



## A Whole Genome Association Study to Detect Single Nucleotide Polymorphisms for Blood Components (Immunity) in a Cross between Korean Native Pig and Yorkshire

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**ABSTRACT:** The purpose of this study was to detect significant SNPs for blood components that were related to immunity using high single nucleotide polymorphism (SNP) density panels in a Korean native pig (KNP)×Yorkshire (YK) cross population. A reciprocal design of KNP×YK produced 249 F<sub>2</sub> individuals that were genotyped for a total of 46,865 available SNPs in the Illumina porcine 60K beadchip. To perform whole genome association analysis (WGA), phenotypes were regressed on each SNP under a simple linear regression model after adjustment for sex and slaughter age. To set up a significance threshold, 0.1% point-wise p value from *F* distribution was used for each SNP test. Among the significant SNPs for a trait, the best set of SNP markers were determined using a stepwise regression procedure with the rates of inclusion and exclusion of each SNP out of the model at 0.001 level. A total of 54 SNPs were detected; 10, 6, 4, 4, 5, 4, 5, 10, and 6 SNPs for neutrophil, lymphocyte, monocyte, eosinophil, basophil, atypical lymph, immunoglobulin, insulin, and insulin-like growth factor-I, respectively. Each set of significant SNPs per trait explained 24 to 42% of phenotypic variance. Several pleiotropic SNPs were detected on SSCs 4, 13, 14 and 15. (**Key Words:** WGA, SNP, Immunity, Korean Native Pig, Yorkshire)

### INTRODUCTION

Disease is one of the important factors in swine production. Due to large-scale intensive production systems, pigs are exposed on various pathogens that cause infectious diseases and pathological symptoms. Especially, porcine epidemic diarrhea (PED) and porcine reproductive and respiratory syndrome (PRRS) cause serious damage in pig farms each year. Once infected by the diseases, the pigs grow slow and yield low productivity, because the nutrients and energy for growth turn into use in defense of the pathogenic microorganisms, which can break out under insanitary environments in large-scale intensive production system (Coates et al., 1963; Roura et al., 1992).

Annual costs for treating epidemics are increasing in pork industry, causing loss of productivity and high

mortality rate. If a disease is heritable, *i.e.* genetic variation exists between individuals, selection of a pig that is resistant to a disease would enable its progeny to be also resistant, decreasing incidences of the disease and increasing productivity. Among selection methods for disease resistance, application of biomarkers, *e.g.* quantitative trait loci (QTL) or SNPs that are related to immune response, can be a good option to avoid damages caused by the disease, and several QTL studies were reported to find QTL for blood components that were related to immune response using microsatellites or high density SNP panels (Cho et al., 2011; Lu et al., 2011; Reiner et al., 2011).

To understand immunity responses, it is necessary to characterize blood components. The serum levels of cytokines and TLRs are important innate immune proteins, which indicate the level of disease resistance (Scheerlinck and Yen, 2005; Shinkai et al., 2006; Yao et al., 2008; Uenishi and Shinkai, 2009). IFNG and IL10 have immunomodulatory functions, possess antiviral activity, protect pig from diseases and modulate the survival time of infected animals (Scheerlinck and Yen, 2005; Danilowicz et al., 2008; Yao et al., 2008). IFNG and IL2 act

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Submitted Aug. 14, 2012; Accepted Sept. 19, 2012; Revised Sept. 20, 2012

synergistically and contribute to immune response. IFNG enhances IL1 secretion by priming monocytes to be more sensitive to an IL1-inducing stimulus (Kruse et al., 2008). IL10 is secreted from lymphocytes and monocytes (Scheerlinck and Yen, 2005).

In this study, we performed whole genome scans to find significant SNPs for blood components that are related to immunity using the Illumina porcine 60K SNP chips in a KNP×Yorkshire F<sub>2</sub> cross population.

## MATERIALS AND METHODS

### Animals and phenotypes

Data were collected from the QTL experimental population that was produced by crossing Korean Native (KNP) boars and Yorkshire (YK) sows at National Livestock Research Institute (NLRI), Cheju, Korea. In the reciprocal cross design of the experiment, three F<sub>0</sub> grand sires of KNP and 14 F<sub>0</sub> grand dams of YK were mated. Among the F<sub>1</sub> offspring, five F<sub>1</sub> sires and 14 F<sub>1</sub> dams were randomly chosen to produce F<sub>2</sub> offspring. Another mating design comprised three F<sub>0</sub> grand sires of YK and nine F<sub>0</sub> grand dams of KNP to generate F<sub>1</sub>s, among which nine F<sub>1</sub> sires and 29 F<sub>1</sub> dams were randomly chosen. The two reciprocal matings produced 249 F<sub>2</sub> individuals. The F<sub>2</sub> individuals were raised under the similar feeding conditions in a private farm in Chungbuk province, and slaughtered at approximately 220±23 d of age.

Nine blood component measures that are related to serum immunity and hormones were measured in the F<sub>2</sub>s; neutrophil (mg/dl), lymphocyte (mg/dl), monocyte (mg/dl), eosinophil (mg/dl), basophil (mg/dl), atypical lymph (mg/dl), immuno globulin (g/dl), insulin (uIU/ml), and insulin-like growth factor-I (ng/ml). Table 1 depicted the mean and standard deviation for the respective blood component traits.

### Molecular data

Using the *Infinium HD Assay Ultra* Protocol (Illumina),

all of the 249 F<sub>2</sub> animals were genotyped with the porcine 60k SNP bead chips (Illumina Inc., USA). Raw data were visualized and analyzed with the Genome Studio software (Illumina). Every SNP from the chip data was screened for GWA tests. Those SNPs on autosomal chromosomes were removed before WGA testing, which met the following three criteria; i) the number of genotype group with one or none (e.g. only AA genotypes and no AB or BB), ii) with a minor allele frequency less than 0.05, and iii) with the proportion of genotyped individuals less than 90%. Among the 62,123 SNPs in the Illumina porcine chip, a total of 46,865 SNPs were selected for WGA tests.

