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Mapping quantitative trait loci for the lysozyme level and immunoglobulin G blocking percentage of classical swine fever virus

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ABSTRACT. Increased disease resistance through improved general immune capacity would be beneficial for the welfare and productivity of farm animals. Classical swine fever (CSF) is a contagious disease in farm animals. The immunoglobulin G (IgG) blocking percentage of CSF virus (CSFV) in serum is an essential diagnostic parameter in veterinary practice. In addition, lysozymes are a part of the innate immune system. To identify quantitative trait loci (QTL) for IgG blocking percentage of CSFV and lysozyme concentration, IgG blocking percentage and lysozyme concentration in serum were measured in a composite pig population before and after challenge with modified live CSF vaccine.

Through genome-wide mapping by MQREML analysis and the SOLAR software, several QTL for the lysozyme concentration and the IgG blocking percentage of CSFV were identified, respectively. Within these QTL regions, some known genes were revealed, and some of them may serve as candidate genes in the pig.

Key words: Lysozyme; Immunoglobulin G; Quantitative trait loci; Classical swine fever; Swine

INTRODUCTION

Classical swine fever (CSF), also known as hog cholera, is a highly contagious viral disease in domestic and wild pigs (Moennig, 2000), which causes devastating financial losses in the pig industry. The causative virus (CSFV) is a member of the genus *Pestivirus* of the family Flaviviridae, and it is closely related to the viruses of bovine viral diarrhea and Border disease. Infected animals develop severe leukopenia and immunosuppression accompanied by hemorrhagic lesions, with a mortality of about 90% in infected young pigs (Moennig and Plagemann, 1992; Moennig et al., 2003).

The immune system plays an essential role in the disease resistance of animals. Immunoglobulin G (IgG) is important in immune responses. IgG is the most common immunoglobulin circulating in the blood. IgG can facilitate the phagocytic destruction of microorganisms that are foreign to the body and can bind to and activate complement to be involved in immune responses (Hamilton, 2001).

Lysozymes are a part of the innate immune system. It damages bacterial cell walls by catalyzing the hydrolysis of 1,4-beta-linkages between *N*-acetylmuramic acid and *N*-acetyl-D-glucosamine residues in a peptidoglycan and between *N*-acetyl-D-glucosamine residues in chitodextrins; therefore, it appears to have a role in host defense (Jolles and Jolles, 1984). Lysozymes are widely distributed in a variety of tissues and body fluids, including the liver, articular cartilage, plasma, saliva, tears, and milk (Reitamo et al., 1978).

Differences in the concentration of peripheral blood lysozyme and blocking percentage of IgG in serum among individuals under the same conditions provide evidence of genetic control on these traits, but little is known about the underlying genetics, especially in swine. Identifying the genetic control of the variation in these traits may help the genetic improvement of immune capacity against CSFV in animals through selection.

Since the first quantitative trait locus (QTL) mapping project in swine (Andersson et al., 1994), more than 5738 QTL have been mapped for more than 558 traits (http://www.animalgenome.org/QTLdb/pig.html). Most QTL studies have focused on performance traits. In recent years, many QTL for health traits have been identified. Currently, almost 500 QTL for immune traits have been determined (Edfors-Lilja et al., 1998, 2000; Reiner et al., 2007, 2008; Zou et al., 2008). However, very few QTL for IgG and no QTL for lysozyme traits in swine have been reported.

In this study, we report the identification of QTL that influence the concentration of peripheral blood lysozyme and blocking percentage of IgG in serum in swine. For this purpose, 206 microsatellite markers were selected in the whole genome. These markers are spaced approximately evenly with an average interval of 12 cM, according to the latest map from the National Center for Biotechnology Information (NCBI). A composite population

with three breeds was used for QTL mapping.

