The receptor locus for Escherichia coli F4ab/F4ac in the pig maps distal to the MUC4-LMLN region

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The receptor locus for *Escherichia coli* F4ab/F4ac in the pig maps distal to the *MUC4–LMLN* region

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Abstract Enterotoxigenic *Escherichia coli* (ETEC) with
fimbriae of the F4 family are one of the major causes of
diarrhea and death among neonatal and young piglets. Bac-
teria use the F4 fimbriae to adhere to specific receptors
expressed on the surface of the enterocytes. F4 fimbriae exist
in three different antigenic variants, F4ab, F4ac, and F4ad, of
which F4ac is the most common. Resistance to ETEC F4ab/
F4ac adhesion in pigs has been shown to be inherited as an
autosomal recessive trait. In previous studies the ETEC
F4ab/F4ac receptor locus (*F4bcR*) was mapped to the q41
region on pig chromosome 13. A polymorphism within an
intron of the mucin 4 (*MUC4*) gene, which is one of the
possible candidate genes located in this region, was shown
earlier to cosegregate with the *F4bcR* alleles. Recently, we
discovered a Large White boar from a Swiss experimental
herd with a recombination between *F4bcR* and *MUC4*. A
three–generation pedigree including 45 offspring was gen-
erated with the aim to use this recombination event to refine
the localization of the *F4bcR* locus. All pigs were pheno-
typed using the microscopic adhesion test and genotyped for
a total of 59 markers. The recombination event was mapped
to a 220-kb region between a newly detected SNP in the
leishmanolysin-like gene (*LMLN* g.15920) and SNP
ALGA0072075. In this study the six SNPs *ALGA0072075*,
ALGA0106330, *MUC13-226*, *MUC13-813*, *DIA0000584*, and
MARC0006918 were in complete linkage disequilibrium
with *F4bcR*. Based on this finding and earlier investigations,
we suggest that the locus for *F4bcR* is located between the
LMLN locus and microsatellite S0283.

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Introduction

Close association to host surface is an essential requirement
for successful induction of disease by a broad variety of
pathogens. This association is often mediated by lectin rec-
ognition of host cell glycoconjugates (Karlsson 1998).
Breeding for genetic resistance is an established routine in
plant sciences and has become an attractive goal in animal
sciences. Thus, selection for absence of receptor molecules
or for genetic variants of receptor molecules to which the
pathogen cannot bind would represent an attractive route to
genetic resistance.

The two most economically dominant porcine enteric
E. coli infections comprise diarrhea induced by diverse
enterotoxins and enterotoxemia mediated by a shigatoxin.
Both diseases have in common that the bacteria have
to colonize the small intestine in high numbers where
they produce the deleterious toxins. To build up huge

populations, the bacteria are forced by the short transit time of ingesta to adhere to the small intestine's inner surface. This adhesion is the result of a highly specific interaction between fimbriae produced by the bacteria and receptors present exclusively on the brush border of small-intestine enterocytes (Bertschinger and Fairbrother 1999). Of the five pig-specific types of fimbriae, designated F4, F5, F6, F18, and F41 (Osek 1999), so far it is known that only two, F18 and F4, are highly prevalent. Antigen variants have been detected for both types of fimbriae. Whereas the two variants F18ab and F18ac recognize the same receptor (Rippinger et al. 1995), the three variants of F4 vary in their receptor specificities (Bijlsma et al. 1982). However, F4ac is by far the most prevalent variant all over the world with the exception of central China, where F4ad is reported to be predominant (Wang et al. 2006). Fortunately, the causative mutation for E. coli F18 susceptibility is known (Meijerink et al. 1997, 2000) and elimination of the E. coli F18 susceptibility allele from the porcine population is currently done in Switzerland and other countries to increase the number of resistant piglets in commercial populations (Luther et al. 2009).

