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# Effects of the polymorphisms of Mx1, BAT2 and CXCL12 genes on immunological traits in pigs

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**Abstract** It is necessary that genetic markers or biomarkers can be used to predict resistance towards a wide range of infectious diseases. In the present study, we estimated the potential markers and measured their relationship with heritabilities of a wide range of immune traits. Polymorphisms in exon 13 of Mx1, intron 25 of BAT2 and intron 3 of CXCL12 were identified by sequencing, and the genotypes were analyzed by PCR-RFLP in a resource population composed of 352 pure breed Landrace piglets at days 0, 17 and 32 after birth. Associations of single-nucleotide polymorphisms (SNPs) in these genes with a variety of immunological traits and antibody levels for pig reproduction and porcine respiratory syndrome virus (PRRSV), pseudorabies virus (PRV) and classical swine fever virus (CSFV) were performed. The performance of GG genotype of BAT2 on hemoglobin concentration (HBG) and hematocrit (HCT) of piglets at day 0 was significantly higher than that of the AA and AG individuals. For Mx1, compared with CT genotype, the pigs with TT or CC generated more PRRS antibody at day 0. The piglets with CT genotype had highly significant difference of PRV antibody from those with CC and TT genotypes at day 0. And the piglets with CC genotype had higher level red blood cell count (RBC), hemoglobin concentration (HBG) and hematocrit (HCT) than those with CT and TT genotypes at day 17. For the C7462G SNP in the intron 3 of CXCL12, the PRV antibody level of piglets with the CG genotype were higher than that of piglets with CC and GG genotypes at day 17, and the mean corpuscular volume (MCV) of GG piglets were larger than that of CC and CG individuals at day 0. At the locus 7331 bp in the intron 3 of CXCL12, there were significantly differences of mean corpuscular hemoglobin concentrations (MCHC) at day 0 and white blood cell count (WBC) at day 32, which showed the trend GG or AG>AA, AA>AG or GG, respectively. The pigs with AA or GG genotype had more platelet distribution width (PDW), mean platelet volume (MPV) and platelet-large cell ratio (PLR) at day 17 than those with AG. The results of this study indicated that polymorphisms in Mx1, BAT2 and CXCL12 genes were significantly associated with the immunological traits in Landrace piglets and had potential application value for marker-assisted selection of pig breeding with disease resistance.

**Keywords** Mx1 · BAT2 · CXCL12 · Landrace pig · Immunological traits · Polymorphism

S.J. Wang and W.J. Liu contributed equally.

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## Introduction

It has been recognized that genetic factors have impacts on resistance to infection and the genes involved in the hosts' responses to challenges by infectious pathogens. The major histocompatibility complex (MHC) is one of the most extensively studied areas in the human genome because of the contribution of multiple variants at this locus to autoimmune responses, infectious and inflammatory diseases, and in organ rejection following transplantation. The swine

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MHC is known as the Swine Leukocyte Antigen complex (SLA), and is located on chromosome 7 spanning thecentromere [1]. The SLA encodes cell surface-expressed, highly polymorphic glycoproteins which present antigenic peptides to T cells and thus initiate specific immune responses [2]. It is involved in graft rejection, production of lymphocytotoxic serum antibodies, immune response (Ir) genes and the stimulation of mixed lymphocyte reactions [3]. The SLA haplotype has been associated with differences in the magnitude of the antibody response to a Bordetella bronchiseptica vaccine [4], a pseudorabies vaccine [5] in commercial pigs and with decreased incidence of encysted Trichinella spiralis larvae in miniature pigs [6]. HLA-B associated transcript 2 (named BAT2) lies within the class III region, and it is conserved between humans and other mammals [7]. Hashimoto et al. [8] reported the BAT2 microsatellite polymorphism was possibly associated with the inflammatory process of pancreatic $\beta$-cell destruction during the development of IDDM (insulin dependant diabetes mellitus).

The Myxovirus resistance protein (Mx1) is first reported to resist to the lethal effects of influenza A virus in A2G mice [9]. Mx1 is present in all vertebrate species, it plays an important role in the immune response, induction of apoptosis, and signal transduction. The presence of Mx1 protein in peripheral blood leukocytes of patients with acute viral infections first indicated the possibility of using this as a biomarker to discriminate between viral and bacterial infections [10]. Haller et al. [11] reported that the Mx1 was the key component of the antiviral state induced by interferons (IFN) in many species. Watanabe's group described a series of Mx alleles in chicken breeds, some of which encode proteins with specific antiviral activity against influenza and vesicular stomatitis virus (VSV) [12]. Thus the gene encoding Myxovirus resistance protein 1 (Mx1) is an interesting candidate for disease resistance in farm animals [13].

Stromal cell-derived factor-1 (SDF-1; CXCL12), a CXC chemokine, binding to CXC chemokine receptor 4 (CXCR4) controls adhesion and transendothelial migration of leukocytes, lymphocytes and monocytes, also induces expression of a number of chemokines including monocyte chemoattractant protein-1, IL-8, and interferon-inducible protein-10, especially during immune and inflammatory reactions [14]. Recent studies report that the chemokines and their receptors played important roles in inflammatory responses, angiogenesis, tumor growth and metastasis [15–17]. They involve in many areas of immunology and human development, including organogenesis, vascularization, hematopoiesis, immune cell homing and trafficking, embryogenesis [18, 19]. CXCL12 and CXCR4 regulate the migration of certain cells in the lymphoid system and also control the migration of tumor cells such as breast cancer cells [20, 21] and melanoma [22]. Locati and Murphy [23] reported that chemokines signal through G protein-coupled seven-transmembrane-domain receptors and were primarily involved in immunosurveillance, activation, and recruitment of specific cell populations during disease progression. CXCL12 was also a key regulator of B-cell lymphopoiesis, hematopoietic stem cell mobilization, and leukocyte migration [23].

BAT2, Mx1 and CXCL12 are considered as promising candidate genes that may play an important role in resistance to infections. We analyzed the association of polymorphisms of Mx1, BAT2 and CXCL12 with immune traits in our resource population, the results will contribute useful information to further research.

