Cross-species hybridizations in situ for identification of Robertsonian translocation in wild boar

MAREK BABICZ¹, BARBARA DANIELAK-CZECH², ANNA KOZUBSKA-SOBOCIŃSKA²
¹Department of Pig Breeding and Production Technology, University of Life Sciences in Lublin
²Department of Animal Genomics and Molecular Biology, National Institute of Animal Production, Balice/Kraków

Abstract: Cross-species hybridizations in situ for identification of Robertsonian translocation in wild boar. Homologies and homeologies between human and pig chromosomes enabled human painting probes to be used for identification of chromosomes involved in homozygous centric fusion in the wild boar (Sus scrofa scrofa) with karyotype 36,XY,rob(15;17), which had been provisionally determined on the basis of G-bands (GTG technique). For interspecies hybridizations two commercial differently labelled human painting probes for chromosome pairs 2 and 20 were used. FISH with the human WCP 20 probe revealed green fluorescence signals on the short arms of the 15;17 translocated chromosomes, and after hybridization with the WCP 2 yellow signals were observed along the long arms of these rearranged acrocentric autosomes as well as on small fragments of the SSC3q arms. The results of cross-species hybridizations in situ have confirmed preliminary cytogenetic evaluation of the karyotype of the Robertsonian translocation-carrying wild boar as well as numerous homologies and homeologies between chromosomes of human and species (Sus scrofa domestica and Sus scrofa scrofa) belonging to the Suidae family. The results obtained have confirmed also the usefulness of commercial human painting probes for identification of chromosome rearrangements in other species that received little study.

Key words: wild pig, Robertsonian translocation, karyotype 36,XY,rob(15;17), ZOO-FISH

INTRODUCTION

The normal karyotype of the domestic pig (Sus scrofa domestica) contains 2n = 38 chromosomes (involving 24 meta- and submetacentric autosomes, 12 acrocentric chromosomes and two XX and XY heterosomes) is almost identical to that of the wild boar (Sus scrofa scrofa) (Gustavsson 1988, 1990).

However, populations of the wild boar frequently demonstrate chromosome number polymorphism caused by chromosome rearrangements of the centric fusion type (Robertsonian translocation), which may lead to a reduction of the chromosome number, but not their arm number (NF), which is characteristic for a given species. The investigations performed on domestic pigs and wild boars showed variation of diploid chromosome numbers of 36-37-38 (Gustavsson et al. 1973, Bosma 1976, Sysa et al. 1984, Troshina et al. 1985, Rejduch et al. 2003, Wnuk et al. 2005).
In pigs, the Robertsonian translocations decrease carriers’ fertility by approx. 5–22%, without any visible phenotypic changes. 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 (Gustavsson 1990). 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 Slota 2008, 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, 14;17 and 15;18) and the unique tandem fusion der(14;17)(14q29;17q10) were identified (Pinton et al. 2012, Danielak-Czech et al. 2016).

The aim of the present study was identification of Robertsonian translocation in wild boar, using two commercial human painting probes in cross-species hybridizations in situ.

MATERIAL AND METHODS

Sus scrofa scrofa has been obtained (after culling) under the planned wildlife management in the Lublin region (Act of 13 November 1995 Hunting Laws, Dz.U. [Official Journal] 1995 No 147, item 713, as amended Dz.U. of 2015, item 2168, of 2016 item 1082). Metaphase chromosome preparations of wild boar studied, were derived from the classical peripheral blood lymphocyte culture. For chromosome staining, conventional Giemsa staining and the standard protocol of GTG-banding technique were applied. The karyotypes were prepared according to the recommendation of the Committee for the Standardized Karyotype of Domestic Pig (Gustavsson 1988).

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 (Rubes et al. 2009, Slota and Danielak 2010, Danielak-Czech et al. 2013a, 2016). Where commercial pig-specific chromosome probes are not available, non-species-specific probes (most often commercial-human probes) could be used (Danielak-Czech et al. 2010, 2013b, Rejduch et al. 2010b).

The aim of the present study was identification of Robertsonian translocation in wild boar, using two commercial human painting probes in cross-species hybridizations in situ.
RESULTS AND DISCUSSION

Microscope analysis of Giemsa stained metaphase spreads revealed that the wild boar studied had 36 chromosomes (2n = 36), of which 26 were meta- and submetacentric chromosomes, 8 acrocentric autosomes and XY heterosomes. The GTG-banding technique performed in this animal proved the existence of the two additional submetacentric chromosomes in his chromosome set, resulting from a centric fusion between acrocentric chromosomes of the pairs 15 and 17 (Fig. 1). Based on this procedure, the karyotype of the studied wild boar with Robertsonian translocation in homozygotic form was defined as 36,XY,rob(15;17) – Figure 2. The diagnosis was unequivocally evidenced by FISH technique with human whole chromosome painting probes. Inter-specific hybridizations in situ with the human WCP 20 probe showed green fluorescence signals on the short arms of the 15;17 translocated chromosomes, and after hybridization with the WCP 2 yellow signals were observed along the long arms of these rearranged acrocentric autosomes and on small fragments of the SSC3q arms (Fig. 3).