In pigs the resistant and susceptible phenotypes for adherence or infection by E. coli with F4ac fimbriae are inherited as a monogenetic trait, the susceptibility allele being dominant over the resistance one (Gibbons et al. 1977). The receptor for F4ac or a closely linked locus binds F4ab as well (Jørgensen et al. 2003; Python et al. 2002). As yet, attempts to develop practical methods for identification of breeding stock that inherit resistance have not been completely successful. Several investigators have mapped the ETEC F4ab/F4ac receptor gene (F4bcR) to SSC13 (Edfors-Lilja et al. 1995; Python et al. 2002). An overview of the marker positions and mapped genes encompassing the F4bcR is given in Fig. 1. Subsequent studies mapped the ETEC F4ab/F4ac susceptibility locus to the interval SW207-S0075 refined in a 5.7-cM region around the F4bcR locus (Joller et al. 2009). Jørgensen et al. (2004) reported an association between resistance and a mutation in intron 7 of the mucin 4 (MUC4 g.8227 G > C) gene. However, data presented later by Rasschaert et al. (2007) as well as observations in Switzerland (Joller 2009) raised doubt as to whether this mutation in the MUC4 gene is in some rare cases not in complete linkage disequilibrium (LD) with the F4ac receptor locus.

Fig. 1 Location of 59 markers in the F4bcR candidate region at SSC13. The gene order and the scale are deduced from the Sscrofa9 assembly. The positions of the microsatellites are shown to the right of the chromosome 13 idiogram. The approximate positions of candidate genes are indicated at the far right side. The number of markers in each gene is given in parenthesis. The position of the six SNPs ALGA0072075, ALGA0106330, MUC13-226, MUC13-813, DIA0000584, and MARC000691, which were in complete LD, are shown in bold
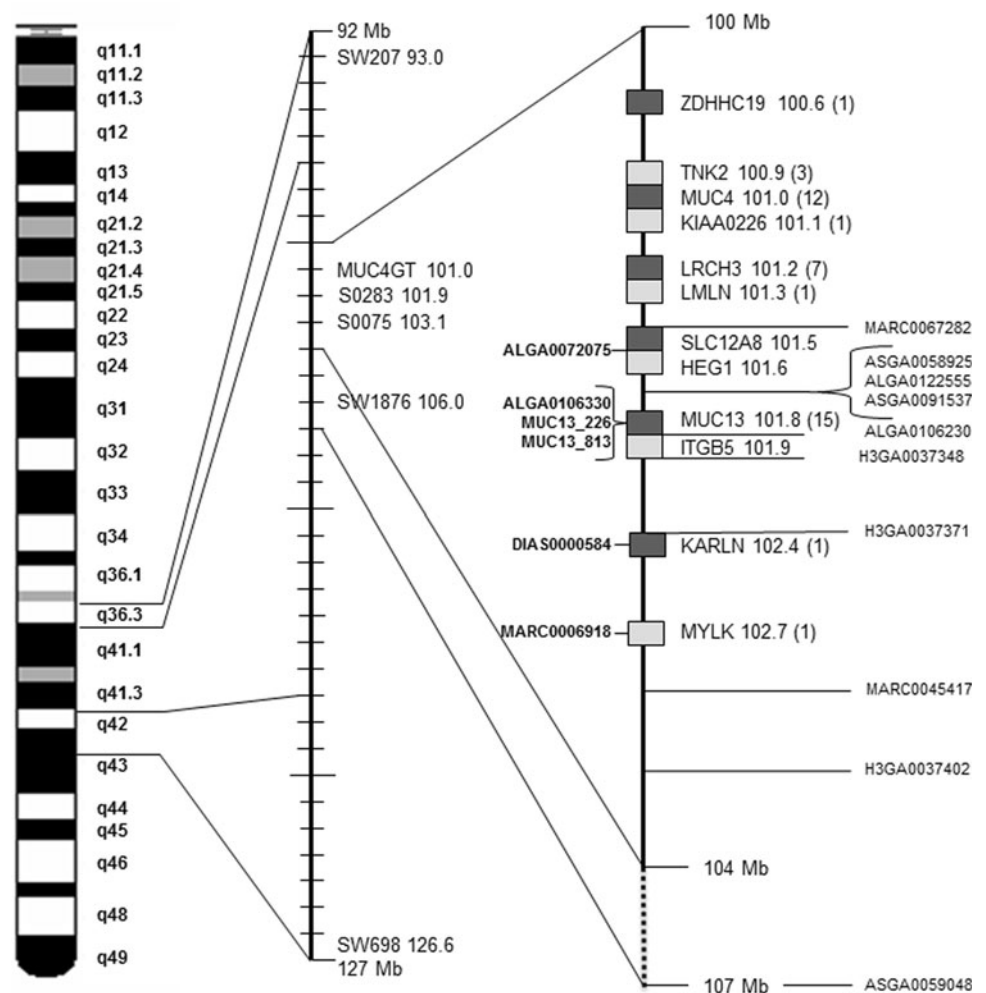
Fig. 2 Diagram of the three-generation family tree and haplotypes of ▶
eight pigs in the *F4bcR* region. SNP names and positions on SSC13
are given at the right side. The animal identity is shown on top of the
haplotypes. The SNP genotypes are depicted on the right side of the
colored columns. The digit 1 corresponds to nucleotide A, 2 to C, 3 to
G, and 4 to T. Microsatellite markers are represented by 5, 6, etc.,
depending on the sizes of the allelic bands. *S* on top of the colored
bars indicates the adhesive and *s* the nonadhesive haplotype. Each
haplotype has its own color. The lines indicate the relationships
between the pigs. The geometric figures above the animal identity
indicate the sex, squares for males and circles for females. Filled
figures indicate F4ab/F4ac susceptible and blank figures resistant pigs

