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Mapping of *ACE* Gene to Pig Chromosome 12 (Brief Report)
(Kartierung des *ACE* Gens auf dem Schweinechromosom 12)

Background: In humans, the Angiotensin I Converting Enzyme (*ACE*) gene is located on the q arm of chromosome 17. It contains 16 exons and 15 introns. The deletion (D) allele of the insertion/deletion polymorphism of the *ACE* gene has been shown to be associated with a higher risk of coronary artery disease (TANIQUCHI et al., 2001) and hypertension (MORSHED et al., 2002), whereas the insertion (I) allele has been found to be associated with a higher risk of Alzheimer's disease (ELKINS et al., 2004). Furthermore, a study (LUFT, 1999) associated *ACE* with longevity. Our hypothesis is that these same genes or regulatory pathways are also important for sow productive life. The objective of this study was to determine the chromosomal location of *ACE* in the pig by linkage and RH mapping.

Procedure:

Primer sequences:

ACE-I12F: 5' TCA TCA TCC AGT TCC AGT TCC 3'

ACE-I12R: 5' GTT CGG CGT CCA GTT GTA CT 3'

The primers were anchored in exon 12 and exon 13 based on pig sequence (NM_001033015) and spanned intron 12 as predicted by human *ACE* (Ensembl ENSG00000159640). Using animals from the F₀ generation (n=11) of the ISU Berkshire x Yorkshire Resource Family (MALEK et al., 2001) and the above primers, we amplified a 540 bp fragment with the following steps: Initial denaturation temperature at 94 °C for 2 min with 36 cycles of 94 °C for 30 sec, 53 °C for 45 sec, 72 °C for 50 sec, and final extension at 72 °C for 5 min. Each PCR was carried out in a 10 µl reaction contained 5 × PCR buffer, 125 µM of dNTPs, 0.25 µM of each primer, 0.25 units of Taq, and 12.5 ng of pig DNA. Resulting PCR products were sequenced. Several single nucleotides polymorphisms (SNPs) were identified, and the exonic C95T SNP which creates a restriction enzyme *AluI* site was selected for genotyping in the ISU Berkshire x Yorkshire Resource Family (MALEK et al., 2001). Two point and multipoint linkage analyses were performed using CRIPMAP software (GREEN et al., 1990). RH mapping was carried out using the IMpRH panel (MILAN et al., 2000). To minimize PCR errors, each clone from the IMpRH panel was amplified twice.

Results: Digestion of the 540 bp PCR product with *AluI* resulted in fragments of 369 bp, 102 bp, 36 bp, and 33 bp (allele C) or 276 bp, 102 bp, 93 bp, 36 bp, and 33 bp (allele T). The *ACE* gene was found to be significantly linked to several markers located on SSC12 including *S0229* (recombination fraction = 0.25, LOD = 19.21), *SW874* (recombination fraction = 0.21, LOD = 12.20), and *S0090* (recombination fraction = 0.25, LOD = 6.25). The best sex-averaged map order produced by multipoint analysis with other linked markers was *S0229* – 16.0 – *ACE* – 22.5 – *SW874* – 12.3 – *S0090* – 14.6 – *S0147* – 22.9 – *SWC23* – 12.2 – *SW2180*, with distances in

Kosambi cM. The chromosomal location of *ACE* was also confirmed by RH mapping using the IMpRH panel (MILAN et al., 2000). Results from two point analysis showed that the most significant linked marker was *SW957* on chromosome 12 (LOD = 8.94). Based on these results and those on a comparative map from National Animal Genome Research Program (NAGRP, <http://www.animalgenome.org>), the gene was mapped where expected. Further research should be conducted to determine the association, if any, between the *ACE* gene and sow reproductive life.

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References

- ELKINS, J.S.; DOUGLAS, V.C.; JOHNSON, S.C.:
Alzheimer disease risk and genetic variation in *ACE*: a meta-analysis, *Neurology* **62** (2004), 363-368
- GREEN, P.; FALLS, K.; CROOKS, S.:
Documentation for CRIMAP, version 2.4. Washington University School of Medicine, St Louis, MO, USA. (1990)
- LUFT, F.C.:
Bad genes, good people, association, linkage, longevity and the prevention of cardiovascular disease, *Clin. Exp. Pharmacol. Physiol.* **26** (1999), 576-579
- MALEK, M.; DEKKERS, J.C.; LEE, H.K.; BAAS, T.J.; ROTHSCHILD, M.F.:
A molecular genome scan analysis to identify chromosomal regions influencing economic traits in the pig. I. Growth and body composition. *Mamm. Genome* **12** (2001), 630-636
- 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.:
pRH server: an RH mapping server available on the Web. *Bioinformatics* **16** (2000), 558-559
- MORSHED, M.; KHAN, H.; AKHTERUZZAMAN, S.:
Association between angiotensin I-converting enzyme gene polymorphism and hypertension in selected individuals of the Bangladeshi population, *J Biochem Mol Biol.* **35** (2002), 251-254
- TANIGUCHI, I.; YAMAZAKI, T.; WAGATSUMA, K.; KURUSU, T.; SHIMAZU, Y.; TAKIKAWA, K.; YOSHIKAWA, M.; KAGEYAMA, S.; MOCHIZUKI, S.:
The DD genotype of angiotensin converting enzyme polymorphism is a risk factor for coronary artery disease and coronary stent restenosis in Japanese patients, *Jpn Circ J.* **65** (2001), 897-900

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