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IDENTIFICATION OF TELOMERIC SEQUENCES IN PIGS WITH REARRANGED KARYOTYPE USING PRINS TECHNIQUE*

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Abstract

Using PRINS technique with oligonucleotide primer complementary to the telomere sequence repeats (TTAGGG)n, the interstitial telomeric signals in 6q22 and 7q13 unstable chromosome regions in pigs with t(7;13)(q13;q46) reciprocal translocation were identified. The intrachromosomal localization of telomeric repeats specific for 7q13 region were revealed to strictly correspond to breakpoint of this translocation as well as several others described earlier. The present study showed the usefulness of PRINS technique for identification of intrachromosomal telomeric sequences in pig genome regions, extremely prone to breakage and predisposed to chromosome reorganizations, especially with regard to the clinical cases of karyotype defects.

Key words: PRINS technique, karyotype rearrangement, interstitial telomeric repeats, reciprocal translocation breakpoints, pigs

Primed *in situ* DNA synthesis (PRINS) is a technique based on *in situ* annealing of unlabelled short oligonucleotides to complementary chromosome sequences and following extension with *Taq* DNA polymerase. This method can be used alternatively to fluorescence *in situ* hybridization (FISH) as a tool for gene mapping and evolutionary studies as well as aneuploidy diagnostics or DNA damage evaluation (Koch, 2006; Pellestor, 2006). In particular, the rapid and sensitive PRINS is very useful to detect the repetitive DNA sequences, such as specific rDNA, heterochromatin or telomere repeats in metaphase chromosomes (de la Sena et al., 1995; Gu et al., 1996; Russo, 2002; Koch, 2006; Wnuk et al., 2008).

The latest studies concentrate on telomeres and the interstitially located (TTAGGG)n repeats due to their role in DNA replication and DNA or chromosomal lesions

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repair leading to chromosomal rearrangements formation and karyotype evolution (Azzalin et al., 2001; Adega et al., 2009; Bolzan, 2012). The main emphasis is being placed on influence of intrachromosomal telomeric sequences on mutagen-induced genome instability, especially chromosome fragility responsible for recurrent chromatid breaking events, aberrations and constitutional rearrangements (Mondello et al., 2000; Adega et al., 2005; Bolzan, 2012). In pigs, it concerns mainly structural karyotype defects, such as highly frequent heritable reciprocal translocations (about 150 cases reported) that are a major breeding problem due to their sharp decreasing effect on reproduction (carrier fertility reduced by 5 to 100% without any visible phenotypic changes) (Ducos et al., 2000, 2007, 2008; Basrur and Stranzinger, 2008; Danielak-Czech and Słota, 2008; Słota and Danielak-Czech, 2010). The majority of reciprocal translocations originate *"de novo"* as a consequence of chromosome breaks resulting from unusual pig genome sensitivity to environmental effects. Most breaks occur in centromeric/pericentromeric or telomeric/subtelomeric areas and within intrachromosomal unstable regions, both notified as recurrent spontaneous breakage sites in at least several translocations and classified as mutagen-induced fragile sites (Danielak-Czech and Słota, 2004; Riggs and Rønne, 2009). A review of numerous reciprocal translocations shows that there is a close correlation between the impact on fertility of these structural changes (smaller litter size by about 50% or above) and the location of breakpoints and size of rearranged chromosome fragments (Basrur and Stranzinger, 2008; Świtoński and Stranzinger, 1998; Villagomez and Pinton, 2008). Therefore, to predict breeding consequences and prevent by early identification, translocations associated with recurrent chromosome breakpoints need to be characterized precisely not only by classical cytogenetic techniques but also with an aid of molecular methods like FISH and PRINS (Basrur and Stranzinger, 2008; Rubes et al., 2009; Słota and Danielak-Czech, 2010). It sufficiently justifies PRINS-based analyses of telomeric repeats and especially their nontelomeric locations in rearranged pig karyotypes as the associated clinical effects still remain to be determined.

The aim of this paper was to examine the telomeric sequences distribution in rearranged pig karyotype using PRINS technique, with special consideration of intrachromosomal telomeric repeats. The experiment was carried out on the basis of reciprocal translocation t(7;13)(q13;q46), identified previously by high-resolution-RBA/GTG banding techniques, meiotic chromosome studies with synaptonemal complex analysis and FISH method with microdissected whole chromosome painting probes (Danielak-Czech et al., 1994, 1996, 1997, 2006; Danielak-Czech and Kaczor, 2010).