We recently discovered a Large White boar that carried
a recombinant chromosome in the interval between *F4bcR*
and *MUC4*. The aim of the study was to characterize the
recombinant haplotype and also to discover more accurate
markers for F4ab/F4ac resistance. Therefore, we tested a
total of 59 markers (53 SNPs and 6 microsatellites) in a
three-generation pedigree. The data indicate that the *F4bcR*
receptor locus is distal to the *MUC4* locus.

Materials and methods

Pigs

The animals used in this study originated from a Swiss
experimental herd (SEH) at the University of Zurich com-
prising Large White, Landrace purebred pigs, and Large
White/Landrace crossbreds. The boar 698B used for
breeding purposes was found to be homozygous for the
susceptibility allele at the *F4bcR* locus but heterozy-
gous in *ZDHHC19* g.4043, *TNK2* g.7075, *TNK2* g.7717,
TNK2 g.11142, *MUC4* g.8227, and *KIAA0226* g.62250,
which were so far in complete LD with the *F4bcR* alleles
(Jacobsen et al. 2010). To be sure that 698B was homozygous
susceptible to ETEC F4ab/F4ac, it was mated to three
resistant sows generating a total of 45 offspring. The
homozygosity for the resistant allele of the sows was proved
by previous matings to resistant boars and confirmed by the
microscopic adhesion test after slaughtering.

To precisely define the location of the recombination
event, a three-generation family was generated, including
boar 698B, its parents (boar 381B and sow 405B), two
siblings of boar 698B (9499 and 9486), and its offspring
(1977, 2122, 2731, and 2734) (Fig. 2).

Sampling of intestinal tissue and microscopic
adhesion test

Pigs ($n = 53$) were slaughtered at about 2 months of age,
at 150 days of age, or when they were eliminated from
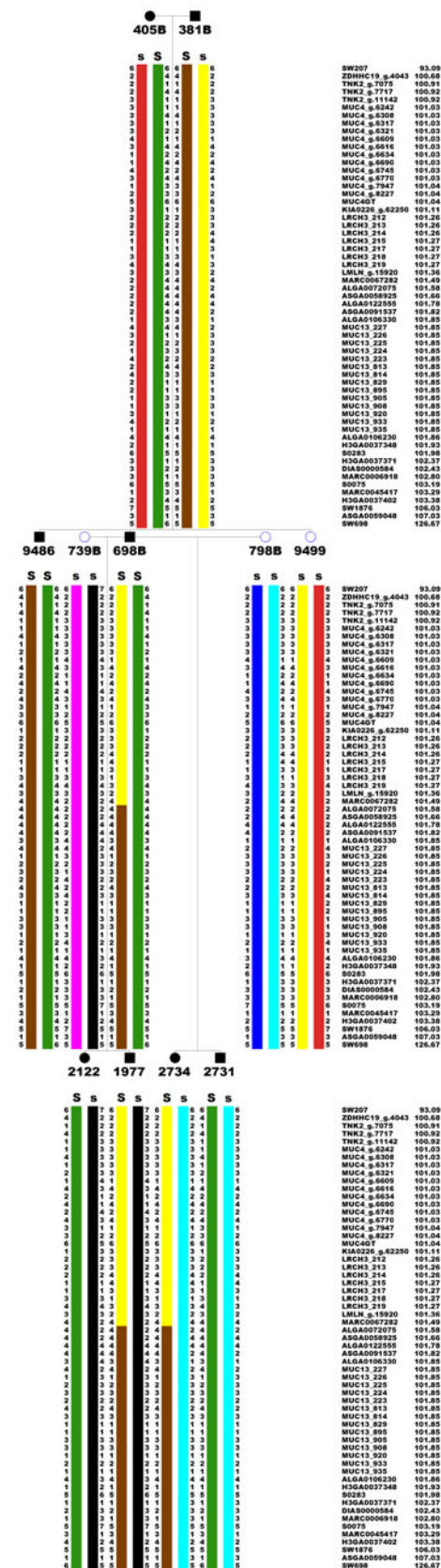
breeding. Samples of blood and intestine were taken at that time. The interval between killing and sampling of intestine was between 10 and 30 min to avoid degeneration of the epithelial intestinal cells.

