

# Genetic and Physical Mapping of the pig Vascular Cell Adhesion Molecule 1 (VCAM1) gene to pig chromosome 4

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**ASL-R1378**

## Summary and Implications

We have developed methods to identify genetic differences at the VCAM1 gene in pigs. We identified significant variability in Chinese and American breeds, with three different forms of this gene and five of the six possible resulting genotypes seen in Duroc and Landrace breeds. This variability was used to map the VCAM1 gene to pig chromosome 4, a chromosome with important quantitative trait loci. We also used our methods to physically map VCAM1 to the end of the long arm of chromosome 4, confirming the genetic linkage assignment.

## Introduction

The vascular cell adhesion molecule 1 (VCAM1) is a cell adhesion molecule required to stop monocytes and lymphocytes near sites of vascular damage or inflammation (Osborn et al., 1989). VCAM1 has important roles during development of blood vessels as well; VCAM1 mutant mice cannot form normal embryonic heart blood vessels nor connect the developing umbilical cord to the placenta (Kwee et al., 1995). Human VCAM1 was mapped to HSA1p21-p34, within a large group of genes conserved across human and several other mammalian species, including mouse, pig and other species (reviewed in DeBry and Seldin, 1996).

Our purpose in this research was to identify genetic differences which exist in the natural population of pigs and use these differences to map the VCAM1 gene in pigs.

## Materials and Methods

We have previously cloned a pig VCAM1 cDNA fragment and identified a *Sac*I polymorphism in the pig

VCAM1 gene using Southern blot hybridization (Helm et al., 1994). This polymorphism segregated in the PiGMaP families, but was not informative enough to allow linkage to the pig genetic map. To develop a PCR-based genotyping method, we sequenced the 3' untranslated region (3'UT) of pig VCAM1. PCR primers were designed and used to PCR amplify a 193 base pair segment. Specific PCR fragments were cloned and sequenced to confirm identity with reported VCAM1 3'UT sequence.

Polymorphism analysis was performed by electrophoresing PCR products on polyacrylamide gels. In different animals, two single bands of differing size and three distinct patterns of multiple bands were observed. The latter patterns were determined to represent homoduplex and heteroduplex bands for three heterozygous types (AB, BC, AC). Although PCR products from AA genotype DNA are larger than the other homozygous genotypes, PCR products produced from BB and CC genotype DNA are not able to be distinguished by electrophoresis. To identify the BB and CC genotypes, equal amounts of the unknown DNA are mixed with known AA, BB, or CC DNA, the DNA is denatured and renatured to anneal the DNA strands, and electrophoresis is then performed to determine the band pattern obtained.

## Results and Discussion

Three VCAM1 alleles in the PiGMaP consortium mapping families and our Iowa State University (ISU) mapping families were identified based on double stranded conformation polymorphisms (DSCP) in the PCR products. Figure 1 shows the results of such gel electrophoresis, demonstrating: a) the five distinct patterns observed originally, b) family material results indicating the apparent double stranded conformation polymorphism observed in products from heterozygote animals, and c) the results of mixing experiments used to distinguish the B and C homozygote genotypes.

Analysis of 53 unrelated animals across five divergent breeds [Duroc (17 pigs); Landrace (10); Large White (12); Meishan (12) and Wild Boar (2)] shows that the allele frequency in commercial breed type animals is allele A, 6%; allele B, 67%, and allele C, 27%. Allele A was seen only in Landrace at 25% frequency and in Meishan, where all animals were of the AA genotype.

Most F1 crosses within the PiGMaP gene mapping families were informative for this marker and VCAM1 was solidly mapped to pig chromosome 4 (SSC4) with linkage to six SSC4 markers. Five of these loci are shown in the map in Figure 2. We note that VCAM1 maps within a 22 cM gap, equally spaced between two

microsatellite markers on the most recent SSC4 map (Archibald et al., 1995). Our work thus improves the marker coverage of this important chromosome, where major quantitative trait loci have been discovered (Andersson et al., 1994).

To confirm this assignment, we physically mapped VCAM1 using a previously characterized panel of pig-hamster or pig-mouse somatic cell hybrids (Robic et al., 1996; Yerle et al., 1996). Genomic DNA from 27 hybrids were typed for VCAM1 by using the pig-specific PCR primers above. Data were analyzed for concordance with chromosome fragments retained in the hybrid cells as described (Robic et al., 1996). VCAM1 was assigned to SSC4q25 with 90% concordancy, at the extreme end of the q arm (Figure 2). This combined genetic and physical mapping data agrees well with the physical mapping reported for S0161 (Robic et al. 1996), where S0161 was localized to SSC4q21-23.

