aJava

Asian Journal of Animal and Veterinary Advances

Academic
Journals Inc.

www.academicjournals.com

Asian Journal of Animal and Veterinary Advances 6 (6): 580-586, 2011
ISSN 1683-9919 / DOI: 10.3923/ajava.2011.580.586

Association Analysis between the Polymorphism of the SLC11A1 Gene and Immune Response Traits in Pigs

Yang Liu, Xiaotian Qiu, Jingjing Xu, Fang Hu, Yuhua Li, Hejun Li, Yuanfang Gong and Qin Zhang

Key Laboratory of Animal Genetics and Breeding of the Ministry of Agriculture, College of Animal Science and Technology, China Agricultural University, Beijing 100193, People's Republic of China

Corresponding Author: Prof. Qin Zhang, College of Animal Science and Technology, China Agricultural University, Beijing 100193, China Tel: 0086-10-62732634 Fax: 0086-10-62732634

ABSTRACT

Solute carrier family 11 member 1 (SLC11A1) plays an important role in the innate immune response against intracellular pathogens. To evaluate effect of SLC11A1 gene on immune response capacity in pigs, one SNP in intron1 was demonstrated by PCR-RFLP and sequencing method. Immune response traits include six normal hematology traits (white blood cell, red blood cell, hemoglobin, granulocyte, lymphocyte and monocyte), four T lymphocyte subpopulations ($CD4^-CD8^-$, $CD4^-CD8^+$, $CD4^+CD8^-$, $CD4^+CD8^+$) and CSFV antibody titer were measured in three pig breeds (Large White, Landrace and Songliao Black) after vaccinated with classical swine fever live vaccine at the 21st day after birth. The further association analysis between the SNP genotype and immune response traits were conducted. The SNP of SLC11A1 gene had significant effect on level of monocyte ($p = 0.010$) and $CD4^-CD8^+$ percentage ($p = 0.041$). The animals with AA genotype had significant higher monocyte and $CD4^-CD8^+$ percentage than that of animals with GG and GA genotype ($p<0.05$). Present results also suggest that SLC11A1 could be a marker gene for genetic selection of disease susceptibility in pigs.

Key words: SLC11A1, polymorphism, PCR-RFLP, pig, immune traits, association analysis

INTRODUCTION

Genetic regulation of immune response and selection for disease resistance in livestock has been documented and should be considered as an economical and prophylactic approach to improve animal health (Stear *et al*., 2001; Crawley *et al*., 2005). In animals, the heritability estimates for antibody and cell-mediated immune responses are moderate to high indicating that genetic selection is feasible (Mallard *et al*., 1989). The advances in animal genomics provide possibilities to determine the genetic markers related to the immune response, which will be greatly helpful to breeding for disease resistance through marker-assisted selection (Gholizadeh *et al*., 2008).

The solute carrier family 11 member 1 (SLC11A1) also called NRAMP1 gene and is the first positional cloned gene related to infectious disease susceptibility in mouse (Vidal *et al*., 1993). In mammals, SLC11A1 gene is associated with the transport of iron and other divalent cations, the transition metal irons are essential for many cellular functions, including regulation of transcription through DNA binding proteins and metal response elements (Blackwell *et al*., 2003). SLC11A1 is also involved in the innate immune response against pathogens (viral, bacterial and protozoan)

(Awomoyi, 2007). The association of the polymorphisms of the SLC11A1 gene with the resistance or susceptibility to some specific diseases has been demonstrated in some species, e.g., pediatric tuberculosis disease (Malik *et al*., 2005) and pulmonary Mycobacterium avium complex infection in human (Tanaka *et al*., 2007); the *Salmonella typhimurium*, *Leishmania donovani* and *Mycobacterium bovis* BCG in mouse (Vidal *et al*., 1993); salmonellosis in chicken (Hu *et al*., 1997); leishmaniasis in dog (Sanchez-Robert *et al*., 2005); rhodococcus equi pneumonia in horses (Halbert *et al*., 2006) and Brucella abortus in water buffalo (Capparelli *et al*., 2007). The cDNA of the porcine SLC11A1 gene has been reported (Tuggle *et al*., 1997) and several SNPs were also detected and some were found to be associated with the fecal bacteria counts of piglets after challenging with Salmonella cholevasuis (Tuggle *et al*., 2005).

Considering its important role on defending bacterial and viral infection of SLC11A1 gene in human and animals, in this study, we analyzed the association between the polymorphisms in intron 1 of the SLC11A1 gene and some indicators of innate immune response to evaluate its potential effects in three pig breeds including Large White, Landrace and Songliao Black pig, one Chinese native breed.