All animals were phenotyped with the antigenic variant F4ac fimbriae by the microscopic adhesion test according to the method of Vögeli et al. (1996). A more detailed description of the preparation of bacterial strains, sampling of intestinal tissues, purification of enterocytes, and the procedure of the microscopic adhesion test can be found in Joller (2009). In brief, starting at the upper mesentery root, the intestine was separated from the mesenterium and an empty segment in the middle part was taken anywhere between 3.5 and 7.5 m in young pigs and between 5 and 10 m in older pigs. Twenty intact enterocytes were scored under an optical microscope by the same person. A pig was classified as adhesive to F4ac if more than 15% of the enterocytes bound more than five bacteria on the brush borders.

Screening for genetic polymorphisms

Genomic DNA was extracted from EDTA-anticoagulated whole blood as described by Vögeli et al. (1994). A total of 59 markers on chromosome 13q41 were used to characterize the three-generation pedigree (53 SNPs listed in Tables 1 and 2) along with 6 microsatellite markers: *SW207*, *MUC4GT*, *S0283*, *S0075*, *SW1876*, and *SW698*. Twenty-nine markers from Table 1 were selected from Jacobsen et al. (2010), nine SNPs in *MUC13* were taken from Zhang et al. (2008), and *LMLN* g.15920 was detected in this study by BLASTing the human sequence (NM_001136049) to the pig genome and by sequencing the genomic porcine homolog. The 14 SNPs listed in Table 2 were analyzed using the Illumina PorcineSNP60 BeadChip (Illumina, Inc., San Diego, CA, USA) (Ramos et al. 2009). The microsatellite markers were selected from Python et al. (2002).

PCR was carried out in a reaction volume of 25 µl containing 100 ng of genomic DNA, 10 mM Tris-HCl (pH 9), 50 mM KCl, 1.5 mM MgCl$_2$, 200 µM of each deoxynucleotide, 0.4 µM of forward and reverse primers, and 1.25 U of *Taq* DNA polymerase. The annealing temperature was chosen according to the melting temperature of the primers. For microsatellites, the PCR consisted of 25 cycles and for the SNPs 35 cycles followed by a final extension at 72°C for 2 min. Thermal cycling conditions were incubation at 94°C for 2 min followed by incubation at the specific annealing temperature for 30 sec and extension at 72°C for 30 sec. The subsequent denaturation was at 94°C for 30 sec. DNA samples were then purified with Montage PCR Centrifugal Filter Devices (p36461) (Millipore, Billerica, MA, USA) and sequenced. Alleles of the microsatellites were analyzed on an ABI377 DNA analyzer (Applied Biosystems, Foster City, CA, USA). Haplotypes were determined using MERLIN (Abecasis et al. 2002) and HaploPainter software (Thiele and Nürnberg 2005).

Results

The distribution of the 59 markers in the candidate region of the *F4bcR* locus is shown in Fig. 1, and the approximate positions of genes and markers are according to the Sscrofa9 assembly and the physical map (http://pre. ensembl.org/Sus_scrofa_map/). These markers cover the 35-Mb interval from microsatellite SW207 to SW698. Fifty-five markers map to the core interval 100–104 Mb on chromosome 13.