## Materials and methods

### Animals and trait data collection

All animal procedures were carried according to protocols approved by Biological Studies Animal Care and Use Committee P. R. China. The polymorphisms and association studies were performed in a resource population composed of 352 pure breed Landrace piglets at days 0, 17 and 32 after birth. These animals with detailed pedigree information were from the Wenshi group in Guangdong province in China. These pigs were immunized with vaccines CSFV (Swine Fever Live, Qilu Animal Health, China) at days 0 and 35 after birth, Mycoplasma (Respi-Sure Mycoplasma, Pfizer, China) and PRRSV (PRRSV NVDC-JXA1 Strain, DaHuaNong, China)/(Ingelvac PRRS MLV, Boehringer Ingelheim, China) at days 7 and 21, respectively. Blood samples were obtained via jugular venipuncture at days 0, 17 and 32, and submitted promptly for haematological analysis using Cell Dyn 3200 hematology cytoanalyzer (Abbott Laboratories, Wiesbaden, Germany). Serum was collected within 2 h and stored at $-80°C$ for detection of antibody levels.

The methods described previously [24] were applied to measure 17 immunological traits including white blood cell count (WBC), red blood cell count (RBC), hemoglobin concentration (HBG), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count (PLT), lymphocyte percentage (LYM), absolute lymphocyte count (ALYM), red cell distribution width (RDW), platelet distribution width (PDW), mean platelet volume (MPV), platelet-large cell ratio (PLR), and antibody levels to pig reproduction and porcine respiratory syndrome virus (PRRSV), pseudorabies virus (PRV) and classical swine fever virus (CSFV).

**Table 1** The primers and amplification condition for PCR-RFLP

| Gene | Primer Name | Primer sequence (5′ → 3′) | Annealing temperature (°C) | Product size (bp) | Restriction enzyme |
|---|---|---|---|---|---|
| BAT2 | BAT2-1L | gcgcacctgtttgtgatc | 55.7 | 146 | Bcl I |
| | BAT2-1R | tctccagctttctgcccta | | | |
| Mx1 | Mx1-1L | agaaggtcagagagaaggacg | 59.7 | 323 | FnuD II |
| | Mx1-1R | ggaaaaccagatgcaagcg | | | |
| CXCL12 | CXCL12-1L | cagtggaagggtcattttcc | 60.7 | 388 | BSTN1 and Bsr I |
| | CXCL12-1R | agagctgggtgacccctaat | | | |
| | CXCL12-2L | aatcattttcactgcagtct | 49.9 | 216 | BsmA I |
| | CXCL12-2R | aaggggcattttccaagta | | | |

### Genomic DNA preparation

Tissue samples were cut into small pieces on ice and then suspended in PBS (10 mM $Na_2HPO_4$, 1.4 mM $KH_2PO_4$, 137 mM NaCl, 2.7 mM KCl, pH 7.4) and centrifuged at 8000 rpm for 10 min. The pellet was suspended in 1× SET (150 mM NaCl, 50 mM Tris–HCl, 1 mM EDTA, pH 8.0) and incubated overnight at 56°C with 0.5% SDS containing 100 μg/ml proteinase K. The DNA used for PCR was extracted by phenol–chloroform–alcohol extraction and ethanol precipitation [25], and the purity and the concentrations of DNA were measured by both spectrophotometry (at 260 and 280 nm absorbance).

### Polymorphism identification and genotyping with PCR-RFLP

The genomic DNA fragments amplified by PCR with specific primers (as shown in Table 1) were purified, sequenced, and Blasted with each other using SeqMan (DNASTAR, Inc., Madison, WI, USA), to identify polymorphisms in BAT2 (LOC100137083), Mx1 (LOC AB259856) and CXCL12 (NCBI GeneID: 494460). The SNP c.12164+79G>A in intron 25 of BAT2 creates a site for the restriction enzyme Bcl I, the SNP c.86172+140C>T in exon 13 of Mx1 creates a site for FnuD II, and the SNPs c.6696+7462C>G, c. 6696+7331A>G and c. 6696+7373/7374AC>CT in intron 3 of CXCL12 creat sites for BsmA I, BSTN1 and Bsr I, respectively. The genotypes of individual animals were identified by PCR-RFLP (PCR restriction fragment length polymorphism), and the DNA products were digested with restriction enzymes (showed in Table 1), then were separated on 8% polyacrylamide gels and visualised by silver-staining (Fig. 1).

### Statistical analysis

Analysis of variance were performed to estimate the effects of the genotypes on each of the immunological traits using the Mixed procedures and Macro statements in SAS 8.0 (SAS Institute Inc. Cary, NC, USA) with REML estimation methods implemented with a Newton–Raphson algorithm. To improve homoscedasticity, arcsine transformation was performed on proportional data. The following mixed models were used to estimate the effects of the genotypes on the immunological parameters.

$$Y_{ijklmn} = \mu + \text{Genotype}_i + \text{Sex}_j + \text{Parity}_k + \text{Environment}_l + \text{Sire}_m + \text{Dam}_n(\text{Sire}_m) + \varepsilon_{ijklmn}$$

where Y is the response vector for the immunological traits above; $\mu$ is the overall mean of each traits; Genotype (i = 3: caused by 1 allelic variation), sex (j = 2), parity (k = 2: first vs. non-first) and environment (L = 2: pigpen 1&2) were considered as fixed effects whereas sire (m = 17) and dam (n = 36) as random effects. In addition, $\varepsilon$ is residual errors; for each immunological trait, the least-square means between genotype classes on each time of blood sampling were computed using lsdmeans statement and compared with a Bonferroni adjustment using pdiff, sderr and adjust = bon options in SAS 8.0 (SAS Institute Inc, Cary, NC).