MATERIAL AND METHODS

Animals and collection of blood samples

The animals consisted of 367 piglets distributed in 5 Landrace boar families (15 sows and 87 piglets), 7 Large White boar families (33 sows and 190 piglets), and 4 Songliao Black Pig boar families (15 sows and 90 piglets). All pigs were raised 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 piglets were vaccinated with 4 doses live CSFV vaccine (rabbit origin, tissue virus ≥0.01 mg/dose) (Qilu Animal Health Products Co., Ltd., Shandong, China) through intramuscular injection. Blood samples were collected from each piglet 1 day before the vaccination inoculation (day 20) and 2 weeks after the vaccination (day 35). The samples were directly injected into VACUETTE® Serum Clot Activator tubes.

Measurement of lysozyme concentration and IgG blocking percentage in serum

Lysozyme concentration in each serum sample was measured using a commercial lysozyme turbidimetric assay kit (Jiancheng Bioengineering Institute, Nanjing, China) according to manufacturer instructions.

The IgG blocking percentage in the serum was measured using the commercial CSFV antibody test kit (IDEXX Laboratories, Liebefeld-Bern, Switzerland) according to manufacturer instructions.

Genetic markers

Two hundred and six microsatellites were selected from NCBI (http://www.ncbi.nlm.nih.gov/), and the latest porcine sex-average linkage map in NCBI was used in statistical analysis. These markers are approximately evenly distributed over all of the 18 autosomes and the X chromosome. The average distance between adjacent microsatellites on the sex-averaged map is 12 cM.

Statistical analysis

On the one hand, interval mapping of QTL was performed using the variance component approach (Grignola et al., 1996a,b; Zhang and Hoeschele, 1998) based on a linear mixed model as follows:

$$\mathbf{y} = \mathbf{Xa} + b\mathbf{c} + \mathbf{Zu} + \mathbf{Tv} + \mathbf{e}$$

$$\mathbf{u} \sim \mathrm{N}(\mathbf{0}, \mathbf{A}\sigma_u^2),\ \mathbf{v} \sim \mathrm{N}(\mathbf{0}, \mathbf{Q}\sigma_v^2),\ \mathbf{e} \sim \mathrm{N}(\mathbf{0}, \mathbf{I}\sigma_e^2),\ Cov(\mathbf{u}, \mathbf{v}') = \mathbf{0}$$

where y is a vector of the phenotypic values for the lysozyme concentration and IgG blocking

percentage on day 35; *a* is a vector of fixed effects including breed (three), sex (two), and sampling batch (three); *c* is a vector of the lysozyme concentration and IgG blocking percentage on day 20; *b* is the regression coefficient; *u* is a vector of residual polygenic effects; *v* is a vector of QTL allelic effects; *e* is a vector of residuals; X, Z, and T are incidence matrices for *a*, *u*, and *v*, respectively; *A* is the additive genetic relationship matrix among all individuals; σ_u^2 is the additive polygenetic variance; *Q* is the identity-by-descent probability matrix among QTL alleles; σ_v^2 is the QTL allelic variance; *I* is a unit matrix; and σ_e^2 is the residual variance.

The QTL analysis was scanned along each chromosome with 1-cM steps. The restricted maximum likelihood (REML) method was used to estimate the three variance components in the model, and the likelihood ratio was calculated as a test statistic for each particular location on the chromosome using the following formula:

$$LR = -2\ln\frac{L_{\mathrm{MAX}} \mid \mathrm{H}_0}{L_{\mathrm{MAX}} \mid \mathrm{H}_\mathrm{A}}$$

where $L_{\mathrm{MAX}}|H_0$ and $L_{\mathrm{MAX}}|H_\mathrm{A}$ are REML functions corresponding to the null hypothesis (there is no QTL) and the alternative hypothesis (there is a QTL), respectively. The MQREML program that was developed by Zhang and Hoeschele (1998) was used for the calculation mentioned.