Boar B698 was mated with three sows resistant to ETEC F4ab/F4ac and hence putative homozygotes for the absence of an adherent ETEC F4ab/F4ac receptor, generating a total of 45 offspring. All of the offspring were adhesive to ETEC F4ab/F4ac according to the microscopic adhesion test. Thus, all the offspring are presumed to have received (from boar B698) a susceptibility allele encoding the presence of a receptor to which *E coli* F4ac can adhere. Therefore, it can be deduced that boar B698 is homozygous for the susceptibility allele. However, the genotyping data showed that 23 piglets (51.1%) possessed the *MUC4* g.8227 *C/C* genotype, which was previously reported to be associated with the absence of F4ab/F4ac adhesion (Jacobsen et al. 2010; Jørgensen et al. 2004), and 22 piglets were *C/G* (48.9%). Haplotype analysis of the genotype data reveals a putative recombination event between *LMLM* g.15920 and *ALGA0072075* SNP marker loci (Fig. 2). The recombinant chromosome was transmitted to 23 of B698's offspring. The recombinant chromosome was identical to all other susceptible haplotypes for the interval *ALGA0072075*–SW698. The *LMLN–S0075* region was also covered with 11 SNPs of the PorcineSNP60 BeadChip (Table 2), 2 microsatellites, and 14 SNPs in *MUC13*. In contrast to the *MUC4* markers, *ALGA0072075*, *ALGA0106330*, *MUC13-226*, *MUC13-813*, *DIA0000584*, and *MARC0006918* were all in complete LD with *F4bcR* in our family (Fig. 2).

Discussion

Previous studies identified *MUC4* as the most probable candidate gene for *F4bcR* based on its chromosomal location and evidence that the molecule to which the *E. coli* F4ac adheres is a mucin-like sialoglycoprotein (Erickson et al. 1994; Jacobsen et al. 2010; Joller et al. 2009; Jørgensen et al. 2004). As reported earlier, the resistance

Table 1 Description of the SNPs

BAC Clone	GenBank reference	SNP name	Alleles[a] R>S	Position on SSC13 (Sscrofa9)	Forward primer	Reverse primer	Amplicon lenght
CH242 182C12	NM_001039617	ZDHHC19 g.4043[b]	C>T	100681071	TCCTACGAGGGCAAGGTATGTG	GAACTGAATCTGGTCTGGAATAGC	171
CH242 221N4	NM_001010983	TNK2 g.7075[b]	C>A	100914782	GGAAGCCACTTGATTGTTCTC	GACAGGGACACCAACAGCTAA	625
		TNK2 g.7717[b]	C>T	100915124	GAAGAGCTGGGTGCTCTGCTT	AGCGGCAGCTGCATACTTGA	551
		TNK2 g.11142[b]	G>A	100924258	CCTGTGAGTGAAGACCAAGACC	CCCCATCTCCTCCTCACTTGA	751
CH242 240L11	NM_004532	MUC4 g.6242[b]	G>A	101034210	GTTACTGGCCTCGACTCTCC	AGGTTGTACCCTTGGCATTC	749
		MUC4 g.6308[b]	G>T	101034276			
		MUC4 g.6317[b]	G>A	101034285			
		MUC4 g.6321[b]	G>C	101034289			
		MUC4 g.6609[b]	T>A	101034577			
		MUC4 g.6616[b]	G>T	101034584			
		MUC4 g.6634[b]	A>C	101034602			
		MUC4 g.6690[b]	A>T	101034658			
		MUC4 g.6745[b]	T>C	101034713			
		MUC4 g.6770[b]	G>T	101034738			
		MUC4 g.7947[b]	A>G	101035915	GGTCCTACGCCTTGTTTCTC	CCTTCATGGGGTTGTTGTAATA	462
		MUC4 g.8227[b]	C>G	101036195	GTGCCTTGGGTGAGAGGTTA	CACTCTGCCGTTCTCTTTCC	367
	NM_001145642	KIAA0226 g.62250[b]	G>A	101112249	GCGGGAGCTGCTAGCCAA	GGGCTTCGGTGGGTTCCT	70
CH242 123C16	NM_032773	LRCH3-212[b]	G>C	101264808	TGCCTGACATTTGCTAACG	CTGCACTTGTGGTGGAGAAC	643
		LRCH3-213[b]	G>C	101264855			
		LRCH3-214[b]	T>C	101264894			
		LRCH3-215[b]	T>A	101265038			
		LRCH3-217[b]	G>A	101270027	TTGAGGAGAGTTGCATGTGTT	TCCTGCTCAGTGGATTAAAGG	405
		LRCH3-218[b]	A>G	101270038			
		LRCH3-219[b]	G>T	101270040			
	NM_001136049	LMLN g.15920[d]	C>G	101361940	GGCACTATCTTACTTAGCAG	TGGTTTGTTGCACATTGT	528
CH242 101B19	NM_033049.2	MUC13-227[b]	C>T	101846954	TGAGCAAGATGAGTGCCCCAGT	TAGCCAGGCAGGCACAAGCA	536
		MUC13-226[b]	G>A	101847134			
		MUC13-225[b]	G>C	101847197			
		MUC13-224[b]	A>G	101847222			
		MUC13-223[b]	T>C	101847300			

