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Association between the Polymorphism in FUT1 Gene and the Resistance to PWD and ED in Three Pig Breeds

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ABSTRACT : Post-weaning diarrhoea (PWD) and oedema disease (ED) caused by *E. coli* F18 always result in economic losses to pig producers, and no effective methods of controlling PWD and ED are presently available. *FUT1* has been identified as a candidate gene controlling the expression of *E. coli* F18 receptor. This study examined the correlation between F18ab and F18ac adhesion phenotypes and the polymorphism at position M307 of the *FUT1* gene in three pig breeds (231 Large White, 107 Landrace and 109 Songliao Black). The results showed: i) Both the susceptible genotypes (GG and GA) and the adhesion phenotypes (adhesive or weekly adhesive) were dominant in all three breeds with frequencies over 95%. ii) Three adhesion patterns of the two F18 variants F18ab and F18ac, i.e., (ab^+, ac^+), (ab^+, ac^-) and (ab^-, ac^-), were found in all three breeds, and there was no significant difference in the distribution of adhesion phenotypes of the two variants (separately or jointly) among the three breeds ($p>0.05$). iii) The *FUT1* M307 genotypes were completely associated with the F18ab adhesion phenotypes and very strongly associated with the F18ac adhesion phenotypes. All individuals of genotype AA were non-adhesive to both F18ab and F18ac. All individuals of genotype GG or GA were adhesive to F18ab, whereas 11% of them were non-adhesive to F18ac. These results suggest that the polymorphism at *FUT1* M307 can be used for marker-assisted selection of PWD and ED resistant pigs. (**Key Words :** Porcine, Post-weaning diarrhea, Oedema Disease, *E. coli* F18, *FUT1*)

INTRODUCTION

Porcine post-weaning diarrhea (PWD) and oedema disease (ED) occurred at age of about 4-12 weeks are common diseases in swine and are responsible for considerable economic losses to pig producers all over the world (Imberechts et al., 1992b). Both PWD and ED are caused by toxins produced by *E. coli* F18, which colonize the surface of the small intestine (Nagy et al., 1992). Bertschinger et al. (1990) reported the detection of adhesive fimbriae on an oedema disease strain of *E. coli* serotype O139:K12(B):H1 and these fimbriae were preliminarily designated as F107. Rippinger et al. (1995) examined the isolates from cases of PWD and ED by serological examination and Western blot techniques, and revealed a high prevalence of F107 fimbriae in those isolates. They designated the F107 fimbriae as F18. There are two variants of the *E. coli* strain F18 (F18ab and F18ac), which possess the variant-specific antigenic determinant "b" and "c", respectively, in addition to a common antigenic determinant "a". Rippinger et al. (1995) also suggested that the receptors of F18ab and F18ac on the surface of the small intestine were probably the same. Earlier studies revealed an association between expression of F18ab fimbriae (VTEC) and ED, and expression of F18ac fimbriae (ETEC) and PWD (Witting et al., 1994; Nagy et al., 1997). Susceptibility of porcine small intestine to *E. coli* is fimbriae-mediated. The pathogenesis appears to be that: *E. coli* adheres and colonizes to the brush border of enterocytes through its fimbriae, then secretes and releases enterotoxin and histamine by osmosis; this causes trauma of the vessel wall and stimulates effusion of much fluid and electrolyte from the small intestine into the gut lumen. Finally the symptoms of PWD and ED appear. An essential step herein is the fimbriae-receptor interaction (Sellwood et al., 1975; Imberechts et al., 1992b; Fairbrother et al., 2005). The adhesion of the fimbriaed *E. coli* to receptors in the brush border membranes of porcine small intestinal mucosa

