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COMPARATIVE MAPPING OF THE UNSTABLE CGG TANDEM REPEATS OF THE HUMAN *FRM1* GENE IN FARM ANIMALS

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Based on in situ PCR technique with primers flanking CGG repeats located in the 5'UTR region of the human FRM1 mRNA, the trinucleotide repeats of the FRM1 gene have been mapped to the structurally unstable X chromosome regions of cattle — BTA Xp13, sheep — OAR Xq22, goats — CHI Xq22 and pigs — Xq26. The experiment confirmed homology of regulatory sequences in CGG region of FRM1 gene and conservation of the corresponding unstable X chromosome regions in mammals. These results can be applied in comparative evolutionary studies and make it possible to ascertain the relationships between region-specific X chromosome instability and phenotypic effects in farm animals.

Key words: TANDEM REPEATS, TRINUCLEOTIDE REPEATS EXPANSION, REPEAT EXPANSION DISEASES, FRM1 GENE, FRAGILE X, FARM ANIMALS

Hypervariable tandem repeat tracts of different sequence, number and locations are a ubiquitous feature of mammalian genomes however their evolution and functional properties need interspecies comparative studies. In humans, long, highly polymorphic repeats are associated with genetic disorders affecting many biological processes. While unstable repeats in the coding sequence can result in toxic proteins, noncoding repeats can have significant effects on chromosome fragility, gene silencing, transcription, translation or splicing modulation and cell architecture (Usdin, 2008). For example, the CGG repeat expansion of the 5'UTR region of human *FRM1* gene, followed by Xq27.3 fragile site expression, leads to severe phenotypic changes including neurodegeneration and ovarian insufficiency (fragile X syndrome) (Pearson et al., 2005; Hagerman, 2006; Wittenberger et al., 2007). Although homologous CGG region of this gene was identified in genomes of several farm animals, its chromosomal location has not been assigned as yet (Deelen et. al, 1994).

The aim of this study was cytogenetic localization of the unstable CGG tandem repeats of the human *FRM1* gene in domestic *Bovids* and pig karyotypes.

Materials and methods

Chromosome preparation: Cattle, sheep, goat and pig metaphase chromosome slides were prepared following classical cytogenetic protocols of lymphocyte culture and banding techniques (GTG, QFQ). Karyotypes were arranged according to the international karyotype standards of the species under study (Gustavsson, 1988; Di Berardino et al, 2001).

IN situ PCR: The primers GCTCAGCTCGGTTTCGGTTTCACTTCCCGT (forward) and AGCCCCGCACTTCCACCACCAGCTCCTCCA (reverse) flanking CGG repeats of the 5'UTR region of human *FRM1* gene (0.3830 kb) (GDB: 187391; c/f) (Fu et al., 1991) were used for *in situ* PCR and biotin-16 dUTP labeling (Troyer et al., 1994) of the homologous sequence directly on microscopic slides with metaphase chromosome spreads (in MJR PTC-100 thermocycler with metal

heating block for glass slides). The reaction was carried out according to the thermal profile: 1 cycle: 94 °C — 3 min, 65 °C — 1 min, 72 °C — 1 min; 30 cycles: 94 °C — 1 min, 65 °C — 1 min, 72 °C — 1 min.

Detection: The amplified and labeled gene fragment was detected by avidin-conjugated FITC, and hybridization signals were analyzed with fluorescence microscope equipped with the computer-assisted image analysis system LUCIA-FISH (Laboratory Imaging Ltd, Prague, Czech Republic).

Results and discussion

Based on combined *in situ* PCR method and GTG/QFQ banding techniques the CGG repeats containing fragment of the human *FRM1* gene was mapped to unstable X chromosome regions of the domestic *Bovids* under study — BTA Xp13, OAR Xq22 and CHI Xq22 (cattle, sheep and goats, respectively) and pigs — SSC Xq26 (Fig. 1 and 2).The results obtained supported the preliminary cytogenetic mapping of bovid X chromosome (FISH, *in situ* PCR) which revealed homologies and divergences between the subfamilies *Bovinae* and *Caprinae*, suggesting transposition of common chromosome X segments during their karyotypic evolution (Iannuzzi et al., 2000; Słota et al., 2007). Additionally, performed comparative interspecies analysis including *Bovids* and pigs proved previous predictions about conservation of the *FRM1* gene locus and equivalent unstable X chromosome regions in more distantly related mammals (Deelen et al., 1994; Danielak-Czech and Słota, 2006; Madsen et al., 2007); Słota et al., 2007).

