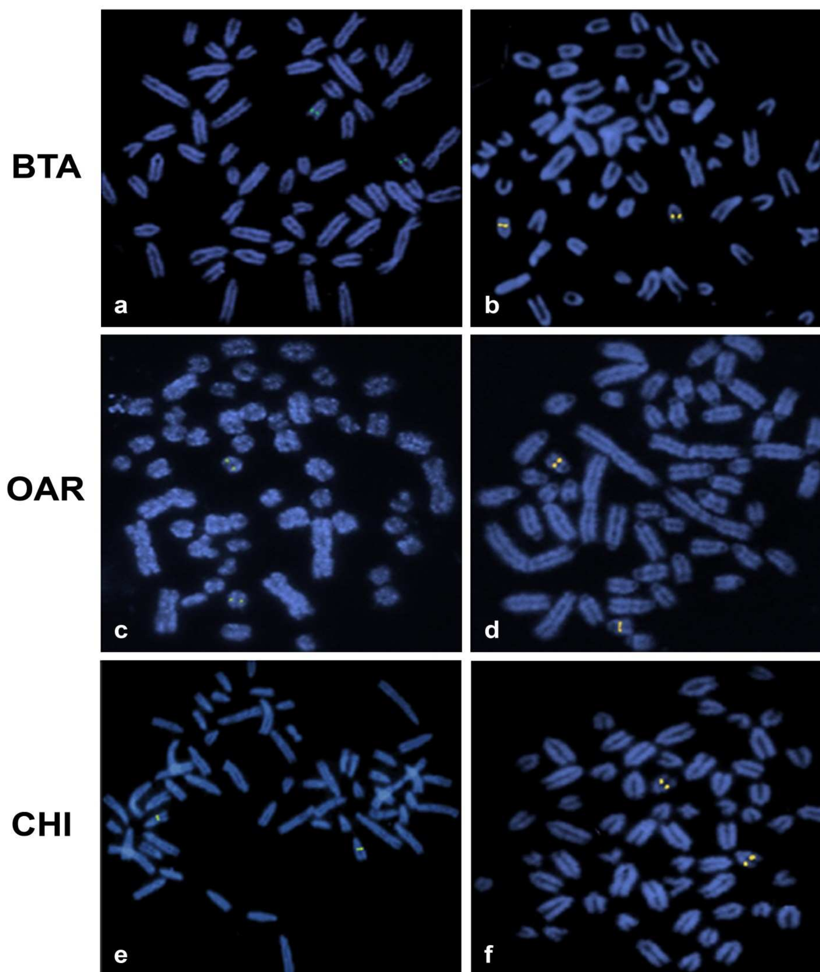
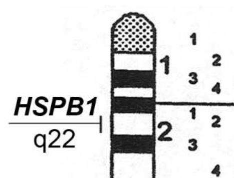


Fig. 1. Cytogenetic localization of the *HSPB1* gene on chromosomes of cattle (BTA), sheep (OAR) and goats (CHI). FISH technique with bovine BAC probe (a, c, e) and human probe specific for HSA7q11.23 chromosome region (b, d, f)
Rys. 1. Cytogenetyczna lokalizacja genu *HSPB1* na chromosomach bydła (BTA), owiec (OAR) i kóz (CHI). Technika FISH z bydlęcą sondą BAC (a, c, e) oraz ludzką sondą specyficzną dla regionu chromosomowego HSA7q11.23 (b, d, f)



BTA25/OAR24/CHI25

Fig. 2. Diagrammatic localization of the *HSPB1* gene on cattle (BTA25), sheep (OAR24) and goat (CHI25) chromosomes
Rys. 2. Diagramatyczna lokalizacja genu *HSPB1* na chromosomach bydła (BTA25), owiec (OAR24) i kóz (CHI25)

The presented study adds further information to the previous cytogenetic maps of several *Bovidae* species (Bovmap, http://dga.jouy.inra.fr/cgi-bin/lgbc/main.pl?BASE=) [Goldammer *et al.* 2009, Schibler *et al.* 2009] and precisely assigns, substantially involved in prion diseases, *HSPB1* gene on chromosomes of cattle, sheep and goat chromosomes, which are the major domestic bovid species of great economic importance. Furthermore, the experiments carried out indicated that FISH mapping, including cross-species *in situ* hybridization method (Zoo-FISH), is still useful to validate the data on physical gene location and improve available genome assemblies of the domestic bovids, e.g. Btau_4.0 containing even now many gaps or errors [De Lorenzi *et al.* 2010]. It is noteworthy, that reported chromosomal assignment of the *HSPB1* gene may be also a basis for identifying new QTL associated with response for TSE in *Bovidae* species.