Table 1 continued

BAC Clone	GenBank reference	SNP name	Alleles[a] R>S	Position on SSC13 (Sscrofa9)	Forward primer	Reverse primer	Amplicon lenght
		MUC13-813[c]	C>T	101853611	ATGTGGAAGAACAGAAACTTGATTGAG	ATAGTCAGGGCGGGGGTATACTACC	176
		MUC13-814[c]	T>G	101853612			
		MUC13-829[c]	C>A	101853627			
		MUC13-895[c]	C>A	101853693			
		MUC13-905[c]	A>G	101853703			
		MUC13-908[c]	A>G	101853706			
		MUC13-920[c]	G>A	101853718			
		MUC13-933[c]	T>C	101853731			
		MUC13-935[c]	C>A	101853733			

The positions of the SNPs are given according to their position in the reference sequence (second column)

[a] R alleles are associated with the resistant and S alleles with the susceptible haplotypes, respectively

[b] SNPs from Jacobsen et al. (2010); [c] SNPs from Zhang et al. (2008); [d] SNP from this study

Table 2 SNP list from the PorcineSNP60 BeadChip

SNP name	Position on SSC13 (Sscrofa9)	Sequence and allelic variants[a]
MARC0067282	101488856	AGAAAAAAGAAACTCTATTTGAATGACGGT **[C/T]** TCAGAGTATACCTGTTCCCTGTTCCACCTA
ALGA0072075	101582070	CAGCTTTGGAAGCCATGCGTTGGAGAATGG **[C/T]** AGAGCCACTCAGAGTATGGAGACCTGAGTG
ASGA0058925	101659492	TGTGTGTCTGTCTCCCGGATGGGGCTCTTC **[C/T]** TCTACAGGAGAGCTGACTGCAGCATGTTGG
ALGA0122555	101783014	CTTGAAGAAGCTAATTGGGCAAACACTGCT **[C/T]** GAAGCCTTAATGTCTTTACTGTTGAATTAG
ASGA0091537	101818006	GATAGGAAAATAACTGATCAACTTTATTTA **[C/T]** TATCCAAATAAGATATTGATAATTAGCTTT
ALGA0106330	101846502	TAACACAGACTTTAAGTACAATGGGAATTC **[A/G]** GGGGCTCAGCTACCTAAAGGTAACATTGTG
ALGA0106230	101860064	CATGTCACATATATCTCAATAAAGCTGTTG **[G/T]** TTTTTTTTAAGAGGAAGGTGGCTGCTCATG
H3GA0037348	101925778	GGAGGCCACCCAGGTATGGAGAGCATTTCG **[C/A]** TGCAGCCTTTTCTAAGTGGCTCTGGCTTCA
H3GA0037371	102371992	CAAAGCAGGCCTGCTTTCAAGTTAAGTCTG **[G/A]** GACCAATACATGGTAAATAAAAGTAAGAA
DIAS0000584	102430792	CCCAGAAACCCCACCACGGACAGTCCTTCT **[G/C]** CAGTGTCTTAGATCACACCCCCCGCTGGAC
MARC0006918	102797817	CATCCAGAAATTGCTGACCCAGCATGCTGA **[G/A]** GTGATTTGTGCAGCCTCTTCTCCCTGTTTT
MARC0045417	103289127	CCTTACCTCCTTCAAGCTTATGTTGGAATC **[A/G]** AGAATAAATCCTGCCCTGAGAAGCAATAAAA
H3GA0037402	103303135	GGCCTTGGTTCACTACCTTTGAAATAATTC **[C/T]** ATCTTCCTTTCAATACGTAAGAGCAGTAAA
ASGA0059048	107026948	TCTAGGCTCATGTTTGTTTTTGAAGGGAAAA **[G/A]** CAGAGAGCTGGCTCCTTTGTGGAAAGCTTT