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(ECF18 receptor) is the precondition leading to PWD and ED, which in turn is due to the existence of ECF18 receptor. Thus, porcine intestinal cell lines could be used as an *in vitro* model for studying ETEC pathogenesis (Koh et al., 2008). Many studies have been conducted on the molecular mechanisms underlying the pathogenesis of the disease. Previous observations have revealed that the presence or absence of the ECF18 receptor is controlled by a single locus (*ECF18R*), with its dominant allele B responsible for the presence and the recessive allele b for the absence (Bertschinger et al., 1993). This locus has been shown to be linked to the blood group inhibitor *S* and the halothane linkage group (*HAL*) on porcine chromosome 6 (Vogeli et al., 1996). The α-1 fucosyltransferase gene (*FUT1*), which was localized on SSC6q11 by the FISH method, has been recognized as the best candidate gene for *ECF18R* (Meijerink et al., 1997). A guanine (G)/adenine (A) polymorphism at nucleotide 307 in the *FUT1* gene (*FUT1* M307) was found to be highly associated with the *ECF18R* genotypes, and could be a good marker for marker-assisted selection of *E. coli* F18-resistant animals (Meijerink et al., 2000). Genotype $M307^{AA}$ is regarded as a resistant genotype and genotypes $M307^{GG}$ and $M307^{GA}$ as susceptible. A PCR-RFLP method has been developed for detecting this mutation (Meijerink et al., 1997; Vogeli et al., 1997).

In this study, PCR-RFLP tests and *in vitro* adhesion assays were undertaken in three pig breeds to confirm the association between the *FUT1* M307 polymorphism and the adhesion phenotypes of F18ab and F18ac.

MATERIALS AND METHODS

Animals

The experimental population comprised 363 piglets and their parents of three breeds with different genetic background, including Large White (LW), Landrace (LR), and Songliao Black (SB). All piglets were raised in the same rearing systems at the Pig Breeding Farm of the Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China. The numbers of animals of each breed are shown in Table 1. All animals (parents and offspring) were ear trimmed and the ear bits were frozen at -20°C for DNA extraction. The piglets were slaughtered at 35-day-old and their jejunum samples were collected for preparation of small intestinal epithelial cells.

Bacterial strains and antiserum

The two variants of the *E. coli* F18 strain, 107/86 (F18ab positive *E. coli* strain, O139:K12:H1) and 2134P (F18ac positive *E. coli* strain O157:H19), and the antiserum containing the common antigenic determinant "a" of F18ab and F18ac were provided by Yangzhou University, Yangzhou, China.

Culture of the bacteria

E. coli strain 107/86 was removed from cryo-storage and then cultured in static Luria-Bertain (LB) medium for 24 h at 37°C for three generations. Likewise, *E. coli* strain 2134P was cultured in static Tryptone Soya Agar (TSA) medium for 24 h at 37°C, and then in static Tryptone Soya Broth (TSB) medium for 24 h at 37°C for two generations. Plate cultures were stored at 4°C for the following experiments. For *in vitro* adhesion assays, they were sub-cultured in shaking LB and TSA medium respectively for 12 h at 37°C to make *E. coli* fimbriated. Then the solution was centrifuged at 4,000 rpm for 15 min to pellet the bacterial cells. The pelleted cells were suspended in phosphate-buffered saline (PBS, pH 7.4) with an optical density of approximately 1.0 at 520 nm (OD_{520} = 1.0). The *E. coli* suspension could be stored at 4°C for about one week, without decrease of activity.

Verification of the F18 variants

Three tests were used to verify whether the two variants we obtained (107/86 and 2134P) were indeed F18ab and F18ac. First, the slide agglutination test was carried out to confirm whether the two variants were the *E. coli* F18 strain (but not to distinguish the two variants). The cultured *E. coli* and the antiserum containing the F18 specific antigenic determinant "a" were mixed and the agglutination reaction was observed. Second, the duplex-PCR test, which was established according to the difference in the major fimbrial subunit genes *fedA* between the F18ab and F18ac variants (Imberechts et al., 1992a; Bosworth et al., 1998), was performed to verify whether the two variants were F18ab and F18ac. Three primers, which were upstream specific to

Table 1. Genotype distributions and allele frequencies of *FUT1* M307 in three pig breeds

Breed	No. pigs	Genotype*			Allele	
		GG	GA	AA	A	G
LW	231	112 (0.48)	108 (0.47)	11 (0.05)	0.28	0.72
LR	107	67 (0.63)	38 (0.35)	2 (0.02)	0.20	0.80
SB	109	85 (0.78)	22 (0.20)	2 (0.02)	0.12	0.88
Total	447	264 (0.59)	168 (0.38)	15 (0.03)	0.22	0.78