MATERIALS AND METHODS

Animals: The pigs used in this study are consisting of 345 pigs, including 77 Landrace, 180 Large White and 88 Songliao Black pigs (a Chinese indigenous breed). All pigs were raised in 2007, 2008 and under standard indoor conditions at the experimental farm of the Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China. At 21 days of age, all pigs were vaccinated with Classical Swine Fever (CSF) live vaccine. The first blood samples were collected from each piglet one day before the vaccination (day 20) and two weeks after the vaccination, the second blood samples were collected (day 35). All blood samples were directly injected into VACUETTE® Serum Clot Activator tubes. Ear tissue samples of all pigs were also collected for DNA extract.

Gene amplification by polymerase chain reaction: Polymerase chain reaction (PCR) was performed to amplify the 935 bp fragment of the SLC11A1 gene which comprises the upstream 5' UTR, exon 1 and intron 1 region, using the forward primer (F 5'- TGAACACTTCATTTAACAGAA GA-3') and the reverse primer (R 5'-GGCTCTGAGCAGGGAAGACT-3') as described by Tuggle *et al*. (2005).

The PCR reactions were carried out in a total volume of 25 μL solution containing 2.5 μL PCR buffer, 200 μM of each dNTP, 25 pmol of each primer and 1.5U Tax polymerase (TaKaRa Biotechnology, China). The PCR program 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 45 sec, annealing at 62°C for 35 sec and extension at 72°C for 1 min with a final extension step at 72°C for 5 min.

Polymorphism detection by PCR-RFLP: The SNP in the 935 bp fragment of the SLC11A1 gene which have been revealed by Tuggle *et al*. (2005) was detected in our experimental pigs. For detection of the SNP, the PCR products (6 μL) were digested with 10 U of SmaI enzyme (TaKaRa Biotechnology, China), mixed with 2 μL of 10×T buffer and 2 μL 0.01% BSA and added water up to 20 μL. The mixtures were incubated at 37°C overnight, then the digested PCR products were analyzed by 3~4% agarose gel electrophoresis and ethidium bromide staining. The gel was

visualized by Gel Doc XR system (Bio-Rad, Hercules, CA, USA). The genotypes were identified according to the restriction fragment patterns and later confirmed by sequencing in ABI PRISM 377 DNA sequencer (Applied Biosystems, CA, USA).

Measurement of clinical phenotypes: For all piglets, the following clinical phenotypes were measured.

CD4/CD8 T lymphocyte subpopulations: The percentages of various CD4/CD8 T lymphocyte subpopulations were obtained by the double cytofluorometric analysis. The blood cells were incubated with 10 μL of mouse anti porcine CD4-FITC (Serotec UK) and 10 μL of mouse anti porcine CD8-RPE (Serotec UK) for 30 min and then washed with 0.1 M PBS (pH 7.2, containing 0.3% bovine serum albumin). The red blood cells were digested by 0.1% ammonium oxalate solution. The stained cells were detected by EPICS Flow Cytometer (Beckman-Coulter Company, USA).

Normal hematology values: The normal hematology values, including white blood cell (WBC), red blood cell (RBC), hemoglobin (HGB), granulocyte (GR), lymphocyte (LY) and monocyte (MO), All blood samples were directly injected into VACUETTE® Serum Clot Activator tubes for detection of blood routine parameters and T lymphocyte subpopulation; at the same time, serum samples were also isolated for lysozyme concentration detection. All these hematological parameters were measured by MEK-6318K type full automatic Hematology Analyzer (Nihon Kohden, Japan).

CSFV antibody titer: The serum samples were tested for the presence of antibodies against CSFV with the commercialized ELISA assays HerdCheck CSFV AB (IDEXX) following the instructions given by the manufacturers. The percentage inhibition (PI) for each sample was calculated by the formula as described by Ghadersohi *et al.* (2005):

$$PI = (OD_N\text{-}OD_T)/(OD_N\text{-}OD_P)$$

where, OD_N, OD_T and OD_P are the OD value of negative, tested and positive sample respectively. The CSFV antibody titer (Y) was estimated using the regression equation:

$$Log_2Y = -7.56 + 56.41PI\text{-}83.85PI^2 + 44.66PI^3 \ (R = 0.993)$$

Statistical analysis: Association analysis between the polymorphisms of the SLC11A1 gene and the clinical phenotypes were performed using PROC MIXED of SAS9.13 (SAS Institute, Cary, NC, USA). The model was as below following:

$$Y_{ijklmno} = \mu + S_i + Sex_j + B_k + G_l + p_{km} + d_{kmn} + y1_{ijklmno} + e_{ijklmno}$$

where, $Y_{ijklmno}$ is the observation on the 35th day immunology traits; μ is the overall mean; S_i is the effect of the ith season; Sex_j is effect of the jth sex; B_k is the effect of the kth breed; G_l is the effect of the lth SmaI-restriction fragment length polymorphism (RFLP) genotype; p_{km} is the random effect of the mth sire within breed; d_{kmn} is the random effect of the nth dam within breed and sire; $y1_{ijklmno}$ is the covariate of the observation on the 20th day immunology traits and $e_{ijklmnop}$ is the random residual effect.

