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Cytogenetic mapping of genes from the family *HSPB* in the pig genome

Cytogenetyczne mapowanie genów z rodziny *HSPB* w genomie świni

Summary. The proteins from the family *HSPB* (small heat shock proteins) play a functional role in the regulation of intracellular processes (apoptosis, inflammatory response, chaperone activity concerning the protein folding and aggregation control) responsible for the protection from environmental stress factors. Mutations of genes encoding these proteins are the reason for neuronal cells dysfunctions, leading to myopathies, motor neuropathies and neurodegenerative disorders. The aim of the study was cytogenetic mapping of the *HSPB* genes in the pig genome with an application of FISH technique and the probes obtained from BAC clone containing sequences of *HSPB1*, *HSPB2*, *CRYAB* (alternative name *HSPB5*), *HSPB6*, *HSPB8* genes, derived from the CHORI-242 Porcine BAC Library. Prior to *in situ* hybridization, carried out on metaphase chromosomes stained by DAPI bands technique, the presence of the studied genes in the selected clone was confirmed by means of PCR method with the use of the gene-specific primers. As a result of the experiments FISH signals in the chromosome regions SSC3p15 (*HspB1*), SSC9p21 (*HspB2* and *CRYAB*), SSC6q12 (*HspB6*) and *HspB8* SSC14q21 (*HspB8*) were obtained, which enabled to designate cytogenetic localization of the studied *HSPB* genes on the domestic pig genome map. The results obtained may help to elucidate the role of the *HSPB* genes in the pathomechanisms of myopathies and neuropathies in breeding animals.

Key words: pig chromosomes, FISH, cytogenetic mapping, small heat shock proteins – *HSPB*, muscle development and function disorders

INTRODUCTION

The ten members of the small heat shock protein family (*HSPB1* – 10) are key players of the protein quality control system and participate, together with other molecular chaperones and co-chaperones, in the maintenance of protein homeostasis. The *HSPB* all

contain a highly conserved sequence (of 80–100 amino acids) called α -crystallin domain and the C-terminal and the N-terminal regions which are both involved in the stabilization of the proteins [Taylor and Benjamin 2005]. This structural domain is responsible for many intra- and inter-molecular interactions leading to the formation of dimers, which are considered as the basic unit of all *HSPB* (but can interact with each other forming higher molecular weight oligomers) [Boncoraglio *et al.* 2012, Arrigo 2013]. The ability of the *HSPB* to form dynamic oligomers of different size might significantly influence their functions, such as role in cytoskeleton stabilization, chaperon function, anti-aggregation and anti-apoptotic activities [Mymrikov *et al.* 2011, Wettstein *et al.* 2012]. Mutation of encoding genes can be the reason of neurological and muscular disorders (protein deposit or conformation diseases) which are characterized by the accumulation of aggregate-prone proteins. However, mechanisms of gain-of-toxic function and/or loss of function which can contribute to these *HSPB*-associated pathologies still remain to clarify [Arrigo 2012, Brownell *et al.* 2012, Dubińska-Magiera *et al.* 2014].

Nowadays, a number of extensive studies was performed the contribution of small heat shock proteins to muscle development and function using developing piglets and adult pigs as models to analyze the multiple stress influence on the *HSPB* expression during the various stages of life – from birth to slaughter. On the basis these experiments it was stated that impairment expression of *HSPB* encoding genes is associated with substantial developmental disorders or death of transported slaughter pigs leading to poor quality of meat [Tallot *et al.* 2003, Nefti *et al.* 2005, David *et al.* 2006, Bao *et al.* 2009, Jensen *et al.* 2012, Liu *et al.* 2014]. Moreover, the recent research evidenced the role of these heat shock proteins in conversion pig muscle to meat and shaping of meat quality traits [Ouali *et al.* 2006, Lomiwes *et al.* 2014].

FISH mapping of the *HSPB* genes is assumed to be a reasonable approach to identify additional QTLs associated with pig stress resistance and product quality as well as improve porcine cytogenetic map as well as enhance the applicability of whole genome assembly [Lewin *et al.* 2009, Hu *et al.* 2013].