* Values in parentheses are percentages.

both *fedA*/F18ab and *fedA/*F18ac, downstream specific to both *fedA*/F18ab and *fedA/*F18ac, and upstream specific to *fedA*/F18ab, respectively (Cheng et al., 2005), were applied together to each of the F18 variants in the duplex-PCR. The bacterial DNA was amplified using a 50 μl reaction volume, containing 5 μl of the bacterial suspension (F18ab or F18ac), 5 μl 10×PCR buffer (containing 15 mmol/L Mg^{2+}), 1.0 μl 10 mmol/L dNTPS, 1.0 μl 25 μmol/L of each primer, and 1 unit of Taq DNA polymerase (2 U/μl). The PCR was carried out on a PCR system PTC-2000 under the follow cycling conditions: an initial denaturation of 3 min at 94°C, followed by 6 cycles of 30 sec at 94°C, 30 sec at 66°C, 60 sec at 72°C, given annealing temperature reduced 1°C every two cycles. Then, 24 cycles of 30 sec at 94°C, 30 sec at 63°C, 60 sec at 72°C, followed by a 10min final extension at 72°C. The PCR products were separated on 1.5% agarose gels. Third, the DNA sequence analysis was conducted to further prove the results of the duplex-PCR test. The PCR products of the two variants from duplex-PCR were sequenced and the sequences were aligned to reveal differences between them.

Preparation of piglet small intestinal epithelial cells

Enterocytes were obtained from the jejunum of weaned pigs (35-day-old). Within two hours after slaughtering, a 10 to 30 cm segment of the jejunum sample was longitudinally opened and washed with cold hypotonic EDTA solution (5 mmol/L, pH 7.4) to remove jejunal contents. The mucosal surface was scraped off the jejunum and the cells were lysed in a hypotonic EDTA solution. After incubation for 30 min at 4°C, the mixture was homogenized at 12,000 rpm for 20 seconds to make the cells evenly disposed. The gross particulates were allowed to settle, and the supernatant was washed twice at low centrifuge speed (1,200 rpm) for 10 min and re-suspended in cold PBS (pH 7.4). Finally, 100 μl gentamicin sulfate (1 mg/ml) and 100 μl sodium azide (3 mmol/L) were added to the cell suspension. The purified cell suspension was stored at 4°C for the following assays (Vogeli et al., 1996; Li et al., 2007).

Microscopical adhesion test

The F18ab and F18ac adhesion phenotypes were tested *in vitro* for all 363 piglets using a microscopical adhesion test. Firstly, 100 μl of enterocyte suspension was mixed with 100 μl of *E. coli* suspension and 1 μl of D-mannose (0.4 mg/ml). Enterocyte brush borders were incubated with fimbriated F18 *E. coli* for 30 min at room temperature. Following incubation, a drop of the mixed suspension was put on a slide under a cover-slip and examined by phase contrast microscopy (Leica). This assay determined whether swine were susceptible (intestinal cells expressed ECF18 receptors and had adhering bacteria) or resistant (intestinal cells lack ECF18 receptors and had no adhering bacteria). For each probe, at least 20 well-maintained enterocytes were inspected. A cell was classified as adhesive if there were more than five bacteria adhering to it, or weakly adhesive if one to five bacteria adhered to it. A pig was regarded as adhesive (named A) if at least 10% of the enterocytes examined were adhesive, or weakly adhesive (named A′) if at least 10% of the enterocytes examined were weakly adhesive or less than 10% of the enterocytes were adhesive, or non-adhesive (named N) otherwise. These classification criteria were based on previous study (Vogeli et al., 1996).