RESULTS AND DISCUSSION

Genotype and allele frequencies: A 935 bp amplified product was obtained by PCR amplification in all animals. For the SNP, there is a restriction site for restriction enzyme SmaI at site 506 which leads to the 935 bp fragment being cut down to a 505 and 430 bp fragment. The A→G transversion at site 738 in intron 1 forms another restriction site for SmaI enzyme. When the A→G mutation is present, the 430bp fragment was further cut down to a 232 and 198 bp fragment. Therefore, three patterns of restriction fragments were clearly observed after digestion with SmaI enzyme (Fig. 1). A total of 345 DNA samples from animal population were genotyped and allele frequencies were determined for each breed (Table 1). Genetic variation analysis demonstrated that the allele G is obviously dominant in three detected breeds. In contrast, the allele A has lower frequencies and AA genotype is not detected in Large White and Songliao Black pig. These results are consisting with that of Tuggle *et al.* (2005) reported.

Association analysis: Association analysis results of immune response traits and SNP genotypes were shown in Table 2, the SNP of SLC11A1 gene had significant effect on level of monocyte ($p = 0.010$) and $CD4^{-}CD8^{+}$ percentage ($p = 0.041$). The monocyte and $CD4^{-}CD8^{+}$ percentage of the AA genotype were significantly higher than those of the GG and GA genotype ($p<0.05$). Tuggle *et al.* (2005) also investigated the association between the SNP and disease resistant traits measured after challenged with Salmonella cholevasuis, however, they did not find any significant association between the SNP and monocyte and lymphocyte. Instead, a significant association between both the SNP and the fecal Salmonella count was found. These disagreements may be due to a different pig population and immune response traits and challenged different pathogen.

Monocytes play critical roles in immunopathology. The increase of the percentage of monocytes in pigs after acute CSFV infection has been reported (Lee *et al.*, 1999). When infected by CSFV, they produce immunomodulatory and vasoactive factors, such as prostaglandin E2 (PGE2), which can enhance the proliferation of lymphocyte (Knoetig *et al.*, 1999) and monocyte precursor cell

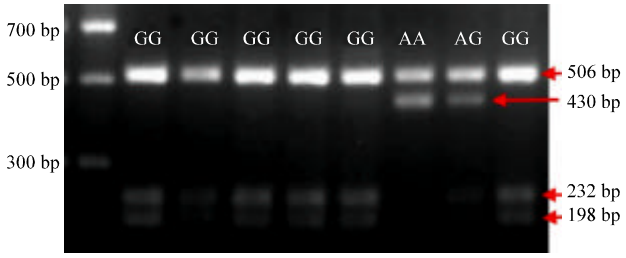

Fig. 1: PCR-SmaI-RFLP analysis of the porcine SLC11A1 gene. The first lane is marker. DNA molecular weight marker; Lane GG, AG and AA represent different genotypes

Table 1: Genotype and allele frequencies of the SLC11A1 gene determined by PCR-RFLP in three pig breeds

		Genotype frequencies			Allele frequencies	
Breed	No.	GG	AG	AA	G	A
Large white	180	0.66	0.34	0.00	0.83	0.17
Landrace	77	0.67	0.20	0.13	0.77	0.23
Songliao black	88	0.78	0.22	0.00	0.89	0.11

Table 2: Association analysis of the SNP genotypes of SLC11A1 gene with immune response traits and antibody level in three pig breeds