The aim of the presented study was cytogenetic mapping of the *HSPB1*, *HSPB2*, *CRYAB* (alternative name *HSPB5*), *HSPB6* and *HSPB8* genes from the family small heat shock protein in the pig genome.

MATERIAL AND METHODS

Pig lymphocytes were cultured and treated with BrdU (10 μ g/ml) and H33258 (20 μ g/ml) (Sigma) 6 h before harvesting to obtain, counterstained by DAPI, late-replicating banded chromosome preparations for FISH detection (according to the protocol reported by Iannuzzi and Di Berardino) [Iannuzzi and Di Berardino 2008]. The porcine Bacterial Artificial Chromosome (BAC) clones, overlapping five small heat shock protein genes: *HSPB1*, *HSPB2*, *CRYAB* (*HSPB5*), *HSPB6* and *HSPB8*, were obtained from the CHORI-242 Porcine BAC Library (<http://www.chori.org/bacpac/porcine242.htm>). The presence of the studied genes in clones, selected based on information about BAC end sequences (BES) (http://www.sanger.ac.uk/Projects/S_scrofa/BES.shtml), was verified by PCR using gene-specific primers (Tab. 1). It was not possible to select separate clones for the closely located in the pig genome *HSPB2* and *CRYAB* (*HSPB5*) genes, therefore the same clone (CH242-333E2) containing sequences of both the genes was used

(<http://www.ncbi.nlm.nih.gov/gene>). The BAC DNA was isolated, labelled with biotin 16-dUTP by random priming and used as probes in the FISH experiments on pig chromosomes. Labelled probes with an excess of porcine competitor DNA were denatured for 10 min at 70°C, preannealed for 30 min at 37°C, and applied onto chromosome preparations. Hybridizations were carried out overnight at 37°C. Signal detection and amplification were performed using avidin-FITC anti-avidin system. Slides were stained by DAPI and analyzed under fluorescence microscope (Zeiss Axio Imager.D2) equipped with computer-assisted image analysis system (Axio Vision). Chromosome identification followed the standard karyotype, according to the international nomenclature for the domestic pig chromosomes [Gustavsson 1988].

Table 1. PCR protocol verifying presence of the *HSPB* genes in BAC clones
Tabela 1. Protokół reakcji PCR weryfikującej obecność genów *HSPB* w klonach BAC

Gene Gen	BAC clone Klon BAC	GenBank Accession numer Numer akce- syjny Gen- Bank	PCR			
			primers (5'-3' sequences) startery (sekwencje 5'-3')	Ta (°C)	product size (bp) dł. produk- tu (pz)	gene fragment fragment genu
<i>HSPB1</i>	CH242- 237N5	AY789513	ctcgaataacacgctgcc ggatggtgatctctgccgac	57	129	exon 3
<i>HSPB2</i>	CH242- 333E2	DN119723	ttgcctcactaagccgaag ggcaccactgagtacgag	58	186	exon 3
<i>CRYAB</i>	CH242- 333E2	DY408556	ccattcacagtgaggacccc ccgcctcttgaccagttc	59	378	exon 1-2
<i>HSPB6</i>	CH242- 173G9	AY574050	tttctcggtgctgctggatg gcatgcacctccacatgttc	59	84	exon 1
<i>HSPB8</i>	CH242- 102C8	AY609863	ctctctgagcctccgtttcc tgctgcttctctctgtgtt	56	429	exon 1

RESULTS

Positive, strong FITC signals (double or single spots on both or single chromosomes or chromatids with frequency varying from 39% to 85%) were obtained after fluorescence *in situ* hybridization with the use of the BAC clones containing the studied genes as probes. The genes were assigned to the following pig chromosome regions: SSC3p15 (*HspB1*), SSC9p21 (*HspB2* and *CRYAB*), SSC6q12 (*HspB6*) and *HspB8* SSC14q21 (*HspB8*) (Fig. 1, Tab. 2). The *HspB2* and *CRYAB*, two small heat shock protein genes located adjacently in the pig genome, were mapped to the same chromosome band – SSC9p21. The assignment of five *HSPB* loci to 3, 9, 6 and 14 chromosome pair extended the cytogenetic maps for these four autosomes of the domestic pig.