***FUT1* M307 genotyping**

To determine the *FUT1* M307 genotype, we used PCR amplification followed by digestion with Hin6I (MBI). Genomic DNA was isolated from the porcine ear sample following standard procedures (Sambrook and Russell, 2002). Sequences of forward and reverse primers were: 5′-CTT CAG CCA GGG CTC CTT TAA G-3′ and 5′-CTG CCT GAA CGT CTA TCA AGA CC-3′ (Meijerink et al., 1997). The PCR reaction system was 25 μl volume, including 100 ng genomic DNA, 0.5 μl 10 μmol/L of each primer, 2.5 μl 10×PCR buffer (containing 15 Mmol/L Mg^{2+}), 1.0 μl 10 Mmol/L dNTPs, 1 μl *Taq* DNA polymerase (2 U/μl), and 18.5 μl ddH_2O. PCR was carried out on a PCR system PTC-2000 with the following procedures: an initial denaturation of 3 min at 94°C, followed by 35 cycles of 45 sec at 94°C, 30sec at 58°C, 45 sec at 72°C, and then a 5 min final extension at 72°C. The amplified DNA (10 μl) was digested at 37°C with 1 unit of Hin6I for 5 h. The digests were separated by electrophoresis on 8% poly-acrylamide gel.

Statistical analysis

Chi-square test of the SAS software package (SAS8.0) was applied to determine the association between *FUT1* genotype and adhesion phenotype.

RESULTS

Verification of the two *E. coli* variants 107/86 and 2134P

The slide agglutination test resulted in obvious floc sediment, indicating a reaction between *E. coli* and the F18 specific antigenic determinant “a” had happened and thus confirming the two *E. coli* variants were indeed F18 strain. The results of the duplex-PCR test are shown in Figure 1. Two fragments (513 bp and 169 bp) were obtained from the 107/86 variant and only one fragment (516 bp) from the 2134P variant. For each variant only the corresponding specific PCR product was obtained, indicating that the two variants were indeed the F18ab and F18ac, respectively, and also they were not polluted. The alignment of the sequences

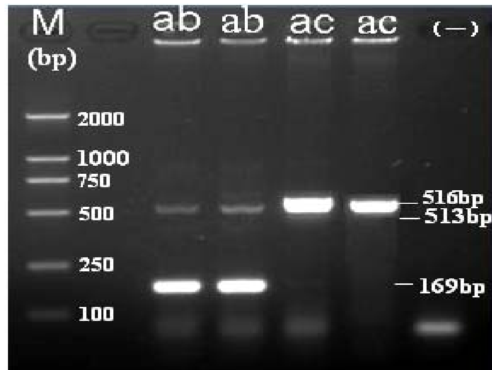

Figure 1. Genotyping of the *fedA* gene by duplex-PCR. Lane M: DNA marker DL2000, lane ab: PCT products for 107/86, lane ac: PCR products for 2134P, lane (-): negative control.

for the two variants is shown in Figure 2. The two sequences showed a high identity of 94.96%, and 20 mutations were found in total. An insertion of CCG at 364 bp in the sequence for variant 2134P (supposed to be F18ac) made the 3′ of the *fedA*/F18ab specific primer fail to combine with *fedA*/F18ac. Therefore, the 169 bp fragment produced in the duplex-PCR test must have been from the variant 107/86, proving it was indeed F18ac, rather than F18ab.

Genotype distribution in the three pig breeds

A 421 bp fragment was amplified from the *FUT1* gene. There were two restriction sites for the enzyme Hin6 I within this fragment, *i.e.* a monomorphic site (present in both of the *FUT1* alleles) and a polymorphic site (present only in allele G). G/A mutation at position 307 eliminated the polymorphic site. The monomorphic site gave two fragments of 328 bp and 93 bp in length, respectively, and the polymorphic site caused the 328 bp fragment to be further digested to a 241 bp fragment and an 87 bp fragment. The three different genotypes (GG, GA and AA) at the Hin6 I-RFLP site of the *FUT1* gene are illustrated in Figure 3. The frequencies of the three genotypes in the three pig breeds were, respectively, 0.48, 0.47, and 0.05 in LW, 0.63, 0.35 and 0.02 in LR, and 0.78, 0.20 and 0.02 in SB (Table 1). Obviously, the GG and AG were dominant in all three breeds with frequencies of 95%, 98% and 98%. However, the allele frequencies were significantly different in the three breeds. The frequency of G in SB (0.88) was