	Genotypes (Least square Means±SE)			
Traits[1]	GG (n = 244)	GA (n = 99)	AA (n = 20)	p-value
WBC (g L^{-1})	18.61±0.81	19.10±0.97	19.05±4.28	0.810
RBC (g L^{-1})	6.30±0.11	6.11±0.16	6.20±0.89	0.461
HGB (g L^{-1})	118.85±2.55	118.10±3.26	119.42±16.16	0.965
GR% (%)	26.12±1.36	24.76±1.78	11.85±9.24	0.232
LY % (%)	59.22±1.28	60.60±1.71	62.84±9.14	0.654
MO % (%)	14.80±0.62^{A}	14.96±0.74^{A}	25.18±3.41^{B}	0.010**
$CD4^{-}CD8^{-}$ (%)	34.41±0.80	33.85±0.94	20.53±4.06	0.082
$CD4^{+}CD8^{-}$ (%)	13.70±0.46	13.64±0.58	9.38±2.88	0.404
$CD4^{-}CD8^{+}$ (%)	39.64±0.75^{a}	40.38±0.92^{a}	58.15±4.26^{b}	0.041*
$CD4^{+}CD8^{+}$ (%)	10.41±0.46	10.43±0.56	11.62±2.49	0.905
CSFV Ab ($\log_2$)	56.64±16.22	58.77±16.72	60.83±34.69	0.810

[1]WBC: White blood cell count; RBC: Red blood cell count; HGB: Hemoglobin; GR%: Neutrophilic granulocyte count percentage; LY%: Lymphocyte count percentage; MO%: Percentage; $CD4^{+/-}CD8^{+/-}$ T-lymphocyte subsets; CSFV Ab, classical swine fever virus antibody titer. ^{a,b}Signed by small letters differ significantly at $p<0.05$. ^{A,B}Means signed by capital letters differ significantly at $p<0.01$

(Sluiter *et al*., 1982). Therefore, the more active the monocytes, the more monocyte precursor cells will be produced. SLC11A1 mediates the iron homeostasis in monocytes/macrophages and hence modulates their response to acute inflammatory stimuli (Wyllie *et al*., 2002). So, the higher percentage of monocytes in animals with AA genotype in our study may imply that the AA genotype may enhance the activity of the monocytes by mediating the iron homeostasis in monocytes.

The $CD4^{-}CD8^{+}$ T Lymphocytes also play an important role in disease resistance. Pauly *et al*. (1995) reported that $CD4^{-}CD8^{+}$ could mediate the activity of the CSFV-specific cytotoxic T lymphocyte. Suradhat *et al*. (2005) also found that $CD4^{-}CD8^{+}$ had an effect on the cytokine production following the CSFV challenge. The differentiation of the T Lymphocyte into CD4/CD8 subpopulation is regulated by some cytokines, such as interferon γ (IFN-γ), interleukin 6(IL-6) and tumor necrosis factor α (TNF-α), secreted by the antigen-presenting cells (APCs) such as macrophages, which in turn is regulated by the SLC11A1 gene (Smit *et al*., 2004). Therefore, the higher $CD4^{-}CD8^{+}$ percentage in animals with AA genotype in our study indicate that AA genotype may enhance the differentiation of the T Lymphocyte into $CD4^{-}CD8^{+}$ by regulating the activity of APCs.

In summary, a SNP of the SLC11A1 gene was identified by PCR-RFLP and the association of polymorphism of the SLC11A1 gene with the percentage of monocyte and $CD4^{-}CD8^{+}$ T lymphocyte subpopulation were firstly found; these results should supply some useful information for understanding the relationship between the SLC11A1 gene and immune responses in pigs. However, the polymorphism analyzed is not functional mutation and the small number pigs with AA genotype of the SNP is found, it should be need to confirm genetic variation among other pig populations in further studies.

ACKNOWLEDGMENTS

This study is supported by National Key Project for Basic Research and Development Plans (Grant No. 2006CB102104), China Postdoctoral Science Foundation Funded Project (Grand No. 20100470412) and National High-Tech Development Project of China (Grant No. 2006AA10Z1D7).

REFERENCES

Awomoyi, A.A., 2007. The human solute carrier family 11 member 1 protein (SLC11A1): Linking infections, autoimmunity and cancer. FEMS Immunol. Med. Microbiol., 49: 324-329.

Blackwell, J.M., S. Searle, H. Mohamed and J.K. White, 2003. Divalent cation transport and susceptibility to infectious and autoimmune disease: Continuation of the Ity/Lsh/Bcg/Nramp1/Slc11a1 gene story. Immunol. Lett., 85: 197-203.

Capparelli, R., F. Alfano, M.G. Amoroso, G. Borriello and D. Fenizia *et al.*, 2007. Protective effect of the Nramp1 BB genotype against Brucella abortus in the water buffalo (Bubalus bubalis). Infect. Immunol., 75: 988-996.

Crawley, A.M., B. Mallard and B.N. Wilkie, 2005. Genetic selection for high and low immune response in pigs: Effects on immunoglobulin isotype expression. Vet. Immunol. Immunopathol., 108: 71-76.