Material and methods

Cytogenetic examination of t(7;13)(q13;q46) carriers without any visible phenotype changes (990 hybrid line sow as well as 5 female and 6 male offspring, randomly chosen from the population of 50 pigs kept in the Pig Hybridization Station of

the National Research Institute of Animal Production) was based on standard protocols of lymphocyte culture, GTG/DAPI banding and FISH techniques, following the procedure described by Danielak-Czech et al. (2006).

GTG-banded chromosomes were scored for recurrent spontaneous chromatid break/gaps (occurring at least twice) and karyotypes were arranged according to the international pig karyotype standard.

The PRINS technique with telomere human commercial kit (Prins-Probe 1409-T, Cambio Ltd., UK) was applied according to the manufacturer's instruction with minor modifications reported by Wnuk et al. (2008). Briefly, after treatment of metaphase spreads with RNase (1 h, 37°C) and pepsin solutions (30 min, 37°C), washing in PBS (pH 7.4) and passing through a methanol series (70, 80, 95%), 25 µl of warmed (5 min, 37°C) reaction mixture (PRINS kit, 0.05 mM biotin –11-dUTP and 2 U Taq polymerase) was added to air-dried slides, which were placed on preheated block (5 min, 94°C) for 30 min (58°C). Subsequently, the reaction was stopped by stop buffer (50 mM NaCL and 50 mM EDTA, pH 8.0) at 58°C (5 min) and next at room temperature (7 min). Finally, slides were dehydrated in ethanol series, placed in blocking solution (3% BSA, 4×SSC, 0.05% Tween 20; 37°C, 30 min) and incubated with the avidin-FITC, anti-avidin antibody, FITC (each at 37°C, 45 min) separated by thrice washing (4×SSC, 0.05% Tween 20; 45°C, 3 min).

The DAPI-banded chromosome slides (mounted in antifade solution) were analysed in Axio Imager.D2 (Zeiss) fluorescence microscope equipped with Axio Vision computer-assisted image analysis system.

Results

Cytogenetic evaluation involved 12 healthy, exterior-normal sows and boars carrying t(7;13)(q13;q46) reciprocal translocation (scheme with breakpoints location and FISH-painted rearranged chromosomes were presented on Figs. 1 A and 1 B). Detailed karyotype analysis displayed increased percentage (2–30%) of chromatid breakages in 1q21, 3p13, 6q22, 7q13, 13q41, 14q21, 15q13, Xq22, Xq26 bands, which were determined as unstable genome regions prone to multiple structural lesions.

The application of PRINS technique gave distinct fluorescence signals on both ends of all chromosomes as well as centromeres of acrocentric chromosomes of pairs 13 to 18. Additionally, PRINS revealed interstitial telomeric signals (ITS) on both homologues of 6 and 7 sub-metacentric chromosome pairs, strictly in the above mentioned 6q22 and 7q13 unstable regions predisposed to repeated structural defects. Moreover, the intrachromosomal 7q13-specific telomeric signals were defined as evidently coincident with one of the t(7;13)(q13;q46) reciprocal translocation breakpoints, which accurately proved their location supplementing rearrangement diagnosis (results of PRINS-detection of telomeric repeats in rearranged karyotype were shown on Fig. 2).

