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Polymorphism of *FUT1* Gene M307 and its Relationship with Partial Immune Indexes and Economic Traits in Yorkshire Pigs

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ABSTRACT

To assess the feasibility of *FUT1* gene M307 as a disease resistance genetic marker in Yorkshire pigs, this experiment adopted PCR-RFLP method to investigate the genetic variations at M307 of *FUT1* gene in purebred Yorkshire (total 218 individuals), determined partial biochemical parameters and important cytokines using the automatic blood cell analyzer and ProCarta immunoassay kits and assayed a number of economic traits including eye muscle thickness, backfat thickness, growth rate and reproductive performance. This study also analyzed the relationship between polymorphism of *FUT1* gene M307 and partial immune indexes, vital economic traits. The results showed that there were three genotypes (AA, AG and GG) in Yorkshire test groups, in which the allele G was dominant one. *FUT1* gene M307 deviated from Hardy-Weinberg equilibrium ($p < 0.01$) in the population. Correlation analysis showed that the level of thrombocytocrit (PCT) in AG, GG genotype individuals were significantly higher than that in AA genotype individuals ($p < 0.05$), while there were no significant difference between AG and GG genotypes ($p > 0.05$). The individuals with GG genotype were significantly higher than those with AG genotype ($p < 0.01$) in IFN- γ , TGF- β and TNF- α . In terms of the age at 100 kg, individuals with AA type were significantly higher than those with AG type ($p < 0.05$), the average age at 100 kg of AA type individuals was 4.23 days less than GG type individuals. From the 3rd-5th parity, the Total Number Born (TNB) and Number Born Alive (NBA) demonstrated a trend of AA>AG>GG, individuals with AA type were significantly higher than those individuals with GG type ($p < 0.05$) in TNB and NBA at 3rd and 5th parities. These results indicated that AA genotype, as an ETEC F18 resistance genotype, would also improve growth rate and reproduction performance of Yorkshire pigs, but reduce their general disease resistance ability.

Key words: Yorkshire pig, *FUT1* gene, immune indexes, economic trait

INTRODUCTION

Alpha (1, 2)-fucosyltransferase gene (*FUT1*) is located in pig chromosome 6q¹¹. It has reported that the *FUT1* polymorphism substitutes an adenine (A) for a guanine (G) at nucleotide 307 of *FUT1* open reading frame, alter alpha (1) fucosyltransferase activity of the pig *FUT1* enzyme and determines susceptibility of small intestinal epithelium to ECF18 adhesion (Vogeli *et al.*, 1997).

Swines with the AA genotype are resistant to ETEC F18, whereas GG or AG genotypes are sensitive to F18 (Meijerink *et al.*, 1997). Hence, through marker assisted selection (MAS), *FUT1* gene has been regarded as an anti-ETEC F18 candidate gene to realize breeding for disease resistance at home and abroad (Vogeli *et al.*, 1997; Meijerink *et al.*, 1997; Bao *et al.*, 2012a). Meanwhile, the study about polymorphism of *FUT1* gene at M307 and its relationship with partial immune parameters or vital economic traits had been published (Bao *et al.*, 2012b; Jiang *et al.*, 2005; Bao *et al.*, 2009; Zhu *et al.*, 2009).

At present, Yorkshire plays an important role in production system of pigs. Using *FUT1* gene M307 to select anti-ETEC F18 groups during the breeding for disease resistance in Yorkshire, it is necessary to pay close attention on whether the molecular breeding based on this locus will generate adverse impacts on general disease resistance ability and important economic traits. Within this context, this research applied PCR-RFLP method to investigate the genetic variations at M307 of *FUT1* gene in purebred Yorkshire pigs, determined the levels of partial biochemical parameters and important cytokines with the automatic blood cell analyzer and ProCarta immunoassay kits during the weaning period when piglets are susceptible the Edema Disease (ED) and Post-Weaning Diarrhea (PWD) and assayed a number of economic traits including eye muscle thickness, backfat thickness, growth rate and reproductive performance. On this basis, this study analyzed the relationship between polymorphism of *FUT1* gene M307 and partial immune parameters, important economic traits, discussed the feasibility about this variation as a genetic marker for disease resistance breeding in Yorkshire.

