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Mapping of the *SMC6* gene to porcine chromosome 3

(Brief report)

(Kartierung des *SMC6*-Gens auf Chromosom 3 beim Schwein)

Background: Among the expressed sequence tags (ESTs) with different expression in *M. biceps femoris* of healthy and splay leg piglets there were several fragments lacking an annotation (MAAK et al., 2001). The aims of this study were (1) to identify the gene represented by one of these anonymous ESTs (GenBank accession no. AJ271018) through inter-species comparison and (2) to determine its chromosomal location in swine by radiation hybrid and in-silico mapping. Identification of the function of the gene and its mapping should clarify whether the gene can be considered a candidate for congenital splay leg.

Procedure:

Primer sequences:
SMCf: 5'-TTCATAAAATAGGCAGTATCAAG-3'
SMCr: 5'-AAAAGAGCCAAAAATGTAGAC-3'

The initial, 280 bp EST isolated from differential display was aligned to the porcine genomic TRACE sequences with BLAST in order to identify exon/intron boundaries within the EST. Primers for RH-mapping were subsequently developed from an overlapping TRACE sequence (TI 862132025; 98% identity in 232 bp). RH mapping was done with the 118 clones of the IMpRH panel (YERLE et al., 1998). The PCR reactions were set up in a total volume of 25 µl containing 25 ng hybrid DNA, 1 µM of each primer and 12.5 µl 2 x Master-Mix (peqlab Biotechnologie, Erlangen, Germany). An initial denaturation step (5 min at 95°C) was followed by 35 cycles with 92°C (1 min), 50°C (1 min) and 70°C (2 min). The amplification products were separated on a 2% agarose gel containing ethidium bromide and were visualized under UV-light. Each of the reactions using either porcine genomic DNA, hamster genomic DNA or water instead of the hybrid DNA served as positive and negative controls. The PCR results were analyzed with the online mapping tool (http://imprh.toulouse.inra.fr/; MILAN et al., 2000).

The human and porcine GenBank databases were searched with different options of BLAST for annotation of the fragment. In-silico mapping was done using the positional information for the human gene for searching the porcine BAC-end sequence (BES) database
(http://www.sanger.ac.uk/cgi-bin/Projects/S_scrofa/BESsearch.cgi).

Furthermore, available information from the porcine BAC-based RH map (MEYERS et al., 2005) and the human- porcine comparative RH map (RINK et al. 2006) was used.

Results: The PCR results using the IMpRH panel assigned the EST AJ271018 to porcine chromosome (SSC) 3. According to the two-point-analysis the most significantly linked marker is SW1327 on this chromosome (distance 21 cR, LOD = 16.37). The corresponding multipoint analysis placed the EST between markers S0002 and SW1327.

BLAST searches revealed eight partial matches (180/280 bp) with sequences from the porcine EST and Trace (EST) sections of GenBank but only one full length match with the porcine EST CF796046 (503 bp). In order to identify the gene represented by our fragment the human databases were screened with that EST. There was one partial match (91% in 378 bp) with the human mRNA for the *SMC6* gene (structural maintenance of chromosomes 6; NM_024624). This gene codes for a component of a multiprotein-complex required for the repair of DNA double-strand breaks during replication or after exposure to DNA damaging agents. Thus, this protein complex is essential for genome stability (TAYLOR et al., 2001; DE PICCOLI et al., 2006). In order to clarify the reason for the only partial match between the porcine ESTs and the human *SMC6* gene we performed a local alignment. Our data demonstrate that the first 180 bp of the mapped EST (AJ271018) consist of sequences corresponding to exons 24 and 25 of the human gene. However, the remaining 100 bp of the EST are not similar to one of the following exons 26-29 of the human *SMC6* gene. In-silico translation revealed a stop-codon at the end of exon 25 leading to a hypothetical protein of 935 aa instead of 1,091 aa as deduced from the full length human mRNA. Since the overlapping EST (CF796046) has essentially the same features in the region 3' of putative exon 25 there is some evidence for a truncated variant of porcine *SMC6*. In contrast, 3 of the further 8 porcine ESTs matching human *SMC6* contain the continuous sequence of exons 21-27, 24-29 and 24-29 indicating the existence of full length trancripts of the porcine *SMC6* gene.

