Journal of Animal and Veterinary Advances 10 (14): 1841-1844, 2011
ISSN: 1680-5593

Polymorphism Identification and Association Analysis of *IRF3* Gene in Pig

Zhihua Cai, Like Wang, Deyi Liu, Shenghe Li, Xunsheng Pang and Yuxin Cheng
College of Animal Science, Anhui Science and Technology University,
233100 Fengyang, P.R. China

Abstract: In this study, *IRF3* gene was chosen as a candidate gene for evaluating its effect on porcine immune traits. A SNP (HQ026024: g.4252T>C) in exon7 of *IRF3* gene was demonstrated by PCR-SSCP analysis and sequencing. Immune traits including IFN-γ and IL10 concentrations in serum were measured when the pigs are at 20th day of age. The further association analysis of SNP genotypes with immune traits were also conducted in 3 pig breeds including Large white, Landraces and Chinese indigenous breed Dingyuan black pig. The results indicated that the SNP of *IRF3* gene had highly significant effects on level of IFN-γ and IL10 (day 20) in serum ($p = 0.0344$; $p = 0.0121$) and ratio of IFN-γ to IL10 (day 20) in serum ($p = 0.0315$). The results suggested that the *IRF3* gene could be regarded as a molecular marker gene for genetic selection of these immune traits in the pig breeding program.

Key words: Pig, *IRF3* gene, polymorphism, PCR-SSCP, association analysis, China

INTRODUCTION

Interferon Regulatory Factors (IRFs) are a family of transcription involved in interferon-inducible genes regulation, viral response, cytokine signaling, cell growth regulation and hematopoietic development (Nguyen *et al*., 1997; Taniguchi *et al*., 1995). The *IRF* gene family consists of nine members encoding transcription factors that share a highly conserved helix-turn-helix DNA-binding domain and a less conserved protein-binding domain (Taniguchi *et al*., 2001). Most IRFs regulate the expression of IFN-α and -β after viral infection. IRFs play important roles in viral immune response, cytokine signaling, cell growth regulation and hematopoietic development (Nguyen *et al*., 1997; Honda and Taniguchi, 2006) thus, IRFs could be important marker genes for porcine immune response and disease susceptibility due to their drivers and potent effects on the immune system.

Cytokines are important mediators of the immune responses and vary with health and disease status. IFN-γ is critical for innate and adaptive immunity against viral and intracellular bacterial infections and for tumor control (Schoenborn and Wilson, 2007). IL-10 has pleiotropic effects in immunoregulation and inflammation. It down-regulates the expression of Th1 cytokines, MHC class II antigens (Redpath *et al*., 1999; Lohoff and Mak, 2005). Differences in levels of these immune traits and their ratios in serum among individuals under same conditions provide evidences for genetic control. Identification of these genes and their potential functional mutation on these immune variants may help to improve immune capacity in pigs. Interferon Regulatory Factor 3 (*IRF3*) gene is one of nine members of IRF-family and pays an important role in IFN regulation. Considering the effects on IFN immune response and cytokines signaling pathway of *IRF3* gene, we firstly investigated polymorphisms of all exons region of porcine *IRF3* gene and then analyzed the association between the SNP and immune traits (IFN-γ, IL-10, IFN-γ/IL-10) in serum to evaluate the possible effect of the *IRF3* gene in 3 pig populations including Large white, Landrace and Chinese indigenous breed Dingyuan black pig.

MATERIALS AND METHODS

Animals and sampling: The animals consisted of 258 pig lets distributed in 3 pig breeds including big white pig (96), Landrace (89), Dingyuan black pig (73). Big white pigs and Landrace were from the experimental farm of the Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China. Dingyuan black pigs were from breeding stock farm in Dingyuan county, Anhui province, China. The blood samples were collected from each piglet 1 day before the vaccination (day 20). All blood samples were directly injected into VACUETTE® serum clot activator tubes. Ear tissue samples of all pigs were also collected for DNA extracting.

Measurement of IFN-γ and IL-10 concentration in serum: Immune traits including IFN-γ and IL-10 concentrations in each serum sample were measured using a commercial ELISA kit (Biosource, Carlsbad, California) according to the manufacturer's instructions.

Corresponding Author: Zhihua Cai, College of Animal Science, Anhui Science and Technology University, 233100 Fengyang, P.R. China

All samples were arranged randomly in each plate and a standard curve was fitted for each plate to calculate IFN-γ and IL-10 concentrations in each serum sample.