MATERIALS AND METHODS

Experimental materials: The experimental Yorkshire pigs (total 218 individuals) were from Kangle (Changzhou, Jiangsu) farming Co., Ltd. Approximately 1.0 g of ear tissue was collected from every individual and placed into a 1.5 mL Eppendoff tube in ice box before taking to the lab. Genomic DNA was extracted from ear notches according to a modified phenol and chloroform method (Sambrook and Russell, 2001). The 1% agarose gel electrophoresis and NanoDrop-1000 spectrophotometer detected the purity and concentration of DNA, respectively. The Genomic DNA was diluted into 100 ng- μL^{-1} and then stored at -20°C .

PCR-RFLP analysis

Primers: 5'-CCAACGCCTCCGATTCCTGT-3' and 5'-GTGCATGGCAGGCTGGATGA-3' were designed according to *FUT1* gene sequence in GenBank (accession number L50534) and the length of the amplified *FUT1* fragment was 161 bp. The specific primers were synthesized by Sangon Biotech (Shanghai) Co., Ltd. Exactly 20 μL of PCR product was used included 100 ng of template DNA, 10 pmol of each primer, 10 μL of PCR Master-Mix (Tiangen Biotech, Beijing) and sterilized distilled water to make up the final volume of 20 μL . PCR condition was carried out by thermal cycle, 95°C for 5 min and then 95°C for 30 sec, 60°C for 30 sec, 72°C for 30 sec, 30 cycles and an extension at 72°C for 10 min. PCR products were checked in 1% agarose gels stained with GoldView. The 5 μL of each PCR products were digested overnight at 37°C by 2 U of *Hin6 I* (Sangon, China) restriction enzyme. The digested fragments were electrophorised in 10% polyacrylamide gels in $1\times\text{TBE}$ at a constant voltage of 180 V, stained by silver and visualized under ultraviolet light using trans-illuminator.

Measurement of immune indexes: Precaval venous blood (5 mL) was collected from 35 days weaning piglets, 200 μL of which was for analysis of biochemical indexes, EDTA was used as

anticoagulant. The residual blood was used to prepare serum. An automatic blood cell analyzer (Sanhe Medical Equipment, China) was used to measure the following 12 indexes: White Blood Cell count (WBC), Red Blood Count (RBC), Hemoglobin (HGB), Hematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Red Blood Cell Distribution Width (RDW), Platelet Count (PLT), Mean Platelet Volume (MPV), Platelet Distribution Width (PDW) and Plateleterit (PCT). In addition, the level of 8 cytokines, including interleukin-1 β (IL-1 β), interleukin-4 (IL-4), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-10 (IL-10), interferon- γ (IFN- γ), transforming growth factor- β (TGF- β) and tumor necrosis factor- α (TNF- α) was determined by Procarta immunoassay kits (Affymetrix, USA).

Measurement of partial economic traits: At 185-195 days, determining the eye muscle thickness, backfat thickness via B-ultrasound and measuring weight. The reproductive performance was relatively stable from 3rd-5th parity, so this study recorded the Total Number Born (TNB) and Number Born Alive (NBA) of those parities detailedly. "Age at 100 kg" and "Backfat thickness at 100 kg" was corrected respectively according to the research (Wang, 2000).

Statistic analysis: The frequencies of gene and genotype were calculated according to Hardy-Weinberg equilibrium principle: The $p = P+H/2$, $q=Q+H/2$, $\chi^2 = \Sigma d^2/e$; $d = e-o$, which is the difference between predicted value and detected value, p and q represent allele frequency at certain position. The General Linear Model (GLM) was established to analyze the genotype effects of *FUT1* gene on measured traits.

The following linear model was used:

$$y_{ij} = \mu + G_i + e$$

where, y_{ij} represented the measured traits, μ was the overall mean, G_i was the genotypic effect of M307 *FUT1* gene and e was the residual error.

These statistical analyses were carried out using the SPSS 16.0 software.

RESULTS AND ANALYSIS

PCR-RFLP analysis: The PCR products were detected with 1% agarose electrophoresis. A clear, specific DNA band was showed at 161 bp position, which agreed with the predicted amplified fragment size (Fig. 1).

