2002

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Abstract

Genus and Species. Sus scrofa. Loci. DNAX-activation protein 10 (DAP10) and natural killer cell receptor D (NKG2D). Source and Description of Primers. Primers were designed from the porcine DAP10 and NKG2D sequences (GenBank accession nos. AF285446 and AF285448, respectively). The DAP10 primers were used to amplify approximately 500 bp of the fragment spanning DAP10 exon 4 and DAP12 exon 5. The DAP10 and DAP12 genes are linked in opposite transcriptional orientation, separated by 152 bp (Yim et al., 2001). The NKG2D primers were used to amplify approximately 700 bp of the fragment spanning NKG2D exons 8 and 9.

Keywords

Pigs, Gene Mapping, Immune System, Natural Killer Cells, Receptors

Disciplines

Agriculture | Animal Sciences | Genetics and Genomics | Microbiology

Comments

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Rapid communication: Linkage mapping of the porcine immunoreceptor *DAP10* and *NKG2D* genes[1]

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Loci. DNAX-activation protein 10 (***DAP10***) and natural killer cell receptor D (***NKG2D***).

Source and Description of Primers. Primers were designed from the porcine *DAP10* and *NKG2D* sequences (GenBank accession nos. AF285446 and AF285448, respectively). The *DAP10* primers were used to amplify approximately 500 bp of the fragment spanning *DAP10* exon 4 and *DAP12* exon 5. The *DAP10* and *DAP12* genes are linked in opposite transcriptional orientation, separated by 152 bp (Yim et al., 2001). The *NKG2D* primers were used to amplify approximately 700 bp of the fragment spanning *NKG2D* exons 8 and 9.

Primer Sequences. The primer sequences for *DAP10* and *DAP12* are as follows: forward, 5′-GCA AAA TCT ACA TCA ACA TGC CG-3′ and reverse, 5′-GAT GTC TAC AGC GAC CTC AAC ACA C-3′. The primer sequences for *NKG2D* are as follows: forward, 5′-TAA TGA GAG CAA GAC CTG GC-3′ and reverse, 5′-GGT TAG GTG AGA GGA TGG AA-3′.

Method of Detection. Both PCR reactions were performed using 12.5 ng of porcine genomic DNA, 1× PCR buffer, 1.5 m*M* $MgCl_2$, 0.125 m*M* dNTP, 3 pmol of each primer, and 0.35 U *Taq* DNA polymerase (Promega, Madison, WI) in a 10-μL final volume. Each PCR profile included 2 min at 94°C, 40 cycles of 30 s at 94°C, 1 min at 54°C, 1.5 min at 72°C, and a final 15-min extension at 72°C in a Robocycler (Stratagene, La Jolla, CA). The *DAP10* and *NKG2D* PCR products were digested with *Xcm*I and *Rsa*I restriction endonucleases, respectively, and incubated overnight at 37°C. The digested fragments were separated by 3% agarose gel electrophoresis.

Description of Polymorphisms. The *Xcm*I digestion of the *DAP10* PCR product produced a 500-bp (allele 1) fragment and 400-bp and 100-bp (allele 2) polymorphic fragments. The *Rsa*I digestion of the *NKG2D* PCR product produced a 700-bp (allele 1) fragment and 500-bp and 200-bp (allele 2) polymorphic fragments (Figure 1).

Patterns of Inheritance. Autosomal segregation of Mendelian inheritance was observed in the three-generation European PiGMaP pedigrees (Archibald et al., 1995), in three families for the *DAP10* and four families for the *NKG2D*.

Allele Frequencies. Allele frequencies were determined by genotyping pigs of several breeds in the Iowa State University herd (Table 1).

Chromosomal Locations. Two-point and multi-point linkage analyses were performed using the genotypes of the PiGMaP families and the CRI-MAP program (Green et al., 1990). The *DAP10* gene was significantly linked to several markers on porcine chromosome 6 (SSC6). The most closely linked markers (LOD score and recombination fraction in parentheses) were *SO220* (13.85, 0) and *GPI* (14.17, 0.04). The best map order of the *DAP10* gene is as follows (with distance in Kosambi cM): *S0300*-2.5-*GPI*-6-*DAP10*-*DAP12*-0-*S0220*-5.7-*PGD*. The *NKG2D* gene was most significantly linked to *S0005* (13.55, 0) and *SW1017* (12.76, 0.03) on SSC5 and the best map order is as follows: *IFNG*-5.8-*SW1071*-1.7-*NKG2D*-0- *S0005*-17.7-*S0018*.

Comments. The porcine *DAP10* and *NKG2D* genes have been physically mapped on SSC6q21 and SSC5q25, respectively (Yim et al., 2001). Our linkage data will improve the comparative map of the regions between humans and pigs. The human NKG2D/DAP10 complex is expressed on gamma/delta T-cells, CD8+ alpha/beta T-cells, and natural killer cells and evokes immune responses against transfectants and epithelial tumor cells expressing MICA, stress-induced or tumor

Table 1. Allele 1 frequencies of the porcine *DAP10* and *NKG2D* polymorphisms in the Iowa State University herd

Breed	No. of animals	DAP10	NKG2D
Landrace	5	0.9	0
Hampshire	6	1	0
Yorkshire	7	0.85	0.64
Meishan	7	0.43	0

[1]This work was supported by PIC International Group and the Iowa Agric. and Home Econ. Exp. Stn., Ames, paper no. J-19293, project no. 3600, as well as by Hatch Act and State of Iowa funds. Support by the EEC for the PiGMaP DNA and bioinformatics support by A. Archibald and associates of the Roslin Institute is greatly appreciated.

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Received April 25, 2001.
Accepted January 16, 2002.

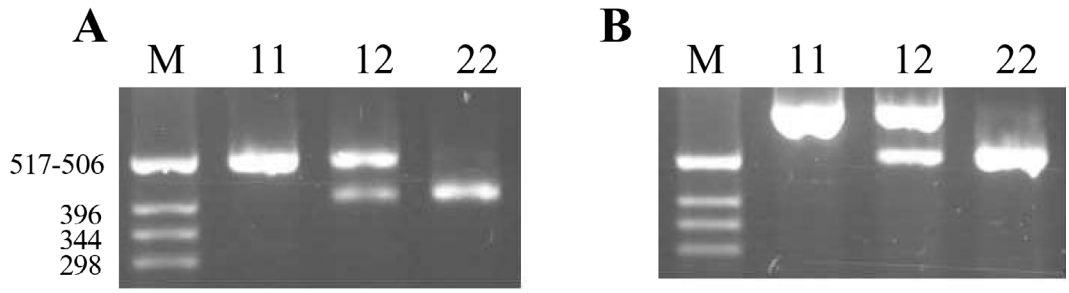

Figure 1. Agarose gel (3%) showing genotypes of the porcine *DAP10* and *NKG2D* polymorphisms. (A) The *Xcm*I PCR-RFLP of porcine *DAP10*. (B) The *Rsa*I PCR-RFLP of porcine *NKG2D*. Molecular marker (M) and each genotype are indicated at the top of the lane.

associated MHC-related molecules (Bauer et al., 1999; Wu et al., 1999). Therefore, the NKG2D/DAP10 complex may play an important role in the animal's immune surveillance system.

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