In summary, we have identified genetic variability in the pig gene for VCAM1. Using this variability, the chromosomal location of VCAM1 was shown to be on pig chromosome 4, a chromosome with important genes for economic traits.

#### Acknowledgments

The authors thank PiGMAP collaborators for supplying the PiGMAP DNA samples used in the linkage studies. This work is part of the European Community BRIDGE Pig Genome Mapping project and was supported in part by a grant from Dalgety Food Technology Centre, Cambridge, UK, and PIC USA, Franklin, KY, USA.

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Figure 1. PCR products of VCAM1 3' untranslated region shows six genotypes.

A. Five patterns were identified among animals. Identical patterns are seen in lanes 1 and 5, lanes 2 and 3, lanes 4 and 10; lanes 6 and 7, and lanes 8 and 9.

B. Pedigree information allowed definition of the A, B and C alleles. For example, in the first pedigree (B1), a new pattern in the offspring is observed (called BC), even though the parentals look identical, indicating two alleles with indistinguishable sizes are present in the parentals; the second pedigree (B2) confirms the conclusions made for the first pedigree as the mating is between an AA animal and a heterozygous BC animal creating two new patterns (AC and AB); the third (B3) and fourth (B4) pedigrees show segregation patterns consistent with AA x CC and AC x AB crosses, respectively.

C. Mixing experiment used to demonstrate heteroduplex polymorphisms and identify the BB and CC genotypes. Known genotypes: Lanes 1-4, AA; Lane 9 and 11, AB; Lane 14, AC; Lane 17, BC. Lanes 5-8 were unknown BB/CC genotype. Lane 10, mix of lane 3 and lane 6; lane 12, mix of lane 7 and lane 1; lane 13, mix of lane 7 and lane 3; lane 15, mix of lane 8 and lane 1; lane 16, mix of lane 8 and lane 3; lane 18, mix of lane 7 and lane 8.

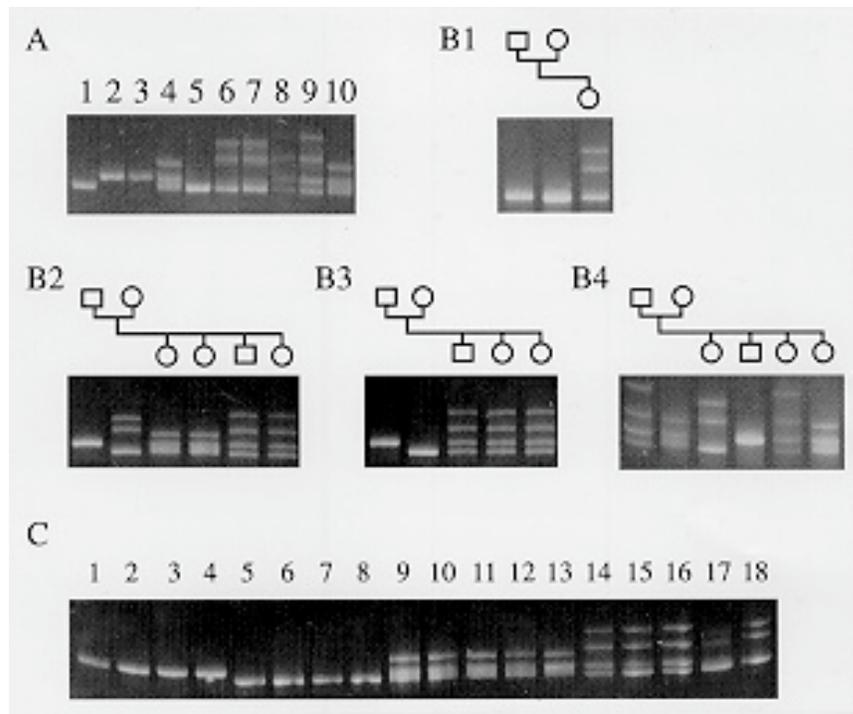


Figure 2. Genetic and Physical Mapping of Pig VCAM1. At left is the map of markers found linked to VCAM1. Distances between genes are shown in centiMorgans (percent recombination). In the center are physical maps of selected loci for SSC4q and SSC6q, and at right is a partial physical map of HSA1p, shown in reverse orientation.

