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Two α(1,2) fucosyltransferase genes on porcine Chromosome 6q11 are closely linked to the blood group inhibitor (*S*) and *Escherichia coli* F18 receptor (*ECF18R*) loci

E. Meijerink,[1] R. Fries,[1,*] P. Vögeli,[1] J. Masabanda,[1] G. Wigger,[1] C. Stricker,[1] S. Neuenschwander,[1] H.U. Bertschinger,[2] G. Stranzinger[1]

[1]Institute of Animal Science, Swiss Federal Institute of Technology, ETH-Zentrum, CH-8092 Zurich, Switzerland
[2]Institute of Veterinary Bacteriology, University of Zurich, CH 8057 Zurich, Switzerland

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Abstract. The *Escherichia coli* F18 receptor locus (*ECF18R*) has been genetically mapped to the halothane linkage group on porcine Chromosome (Chr) 6. In an attempt to obtain candidate genes for this locus, we isolated 5 cosmids containing the α(1,2)fucosyltransferase genes *FUT1*, *FUT2*, and the pseudogene *FUT2P* from a porcine genomic library. Mapping by fluorescence in situ hybridization placed all these clones in band q11 of porcine Chr 6 (SSC6q11). Sequence analysis of the cosmids resulted in the characterization of an open reading frame (ORF), 1098 bp in length, that is 82.3% identical to the human FUT1 sequence; a second ORF, 1023 bp in length, 85% identical to the human FUT2 sequence; and a third FUT-like sequence thought to be a pseudogene. The *FUT1* and *FUT2* loci therefore seem to be the porcine equivalents of the human blood group H and Secretor loci. Direct sequencing of the two ORFs in swine being either susceptible or resistant to adhesion and colonization by F18 fimbriated *Escherichia coli* (ECF18) revealed two polymorphisms at bp 307 (M307) and bp 857 (M857) of the *FUT1* ORF. Analysis of these mutations in 34 Swiss Landrace families with 221 progeny showed close linkage with the locus controlling resistance and susceptibility to *E. coli* F18 adhesion and colonization in the small intestine (*ECF18R*), and with the locus of the blood group inhibitor *S*. A high linkage disequilibrium of *M307–ECF18R* in Large White pigs makes the M307 mutation a good marker for marker-assisted selection of *E. coli* F18 adhesion-resistant animals in this breed. Whether the *FUT1* or possibly the *FUT2* gene products are involved in the synthesis of carbohydrate structures responsible for bacterial adhesion remains to be determined.

Introduction

Edema disease and post-weaning diarrhea in swine is associated with the colonization of the small intestine with toxigenic *Escherichia coli* strains of a limited number of serotypes. Colonization depends on adherence of the bacteria, which is mediated by bacterial fimbriae F18 or F4 (Sellwood et al. 1974; Bertschinger et al. 1990; Rippinger et al. 1995). Several candidate structures have been proposed as receptors for fimbriae F4 (Grange and Mouricout 1996), whereas receptors for F18 have not yet been characterized. Susceptibility to adhesion, meaning expression of receptors mediating the binding to the bacterial fimbriae F18 formerly designated fimbriae F107, has been shown to be genetically controlled by the host and is inherited as a dominant trait (Bertschinger et al. 1993) with *B* being the susceptibility allele and *b* the resistance allele. The genetic locus for this *E. coli* F18 receptor (*ECF18R*) has been mapped to porcine Chr 6 (SSC6), based on its close linkage to the *S* locus and other loci of the halothane (*HAL*) linkage group (Vögeli et al. 1996). The epistatic *S* locus suppresses the phenotypic expression of the A-0 blood group system when being S^sS^s (Vögeli et al. 1983) and allows expression when being S^SS^-. Likewise, the expression of the AB0 and Lewis (Le) blood group systems in humans is influenced by the blood group *H* and by the Secretor (*Se*) locus. Inactivating mutations in these genes prevent the expression of the precursor H determinant, and thus of A and B (Kelly et al. 1994) and Le^b determinants. The expression of Lewis antigen in the small intestine of humans was shown to be under control of the Lewis gene (α1,3/4 fucosyltransferase) and the Secretor gene (Björk et al. 1987; Henry et al. 1994) with the type of Lewis antigen (Le^a or Le^b) determined by the Secretor locus (*se* or *Se*, respectively).

