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GENETIC CONSERVATION OF THE *INS-IGF2-H19* GENE CLUSTER IN PIGS

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The aim of this study was to analyse genetic conservation of the telomeric region containing the INS-IGF2-H19 gene cluster in pigs using FISH technique. Human band specific probe (HSA 11p15) was used for hybridization with pig chromosomes. The results obtained showed telomeric regions of telomeric regions of pig chromosomes 2 (band p17). Some aspects of genetic conservation of the human and pig genome have been discussed.

Key words: HUMAN, PIG, *INS-IGF2-H19* GENE CLUSTER, SPECIFIC BAND PROBE, FISH TECHNIQUE.

The gene cluster *INS-IGF2-H19* contains three functionally important genes — insulin, insulin-like growth factor 2 and *H19*. Among them, the insulin-like growth factor 2 (*IGF2*) is paternally expressed and was identified as the major candidate gene for the QTLs because of its involvement in muscle growth and differentiation (Florini et al., 1995). In pigs the *IGF2* gene is paternally expressed, which makes possible restrictive selection for carcass lean (Nezer et al., 1999). Moreover, recent Swedish studies showed that (G→A) transition in intron 3-G3072A of the gene (in conservative region of CpG islet) is related to an increase of about 10–20% in the muscle weight in pigs. This mutation was transmitted to the F_1 generation (created for experimental purposes) by sires — homozygous or heterozygous carriers of the changed gene (Van Laere et al., 2003).

The *IGF2* gene is flanked on its 5' and 3' sides by the insulin (*INS*) and *H19* genes, respectively, and these three genes cover a region of about 150 kb on human chromosome 11p15 (Zemel et al., 1992). Bidirectional chromosome painting established a correspondence between HSA11pter-q13 and SSC2p (Rettenberger et al., 1995; Goureau et al., 1996) Jeon et al. (1999) indicated that *IGF2* in pigs is located close to telomere (band 2p17).

The aim of this study was to analyse genetic conservation of the telomeric region containing the *INS-IGF2-H19* gene cluster in humans and pigs using FISH technique.

Materials and methods

Chromosome spreads of the pig were prepared from pokeweed mitogen stimulated peripheral blood lymphocyte culture according to the routine protocol.

Human band specific probe (HSA 11p15) was bought from Q-BIOgene company (United Kingdom). The probe was prepared after microdissection and labelled with rhodamine (R110). In situ hybridization was performed according to the manufacturer's procedure. For precise identification of chromosome region the metaphases were read out using DAPI technique. Intense fluorescent signals were observed under an OPTON-Axiophot fluorescent microscope using double attenuation filters — DAPI/FITC. Selected cells were recorded and evaluated using the image analysis program LUCIA — FISH (Laboratory Imaging Ltd, Prague, Czech Republic).

Results and discussion

The results presented in Figure 1 showed strong signals indicating telomeric regions of pig chromosomes 2 (band p17).

