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DIAGNOSIS OF TANDEM FUSION TRANSLOCATION IN THE BOAR USING FISH TECHNIQUE WITH HUMAN PAINTING PROBES*

Barbara Danielak-Czech, Anna Kozubska-Sobocińska, Barbara Rejduch

Department of Animal Immuno- and Cytogenetics, National Research Institute of Animal Production, 32-083 Balice n. Kraków, Poland

Abstract

Interspecies fluorescence *in situ* hybridizations (FISH) with human chromosome painting probes: WCP 10, 12, 20 were used to confirm the diagnosis of the tandem fusion translocation der(14;17)(14q29;17q10) in a boar, which had previously been identified using high-resolution HRBT-GTG banding technique. The experiment showed the usefulness of human painting probes for precise diagnosis of translocations or other chromosomal rearrangements in pigs using FISH technique.

Key words: pigs, tandem fusion translocation, chromosome aberration, FISH technique, human painting probes

Constitutional translocations (reciprocal, Robertsonian and tandem) are the most common chromosome abnormalities occurring in farm animals with fertility impairment or clinical disorders.

Tandem translocations involve the centromere–telomere fusion of two heterologous chromosomes which, similarly to centric fusions, leads to the formation of a long derivative chromosome followed by change of chromosome number in karyotype. In consequence, the animals affected demonstrate reproductive disturbances due to the irregularities in chromosome segregation during meiosis (Villagómez and Pinton, 2008).

In pigs, the major breeding problem are reciprocal and centric fusions – Robertsonian translocations which drastically decrease carriers' fertility by approx. 5–100% and 5–22%, respectively, without any visible phenotypic changes (Gustavsson, 1990). Therefore, such karyotype defects can be widespread in many populations, especially as a result of intensive use of sires affected in artificial insemination (AI) and cause considerable economic losses to breeding organizations (Long, 1991).

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For this reason, several European countries, including Poland, have developed chromosomal screening programmes involving hypoprolific sires and, recently, young AI boars analysed before reproduction (Danielak-Czech and Słota, 2008 a; Ducos et al., 2008). In cytogenetic monitoring of breeding pigs performed recently in the French and Polish specialist laboratories, in addition to new reciprocal translocations also rare centric fusions (13;17, 14;15 and 14;17) (Danielak-Czech and Słota, 2007; Ducos et al., 2007) and the unique tandem fusion der(14;17)(14q29;17q10) were identified (Danielak-Czech and Słota, 2008 b; Danielak-Czech et al., 2010).

In order to predict breeding consequences and prevent the occurrence with early diagnosis, translocations need to be characterized precisely using not only classical cytogenetic techniques but also molecular methods, particularly fluorescence *in situ* hybridization. Where commercial pig-specific chromosome probes are not available, non-species-specific FISH probes (most often commercial human probes) could be used (Rejduch et al., 2004).

In the present paper we report the diagnosis of the first case of tandem fusion translocation in the boar using FISH technique with human painting probes.

Material and methods

A tandem fusion translocation carrier was identified in the boar population of the Animal Breeding and Insemination Station in Bydgoszcz (north-eastern Poland), covered by cytogenetic screening programme in the National Research Institute of Animal Production. The 7-month-old hybrid line boar affected demonstrated normal body conformation and semen parameters. The karyotype formula 37 XY, der(14;17)(14q29;17q10) was defined with the use of classical cytogenetic methods, following the standard protocols of lymphocyte culture, Giemsa staining, and G- and C-banding techniques (Danielak-Czech and Słota, 2008 b).

In this paper we present confirmation of the previous diagnosis by FISH technique with three commercial human whole chromosome painting probes (Cambio): Human WCP FITC Chromosome 10 – Cat. No. 1083-10F-01; Human WCP Individual DIG Labelled Probe – Cat. No. T1-12-SP; Human WCP Cy3 Chromosome 20 – Cat. No. 1153-20Cy3-01 (Pinkel et al., 1986; Solinas-Toldo et al., 1995; Danielak-Czech et al., 2010). The karyotypes, based on GTG and QFQ banded chromosomes, were arranged according to the international pig karyotype standard (Caspersson et al., 1970; Wang and Fedoroff, 1972; Gustavsson, 1988).

Chromosome analyses were carried out under an OPTON-Axiophot fluorescent microscope equipped with a CCD camera and Lucia software.

Results

The phenomenon of genetic conservation between human and pig chromosomes enables human painting probes (WCP) to be used for identification of particular

chromosomes and chromosome rearrangements in *Suidae* species (Frönicke et al., 1996; Jiang and Rothschild, 2007; Rejduch et al., 2004; Rejduch et al., 2010).

In our studies, FISH with human WCP 10 probe showed yellow fluorescence signals involving the SSC14q22→tel regions of rearranged and normal chromosome and SSC10q arms. The second human probe, WCP 12, revealed red fluorescence signals including homeologic segment SSC14q14→16 of changed and normal chromosome and almost whole SSC5 (p14→qtel). The third probe, WCP 20, hybridized to a fragment of derivative SSC14 corresponding to SSC17q10→tel and whole normal SSC17 (green signals) (Figure 1).