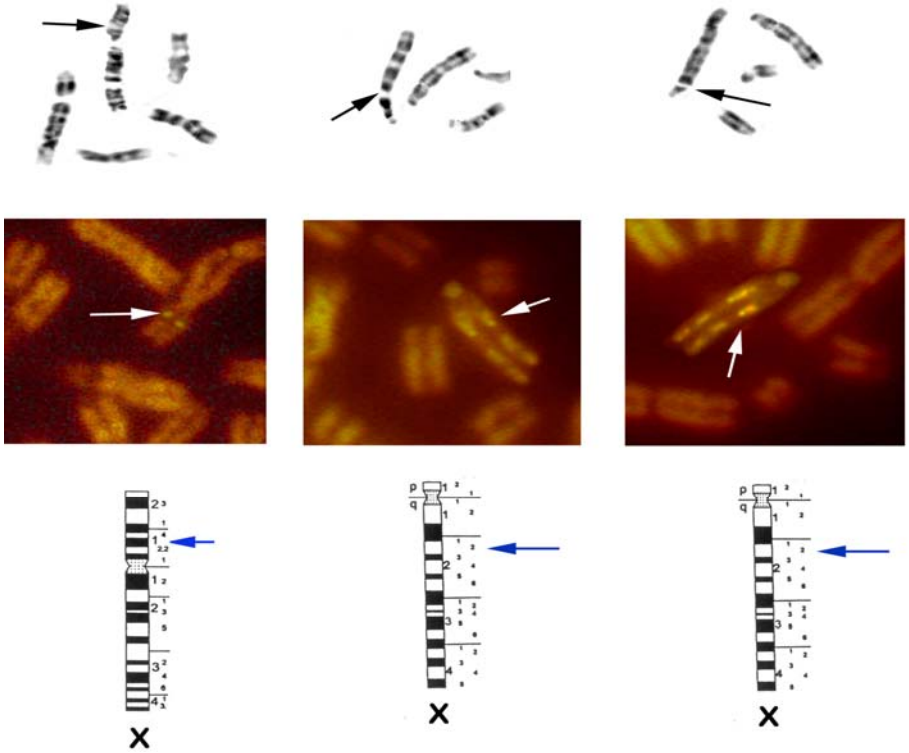

Figure 1. Unstable chromosome regions and corresponding FITC signals specific to the CGG tandem repeats of the human *FRM1* gene Unstable chromosome regions and corresponding on GTG/QFQ banded X chromosomes of domestic *Bovids*: cattle — BTA Xp13 (left); sheep — OAR Xq22 (middle); goats — CHI Xq22 (right).

