Assignment¹ of the scavenger receptor class B, member 2 gene (*SCARB2*) to porcine chromosome 8q11→q12 by somatic cell and radiation hybrid panel mapping

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Rationale and significance

The identification of genes controlling infectious disease is an important step towards increasing disease resistance in the pig. Infectious diseases increase the cost of production (Wegener et al., 2003), and, for zoonotic pathogens, also impact food safety (Beloeil et al., 2004). Disease resistance has genetic heritability, and the mapping of genes involved in natural resistance (innate immunity) has been reported (Tuggle et al., 1997; Sun et al., 1998). Quantitative trait loci (QTLs) for immune parameters (Edfors-Lilja et al., 1998) and immune capacity traits have been reported (Edfors-Lilja et al., 2000). The objective of this research was to map a gene identified as having altered gene expression patterns within the mesenteric lymph nodes of swine during infection with *Salmonella enterica* serotype Choleraesuis. The *SCARB2* gene has been shown to increase RNA expression within 24 h post-infection, and was identified using Suppression Subtractive Hybridization (SSH) (Uthe et al., manuscript in preparation). Overall, this gene is an excellent candidate for participating

in the host response to infection and could therefore be useful in improving disease resistance through marker-assisted selection.

Materials and methods

The PCR primers for the *SCARB2* sequence were designed using Oligo 5.0 software (MBI Cascade, Co, USA) and supplied from Integrated Technologies Inc. (Coralville, IA, USA). The PCR primers are F 5'-AAGTGTCTGCGTCTCAACT-3' and R 5'-TCTGTCAGGGGACACTGGCT-3'. The PCR was performed in a 5-μl reaction volume of 1 × PCR buffer, 1.5 mM MgCl₂, 0.5 μM of each primer, 200 μM dNTP, 2 U *Taq* polymerase, and 10 ng of genomic DNA of the porcine somatic cell hybrid panel (SCHP) (Yerle et al., 1996) or the full IMpRH panel (Yerle et al., 1998; Hawken et al., 1999). The PCR reaction was preheated at 94°C for 3 min, followed by 1 cycle (94°C, 72°C to 61°C, 72°C) at 30 s for each 1°C drop in temperature, then followed by 40 cycles at 94°C for 30 s, 60°C for 30 s, and 72°C for 30 s, and completed at 72°C for 5 min. Unique PCR products were confirmed by 3% agarose gel electrophoresis. In all cases, the primers listed above only amplified pig genomic DNA, and not mouse or hamster controls. This gene was physically assigned with the SCHP using software at the INRA web site (<http://www.toulouse.inra.fr/lgc/pig/hybrid.htm>). The gene was also typed in duplicate with the IMpRH panel as directed (Yerle et al., 1998). Two-point results were calculated and multipoint radiation hybrid maps were built with IMpRH server (<http://imprh.toulouse.inra.fr/>). Linkage groups were assigned using a minimum LOD score threshold of 5.0.

Results

The 27 clones of the somatic cell hybrid panel showed the following vector for *SCARB2*: 01000 11101 11011 11001 01100 10. This gene was assigned to SSC8q11→q12 with 88% probability and 0.80 correlation.

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Typing of the 118 clones of the IMpRH panel for *SCARB2* resulted in the following vector: 00011 00011 00100 00110 00100 10000 10011 00010 00000 11001 11000 11110 11110 10110 00011 01010 01100 00011 01110 10011 10011 11111 11111 110. The retention fraction for *SCARB2* in the RH panel was 35%. Two-point analysis indicated *SCARB2* is located on SSC8q11→q12 with the closest marker being SW1679; highest two-point LOD score for this locus was 11.73. The SW1679 locus has been linkage mapped 2.8 cM from S0086 which has been physically mapped to SSC8q11→q21. Thus our *SCARB2* mapping data is consistent with the known conserved synteny of the region containing *SCARB2* (SSC8q11→q12) with the human chromosome region (HSA4q21.1) to which human *SCARB2* has been mapped (Calvo et al., 1995).

The *SCARB2* gene acutely increases expression in response to *Salmonella* infection (Uthe et al., manuscript submitted), and *SCARB2* (also known as *LIMP-II*) is highly expressed in late lysosomes, where it functions in both biogenesis and maintenance of such structures (reviewed in Eskelinen et al., 2003). Thus *SCARB2* may be involved in the macrophage phagolysosome response to infection. Interestingly, *SCARB2* may be a candidate gene for a QTL that affects the number of lymphocytes following mixing and transport of pigs, which has been mapped between S0086 and S0069 on SSC8q11→q12 (Edfors-Lilja et al., 2000). We mapped *SCARB2* to virtually the same region and flanking markers. The STS described here can be used to develop SNPs at *SCARB2* to determine the association to this QTL.

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