Mapping of the Porcine CCS and CCR7 Genes

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Abstract


CCS (cooper chaperon for superoxide dismutase1 - SOD1) and CCR7 (chemokine (C-C motif) receptor 7 were studied as potential candidate genes associated with sow stress and were subsequently linkage mapped in the swine genome. Primers were designed for each gene from the pig CCS and CCR7 sequences found at the National Center for Biological Information. The CCS primer pair amplifies a 160bp amplicon, while the CCR7 primer pair amplifies a 385bp amplicon. Polymerase chain reaction-restriction fragment length polymorphisms (PCR-RFLP) tests with Nci I (New England Biolabs) for CCS and with HpyCh4 III (New England Biolabs) for CCR7 were developed for genotyping polymorphisms. Pig CCS was mapped with strong linkage to SSC 2 markers SW2623, SWC9, SW2443, and SW2445 (LOD>10). Pig CCR7 was mapped with strong linkage to SSC 12 markers SW874, S0090, S0147, and S0229 (LOD>9). The present study of mapping the pig CCS and CCR7 genes increases information for SSC 2 and SSC 12 and it is a base to study CCS and CCR7 genes as candidate genes for sow stress and longevity.

Key words: CCS, CCR7, pigs, markers, linkage analysis

Introduction

CCS (cooper chaperon for superoxide dismutase1 - SOD1) is one of three chaperones with carrier function that attaches and carries cooper ions to the specific target enzymes within the cell. The CCS gene is necessary for expression of an active copper bound form of superoxide dismutase (Furukawa et al., 2004, Puig and Thiele, 2002, Rae et al., 1999). In mice, targeted deletion of the CCS gene leads to viable offspring but with a reduction in SOD activity and reduced female fertility (Wong et al., 2000). The porcine CCS gene mapping position was previously known from physical mapping to be on the short arm of swine chromosome 2 with localization to 2p14-p17 (Silahtaroglu et al., 2004).

The system of chemokines and their receptors play pivotal roles in lymphocyte biology, lymphocyte trafficking, recruiting and recirculation and in this way in the...
regulation of normal and pathologic immune response (Christoferson II and Hromas, 2001; Comerford and Nibbs, 2005; Mahalingam and Karupiah, 1999; Seder and Ahmed, 2003). The CCR7 (chemokine (C-C motif) receptor 7) was identified in gene-targeted mice as an important organizer of primary immune response for the rapid initiation of an adaptive immune response (Foster et al., 1999).

In humans, CCR7 gene is associated with a broad range of cardiovascular, hematological, neurological, urological, dermatological and cancer disorders and with different infective, gastrointestinal, liver and respiratory diseases (Golz et al., 2004). The porcine CCR7 gene was mapped to swine chromosome 12 - 12p13-p11 by FISH analysis (Shinkai et al., 2003).

The objectives of the present study were to identify polymorphisms and subsequently map porcine CCS and CCR7 genes as a beginning for the study of their role and association with sow stress and longevity.

**Materials and Methods**

Primer sequences: Pig CCS and CCR7 gene sequences (GenBank accession nos. NM_001001866.1 and AB090356 respectively) were queried from the NCBI Nucleotide database (National Center for Biotechnology Information, Bethesda, Maryland) and used to design primers for each gene using Primer3 (Whitehead Institute for Biomedical Research, Cambridge, MA). Primer sequences are CCS Forward 5’ TTT CCT AAC AGG TCC ACT GTC TG 3’, CCS Reverse 5’ AGA GCG CTC ACC TCT CTC C 3’, CCR7 Forward 5’ AAG TCC TGG GTC TTC GGA GT 3’, and CCR7 Reverse 5’ GGA TGA TGA CGA GGT AGC AGA 3’. The CCS primer pair amplifies a 160bp amplicon while the CCR7 primer pair amplifies a 385bp amplicon. PCR conditions are as follows for both primer sets: 10 µL PCR volume contained 12.5 ng genomic porcine DNA, 2.5 pmol of each primer, 1.25 mM dNTP, 2 µL GoTaq buffer (Promega), 0.25 U GoTaq polymerase (Promega), and distilled water to equal 10 µL. The thermal cycling protocol was 2 min. at 94° C, 35 cycles of 94° C for 30 sec., 61° C primer annealing temperature for 45 sec., and 72° C for 45 sec., with a final extension of 72° C for 10 min.