In-silico mapping was applied to confirm our RH-mapping result for porcine *SMC6*. The genomic region covering the complete human *SMC6* locus was searched against the porcine, fingerprinted BES-database and matched clones were re-checked on their positions within the *SMC6* gene with the bl2seq-option of BLAST. Among the 15 matched porcine clones in this region (contig 3009) one end sequence (CT375352) is highly similar (95%) to exon 26 of the human *SMC6* gene. This similarity is supported by further hits in several exons thus providing evidence for the localization of the porcine homolog of *SMC6* in this region on SSC3. The framework marker CL379758 overlaps with the clone containing BES CT375352 and subsequently links the porcine *SMC6* gene to this region. MEYERS et al. (2005) assigned CL379758 to the region between markers S0002 and SW1327 by RH-mapping. Furthermore, an EST (BI132553) was RH-mapped between both markers by RINK et al. (2006). This EST corresponds to the human gene for visinin-like1 (*VSNL1*) - the direct neighbor of *SMC6* on HSA2. In summary, our result is concordant with all available mapping data for this region. Although SSC 3 harbors a putative QTL for congenital splay leg (SCHWARZ, 2002), neither the position of *SMC6* nor the function of the gene product supports a role of porcine *SMC6* in the etiology of the disease.

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References

DE PICCOLI, G.; CORTES-LEDESMA, F.; IRA, G.; TORRES-ROSELL, J.; UHLE S.; FARMER S.; HWANG, J. Y.; MACHIN, F.; CESCHIA, A.; MC ALEENAN, A.; CORDON-PRECIADO, V.; CLEMENTE-BLANCO, A.; VILELLA-MITJANA, F.; ULLAL, P.; JARMUZ, A.; LEITAO, B.; BRESSAN D.; DOTIWALA, F.; PAPUSHA, A.; ZHAO, X.; MYUNG, K.; HABER, J. E.; AGUILERA, A.; ARAGON, L.:
Smc5-Smc6 mediate DNA double-strand-break repair by promoting sister-chromatid recombination. Nat. Cell Biol. **8** (2006), 1032-1034

MAAK, S.; JAESERT, S.; NEUMANN, K.; YERLE, M.; LENGERKEN, G. v.:
Isolation of expressed sequence tags of skeletal muscle of neonatal healthy and splay leg piglets and mapping by somatic cell hybrid analysis. Anim. Genet. **32** (2001), 303-307.

MEYERS, S. N.; ROGATCHEVA, M .B.; LARKIN, D. M.; YERLE, M.; MILAN, D.; HAWKEN, R. J.; SCHOOK, L. B.; BEEVER, J. E.:
Piggy-BACing the human genome II. A high-resolution, physically anchored, comparative map of the porcine autosomes. Genomics **86** (2005), 739-752.

MILAN, D.; HAWKEN, R.; CABAU, C.; LEROUX, S.; GENET, C.; LAHBIB, Y.; TOSSER, G.; ROBIC, A.; HATEY, F.; ALEXANDER, L.; BEATTIE, C.; SCHOOK, L.; YERLE, M.; GELLIN, J.:
IMpRH server: an RH mapping server available on the Web. Bioinformatics **16** (2000), 558-559

RINK, A.; EYER, K.; ROELOFS, B.; PRIEST, K. J.; SHARKEY-BROCKMEIER, K. J.; LEKHONG, S.; KARAJUSUF, E. K.; BANG, J.; YERLE, M.; MILAN, D.; LIU, W.S.; BEATTIE, C. W.:
Radiation hybrid map of the porcine genome comprising 2035 EST loci. Mamm. Genome. **17** (2006), 878-885.

SCHWARZ, S.:
Kartierung von Loci für kongenitales Ausgrätschen beim neugeborenen Ferkel. *Thesis*, Kiel, Germany http://e-diss.uni-kiel.de/diss_827/ (2002).

TAYLOR, E. M.; MOGHRABY, J. S.; LEES, J. H.; SMIT, B.; MOENS, P. B.; LEHMANN, A. R.:
Characterization of a novel human SMC heterodimer homologous to the Schizosaccharomyces pombe Rad18/Spr18 complex. Mol. Biol. Cell **12** (2001), 1583-1594

YERLE, M.; PINTON, P.; ROBIC, A.; ALFONSO, A.; PALVADEAU, Y.; DELCROS, C.; HAWKEN, R.; ALEXANDER, L.; BEATTIE, C.; SCHOOK, L.; MILAN, D.; GELLIN, J.:
Construction of a whole-genome radiation hybrid panel for high-resolution gene mapping in pigs. Cytogenet. Cell Genet. **82** (1998), 182-188

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