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Mapping of *CARD4* gene to pig chromosome 18 (Brief report)

(Kartierung des *CARD4* Gens auf dem Schweinechromosom Nr. 18)

Background: The caspase recruitment domain family, member 4 gene (*CARD4*) is a member of the CATERPILLER (caspase recruitment domain, transcription enhancer, r (purine)-binding, pyrin, lots of leucine repeats) family. In humans, *CARD4* has been shown to be involved in the innate immune system responsible for cytosolic recognition of bacteria. This gene also contributes to the inflammation processes seen in asthma (HYSI et al., 2005) and intestinal bacterial infections (KIM et al., 2004). The human *CARD4* gene is located on chromosome 7. It contains 14 exons and spans approximately 4.4 kb. The objective of this study was to determine the chromosomal location of *CARD4* in the pig by linkage and RH mapping.

Procedure:

Primer sequences:
CO1F: 5´-TTG TTG TTG TCC AGG TCG AG-3´
CO1R: 5´-ATC ATG GAG CTG GTG GAC TT-3´
CO2F: 5´-TCC TCT GGA AGA AGG TGC TC-3´
CO2R: 5´-CAC CTC CTC CTG GTT GAA AA-3´

Initially, primers were designed using a stretch of sequence from a porcine BAC clone (AC091756) that was 86% homologous to exon 6 of human *CARD4*. Primers (C01F & C01R) were used to amplify a 1654 bp fragment of the porcine *CARD4* gene. DNA pools consisting of the Iowa State University Berkshire x Yorkshire founder animals (MALEK et al., 2001) were sequenced and polymorphisms identified by looking at sequence differences between the Berkshire and Yorkshire breeds. The sequence was analyzed using Sequencher software version 3.0 (Gene Codes, Ann Arbor, MI, USA) and the annotated sequence submitted to GenBank (DQ388480). After polymorphisms were identified, new primers (C02F & C02R) amplifying a 425 bp fragment were designed to create a PCR-RFLP test. The PCR reactions were carried out in 10 µl reactions using 12.5 ng of DNA, 10x PCR buffer, 2.0 mM $MgCL_2$, 0.125 mM dNTPs, 0.3 µM of each primer, and 0.35 U Taq DNA polymerase (Promega, Madison, WI, USA). The PCR conditions consisted of an initial 3 minute denaturation step at 94 ºC, followed by thirty-five cycles at 94 ºC for 30 sec, 57.8 ºC for 35 sec, and 72 ºC for 30 sec. This test was used to genotype the entire ISU Berkshire x Yorkshire pedigree (MALEK et al., 2001). Digestions were performed using restriction endonuclease *Nci*I. Linkage mapping of *CARD4* was performed using two- and multi-point analyses, using CRIMAP version 2.4 (GREEN et al., 1990). RH mapping was also completed using the IMpRH panel (MILAN et al., 2000). The primers and reaction conditions used in RH mapping were the same as those used for the PCR-RFLP test. Each clone on the RH panel was genotyped in duplicate to minimize genotyping errors.

Results: Out of several SNPs discovered, one non-synonymous SNP was selected for genotyping, and a PCR-RFLP test was designed. The A60G SNP was recognized by *NciI* and following digestion of the PCR products produced fragments of 425 bp

(allele A) and 366 bp and 59 bp (allele G). Allele A produces an arginine and has an allelic frequency of 0.30 and allele G generates a glycine and has an allelic frequency of 0.70. Genetic linkage was confirmed between *CARD4* and microsatellites *S0062* (recombination fraction = 0.02; LOD = 65.09) and *SW787* (recombination fraction = 0.07; LOD = 56.18). Both microsatellites, *S0062* and *SW787*, had been previously mapped on chromosome 18. *CARD4* also showed linkage with microsatellites *S0120* (recombination fraction = 0.11; LOD = 84.86) and *SW1984* (recombination fraction = 0.08; LOD = 77.08). The best map order of the *CARD4* gene produced by multipoint linkage analysis with other linked markers was (with distance in Kosambi cM): *SW1984*-1.5-*SW787*-8.0-*CARD4*-1.7-*S0062*-2.3-*S0120*. The chromosomal location of *CARD4* was also confirmed by RH mapping using the IMpRH panel. Results from 2 point analysis show that the most significantly linked marker was S0062 on chromosome 18 (26 cR; LOD =14.71) (5).
Based on these results and those from a recent comparative map (MEYERS et al., 2005), the *CARD4* gene mapped where expected. Further research should be conducted to determine if there is an association between *CARD4* and any growth or immune response traits in the pig.

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