**SNP detection and polymorphisms:** Single nucleotide polymorphisms were detected using a pooled DNA sample from the two Berkshire sires and three pooled samples of the nine Yorkshire dams from Iowa State University’s Berkshire X Yorkshire resource population described in Malek et al. 2001. The sequence data were analyzed using Sequencher software (Gene Codes Corporation, Ann Arbor, MI). An A/C SNP was identified in CCS at 132bp of the amplified fragment and a C/T SNP was identified in CCR7 at position 147bp. A PCR-RFLP test for the CCS SNP was designed by incubating the cocktail of 3 µL of PCR product, 3 units of Nci I (New England Biolabs), 1 µL NEB buffer 4, and 5.85 µL distilled water at 37° C according to manufacturer’s directions for at least 5 hr. A PCR-RFLP test for the CCR7 SNP was designed by incubating the cocktail of 3 µL of PCR product, 3 units of HpyCh4 III (New England Biolabs), 1 µL NEB buffer 4, and 5.6 µL distilled water at 37° C according to manufacturer’s directions for at least 5 hr. The digested PCR products were separated on agarose gels stained with ethidium bromide. The resulting banding patterns for the CCS 11 genotype are 103 and 57bp;
the 12 genotype are 103, 57, 29, and 28bp, and the 22 genotype are 103, 29, and 28bp. The resulting banding patterns for the CCR7 11 genotype are 385bp; the 12 genotype are 385, 240, and 145bp, and the 22 genotype are 240 and 145bp.

Mapping: The ISU Berkshire x Yorkshire resource population (Malek et al., 2001) was genotyped using PCR-restriction fragment length polymorphism (RFLP) tests under reaction and cycling conditions listed above using designated primers and restriction enzymes. Two point and multipoint linkage analyses of the genotype results were completed using CRI-MAP software (Green et al., 1990).

Results and Discussion

Results of two point linkage analysis (Table 1) produced significant LOD scores between CCS and the previously mapped close markers on SSC2 (two point log of the odds (LOD) score in parentheses): SW2623 (24.39), SWC9 (10.15), SW2443 (16.62), and SW2445 (12.97). The multipoint linkage analysis determined the linear marker position of the CCS gene in relation to the 13 previously mapped markers (Figure 1). The predicted map placed it in a position between markers SW2623 and SW2445 and the cumulative map distance from the first marker was 22.8 cM.

Table 1
Linkage between the CCS locus and gene markers on Porcine Chromosome 2

<table>
<thead>
<tr>
<th>Marker</th>
<th>Sex averaged recombination rate</th>
<th>LOD score</th>
</tr>
</thead>
<tbody>
<tr>
<td>SW2443</td>
<td>0.17</td>
<td>16.62</td>
</tr>
<tr>
<td>SW2445</td>
<td>0.18</td>
<td>12.97</td>
</tr>
<tr>
<td>SW2623</td>
<td>0.09</td>
<td>24.39</td>
</tr>
<tr>
<td>SWC9</td>
<td>0.14</td>
<td>10.15</td>
</tr>
</tbody>
</table>

Fig. 1. Sex average linkage map of CCS gene and other genetic markers on SSC2
The cumulative map distance for all the 14 loci involved in the analysis was 159.3 cM.

For the CCR7 gene, two point linkage analyses (Table 2) found significant linkage between the CCR7 gene and several previously mapped microsatellite markers on porcine chromosome 12. The closely linked markers to the CCR7 gene (LOD) were SW874 (52.99), S0090 (26.88), S0147 (11.26) and S0229 (9.43). The multipoint linkage analysis placed the linear marker position of CCR7 between the other 6 previously mapped markers (Figure 2). The best map position of the CCR7 gene was on the second place between marker S0229 and SW874. The cumulative map distance for all 7 markers involved in the analysis was 97.7 cM.

### Table 2

<table>
<thead>
<tr>
<th>Marker</th>
<th>Sex average recombination rate</th>
<th>LOD score</th>
</tr>
</thead>
<tbody>
<tr>
<td>S0090</td>
<td>0.13</td>
<td>26.88</td>
</tr>
<tr>
<td>S0147</td>
<td>0.24</td>
<td>11.26</td>
</tr>
<tr>
<td>S0229</td>
<td>0.29</td>
<td>9.43</td>
</tr>
<tr>
<td>SW874</td>
<td>0.05</td>
<td>52.99</td>
</tr>
</tbody>
</table>

**Table 2**

**Linkage between the CCR7 locus and gene markers on Porcine Chromosome 12**

Conclusions

Single nucleotide polymorphisms were identified in CCS and CCR7 genes. The single nucleotide polymorphisms allowed for the opportunity to obtain the map linkage positions of the two genes. The linkage map placed the CCS gene on porcine chromosome 2 (SSC2) in a position between markers SW2623 and SW2445, which is in agreement with the physical mapping of Silahtaroglu et al. (2004) and the cumulative map distance from the first marker on SSC2 to the CCS locus was 22.8 cM. The cumulative map distance for all the 14 loci involved in the analysis was 159.3 cM.

The best map position of the CCR7 gene on porcine chromosome 12 (SSC12) was found to be between markers S0229 and SW874, which is in agreement with the FISH results produced by Shinkai et al. (2003) and the cumulative map distance from the first marker on SSC12 to the CCR7 locus was 30.5 cM. The cumula-
tive map distance for all 7 markers involved in the analysis was 97.7 cM. Mapping of the pig CCS and CCR7 genes increases information for SSC2 and SSC12 and is a good base to study them as candidate genes for sow stress and longevity.

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References


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