Brief Gene Mapping Report

Cytogenet Genome Res 109:533B (2005)
DOI: 10.1159/000084216

Cytogenetic and Genome Research

Assignment[1] of the equine *S100A7* gene (psoriasin 1) to chromosome 5p12→p13 by fluorescence in situ hybridization and radiation hybrid mapping

T. Leeb,[a] O. Bruhn,[b] U. Philipp,[a] H. Kuiper,[a] P. Regenhard,[b] S. Paul,[b] O. Distl,[a] B.P. Chowdhary,[c] E. Kalm,[b] and C. Looft[b]

[a] Institute of Animal Breeding and Genetics, School of Veterinary Medicine Hannover, Hannover;
[b] Institute of Animal Breeding and Husbandry, University of Kiel, Kiel (Germany);
[c] Dept. of Veterinary Anatomy and Public Health, College of Veterinary Medicine, Texas A&M University, College Station, TX (USA)

Manuscript received 5 October 2004; accepted for publication by M. Schmid 21 October 2004.

[1] To our knowledge this is the first time this gene has been mapped in horse.

Rationale and significance

Psoriasin *(S100A7)* is a member of the S100 gene family and was discovered as a calcium-binding protein with a molecular weight of 11.4 kDa. Psoriasin was first identified as a secreted protein expressed in human skin (keratinocytes) involved in psoriasis (Celis et al., 1990). It was subsequently shown that psoriasin is a potent and selective chemotactic inflammatory protein for CD4(+) T-lymphocytes and neutrophils (Jinquan et al., 1996). Additionally psoriasin was identified in a fraction also containing lysozyme, and it is possible that psoriasin could be a potential antimicrobial peptide. Gläser et al. (2001) found psoriasin to exhibit antibacterial activity, indicating that psoriasin also contributes to the antimicrobial activity in vernix. The mapping of the equine *S100A7* gene is the first step for further investigations to understand the biological role of S100A7 in epithelial defense in regard to equine health.

This work was supported by the German Research Foundation (DFG) and is part of SFB617.

Request reprints from Christian Looft, Institute of Animal Breeding and Husbandry
Christian Albrechts University
Olshausenstrasse 40, D–24098 Kiel (Germany)
telephone: +49 431-880-1453; fax: +49 431-880-2588
e-mail: clooft@tierzucht.uni-kiel.de

Materials and methods

Identification of an equine S100A7 EST

The EST with the accession AW260999 describes an equine mRNA sequence encoding a protein similar to the human S100 calcium-binding protein A7. Based on this information a primer pair was constructed and 5′ and 3′ RACE with cDNA from equine skin was performed. Sequencing of the 5′ and 3′ RACE products allowed us to establish the complete equine *S100A7* cDNA sequence.

RNA isolation, 5′ RACE and 3′ RACE

Total RNA from equine skin was isolated with the RNeasy Protect Mini Kit (Qiagen, Hilden, Germany) after rotor-stator homogenization with a Polytron homogenizer. For the RT-reaction the Superscript transcriptase (Invitrogen, Carlsbad, USA), a cap-finder-primer with three guanosine-ribonucleotides at the 3′ end (5′-GAG AGA ACG CGT GAC GAG AGA CTG ACA rGrGrG-3′) and an oligo dT-Bio primer labeled with biotin at the 5′ end were used (Schramm et al., 2000). For purification the samples were transferred into streptavidin coated tubes (Abgene, Surrey, UK) to bind the biotin labeled mRNA. 5′ end amplification was performed with a cap-primer (5′-GAG AGA ACG CGT GAC GAG AGA CTG ACA G-3′) and the reverse primer 5′-TCC CCC AGC AAA GAG A-3′. 3′ end amplification was performed with a forward primer 5′-AAG CCA AGA TGA GCG AAA C-3′ and a tail primer 5′-ACT CTA TGA GAA TTC GAT GAG CGA TCT G-3′. The sequence was determined by using an ABI Prism Big Dye sequencing kit 3.1 (Applied Biosystems, Foster City, USA) according to the manufacturer's instructions. The PCR products of approximately 190 bp (5′ RACE) and 380 bp (3′ RACE) were sequenced with an ABI Prism 377 DNA sequencer.

Isolation and characterization of the genomic S100A7 clone

A genomic DNA clone (CH241-159O24) of approximately 230 kb was isolated from the equine CHORI-241 BAC library after screening high density BAC filters according to the CHORI protocols (http://www.chori.org/bacpac/). Two 40-bp overgo probes were designed from the publicly available equine *S100A7* EST sequence (acc. AW260999) for the library screening.