[a] The SNP is given in bold and in square brackets. The first nucleotide of the SNP indicates the resistant and the second the susceptible allele

allele is presumed to represent the ancestral allele and is associated with greater haplotype heterogeneity (see 798B and 739B, Fig. 2) (Jacobsen et al. 2010). The large shared haplotype block associated with the susceptibility allele handicaps efforts to locate and identify the causative mutation. In addition, determination of the *F4bcR* genotypes is time-consuming as it has to be done by progeny phenotyping which leads to the loss of potential breeding animals. Thus, when we detected a boar with a recombination between the *MUC4* markers and *F4bcR*, it represented an opportunity to refine the search for the causative mutation. Therefore, we mated this boar to homozygous resistant sows and analyzed more markers distal to *MUC4*.

Boar 698B inherited a recombinant haplotype from its father, boar 381B. The recombinant allele was transmitted from 698B to half of its progeny, as seen in pigs 1977 and 2734 (Fig. 2), and was not found in other pigs from the same herd. The recombination in 698B occurred in an interval of about 220 kb, between SNPs *LMLN* g.15920 and *ALGA0072075*. Boars 381B and 698B were found to be homozygous for the SNP *MARC0067282*, making it impossible to determine the position of the crossing over in 698B more precisely.

These results support the hypothesis that the locus for *F4bcR* is located distal to *MUC4*. Besides the experimental pigs, we phenotyped 78 unrelated pigs of 38 litters of the Swiss performing station and determined their *MUC4* g.8227 genotype (Joller 2009). Five pigs were phenotyped as resistant, whereas the genotype was *MUC4* g.8227 *C/G* and one susceptible pig was *MUC4* g.8227 *C/C*. These pigs were typed with the markers used in this study and the resistant pigs were homozygous *A/A* in *ALGA0106330*, *G/G* in *MUC13-226*, and *C/C* in *MUC13-813*, consistent with a resistant genotype. The susceptible pig was heterozygous *A/G*, *A/G*, and *C/T*, respectively, for the same markers. These results suggest stronger LD between these markers and *F4bcR* than between *MUC4* g.8227 and *F4bcR* and give further evidence that *F4bcR* is located distal to *MUC4*.

BLAST analyses of the markers that were in complete LD with *F4bcR* showed that only *MUC13-813* was located in an exon. However, this C > T transition is silent. *ALGA0072075* was mapped between *SLC12A8* and *HEG1*. *ALGA0106330* was located in an intron of *MUC13*. *DIAS0000584* was mapped to an intron of gene *KALRN* and *MARC0006918* to an intron of *MYLK*. These findings indicate that these markers may not be causative for ETEC F4ab/F4ac adhesion.

Joller et al. (2006) had initially mapped the most probable position of the *F4bcR* locus in the *SW207–S0283* interval. Unfortunately, the family in this study did not allow the identification of a distal border closer to the unknown *F4bcR* than microsatellite *S0283*. Therefore, we propose the interval between *LMLN* g.15920 and *S0283*, consisting of around 620 kb, to be the most probable region for *F4bcR*.

Mucin-type proteins and lipids are present on almost every epithelial tissue and are important for defense against pathogens. However, mucins and other glycosylated structures are also targets for microbial attachment as they contain a variety of oligosaccharide structures providing binding sites for bacteria. In the case of bacteria expressing the F18-type fimbriae, the adhesion is dependent on the activity of fucosyltransferase 1 and the presence of a yet unknown fucosylated structure (Meijerink et al. 2000). Although the causative mutation in F4ac is still unknown, the genetic analyses performed in 698B's family have determined that the causal mutation for ETEC F4ab/F4ac susceptibility is downstream of the gene *LMLN* and probably may be located around the region containing the *MUC13* gene.

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