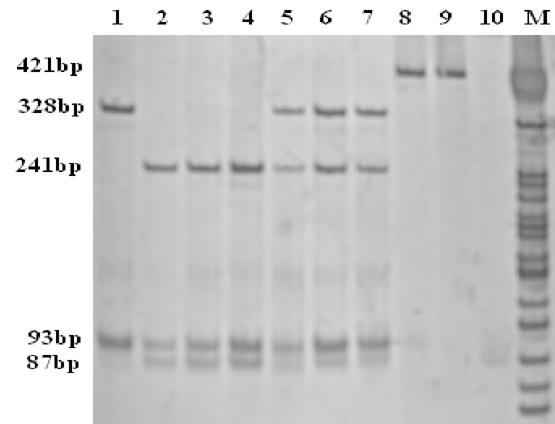

Figure 3. Genotyping result of *FUT1* gene by PCR-RFLP. Lane M: pBR322 DNA/MspI marker, lane 1: genotype AA, lane 2, 3, and 4: genotype GG, Lane 5, 6 and 7: genotype AG, lane 8 and 9: PCR products, lane 10: negative control.

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F18ab  ATGAAAAGACTAGTGTTTATTTCTTTTGTTGCTCTGTCCATGACAGCGGGTTCCGCAATG 60
F18ac  ATGAAAAGACTAGTGTTTATTTCTTTTGTTGCGCTGTCCATGACAGCGGGTTCCCCAATG 60
F18ab  GCTCAGCAAGGGGATGTTAAATTCTTTGGTAACGTATCAGCAACTACCTGTAATTTGACA 120
F18ac  GCTCAGCAAGGGGATGTTAAATTCTTTGGTAGCGTATCAGCAACTACCTGTAATTTGACA 120
F18ab  CCACAAATAAGTGGCACTGTAGGAGATACCATTCAGCTTGGTACTGTTGCACCAAGCGGA 180
F18ac  CCACAAATAAGTGGCACTGTAGGAGATACCATTCAGCTTGGTACTGTTACACCAAGCGGA 180
F18ab  ACTGGTAGTGAAATTCCTTTTGCACTGAAGGCTTCTTCAAATGTTGGCGGTTGTGCTTCC 240
F18ac  ACTGGTAGTGAAATTCCTTTTGCACTGAAGGCTTCTTCAGCTACTGGCGGTTGTGCTTCC 240
F18ab  TTGTCCACTAAAACAGCTGATATAACTTGCACCGGGCAGTTAACCGAAAAAGGTTTTCCT 300
F18ac  TTGTCCAATAAAACAGCTGATATAACTTGGAGCGGGCAGTTAACCGAAAAAGGTTTTGCT 300
F18ab  AATCAAGGGGGGGTGGCAAATGATTCATATGTCCCTCTGAAAACCGTGAACGGTAAAACA 360
F18ac  AATCAAGGGGGAGTGCCAAGAGATTCATATGTTGCTCTGAAAACCGTGAACGGTAAAACA 360
F18ab  CAG---GGGCAGGAGGTTAAGGCGTCGAATAGCACTGTAAGTTTCGATGCATGAAAAGCA 417
F18ac  CAGCCGGCGCAGGAGGTTAAGGCGTCGAATAGCACTGTAAATTTCGATGCATCAAAAGCA 420
F18ab  ACTACGGAAGGTTTGAAATTTACTGCTCAACTGAAAGGTGGTCAAACCCCGGGTGACTTC 477
F18ac  ACTACGGAAGGTTTCAAATTTACTGCTCAACTGAAAGGTGGTCAAACCCCGGGTGACTTC 480
F18ab  CAGGGGGCAGCGGCTTACGCGGTTACTTACAAGTAA                         513
F18ac  CAGGGGGCAGAGGCTTACGCGGTTACTTACAAGTAA                         516
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Figure 2. Alignment of the nucleotide sequence of *fedA*/F18ab and *fedA*/F18ac. The mutations are underlined.

