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Rapid Communication: Assignment of Porcine Serotonin Receptor Subtype 2 Alpha and Endothelin-B Receptor to Chromosome 11 by Linkage Analysis

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Abstract

Genus and Species. Sus scrofa. Locus. Pig serotonin receptor subtype 2 alpha (HTR2A) and pig endothelin-B receptor (EDNRB) . Source and Description of Primers. Heterologous primers for EDNRB were obtained from Leslie Lyons through the CATS project (Lyons et al., 1997) and primers for HTR2A were designed from highly conserved human (M86841) and mouse sequences (X72222). Primers (EDNRB forward: 5¢-AATTGTTTAATTTGGGTGGTCTC- 3¢ and EDNRB reverse: 5¢-AGCCACCAGTCTTTAGCTGTC-3¢; HTR2A forward: 5¢-CCCTAGAGAAAAAGCTGCAGA-3¢ and HTR2A reverse: 5¢-GACACGGGCATGACAAGGA-3¢) were used to amplify pig homologous fragments by standard PCR. Pig genomic fragments amplified with heterologous primers were sequenced to confirm homology. An 81% similarity over 125 bp was found in the exon 3 region of the EDNRB gene between human and the new pig sequence-tagged sites (STS) . A 100% sequence similarity was found for 115 bases of the exon 1 region of HTR2A gene between our STS and a pig HTR2A cDNA sequence (accession no. S78208). Pig-specific primers were designed from the pig STS obtained in this study (HTR2A forward: 5¢-CCCTAGAGAAAAAGCTG CAGA-3¢ and HTR2A reverse: 5¢-GCAGAGGCCACCGGTA-3¢) to increase the PCR efficiency.

Keywords

Pigs, PCR-RFLP, Linkage, Gene Mapping, Pig Chromosome 11

Comments

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Rapid Communication: Assignment of Porcine Serotonin Receptor Subtype 2 Alpha and Endothelin-B Receptor to Chromosome 11 by Linkage Analysis[1]

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Genus and Species. *Sus scrofa*.

Locus. Pig serotonin receptor subtype 2 alpha (*HTR2A*) and pig endothelin-B receptor (*EDNRB*).

Source and Description of Primers. Heterologous primers for *EDNRB* were obtained from Leslie Lyons through the CATS project (Lyons et al., 1997) and primers for *HTR2A* were designed from highly conserved human (M86841) and mouse sequences (X72222). Primers (*EDNRB* forward: 5′-AATTGTTTTAATTTGGGTGGTCTC-3′ and *EDNRB* reverse: 5′-AGCCACCAGTCTTTAGCTGTC-3′; *HTR2A* forward: 5′-CCCTAGAGAAAAAGCTGCAGA-3′ and *HTR2A* reverse: 5′-GACACGGGCATGACAAGGA-3′) were used to amplify pig homologous fragments by standard PCR. Pig genomic fragments amplified with heterologous primers were sequenced to confirm homology. An 81% similarity over 125 bp was found in the exon 3 region of the *EDNRB* gene between human and the new pig sequence-tagged sites (**STS**). A 100% sequence similarity was found for 115 bases of the exon 1 region of *HTR2A* gene between our STS and a pig *HTR2A* cDNA sequence (accession no. S78208). Pig-specific primers were designed from the pig STS obtained in this study (*HTR2A* forward: 5′-CCCTAGAGAAAAAGCTG CAGA-3′ and *HTR2A* reverse: 5′-GCAGAGGCCACCGGTA-3′) to increase the PCR efficiency.

Method of Detection. Standard *Taq* polymerase (Promega, Madison, WI) with 1.5 m*M* $MgCl_2$ was used for both amplifications. The annealing temperature of *HTR2A* and *EDNRB* is 57°C and 60°C, respectively. Detailed conditions can be found in the STS entries for each locus. The sizes of PCR-amplified pig *HTR2A* and *EDNRB* fragments are 3 and 2.3 kb, and RFLP within the PCR products was detected using *Hin*dIII and *Dpn*II enzymes, respectively (Figure 1).

Description of Polymorphism. Two alleles were detected for each locus. For the *EDNRB* marker, eight monomorphic fragments and three polymorphic fragments with sizes of 240 and 150 bp (Allele A) or 390 bp (Allele B) were detected among all breeds (Figure 1A). For the *HTR2A* marker, the PCR product of 3 kb was uncut (allele A) or was cut into two fragments of 1.6 and 1.4 kb (allele B) (Figure 1B).

Inheritance Pattern. Individuals from the PiGMaP reference families (Archibald et al., 1995) were genotyped for *HTR2A* and *EDNRB* markers. Informative meioses totaling 144 and 148 were detected for *HTR2A* and *EDNRB*, respectively. Linkage analysis was done by using the CRIMAP program (Green et al., 1990) against currently available information in the PiGMaP database.

Chromosomal Location. The *HTR2A* locus was linked to SSC11 markers S0071 and S0230 with the recombination frequencies of .26 (LOD = 3.85) and .28 (LOD = 3.36), respectively. Even though *EDNRB* was linked to several SSC11 markers, tight linkage was found between *EDNRB* and EAMJ, S0230, and S0009 with the recombination frequencies of 0 (LOD = 5.42), .05 (LOD = 23.91), and .07 (LOD = 16.63), respectively. A multipoint linkage map for *HTR2A* and *EDNRB* was constructed using several chromosome 11 markers (Archibald et al., 1995) as a framework map. The best-ordered map of *HTR2A*-S0071-*EDNRB*-S0230-*EAMJ*-S0009 is given in Figure 2.