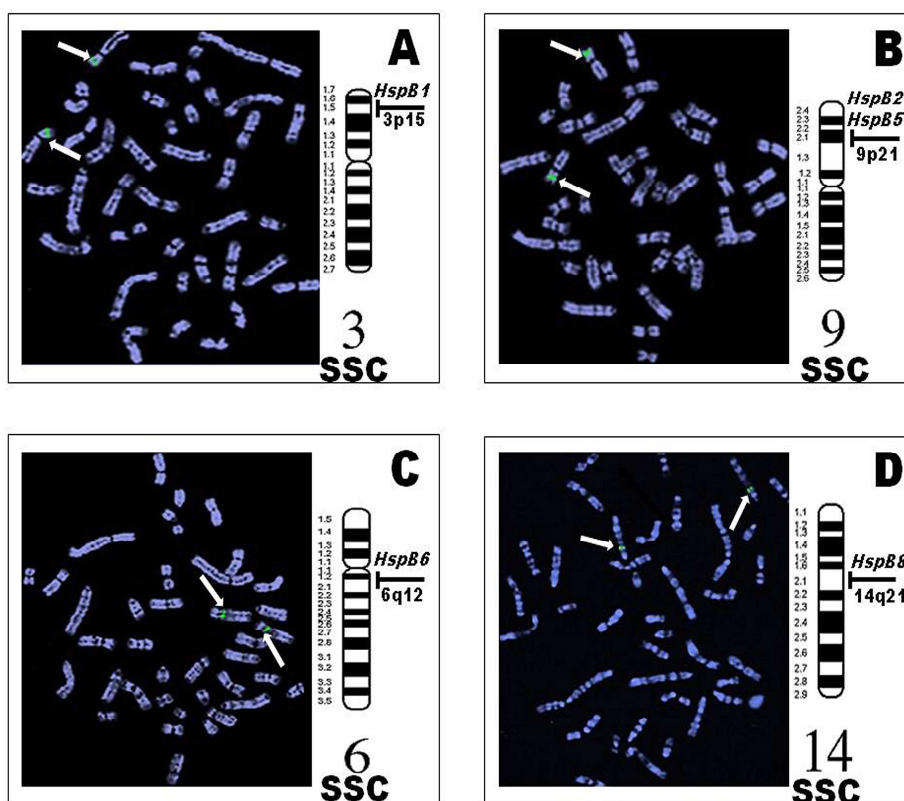


Fig. 1. Cytogenetic localization of the *HSPB* genes on pig chromosomes
Rys. 1. Cytogenetyczna lokalizacja genów *HSPB* na chromosomach świni

Table 2. Cytogenetic localization of the studied *HSPB* genes on pig chromosomes (SSC) (in relation to their location in human genome – HSA) and functions of encoded proteins
Tabela 2. Cytogenetyczna lokalizacja badanych genów *HSPB* na chromosomach świni (SSC) (w odniesieniu do ich lokalizacji w genomie człowieka – HSA) oraz funkcje kodowanych białek

Gene symbol Symbol genu	Gene name Nazwa genu	Protein function Funkcja białka	Cytogenetic localization Cytogenetyczna lokalizacja	
			BTA	HSA
<i>HSPB1</i>	heat shock 27 kDa protein 1	stress resistance, actin organization	3p15	17q11.23
<i>HSPB2</i>	heat shock 27kDa protein 2	stress response, somatic muscle development	9p21	11q22.3-23.1
<i>CRYAB</i>	crystallin, alpha B	anti-apoptosis, muscle organ development, response to heat, negative regulation of intracellular transport, camera-type eye development, structural constituent of eye lens, protein homooligomerization activity, unfolded protein binding	9p21	11q22.3-23.1
<i>HSPB6</i>	heat shock protein beta-6	stress response, protein homodimerization activity, structural constituent of eye lens	6q12	19q13.12
<i>HSPB8</i>	heat shock 22kDa protein 8	stress response, chaperone activity, identical protein binding	14q21	12q24.23