Recently, *HSPB1* gene has been analyzed as possible candidate gene (other than the *PRNP* gene) regulating response to spongiform encephalopathy (incubation period) in the domestic bovids, chosen for its role as chaperone or apoptosis modulators as well as due to protective effect against the stress-related infectious protein aggregation and neuronal degeneration in prion diseases [Sawiris *et al.* 2007, Serrano *et al.* 2011, Brownell *et al.* 2012, Brown *et al.* 2014]. In general, it has been shown that increased *HSPB1* locus expression is associated with prevention or degradation of prion protein aggregates and the presence of reactive astrocytosis in natural scrapie [Serrano *et al.* 2011]. Actually, the other studies also effectively proved that low expression level of this gene contribute to the development of the prion protein deposits and morphological lesions such as spongiosis or gliosis of classical scrapie in sheep [Vidal *et al.* 2009, Brown *et al.* 2014]. The similar investigations revealed as well increased *HSPB1* expression as a stress response of the central nervous system in a mouse model of BSE [Tortosa *et al.* 2008]. However, there have been no currently published findings determining whether loss or gain, likewise mutations of this gene would have an effect on TSE development in domestic bovids.

CONCLUSIONS

The experiments performed will make it possible to identify and determinate of new locus controlling susceptibility/resistance to prion diseases, important from viewpoint of domestic bovids farming.

Physical localization of the *HSPB1* gene in the cattle, sheep and goats genomes assigned its attachment to the linkage and syntenic groups of genes, which is essential for the expectation of the genetic selection effects.

The reported studies will broaden our knowledge of genome organization and extend the physical maps in *Bovidae,* enabling better understanding of the evolutionary processes in this mammalian family.

REFERENCES

Acunzo J., Katsogiannou M., Rocchi P., 2012. Small heat proteins HSP27 (HspB1), αβ-crystallin (HspB5) and HSP22 (HspB8) as regulators of cell death. Int. J. Biochem. Cell B. 44, 1622–1631.

Arrigo A.P., 2012. Pathology-dependent effects linked to small heat shock proteins expression: an update. Scientifica, ID 185641, doi: org/10.6064/2012/185641.

Arrigo A.P., 2013. Human small heat shock proteins: Protein interactomes of homo- and hetero-oligomeric complexes: An update. FEBS Lett. 587, 1959–1969.

Bae S.E., Jung S, Kim H.Y., Son H.S., 2012. Correlation analysis for the incubation period of prion disease. Prion 6, 276–281.

Boncoraglio A., Minoia M., Carr S., 2012. The family of mammalian small heat shock proteins (HSPB*s*): Implications in protein deposit diseases and motor neuropathies. Int. J. Biochem. Cell Biol. 44, 1657–1669.

Brown C.A., Schmidt C., Poulter M., Hummerich H., Klöhn P.C., Jat P., Mead S., Collinge J., Lloyd S.E., 2014. *In vitro* screen of prion disease susceptibility genes using the scrapie cell assay. Hum. Mol. Genet. 2, 5102–5108.

Brownell S.E., Becker R.A., Steinman L., 2012. The protective and therapeutic function of small heat shock proteins in neurological diseases. Front. Immunol. 3, 74, doi: 10.3389/ fimmu.2012.00074.

Chowdhary B.P., Fronicke L., Gustavsson I., Scherthan H., 1996. Comparative analysis of the cattle and human genomes: detection of ZOO-FISH and gene mapping-based chromosomal homologies. Mamm. Genome 7, 297–302.

Danielak-Czech B., Kozubska-Sobocińska A., Bąk A., 2014a. FISH-based comparative mapping of the *Hsp27* gene on chromosomes of the domestic Bovids. Chromosome Res. 22, 414–414.

Danielak-Czech B., Kozubska-Sobocińska A., Kruczek K., 2014 b. Chromosomal assignment of the small heat protein genes in the sheep genome. Chromosome Res. 22, 412–413.

Darlymple B.P., Kirkness E.F., Nefedov M., McWillam S., Ratnakumar A., Barris W., Zhao S., Shetty J., Maddox J.F., O'Grady M., Nicholas F., Crawford A.M., Smith T., de Jong P.J., McEwan J., Oddy V.H., Cockett N.E., International Sheep Genomics Consortium, 2007. Using comparative genomics to reorder the human genome sequence into a virtual sheep genome. Genome Biol. 8, R152, doi: 10.1186/gb-2007-8-7-r152.

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

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

Everts-van der Wind A., Kata S.R., Band M.R., Rebeitz M., Larkin D.M., Everts R.E., Green C.A., Liu L., Natarajan S., Goldammer T., Lee J.H., McKay S., Womack J.E., Lewin H.A., 2004. A 1463 gene cattle-human comparative map with anchor points defined by human genome sequence coordinates. Genome Res. 14, 1424–1437.

Goldammer T., Di Meo G.P., Lühken G., Drögemüller C., Wu C.H., Kijas J., Dalrymple B.P., Nicholas F.W., Maddox J.F., Iannuzzi L., Cockett N.E., 2009. Molecular cytogenetics and gene mapping in sheep (*Ovis aries*, 2n = 54). Cytogenet. Genome Res. 126, 63–76.