In addition, in order to avoid the increased false-positive rate caused by multiple tests, the false-discovery rate (FDR) control approach (Benjamini and Hochberg, 1995; Weller et al., 1998) was adopted to further test the detected QTL. If *m* is the total number of tests involved in the analysis and $\mathrm{P}_1 \leq \mathrm{P}_2 \leq ... \leq \mathrm{P}_\mathrm{m}$ is the ordered observed P values corresponding to the χ^2 distribution for the m tests, the FDR for P_i (i = 1, 2, ..., m) is calculated as

$$FDR = \frac{mP_i}{i}$$

In this study, there were 2 phenotypic traits and 206 markers, leading to the total number of tests m = 2 x 206 = 512.

On the other hand, QTL analysis was performed along each chromosome at 1-cM steps using the SOLAR software (Almasy and Blangero, 1998) with the multipoint command to search for possible multiple QTL by repeated scans of all chromosomes. In each scan, all QTL that were revealed in the previous scans will be fixed, and a new QTL will be identified if the highest logarithm of the odds (LOD) score exceeds the given threshold. Interval mapping of QTL was performed using the variance component approach (Grignola et al., 1996a,b) based on a linear mixed model, which included four covariates (the IgG blocking percentage on day 20, breed, gender, and sampling batch), the random QTL allelic effect, and the residual polygenic effect. In this study, an LOD threshold of 3 was given for the first scan, and a threshold of 2 was given for the subsequent scans. The LOD drop-off method (Lander and Botstein, 1989) was used to estimate the confidence intervals of the QTL positions.

RESULTS

In the interval mapping of QTL using the MQREML program, one QTL for lysozyme with an FDR value <0.05 was identified (Table 1). This significant QTL was found approximately 84 cM from the proximal end of SSC11 (between markers SW1494 and SW2413) with a 95% confidence interval of 3 cM. However, in the QTL analysis by the SOLAR software, no QTL with an LOD threshold >3 was found.

Table 1. Results of QTL mapping for the lysozyme level and the immunoglobulin G blocking percentage to CSFV in serum by MQREML analysis.

Trait	Chromosome	Position (cM)	LR value	P value	95% Confidence interval	Flanking markers	
						Left	Right
IgG	ssc2	31.6	28.19	4.46E-05	29.6-33.6	SW256	S0141
	ssc3	33.8	20.11	1.61E-03	31-35	SE47329	SW1443
	ssc4	80.0	34.28	2.96E-06	78-81	S0023	SW512
	ssc5	107.4	21.53	8.68E-04	107-110	SW152	IGF1
	ssc6	115.0	25.45	1.36E-04	113-116	S0031	S0121
	ssc7	59.0	39.69	2.48E-07	58-61	SWR74	SW0102
	ssc9	37.0	41.53	1.32E-07	36-39	SW911	ESTMS20
	ssc12	84.6	27.77	4.80E-05	75.6-87.6	SWC62	SWC23
	ssc15	4.0	17.84	4.58E-03	3-6	KS169	S0355
	ssc16	24.5	43.35	7.95E-08	23-26	KS601	S0363
	ssc17	57.0	46.20	3.83E-08	54-58	SW1920	SW1031
	sscX	72.2	31.58	9.54E-06	70.2-75.2	SWR1861	SE15078
Lysozyme	ssc11	83.9	12.79	5.00E-02	82-84	SW1494	SW2413

LR = likelihood ratio.

In the interval mapping of QTL using the MQREML program, 12 QTL for IgG were identified with FDR values <0.05 (Table 1). In the QTL analysis by the SOLAR software, one IgG QTL with an LOD score of 3.62 was identified. This QTL was mapped 85 cM from the proximal end of SSC11 (between SW1494 and SW2413) with a confidence interval of 7 cM. A putative QTL with an LOD score of 2.97 was mapped 150 cM from the proximal end of SSC6 (between SW322 and SW2419) with a confidence interval of 13 cM.