The blood group H and Se loci have been mapped genetically (Oriol et al. 1984; Reguigne-Arnould et al. 1995) and physically (Rouquier et al. 1995) to human Chr 19q13.3. This region is evolutionarily conserved, containing homologous genes from the *HAL* linkage group in pigs. Conservation was shown by ZooFISH analysis (Rettenberger et al. 1995) and earlier by the genetic and physical mapping of genes such as glucosephosphate isomerase (GPI; Davies et al. 1988), phosphogluconate dehydrogenase (PGD) and apolipoprotein E (APOE) in both chromosomal segments (Gedde-Dahl et al. 1984; Vögeli et al. 1988; Clamp et al. 1993; Rouquier et al. 1994). Larsen and associates (1990) characterized the blood group H encoding gene (FUT1). The structure and expression of the Se gene (FUT2) was reported by Kelly et al. (1995) and Rouquier et al. (1995). FUT1 determines H antigen expression in the erythroid cell lineage, whereas FUT2 regulates expression of the H antigen in secretory epithelia and saliva. Conservation of the *FUT1* gene has been shown in lower mammals such as rat, rabbit, and pig, and mRNA expression has been found in rabbit brain tissue (Hitoshi et al. 1995) and rat colon (Piau et al. 1994). In all these species, two types of α(1,2)fucosyltransferase genes have been reported that are structurally very similar to the human FUT1 and FUT2 genes, but in particular the FUT1 homologous genes show a species-specific expression pattern. In humans the FUT1 gene is responsible for synthesis of H antigens in the precursors of erythrocytes (Watkins 1980). However, in pigs erythrocytes passively adsorb H-like antigens from the serum (Oriol 1987), as is the case for the human Lewis antigens. In pigs, all H-like antigens detected by Oriol and colleagues (1993) were related to exocrine secretory tissues, and expression of the FUT2

Correspondence to: P. Vögeli

* *Present address:* Chair of Animal Breeding, Technical University Munich, D-85350 Freising-Weihenstephan, Germany

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(Secretor) gene is seen in secretory tissues of other animal species (Piau et al. 1994; Rouquier et al. 1995). Therefore, expression of the porcine A-0 blood group determinants, which cross-react with anti-human blood group H and A antibodies (King and Kelly, 1991), might be influenced by the FUT2 gene.

Carbohydrate structures of blood group antigens have been shown to mediate the adhesion of some pathogenic microorganisms to host tissues; for example, *Helicobacter pylori* adheres to Lewis^b blood group antigens (Borén et al. 1993), and *E. coli* strains causing urinary tract infections adhere to P blood group substance (Källenius et al. 1980; Svenson et al. 1983). Genes encoding glycosyltransferases that are responsible for the formation of the blood group-specific carbohydrate structures therefore represent candidate genes for the control of bacterial colonization by the host. It was on this basis that we attempted to clone porcine fucosyltransferase genes. These genes can be considered as positional candidates, since their localization was expected to be in the same chromosomal region as the locus responsible for adhesion/non-adhesion of F18-positive *E. coli* in the small intestine. The detection of single nucleotide polymorphisms in one of the cloned fucosyltransferase (FUT1) genes allowed us to study linkage between this gene, the S-system, the *ECF18R* locus, and other loci of the *HAL*-linkage group.

The chromosomal localization of the fucosyltransferase genes in the *ECF18R* region was confirmed by fluorescence in situ hybridization and by linkage analysis.

Materials and methods

Primers (Table 1) derived from the human FUT1 gene were used for the amplification of its porcine counterpart from genomic DNA. From the resulting porcine sequences, specific primers were designed that were used in further amplification and sequencing reactions.

A porcine genomic library, constructed in SuperCos 1 (Stratagene, La Jolla, Calif., USA), was screened with an α^{32}P dATP-labeled (Prime It II, Stratagene) FUT1 probe obtained from porcine genomic DNA with primers P7 and P10. After hybridization of replica filters at 42°C for 15 h (50% formamide, 6 × SSC, 5 × Denhardt's, 0.5% SDS, 0.1 mg/ml salmon sperm) and washing twice at 65°C for 30 min (1 × SSC, 0.1% SDS), positive colonies could be identified after exposure (15 h, −80°C) to X-ray film.

Cosmid clones ETHs1, ETHs2, ETHs3, ETHs4, and ETHs6 were subjected to fluorescence in situ hybridization (FISH) on porcine metaphases. Metaphase chromosomes were Q-banded and photographed before hybridization. The probes were labeled by random priming with biotin-16-dUTP. Signal detection and amplification were performed with avidin-FITC and biotinylated anti-avidin. The chromosomes were counterstained with 4,6-diamidino-2-phenylindole, and the relative positions of the cosmids were determined as described by Solinas Toldo and coworkers (1993).

