Genetics and Molecular Research

Differential expression of *FUT1* and *FUT2* in Large White, Meishan, and Sutai porcine breeds

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ABSTRACT. To assess the relationship between the expression of α(1,2)-fucosyltransferase (*FUT1* and *FUT2*) genes and resistance to *Escherichia coli* F18 in weaned pigs, *FUT1* and *FUT2* expression levels in Large White, Meishan, and Sutai pigs (with resistance to *E. coli* F18) were determined using real-time PCR. The results revealed that *FUT1* and *FUT2* expression levels were higher in the liver, lungs, kidneys, stomach, duodenum, and jejunum than in the muscle and heart. Medium *FUT2* expression levels were detected in the spleen, thymus, and lymph nodes. Intestinal *FUT1* expression levels were higher in Sutai pigs than in Large White and Meishan pigs ($P < 0.05$). However, intestinal *FUT2* expression levels were lower in Sutai pigs than in Large White and Meishan pigs ($P < 0.05$). *FUT1* and *FUT2* expression levels did not differ between Large White and Meishan pigs ($P > 0.05$). The results revealed that high *FUT1* expression levels and low *FUT2* expression levels in the intestines of Sutai

pigs affected FUT1 and FUT2 enzymes, the synthesis of type 2 H and type 1 H antigens, and *E. coli* F18 adhesion. Moreover, low *FUT2* expression levels conferred resistance to *E. coli* F18.

Key words: Pig; *FUT1*; *FUT2*; mRNA expression

INTRODUCTION

Porcine post-weaning diarrhea (PWD) is a common disease that contributes to significant financial losses in the swine industry. *Escherichia coli* causing PWD and *E. coli* F18 is the most predominant and pathogenic bacteria in pigs (Van den Broeck et al., 2000; Boldin, 2008). Studies showed that the pathogenicity of *E. coli* F18 was dependent on the presence of specific receptors expressed on the small intestinal surface of piglets (Bertin and Duchet-Suchaux, 1991). The α1-fucosyltransferase gene (*FUT1*) plays a major role in the synthesis of the *E. coli* F18 receptor (Vögeli et al., 1997). Meijerink et al. (1997, 2000) reported that a mutation in the M307 locus of *FUT1* conferred resistance to *E. coli* F18 infections. However, genotypes at this locus have highly skewed distributions among Chinese porcine breeds, so the gene is not suitable for the breeding of disease resistance (Yan et al., 2003; Bao et al., 2008).

The participation of the FUT1 enzyme in the regulation of the ABH blood group antigen has been assessed by thin layer chromatography, negative ion mass spectrum, and proton magnetic resonance. Studies showed that the *E. coli* F18 receptor determinant is a type 1 H antigen of the ABH blood group (Coddens et al., 2009). The ABH blood group antigen is a glycosphingolipid on the plasmalemma of red blood cells, and the ABH blood group is determined by the types of sugars present in the glycosphingolipid. The precursor of the ABH blood group antigen is the H antigen. Moreover, the H enzyme is α(1,2)-fucosyltransferase, which is coded by *FUT1* and *FUT2*, and this enzyme catalyzes the synthesis of the H antigen by connecting fucose with the H antigen precursor. Therefore, *FUT1* and *FUT2* play important roles in conferring resistance to *E. coli* F18 in Sutai pigs (Duroc x Meishan; Bao et al., 2012).

In this study, 35-day-old Large White (foreign porcine breed), Meishan (domestic porcine breed), and Sutai pigs (with resistance to *E. coli* F18) were used. The expression profiles of *FUT1* and *FUT2* in 11 tissues of the porcine breeds were determined using real-time PCR. The correlation between *FUT1* and *FUT2* expression levels and resistance against *E. coli* F18 in piglets was calculated.

MATERIAL AND METHODS

Animals and sample collection

Four *E. coli* F18-resistant Sutai piglets (Sutai Pig Breeding Center, Suzhou, Jiangsu) were selected based on birth weight, weaning weight, shape, and coat color similarities (Wu et al., 2007). Four Large White and four Meishan pigs (35 days old) from Kang Le Farming Co., Ltd. (Changzhou) and Meishan Pig Conservation Breeding Co. (Kunshan), respectively, were selected and slaughtered. Tissue samples (heart, liver, spleen, lung, kidney, stomach, muscle, thymus, lymph nodes, duodenum, and jejunum) were collected, frozen in liquid nitrogen, and stored at -70°C.