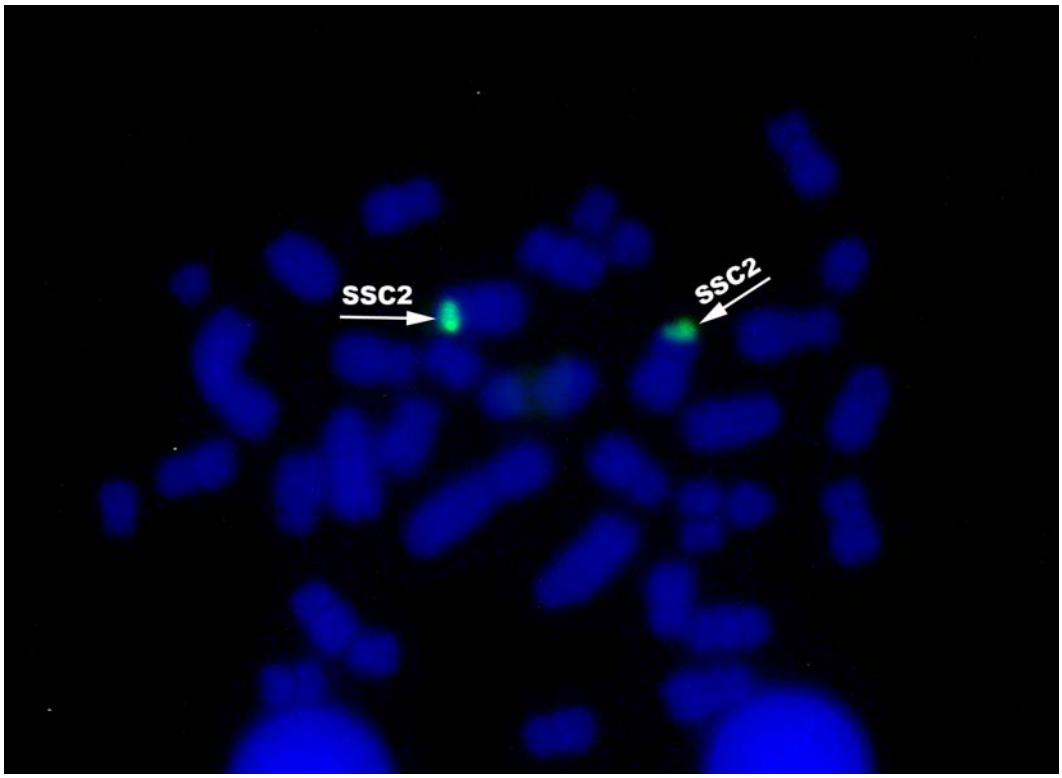

Figure 1. FISH technique — DAPI stained pig chromosomes. Arrows point to signals in telomeric region of pair 2 chromosomes.

These results corresponded to physical and genetic localization of the *IGF2* gene on pig chromosome 2 (Jeon et al., 1999).

A comparative map of the porcine and human genomes prepared using ZOO-FISH technique (Frönike et al., 1996) identified 47 homologous segments on pig metaphase chromosomes. The porcine karyotype was nearly completely covered with homologous human segments. The results obtained by these authors showed that four porcine chromosomes: SSC1, 6, 7 and 14, were composed of homologous segments originating from five human chromosomes (HAS6, 9, 14, 15 and 18). Porcine SSC2 and 9 were composed of homologous segments corresponding to three different human chromosomes each (HSA5, 7 and 11), while the remaining porcine chromosomes showed segmental homology to only one to two human chromosomes. The results of these authors also showed that human probe for chromosome 11 painted whole p arms of SSC2 and 9 porcine chromosomes. In the present study HSA11p15 specific band probe hybridized with telomeric segment of porcine SSC2 only. These results did not confirm the suggestion about localization of human subband 11p15.5 (containing *IGF2* gene) on porcine chromosome 9 (Frönike et al., 1996).

The results shown in this paper correspond to the study by Amarger et al. (2002) comprising the comparative analysis of about 90 kb fragment from the region containing the *INS-IGF2-H19* genes in humans, pigs and mice. The authors showed that the sequence similarity is high in exsons as well as in introns and intergenic regions (CpG islet). The general trend was for a higher sequence similarity between pig and human than between these species and mouse as expected from the phylogenetic relationship. The *IGF2* exon 3 was well conserved between pig and human (74 %), but not in mouse (< 50 % identity compared with human and pig). The 5' flanking region of *IGF2*

exon 5 as well as part of this exon was well conserved between pig and mouse (> 75 %), but not in humans (< 50 %).

Our results (as well as the paper by Amarger et al., 2002) illustrate how a comparative study based on different modern techniques (sequence analysis as well as FISH) and carried out on different species can add power to the interpretation of genome evolution.

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KONSERWATYZM GENETYCZNY KLASTERU GENÓW INS-IGF2-H19

S t r e s z c z e n i e

Celem badań była analiza konserwatyzmu genetycznego w regjonie telomerowym zawierającym klaster genów *INS-IGF2-H19* u świń, przy wykorzystaniu techniki FISH. W hybrydyzacji z chromosomami świń wykorzystano specyficzną ludzką sondę molekularną (HSA11p15). Silnie świecące fluorescencyjne sygnały wskazały regiony telomerowe na chromosomach 2 pary u świń (prążek p17). W pracy omówiono problem homologii ludzkiego i świńskiego genomu

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КОНСЕРВАЦІЯ ГЕНІВ СВИНЕЙ КЛАСТЕРУ ГЕНІВ INS-IGF2-H19

Р е з ю м е

Метою цього дослідження був аналіз консервації генів теломерної ділянки, що містить кластер гена INS-IGF2-H19 у свиней, використовуючи техніку FISH. Для гібридизації з хромосомами свиней використали людський стрічковий специфічний зонд (HSA 11p15). Отримані результати показали сильні сигнали теломерних ділянок хромосом свиней 2 (band p17). Обговорили деякі аспекти консервації генів людини і свиней.

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А н н о т а ц и я

Целью этого исследования был анализ консервирования генов теломерного участка, что включает INS-IGF2-H19 кластер генов у свиней, используя технику FISH. Для гибридизации с хромосомами свиней использовали человеческий ленточный специфический зонд (HSA 11p15). Полученные результаты показали сильные сигналы теломерних участков хромосом свиней 2 (band p17). Обсудили некоторые аспекты консервирования генов человека и свиней.

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