*Ksp*I-, *Eco*RI- and *Ksp*I/*Eco*RI digests of all cosmids were separated on a 0.8% agarose gel and transferred to a Hybond N nylon membrane. This blot was hybridized with α^{32}P dATP-labeled porcine *FUT1* PCR products (primers P6–P11 and P7–P10). Based on the autoradiographic signals, ETHs1, -s2, and -s3 were subjected to further subcloning into pBluescript SK- (Stratagene), and FUT sequences were determined from subclones. The sequences of two FUT-like ORFs (*FUT1* and *FUT2*) (Fig. 3) obtained from cosmids ETHs2 and -s3 were compared in ECF18R-positive (BB/Bb) and -negative (bb) animals by direct sequencing of PCR products.

Using the Perkin Elmer Ready Reaction Dye Terminator kit (Perkin Elmer Cetus, Norwalk, CT, USA) and 10 pmol of primer, cycle sequencing was performed with a thermal program consisting of an initial denaturation of 5 min at 95°C, followed by 25 cycles of 30 s at 95°C, 15 s at 50°C, and 4 min at 60°C. Primers used for amplification and sequencing of the porcine α(1,2)fucosyltransferase genes are listed in Table 1. Additional primers were designed on the sequences shown in Fig. 3, taking the possibility of cross-annealing of primers owing to the high similarity of *FUT1, FUT2*, and the *FUT2* pseudogene into account. Samples were analyzed on a 373A ABI sequencer (Applied Biosystems Inc.), and sequence analysis was performed with the GCG package (Devereux et al. 1984).

Single nucleotide polymorphisms were analyzed in 221 Swiss Landrace pigs produced from 4 boars and 16 sows, and in 29 Large White pigs produced from 9 unrelated matings. In order to produce a large number of informative offspring for the examination of linkage between porcine genes encoding ECF18 receptors and selected polymorphic loci, only informative Swiss Landrace matings of the type *B*/*b* × *b*/*b* were produced for this study. In a study of Bertschinger et al. (1993), the above-mentioned Swiss Landrace pigs were also tested for *ECF18* susceptibility in a colonization test. For this, pigs were inoculated shortly after weaning with bacteria of *E. coli* strain 124/76 of serotype O139:K12(B):H1:F(18). Fecal shedding of the bacteria was monitored daily. The extent of colonization was calculated as the mean of the two highest fecal scores. Pigs with a mean fecal score of 3.5, corresponding to 6.7 log colony forming units (CFU)/g or more, were considered susceptible to colonization. This limit was based on a lack of mortality below this value, and on scores obtained from completely resistant litters.

Table 1. Sequences of forward- (F) and reverse- (R) primers and their relative position to the porcine *FUT1* and *FUT2* start codons. Primers FUT1 P10 and FUT1 P11 are derived from the human *FUT1* gene.

Primer Name	Primer Sequence	Position
FUT1 P6 (R)	5′-CTTCAGCCAGGGCTCCTTTAAG-3′	+489
FUT1 P7 (F)	5′-TTACCTCCAGCAGGCTATGGAC-3′	+720
FUT1 P10 (R)	5′-TCCAGAGTGGAGACAAGTCTGC-3′	+1082
FUT1 P11 (F)	5′-CTGCCTGAACGTCTATCAAGATC-3′	+69
FUT1 P16 (F)	5′-AGAGTTTCCTCATGCCCACAGG-3′	−90
FUT1 P18 (R)	5′-CTGCTACAGGACCACCAGCATC-3′	+1203
FUT1 PBEST (R)	5′-ACCAGCAGCGCAAAGTCCCTGAC GGGCACGGCCTC-3′	+893
FUT2 P16 (R)	5′-CTCCCTGTGCCTTGGAAGTGAT-3′	+1094
FUT2 P17 (F)	5′-AACTGCACTGCCAGCTTCATGC-3′	−83

The results of the single nucleotide polymorphisms were compared with typing data for ECF18R, which were identified in an in vitro adhesion assay described by Vögeli and associates (1996), and with typing data for the *GPI-*, *PGD-*, α-1-B-glycoprotein- (*A1BG*), ryanodine receptor (*RYR1*), *EAH-* and *S-* loci as published by Vögeli and colleagues (1996). Pairwise linkage analysis and calculation of recombination fractions was performed with the CRI-MAP version 2.4 program (Green et al. 1990). Multipoint linkage analysis was performed by sequential insertion of above loci into the map. Haplotype frequencies were calculated from the parental animals in the Swiss Landrace families and from the 8 parental Large White animals, which were haplotyped for *ECF18R* from progeny information. Tetrachoric correlations of ECF18R and mutations in *FUT1* (FUT1/M307) were calculated on all Swiss Landrace and Large White progeny.