There is a restriction recognition site for *Hin6* I at position M307 in *FUT1* gene, which can be completely digested by *Hin6* I to produce the GG genotype (117/44 bp). If this position has a G/A mutation, *Hin6* I enzyme would not be able to digest the fragment, resulting in an AA type (161 bp). If two alleles both exist, it produces an AG type (161/117/44 bp). The restriction map is shown in Fig. 2.

Genotype distribution and gene frequency: Among the test group of 218 Yorkshire pigs, there were 20 AA types, 127 AG types and 71 GG types after digestion with *Hin6* I at M307 in *FUT1* gene. The statistic analysis for PCR-RFLP genotypes and their frequencies in Yorkshire pigs *FUT1* gene indicated that the frequencies of AA, AG and GG genotypes were 0.092, 0.582 and 0.326,

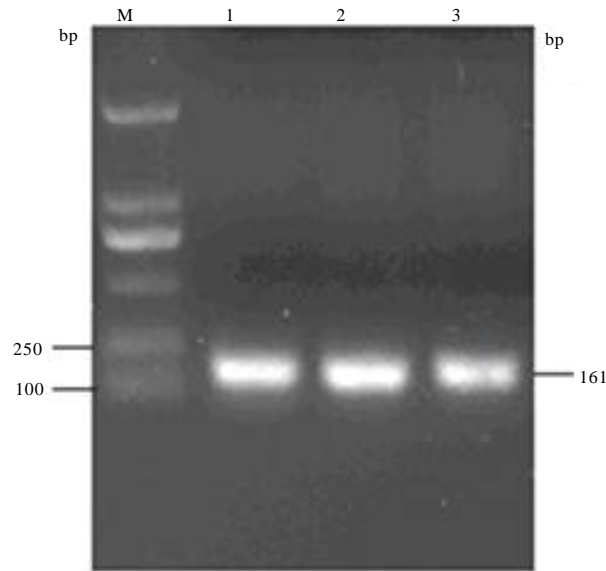


Fig. 1: Agarose gel electrophoresis for PCR products from the *FUT1* gene, M: DL2000 marker, 1-3: PCR products

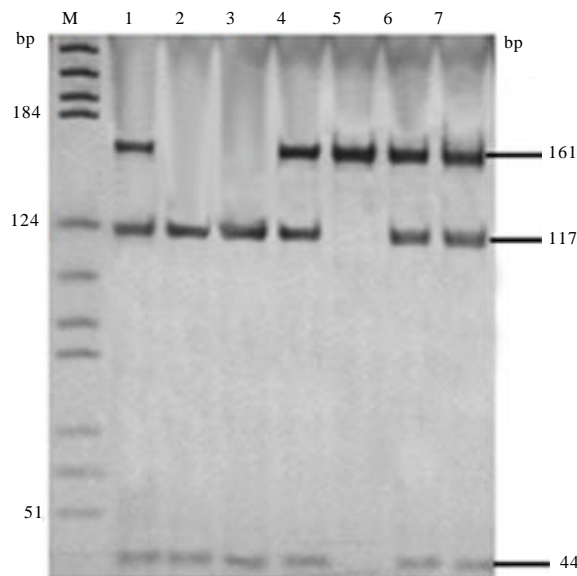


Fig. 2: PCR-RFLP results of *FUT1* gene M307 variation. M: pBR322 DNA/*BsuR* (*HaeIII*) marker, 1, 4, 6, 7: AG genotype, 2, 3: GG genotype, 5: AA genotype

respectively. Allele G was the dominant allele. According to the Chi-square (χ^2) fitness analysis, the group of Yorkshire showed a significant deviation from Hardy-Weinberg equilibrium ($p < 0.01$) (Table 1).

Relationship between *FUT1* gene polymorphism and immune indexes: The determination results of biochemical indexes and cytokines of different genotypes in Yorkshire pigs were showed in Table 2 and 3. The individuals with AG, GG genotypes were significantly higher than those with AA genotype ($p < 0.05$) in PCT, while there were no significant difference between AG and GG genotypes ($p > 0.05$). Individuals with GG genotype were significantly higher than those with AG genotype ($p < 0.01$) in the content of IFN- γ , TGF- β and TNF- α , while there were no significant difference between AA and AG, GG genotypes ($p > 0.05$). Due to the long experimental period and factors such as disease death, subjective elimination and so on, the total number of Yorkshire was less than 218 in the end. Accurate number had been exhibited in each table.

