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A Polymorphism in the IFN-γ Gene is Associated with Immune Response and Economic Traits in Landrace Pig

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Abstract: Interferon-gamma (IFN-γ) is essential for modulating immune responses in mammals. In this study, one Single Nucleotide Polymorphism (SNP) of porcine IFN-γ (Po IFN-γ) was identified at position 1165 in intron I (deletion or insertion of A). Blood samples from 306 piglets were collected. The blood parameters, antibody levels of PRRSV, CSFV and PRV were measured for all piglets at 1, 17, 32 days ages, respectively. In addition, 11 growth traits were also measured for all pigs. Association analysis between the IFN-γ- + 1165 + A polymorphism and antibody levels, blood parameters and growth traits were conducted. Significant effects of the SNP genotype was observed on 1st day PRV Ab, muscle depth ($p<0.01$), 1st day CSFV Ab, 32nd day MCHC, hucklebone width and daily gain from birth to 100 kg ($p<0.05$). Thus, the single nucleotide deletion polymorphism in IFN-γ could be an important genetic marker for both immune response and growth traits and can be potentially used in pig breeding.

Key words: Landrace pig, immune response, growth traits, IFN-γ, SNP, disease resistance

INTRODUCTION

Interferon-gamma (IFN-γ, also termed immune interferon or type α interferon) is a pleiotropic cytokine produced by T lymphocytes and Natural Killer (NK) cells and plays instructive roles not only in antiviral activity (Charley *et al*., 1988; Sammuel, 1991; Sadler *et al*., 2008) but also in modulating immune responses via its immunoregulatory activities (Charley *et al*., 1988; Farrar and Schreiber, 1993; Williams *et al*., 1993; Weining *et al*., 1996; Fromm and Ehrlich, 2001). In human, previous study discovered that different IFN-γ genotypes significantly influence the disease susceptibility of IgA nephropathy in Japanese patients (Masutani *et al*., 2003). Single base change polymorphic variants with pulmonarty tuberculosis susceptibility patients was also identified (Lopez-Maderuelo *et al*., 2003).

Other studies suggested an association between the genetic ability to produce levels of IFN-γ and susceptibility to develop chronic HBV infection (Ben-Ari *et al*., 2003; Lio *et al*., 2002, Lu *et al*., 2002). In pig, experiments *in vivo* and *in vitro* indicated that porcine interferon have the effect of defense and depression to some infectious diseases threatening the animal husbandry production. *In vitro* studies indicated pretreatment with IFN-γ profoundly inhibited PRRSV replication in porcine macrophages (Bautista and Molitor, 1999). Pigs pretreated with rPoIFN-γ were protected from virulent FMDV attack or had delayed appearance of clinical signs since this effect is dose dependent (Yao *et al*., 2008). An *in vivo* experiment also suggested that injecting both CSFV vaccine and IFN-γ could strengthen porcine defense ability against CSFV (Suradhat *et al*., 2001).

Porcine IFN-α/β can suppress effectively the replication of foot-and-mouth disease virus (Chinsangaram *et al*., 1999). Based on the extensive anti-virus activity, there were studies focused on cloning interferon genes and constructing bioreactors recently. Cheng *et al*. (2007) discovered that porcine IFN-α is a powerful adjuvant for recombinant FMD protein vaccine and could aid in vaccination against FMDV in swine.

However, few reports were found on the SNP identification and association studies of porcine IFN-γ. Owing to the importance of porcine IFN-γ in antiviral effect and immune response, the aim of the study was to

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detect its Single Nucleotide Polymorphisms (SNPs) and to carry out association analysis between IFN-γ and various immunological parameters as well as growth traits.

MATERIALS AND METHODS

SNP detection: Chen *et al.* (2008) discovered this SNP by sequencing of IFN-γ PCR products in different porcine breeds (Landrace, Yorkshire, Duroc, Pietrain, Synthethic and Erhualian). Polymerase Chain Reaction (PCR) primers were designed from the sequence of *S. scrofa* DNA for IFN-γ (GenBank accession number X53085). The PCR primers were as follows: IFNγ_F: 5'-ATTTTCTTTTCCTTATTATACTTGTTT-3' and IFNγ_R:5'-TTTTCTCTTCCACCCTCTGTT-3'. The PCR reactions were performed in a 10 μL reaction mixture containing 10 ng of porcine genomic DNA, 5 μL of 2×PCR Reaction Mix, 5 μL ddH_2O, 0.2 μL of 10 μM each primer, 0.1 μL of Taq polymerase (2.5 U μL^{-1}). Amplification conditions were: 94°C for 5 min, followed by 30 cycles of 94°C for 30 sec, 53°C for 30 sec and 72°C for 30 sec and a final extension step at 72°C for 5 min. This primer pair amplifies a 200 bp PCR fragment. PCR reactions were performed on Thermal cycler and the PCR products were examined in a 1% agarose gel.