It is worth noting that similarities between chromosomes of different Suidae species were shown many times in hybridizations in situ using probes obtained from Sus scrofa domestica chromosomes by flow-sorting and microdissection of whole chromosomes or their fragments as well as by PCR from genomic DNA using appropriate primers (Slota and Danielak-Czech 2010, Doležel et al. 2012, Danielak-Czech et al. 2013a, 2016). On the other hand, the phenomenon of genetic

However, it must be stated that the use of molecular methods for karyotype evaluation is still little developed in pigs because the commercial painting probes for this species are almost not available (Rubes et al. 2009, Barasc et al. 2014, Danielak-Czech et al. 2016). For this reason it is often necessary to perform interspecies hybridization in situ, 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 (Goureau et al. 1996, Frönicke et al. 1996, Yerle et al. 1996, Jiang and Rothschild 2007).

It should be added that the numerous comparative mapping studies (genome

FIGURE 3. The cross-species hybridizations in situ with human painting probes (WCP 2 and WCP 20) for identification of Robertsonian translocation in wild boar with karyotype 36,XY,rob15;17. Yellow fluorescence signals (A) after hybridization with the WCP 2 probe were observed along the long arms of the 15;17 translocated chromosomes and on small fragments of the SSC3q arms. Green signals (B) after hybridization with the WCP 20 probe were observed on the short arms of the 15;17 rearranged acrocentric autosomes.
Cross-species hybridizations also definitely proved that porcine karyotype was nearly completely covered with homologous human segments (Danielak-Czech et al. 2010, 2013b, Rejduch et al. 2010a, Kozubskas-Sobocińska et al. 2014, 2015). The results obtained in our comparative studies showed conserved segments between human autosome 2 and porcine SSC15, SSC3, as well as between HSA20 and SSC17. Interspecies homologies concerning the largest of these segments served as a basis for choosing human WCP 2 and 20 probes for our experiment, with the aim of molecular identification of the Robertsonian translocation in the wild boar with karyotype 36,XY,rob(15;17) – Figure 3.

In general, our results illustrate how comparative study based on different modern techniques (as well as FISH) and carried out on different species can add power to precise interpretation of genome rearrangements, including structural changes like centric fusion described here. Moreover, our cross-species hybridization in situ experiments substantially prove conservation of the linkage groups and high degree of homology and homeology of chromosome regions in human and the domestic and wild pig genomes.

CONCLUSION

The results of FISH analysis have confirmed preliminary cytogenetic evaluation of the karyotype of the Robertsonian translocation carrying wild boar as well as numerous homologies and homeologies between human and Sus scrofa scrofa chromosomes.

REFERENCES


Streszczenie: Międzygatunkowe hybrydyzacje in situ do identyfikacji translokacji Robertsonowskiej u dzika. Homologie i homeologie między chromosomami człowieka i świń domowej umożliwiły zastosowanie ludzkich sond malujących do identyfikacji chromosomów zaangażowanych w homozygotyczną fuzję centryczną u dzika (Sus scrofa scrofa) o kariotypie 36,XY,rob(15;17), który został wstępnie zdiagnozowany na podstawie prążków G (technika GTG). Do międzygatunkowych hybrydyzacji wykorzystano dwie komercyjne, różnie znakowane ludzkie sondy malujące dla chromosomów par 2 i 20. FISH z ludzką sondą WCP 20 ujawniła zielone sygnały hybrydyzacyjne na krótkich ramionach translokowanych chromosomów 15;17, a po hybrydyzacji z WCP 2 żółte sygnały obserwowane były wzdłuż długich ramion tych zreorganizowanych akrocenncyjnych autosomów. Wyniki międzygatunkowych hybrydyzacji in situ potwierdziły wstępną cytogenetyczną ocenę kariotypu dzika – nosiciela translokacji robertsonowskiej, a także liczne homologie i homeologie między chromosomami człowieka i gatunków należących do rodziny Suidae (Sus scrofa domestica i Sus scrofa scrofa).

Słowa kluczowe: dzik, translokacja Robertsonowska, kariotyp 36,XY,rob(15;17), ZOO-FISH

MS received 23.10.2016
MS accepted 14.03.2017

Authors’ address:
Marek Babicz
Katedra Hodowli i Technologii Produkcji Trzody Chlewnej
Uniwersytet Przyrodniczy w Lublinie
Akademicka 13, 20-950 Lublin
Poland
e-mail: marek.babicz@up.lublin.pl