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Associations of Polymorphisms in the *Mx*1 Gene with Immunity Traits in Large White×Meishan F_2 Offspring

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ABSTRACT : The mouse myxovirus resistance protein 1 (*Mx*1) is known to be sufficient to confer resistance to influenza viruses, and the gene encoding *Mx*1 is, therefore, an interesting candidate gene for disease resistance in farm animals. The porcine *Mx*1 gene has already been identified and characterized based on its homology with mouse *Mx*1; the full-length coding region of the pig *Mx*1 gene spans 2,545 bp (M65087) and is organized into 17 exons compared with the human ortholog mRNA. In this study, the exons 9, 10 and 11 and introns 6 and 9 of the porcine *Mx*1 gene were cloned and sequenced. Two SNPs were identified in exons 9, 10 and 11 but none of the SNPs led to an amino acid exchange, and the other eleven variants were detected in introns 6 and 9, respectively. Differences in allele frequency between Meishan and other pig breeds were observed within intron 6, of which an A→G substitution at position 371 was detected as an *SnaB*I PCR-RFLP. The association analysis using the Large White×Meishan F_2 offspring suggested that the *Mx*1 genotype was associated with variation in several immunity traits that are of interest in pig breeding. However, further investigations in more populations are needed to confirm the above result. (**Key Words** : Pigs, *Mx*1 Gene, *SnaB*I Locus, Immunity Traits)

INTRODUCTION

The myxovirus resistance protein (Mx) is the key component of the antiviral state induced by type I (a/b) interferons (IFN) in many species (Haller et al., 1998), and the *Mx*1 gene plays an important role in immune response, induction of apoptosis, and signal transduction. They were discovered 20 years ago in an inbred mouse strain that showed an unusually high degree of resistance towards infection with influenza A virus (FLUAV) (Horisberger et al., 1983). Early experiments demonstrated that the mouse Mx1 protein had intrinsic antiviral activity and was the sole mediator of innate immunity against FLUAV in mice (Staeheli et al., 1986; Arnheiter et al., 1990). Later, Mx proteins were characterized as high-molecular-weight GTPases (Horisberger et al., 1990; Nakayama et al., 1991) with significant homology to mammalian dynamins and yeast VPS-1 (Obar et al., 1990; Rothman et al., 1990; Staeheli et al., 1993). In humans, two distinct Mx GTPases are encoded on chromosome 21, called MxA and MxB. Only MxA has detectable antiviral activity. Human MxA has a comparatively wide antiviral spectrum against different types of viruses, irrespective of their intracellular replication site. MxA-sensitive viruses include members of the bunyaviruses, orthomyxoviruses, paramyxoviruses, rhabdoviruses, togaviruses, picornaviruses, and hepatitis B virus, a DNA virus with a genomic RNA intermediate (Haller et al., 1998; Landis et al., 1998; Chieux et al., 2001; Gordien et al., 2001). In the mouse, loss of resistability to influenza virus has been shown to be due to specific polymorphisms in the *Mx* gene. This gene is, therefore, an interesting candidate gene for disease resistance in farm animals (Morozumi et al., 2001). Immunity traits are as important as growth rate and body composition which are important characteristics in pig production. Efficient pig production systems not only demand lean individuals with high growth rate and a low conversion of feed to meat but also high resistance to disease (Wang et al., 2006). However, until now no evidence of association between polymorphisms and economic traits in the porcine *Mx* gene has been reported. The objectives of this research were to screen the gene for polymorphisms, thereby enabling further study of the functions of the *Mx*1 gene in immune competence, including considering it as candidate gene for immune capacity (disease-resistant) QTL.

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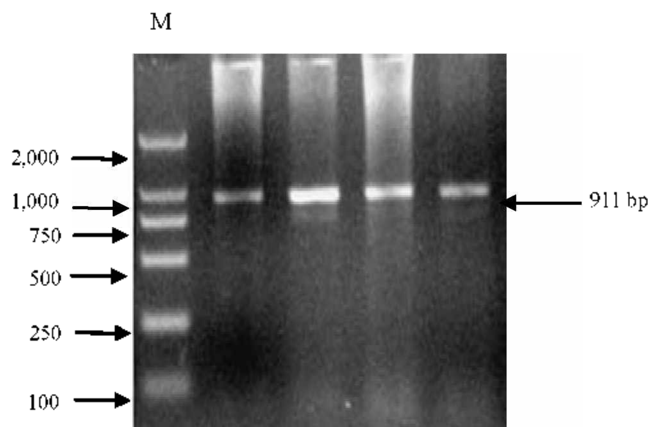

Figure 1. Agarose gel electrophoresis (1.5%) showing the PCR amplification results of intron 6 of pig *Mx*1 gene. M, marker DL 2000 DNA Ladder.