Ghadersohi, A., Z. Fayazi and R.G. Hirst, 2005. Development of a monoclonal blocking ELISA for the detection of antibody to Mycoplasma bovis in dairy cattle and comparison to detection by PCR. Vet. Immunol. Immunopathol., 104: 183-193.

Gholizadeh, M., G.R. Mianji and H.S. Zadeh, 2008. Potential use of molecular markers in the genetic improvement of livestock. AJAVA., 3: 120-128.

Halbert, N.D., N.D. Cohen, N.M. Slovis, J. Faircloth and R.J. Martens, 2006. Variations in equid SLC11A1 (NRAMP1) genes and associations with Rhodococcus equi pneumonia in horses. J. Vet. Intern. Med., 20: 974-979.

Hu, J., N. Bumstead, P. Barrow, G. Sebastiani, L. Olien, K. Morgan and D. Malo, 1997. Resistance to salmonellosis in the chicken is linked to NRAMP1 and TNC. Genome Res., 7: 693-704.

Knoetig, S.M., A. Summerfield, M. Spagnuolo-Weaver and K.C. McCullough, 1999. Immunopathogenesis of classical swine fever: Role of monocytic cells. Immunology, 97: 359-366.

Lee, W.C., C.S. Wang and M.S. Chien, 1999. Virus antigen expression and alterations in peripheral blood mononuclear cell subpopulations after classical swine fever virus infection. Vet. Microbiol., 67: 17-29.

Malik, S., L. Abel, H. Tooker, A. Poon and L. Simkin *et al.*, 2005. Alleles of the NRAMP1 gene are risk factors for pediatric tuberculosis disease. Proc. Natl. Acad. Sci., 102: 12183-12188.

Mallard, B.A., B.N. Wilkie and B.W. Kennedy, 1989. Genetic and other effects on antibody and cell mediated immune response in Swine Leucocyte Antigen (SLA)-defined miniature pigs. Anim. Genet., 20: 167-178.

Pauly, T., K. Elbers, M. Konig, T. Lengsfeld, A. Saalmuller and H.J. Thiel, 1995. Classical swine fever virus-specific cytotoxic T lymphocytes and identification of a T cell epitope. J. Gen. Virol., 76: 3039-3049.

Sanchez-Robert, E., L. Altet, A. Sanchez and O. Francino, 2005. Polymorphism of Slc11a1 (Nramp1) gene and canine leishmaniasis in a case-control study. J. Hered., 96: 755-758.

Sluiter, W., D. van Waarde and R. van Furth, 1982. Regulation of monocyte precursor cell proliferation by two endogenous factors. Immunobiology, 161: 219-226.

Smit, J.J., G. Folkerts and F.P. Nijkamp, 2004. Ramp-ing up allergies: Nramp1 (Slc11a1), macrophages and the hygiene hypothesis. Trends Immunol., 25: 342-347.

Stear, M.J., S.C. Bishop, B.A. Mallard and H. Raadsma, 2001. The sustainability, feasibility and desirability of breeding livestock for disease resistance. Res. Vet. Sci., 71: 1-7.

Suradhat, S., W. Sada, S. Buranapraditkun and S. Damrongwatanapokin, 2005. The kinetics of cytokine production and CD25 expression by porcine lymphocyte subpopulations following exposure to Classical Swine Fever Virus (CSFV). Vet. Immunol. Immunopathol., 106: 197-208.

Tanaka, G., J. Shojima, I. Matsushita, H. Nagai and A. Kurashima *et al.*, 2007. Pulmonary Mycobacterium avium complex infection: association with NRAMP1 polymorphisms. Eur. Respir. J., 30: 90-96.

Tuggle, C.K., C.B. Schmitz and D. Gingerich-Feil, 1997. Rapid communication: Cloning of a pig full-length NRAMP1 cDNA. J. Anim. Sci., 75: 277-277.

Tuggle, C.K., L. Marklund, T.J. Stabel, M.A. Mellencamp and A. Stumbaugh, 2005. Genetic markers for screening animals for improved disease resistance (nramp). United States Patent, 6844159B2. http://ddr.nal.usda.gov/handle/10113/6983.

Vidal, S.M., D. Malo, K. Vogan, E. Skamene and P. Gros, 1993. Natural resistance to infection with intracellular parasites: Isolation of a candidate for Bcg. Cell, 73: 469-485.

Wyllie, S., P. Seu and J.A. Goss, 2002. The natural resistance-associated macrophage protein 1 Slc11a1 (formerly Nramp1) and iron metabolism in macrophages. Microbes Infect., 4: 351-359.