Pig KALRN, MYH1, MLC2V, SNX13, AK1, and PPIA loci RH mapping and chromosome position refining

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Received May 8, 2008
Accepted July 1, 2008
Published October 7, 2008

ABSTRACT. The suppressive subtractive hybridization technique was previously used by the authors to identify candidate genes for meat quality in pig. A set of ESTs homologous (>95%) to genes involved in muscle metabolism is reported in the present paper. Four ESTs homologous to MYH1, KALRN, MLC2V, and SNX13 genes plus two genes (AK1, PPIA) used as housekeeping for muscle tissue were assigned to porcine chromosomes using the INRA-Minnesota 7000 rads radiation hybrid panel (IMpRH). Our data confirm and refine the cytogenetic position of the KALRN, AK1, PPIA genes, improve the existing physical map of MYH1 and assign two new genes (MLC2V and SNX13) to swine chromosomes.

Key words: RH mapping; Pig; Meat quality
Mapping and chromosome position refining of six porcine genes

INTRODUCTION

During the last decades, research has focused on genetic improvement of pork quality, and studies on the RN (Rendement Napole; Le Roy et al., 1990) and halothane (Fujii et al., 1991) genes have already demonstrated the importance of allelic variation in economically important traits such as meat quality. Despite its economic importance and the number of ongoing studies, information regarding genes involved in pork quality is still poor. To identify candidate genes for pork quality, suppressive subtractive hybridization libraries (Diatchenko et al., 1996) starting from skeletal muscle tissue samples and a commercial population were created (Gorni C, Stella A, Restelli GL, Iacuaniello S, et al., unpublished data). Four ESTs homologous to genes involved in muscle metabolism, skeletal muscle myosin heavy polypeptide 1 (MYH1), kalirin (KALRN), myosin regulatory light chain (MLC2V), and sorting nexin 13 (SNX13), were identified and mapped to porcine chromosomes using the INRA-Minnesota 7000 rads radiation hybrid panel (IMpRH; Yerle et al., 1998). Two additional housekeeping genes for muscle tissue, adenylate kinase 1 (AK1) and cyclophilin A (PPIA), were also mapped.

MATERIAL AND METHODS

Primer pairs were designed using PrimerExpress 3.0 (Applied Biosystems) and were based on the EST sequences produced in the present study. AK1 and PPIA specific primers were designed based on mRNA pig sequences downloaded from GenBank (AY610127 and AY008846). Each primer pair was tested against hamster and pig control DNAs. Amplification conditions were optimized to produce single amplicons.

Following optimization, polymerase chain reactions were performed at 95°C for 15 min followed by 40 cycles of 94°C for 15 s, 60°C for 30 s, and 72°C for 30 s. Amplification products were visualized after electrophoresis on ethidium bromide-stained 2% agarose gels. Positive clones in the IMpRH panel were scored and polymerase chain reactions were repeated twice. Data were submitted to the IMpRH database (http://www.toulouse.inra.fr/lgc/pig/RH/IMpRH.htm) to assess map positions.

RESULTS AND DISCUSSION

As summarized in Table 1, all our sequences were successfully mapped at LOD >4 to pig genome, and all assignments are in accordance with in-silico human-pig comparative mapping data available at the PigQTL database (http://www.animalgenome.org/QTLdb/pig.html; Hu et al., 2005).

In particular, for three (KALRN, AK1, PPIA) of the six genes, the reported map position refines the existing cytogenetic chromosomal assignments. KALRN position was reported by GenBank as unpublished cytogenetic mapping data (13q41; Tang H, Ren J, Huang X, Yan X, et al., 2007, unpublished data) and our data assign this locus to SSC13 linked to S0075 marker at 34 cR. AK1 and PPIA genes had been previously mapped using a somatic cell hybrid panel to position 1q28-q213 and 18q24, respectively (Davoli et al., 2002).

The present study refines the position of AK1, by linking it to SW1301 (20 cR), and confirms the cytogenetic position of PPIA on SSC18 linked to S0177 at 50 cR distance.

Moreover, our data allow us to merge the two linkage groups (map1 and map2) identi-
fied by Shimogiri and co-workers (2006) as in our MYH1 study, which had been previously mapped to the linkage group “map2”, is linked to the SWR1021 marker, which had been previously mapped to the linkage group “map1”. Finally, our data, confirm the orientation of Shimogiri’s map and the relative marker position.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Human accession No.</th>
<th>Human location</th>
<th>Porcine accession No.</th>
<th>Chromosome position</th>
<th>Closest marker</th>
<th>Distance (cR)</th>
<th>LOD score</th>
<th>Retention (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KALRN</td>
<td>NM_001024660</td>
<td>3q21</td>
<td>EV825903</td>
<td>13q41</td>
<td>S0075</td>
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<td>NM_005963</td>
<td>17p13.1</td>
<td>AB025262</td>
<td>12q15</td>
<td>SWR1021</td>
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<td>4.47</td>
<td>11</td>
</tr>
<tr>
<td>MLC2V</td>
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<td>12q24.1</td>
<td>AJ487671</td>
<td>14q15</td>
<td>CATP3</td>
<td>19</td>
<td>15.15</td>
<td>22</td>
</tr>
<tr>
<td>SNX13</td>
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<td>7p21.1</td>
<td>DY404375</td>
<td>9q14</td>
<td>SW1006</td>
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<td>4.70</td>
<td>41</td>
</tr>
<tr>
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<td>9q34.1</td>
<td>AY610127</td>
<td>1q213</td>
<td>SW1301</td>
<td>20</td>
<td>14.13</td>
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</tr>
<tr>
<td>PPIA</td>
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<td>7p13</td>
<td>AY008846</td>
<td>18q24</td>
<td>S0177</td>
<td>50</td>
<td>6.13</td>
<td>60</td>
</tr>
</tbody>
</table>

1Position inferred from the linked markers in the porcine cytogenetic map.

Two new genes were added to the swine physical map: MLC2V, which maps to SSC14 linked to CATP3 at 19 cR distance, and SNX13 located on SSR9 at 65 cR from SW1006.

In the same chromosomal regions where the six genes have been mapped, QTLs for ham weight (Milan et al., 2002; Yue et al., 2003), musculus biceps femoris and dissected ham ratio (Karlskov-Mortensen et al., 2006), conductivity 45 min post-mortem (Yue et al., 2003), average backfat (Rohrer and Keele, 1998) and shoulder weight (Milan et al., 2002) have been previously identified, suggesting these genes as positional candidate genes for swine meat quality. All genes investigated in the present paper are functional candidate genes for muscle metabolism: KALRN and MLC2V play an important role in muscular hypoxia and calcium ion binding pathways (Wardle et al., 2007; Smolensky et al., 2007); MYH1 is involved in oxidative muscle metabolism (Lefaucheur et al., 2004); MLC2V plays a role in calcium ion binding pathways (Smolensky et al., 2007), and SNX13 is one of the SNX family members involved in intracellular and vesicular trafficking (Carlton and Cullen, 2005).

ACKNOWLEDGMENTS

We are grateful to Dr. M. Yerle who provided the IMpRH panel. Research supported by the Ministry of Education, University and Scientific Research (FIRB MIUR RBNE01MMHS).

REFERENCES

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