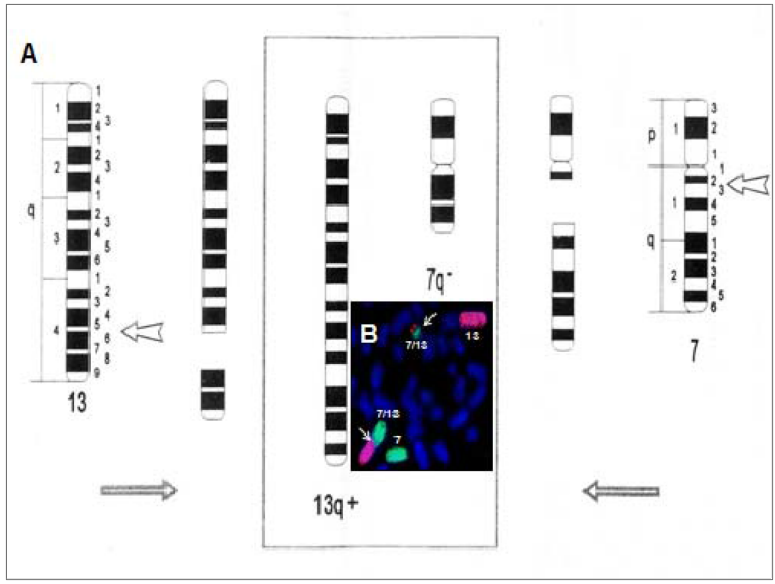

Figure 1. Schematic presentation of t(7;13)(q13;q46) reciprocal translocation with breakpoints location and FISH-painted rearranged chromosomes

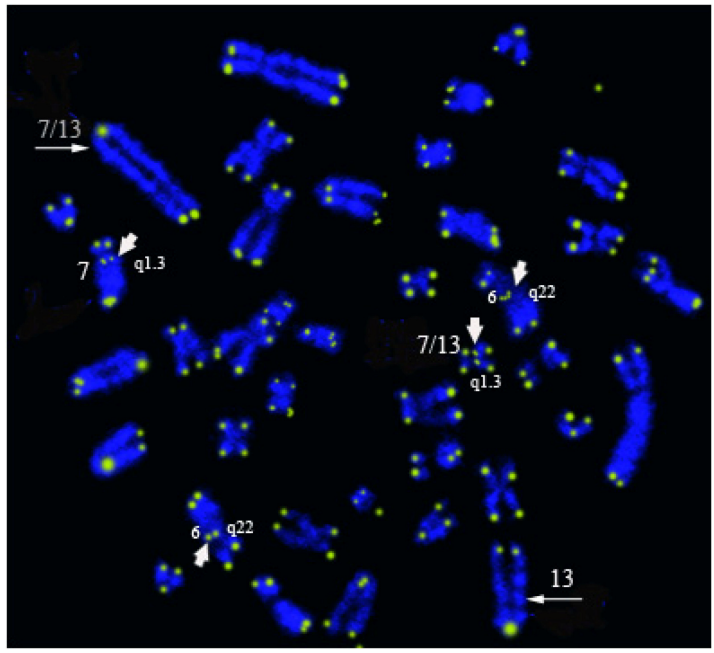

Figure 2. Results of PRINS detection of telomeric repeats in karyotype of t(7;13)(q13;q46) carrier: fluorescence signals in telomeres of all chromosomes and centromeres of acrocentric chromosomes of 13–18 chromosome pairs as well as interstitial telomeric signals (ITS) in 6q22 and 7q13 chromosome regions (arrows show 7q13-specific ITS in rearranged and normal chromosome 7)

Discussion

The results of PRINS-detection performed in our study generally confirmed preliminary *in situ* investigations carried out by FISH or PRINS methods with telomeric DNA probe or oligonucleotide primers (de la Sena et al., 1995; Gu et al., 1996; Wnuk et al., 2008). However, unlike such previous molecular experiments which concerned quite normal pig karyotype, our present examination was aimed to determine distribution of telomeric repeats in pig rearranged karyotype, based on reciprocal translocation t(7;13)(q13;q46). Analyses performed with special consideration of interstitial telomeric repeats and their clinical effects showed location of ITS in 6q22 and 7q13 regions of pig abnormal karyotype and coexistence with one of the t(7;13)(q13;q46) translocation breakpoints.

According to the newest studies in pigs and other species, these nontelomeric (TTAGGG)n sites (detected in pericentromeric or internal chromosomal areas) are consistent with cryptic GC-rich intrachromosomal constitutive heterochromatin bands in G-light-stained genome regions. Furthermore, due to their heterochromatic nature, such interstitial repeated sequences are considered to be responsible for transcriptional silencing of nearby genes or suppression of recombination (including the process of DNA damage repair) resulting in chromosome aberrations and karyotype changes (Russo, 2002; Adega et al., 2005, 2009).