Results

Cosmids ETHs1, -s2, -s3, -s4 and -s6 were identified after screening of the cosmid library with a FUT1 probe obtained from porcine genomic DNA with primers P7 and P10 and were mapped by FISH to Chr 6 in band q11 (Fig. 1).

When hybridizing with radiolabeled porcine *FUT1* P6–P11 and P7–P10 PCR fragments, Southern blot analysis of *Ksp*I, *Eco*RI, and *Ksp*I/*Eco*RI cosmid digests revealed identical autoradiography signals for ETHs2, -s4 and -s6, whereas different signals were obtained from cosmids ETHs1 and -s3 (Fig. 2A,B). From ETHs2, we isolated *Ksp*I subclones 940 bp and 6.2 kb in length, corresponding to the estimated length of hybridizing *Ksp*I fragments on the Southern blot. The sequence results of both subclones were combined to yield a 1501-bp sequence, which was in agreement with results of direct sequencing of genomic PCR products. The 1501-bp sequence contains an ORF of 1098 bp corresponding to the human *FUT1* ORF, with 82.3% nucleotide and 80.8% amino acid identity.

ETHs1 shows one fragment (2.7 kb) (Fig. 2B) hybridizing to *FUT1* sequences, whereas ETHs3 shows two (2.7 kb and 8.2 kb). Subcloning and partial sequencing of the 2.7-kb *Eco*R1 fragments of ETHs1 and -s3 confirmed that these two fragments are identical. The sequence is highly similar to the human FUT2, but shows several changes in the NH_2- and -COOH terminal regions. These lead to frame shifts that are not compatible with a conserved ORF; therefore, we assume the sequence obtained from the 2.7-kb fragment to represent a pseudogene (*FUT2P*). After subcloning of

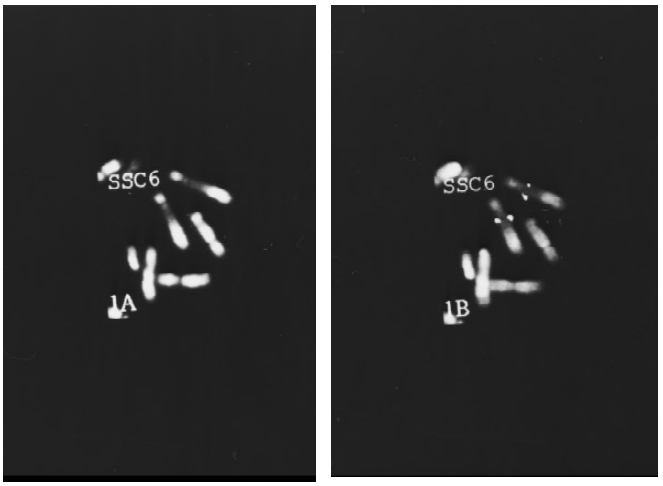


Fig. 1. (A) Q-banding pattern of porcine metaphase chromosomes and **(B)** fluorescence in situ hybridization (FISH) with biotin-labeled cosmid DNA, assigned the highly specific signals to the chromosomal region 6q11.

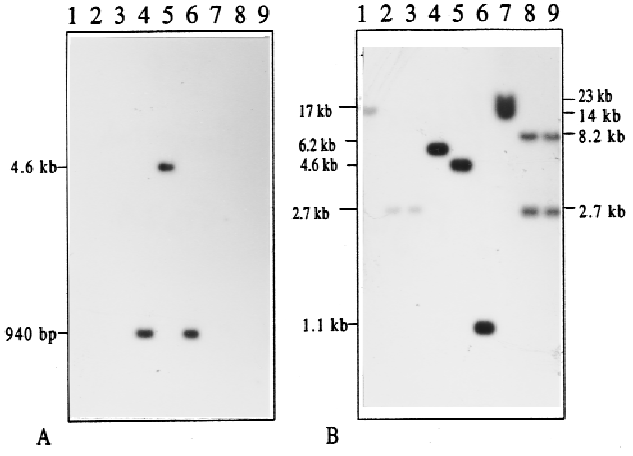


Fig. 2. Southern blot analysis of cosmids ETHs1 (1–3), ETHs2 (4–6), and ETHs3 (7–9) after digestion with enzymes *Ksp*I (1, 4, 7), *Eco*RI (2, 5, 8), and *Ksp*I/*Eco*RI (3, 6, 9) and separation on a 0.8% agarose gel. In Fig 2A is seen that hybridization with an α^{32}P dATP labeled 5′ *FUT1* fragment (primers P6–P11) results in the same hybridizing 940-bp band in both the *Ksp*I digest (lane 4) and the *Ksp*I/*Eco*RI digest (lane 6). In Fig. 2B, however, hybridization with a 3′ *FUT1* fragment (primers P7–P10) shows a 6.2-kb *Ksp*I band in lane 4 and a 1.1-kb *Ksp*I/*Eco*RI band in lane 6. Both the 5′ and 3′ *FUT1* fragments hybridize to the same 4.6 kb *Eco*RI fragment in lanes 5 (Fig. 2A, 2B). This indicates a *Ksp*I site in the *FUT1* gene contained in cosmid ETHs2. In Fig. 2B, cross-hybridization of the 3′ *FUT1* fragment detects 2.7-kb (lanes 2, 3, 8, and 9) and 8.2-kb (lanes 8 and 9) bands, containing the *FUT2* pseudogene and the *FUT2* gene sequences, respectively.