## Results

### Identification of polymorphisms and analysis of Genotyping and frequencies in the three genes

Five SNPs in BAT2, Mx1 and CXCL12 genes were identified using direct sequencing of PCR products in Landrace pigs, the genotypes of the above five SNPs were detected by PCR-RFLPs. The fragments of BAT2, Mx1 and CXCL12 were amplified from swine genomic DNA. The PCR reaction yielded a 146 bp product in intron 25 of BAT2, and a mutation c.12164+79G>A was identified by sequencing directly, which was digested by endonuclease Bcl I, and the result showed 3 genotypes named AA (131/15 bp), AG (146/131/15 bp) and GG (146 bp) (Fig. 1a).

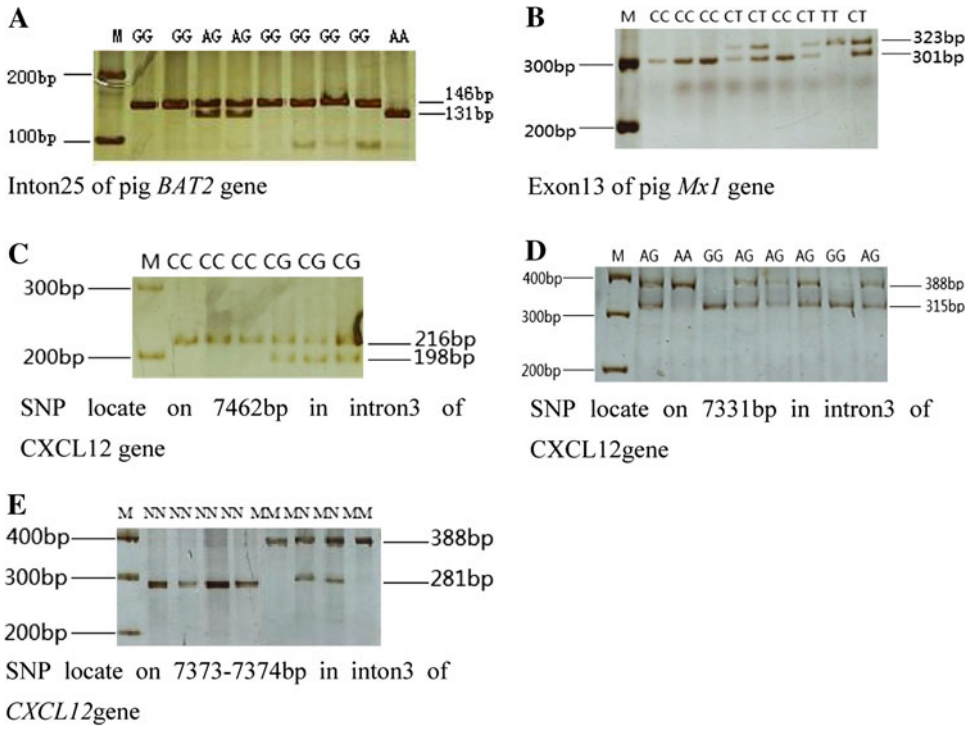


**Fig. 1** PCR-RFLP genotypes of SNP of gene on 8% PAGE gel

**Table 2** The frequencies of genotype and allele of three genes in Landrace pig

| Gene | Number of pig | Genotypic frequencies | | | Allelic frequencies | |
|---|---|---|---|---|---|---|
| BAT2 | 280 | GG | GA | AA | G | A |
| | | 0.236 (66) | 0.518 (145) | 0.246 (69) | 0.495 | 0.505 |
| Mx1 | 225 | TT | TC | CC | T | C |
| | | 0.062(14) | 0.147 (33) | 0.791 (178) | 0.136 | 0.864 |
| CXCL12 | 324 (locate7462 bp) | CC | CG | GG | C | G |
| | | 0.302 (98) | 0.435 (141) | 0.263 (85) | 0.520 | 0.480 |
| | 352 (locate 7331 bp) | AA | AG | GG | A | G |
| | | 0.088 (31) | 0.409 (144) | 0.503 (177) | 0.293 | 0.707 |
| | 350 (locate 7373–7374 bp) | MM | MN | NN | M | N |
| | | 0.254 (89) | 0.509 (178) | 0.237 (83) | 0.509 | 0.491 |

The PCR reaction yielded a 323 bp product in exon 13 of Mx1, and a mutation c.86172+140C>T was identified by sequencing directly, which was digested by endonuclease FnuD II, and the result showed 3 genotypes named CC (301/21 bp), CT (323/301/21 bp) and TT (323 bp) (Fig. 1b). The PCR reaction yielded a 216 bp product in intron 3 of CXCL12, and a mutation c.6696+7462C>G was identified by sequencing directly, which was digested by endonuclease BsmA I, and the result showed 3 genotypes named CC (216 bp), CG (216/198/18 bp) and GG (198/18 bp) (Fig. 1c). Another PCR reaction yielded a 388 bp product in intron 3 of CXCL12, and the mutations c. 6696+7331A>G and c. 6696+7373/7374AC>CT were identified by sequencing directly, which was digested by endonuclease BSTN1 and Bsr I, respectively, and the results showed 6 genotypes named AA (388 bp), AG (388/315/73 bp) and GG (315/73 bp) in the SNP located at 7331 bp of CXCL12 (Fig. 1d), and MM (388 bp), MN (388/281/107 bp) and NN (281/107 bp) (Fig. 1e) in the SNPs located at 7373–73374 bp of CXCL12. The genotypic and allelic frequencies of the five SNPs in Landrace are described in Table 2.

## Association of SNPs and immunological traits

Association studies were performed in Landrace resource population. These results indicated that the polymorphisms were associated with some immunological traits at various levels.

Table 3 showed the results of association analysis between BAT2 genotypes and immunological traits. The pigs with GG genotype had a significant higher level of HBG and HCT at day 0 than those with AG or AA ($P < 0.05$), while there were no differences at days 17 and 32.

These results of association analysis between Mx1 genotypes and immunological traits were given in Table 4. Compared with CT genotype, the pig with TT or CC generated more PRRSV antibody at day 0 ($P < 0.05$). There was a highly significant difference of PRV antibody at day 0 among pigs with CT and CC, TT genotype ($P < 0.01$). And the levels of RBC, HBG and HCT of piglets with CC, CT or TT genotype at day 17 were significantly different ($P < 0.01$, or $P < 0.05$).