Animal samples and DNA preparation: In total, 306 DNA samples of Landrace piglets, 17 DNA samples of sires and 36 DNA samples of dams were genotyped. The fragments amplified were analyzed by the Single Strand Conformation Polymorphism (SSCP) technique (Fig. 1). The PCR product was mixed (v/v = 1:1.5) with a 6 x loading buffer (30 mM Ethylenediaminetetraacetic Acid (EDTA) pH8.0, 36% Glycerol, 0.05% Xylene Cyanol FF and 0.05% Bromophenol Blue), denatured at 95°C for 10 min, chilled on ice and loaded on 12% polyacrylamide gels (29 acrylamide: 1 bis-acrylamide) containing 1 x TBE (Tris base-boric acid-EDTA) buffer. Gels were run at 140 V for 20 h at constant temperature (4°C) in 1×TBE buffer. The fragments were visualized with a silver staining technique and the PCR products of different SSCP patterns were analyzed in a 12% polyacrylamide gels (Fig. 1). The distribution of IFN-γ allele frequency in 306 Landrace piglets was analyzed by PopGene software (Yeh *et al.*, 1999).

Phonotype collection: The traits measured in the association analysis of porcine IFN-γ included three antibody levels, 18 blood parameters and 11 growth indexes, namely, Reproductive and Respiratory Syndrome Virus Antibody (PRRSV Ab), Classical Swine Fever Virus Antibody (CSFV Ab), Pseudorabies Virus Antibody (PRV Ab), White Blood Cell Count (WBC), Red Blood Cell Count (RBC), Hemoglobin Concentration (HGB), Haematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Platelet Count (PLT), Lymphocyte percentage (LYM%), Monocyte percentage (MXD%), Neutrophil percentage (NEUT%), absolute Lymphocyte count (LYM#), absolute Monocyte count (MXD#), absolute Neutrophil count (NEUT#), Red Blood cell Distribution Width (RDW), Platelet Distribution Width (PDW), Mean Platelet Volume (MPV), Platelet Large Cell Ratio (P_LCR), body length, body height, hucklebone width, hind quarters score, muscle depth, percentage of lean meat, live backfat at 100 kg, daily gain from birth to 100 kg, daily gain from 30-100 kg, estimated breeding value of daily gain from birth to 100 kg, estimated breeding value of live backfat at 100 kg.

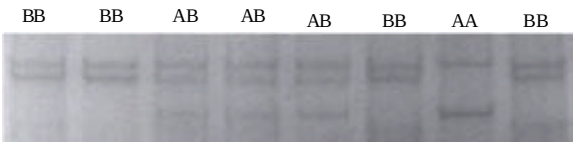

Fig. 1: Polyacrylamide gel electrophoresis (12%) showing polymorphism of the pig IFN-γ gene. Genotypes are indicated at the top of each lane

Statistical analysis: The association analysis between genotypes and traits was performed using the Mixed procedure in SAS software package (SAS Institute Inc. Cary, NC, USA) (Ma *et al.*, 2008), according to the following model:

$$Y = X\beta + Z\,b + \varepsilon$$

Where:

- Y = The response vector for observation traits
- X = The model matrix for the fixed effects for observations
- β = The 4×1 vector of fixed-effect coefficients (genotypes, sex, parity and environment)
- Z = The model matrix for the random effects for observations
- b = The 2×1 vector of random-effect coefficients (sire, dam (sire))
- ε = The vector of errors for observations; mean of b and ε are 0, think of b being constant over subjects, the ε as independent between subjects

RESULTS AND DISCUSSION

Three genotypes were detected by PCR-SSCP for the SNP, which were AA (homozygote for insertion of A), BB (homozygote for deletion of A) and heterozygote AB

Table 1: Distribution of PCR-SSCP-IFN-γ polymorphism in 306 Landrace piglets

Genotype	Allele frequency	χ^2	Pdf = 1
AA	133	0.194	0.6596
AB	135		
BB	38		
A	0.6552		
B	0.3448		