Comments. Previous work done using chromosome painting techniques showed a complete synteny conservation of HSA13 in SSC11 (Goureau et al., 1996). We are interested in mapping additional anchored Type I loci in the pig genome and also in further examining the gene order within this conserved synteny group. The pig STS for *HTR2A* (dbSTS: 49066-7; GenBank: G32117-8) and *EDNRB* (dbSTS: 44871-2; GenBank: G30674-5) and the linkage map we present here are part of our ongoing project to develop a comparative map between human chromosome 13 (HSA13) and pig chromosome 11. Even though this highly conserved syntony group has broken into at least four segments in the mouse (i.e., MMU1, 5, 8, 14), the relative positions of *HTR2A* and *EDNRB* seem to be preserved among HSA13, SSC11, and MMU14.

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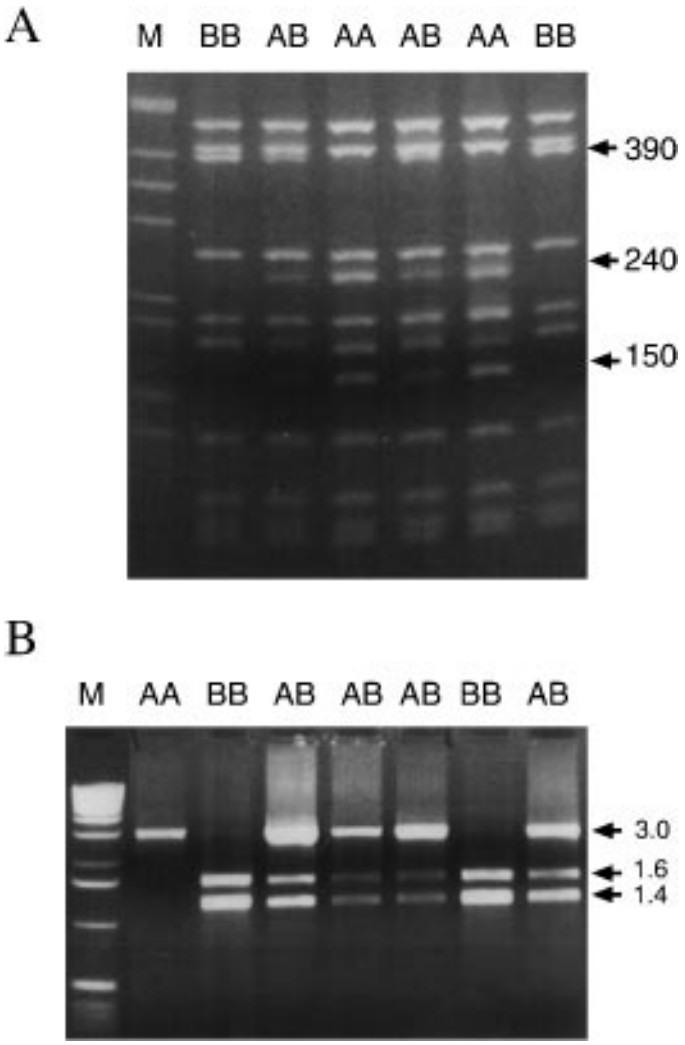

Figure 1. Polymorphisms observed at the *EDNRB* (A) and *HTR2A* (B) loci. The first lane (M) in each gel picture is the 1-kb DNA ladder (BRL, Life Technologies, Gaithersburg, MD) used as a molecular weight standard. Each lane is marked with the scored genotype, and at the right of each gel the approximate length of the polymorphic fragments is indicated.

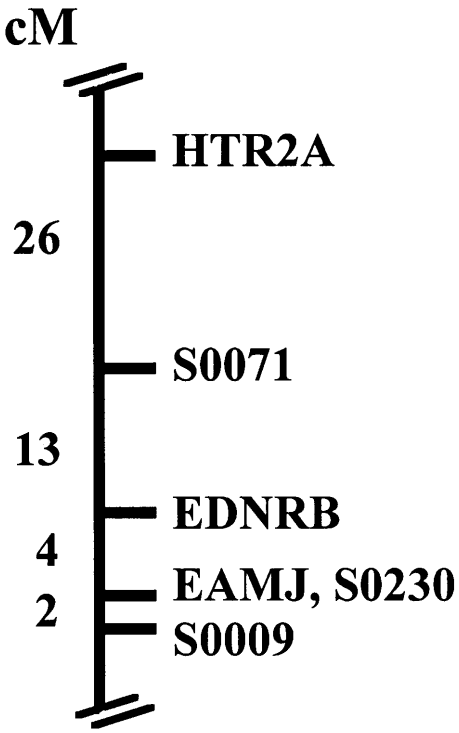

Figure 2. Partial linkage map of pig chromosome 11 (SSC11) demonstrates the positions of *HTR2A* and *EDNRB* relative to other linked markers on SSC11. Distance (centimorgans, cM) was calculated by multipoint linkage analysis (CRIMAP; Green et al., 1990).

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