Design and synthesis of real-time PCR primers

Real-time PCR primers were designed using the Primer-BLAST software based on the

published porcine *FUT1* and *FUT2* sequences in GenBank (Table 1), and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), transferrin-binding protein 1 (*TBP1*), and beta-actin (*ACTB*) genes were used as references. Primers were synthesized by Shanghai Invitrogen Biotechnology Co., Ltd.

Table 1. Real-time PCR primers and their sequences.

Gene	Accession No.	Primer sequences (5'→3')	Length (bp)
FUT1	U70883	F: TTTTAAGCCCCCAAACTGCC	126
		R: TAAATCGACCCCATCAGCCTC	
FUT2	U70881.2	F: AATCCCTGACCTCACTCCGTG	123
		R: CGGAACTACAACTGCTGGCC	
GAPDH	AF017079.1	F: ACATCATCCCTGCTTCTACTGG	187
		R: CTCGGACGCCTGCTTCAC	
TBP1	DQ845178.1	F: AACAGTTCAGTAGTTATGAGC	153
		R: AGATGTTCTCAAACGCTTCG	
ACTB	XM_003124280.3	F: TGGCGCCCAGCACGATGAAG	149
		R: GATGGAGGGGCCGGACTCGT	

Total RNA extraction, reverse transcription, and real-time PCR

Total RNA was extracted from the 11-tissue samples using Trizol (TaKaRa, China). The integrity, purity, and concentration of total RNA samples were determined using 2.2% formaldehyde denaturalization gel electrophoresis and UV spectrophotometry. Total RNA was stored at -70°C prior to conversion to cDNA via reverse transcription.

The reverse transcription reaction mixture (10 µL total) consisted of 2 µL 5X PrimerScript buffer reaction solution, 0.5 µL PrimerScript RT enzyme mix I, 0.5 µL oligo dT, 0.5 µL random 6-mers, 500 ng total RNA, and RNase-free H_2O. The reverse transcription conditions were set at 37°C for 15 min and 85°C for 5 s. The resulting cDNA was stored at 4°C.

cDNA was subsequently amplified using real-time PCR, and the real-time PCR mixture (20 µL total) consisted of 1 µL template, 0.4 µL 10 µM primers (forward and reverse), 0.4 µL 50X ROX Reference Dye II, 10 µL 2X SYBR Green Real-Time PCR Master Mix, and 7.8 µL ddH_2O. The real-time PCR conditions were as follows: one cycle at 95°C for 30 s; 40 cycles at 95°C for 5 s and at 60°C for 34 s; and a final step at 4°C. The dissociation curve was analyzed following amplification, and a dissociation curve Tm of 85 ± 0.8°C was used to determine the specificity of the amplification. Each sample was amplified three times, and the average was calculated.

Statistical analysis

The real-time PCR results were analyzed using the $2^{-\Delta\Delta Ct}$ method, where $\Delta\Delta Ct$ = (average Ct value of target genes in the test group - geometric mean value of Ct values of reference genes in the test group) - (average Ct value of target genes in the control group - geometric mean value of Ct values of reference genes in the control group). Statistical analyses were performed with the SPSS 15.0 software, and differences in *FUT1* and *FUT2* expression levels among the porcine breeds were analyzed using LSD multiple comparisons.

RESULTS

Melting and amplification curves from real-time PCR analyses

The real-time PCR results were analyzed with the Applied Biosystems Sequence detec-

tion software (7500 Fast System SDS Software version 1.4). Both *FUT1* and *FUT2* exhibited a specific peak, and there were no primer dimers or non-specific products, which suggested that the amplification was specific. The *FUT1* and *FUT2* melting and amplification curves are shown in **Figure S1**.

FUT1 and *FUT2* expression levels in the 11 tissues of the three porcine breeds

FUT1 and *FUT2* were expressed in the 11 tissues of the three porcine breeds. Furthermore, the expression levels of the two genes were somewhat similar. The expression levels of both *FUT1* and *FUT2* were high in the liver, lungs, kidneys, stomach, duodenum, and jejunum, and they were low in the heart and muscle. *FUT2* had medium expression levels in the spleen, thymus, and lymph nodes (Figures 1-3).

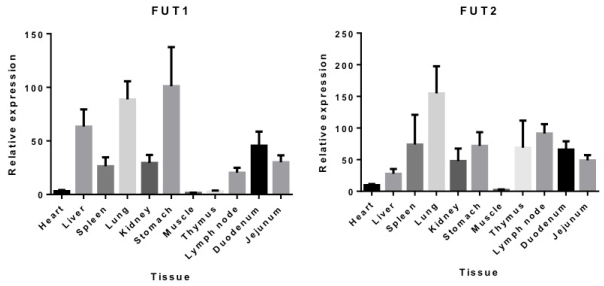

Figure 1. Expression levels of *FUT1* and *FUT2* in 11 tissues of Large White pigs.