Table 2: Association analysis of the IFN-γ SNP with immune response and growth traits

Trait	Genotype (n)	Lsmean±SE	p-value
1-day CSFV Ab	AA (130)	71.3918±4.6765^{a}	0.0186
	AB (134)	70.0143±4.616^{ab}	
	BB (38)	63.7308±5.0024^{B}	
1-day PRV Ab	AA (130)	0.07981±0.03324^{B}	0.0028
	AB (134)	0.1073±0.03168^{B}	
	BB (38)	0.2043±0.03975^{a}	
32-day MCHC	AA (113)	266.68±2.5319^{ab}	0.0346
	AB (119)	268.98±2.4027^{a}	
	BB (33)	263.13± 3.0281^{b}	
Hucklebone width	AA (61)	17.335±0.1158^{ab}	0.0337
	AB (52)	17.1574±0.1039^{b}	
	BB (14)	17.555±0.1629^{a}	
Daily gain from	AA (61)	648.76±9.3921^{b}	0.0337
birth to 100 kg	AB (52)	668.75±8.4259^{a}	
	BB (14)	677.66±13.4086^{a}	
Muscle depth	AA (61)	51.5607±1.0441^{B}	0.0034
	AB (52)	51.4193±0.9367^{B}	
	BB (14)	56.2760±1.4835^{a}	

The lowercase letter (a, b) indicates significant difference level at ($p<0.05$), the capital letter (B) indicates significant difference level at $p<0.01$

(Fig. 1). The numbers of genotypes AA, AB and BB were 133, 135 and 38, respectively. The IFN-γ- +1165 + A allele gained advantage and its frequency was 0.6552. Analysis in PopGene showed that the observed genotypic values of 306 piglets were not statistically different from the expected values based on the Hardy-Weinberg equilibrium ($p = 0.6596$, $p<0.05$) (Table 1). The results indicated that different genotypes of the SNP in IFN-γ intron I was significantly associated ($p<0.05$) with 1st day CSFV Ab, PRV Ab and 32nd day MCHC (Table 2). Further analysis also showed this SNP is associated ($p<0.05$) with hucklebone width, daily gain from birth to 100 kg and muscle depth (Table 2). Moreover, the CSFV Ab (1 day) with AA, MCHC (32 days) with AB, PRV Ab (1 day), hucklebone width daily gain from birth to 100 kg and muscle depth with BB genotype pigs were highest, respectively (Table 2). There was a significant difference for CSFV Ab (1 day), MCHC (32 days) and hucklebone width between three genotypes, respectively.

Blood parameters and antibody levels can reflect the immune competence of the animals to some extent. MCHC is a very important index in the diagnosis of human hemophthisis, such as IDA (iron deficiency anemia) and MA (megaloblastic anemia). CA short tandem repeat polymorphism in the first intron of IFN-γ is associated with the susceptibility of human aplastic anemia but has no relation to the severity of the human aplastic anemia (Zhang *et al*., 2008).

Classical Swine Fever (CSF) and Pseudorabies (PR) are economically important diseases. Proper antibody levels are important to protect the pig from infection of these viruses. Higher secondary antibody avidity was noted in higher response pigs and it was positively correlated with antibody to Hen Egg-White Lysozyme (HEWL) (Appleyard *et al*., 1992). The pigs vaccinated with CSFV vaccine had significantly higher CSFV-specific IFN-γ secreting cells than the unvaccinated pigs (Suradhat *et al*., 2001). A missense polymorphism Q67R (PoIFN-γ cDNAs of Duroc breed and Landrace/Duroc hybrid encoded Q67 and R67, respectively) could markedly reduce antiviral activity of PoIFN-γ protein and a PRV-based plaque inhibition assay was established to determine antiviral activity of PoIFN-γ (Fan *et al*., 2007). Thus, IFN-γ may be important in both CSFV antibody and PRV antibody responses.

CONCLUSION

Differences in pig growth traits may relate to its health and immune response in different individuals. We found a single nucleotide deletion or insertion of porcine IFN-γ is associated with the daily gain from birth to 100 kg, muscle depth and hucklebone width of pigs. This is the first report on the association between the IFN-γ gene and these three growth traits, so the single nucleotide deletion polymorphism of IFN-γ could be an important genetic marker for both immune response and growth traits and can be potentially used in pig breeding. These association results suggest that this SNP may simply link to quantitative trait loci of growth traits. However, the mechanism on why this SNP was associated with the above-mentioned traits needs to be further investigated.

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