Polymorphism detection and PCR-SSCP: Genomic DNA was isolated from the ear tissue sample using phenol/chloroform extraction and ethanol precipitation (Sambrook *et al*., 1989). Base on the cloned porcine IRF3 genomic DNA sequence (GenBank Accession No.: HQ026024), seven specific primers which spanned seven exons region of the *IRF3* gene were designed by software Oligo6.0 (Table 1). The PCR was carried out in a total volume of 25 μL including 50-80 ng of template DNA, 10 pmol of each primer, 250 μM dNTPs, 2.5 μL 10×PCR buffer (with $MgCl_2$) and 1.5 U Taq polymerase (TaKaRa Biotechnology, China). PCR was performed in a MJ research PTC-200 Thermal Cycler (BIO-RAD, USA) under the following reaction procedure: initial denaturation at 94°C for 5 min followed by 35 cycles of denaturation at 94°C for 1 min, annealing at X°C for 45 sec (X was shown in Table 1) and extension at 72°C for 1 min with a final extension step at 72°C for 5 min. PCR-SSCP method was used to find the mutation site in the amplified regions. Aliquots of 6 μL PCR products were mixed with 6 μL denaturing solution (95% formamide, 25 mM EDTA, 0.025% xylene-cyanole and 0.025% bromophenol blue), heated at 98°C for 10 min and then chilled in ice immediately. Denatured DNA was subjected to 12% Polyacrylamide Gel Electrophoresis (PAGE) in 1×TBE buffer and constant Voltage (130 V) for 15 h at a constant temperature of 4°C. After the process above, the gels were stained with 0.1% silver nitrate and visualized with 2% NaOH solution (containing 0.1% formaldehyde). The PCR products which represented different PCR-SSCP genotypes including both homozygous and heterozygous genotypes were purified with the Gel Midi PCR DNA purification kit (Tiangen Biotechnology, China) and sequenced using the ABI377 sequencer. Those tested sequences were aligned by using the DNAMAN software (Version 5.2.2) to identify the mutation site.

Table 1: Specific primers used for SNP detection of the porcine *IRF3* gene

Fragments	Primers sequence (5'~3')	Product size (bp)	Annealing Tm (X°C)
IRF3-Exon1	GAAGATGCCGAAGCCTCCT CCCATGGGAACTCAGAAGC	170	60.5
IRF3-Exon2	GAAATCCCATCCCTCCAAG CCTGGGGGCAAGATTTAAG	270	59.0
IRF3-Exon3	GATGCAGAAAGGGCATGAG AAGTGAGGCTTTGCTTGGAG	205	59.2
IRF3-Exon4	GCACTCACCGTCGTCATTC CAGAAAAGGCCGTGGAAATA	243	60.0
IRF3-Exon5	CGCACTCACCTTCGATGA GCTCAAGAGTGGGAGTTCCAG	300	59.5
IRF3-Exon6	GATCGTCCCTCCACTCCAG TAGAGGGCCTTCTTCCATCC	200	60.3
IRF3-Exon7	GAAGCCGTTTATTGGTCGAG AGGTTGTCCCCATGTGTCTC	221	59.0

Association analysis: Association analysis between genotypes of the SNP and immune traits were tested by using SAS9.1.3 software based on the general linear model. The general linear model is as follows:

$$y_{ijkl} = u+B_i+M_j+G_k+p_l+e_{ijkl}$$

Where:

y_{ijkl} = The vector of the phenotypic value for immune traits of pig k
B_i = The vector of the breed effect
M_j = The vector of the ELISA plate effect
G_k = The vector of the fixed effect corresponding to genotypes of the SNP
p_l = The random environmental effect
e_{ijkl} = The random residuals

RESULTS AND DISCUSSION

SNP identification and genetic variation analysis: Sequence comparisons among 3 pig breeds revealed one SNP (HQ026024: g.4252T>C) in exon 7 of the porcine *IRF3* gene and the three genotypes of the SNP were also clearly obtained (Fig. 1). The SNP (HQ026024: g.4252T>C) of IRF3 is not missense mutation and not to induce amino acid substitution. A total of 258 DNA samples from animal populations were genotyped and allele frequencies were determined for each breed (Table 2). Genetic variation analysis demonstrated that

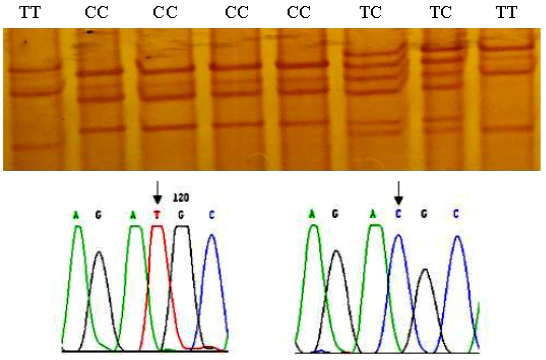

Fig. 1: The PCR-SSCP electrophoresis patterns for the SNP (HQ026024: g.4252T>C) of porcine *IRF3* gene. Lane TT, TC and CC represent different genotypes