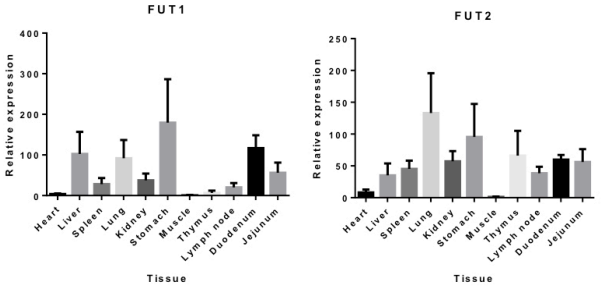

Figure 2. Expression levels of *FUT1* and *FUT2* in 11 tissues of Sutai pigs.

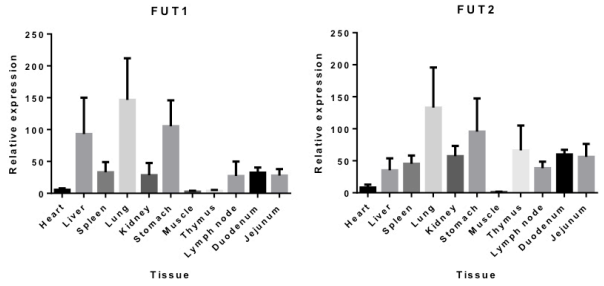

Figure 3. Expression levels of *FUT1* and *FUT2* in 11 tissues of Meishan pigs.

FUT1 and *FUT2* expression levels in the duodenum and jejunum

E. coli adheres to receptors expressed on the surface of intestinal cells. Therefore, in this experiment, we analyzed the expression levels of *FUT1* and *FUT2* in the duodenum and jejunum. Sutai pigs had higher *FUT1* expression levels than Large White and Meishan pigs ($P < 0.05$). However, *FUT2* expression levels were lower in Sutai pigs than in Large White and Meishan pigs ($P < 0.05$). There were no significant differences in *FUT1* and *FUT2* expression levels between Large White and Meishan pigs ($P > 0.05$; Figures 4 and 5).

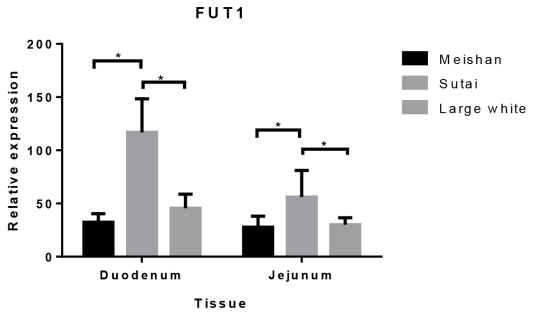

Figure 4. Differential expression analysis of *FUT1* in duodenum and jejunum.

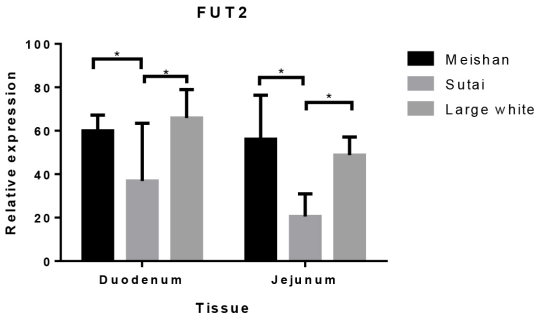

Figure 5. Differential expression analysis of *FUT2* in duodenum and jejunum.

DISCUSSION

PWD in piglets is caused by enterotoxins produced by *E. coli* F18 bacteria after binding to receptors on the small intestinal brush border. Therefore, *E. coli* F18 pathogenicity is dependent on the presence of specific intestinal receptors (Bertin and Duchet-Suchaux, 1991; Berschinger et al., 1990, 1993). Coddens et al. (2007) reported that *E. coli* F18 receptor expression levels increased in Landrace pigs from 0 to 3 weeks of age, and they stabilized between 3 and 23 weeks of age. Unweaned piglets are not susceptible to *E. coli* because the milk antibodies confer protection against enteric infections (Deprez et al., 1986). In this study, the piglet specimens had been weaned for 1 week, and weaned piglets are very susceptible to *E. coli* F18 infections at this age (35 days old). The *E. coli* F18 receptor is a type 1 H antigen of the ABO blood group and a glycosphingolipid derivative (Coddens et al., 2009). Studies reported that ABH blood group antigens