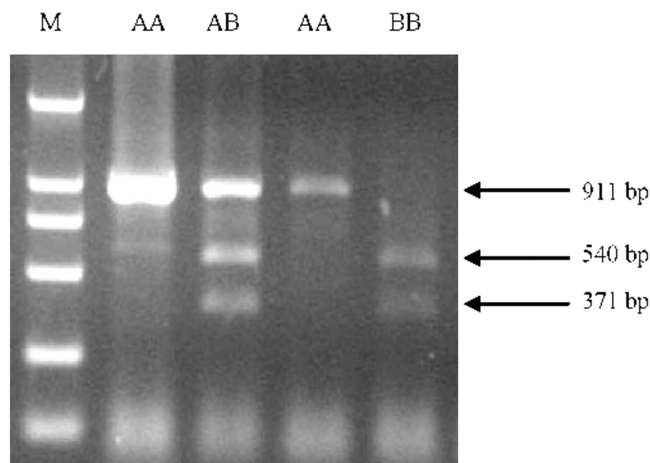

Figure 2. Agarose gel electrophoresis (1.5%) showing polymorphisms in PCR fragments of *Mx*1 after digestion with *SnaB*I. The genotype (AA, AB and BB) are shown at the top of the lanes. M, marker DL 2000 DNA Ladder.

MATERIALS AND METHODS

Pig populations and DNA preparation

A three-generation resource family was investigated, and 109 F_2 offspring with immunity traits were used in this study. All the pigs were bred and raised at the genetic nucleus station of Huazhong Agricultural University, China.

Blood was collected in 50 mM EDTA at pH 8.0 to prevent coagulation, and genomic DNA was extracted from white blood corpuscles. DNA extraction was carried out according to the procedure described by Xiong and Deng (1999).

Traits

Immunity traits components: total erythrocytes (RBC), leukocyte counts (WBS), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and red (cell) distribution width (RDW) were determined on blood corpuscle analyzing machine MEK-5216K (made in Japan). Albumin and total protein were tested on automatic biochemical analyzer. The effect of reducing reaction of nitroblue tetrazolium (NBT) on neutrophil was measured according to the procedure described by Shimizu et al. (1977). All the immunity traits were determined at the end of the 60th day.

Genomic DNA amplification and sequence analysis

PCR primers: intron 1, forward primer 1 F: 5'-GCATTTCTGCTGACGGG-3', reverse primer 1 R: 5'-AATAAACCATCTTCCTCCTT-3'; intron 6, forward primer 6 F: 5' -TAGGCAATCAGCCATACG-3', reverse primer 6 R: 5'-GGTCCTGTCTCCTTCGG-3'; intron 9, forward primer 9 F: 5'-CCAGAAAATAACAGAGGAGT-3', reverse primer 9 R: 5'-TCGCATCTTGGTAAACAG-3'. The polymerase chain (PCR) reactions were carried out in 25 μl volumes containing the following reagents: 1.5 μl of porcine genomic DNA (100-250 ng μl^{-1}), 0.5 μl of dNTPs (10 mM each), 20 pmols of each primer, 2.5 μl of 10× buffer, and 1 unit of Taq DNA polymerase. PCR was run in the GeneAmp PCR system 9600 (Perkin-Elmer Co., Norwalk, CT, USA) thermocycler as follows: initial denaturation at 94°C for 4 min, 35 cycles for 94°C for 45 s, optimal temperature for 45 s, 72°C for 1 min, and a final extension time of 10 min at 72°C.

The primer pairs 1, 6, and 9 produced 1,045 bp, 911 bp, and 589 bp fragments, respectively. The amplified fragment was cloned into the pGEM-T vector (TaKaRa, Dalian of China) and was sequenced using standard M13 primers. Sequence analysis revealed seven SNPs within intron 6, of which G371A can be detected as an *SnaB*I PCR-RFLP. For the PCR-RFLP assays, 7.5 μl of PCR products were digested with 5 U *SnaB*I (TaKaRa) in 1× digestion buffer with 1× BSA added in a total volume of 10 μl. Following digestion for 4 h at 37°C, the digested products were separated by electrophoresis on a 1.5% agarose gel in 1×TAE buffer and stained with 0.5 μg ml^{-1} ethidium bromide. The 911 bp PCR product (Figure 1) was digested into two fragments of 371 bp and 540 bp. The result is shown in Figure 2.

Allele frequencies for the *Mx*1 *SnaB*I polymorphism (Table 1) are significantly different in native Chinese Meishan breed compared with western Large White and Landrace breeds. Allele *B* was found only in Meishan pigs, with frequency of 0.4444.