DISCUSSION

FISH-mapping presented in this paper facilitated the precise assignment of five *HSPB* genes (*HSPB1*, *HSPB2*, *CRYAB* (*HSPB5*), *HSPB6*, *HSPB8*) to the 3p15, 9p21, 6q12, 14q21 porcine chromosome regions. The *HspB2* and *CRYAB* (*HspB5*) loci, clustered at the distance of 0.863 kb in the pig genome (<http://www.ncbi.nlm.nih.gov/gene/>), were identified in the same SSC9p21 chromosome region. Similarly in humans, these two related genes are located in homologous HSA11q22-q23 genome region and arranged in a head-to-head manner with an intergenic sequence of less than 0.9 kb. Such trait gene pair linkage raises a possibility of shared regulatory elements for their expression and is generally regarded as a conserved feature of the mammalian genomes [Iwaki *et al.* 1997, Doerwald *et al.* 2004].

The studied small heat shock protein genes were mapped earlier by the linkage mapping approach to a specific pig chromosome, but band-specific location was not determined [Humphray *et al.* 2007, Jiang and Rothschild 2007, Vingborg *et al.* 2009]. The physical assignments of five *HSPB* genes presented in this study correspond with these primary findings and are in agreement with cytogenetic localization in the human genome, if human-pig comparative chromosome painting data are considered (<https://www-lgc.toulouse.inra.fr/pig/compare/HSA.htm>) [Goureau *et al.* 1996]. Furthermore, the results obtained are consistent with our previous provisional comparative mapping of these genes in the genomes of domestic and wild pig species [Danielak-Czech *et al.* 2014].

Overall, the reported experiments reported in this paper proved that FISH mapping is constantly useful method to validate the data on physical localization of genes due to existence of many gaps or errors in currently available assembled genome sequences (e.g. Sscrofa10) (<http://www.ncbi.nlm.nih.gov/projects/genome/guide/pig/>). The studies confirmed also that verification of gene location by FISH is still a good tool to improve pig physical, integrated and QTLs maps (<http://www.animalgenome.org/QTLdb/pig.html>) in order to enhance the quality and applicability of whole genome sequences for genetic analysis [Rothschild *et al.* 2007, Hu *et al.* 2009, 2013, Lewin *et al.* 2009, Jiang *et al.* 2014]. Additionally, the research described in this paper showed that cytogenetic mapping of the *HSPB* loci may contribute to the identification of new QTLs associated with pig stress and disease resistance, feed efficiency, product quality and reproductive performance. Furthermore, these findings may be also a basis for developing genetically modified strains with improved production traits or providing transgenic model animals for human diseases and therapy [Whyte and Prather 2011, Hu *et al.* 2013].

At present, expression of six members of the small heat shock protein family (*HSPB1*, *HSPB2*, *CRYAB*, *HSPB6*, *HSPB7* and *HSPB8*) has been analyzed in the nervous and non-nervous tissues (lens, brain, heart, liver, kidney, lung, skeletal muscle, stomach, colon) of the developing pigs (from full-term fetuses to three years old adult), which were used as models to study the impact of different forms of stress (hypoxia, bacterial infection, physical activity, transport) on their postnatal expression [David *et al.* 2000, 2006, Tallot *et al.* 2003, Verschuure *et al.* 2003, Chiral *et al.* 2004, Golenhofen *et al.* 2004, Nefti *et al.* 2005, Bao *et al.* 2008, 2009, Jensen *et al.* 2012, Liu *et al.* 2014]. Likewise, the latest research performed on a swine-specific *in vitro* infection model have been focused on variable expression of certain *HSPB* genes in intestinal porcine epithelial cells of newborn and weaning piglets, induced by probiotics which counteract the patho-