The associations between immunological traits and the three SNPs of CXCL12 were different. For the SNP located at 7462 bp, the MCV level of GG pigs at day 0 were significantly higher than that of CC or CG ($P < 0.01$), the pigs with CG generated more PRV antibody at day 17 ($P < 0.05$) (Table 5). In the SNP located at 7331 bp, there were significantly differences in MCHC at day 0 and WBC at day 32 ($P < 0.05$), which showed the trend GG or AG>AA, AA>AG or GG, respectively, the pigs with AA or GG genotype had more PDW, MPV and PLR at day 17 than that with AG ($P < 0.01$) (Table 6). However, no significant difference was found at locus 7373–7374 bp.

## Discussion

The BAT2 gene is located in the HLA (SLA in swine) class III region between the class I and class II regions, and the class III region has the highest gene density of the three HLA regions. Population studies suggest that susceptibility to a number of autoimmune diseases is associated with certain HLA haplotypes [26]. Some polymorphic markers located within the areas surrounding the complement factors (C2, C4, Bf), HSP70 and TNF genes, including the BAT2 and TNF-linked microsatellite loci in the class III region, are known to be associated with multiple HLA class II- or class I-linked diseases. Singal et al. [27] reported that microsatellite BAT2 138 allele defined the risk for development of rheumatoid arthritis (RA). Hashimoto et al. [8] reported that the BAT2 microsatellite polymorphism was also associated with the age-at-onset of IDDM and possibly with the inflammatory process of pancreatic $\beta$-cell destruction during the development of IDDM. QTLs (quantitative trait locus) for WBC, RDW, HBG, PLT, MCV, MCH, and NGC (genotyping center) have previously been located on swine chromosome 7 [28], and BAT2 is also located at swine chromosome 7, the genetic mutation of BAT2 may effect some routine blood indexes. In the present study, the polymorphism in intron 25 of BAT2 was demonstrated by PCR-RFLP, the association analysis showed that the different genotypes of BAT2 had significant effect on HBG and HCT at age of day 0 in Landrace ($P < 0.05$). The results indicated GG genotype had significant effect on HBG and HCT, The GG genotype is a dominant genotype to improve organism immunity or/and the disease resistance in pigs.

The Mx1 is regulated by IFN $\alpha/\beta$, and the mutation of Mx1 influence the nonspecific immunity and specific immunity by IFN-mediated, as well as the system of alpha/beta interferons (IFN $\alpha/\beta$) is a central part of the host innate immune defense system. Some studies showed that IFN $\alpha/\beta$ were crucial for limiting early replication and spread of viruses [29]. They induce a number of proteins involved in antiviral actions, such as the 2′,5′-oligoadenylate synthetase, the double-stranded RNA-activated protein kinase, and the Mx proteins (Mx1 in mice and MxA in humans) [30]. Muller-Doblies et al. [31] reported the Mx1 mRNA and protein were up-regulated by IFN $\alpha$-mediated in bovine cells in a virus- and host cell-specific manner. The present study confirmed the association between Mx1 and immune function. The pigs with TT or CC genotype had more PRRS antibody than those with CT genotype at day 0. However, the pigs with CT genotype had more PRV antibody than those with TT or CC genotype at day 0. It is well-know that the antibodies in newborn piglets from maternal antibodies were main source, and could protect themselves against the pathogen and enhance the immunity. The previous study demonstrated that Mx proteins are interferon (IFN)-induced and they exhibited a range of antiviral capabilities. The murine Mx1 protein was critical for effective innate immune defense against influenza [32] and the human MxA protein had a broad antiviral spectrum against many families of RNA viruses, including Orthomyxoviridae, Rhabdoviridae, and Bunyaviridae [33]. Pig Mx1 had the resistance to vesicular stomatitis virus and inhibition to the replication of influenza A virus [34, 35]. Zhang et al. [36] had reported that the PRRSV infection could induce porcine Mx1 expression. In the present study, the mutation of Mx1 caused an amino acid change from Gly to Val which had a significant effect on PRRSV and PRV antibody at day 0 in Landrace. Mx1 protein might interact with IFN to improve the immune responses to PRRV and PRV.

The chemokine CXCL12 and its receptor, CXCR4, have well known roles in the patterning and function of the immune systems [37]. CXCL12 is originally identified as a bone marrow stromal cell-derived chemoattractant proliferative factor for B cell precursors [38, 39]. Studies have demonstrated roles for CXCL12 and CXCR4 in multiple aspects of B and T cell development and in effector immune responses [40–42]. Rossi and Zlotnik [43] reported chemokines affected