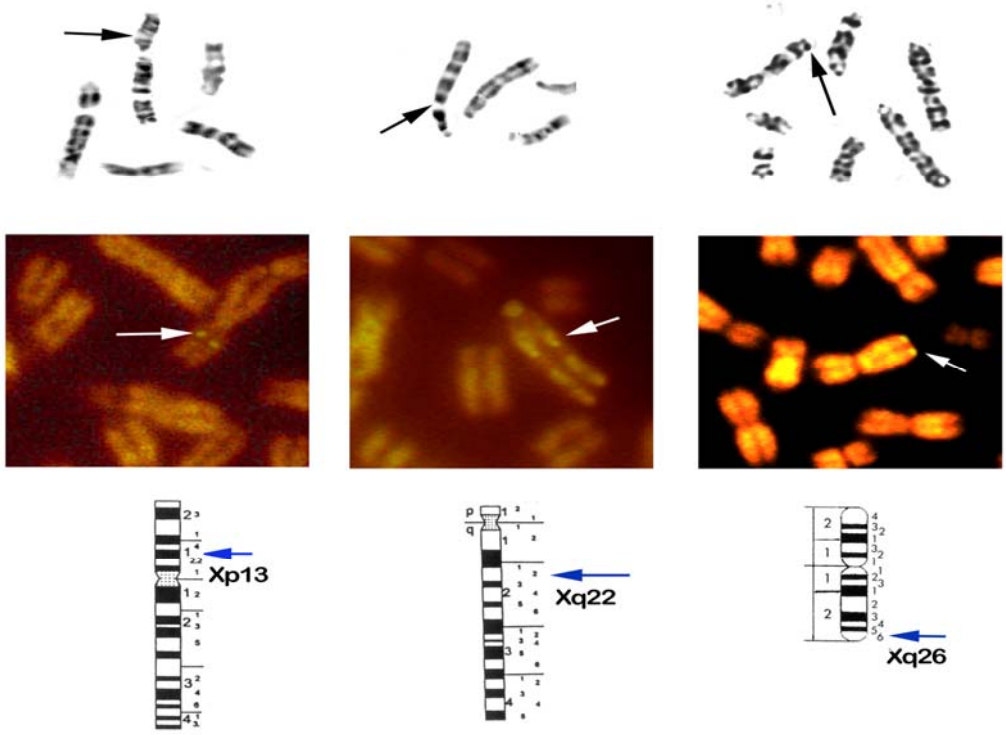

Figure 2. Unstable chromosome regions and corresponding FITC signals specific to the CGG tandem repeats of the human *FRM1* gene (shown by arrows) on GTG/QFQ banded X chromosomes of *Bovids* and pigs: cattle — BTA Xp13 (left); sheep — OAR Xq22 (middle); pigs — SSC Xq22 (right).

The experiments reported in this paper confirmed strong sequence homology of CGG tandem repeats in the 5'UTR region of *FRM1* gene in numerous mammalian species. The presence of such trinucleotide repeat loci like *FRM1* in farm animals' genomes with tract lengths similar to that present in humans support future exploration of the *Bovids* or pigs as natural animal models to study tandem repeat variability and function. In conclusion, the results obtained can be applied in further comparative evolutionary studies and make it possible to ascertain the relationships between tandem repeat loci mutability or region-specific X chromosome instability and phenotypic effects in farm animals.

S u m m a r y

Applying *in situ* PCR method and GTG/QFQ banding techniques the location of CGG repeats containing fragment of the human *FRM1* gene was assigned to unstable X chromosome regions of cattle — BTA Xp13, sheep — OAR Xq22, goats — CHI Xq22 and pigs — SSC Xq26. The results obtained confirmed homology and conservation of CGG regulatory sequence in the 5'UTR of *FRM1* gene in mammals, which can be a basis for further comparative and evolutionary studies.

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

Stosując metodę *in situ* PCR oraz techniki prążkowe GTG/QFQ określono lokalizację fragmentu ludzkiego genu *FRM1* zawierającego powtórzenia CGG w niestabilnych regionach chromosomu X bydła — BTA Xp13, owiec — OAR Xq22, kóz — CHI Xq22 i świń — SSC Xq26. Uzyskane wyniki potwierdziły homologię i konserwatyzm sekwencji regulatorowej CGG w regionie 5'UTR genu *FRM1* u ssaków, co może stanowić podstawę dalszych badań porównawczych i ewolucyjnych.

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ПОРІВНЯЛЬНА КАРТОГРАФІЯ НЕСТІЙКИХ CGG ТАНДЕМІВ ЛЮДСЬКОГО ГЕНА FRM1 У СІЛЬСЬКОГОСПОДАРСЬКИХ ТВАРИН

Р е з ю м е

За технікою PCR in situ з первинним флангом повторень CGG, розташованих у ділянці 5'UTR людського FRM1 mRNA, тринуклеотидні повторення гена FRM1 проектувалися на структурно нестійкі ділянки Х хромосоми великої рогатої худоби — BTA Xp13, вівці — OAR Xq22, кози — CHI Xq22 і свині — Xq26. Експеримент підтвердив гомологію регулярних послідовностей в CGG ділянці гена FRM1 і збереження відповідних нестійких ділянок Х хромосоми у ссавців. Ці результати можна застосувати у порівняльних еволюційних дослідженнях та дають можливість перевірити наявність взаємозв'язків між нестабільністю Х хромосоми у певних ділянках та фенотипними наслідками у сільськогосподарських тварин.

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СРАВНИТЕЛЬНАЯ КАРТОГРАФИЯ НЕУСТОЙЧИВЫХ CGG ТАНДЕМОВ ЧЕЛОВЕЧЕСКОГО ГЕНА FRM1 У СЕЛЬСЬКОХОЗЯЙСТВЕННЫХ ЖИВОТНЫХ

А н н о т а ц и я

По технике PCR in situ с первичным флангом повторений CGG, расположенных в участке 5'UTR человеческого FRM1 mRNA, повторения тринуклеотидни гена FRM1 проектировались на структурно неустойчивые участки Х хромосомы крупного рогатого скота — BTA Xp13, овцы — OAR Xq22, козы — CHI Xq22 и свиньи — Xq26. Эксперимент подтвердил гомологию регулярных последовательностей в CGG участку гена FRM1 и сохранение соответствующих неустойчивых участков Х хромосомы у млекопитающих. Эти результаты можно применить в сравнительных эволюционных исследованиях и дают возможность проверить наличие взаимосвязей между нестабильностью Х хромосомы в определенных участках и фенотипними последствиями у сельскохозяйственных животных.

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