ETHs3 *Bam*HI digests, the hybridizing sequences contained in the 8.2 kb *Eco*RI fragment could be identified. The sequence of the obtained subclones represents a 1023-bp ORF and is 85% identical at the nucleotide, and 83% identical at the amino acid level to the human FUT2 sequence. Many differences in the NH_2- and -COOH terminal regions were observed between the porcine *FUT2* sequence and the *FUT2P* sequence derived from the 2.7-kb fragment. The predicted amino acid sequence corresponds to the partially determined amino acid sequence of the porcine *Secretor* enzyme (Thurin and Blaszczyk-Thurin 1995) (Fig. 3). The obtained porcine *FUT1, FUT2,* and *FUTP* sequences are submitted to GenBank and have accession numbers U70883, U70881, and U70882, respectively.

Direct sequencing of porcine *FUT1* and *FUT2* sequences and their flanking regions in animals of different ECF18R genotype (*Bb, bb*) resulted in the identification of two G → A transitions at positions 307 and 857 (termed *M307* and *M857,* respectively) of the *FUT1* ORF. The *M307* transition eliminates a restriction site for *Cfo*I. Amplification with primers P6 and P11 (3 min at 95°C, 30 cycles of 30 s at 95°C, 30 s at 56°C, and 30 s at 72°C, followed by a 7-min final extension at 72°C) followed by *Cfo*I digestion and separation on a 3% agarose gel results in a restriction fragment length polymorphism (RFLP; Fig. 4). Homozygous $M307^{A/A}$ animals show 93- and 328-bp fragments where homozygous $M307^{G/G}$ animals show 93-, 241- and 87-bp fragments. Heterozygous animals show all four fragments.

The *M857* transition eliminates an *Aci*I site. Primer PBEST was designed to mismatch two additional *Aci*I sites at positions 866 and 872. PCR with primers P7 and PBEST (3 min at 95°C, 30 cycles of 30 s at 95°C, 30 s at 56°C, and 30 s at 72°C, followed by a 7-min final extension at 72°C) followed by *Aci*I digestion enables PCR-RFLP analysis on a 3% agarose gel. Homozygous $M857^{A/A}$ animals show a 174-bp fragment, while amplification products of $M857^{G/G}$ animals show 136- and 38-bp fragments (Fig. 4).

In Swiss Landrace families, recombination events between *M307* and the loci of the HAL linkage group (*S, ECF18R, RYR1, GPI, PGD*) revealed recombination fractions $\theta < 0.04$ (Table 2). The lodscores Z for the overall recombination fractions were between 24.5 and 50.6, showing strong evidence for linkage between these loci. These data allow genetic mapping of the *FUT1* gene to the HAL linkage group in close proximity of *S* and *ECF18R*. Owing to low recombination rates, however, no locus order could be determined as being significantly better than others.