**Table 3** Association of *BAT2* polymorphism with immunological traits

| Trait | 0 days | | | 17 days | | | 32 days | | |
|---|---|---|---|---|---|---|---|---|---|
| | AA | AG | GG | AA | AG | GG | AA | AG | GG |
| PRRSV antibody | 1.20 ± 0.17 | 1.26 ± 0.16 | 1.31 ± 0.18 | 0.94 ± 0.17 | 0.96 ± 0.17 | 0.10 ± 0.18 | 0.67 ± 0.13 | 0.63 ± 0.12 | 0.60 ± 0.13 |
| CSFV antibody | 69.31 ± 4.83 | 70.09 ± 4.70 | 72.92 ± 4.95 | 55.68 ± 4.39 | 57.56 ± 4.27 | 58.15 ± 4.47 | 50.06 ± 4.21 | 48.35 ± 4.07 | 49.93 ± 4.30 |
| PRV antibody | 0.13 ± 0.04 | 0.10 ± 0.04 | 0.06 ± 0.05 | 0.07 ± 0.01 | 0.07 ± 0.01 | 0.07 ± 0.01 | 0.12 ± 0.02 | 0.12 ± 0.02 | 0.14 ± 0.02 |
| WBC | 10.68 ± 1.18 | 10.20 ± 1.13 | 11.30 ± 1.24 | 10.38 ± 0.57 | 9.85 ± 0.47 | 10.06 ± 0.62 | 16.79 ± 0.99 | 17.20 ± 0.85 | 18.78 ± 1.05 |
| RBC | 3.90 ± 0.14 | 3.90 ± 0.13 | 4.19 ± 0.15 | 4.96 ± 0.13 | 5.00 ± 0.11 | 5.05 ± 0.14 | 6.65 ± 0.14 | 6.48 ± 0.13 | 6.59 ± 0.15 |
| HBG (g/dl) | 85.52 ± 3.19[a] | 85.38 ± 2.90[a] | 92.66 ± 3.43[b] | 106.39 ± 2.60 | 107.32 ± 2.31 | 107.78 ± 2.76 | 126.71 ± 3.58 | 123.04 ± 3.18 | 123.47 ± 3.74 |
| HCT (%) | 30.83 ± 1.21[a] | 30.70 ± 1.11[a] | 33.52 ± 1.30[b] | 39.29 ± 0.92 | 39.95 ± 0.82 | 40.16 ± 0.98 | 47.10 ± 0.10 | 46.00 ± 0.89 | 46.20 ± 1.04 |
| MCV (fl) | 79.10 ± 1.09 | 78.92 ± 1.02 | 79.02 ± 1.17 | 79.30 ± 1.30 | 80.31 ± 1.14 | 79.47 ± 1.40 | 70.47 ± 1.49 | 70.24 ± 1.33 | 69.64 ± 1.56 |
| MCH (pg) | 21.86 ± 0.39 | 21.89 ± 0.37 | 21.81 ± 0.41 | 21.43 ± 0.42 | 21.41 ± 0.35 | 21.64 ± 0.46 | 19.08 ± 0.26 | 19.14 ± 0.23 | 18.80 ± 0.27 |
| MCHC (g/dl) | 273.11 ± 4.38 | 276.73 ± 3.89 | 275.13 ± 4.79 | 270.60 ± 2.12 | 268.46 ± 1.93 | 267.70 ± 2.34 | 269.68 ± 2.51 | 269.59 ± 2.25 | 268.24 ± 2.61 |
| PLT | 221.46 ± 20.76 | 241.35 ± 18.88 | 255.14 ± 22.47 | 433.80 ± 29.77 | 421.29 ± 25.64 | 449.03 ± 32.01 | 427.46 ± 35.22 | 470.97 ± 29.68 | 466.50 ± 37.27 |
| LYM | 75.47 ± 5.81 | 77.79 ± 4.98 | 88.05 ± 6.52 | 71.25 ± 3.96 | 74.19 ± 3.31 | 74.62 ± 3.95 | 75.10 ± 5.55 | 78.89 ± 5.22 | 75.28 ± 5.66 |
| LYM | 7.67 ± 1.58 | 7.91 ± 1.41 | 11.12 ± 1.74 | 6.59 ± 0.66 | 7.08 ± 0.54 | 6.99 ± 0.66 | 12.49 ± 1.19 | 13.37 ± 1.04 | 14.61 ± 1.23 |
| RDW (%) | 14.59 ± 0.32 | 14.41 ± 0.31 | 14.39 ± 0.34 | 17.92 ± 0.62 | 17.69 ± 0.58 | 18.63 ± 0.65 | 17.81 ± 0.66 | 18.19 ± 0.62 | 18.99 ± 0.68 |
| RDW (%) | 15.72 ± 0.71 | 16.37 ± 0.58 | 16.70 ± 0.72 | 14.27 ± 0.39 | 13.94 ± 0.33 | 14.05 ± 0.42 | 14.95 ± 0.46 | 15.09 ± 0.40 | 14.99 ± 0.52 |
| MPV (%) | 9.96 ± 0.34 | 10.32 ± 0.27 | 10.77 ± 0.33 | 9.93 ± 0.16 | 9.91 ± 0.14 | 9.81 ± 0.17 | 10.22 ± 0.17 | 10.29 ± 0.15 | 10.13 ± 0.19 |
| PLR | 33.52 ± 1.64 | 35.00 ± 1.37 | 35.89 ± 1.68 | 29.99 ± 1.29 | 29.64 ± 1.12 | 29.07 ± 1.39 | 33.17 ± 1.55 | 33.74 ± 1.36 | 32.38 ± 1.72 |

*Note* Values in each line with different case superscripts are at $P < 0.05$; values in each line with the same case superscripts are at $P > 0.05$