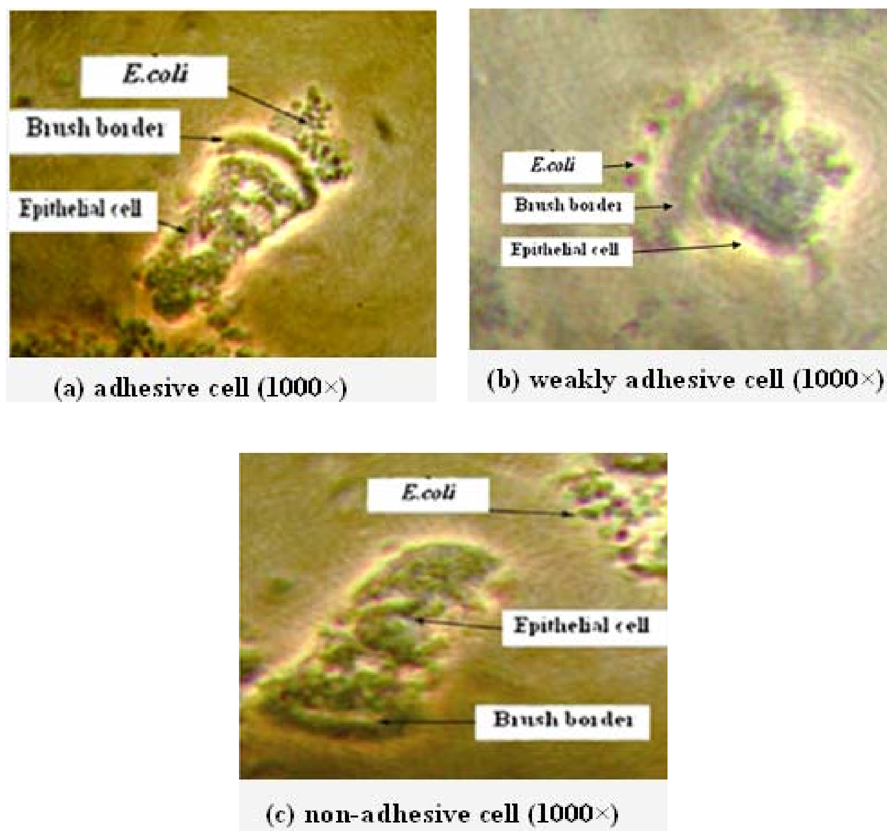

Figure 4. Adhesive small intestinal cell (a), weakly adhesive small intestinal cell (b), and non-adhesive small intestinal cell (c).

significantly higher than that in LW (0.72, $p<0.01$) and LR (0.80, $p<0.05$), and the frequency of G in LR was significantly higher than that in LW ($p<0.01$).

Adhesion phenotypes of F18ab and F18ac in piglets

All of the three adhesion phenotypes, i.e., adhesive (A), weakly adhesive (A′) and non-adhesive (N), for F18ab and F18ac were detected in the three pig breeds (Figure 4). The frequencies of the three phenotypes in the three pig breeds are given in Table 2. For F18ab, the phenotype A (75%) was dominant over the other two phenotypes, while for F18ac the phenotype A′ (82%) was dominant. There were no significant differences ($p>0.05$) among different breeds within the same F18 variant.

When looking at the adhesion phenotypes of F18ab and F18ac jointly and regarding the phenotype A and A′ both as positive (+) and the phenotype N as negative (-), the frequencies of the four possible adhesion patterns are shown in Table 3. It can be seen that more than 80% of piglets were positive to both F18ab and F18ac (ab^+, ac^+), less than 5% of piglets were negative to both F18ab and F18ac (ab^-, ac^-), about 10% of piglets were positive to F18ab but negative to F18ac (ab^+, ac^-), and no piglet with pattern (ab^-, ac^+) was found. Chi-square test showed that there were no significant differences ($p>0.05$) in the adhesion pattern distributions among the three pig breeds.

Association between *FUT1* M307 genotypes and adhesion phenotypes

The distributions of adhesion phenotypes (with adhesive and weakly adhesive phenotype grouped as positive) within each *FUT1* M307 genotype are shown in Table 4. For

Table 2. Distribution of the F18ab and F18ac adhesion phenotypes in three pig breeds

Breed	No. pigs	F18ab*			F18ac*		
		A	A′	N	A	A′	N
LW	187	135 (0.72)	45 (0.24)	7 (0.04)	9 (0.05)	149 (0.80)	29 (0.15)
LR	86	65 (0.76)	19 (0.22)	2 (0.02)	4 (0.05)	71 (0.82)	11 (0.13)
SB	90	71 (0.79)	17 (0.19)	2 (0.02)	2 (0.02)	79 (0.88)	9 (0.10)
Total	363	271 (0.75)	81 (0.22)	11 (0.03)	15 (0.04)	299 (0.82)	49 (0.14)

* A: adhesive, A′: weakly adhesive, N: non-adhesive. Values in parentheses are percentages.