Table 1: Genotype and allele frequency of *FUT1* M307 in Yorkshire

Sample No.	Genotype frequency			Allele frequency		χ^2 value
	AA (n = 20)	AG (n = 127)	GG (n = 71)	A	G	
218	0.092	0.582	0.326	0.383	0.617	11.794

χ^2 value means the test values of different genotypes to Hardy-Weinberg balance, $\chi^2_{0.05}(1) = 3.84$, $\chi^2_{0.01}(1) = 6.63$

Table 2: Correlation analysis between the polymorphism of *FUT1* M307 and partial biochemical indexes in Yorkshire

Biochemical index	Genotype		
	AA (n = 20)	AG (n = 127)	GG (n = 71)
White Blood Cell count (WBC)	14.97±3.57	15.89±4.31	14.78±3.35
Red Blood Count (RBC)	6.46±1.12	6.37±1.00	6.01±0.76
Hemoglobin (HGB)	108.50±22.30	115.22±27.68	108.21±19.02
Hematocrit (HCT)	35.65±6.55	36.01±6.97	33.49±5.37
Mean Corpuscular Volume (MCV)	55.32±4.80	56.60±5.32	55.85±5.70
Mean Corpuscular Hemoglobin (MCH)	16.74±1.76	17.96±2.58	17.97±2.33
Mean Corpuscular Hemoglobin Concentration (MCHC)	305.86±24.25	314.04±24.84	322.21±20.35
Red blood cell distribution width (RDW)	20.14±3.32	20.19±2.97	20.08±4.03
Platelet count (PLT)	302.25±123.71	362.14±113.32	386.41±129.44
Mean Platelet Volume (MPV)	7.66±0.80	7.71±0.73	7.88±0.88
Platelet Distribution Width (PDW)	15.24±0.80	15.22±0.70	15.37±0.78
Plateletcrit (PCT)	0.22±0.05 ^a	0.30±0.08 ^b	0.30±0.09 ^b

Different superscript letters in the same row means significant difference ($p < 0.05$)

Table 3: Correlation analysis between the polymorphism of *FUT1* M307 and partial cytokines in Yorkshire

Cytokines (pg•mL ⁻¹)	Genotype		
	AA (n = 20)	AG (n = 127)	GG (n = 71)
IL-1 β	17.25±1.83	16.69±1.78	17.47±2.49
IL-4	10.19±1.25	9.09±2.83	9.60±1.63
IL-6	29.43±11.27	25.40±12.70	23.05±6.52
IL-8	46.25±32.52	42.46±21.48	50.80±25.01
IL-10	7.75±1.83	7.51±2.08	8.29±1.56
IL-12	47.27±29.49	49.72±23.76	59.70±18.19
IFN- γ	7.19±0.75 ^{ab}	7.08±0.42 ^a	7.65±0.85 ^b
TGF- β	10.25±2.51 ^{ab}	9.46±1.98 ^a	11.32±2.46 ^b
TNF- α	9.88±2.36 ^{ab}	9.37±0.96 ^a	10.48±1.77 ^b

Different superscript letters in the same row means significant difference ($p < 0.01$)

Table 4: Correlation analysis between the polymorphism of *FUT1* M307 and partial production traits in Yorkshire

Production trait	Genotype		
	AA (n = 17)	AG (n = 115)	GG (n = 62)
Eye muscle thickness (cm)	5.96±0.78	10.70±1.12	6.03±0.68
Backfat thickness at 100 kg (mm)	10.65±2.47	5.96±0.47	10.45±1.78
Age at 100 kg (day)	185.17±11.29 ^a	194.44±12.03 ^b	189.40±8.73 ^a

Different superscript letters in the same row means significant difference (p<0.01)

Table 5: Correlation analysis between the polymorphism of *FUT1* M307 and partial reproductive performances in Yorkshire