**Table 4** Association of *Mx1* polymorphism with immunological traits

| Trait | 0 days | | | 17 days | | | 32 days | | |
|---|---|---|---|---|---|---|---|---|---|
| | TT | CT | CC | TT | CT | CC | TT | CT | CC |
| PRRSV antibody | 1.27 ± 0.21[a] | 0.97 ± 0.19[b] | 1.16 ± 0.19[a] | 0.81 ± 0.19 | 0.79 ± 0.17 | 0.85 ± 0.16 | 0.66 ± 0.17 | 0.58 ± 0.14 | 0.59 ± 0.13 |
| CSFV antibody | 71.38 ± 5.81 | 64.76 ± 5.17 | 70.28 ± 4.85 | 56.51 ± 5.11 | 55.33 ± 4.68 | 56.93 ± 4.42 | 47.83 ± 5.79 | 51.15 ± 5.31 | 47.93 ± 4.95 |
| PRV antibody | 0.07 ± 0.05[A] | 0.19 ± 0.03[B] | 0.08 ± 0.02[A] | 0.07 ± 0.01 | 0.08 ± 0.01 | 0.08 ± 0.01 | 0.18 ± 0.03 | 0.16 ± 0.03 | 0.12 ± 0.02 |
| WBC | 8.33 ± 1.51 | 9.74 ± 1.21 | 9.90 ± 1.01 | 9.67 ± 1.02 | 9.95 ± 0.73 | 10.12 ± 0.47 | 16.94 ± 1.73 | 17.66 ± 1.33 | 19.08 ± 1.01 |
| RBC | 3.46 ± 0.24 | 3.91 ± 0.18 | 3.89 ± 0.13 | 4.63 ± 0.19[a] | 4.90 ± 0.15[ac] | 5.05 ± 0.11[c] | 6.60 ± 0.23 | 6.43 ± 0.19 | 6.63 ± 0.16 |
| HBG (g/dl) | 75.67 ± 5.60 | 88.08 ± 4.24 | 85.54 ± 3.19 | 97.47 ± 4.02Aa | 105.41 ± 3.06b | 107.33 ± 2.36B | 123.05 ± 5.83 | 123.08 ± 4.67 | 126.00 ± 3.69 |
| HCT (%) | 26.89 ± 2.06 | 31.47 ± 1.57 | 30.78 ± 1.22 | 36.11 ± 1.38Aa | 38.96 ± 1.07b | 39.78 ± 0.85B | 45.83 ± 1.61 | 45.33 ± 1.30 | 46.62 ± 1.04 |
| MCV (fl) | 78.16 ± 1.55 | 80.58 ± 1.32 | 79.15 ± 1.20 | 78.45 ± 1.84 | 79.73 ± 1.44 | 78.80 ± 1.16 | 69.84 ± 2.31 | 70.55 ± 1.80 | 69.84 ± 1.35 |
| MCH (pg) | 21.90 ± 0.51 | 22.53 ± 0.45 | 21.96 ± 0.41 | 21.10 ± 0.53 | 21.60 ± 0.40 | 21.25 ± 0.31 | 18.77 ± 0.40 | 19.27 ± 0.33 | 19.03 ± 0.26 |
| MCHC (g/dl) | 278.15 ± 8.73 | 281.50 ± 6.92 | 275.29 ± 5.68 | 269.91 ± 3.52 | 271.09 ± 2.78 | 270.09 ± 2.30 | 269.33 ± 3.66 | 272.76 ± 2.95 | 271.35 ± 2.37 |
| PLT | 226.73 ± 32.99 | 224.44 ± 24.79 | 230.36 ± 18.28 | 450.21 ± 45.87 | 417.20 ± 33.63 | 431.29 ± 23.70 | 480.11 ± 54.53 | 475.00 ± 41.93 | 486.48 ± 30.90 |
| LYM | 85.59 ± 7.85 | 76.10 ± 6.57 | 80.67 ± 5.05 | 83.10 ± 6.14 | 73.07 ± 4.82 | 76.53 ± 3.61 | 80.86 ± 8.06 | 76.21 ± 6.95 | 77.61 ± 6.05 |
| LYM | 7.23 ± 2.09 | 8.34 ± 1.78 | 7.56 ± 1.43 | 7.47 ± 1.13 | 6.18 ± 0.85 | 7.18 ± 0.56 | 12.81 ± 1.94 | 12.63 ± 1.54 | 14.36 ± 1.17 |
| RDW (%) | 14.11 ± 0.46 | 13.83 ± 0.38 | 14.18 ± 0.32 | 17.97 ± 1.00 | 17.82 ± 0.81 | 17.69 ± 0.69 | 18.04 ± 1.06 | 17.91 ± 0.89 | 18.12 ± 0.75 |
| RDW (%) | 17.54 ± 1.00 | 16.21 ± 0.76 | 16.09 ± 0.63 | 12.85 ± 0.60 | 13.63 ± 0.45 | 13.71 ± 0.37 | 14.95 ± 0.66 | 14.61 ± 0.55 | 14.84 ± 0.43 |
| MPV (%) | 10.94 ± 0.41 | 10.36 ± 0.31 | 10.32 ± 0.23 | 9.55 ± 0.24 | 9.67 ± 0.18 | 9.77 ± 0.15 | 10.12 ± 0.25 | 10.13 ± 0.22 | 10.12 ± 0.19 |
| PLR | 38.43 ± 2.34 | 34.44 ± 1.86 | 34.65 ± 1.50 | 26.31 ± 1.91 | 27.67 ± 1.44 | 28.66 ± 1.22 | 32.00 ± 2.24 | 32.34 ± 1.96 | 32.16 ± 1.67 |

*Note* Values in each line with different case superscripts are at $P < 0.05$; values in each line with the same case superscripts are at $P > 0.05$ and different big letters in each line show that the values differ very significantly ($P < 0.01$)