DISCUSSION

Lysozyme concentration in serum QTL

Lysozyme is found in monocytes and neutrophils (Hansen, 1975; Lappin et al., 1986; Lewis et al., 1990; Panarelli et al., 1994). It is also a leukocyte-derived granular protein. There are some close ties between the lysozyme concentration, number of leukocytes, and shares of monocytes and neutrophils in serum. In this study, one significant QTL for lysozyme was found on SSC11. Three QTL with effects on white blood cell numbers have been reported on SSC11, but no QTL for lysozyme in the regions of QTL for leukocytes was found in this study, suggesting that lysozyme and leukocytes are controlled by different QTL.

Through comparisons with human chromosomes via radiation hybrid (RH) mapping, we found the ephrin-B2 (*EFNB2*) gene (located at 43 Mb on SSC11), which has a direct or indirect relationship with lysozymes, lysozyme-producing cells, and bacterial infections, in the region harboring the QTL that was identified in this study. Negrete et al. (2005) reported that EFNB2 is

the entry receptor for Nipah virus, which is an emergent deadly paramyxovirus. EFNB2 was predominantly expressed in macrophage-like cells in hyperinflammatory lesions (Yuan et al., 2004).

IgG blocking percentage in serum QTL

A total of 12 QTL for IgG with FDR values <0.05 were identified by MQREML, but just one IgG QTL with an LOD score of 3.62 was identified by the SOLAR software. Comparing the results of QTL mapping for IgG by MQREML and SOLAR, the most prominent QTL on SSC11 by SOLAR analysis is just a putative QTL (FDR < 0.1) by MQREML analysis. The IgG QTL on SSC6 (150 cM) that was identified by SOLAR analysis is not in the region of the QTL for IgG on SSC6 (115 cM) that was found using MQREML analysis.

In swine, several QTL for IgG titers were reported. Edfors-Lilja et al. (1998) reported a putative QTL that affected the serum IgG titer in *Escherichia coli* K88 on SSC6, which is close to the QTL that we found on SSC6 in this study.

There are some close ties between other immune traits and the blocking percentage of IgG in serum. Kramer et al. (1980) reported that IgG can inhibit leukocyte motility specifically and irreversibly. Edfors-Lilja et al. (1998) mapped a QTL that affected leukocyte counts on SSC6 in the region that also harbors the QTL that we found on SSC6 for the IgG blocking percentage. Interleukin-2 (IL-2) is vital to elicit and amplify the cellular and humoral immune responses to foreign antigens, which is extensively utilized in the control of infectious disease. IL-2 promotes the secretion of IgM and IgG (Gearing et al., 1985). QTL on SSC6 that were related to spontaneous IL-2 activity and PHA-induced IL-2 activity were identified in pigs after population mixing and transport (Edfors-Lilja et al., 2000). The QTL that we found on SSC6 is also close to these two QTL.

Through comparisons with human chromosomes via radiation hybrid mapping, we found that the tumor necrosis factor (ligand) superfamily, member 13b (*TNFSF13B*) gene, which is located at 44 Mb on SSC11 and is in the region harboring the identified QTL, has a direct or indirect relationship with IgG and may serve as a candidate gene for IgG blocking percentage. Tribouley et al. (1999) showed that soluble TNFSF13B induces apoptosis in activated T cells. Seyler et al. (2005) proposed that TNFSF13 and TNFSF13B regulate B cell and T cell function and have both pro- and anti-inflammatory effects in rheumatoid arthritis. Acevedo et al. (2009) found that the *TNFSF13B* gene was associated positively with IgG levels against *Ascaris* extract.

The physiological similarity between swine and humans has resulted in a substantial increase in research efforts on the immune system of swine during the past few years because swine can be used as a large animal model for biomedical research (Pescovitz et al., 1985; Saalmüller and Bryant, 1994; Saalmüller et al., 1994). Our analysis is the first step for identifying significant candidate genes that control the lysozyme level and the IgG blocking percentage of CSFV. The results from this study increase our understanding of the genetic control of lysozyme and IgG, but further studies are needed to identify the genes underlying these QTL.

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