were primarily synthesized by the globo- and lactose-series of glycosphingolipid biosynthetic pathways (Hakomori, 2000). Moreover, H antigens were synthesized during the glycosphingolipid biosynthetic pathway, and neutral-, lactose-, and novel lactose-series glycosphingolipid biosynthetic pathways were utilized based on the glycosphingolipid biosynthesis-globo series pathway (Kannagi et al., 1983). Therefore, the glycosphingolipid biosynthesis-globo-series pathway plays an important role in the synthesis of the *E. coli* F18 receptor. According to the glycosphingolipid biosynthesis-globo-series pathway (**Figure S2**), *FUT1* and *FUT2* are involved in the synthesis of the intestinal receptor. In this study, 35-day-old Large White (foreign porcine breed), Meishan (domestic porcine breed), and Sutai pigs (with resistance to *E. coli* F18) were used, and *FUT1* and *FUT2* expression levels in 11 tissues of the three porcine breeds were analyzed using real-time PCR. Additionally, the correlation between *FUT1* and *FUT2* expression levels and resistance to *E. coli* F18 was calculated.

The expression profiles of the two genes of interest were similar among the breeds. Furthermore, the expression levels of *FUT1* and *FUT2* were higher in the liver, lungs, kidneys, stomach, duodenum, and jejunum than in other tissues. Saccharides are often the structural components of hormones, enzymes, cell surface receptors, the cell matrix, and connective tissue. Accordingly, *FUT1* and *FUT2* were expressed in the liver, lungs, kidneys, stomach, and intestinal tissue. It is noteworthy that *FUT2* had medium expression levels in the spleen, thymus, and lymph nodes (i.e., organs where lymphocyte and the other immune cells differentiate).

Studies reported that *FUT1* controls the adhesion of *E. coli* F18 (Imberechts et al., 1996; Meijerink et al., 1997, 2000). However, Coddens et al. (2009) observed that the *E. coli* F18 receptor is a type 1 H antigen catalyzed by α2-fucosyltransferase that is encoded by *FUT2* as opposed to a type 2 H antigen catalyzed by α1-fucosyltransferase that is encoded by *FUT1*, and Moonens et al. (2012) and Lonardi et al. (2013) validated these results. Additionally, FUT1 glycosyltransferase converts type 1 H precursors into type 1 H blood group antigens (Kyprianou et al., 1990; Liu et al., 1998; Mathieu et al., 2004). *FUT2* expression levels were significantly lower in *E. coli* F18-resistant piglets than those in *E. coli* 18-sensitive piglets, and Meijerink et al. (2000) reported that *FUT2* expression levels were high in sensitive individuals and insignificant in resistant subjects. The high *FUT2* expression levels contributed to high levels of fucosyltransferase in *E. coli* F18-sensitive piglets. In this study, *FUT1* expression levels were significantly higher in Sutai pigs than in Large White and Meishan pigs ($P < 0.05$). On the other hand, *FUT2* expression levels in Sutai pigs were significantly lower than those detected in Large White and Meishan pigs ($P < 0.05$). Epidemiological data revealed that morbidity rates associated with *E. coli* F18 infections did not differ significantly between the domestic and foreign breeds. Additionally, the results revealed that there were no significant differences in *FUT1* and *FUT2* expression levels between Large White and Meishan pigs ($P > 0.05$). Therefore, the high expression of *FUT1* and the low expression of *FUT2* in Sutai pigs with resistance to *E. coli* F18 affected the activity of FUT1 and FUT2 enzymes, respectively. Furthermore, the formation of type 1 H antigens and type 2 H antigens reduced the adhesion of *E. coli* F18. Low *FUT2* expression levels contributed to *E. coli* F18 resistance in Sutai pigs. Therefore, further studies should evaluate the antagonistic relationship between *FUT1* and *FUT2* expression levels, and the relationship between *FUT1*, *FUT2*, and other genes in the glycosphingolipid biosynthetic pathway should also be examined.

Conflicts of interest

The authors declare no conflict of interest.

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Supplementary material

Figure S1. *FUT1* and *FUT2* melting and amplification curves in different tissues.

Figure S2. Gene interaction network of seven glycosylation transferase enzymes in the STRING database.

http://www.geneticsmr.com/year2016/vol15-1/pdf/gmr7613_supplementary.pdf