Table 3. Adhesion patterns of F18ab and F18ac and their frequencies in three pig breeds

Breed	No. pigs	Adhesion pattern*			
		(ab^+, ac^+)	(ab^+, ac^-)	(ab^-, ac^+)	(ab^-, ac^-)
LW	187	158 (0.84)	22 (0.12)	0 (0)	7 (0.04)
LR	86	75 (0.88)	9 (0.10)	0 (0)	2 (0.02)
SB	90	81 (0.90)	7 (0.08)	0 (0)	2 (0.02)
Total	363	314 (0.87)	38 (0.10)	0 (0)	11 (0.03)

* "+": positive (adhesive or weakly adhesive), "-": negative (non-adhesive). (ab^+, ac^+) represents the adhesion pattern of being positive to both F18ab and F18ac. Analogical for other patterns.

F18ab, all individuals with genotype GG or GA were adhesive and all with AA were non-adhesive, whereas for F18ac, about 89% of the piglets with genotype GG or GA were adhesive and all with AA were non-adhesive. Chi-square test showed the association between *FUT1* M307 genotypes and the adhesion phenotypes was highly significant ($p<0.001$).

DISCUSSION

Reduction of costs in swine production is a goal of breeders, and keeping the pigs disease-free is the major problem to be resolved. However, PWD and ED always lead to tremendous economic losses and effective prevention remains an unsolved problem. Traditional methods for disease prevention and treatment included segregation, slaughter, immuno-prophylaxis with live or subunit vaccines, use of preventive feed medication (organic acids, proteases, bacterial probiotics and phages), and administration of specific egg yolk antibodies and so on (Fairbrother et al., 2005). Researchers have paid great attention to vaccination to prevent PWD and ED. Although vaccines should activate the mucosal immune system and evoke antigen-specific IgA or IgM responses in order to induce a protective mucosal immunity, the cross-protection between strains with fimbrial variants F18ab and F18ac may not be very high (Bertschinger et al., 2000). Verdonck et al. (2007) found that it is difficult to induce protective immunity against $F18^+$ *E. coli* through mucosal immunization of piglets with the whole F18 fimbriae. Tiels et al. (2008) revealed that almost no FedF specific-response could be detected after oral immunization to piglets with a subunit vaccine containing the F18 fimbrial adhesion FedF. Up to now, no well-designed vaccines are available and it is unlikely to be a preferred method because of difficulties in administering live vaccine orally to piglets and because of regulatory restrictions. In summary, traditional methods cannot deal with the problem effectively and thus new methods are needed. In essence, breeding resistant animals is an attractive approach to prevent PWD and ED.

The purpose of breeding for disease resistance is to increase genetically the disease resistance ability of animals though eliminating genetically susceptible animals from the herds. For this purpose, large scale selection of resistant pigs is a prerequisite. Current methods for identifying pigs resistant to PWD and ED caused by F18ab and F18ac include: i) microscopical adhesion test, ii) colonization test (challenge the animals with virulent bacteria), iii) blood typing of the A-O(S) blood group, and iv) detecting the *FUT1* polymorphism (Bosworth and Vogeli, 2002). The colonization test is too laborious and expensive (Bertschinger et al., 1993). Although the *in vitro* adhesion assay is more reliable than the colonization test, it is not applicable for breeding because the pigs slaughtered for the collection of enterocytes are no longer available for breeding purposes (Vogeli et al., 1996). The blood typing method does identify resistant pigs, but is not able to determine whether susceptible pigs are homozygous or heterozygous. Compared with the first three methods, detecting the *FUT1* polymorphism has many advantages, including ease of implementation and giving distinct and reliable results. It is a non-invasive method to genetically differentiate susceptible pigs from resistant ones with a high level of sensitivity and specificity, and ultimately will make it possible to keep swine disease-free. In addition, this method is useful to develop drugs for treatment.