Table 2: Genotype frequencies and allelic frequencies of the *IRF3* gene determined by PCR-SSCP in 3 pig populations

		Genotype frequencies			Allele frequencies	
Breeds	Number	TT	TC	CC	T	C
Large white	96	67	16	13	0.78	0.22
Landrace	89	48	31	10	0.71	0.29
Dingyuan black	73	51	22	0	0.85	0.15

Table 3: Association analysis of the SNP genotypes of *IRF3* gene with immune traits in 3 pig populations

	Genotypes (Least sequares mean±SE)			
Traits	TT (n = 166)	TC (n = 69)	CC (n = 23)	p value
IFN-γ (day 20)	29.403±4.413^{A}	94.032±8.378^{B}	101.134±33.576^{B}	0.0344
IL-10 (day 20)	151.462±13.673^{a}	146.516±28.861^{a}	89.874±42.462^{b}	0.0121
IFN-γ/IL-10 (day 20)	0.863±0.147^{a}	1.553±0.316^{b}	1.147±1.265^{b}	0.0315

^{a,b}Signed by small letters differ significantly at $p<0.05$; ^{A,B}means signed by capital letters differ significantly at $p<0.01$

allele frequencies were not significantly different among 3 pig breeds. The allele T is obviously dominant in 3 detected breeds. In contrast, the allele C has lower frequencies and CC genotype is not detected in Chinese indigenous breed Dingyuan black pig.

Association analysis of immune traits and SNP genotypes: We performed an association study to determine whether the SNP affected immune traits in 3 pig populations. Association analysis results showed the SNP of *IRF3* gene had significant effects on level of IFN-γ and IL10 (day 20) in serum ($p = 0.0344$; $p = 0.0121$) and ratio of IFN-γ to IL10 (day 20) in serum ($p = 0.0315$) (Table 3). Further, analysis results also showed that IFN-γ and IL10 level in serum of pigs with TT genotype were significantly higher than those of pigs with CC genotype ($p<0.05$) but ratio of IFN-γ to IL10 with CC genotype were significantly higher than those of pigs with TT genotype ($p<0.05$). The ratio of IFN-γ/IL-10 production reflects the capacity to activate or inhibit monocytic and T lymphocytic functions and a higher ratio has also been shown to be associated with depressive disorders (Maes, 1999).

The lower ratio of IFN-γ to IL-10 of Dingyuan black pig should be explained for Chinese indigenous breed may have better T lymphocytic balance and immune capacity than western commercial pig breeds. The results also implied that genetic background is one of the most important influences of immune traits. Interferon Regulatory Factor 3 (IRF3) plays an important role in initiating cellular interferon-stimulated gene-mediated antiviral responses. IRF 3 contributes to the host response during pseudomonas aeruginosa lung infection in mice (Carrigan *et al*., 2010).

In human, IRF3-dependent pathways are critical for control of herpes simplex virus type 1 central nervous system infection (Menachery *et al*., 2010); *IRF3* gene are also revealed negative regulation of intracellular hepatitis C virus replication (Yamashiro *et al*., 2006) and polymorphisms of *IRF3* gene are also association with lung cancer risk, systemic lupus erythematosus (Akahoshi *et al*., 2008; Shen *et al*., 2009).

In pig, another INF regulatory factor (IRF1) has been reported significantly associated with cytokine traits (Liu *et al*., 2009) and Classical swine fever virus Npro interacts with *IRF3* gene and induces its proteasomal degradation (Bauhofer *et al*., 2007). While the SNP (HQ026024: g.4252T>C) of IRF3 in this study is not missense mutation or not to induce amino acid substitution but synonymous SNPs can affect protein expression by alteration or increase in the stability of the mRNA (Capon *et al*., 2004) and a silent polymorphism changes substrate specificity (Kimchi-Sarfaty *et al*., 2007).

The immune system plays an essential role in disease resistance of animals. The genes affect the response of immune system could be researched as the candidate genes. The results indicated that the SNP of IRF3 may be regarded as a genetic marker gene for immune traits with effects on IFN-γ, IL-10 and ratio of IFN-γ to IL-10 in serum. However, the number of pigs analyzed is limited further investigation is also required among other populations of pigs to confirm the association between the SNP and immune traits.