genic effects of enterotoxigenic bacteria [Liu *et al.* 2014, 2015]. In turn, differing expression levels of some *HSPB loci*, analyzed in studies on adult slaughter pigs, had been considered as the reason of increased susceptibility to acute heart failure and the sudden death syndrome in transported pigs [Bao *et al.* 2008, 2009]. Overall, these experiments revealed that impairment of *HSPB* genes expression affects stress response and result in severe adverse developmental outcome, neonatal morbidity and mortality as well as death syndrome of transported slaughter pigs and poor eating quality of meat.

On the other hand, the recent investigations evidenced chaperone and anti-apoptotic role of *HSPB* proteins during conversion pig muscle to meat which is believed to ultimately influence meat quality (the lower concentrations of these proteins are consistent with increased meat tenderness, juiciness and flavor, while the higher ones correspond with darker meat color and cooking loss) [Lametsch and Bendixen 2001, Hwang *et al.* 2005, Herrera-Mendez *et al.* 2006, Ouali *et al.* 2006, Kwasiborski *et al.* 2008, Laville *et al.* 2009, Lomiwes *et al.* 2014]. It is also noteworthy, that four of the studied *HSPB* genes, are located within or near many QTLs for meat and carcass quality traits, such as flavor, color, odor, pH, stiffening and texture. The reported chromosomal localizations of the small heat shock protein genes may be a basis for identifying new QTL associated with meat quality.

CONCLUSIONS

The cytogenetic mapping of the small heat shock protein genes in the pig genome is of great importance for improving the physical and integrated maps of this species.

In view of the biological function of encoded proteins and their location overlapping QTL regions for the pig meat quality traits, the studied *HSPB* genes can be considered as candidates for such traits.

The identification of porcine stress protein genes controlling stress and diseases resistance, such as *HSPB*, is relevant due to the fact that pigs are good model animals for studying human diseases, involving therapy and prevention.

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Streszczenie. Białka z rodziny *HSPB* (małe białka szoku cieplnego) odgrywają funkcjonalną rolę w regulacji wewnątrzkomórkowych procesów (apoptoza, odpowiedź na stany zapalne, aktywność chaperonowa dotycząca kontroli fałdowania i agregacji białek) odpowiedzialnych za ochronę przed stresowymi czynnikami środowiskowymi. Mutacje genów kodujących te białka są przyczyną dysfunkcji komórek neuronowych, prowadzących do miopatii, neuropatii motorycznych i chorób neurodegeneracyjnych. Celem badań było cytogenetyczne mapowanie genów *HSPB* w genomie świni z zastosowaniem techniki FISH i sond uzyskanych z klonów BAC zawierających sekwencje genów *HSPB1*, *HSPB2*, *CRYAB* (alternatywna nazwa *HSPB5*), *HSPB6*, *HSPB8*, pochodzących z biblioteki genomowej CHORI-242 Porcine BAC Library. Przed hybrydyzacją *in situ*, przeprowadzoną na chromosomach metafazowych barwionych techniką prążków DAPI, potwierdzono obecność badanych genów w wyselekcjonowanym klonie metodą PCR z wykorzystaniem genowo specyficznych starterów.

W wyniku przeprowadzonych eksperymentów uzyskano sygnały FISH w regionach chromosomów SSC3p15 (*HspB1*), SSC9p21 (*HspB2* and *CRYAB*), SSC6q12 (*HspB6*) i *HspB8* SSC14q21 (*HspB8*), co umożliwiło określenie fizycznej lokalizacji badanych genów *HSPB* na mapie genomowej świni domowej. Uzyskane wyniki mogą przyczynić się do wyjaśnienia roli genów *HSPB* w patomechanizmach miopatii i neuropatii u zwierząt hodowlanych.

Słowa kluczowe: chromosomy świni, FISH, mapowanie cytogenetyczne, małe białka szoku cieplnego – *HSPB*, zaburzenia rozwoju i funkcji mięśni