KARGER
Fax + 41 61 306 12 34
E-mail karger@karger.ch
www.karger.com

1424–8581/05/1094–0533$22.00/0

Accessible online at:
www.karger.com/cgr

DNA of the hybridization-positive BAC clone CH241-159O24 was isolated using the Qiagen plasmid midi kit (Qiagen, Hilden, Germany). BAC ends were sequenced with the ThermoSequenase kit (Amersham Biosciences, Freiburg, Germany) on a LI-COR 4200L-2 automated sequencer (MWG Biotech, Ebersberg, Germany). The end sequences were deposited under accessions AJ786151 and AJ786152 in the EMBL nucleotide database.

Fluorescence in situ hybridization (FISH) analysis

The equine genomic BAC clone CH241-159O24 containing the *S100A7* gene was labeled with digoxigenin by nick translation using a Nick-Translation-Mix (Boehringer Mannheim, Mannheim, Germany). FISH on GTG-banded horse chromosomes was performed using 750 ng of digoxigenin-labeled BAC DNA. In this experiment 24 µg sheared total equine DNA and 10 µg salmon sperm were used as competitors. After hybridization over night, signal detection was performed using a Digoxigenin-FITC Detection Kit (Quantum Appligene, Heidelberg, Germany). The chromosomes were counterstained with DAPI and propidium iodide and embedded in antifade solution. Thirty metaphases that had previously been photographed were re-examined after hybridization with a Zeiss Axioplan 2 microscope equipped for fluorescence.

Radiation hybrid (RH) mapping

A primer pair of 183 bp for the RH-mapping of *S100A7* was developed from the 3′ UTR of the equine *S100A7* cDNA. The primers were S100A7_F (5′-CTT GGC CAA TGT CTT TGA G-3′) and S100A7_R (5′-GTT TCT TGC GGT CTC TGG-3′). Amplification of the marker was done on the TAMU equine radiation hybrid panel (Chowdhary et al., 2002) using an annealing temperature of 58 °C and 38 cycles of PCR. To ascertain the chromosomal location of *S100A7* a two point linkage analysis (Slonim et al., 1997; http://equine.cvm.tamu.edu/cgi-bin/ecarhmapper.cgi) was conducted to find associations between *S100A7* versus the markers of the first generation whole genome RH map (Chowdhary et al., 2003).

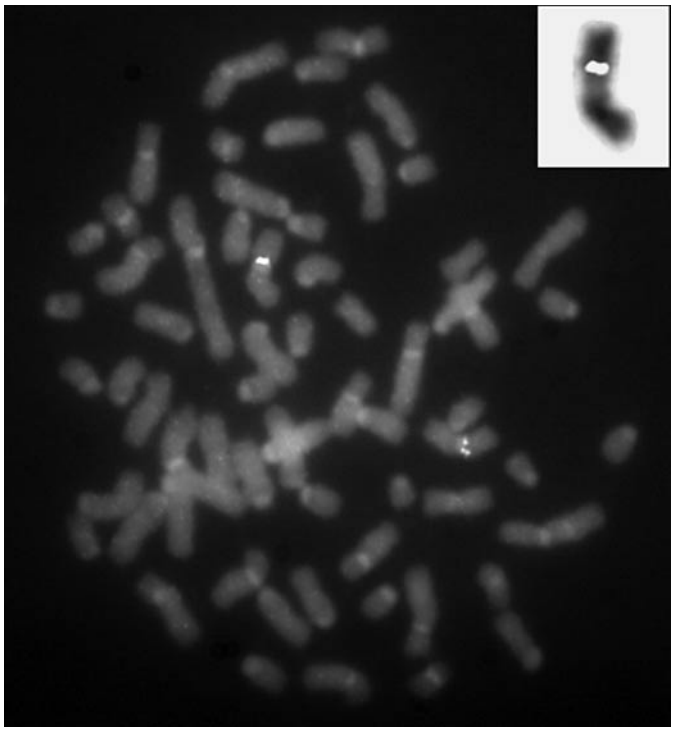

Fig. 1. Chromosome assignment of the equine *S100A7* gene by FISH analysis on an equine metaphase spread. The inset shows an overlay of the hybridization signals on one of the GTG-banded ECA5 homologs. The digoxigenin-labeled BAC clone CH241-159O24 containing the equine *S100A7* gene was hybridized to GTG-banded metaphase chromosomes of a normal horse. Double signals are visible on both chromosomes 5p13→p12. The chromosomes were counterstained with propidium iodide and subsequently identified by DAPI staining.

Results and discussion

By comparing the two sequences determined by 5′ and 3′ RACE the complete mRNA sequence was constructed. Start and stop (TGA) codons were located. The length of the open reading frame was determined with 306 bp encoding a protein of 101 amino acids. Some inconsistencies to the previously published sequence AW260999 were clarified by multiple sequencing from both ends of PCR products (AJ781506).