Association analysis

To study the association between carriers of different

Table 1. Distribution of *SnaB*I-RFLP genotype and allele frequencies among pig breeds

Breeds	N	Genotype			Allele frequency	
		AA	AB	BB	A	B
Large White	148	148	0	0	1	0
Landrace	20	20	0	0	1	0
Meishan	27	10	10	7	0.5556	0.4444

Table 2. Statistical analysis of *Mx*1 *SnaB*I-RFLP genotypes with immunity traits

Traits	*Mx1* genotype ($\bar{x}$±SE)			Effect ($\bar{x}$±SE)	
	AA	AB	BB	Additive	Dominance
WBC (T/L)	26.6457±2.1418	26.7110±4.8948	22.0421±5.2833	-2.3018±2.8503	-1.1835±2.8333
RBC (G/L)	7.9086±0.1304^{Aa}	8.9171±0.3000^{Bb}	8.6477±0.324^{ABb}	0.3695±0.1746*	-0.3195±0.1736
TP (g/L)	71.4448±1.4731	74.8189±3.3890	70.8127±3.6589	-0.3160±1.9721	-1.8451±1.9613
ALB (g/L)	32.7331±0.5286	33.9652±1.2161	32.6263±1.3129	-0.0534±0.7076	-0.6428±0.7038
NBT (%)	11.0146±0.3736	11.3973±0.8915	10.6461±0.9280	-0.1843±0.5002	-0.2835±0.5112
HGB (mmol/L)	9.0038±0.1009	8.8552±0.2306	9.0040±0.2490	0.0001±0.1343	0.0743±0.1335
HCT (%)	50.2767±0.8753^{a}	55.7680±2.0137^{b}	52.7808±2.1741^{ab}	1.2521±1.1718	-2.1196±1.1654
MCV (fL)	63.5677±0.4853^{a}	62.426±1.1166^{ab}	60.8348±1.2055^{b}	-1.3665±0.6497*	-0.1125±0.6462
MCH (Pg)	1.1907±0.0322^{a}	0.9644±0.0822^{b}	1.1157±0.0556^{ab}	-0.0428±0.0204*	0.0343±0.0198
MCHC (mmol/L)	18.0389±0.4795^{a}	14.3222±1.2254^{b}	19.3722±0.8279^{a}	-0.3040±0.2824	0.5601±0.2806*
RDW (% CV)	16.8931±0.1216^{a}	17.357±0.2798^{ab}	17.5346±0.3231^{b}	0.3208±0.1628	- 0.0716±0.1620

All the data in the table are least square means±standard error. Values in each line with different lower-cased superscripts are significantly different at $p<0.05$, with upper-cased superscripts different at $p<0.01$. * $p<0.05$ and ** $p<0.01$.

WBC = White blood cell counts; RBC = Red blood cell counts; TP = Total protein; ALB = Albumin; NBT = Nitrobiue tetrazolium; HGB = Hemoglobin; HCT = Hematocrit; MCV = Mean corpuscular volume; MCHC = Mean corpuscular hemoglobin concentration; MCH = Mean corpuscular hemoglobin; RDW = Red (cell) distribution width.

genotypes and the trait values, the *SnaB*I PCP-RFLP was tested in 109 F_2 pigs of a Large White×Meishan reference family (Zhang et al., 2005). The animals were born and raised in Huazhong Agricutural University Jingpin pig station. They were given twice daily diets formulated according to age under a standardized feeding regimen and free access to water (Zhang et al., 2006; Wang et al., 2007). The association between genotype and immunity traits was performed with the least squares method (GLM procedure, SAS version 8.0), and the model used to analyze the date was assumed to be:

$$Y_{ij} = \mu+S_i+G_j+b_{ij}X_{ij}+e_{ij}$$

where, Y_{ij} is the observation of the trait; μ is the least square mean; S_i is the effect of *i*th sex (*i* = 1 for male and 0 for female); Gj is the effect of *j*th genotype (j = AA, AB or BB); b_{ij} is the regression coefficient of the carcass weight, and e_{ij} is the random residual.

At this locus, the number of animals of genotypes AA, AB, and BB was 82, 15, and 12, respectively. According to the method of Liu (1998), both additive and dominance effects were also estimated using REG procedure of SAS version 8.0, where the additive effect was denoted as -1, 0, and 1 for AA, AB, and BB, respectively, and the dominance effect was denoted as 1, -1, and 1 for AA, AB, and BB, respectively.

RESULTS AND DISCUSSION

The results of tests for *Mx*1 genotypes and immunity traits are given in Table 2.

In Table 2, statistically significant associations with RBC, HCT, MCV, MCH, MCHC, and RDW were found, but no significant conclusion was made on other immunity traits. Results of the single marker analysis revealed pigs with the AB genotype had significantly higher RBC and HCT, and lower MCH and MCHC when compared with genotypes AA or BB (Table 2). Meanwhile, allele A seemed to be associated with increase in MCV and decrease in RDW. However, the present estimations are based on a weaker data structure, as BB genotype is carried only by the native Meishan pigs and the genotype AA mainly by western Large White and Landrace breeds. It can be assumed that the effects of genetic background influenced the results. Moreover, it is also likely that the intronic mutations are unlikely to be directly responsible for the effects on porcine immunity traits, therefore, the associations observed may simply reflect linkage disequilibrium between the *Mx*1 polymorphisms and casual genetic variation in some other gene distant from *Mx*1 or the chromosomal interval and gene interactions. According to the results obtained, analysis of more animals is necessary to confirm the association between the *Mx*1 genotype and immunity traits in F_2 intercross pedigree pigs.

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