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Rapid communication: Mapping of the Ca\(^{2+}\) ATPase of fast twitch 1 skeletal muscle sarcoplasmic reticulum (ATP2A1) gene to porcine chromosome 3

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Locus Name. Ca\(^{2+}\) ATPase of fast twitch 1 skeletal muscle sarcoplasmic reticulum.

Species. Sus scrofa.

Locus Symbol. ATP2A1.

Source of Primers. Two sets of PCR primers were designed based on human ATP2A1 sequences (GenBank accession numbers U96773 and U96777) available in the GenBank database. Using pig genomic DNA as a template we used set A primers to amplify approximately 1,100 bp spanning exon 1 to exon 2 and set B primers to amplify 1,200 bp gene segment covering some of exon 8 through part of exon 9. These sequences were deposited in GenBank, accession number AY027797-AY027799.

Primer Sequences. Primers derived from the porcine sequences were as follows: Set A: forward, 5′-GTC TCA GCC AGC CAA TCC CT-3′ and reverse, 5′-AAG GAA ATG CAT GCC AG-3′; Set B: forward, 5′-GGG CAC CAA CAT TGC AGC C-3′ and reverse, 5′-CTT GCA GAC AGA CAT CTG GTT-3′.

Method of Detection. The PCR was performed in 10-μL reactions that included Taq DNA polymerase (Promega, Madison, WI), 1× PCR buffer, 1.5 mM MgCl\(_2\), 200 μM each dNTP, 3 pmol each primer, and 12.5 ng of genomic DNA. The PCR was carried out in a PTC-200 thermocycler (MJ Research, Watertown, MA). The cycling conditions included initial denaturation at 95°C for 4 min followed by 30 cycles at 95°C for 45 s, 60°C for 1 min, 72°C for 1.5 min, and final extension at 72°C for 12 min. For the PCR-RFLP we used the set B forward primer and following as reverse, 5′-GAT CGT TGA AGT GGC CGA TGT-3′. The PCR product generated by these primers was digested overnight with DpnII at 37°C. For the physical mapping the set A forward primer and the following pig specific reverse primer were used: 5′-CGT CTG GGT TTC TTC CTC TCT-3′ in order to obtain a pig-specific amplicon only. All the PCR products were separated by agarose electrophoresis and visualized by ethidium bromide staining.

Description of Polymorphisms. The PCR products from several individuals from each of the commercial lines of pigs (Landrace, Large White, Duroc, Hampshire, and Berkshire) were directly sequenced using dye terminators and an ABI 377 sequencer (Perkin Elmer, Foster City, CA). We initially found four single nucleotide polymorphisms (SNP) and one microsatellite polymorphism in the gene segment covering exon 8 to exon 9. One SNP, present in exon 8, was confirmed by enzyme digestion by PCR-RFLP. This polymorphism (C to T substitution at position 20 in AY027798) determines a restriction site for the enzyme DpnII and was found to segregate in three of the PiGMaP families (Archibald et al., 1995). The fragment sizes generated by the DpnII

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Table 1. Genotype and allelic frequency for the porcine ATP2A1 DpnII PCR-RFLP site

<table>
<thead>
<tr>
<th>Commercial population</th>
<th>n</th>
<th>1/1</th>
<th>1/2</th>
<th>2/2</th>
<th>Allele 1 frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large White</td>
<td>50</td>
<td>33</td>
<td>15</td>
<td>2</td>
<td>0.81</td>
</tr>
<tr>
<td>Landrace</td>
<td>50</td>
<td>8</td>
<td>17</td>
<td>25</td>
<td>0.33</td>
</tr>
<tr>
<td>Hampshire</td>
<td>50</td>
<td>36</td>
<td>13</td>
<td>1</td>
<td>0.85</td>
</tr>
<tr>
<td>Duroc</td>
<td>50</td>
<td>3</td>
<td>35</td>
<td>12</td>
<td>0.41</td>
</tr>
<tr>
<td>Berkshire</td>
<td>50</td>
<td>3</td>
<td>20</td>
<td>27</td>
<td>0.26</td>
</tr>
</tbody>
</table>

digestion were 70 bp (allele 1) and 61 bp and 9 bp (allele 2) as well as the 140-bp monomorphic fragment (Figure 1).

**Inheritance Pattern.** In three PiGMaP families the segregation of the DpnII polymorphism followed a Mendelian autosomal inheritance pattern.

**Allele Frequency.** Alleles and genotypes frequencies were estimated in several commercial pig populations and these are shown in Table 1.

**Chromosomal Location.** We used a pig/rodent somatic cell hybrid panel (SCHP) including 27 cell lines (Yerle et al., 1996) for physical mapping of the pig ATP2A1 gene. The PCR results were submitted and analyzed as described on the Web site http://www.toulouse.inra.fr/lgc/pig/hybrid.htm. The results of the SCHP analysis revealed that the ATP2A1 gene is located on pig chromosome 3p16–17. Linkage mapping was then performed using two-point linkage analysis, using the genotypes revealed by DpnII PCR-RFLP analysis of three PiGMaP families (Archibald et al., 1995) and employing the CRIMAP program (Green et al., 1990). The ATP2A1 gene is significantly linked with several markers (the two-point recombination frequencies and LOD scores with values greater than 5.00 are given in parentheses): SW2527 (0.06, 8.30), SW833 (0.12, 7.87), S0151 (0.07, 6.91), SW72 (0.06, 6.88), S0032 (0.12, 5.72), and S0206 (0.06, 5.69). These results and multipoint linkage analysis show that the ATP2A1 gene is most likely located between SW2527 and SW833, confirming the physical localization.

**Comments.** The ATP2A1 gene encodes SERCA1, which is the fast-twitch skeletal muscle sarcoplasmic reticulum Ca\(^{2+}\) ATPase. Recently, mutations in the ATP2A1 gene were reported to be associated with Brody disease in humans (Odermatt et al., 2000).

The human ATP2A1 gene maps to human chromosome 16p12.1. There is a conserved synteny between HSA16p and SSC3p as demonstrated by the bidirectional chromosome painting analysis (Goureau et al., 1996; http://www.toulouse.inra.fr/lgc/pig/compare/SSCHTML/SSC3B.HTM). The physical localization of the pig ATP2A1 gene on SSC3p16–17 was confirmed also by linkage mapping, and these results are in accordance with the across-species studies and add another locus to this conserved segment of synteny.

**Literature Cited**


Green, P., K. Falls, and S. Crooks. 1990. Documentation for CRIMAP, version 2.4. Washington Univ. School of Medicine, St. Louis, MO.


**Key Words:** Pigs, Polymorphism, Gene Mapping, Adenosinetriphosphatase