**Table 5** Association of SNP located at *7462* bp of *CXCL12* with immunological traits

| Trait | 0 days | | | 17 days | | | 32 days | | |
|---|---|---|---|---|---|---|---|---|---|
| | CC | CG | GG | CC | CG | GG | CC | CG | GG |
| PRRSV antibody | 1.25 ± 0.17 | 1.20 ± 0.17 | 1.19 ± 0.18 | 0.96 ± 0.17 | 0.97 ± 0.17 | 0.99 ± 0.18 | 0.64 ± 0.12 | 0.54 ± 0.12 | 45.74 ± 4.35 |
| CSFV antibody | 72.49 ± 4.88 | 70.66 ± 4.83 | 72.73 ± 5.02 | 57.53 ± 4.47 | 56.33 ± 4.43 | 57.66 ± 4.61 | 47.83 ± 4.20 | 49.28 ± 4.15 | 45.74 ± 4.35 |
| PRV antibody | 0.09 ± 0.04 | 0.09 ± 0.04 | 0.10 ± 0.04 | 0.07 ± 0.01ab | 0.08 ± 0.01a | 0.07 ± 0.01b | 0.12 ± 0.02 | 0.13 ± 0.02 | 0.11 ± 0.02 |
| WBC | 9.83 ± 1.20 | 10.55 ± 1.18 | 10.50 ± 1.27 | 10.65 ± 0.57 | 9.93 ± 0.52 | 10.99 ± 0.66 | 17.71 ± 1.01 | 18.47 ± 0.95 | 18.27 ± 1.13 |
| RBC | 3.76 ± 0.14 | 3.87 ± 0.14 | 3.86 ± 0.16 | 4.99 ± 0.12 | 5.04 ± 0.11 | 5.13 ± 0.14 | 6.54 ± 0.14 | 6.60 ± 0.13 | 6.40 ± 0.16 |
| HBG (g/dl) | 83.16 ± 3.36 | 84.27 ± 3.24 | 85.34 ± 3.69 | 107.07 ± 2.59 | 106.95 ± 2.42 | 110.13 ± 2.90 | 123.75 ± 3.21 | 124.95 ± 3.03 | 121.93 ± 3.56 |
| HCT (%) | 29.85 ± 1.25 | 30.36 ± 1.21 | 30.89 ± 1.37 | 39.95 ± 0.91 | 39.64 ± 0.86 | 40.32 ± 1.01 | 46.19 ± 1.02 | 46.44 ± 0.96 | 45.17 ± 1.14 |
| MCV (fl) | 78.80 ± 1.10A | 78.17 ± 1.09A | 79.92 ± 1.16B | 80.78 ± 1.13 | 78.64 ± 1.03 | 78.00 ± 1.29 | 69.60 ± 1.21 | 69.76 ± 1.10 | 69.54 ± 1.38 |
| MCH (pg) | 21.94 ± 0.38 | 21.77 ± 0.38 | 22.14 ± 0.40 | 21.84 ± 0.39 | 20.96 ± 0.35 | 21.33 ± 0.46 | 18.87 ± 0.29 | 18.81 ± 0.27 | 18.83 ± 0.32 |
| MCHC (g/dl) | 276.66 ± 4.33 | 277.91 ± 4.18 | 269.58 ± 4.78 | 267.79 ± 2.25 | 269.48 ± 2.16 | 272.71 ± 2.47 | 267.81 ± 2.76 | 268.29 ± 2.64 | 269.18 ± 3.02 |
| PLT | 214.01 ± 20.96 | 232.99 ± 20.41 | 201.36 ± 22.73 | 441.11 ± 27.05 | 418.35 ± 25.19 | 422.09 ± 30.41 | 497.57 ± 34.08 | 498.33 ± 31.29 | 486.85 ± 38.65 |
| LYM | 83.58 ± 5.11 | 79.60 ± 5.05 | 82.35 ± 5.83 | 75.06 ± 3.81 | 75.92 ± 3.48 | 79.38 ± 4.32 | 80.08 ± 5.36 | 76.94 ± 5.24 | 75.63 ± 5.57 |
| LYM | 8.04 ± 1.60 | 9.32 ± 1.59 | 8.84 ± 1.79 | 7.39 ± 0.66 | 7.35 ± 0.59 | 8.48 ± 0.76 | 13.46 ± 1.16 | 13.71 ± 1.09 | 13.40 ± 1.27 |
| RDW (%) | 14.28 ± 0.31 | 14.53 ± 0.31 | 14.64 ± 0.33 | 18.01 ± 0.65 | 17.71 ± 0.63 | 17.65 ± 0.70 | 18.85 ± 0.69 | 18.34 ± 0.67 | 17.90 ± 0.74 |
| RDW (%) | 16.28 ± 0.72 | 16.76 ± 0.67 | 15.79 ± 0.82 | 14.37 ± 0.39 | 13.80 ± 0.37 | 14.00 ± 0.44 | 14.79 ± 0.44 | 14.77 ± 0.43 | 14.62 ± 0.49 |
| MPV (%) | 10.14 ± 0.35 | 10.44 ± 0.33 | 9.91 ± 0.41 | 10.01 ± 0.16 | 9.74 ± 0.15 | 9.81 ± 0.18 | 10.21 ± 0.18 | 10.21 ± 0.17 | 10.23 ± 0.19 |
| PLR | 34.43 ± 1.76 | 34.80 ± 1.64 | 33.17 ± 1.97 | 30.64 ± 1.28 | 28.37 ± 1.22 | 29.10 ± 1.42 | 32.85 ± 1.56 | 32.86 ± 1.52 | 32.79 ± 1.69 |

*Note* Values in each line with different case superscripts are at $P < 0.05$; values in each line with the same case superscripts are at $P > 0.05$ and different big letters in each line show that the values differ very significantly ($P < 0.01$)