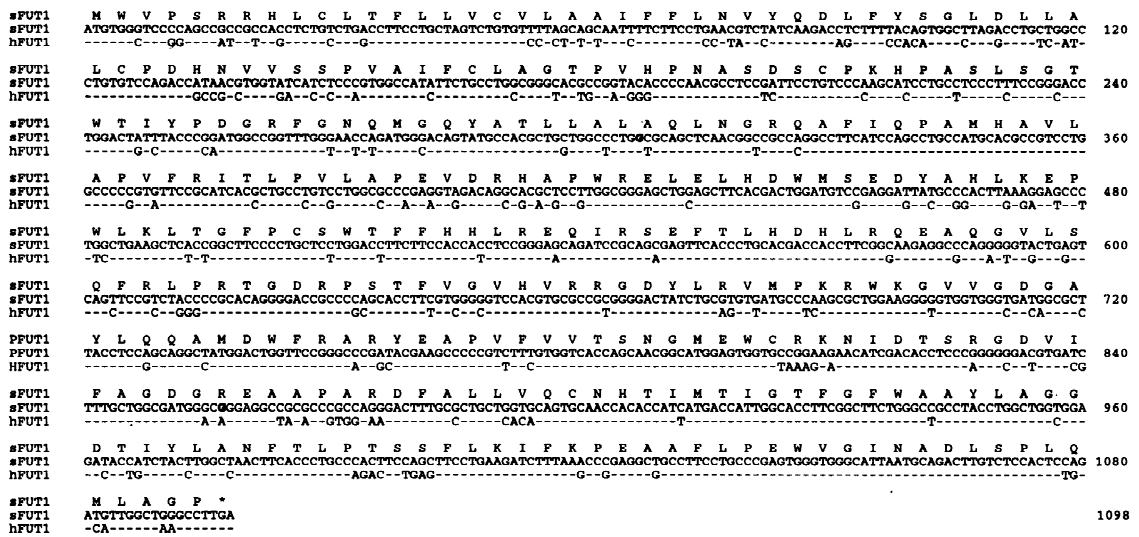
The haplotype frequencies are presented in Table 3. In Swiss Landrace (SL) and Large White (LW) parental pigs, $ECF18R^b$ (the edema and post-weaning diarrhea resistance allele) is 100% associated with $M307^A$, and $ECF18R^B$ (the edema and post-weaning diarrhea susceptibility allele) 100% with $M307^G$. It is worth while mentioning that in SL pigs the $RYR1^T$ (the malignant hyperthermia susceptibility allele) accounted for 93% (28/30) of all $ECF18R^b$ haplotypes. In SL pigs 88% (30/34) of S^s accounted for all $ECF18R^b$ and $M307^A$ haplotypes, respectively. The corresponding values for both the S^s-$ECF18R^b$ and S^s-$M307^A$ haplotypes were 82% (9/11) in Large White pigs. In our experimental SL families the occurrence of the $M857^A$ allele at *FUT1* was low and even absent in LW pigs. Therefore, a significant gametic association could not be observed between the alleles of *M857* and the alleles of the flanking genes.

Table 4 shows that the distribution of *FUT1* genotypes at nucleotide 307 among *ECF18R* types was significantly different from the expected ratio. Of the 119 edema and post-weaning diarrhea-resistant $ECF18R^{b/b}$ animals, 118 were determined as $M307^{A/A}$ in the DNA-based test. One resistant animal had the genotype $M307^{A/G}$. Of the 131 susceptible pigs, 130 were $M307^{A/G}$ or $M307^{G/G}$. One animal, susceptible to *E. coli* adhesion, was shown to be homozygous $M307^{A/A}$ by the DNA test.

Discussion

The genetic and physical mapping of the identified *FUT1* ORF is in agreement with our expectations showing that the porcine *FUT1* homolog is located on SSC6q11. Similarities in length, nucleotide- and amino acid sequence with the human FUT1 suggest that the established ORF in our study represents the porcine *FUT1*. Furthermore, except for nucleotides 229 and 714, our sequence is identical to the recently published sequence of Cohney and coworkers (1996), who showed that the *FUT1* gene product exhibits α(1,2)fucosyltransferase activity. We found two *FUT2*-like sequences, one representing the porcine *FUT2* and the other one being a pseudogene (*sFUT2P*). As in humans, the α(1,2)fucosyl-