Using primers deduced from the cDNA sequence, a genomic DNA fragment of 1300 bp was amplified by a PCR reaction with genomic DNA isolated from blood cells. The size of this PCR product indicates that an intron of approximately 1100 bp separates two exons containing the coding sequence of the equine *S100A7* gene.

Comparative studies with sequences from different species showed 80% identity with the *S100A7* cDNA of *Bos taurus* (NM_174596) and 85% identity with the human *S100A7* cDNA (NM_002963).

The equine BAC clone CH241-159O24 has been retrieved from the CHORI-241 BAC library with equine *S100A7* overgo probes. Colony PCR with primers derived from the 3′ UTR of the *S100A7* gene indicated that the clone indeed contained the desired insert. Additional support for the correct identity of the BAC clone was gained by the analysis of the BAC end sequences. The T7 end sequence had a BLAST hit to the human genome (build 35.1) at 150.12 Mb on HSA1 ($E = 3 \cdot 10^{-8}$) which is about 120 kb proximal of the human *S100A7* gene. Given the large insert size of 230 kb of the isolated BAC clone this is another confirmation that CH241-159O24 contains the equine *S100A7* gene. The chromosomal location of the equine *S100A7* gene was determined by FISH of the BAC clone to metaphase chromosomes (Fig. 1). This *S100A7* BAC clone has been assigned to ECA5p13→p12.

Mapping data

Location: ECA5p13→p12

Number of cells examined: 30

Number of cells with specific signals: 0 (0), 1 (0), 2 (2), 3 (3), 4 (26) chromatids per cell

Most precise assignment: ECA5p13→p12

Location of background signals (site with >2 signals): none observed

Additionally, the localization of the *S100A7* gene was verified by RH mapping on the TAMU equine radiation hybrid panel. Two-point analysis revealed that *S100A7* is linked to the marker GBA on ECA5 with a distance of 19 cR and a two-point Lod score greater than 12. The RH results were thus consistent with the cytogenetic localization of *S100A7* at ECA5p13→p12. The human *S100A7* ortholog is located on HSA1q21 (Schäfer et al., 1995). This localization corresponds well to the synteny data of the equine RH map (Chowdhary et al., 2003).

Acknowledgements

We wish to thank Prof. Jens Michael Schröder for initiating this work and Diana Seinige for expert technical assistance. We would also like to thank Pieter de Jong and his lab for providing the CHORI-241 BAC library.

References

Celis JE, Crüger D, Kiil J, Lauridsen JB, Ratz G, Basse B, Celis A: Identification of a group of proteins that are strongly up-regulated in total epidermal keratinocytes from psoriatic skin. FEBS Lett 262:159–164 (1990).

Chowdhary BP, Raudsepp T, Honeycutt D, Owens EK, Piumi F, Guérin G, Matise TC, Kata SR, Womack JE, Skow LC: Construction of a 5000 rad whole-genome radiation hybrid panel in the horse and generation of a comprehensive and comparative map for ECA11. Mamm Genome 13:89–94 (2002).

Chowdhary BP, Raudsepp T, Kata SR, Goh G, Millon LV, Allan V, Piumi F, Guérin G, Swinburne J, Binns M, Lear TL, Mickelson J, Murray J, Antczak DF, Womack JE, Skow LC: The first-generation whole-genome radiation hybrid map in the horse identifies conserved segments in human and mouse genomes. Genome Res 13:742–751 & Erratum in Genome Res 13:1258 (2003).

Gläser R, Harder J, Bartels J, Christophers E, Schröder J-M: Psoriasin (S 100a7) is a major and potent *E. coli*-selective antimicrobial protein of healthy human skin. J Invest Dermatol 117:768 (2001).

Jinquan T, Vorum H, Larsen CG, Madsen P, Rasmussen HH, Gesser B, Etzerodt M, Honoré B, Celis JE, Pederson KT: Psoriasin, a novel chemotactic protein. J Invest Dermatol 107:5–10 (1996).

Schäfer BW, Wicki R, Engelkamp D, Mattei MG, Heizmann CW: Isolation of a YAC clone covering a cluster of nine S100 genes on human chromosome 1q21: Rationale for a new nomenclature of the S100 calcium-binding protein family. Genomics 25:638–643 (1995).

Schramm G, Bruchhaus I, Roeder T: A simple and reliable 5′-RACE approach. Nucleic Acids Res 28:96 (2000).

Slonim D, Kruglyak L, Stein L, Lander E: Building human genome maps with radiation hybrids. J Comput Biol 4:487–504 (1997).