CONCLUSION

In this study, a SNP in exon 7 of the *IRF3* gene was identified by PCR-SSCP method. Further association analysis between the SNP and immune traits was also performed, the results showed that the SNP of *IRF3* gene was significantly associated with IFN-γ to IL-10 level of serum in 258 pigs from 3 pig populations. All these results will provide a foundation for further studies of the porcine *IRF3* gene and may as a candidate gene for marker-assist selection in pig disease resistance breeding program.

ACKNOWLEDGEMENT

This study was supported by Scientific Technology Plan of Education Department of Anhui province (Grand No. 2006KJ034C).

REFERENCES

Akahoshi, M., H. Nakashima, A. Sadanaga, K. Miyake and K. Obara *et al*., 2008. Promoter polymorphisms in the IRF3 gene confer protection against systemic lupus erythematosus. Lupus, 17: 568-574.

Bauhofer, O., A. Summerfield, Y. Sakoda, J.D. Tratschin, M.A. Hofmann and N. Ruggli, 2007. Classical swine fever virus N^{pro} interacts with interferon regulatory factor 3 and induces its proteasomal degradation. J. Virol., 81: 3087-3096.

Capon, F., M.H. Allen, M. Ameen, A.D. Burden, D. Tillman, J.N. Barkerand and R.C. Trembath, 2004. A synonymous SNP of the corneodesmosin gene leads to increased mRNA stability and demonstrates association with psoriasis across diverse ethnic groups. Hum. Mol. Genet., 13: 2361-2368.

Carrigan, S.O., R. Junkins, Y.J. Yang, A. Macneil, C. Richardson, B. Johnston and T.J. Lin, 2010. IFN regulatory factor 3 contributes to the host response during Pseudomonas aeruginosa lung infection in mice. J. Immunol., 185: 3602-3609.

Honda, K. and T. Taniguchi, 2006. IRFs: Master regulators of signaling by Toll-like receptors and cytosolic pattern-recognition receptors. Nat. Rev. Immunol., 6: 644-658.

Kimchi-Sarfaty, C., J.M. Oh, I.W. Kim, Z.E. Sauna, A.M. Calcagno, S.V. Ambudkar and M.M. Gottesman, 2007. A silent polymorphism in the *MDR*1 gene changes substrate specificity. Sciences, 315: 525-528.

Liu, Y., X. Lu, Y.R. Luo, J.P. Zhou, X.Y. Liu, Q. Zhang and Z.J. Yin, 2009. Effect of single nucleotide polymorphism of IRF1 gene on cytokine traits in three pig breeds. J. Anim. Vet. Adv., 18: 2346-2350.

Lohoff, M. and T.W. Mak, 2005. Roles of interferon-regulatory factors in T-Helper-cell differentiation. Nat. Rev. Immunol., 5: 125-135.

Maes, M., 1999. Major depression and activation of the inflammatory response system. Adv. Exp. Med. Biol., 461: 25-46.

Menachery, V.D., T.J. Pasieka and D.A. Leib, 2010. Interferon regulatory factor 3-dependent pathways are critical for control of herpes simplex virus type 1 central nervous system infection. J. Virol., 84: 9685-9694.

Nguyen. H., J. Hiscott and P.M. Pitha, 1997. The growing family of interferon regulatory factors. Cytokine Growth Factor Rev., 8: 293-312.

Redpath, S., A. Angulo, N.R.J. Gascoigne and P. Ghazal, 1999. Murine cytomegalovirus Infection down-regulates MHC class II expression on macrophages by induction of IL-10. J. Immunol., 162: 6701-6707.

Sambrook, J., E.F. Fritsch and T. Maniatis, 1989. Molecular Cloning: A Laboratory Manual. 2nd Edn., Cold Spring Harbor Laboratory Press, New York.

Schoenborn, J.R. and C.B. Wilson, 2007. Regulation of interferon-γ during innate and adaptive immune responses. Adv. Immunol., 96: 41-101.

Shen, M., R. Vermeulen, P. Rajaraman, I. Menashe and X. He *et al*., 2009. Polymorphisms in innate immunity genes and lung cancer risk in Xuanwei, China. Environ. Mol. Mutagen., 50: 285-290.

Taniguchi, T., H. Harada and M. Lamphier, 1995. Regulation of the interferon system and cell growth by the IRF transcription factors. J. Cancer Res. Clin. Oncol., 121: 516-520.

Taniguchi, T., K. Ogasawara, A. Takaoka and N. Tanaka, 2001. IRF family of transcription factors as regulators of host defense. Annu. Rev. Immunol., 19: 623-655.

Yamashiro, T., N. Sakamoto, M. Kurosaki, N. Kanazawa and Y. Tanabe *et al*., 2006. Negative regulation of intracellular hepatitis C virus replication by interferon regulatory factor 3. J. Gastroenterol., 41: 750-757.