Table 4. Numbers and percentages of adhesive and non-adhesive pigs with respect to different *FUT1* M307 genotypes

Genotype	No. pigs	F18ab		F18ac	
		Adhesive*	Non-adhesive	Adhesive*	Non-adhesive
GG	222	222 (100%)	0 (0)	199 (89.64%)	23 (10.36%)
GA	130	130 (100%)	0 (0)	115 (88.46%)	15 (11.54%)
AA	11	0 (0)	11 (100%)	0 (0)	11 (100%)
Total	363	352	11	314	49

* Including weakly adhesive.

In our research population, the frequency of allele G at *FUT1* M307 in Songliao Black (0.88) was higher than in Large White (0.72, p<0.01) and Landrace (0.80, p<0.05). The frequency of genotype AA was very low in all three breeds (<5%), which was similar to that reported by Vogeli et al. (1997) in Duroc, Hampshire, Pietrain, Large White and Landrace populations, in which the frequency of AA was less than 5%. Klukowska et al. (1999) investigated the *FUT1* M307 polymorphism in four Polish pig breeds, and revealed that the frequency of allele A was high, up to 0.63, and they suggested that Polish indigenous pig breeds could be a useful resource for breeding an *E. coli* F18 resistant pig line. China has abundant native pig breeds. Shi et al. (2003) and Yan et al. (2003) investigated the *FUT1* M307 polymorphism in some Chinese pig breeds (Ningxiang, Shaziling, Daweizi, Lingao, Erhualian and others) and the polymorphism was only detected in the Lingao breed, which had two genotypes, GG and GA. Bao et al. (2008) investigated the gene variation at *FUT1* M307 by PCR-RFLP in an Asian wild pig breed, four Western commercial pig breeds and 16 Chinese native pig breeds, and discovered that only Western pig breeds had the resistant AA genotype, while the Asian wild pig and the Chinese native pig breeds had only susceptible genotypes (GG and GA), with most individuals of the Chinese breeds being homozygous GG. Songliao Black used in this study is a hybrid breed produced by crossing Duroc, Large White and a Chinese native pig breed in Jilin province. Thus the low frequency of allele A in Songliao Black (0.12) is understandable, and it may be inferred that allele A of *FUT1* M307 originated from Western pig breeds. However, the epidemiological investigation of PWD and ED in 10 provinces in China showed that the morbidity of Western pig breeds and hybrids was higher than that of Chinese native pig breeds (He et al., 2001). This phenomenon is not in accordance with the fact that no resistant genotype AA was found in any Chinese native pig breeds so far. It is still a confused problem.

The results of this study reveal a complete relationship between the *FUT1* M307 genotypes and the F18ab adhesion phenotypes. All individuals of genotype GG or GA were adhesive to F18ab, whereas all individuals of genotype AA were non-adhesive, which confirms the results of Meijerink et al. (1997) and Frydendahl et al. (2003). However, for F18ac, the relationship, although highly significant, is incomplete since about 11% pigs with genotypes GG or GA were non-adhesive to F18ac. This indicates that the F18ac receptor may be controlled by a different gene from that for the F18ab receptor, which needs further study.

The relationship of the *FUT1* M307 polymorphism and economic traits has been studied by several researchers. Horak et al. (2005) reported a significantly lower number of piglets born alive and number of weaned piglets from sows of genotype AA in a population of Přeštice Black-Pied sows. Jiang et al. (2005) found that genotype AA was the advantaged genotype with respect to some meat quality (better pH, color and water holding capacity) and carcass traits (thiner backfat) in hybrid pigs from Large-white×Meishan. Some contradictory results to those of Jiang et al. (2005) were observed by Kadarmideen (2008) in Swiss pigs, who revealed a positive effect of allele A on growth but negative effect on carcass traits (increasing backfat and decreasing proportion of premium cuts), and no significant effect on meat quality traits. Thus, the association between *FUT1* M307 polymorphism and production performance needs to be further confirmed.

In conclusion, the *FUT1* M307 polymorphism can be used as a predictor for selection of PWD- and ED-resistant swine. Furthermore, when selecting PWD- and ED-resistant pigs according to the *FUT1* M307 genotype, we should take into consideration its effect on reproduction and production traits to achieve balanced breeding.

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