Hernandez-Sanchez J., Waddington D., Wiener P., Haley C.S., Williams J.L., 2002. Genome-wide search for markers associated with bovine spongiform encephalopathy. Mamm. Genome 13, 164–168.

Hu Z.L., Park C.A., Wu X.L., Reccy J.M., 2013. Animal QTLdb: an improved database tool for livestock animal QTL/association data dissemination in the post-genome era. Nucleic Acids Res. 41, 871–879, doi: 10.1093/nar/gks1150.

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

Iannuzzi L., King W.A., Di Berardino D., 2009. Chromosome evolution in domestic bovids as revealed by chromosome banding and FISH-mapping techniques. Cytogenet. Genome Res. 126, 49–62.

Lewin H., Larkin D.M., Pontius J., O'Brien S.J., 2009. Every genome sequence needs a good map. Genome Res. 19, 1925–1928.

Moreno C.R., Cosseddu G.M., Schibler L., Roig A., Moazami-Goudarzi K., Andreoletti O., Eychenne F., Lajous D., Schelcher F., Cribiu E.P., Laurent P., Vaiman D., Elsen J.M., 2008. Identification of new quantitative trait *loci* (other than the *PRNP* gene) modulating the scrapie incubation period in sheep. Genetics 179, 723–726.

Moreno C.R., Moazami-Goudarzi K., Briand S., Robert-Granié C., Weisbecker J.L., Laurent P., Cribiu E.P., Haley C.S., Andreoletti O., Bishop S.C., Pong-Wong R., 2010. Mapping of quantitative trait *loci* affecting classical scrapie incubation time in a population comprising several generations of scrapie-infected sheep. J. Gen. Virol. 91, 575–579.

Sawiris G.P., Becker K.G., Elliott E.J., Moulden R., Rohwer R.G., 2007. Molecular analysis of bovine spongiform encephalopathy infection by cDNA arrays. J. Gen. Virol. 88, 1356–1362.

Schibler L., Di Meo G.P., Iannuzzi L., 2009. Molecular cytogenetics and comparative mapping in goats (*Capra hircus*, 2n = 60). Cytogenet. Genome Res. 126, 77–85.

Serrano C., Bolea R., Lyahyai J., Filali H., Varona L., Marcos-Carcavilla A., Acin C., Calvo J.H., Serrano M., Badiola J.J., 2011. Changes in *HSP* gene and protein expression in natural scrapie with brain damage. Vet. Res. 42, 13, doi: 10.1186/1297-9716-42-13.

Tortosa R., Vidal E., Costa C., Alamillo E., Torres J.M., Ferrer I., Pumarola M., 2008. Stress response in the central nervous system of a transgenic mouse model of bovine spongiform encephalopathy. Vet. J. 178, 126–129.

Vidal E., Acín C., Foradada L., Monzón M., Márquez M., Monleon E., Pumarola M., Badiola J.J., Bolea R., 2009. Immunohistochemical characterization of classical scrapie neuropathology in sheep. J. Comp. Pathol. 141, 135–146.

Wettstein G., Bellaye P.S., Micheau O., Bonniaud P., 2012. Small heat shock proteins and the cytoskeleton: An essential interplay for cell integrity? Int. J. Biochem. Cell B. 44(10), 1680–1686.

Zhang C., de Koning D.J., Hernandez–Sanchez J., Haley C.S., Williams J.L., Wiener P., 2004. Mapping of multiple quantitative trait *loci* affecting bovine spongiform encephalopathy. Genetics 167, 1863–1872.

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Streszczenie. Białko HSPB1, z rodziny małych białek szoku cieplnego (sHsps), pełni funkcjonalną rolę w regulacji wielu procesów wewnątrzkomórkowych oraz ochronie przed stresowymi czynnikami środowiskowymi. Mutacje genu *HSPB1* są przyczyną dysfunkcji komórek neuronowych, związanych z miopatiami, neuropatiami motorycznymi i zaburzeniami neurodegeneracyjnymi, w tym również z chorobami prionowymi. Precyzyjna, chromosomowa lokalizacja tego genu może przyczynić się do identyfikacji nowego QTL skorelowanego z opornością/podatnością na choroby prionowe u bydłowatych. W wyniku porównawczego mapowania przeprowadzonego techniką FISH z gatunkowo specyficznymi i heterologicznymi sondami molekularnymi określono lokalizację genu *HSPB1* w regionie 25q22 genomu bydła i kóz oraz 24q22 owiec. Fizyczna lokalizacja tego genu w genomach badanych gatunków określiła jego przynależność do grup sprzężeniowych i syntenicznych, co jest istotne dla przewidywania skutków selekcji genetycznej.

Słowa kluczowe: bydło, owce, kozy, chromosomy, technika FISH, gen *HSPB1*, małe białka szoku cieplnego, choroby prionowe