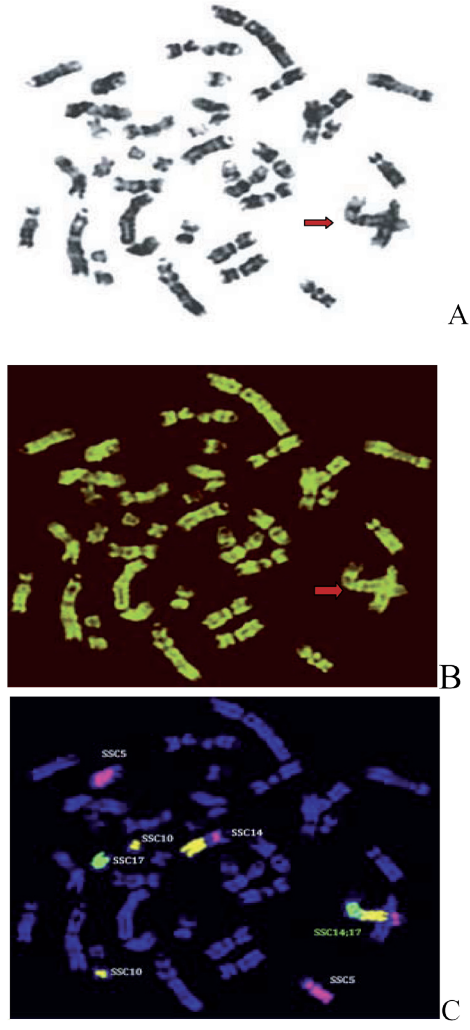

Figure 1. Metaphase chromosomes of the der(14;17)(14q29;17q10) carrier boar:
A – GTG-banded karyotype (arrow indicates derivative chromosome 14);
B – QFQ-banded karyotype (arrow indicates derivative chromosome 14);
C – FISH signals: yellow→ human WCP 10; red→ human WCP 12; green→ human WCP 20

Chromosome painting confirmed the der(14;17)(14q29;17q10) identification carried out previously with the use of classical and high-resolution banding techniques (Danielak-Czech and Słota, 2008 b; Danielak-Czech et al., 2010).

Discussion

Our study showed that the chromosome painting method could effectively supplement classical banding techniques in diagnosis of chromosomal rearrangements. However, the use of molecular methods for karyotype evaluation is still little developed in pigs because the commercial painting probes for this species are not available (Danielak-Czech et al., 2006). For this reason it is often necessary to perform interspecies *in situ* hybridization, especially with human chromosome probes, because the pig genome is of similar size, complexity and genetic information as the human genome. Although some discrepancies exist among the human and pig genome maps, they have contributed to an identification of over 170 conserved segments between genomes of these two species, which have helped to further determine the evolutionary relationship between them (Frönicke et al., 1996; Rejduch et al., 2004; Jiang and Rothschild, 2007). Comparative mapping studies reported by Yerle et al. (1998), Hawken et al. (1999), Jiang and Rothschild (2007) and Rejduch et al. (2010), involving genome linkage, ZOO-FISH and radiation hybrid maps (RH), proved that porcine karyotype was nearly completely covered with homologous human segments. The results obtained by these authors showed conserved segments between human 1, 4, 8, 9, 10, 12, 22 autosome and porcine SSC14, as well as between HSA 4, 8, 20 and SSC17. Interspecies homologies concerning the largest of these segments served as a basis for choosing human WCP 10, 12 and 20 probes for our experiment, with the aim of molecular identification of the first case of tandem fusion translocation in pigs (Figure 1). Our results illustrate how comparative study based on different modern techniques (sequence analysis as well as FISH) and carried out on different species can add power to precise interpretation of genome rearrangements, including structural changes like tandem fusion described here.

It must be assumed that the presented der(14;17)(14q29;17q10) tandem fusion diagnosed in the insemination boar would have almost the same impact on carrier's fertility as 13;17 centric fusion discussed above. Furthermore, global breeding and economic consequences resulting from the use of affected offspring in reproduction are expected to be much higher owing to the accumulation of an individual effect in large populations, as is generally the case for AI sires. However, the massive spread of this abnormality will be avoided by early identification and culling of affected animals thanks to the chromosomal control programme of young AI boars, developed by the National Research Institute of Animal Production in Poland in 2007 (Danielak-Czech and Słota, 2007, 2008 a, 2008 b).

The results of FISH analysis, performed for improved diagnosis, have confirmed previous cytogenetic evaluation of the karyotype of the tandem fusion carrying boar, as well as numerous homologies and homeologies between human and porcine chro-

mosomes. The experiment proved also the usefulness of commercial human painting probes for identification of chromosome rearrangements in other species that received little study.

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BARBARA DANIELAK-CZECH, ANNA KOZUBSKA-SOBOCIŃSKA, BARBARA REJDUCH

Diagnoza tandem fuzji-translokacji u knura przy wykorzystaniu techniki FISH z ludzkimi sondami malującymi

STRESZCZENIE

Przy wykorzystaniu międzygatunkowych hybrydyzacji *in situ* z ludzkimi chromosomowymi sondami malującymi: WCP 10, 12, 20 potwierdzono diagnozę tandem fuzji-translokacji der(14;17)(14q29;17q10) u knura, zidentyfikowanej wcześniej wysoko rozdzielczą techniką prążkową HRBT-GTG. Eksperyment wykazał przydatność ludzkich sond malujących do precyzyjnej diagnozy techniką FISH translokacji i innych chromosomowych rearanżacji u świń.