In farm animals, the intrachromosomal (TTAGGG)n repeats corresponding to heterochromatin DNA satellite sequences are thought to be 'hot spots' for recurrent genome lesions, chromosome fragility and structural rearrangements related to clinical consequences, reproductive failure, hypoprolificacy and following financial losses in breeding (Basrur and Stranzinger, 2008; Ducos et al., 2008; Adega et al., 2009; Riggs and Ronne, 2009). As regards pigs, our study confirmed the correlation between identified 6q22 and 7q13 region-specific interstitial telomeric repeats and reciprocal translocation breakpoints as well as a scale of reproductive consequences expressed by reduced litter size (Świtoński and Stranzinger, 1998; Adega et al., 2005, 2009; Villagomez and Pinton, 2008). The first one is parallel to 6q22 breakpoint in t(1;6)(q12;q22) translocation, reported as a cause of 56% fertility decrease of boar-carrier (Ducos et al., 2000). The second intrachromosomal 7q13-specific telomeric site is strictly coincident with t(7;13)(q13;q46) translocation breakpoint which was evidently documented in the present paper. In that case, a considerable impact on fertility was a result of 7q13 breakpoint location and clearly unsymmetrical size of rearranged chromosome fragments as factors determining the course of chromosome pairing, crossing-over and meiotic segregation leading to production of gametes with deletions/duplications, which increased embryo losses due to an unbalanced chromosome number (Danielak-Czech et al., 1994, 1997). As a result, t(7;13)(q13;q46) translocation was proved to decrease mean litter size by 48% and cause significant financial losses related to using the boar-carrier in a commercial herd, estimated (on the basis of simulation account) at about USD8,000 for natural mating and about USD162,000 for artificial insemination in the active Polish pig population (Danielak-Czech et al., 1996). Furthermore, it is necessary to mention that the same 7q13 breakpoint was identified also in the three other reciprocal translocations (7;8)

(q13;27), (7;10)(q13;q11), (1;7)(q17;q13) associated with significant hypoprolificacy of breeding boars (Ravaoarimanana et al., 1992; Ducos et al., 2007). Summing up, we can state that 7q13 intestitial telomeric site is one of the pig chromosome regions prone to breakage and reorganization, which may be repeatedly used in many clinical cases of karyotype defects and during the evolutionary process.

It is worth noting that the pig karyotype displays really great variation, with a prevalence of 1/200 of structural chromosomal rearrangements, mainly reciprocal translocations with reiterating chromatid breakpoints involving analogical unstable regions (specific tandemly repeated DNA sequences or fragile sites), associated with several clinical defects such as congenital malformations, intersexuality or reproductive dysfunction observed by reduction of the fertility/prolificacy of the carriers and/or their mates (Ducos et al., 2000, 2007, 2008; Danielak-Czech and Słota, 2008; Słota and Danielak-Czech, 2010). Therefore, future cytomolecular studies of relevant repetitive sequences promoting chromosome aberrations can be essential to identify crucial agents predisposing for recurrent reciprocal translocations and predict their clinical consequences before use of young carriers in reproduction, prevent genetic defects from spreading in breeding populations and limit economic effects.

In conclusion, the results obtained suggest that interstitial telomeric sequences coincident with repeating spontaneous breakages and reciprocal translocations breakpoints can be a molecular signature of predisposition for iterative pig chromosome aberrations related to loss of productivity. The findings can be applied in further comparative cytomolecular studies aimed to ascertain functional significance of repetitive DNA sequences in mechanisms of multiple karyotype rearrangements and genome remodelling with regard to clinical, breeding and evolutionary effects in farm animals.

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Identyfikacja sekwencji telomerowych u świń z rearanżacją kariotypu przy wykorzystaniu techniki PRINS

STRESZCZENIE

Stosując technikę PRINS z oligonukleotydowym primerem komplementarnym do telomerowej sekwencji powtarzalnej (TTAGGG)n w niestabilnych regionach chromosomowych 6q22 i 7q13 świń z translokacją wzajemną t(7;13)(q13;q46) zidentyfikowano interstycjalne sygnały telomerowe. Wykazano, że wewnątrzchromosomowa lokalizacja powtórzeń telomerowych specyficznych dla regionu 7q13 dokładnie odpowiada punktowi pęknięć tej translokacji, jak również kilku innych opisanych wcześniej. Przeprowadzone badania wykazały przydatność techniki PRINS do identyfikacji wewnątrzchromosomowych sekwencji telomerowych w regionach genomu świni, wyjątkowo podatnych na pęknięcia i predysponowanych do reorganizacji chromosomowych, szczególnie w aspekcie klinicznych przypadków wad kariotypu.