3A



3B

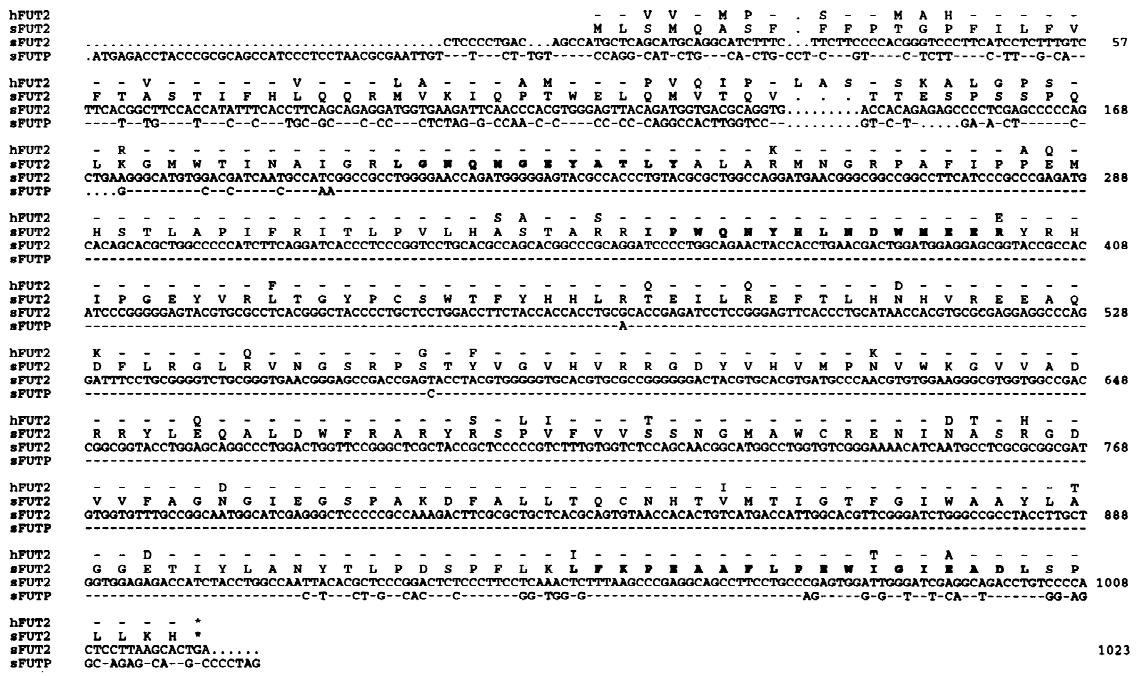


Fig. 3. (A) Alignment of the porcine (sFUT1) and human (hFUT1) FUT1 coding regions. Amino acid translation is given for the porcine FUT1 (upper row). At nucleotides 307 (M307) and 857 (M857) of the porcine FUT1 G→A transitions were observed (bold printed). **(B)** Alignment of the porcine *FUT2* gene (sFUT2) and *FUT2* pseudogene (sFUTP) by the Pileup function of the GCG sequence analysis program. Basepair count is given for the porcine *FUT2*, starting from the ATG startcodon. In the upper two rows, amino acid translations of human- (hFUT2) and porcine- (sFUT2) *FUT2* coding regions are given. Similarities in nucleotide- and amino acid sequences are indicated by dashes. Bold printed amino acid sequences are identical to the partial amino acid sequence of porcine Secretor type α(1,2)fucosyltransferase (Thurin and Blaszczyk-Thurin 1995).

transferase genes in pigs seem to form a cluster of functional genes and pseudogenes. However, in human the pseudogene seems to be derived from the FUT1 gene, whereas in pigs it is most similar to the *FUT2* gene. Linkage was demonstrated between inheritance of the alleles of the *ECF18R* gene and polymorphisms in the *FUT1* (*M307*/*M857*), *S*, *RYR1* (the halothane gene), *GPI*, and *PGD* genes. The mutation of *M307* at *FUT1* is linked to *ECF18R* with a recombination fraction of 0.01 in 221 informative SL meiosis. In both recombinants where lack of association between *M307* and *ECF18R* was found (Table 4), adhesion status, although determined by the microscopic adhesion test, may have been falsely diagnosed. Indeed, the fecal score in the colonization test was 5.5 for the $M307^{A/G}$ animal classified as resistant ($ECF18R^{b/b}$) and 2.3 for the $M307^{A/A}$ animal classified as susceptible ($ECF18R^{B/b}$). Animals with a fecal score of 3.5 or greater are considered to be susceptible (see Materials and methods). Therefore, if the resis-

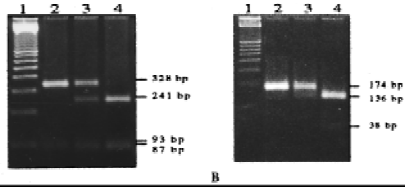


Fig. 4. Detection of **(A)** the M307 G to A and **(B)** the M857 G to A mutation in the porcine *FUT1* gene. Digestion of amplified *FUT1* fragments with *Cfo*I **(A)** and *Aci*I **(B)** results in a restriction fragment length polymorphism. First lane: 100-bp marker. Fragment lengths are indicated in basepairs. **(A)** The $M307^{A/A}$ genotype (lane 2) generates 328 and 93 bp restriction fragments, while the $M307^{G/G}$ genotype (lane 4) generates 93, 241, and 87 bp fragments and heterozygous $M307^{A/G}$ genotypes (lane 3) show all four fragments. **(B)** Digestion of the $M857^{A/A}$ genotype (lane 2) generates 174-bp fragments while it generates 136- and 38-bp fragments in the $M857^{G/G}$ genotype (lane 4), and in $M857^{A/G}$ genotypes (lane 3) all three fragments are generated.

Table 2. Overall recombination fractions (θ), lodscores (Z), and number, of informative animals (N) for *M307* and loci of the *HAL* linkage group in the Swiss Landrace experimental population.