Reproductive performance	Genotype		
	AA (n = 15)	AG (n = 108)	GG (n = 57)
TNB (Parity)			
3rd	12.27±2.69 ^a	10.68±2.75 ^{ab}	10.11±3.16 ^b
4th	11.18±1.99	10.48±2.94	10.26±2.74
5th	11.90±2.02 ^a	11.40±2.19 ^a	10.04±2.51 ^b
NBA (Parity)			
3rd	11.91±2.84 ^a	10.47±2.75 ^{ab}	9.91±3.05 ^b
4th	11.08±1.87	10.28±2.97	9.97±2.42
5th	11.40±1.65 ^a	11.09±2.11 ^a	9.71±2.54 ^b

Different superscript letters in the same row means significant difference (p<0.05)

Relationship between *FUT1* gene and vital economic traits: The relationship between *FUT1* gene polymorphism and economic traits was showed in Table 4 and 5. For the age at 100 kg, individuals with AA type were significantly higher than those with AG type (p<0.05). In addition, the average age at 100 kg of AA type individuals was 4.23 days less than GG type individuals. While there were no significant difference on eye muscle thickness and backfat thickness at 100 kg among the individuals of different genotypes. From the 3rd-5th parity, the TNB and NBA demonstrated a trend of AA>AG>GG, individuals with AA type were significantly higher than those individuals with GG type (p<0.05) in terms of TNB and NBA at third and fifth parities. Individuals with AG type were significantly higher than those individuals with GG type (p<0.05) in TNB and NBA at fifth parity.

DISCUSSION

Research scholars found that western swine breeds possessed three different genotypes (AA, AG and GG) and Lingao swine breed had two susceptible genotypes GG and AG, while all the other Chinese native swine breeds only presented the susceptible GG genotype (Meijerink *et al.*, 1997; Yan *et al.*, 2003; Shi *et al.*, 2003; Bao *et al.*, 2008). After analyzing the polymorphism of *FUT1* gene at M307, this experiment also detected three kinds of genotypes in Yorkshire test group, G was the dominant allele. Chi-square (χ^2) fitness analysis showed that this locus deviated from Hardy-Weinberg equilibrium (p<0.01).

Until now, correlation analysis with *FUT1* gene and immune traits were rarely reported. Our laboratory analyzed the genetic effects of *FUT1* gene M307 on some immune indexes in foundation population of Sutai ETEC F18 resistant breeding pigs, the results demonstrated that the resistant populations to ETEC F18 with AA genotype show fine characters of stress resistance, AA pigs not only were resistant to ED and PWD in piglets but also had relatively strong resistance to disease in general (Bao *et al.*, 2012b). PCT is the percentage of platelets in blood volume and it is related to the quantity and volume of platelets. According to research, platelets, bacterial products,

endothelial cells and immune cells interact with each other. The platelets enhance the antimicrobial capacity of leukocytes, then the leukocytes generate and release cytokines and chemokines in blood and adaptive immune response is formed at last (Yeaman and Bayer, 2000). The other study considers platelets could reinforce the elimination of pathogen in blood system via the direct or indirect effect on microorganism. Through the stimulation or signal with platelets, the microorganism produces active oxygen which can play direct antimicrobial activity (Xiang and Liu, 2013). The result of this study showed individuals with AG, GG genotypes were significantly higher than those individuals with genotype AA ($p < 0.05$) in PCT, while there were no significant difference between AG and GG genotypes ($p > 0.05$), so it was possible that individuals with AG, GG genotypes might have stronger ability of immunoregulation and anti-microbial. IFN- γ is a pleiotropic cytokine that play an important role in regulating the development and function of the immune system. It is mostly produced by the T cells and Natural Killer (NK) cells. Besides the function of antiviral, anti-intracellular bacteria and parasitic protozoa, anti-tumor, IFN- γ has also the distinct immune regulation function. It can promote expression of MHC II antigens, enhance the interaction between Antigen Present Cells (APC) and T cells, accelerate differentiation of the cells and Cytotoxicity T Lymphocyte (CTL) (Chevallard *et al.*, 2002; Pan *et al.*, 2008). TGF- β is a potent regulatory cytokine with diverse effects on hemopoietic cells. The pivotal function of TGF- β in the immune system is to maintain tolerance via the regulation of lymphocyte proliferation, differentiation and survival. Besides, TGF- β controls the initiation and resolution of inflammatory responses through the regulation of chemotaxis, activation and survival of lymphocytes, NK cells, dendritic cells, macrophages, mast cells and granulocytes (Li *et al.*, 2006). TNF can prompt apoptosis; prevent tumorigenesis, virus replication and the proliferation of pathogen by means of inducing IL-1, IL-2 and IL-6 to form inflammation. As vital part of TNF, TNF- α is the essential cytokine of maintaining internal stability and resisting pathogenic factor (Tracey *et al.*, 1990). Immune parameters may represent the body's immune function and thus indirectly reflect the disease resistance of body (Ouyang *et al.*, 2004). In this study, individuals with GG genotype were significantly higher than those with AG genotype ($p < 0.01$) in IFN- γ , TGF- β and TNF- α , while there were no significant difference between AA and AG, GG genotypes ($p > 0.05$). The results above indicated that the anti-viral and immunoregulation ability of GG genotype individuals may be stronger. Considering the correlation between the polymorphism of *FUT1* gene M307 and immune parameters, it could be inferred that individuals with GG genotype possessed stronger general disease resistance ability.

