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SNP analysis, genotyping and mapping of the porcine *PTHR1* gene to chromosome 13 (Brief report)

(SNP-Analyse, Genotypisierung und Kartierung des porcinen *PTHR1* Gens auf Chromosom 13)

Background: The parathyroid hormone/parathyroid hormone like hormone type I receptor (PTHR1) belongs to the family of G protein-coupled receptors for peptide hormones, including parathyroid hormone (PTH) and parathyroid hormone like hormone (PTHLH), which participate in epithelial-mesenchymal interactions during the formation and differentiation of epithelial organs (FOLEY et al., 2001; CHOMDEJ et al., 2004). The function of PTHR1 and its ligands suggest its candidacy for traits related to the development of bones and joints but also of mammary gland. The porcine gene was screened for SNPs and assigned to SSC13.

Procedures:
Primer sequences:
PTHR-F: 5'-GCTATGGTCCGATGGTGTCT-3'
PTHR-R: 5'-ACTGTCTCCCACTCCTCCTG-3'
SNP analysis, genotyping and mapping:
The *PTHR1* gene sequence (GenBank accession no. NM_214382) was used to derive homologous primers every 400-500 bp of the entire mRNA to screen for polymorphisms.
The PCR fragments were amplified and sequenced from 5 pig breeds (Duroc, Hampshire, German Landrace, Pietrain and Berlin-Miniature pig) using standard lab protocols. PCR programs were: initial denaturation for 5 min 95 °C, 40 cycles each 30 s at 95 °C, 30 s at 64 °C and 1 min at 72 °C and 5 min final extension at 72 °C. Genotyping was performed using the primers PTHR-F and PTHR-R for amplification and *Mbo*II (Fermentas, Leon-Rot, Germany) for restriction digestion. Physical mapping was achieved by screening of the IMpRH panel using the same primers for PCR and by analysis of the results using twopoint and multipoint analysis option of the IMpRH mapping tool (http://www.toulouse.inra.fr). For linkage mapping 19 informative families (n=313) of the DUMI F_2 resource population were genotyped (HARDGE et al., 1999). Multipoint linkage map was established using the BUILD option of the CRIMAP 2.4 package.

Results: A C/T non-synonymous SNP (L556F) was detected at nucleotide position 1819 in the *PTHR1* gene. Within the DUMI F_1 generation (n=14) we observed 11 heterozygous animals and 3 homozygous for allele C; in the DUMI F_2 generation 116 animals were homozygous for allele C, 149 animals were heterozygous and 48 animals were homozygous for allele T. The *PTHR1* gene was assigned to SSC13 in close proximity to marker SW1400 by twopoint analysis (20cR; LOD 13.05) using IMpRH panel (Vector: 1101110000 0000000001 0000000000 0000001000 0001000000

1000010001 0100000001 0000000100 0000000000 0101000011 1011000000 01010000). This assignment was further confirmed by genetic mapping of *PTHR1* using multipoint and twopoint analysis that revealed linkage to S0219 (proximal) and SW344 (distal) with distances of 38.4 cM (recombination fraction=0.31, LOD=1.83) and 15.5 cM (recombination fraction=0.15, LOD=12.69), respectively, on the sex averaged map. Our assignment of *PTHR1* to the q arm of SSC13 is in agreement with the published physical and genetic maps.

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