Locus Pair	N	θ	Z
S–ECF18R	183	0.01	50.6
M307–S	183	0.01	50.6
M307–ECF18R	216	0.01	57.1
M307–RYR1	198	0.02	47.2
M307–GPI	147	0.03	34.2
M307–PGD	147	0.04	24.5

Table 3. Haplotype frequencies (%) at the four loci (*S-FUT1* (*M307,M857*)-*ECF18R-RYR1*) in the Swiss Landrace (SL) experimental population and randomly selected Large White (LW) pigs.

Breed	Haplotype at S, *FUT1*(*M307, M857*), *ECF18R, RYR1*[a]	Frequency[b] (Number)
SL	*sAGbT*	70 (28)
	sAGbC	5 (2)
	SGGBC	15 (6)
	sGABC	10 (4)
LW	*sAGbC*	56 (9)
	SGGBC	31 (5)
	sGGBC	13 (2)

[a] S: Suppressor locus for A and 0 blood types (*S* and *s*). *FUT1*(*M307*); alteration of adenine (A) to guanine (G) at nucleotide 307 of the α(1,2)fucosyltransferase (*FUT1*) gene. *FUT1*(*M857*); alteration of adenine (A) to guanine (G) at nucleotide 857 of the *FUT1* gene. *ECF18R:E. coli* F18 receptor. The dominant susceptible allele is indicated by *B* and the resistant allele by *b*. *RYR1:* skeletal muscle ryanodine receptor. *C* (cytosine) is the dominant resistant and *T* (thymine) the susceptible allele for malignant hyperthermia.
[b] Haplotype frequencies in % and absolute number of haplotypes between brackets.

tance/susceptibility status was determined on the basis of the colonization test, there would be no recombinants. Conflicting results may be due to heterogeneity in the genetic control of the F18 receptor phenotypes.

The discovery of the close linkage of *M307* at *FUT1* and *ECF18R* has made it possible to develop a diagnostic test for *E. coli* F18 adhesion resistant, heterozygous (carrier) and homozygous susceptible animals in SL and LW. In Duroc, Hampshire, and Pietrain pigs, we observed the same polymorphism at *M307* as in the SL and LW (data not shown). Extended studies yield preliminary evidence that no more than 5–10% of Swiss Landrace, Large White, and Duroc pigs are resistant to infection with highly toxigenic. *E. coli* F18. The availability of a diagnostic test for the *M307* mutation provides breeders with the opportunity to eliminate the $ECF18R^B$ allele from their herds, thereby eliminating a prerequisite for *E. coli* F18 bacterial adhesion causing edema and post-weaning diarrhea. In such a selection program, identification of the 1843C to 1843T mutation in the *RYR1* gene has to be applied for positive diagnosis of malignant hyperthermia susceptibility in order to prevent a rise of the *RYR1* susceptibility allele in the population.

Table 4. Distribution of the genotypes, tetrachoric correlation (r) and significance of the association (χ^2 and $w \times \chi^2$) of the associated polymorphic *FUT1*(*M307*) and *ECF18R* loci in Swiss Landrace (SL) experimental population and randomly selected Large White (LW) pigs.

Breed	Locus	FUT1/M307 genotype	A/G	A/A	r	χ^2	$\chi^2 \times w$[a]
SL	[b]*ECF18R*	*b/b*	1	113	0.98	213.1	42.6***
		B/b	106	1			
		genotype	A/G,G/G	A/A			
LW	[b]*ECF18R*	*b/b*	0	5	1.00	29.0	11.6***
		B/b,B/B	24	0			

[a] A weight factor of w = 0.2 (SL) and 0.4 (LW) was applied to correct the lack of precision resulting from inclusion of related animals in the data according to Cotterman (1947).
*** $p < 0.001$.
[b] Animals of genotype *b/b* at the *ECF18R* locus are resistant and those of genotype *B/b* and *B/B* are susceptible to adhesion of F18ab *E. coli* bacteria.

The functional relation of the *FUT1* or the *FUT2* gene to the *ECF18* receptor needs to be elucidated by studying the expression of these genes and the implications for bacterial adhesion. If *FUT1* is expressed in pigs, the observed mutations might reflect on enzyme activity and change the phenotypic expression, as seen in the Bombay and para-Bombay phenotypes in humans (Kelly et al. 1994). The G → A changes at bp +307 and bp +857 that we observed in our material result in a predicted amino acid threonine (neutral-polar) instead of alanine (neutral-nonpolar), and glutamine (neutral-polar) instead of an arginine (basic), respectively, and may indeed have functional consequences.

Although we did not find mutations in the *FUT2* sequence, *FUT2* cannot be ruled out to be involved in the expression of the adhesion and resistance phenotypes, respectively. We are presently screening the FUT2 promoter region for potentially causal mutations.

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