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SNP analysis, genotyping and mapping of the porcine *GPCR142* gene
(Brief report)

(SNP-Analyse, Genotypisierung und Kartierung des porcinen *GPCR142* Gens)

Background: The *RLN3* gene has been recently reported as a ligand for two related orphan G protein-coupled receptors, *GPCR135* and *GPCR142* (BATHGATE et al., 2002; LIU et al., 2003a; LIU et al., 2003b; CHEN et al., 2005). Pharmacological studies of the *GPCR142* gene indicated that the *RLN3* gene is the only member of the *RLN* family showing the ability to activate this gene (BATHGATE et al., 2002). The *GPCR142* gene contains a single exon and is located on chromosome 1 (1q22) in human (FREDRIKSSON et al., 2003). It is expressed abundantly in the hypothalamus with discrete expression in the paraventricular nucleus of the hypothalamus and supraoptic nucleus; as well as in the cortex, septal nucleus and preoptical area (LIU et al., 2003b). The functional roles of the *RLN3* gene and its receptors within the mammalian physiology are still unclear.
The objectives of this study were to characterize the *GPCR142* gene in pigs, to determine the chromosomal location and to analysis the expression profile in pigs.

Procedures: Primers were designed from the published sequence (acc.-no. AY633768) of the porcine *GPCR142* gene. The forward 5'–ctttctgggtcaatgcgtct–3' and the reverse 5'–cttcctttagggccacctgt–3' primers were used to screen for polymorphisms within an 1,002 bp fragment in the sequence of the porcine *GPCR142* gene.
Genotyping was performed by PCR-RFLP using the *Hae*III (Invitrogen, Karlsruhe, Germany) to verify the detected polymorphism. The PCR and PCR-RFLP products were run in 1.5% and 3.0% agarose gels, stained with ethidium bromide, and visualized under UV irradiation. Total RNA was isolated from different tissue of two male pigs (muscle, heart, spleen, lymph nodes, skin, brain, teat, lung, testis, tonsil, and kidney) and one sow (uterus and inverted teat) using Trizol® (Gibco BRL). The following forward primer 5'–ctg–ctg–gtt–ctc–tac–ctg–3' and reverse primer 5'–gtt–cat–gga–cgt–cac–tac–gg–3' were used to analyse the expression pattern in different tissues following the protocol for semi-quantitative RT-PCR. The expression of *18S* rRNA was used as an internal reference.
For genetic mapping animals of an experimental pig population were genotyped. The multipoint linkage map was established using the BUILD, TWOPOINT and FIXED options of the CRIMAP 2.4 package against microsatellite markers on the different chromosomes (GREEN, 1992).

Results: Comparative sequencing of the amplified fragment revealed one SNP (G to A) within the single exon of the porcine *GPCR142* gene. Digestion of the resulting 1,002 bp product using *Hae*III validated the polymorphism. Two alleles could be detected, allele A included a 153 bp fragment, whereas allele G yielded one additional cutting site for the enzyme and two fragments (126 and 27 bp) were seen after digestion.

Monomorphic fragments of 222, 170, 109 and 96 bp were also found in both alleles. The *GPCR142* gene was assigned to SSC4 in close proximity to marker S0214 and S0001 using the twopoint option of CRIMAP. The results were in agreement with the published comparative porcine-human map of HSA1 with the most evidence for an assignment to SSC4 (MEYERS et al., 2005). *GPCR142* is highly expressed in spleen, tonsil and lung, moderate in muscle, heart, and inverted teat, lower in skin, testes and uterus.

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References

BATHGATE, R.A.D.; SAMUEL, C.S.; BURAZIN, T.C.D.; LAYFIELD, S.; CLAASZ, A.A.; REYTOMAS I.G.T.; DAWSON, N.F.; ZHAO, C.; BOND, C.; SUMMERS, R.J.; PARRY, L.J.; WADE, J.D.; TREGEAR, G.W.:
Human Relaxin Gene 3 (H3) and the Equivalent Mouse Relaxin (M3) Gene. novel members of the relaxin peptide family. J. Biol. Chem. **277** (2002), 1148-1157

CHEN, J.; KUEI, C.; SUTTON, S.W.; BONAVENTURE, P.; NEPOMUCENO, D.; ERISTE, E.; SILLARD, R.; LOVENBERG, T.W.; LIU, C.:
Pharmacological Characterization of Relaxin-3/INSL7 Receptors GPCR135 and GPCR142 from Different Mammalian Species. J Pharm. Exp. Ther. **312** (2005), 83-95

FREDRIKSSON, R.; HOGLUND, P.J.; GLORIAM, D.E.I.; LAGERSTROM, M.C.; SCHIOTH, H.B.:
Seven evolutionarily conserved human rhodopsin G protein-coupled receptors lacking close relatives. FEBS Letters **554** (2003), 381-388

GREEN, P.:
Construction and Comparison of Chromosome-21 Radiation Hybrid and Linkage Maps Using Cri-Map. Cytogenetics and Cell Genetics **59** (1992), 122-124

LIU, C.; CHEN, J.; SUTTON, S.; ROLAND, B.; KUEI, C.; FARMER, N.; SILLARD, R.; LOVENBERG, T.W.:
Identification of Relaxin-3/INSL7 as a Ligand for GPCR142. J. Biol. Chem. **278** (2003a), 50765-50770

LIU, C.; CHEN, J.; SUTTON, S.; ROLAND, B.; KUEI, C.; FARMER, N.; SILLARD, R.; LOVENBERG, T.W.:
Identification of Relaxin-3/INSL7 as an Endogenous Ligand for the Orphan G-protein-coupled Receptor GPCR135. J. Biol. Chem. **278** (2003b), 50754-50764

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

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