**Table 6** Association of SNP located at at 7331 bp *CXCL12* with immunological traits

| Trait | 0 days | | | 17 days | | | 32 days | | |
|---|---|---|---|---|---|---|---|---|---|
| | AA | AG | GG | AA | AG | GG | AA | AG | GG |
| PRRSV antibody | 1.12 ± 0.19 | 1.19 ± 0.17 | 1.27 ± 0.17 | 0.99 ± 0.19 | 1.01 ± 0.17 | 0.95 ± 0.17 | 0.69 ± 0.14 | 0.59 ± 0.12 | 0.64 ± 0.12 |
| CSFV antibody | 71.54 ± 5.28 | 69.90 ± 4.74 | 71.45 ± 4.68 | 58.23 ± 4.81 | 56.42 ± 4.39 | 56.46 ± 4.33 | 48.45 ± 4.72 | 48.62 ± 4.14 | 47.48 ± 4.10 |
| PRV antibody | 0.08 ± 0.05 | 0.09 ± 0.04 | 0.10 ± 0.03 | 0.06 ± 0.01 | 0.08 ± 0.01 | 0.07 ± 0.01 | 0.10 ± 0.03 | 0.13 ± 0.02 | 0.12 ± 0.02 |
| WBC | 11.75 ± 1.45 | 10.87 ± 1.34 | 10.04 ± 1.09 | 10.98 ± 0.74 | 9.82 ± 0.49 | 10.34 ± 0.45 | 20.16 ± 1.26a | 17.50 ± 0.82b | 16.87 ± 0.78b |
| RBC | 3.90 ± 0.19 | 4.00 ± 0.13 | 3.85 ± 0.12 | 5.11 ± 0.15 | 5.02 ± 0.11 | 4.91 ± 0.11 | 6.34 ± 0.18 | 6.54 ± 0.13 | 6.49 ± 0.12 |
| HBG (g/dl) | 86.35 ± 4.48 | 87.61 ± 3.15 | 84.80 ± 2.95 | 111.94 ± 3.25 | 106.40 ± 2.33 | 105.15 ± 2.18 | 121.90 ± 4.66 | 122.83 ± 3.13 | 122.30 ± 3.01 |
| HCT (%) | 31.21 ± 1.63 | 31.61 ± 1.15 | 30.53 ± 1.09 | 41.36 ± 1.13 | 39.55 ± 0.83 | 39.25 ± 0.78 | 45.29 ± 1.32 | 45.93 ± 0.91 | 45.93 ± 0.87 |
| MCV (fl) | 80.59 ± 1.31 | 78.95 ± 1.08 | 78.71 ± 1.05 | 80.60 ± 1.57 | 79.04 ± 1.11 | 80.59 ± 1.04 | 70.51 ± 1.62 | 69.97 ± 1.05 | 70.12 ± 1.00 |
| MCH (pg) | 22.31 ± 0.45 | 21.90 ± 0.37 | 21.82 ± 0.36 | 21.68 ± 0.54 | 20.97 ± 0.36 | 21.63 ± 0.33 | 19.07 ± 0.37 | 18.78 ± 0.26 | 18.81 ± 0.25 |
| MCHC (g/dl) | 263.49 ± 5.63$^{A}$ | 278.22 ± 3.98$^{B}$ | 278.42 ± 3.75$^{B}$ | 270.50 ± 2.81 | 267.83 ± 2.17 | 267.87 ± 2.08 | 269.03 ± 3.46 | 267.56 ± 2.49 | 266.15 ± 2.41 |
| PLT | 251.97 ± 27.64 | 240.00 ± 20.28 | 232.68 ± 19.30 | 391.05 ± 36.04 | 465.70 ± 25.61 | 430.41 ± 23.92 | 442.37 ± 47.09 | 482.84 ± 31.06 | 485.46 ± 29.76 |
| LYM | 89.35 ± 7.25 | 80.22 ± 5.24 | 81.86 ± 4.65 | 78.51 ± 4.93 | 75.02 ± 3.41 | 75.21 ± 3.22 | 80.13 ± 6.36 | 77.31 ± 5.28 | 77.26 ± 5.22 |
| LYM | 8.44 ± 2.08 | 9.65 ± 1.60 | 8.47 ± 1.49 | 7.93 ± 0.88 | 6.99 ± 0.57 | 7.26 ± 0.53 | 13.81 ± 1.56 | 13.91 ± 1.06 | 13.50 ± 1.04 |
| RDW (%) | 14.68 ± 0.38 | 14.45 ± 0.30 | 14.40 ± 0.29 | 17.03 ± 0.75 | 17.95 ± 0.59 | 18.15 ± 0.57 | 17.87 ± 0.82 | 18.43 ± 0.62 | 18.70 ± 0.61 |
| RDW (%) | 17.93 ± 0.96 | 16.29 ± 0.59 | 15.87 ± 0.53 | 14.43 ± 0.47$^{A}$ | 13.25 ± 0.37$^{B}$ | 14.27 ± 0.35$^{A}$ | 15.04 ± 0.57 | 14.81 ± 0.41 | 14.73 ± 0.39 |
| MPV (%) | 10.92 ± 0.50 | 10.30 ± 0.30 | 10.13 ± 0.27 | 10.15 ± 0.20$^{A}$ | 9.66 ± 0.16$^{B}$ | 9.94 ± 0.15$^{A}$ | 10.24 ± 0.22 | 10.17 ± 0.17 | 10.20 ± 0.16 |
| PLR | 38.15 ± 2.28 | 34.48 ± 1.44 | 33.74 ± 1.31 | 31.55 ± 1.58$^{A}$ | 27.41 ± 1.25$^{B}$ | 30.12 ± 1.19$^{A}$ | 33.01 ± 1.93 | 32.69 ± 1.47 | 32.83 ± 1.43 |

*Note* Values in each line with different case superscripts are at $P < 0.05$; Values in each line with the same case superscripts are at $P > 0.05$ and different big letters in each line show that the values differ very significantly ($P < 0.01$)

on the control of leukocyte chemotaxis under normal circumstances and the context of inflammation. The present study demonstrated the CXCL12 gene was associated with immunological traits in Landrace. The mutation (C/G) at 7462 bp of intron3 significantly influenced the level of PRV antibody at day 17 and MCV at day 0. Vaccination rests on the generation of memory B-ell and T cells. CXCL12 and CXCR4 were involved in multiple aspects of B- and T-cell development as well as in the regulation of immune responses, and CXCL12 protein is important to control adhesion and transendothelial migration of leukocytes, lymphocytes, and monocytes, especially during immune and inflammatory reactions [14, 23]. Therefore, the CXCL12 gene mutation (C/G) at 7462 bp of intron3 can influence the antibody titer especially for PRV, which can enhance the immunity and resistance. CXCL12 and CXCR4 have been demonstrated as important regulators of a number of fetal and adult cell functions, including migration/homing of hematopoietic stem and progenitor cells [44]. In the present study, the levels of MCHC at day 0, PDW, MPV and PLR at day 17, and WBC at day 32 were significantly different among pigs with different genotypes of the mutation (7331 A/G) at intron 3 of CXCL12. The previous study demonstrated that white blood cells play an essential resistant role in fighting infection and inflammation. High percentage of white blood cells indicates infection, inflammation and some forms of cancer, which is an important innate immune parameter for disease prognosis, low percentage of white blood cell count indicates viral infections, overwhelming infections and sepsis. The piglets are more prone to iron deficiency anemia and weak in immune system. The association analysis indirectly confirmed that CXCL12 gene play an important role in immune system and hematopoietic system. And CXCL12 gene may serve as a genetic marker in breeding with disease resistance in pig.

In conclusion, the mutations of Mx1, BAT2 and CXCL12 genes were associated with PRRSV and PRV antibody, HBG, HCT, RBC, PDW, MPV and PLR in Landrace. These might be an important molecular basis to assist selection of pig breeding with disease resistance, conduct further studies of gene functions and regulatory mechanisms. Nevertheless, whether these effects association with the later resistance of piglets, is worth further investigation.

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