For investigating the effects of *FUT1* gene on economic traits, a total of 139 hybrids finishing pigs from Yorkshire \times Meishan were slaughtered at about 88 kg bodyweight, fourteen meat quality traits and eight carcass traits were assayed for each pig and *FUT1* gene was detected by PCR-RFLP. The results indicated that *FUT1* gene M307 had good genetic effects on meat quality and carcass traits of pig (Jiang *et al.*, 2005). Bao *et al.* (2009) and Zhu *et al.* (2009) analyzed the *FUT1* gene polymorphism of Duroc, Hebao pig and its relationship with litter size, respectively. Both of those results showed that the AA type is an effective genotype for litter trait. This study analyzed the age at 100 kg of different genotypes in Yorkshire and the result showed that individuals with AA type were significantly higher than those with AG type ($p < 0.05$). In addition, the average age at 100 kg of AA type individuals was 4.23 days less than GG type individuals. While there were no significant difference on eye muscle thickness and backfat thickness at 100 kg among the individuals of different genotypes, in other words, the polymorphism of this locus had no significant effect on lean meat percentage. From the 3rd-5th parity, the individuals with

AA type were significantly higher than those individuals with GG type ($p < 0.05$) in terms of TNB and NBA at third and fifth parities, individuals with AG type were significantly higher than those individuals with GG type ($p < 0.05$) in TNB and NBA at fifth parity. Although there were no significant difference on this traits of different genotypes at fourth parity ($p < 0.05$), the total TNB and NBA demonstrated a trend of AA>AG>GG, this was in agreement with previous reports (Bao *et al.*, 2009; Zhu *et al.*, 2009). The TNB and NBA of continuous parities showed the same trend in Yorkshire, so it was clear that individuals with AA genotype have higher litter size.

The general or special disease resistance ability of livestock and poultry usually exist genetic antagonism with production traits, a negative correlation was found among them (Tang *et al.*, 2002). This experiment also indicated that Yorkshire pigs with the AA genotype in M307 of *FUT1* gene have higher growth rate and litter size, while the general disease resistance ability was relatively poor. So, when launching the breeding practice of anti-ETEC F18 in Yorkshire, it required great attentions. To avoid the stress resistance of breeding group being affected observably, during the process of MAS based on genetic marker of the *FUT1* gene M307, the immune responses ability of breeding group in Yorkshire could be improved by methods such as selective breeding, nutritional regulation and so on.

CONCLUSION

In conclusion, this study preliminarily demonstrated that Yorkshire pigs with AA genotype (resistant to ETEC F18) at position 307 in *FUT1* gene not only have excellent genetic characteristics of faster growth rate, but also have some higher production performances, but were relatively weak in resisting general diseases. It is necessary to pay close attention to the immune responses ability of test group during the process of utilizing the loci of *FUT1* at M307 to carry out MAS, in case the stress resistance of groups would be affected significantly.

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