### Statistical analysis

A SAS GLM procedure (SAS version 9.1) was applied to pre-adjust the animal phenotypes before WGA testing. Sex and slaughter age were fitted as a fixed effect and a covariate, respectively. Then, the residuals of each phenotype were regressed for each SNP under a simple linear regression additive model. In the model, SNP genotypes with AA, AB and BB were assigned as 1, 0, and -1 for the additive effect, respectively, reflecting a substitution effect of allele A on allele B. For significance threshold, 0.1% point-wise p value from *F* distribution was applied for each SNP test.

Among the significant SNPs, the best set of markers for each trait was selected by applying a stepwise regression procedure (Neter et al., 1990), because some of the significant SNPs would yield redundant information due to linkage disequilibrium (LD) between closely linked SNPs, i.e. a non-random association between alleles of different SNPs. Inclusion and exclusion of each SNP out of the model was determined at 0.001 level.

The variation explained by each (*j*<sup>th</sup>) SNP ( $S^2_{SNPj}$ ) was calculated as  $\sum_{i=1}^3 \alpha_i^2 f_i - \mu^2$ , where *i* denotes each genotype,  $\alpha_i$  is allele substitution effect (=  $-\hat{a}$ , 0, and  $+\hat{a}$  for BB, AB, and AA, respectively, in which  $\hat{a}$  was estimated from the simple regression analysis for the SNP),  $f_i$  is the frequency of *i*<sup>th</sup> genotype,  $\mu$  is the population mean that can

**Table 1.** The means and standard deviations for blood components measured in F<sub>2</sub> individuals from the cross between Korean native pig (KNP) and Yorkshire (YK)

Trait	N	Mean	SD	Minimum	Maximum	CV <sup>a</sup>
Neutrophil (mg/dl)	209	54.1	13.0	7	85	30.9
Lymphocyte (mg/dl)	209	41.2	12.7	11	86	65.7
Monocyte (mg/dl)	209	3.7	2.4	0	12	257.4
Eosinophil (mg/dl)	209	0.3	0.9	0	8	427.6
Basophil (mg/dl)	209	0.1	0.3	0	2	168.5
Atypical lymph (mg/dl)	209	0.6	1.0	0	6	71.4
Immuno globulin (g/dl)	217	2.0	0.4	1	4	24.0
Insulin (uIU/ml)	217	7.0	5.6	2	35	15.4
Insulin-like growth factor-I (ng/ml)	217	198.2	71.3	715	534	79.4

<sup>a</sup> Coefficient of variation: 100×SD/mean.

be expressed as  $(f_{AA}-f_{BB})\hat{a}$  (Falconer and Mackay, 1996). Proportion of phenotypic variance due to the  $j^{th}$  SNP was then estimated as  $S_{SNP_j}^2 / S_p^2$ , in which  $S_p^2$  was obtained from residual values of the trait after adjusting fixed and/or covariate effects. Therefore, the estimate of the proportion of phenotype variance due to all of the significant SNPs was  $\sum S_{SNP_j}^2 / S_p^2$ .

## RESULTS AND DISCUSSION

A set of 46,865 SNPs was chosen from the 62,163 SNPs in Illumina Porcine SNP 60K Beadchip (Table 2). The number of SNPs (6,622) was the greatest in *Sus scrofa* chromosome (SSC) 1, while SSC12 had the smallest number of SNPs (1,098). The SSCs 4, 7, 13, and 14 had more than 3,000 SNPs. The physical map with all of the available SNPs spanned about 2,424 Mb with an average distance of  $54.0 \pm 143.6$  Kb between adjacent SNPs. However, the average distances were very variable between chromosomes, ranging from 35.6 Kb in SSC17 to 87.3 Kb in SSC6. Only 75% of the SNPs that were embedded in the porcine chip were available in this study, partly due to ascertainment bias, *i.e.* the Illumina porcine SNP 60K Beadchip was not based on the genetics of Korean native pigs, but other commercial breeds.

A total of 54 SNPs were detected in various chromosomes by the stepwise regression procedures, and the SNPs for each trait explained great proportions of phenotypic variance, ranging from 24% for eosinophil to 42% for neutrophil (Table 3).

The white blood cell (WBC) components such as neutrophil, lymphocyte, monocyte, eosinophil, and basophil, are essential for immune responses in mammalian species. We identified ten SNP (QTL) for neutrophil on SSCs 1, 4, 8, 11, 13, 14 and 15 in a KNP×YK cross population (Table 3). Among the QTL, three QTL on SSCs 4, 13 and 14 had pleiotropic effects, *i.e.* for lymphocyte, for which additional three QTL were detected on SSC1, 11, and 18 (Table 3). For monocytes, four QTL were detected on SSCs 4, 5, 6 and 10, and for eosinophils four significant SNPs on SSCs 2, 4, 11 and 15 (Table 3). A total of five QTL for basophil were found, among which the QTL on SSC13 was resided closely with the lymphocytes QTL (Table 3).

Neutrophil, a leukocyte WBC component, engulfs, kills and digests microorganisms. Interleukins or other cytokines are, in general, produced in lymphocytes or monocytes. Li et al. (2010) reported that a QTL for neutrophil was found at 29.8 cM on SSC1 in Landrace, while we detected a neutrophil QTL at 30.8 Mb (Table 3). A protein coding gene, MAP3K5, was located around the QTL (Ensembl). DARC gene, which was reported to regulate neutrophil production and migration in human (Dawson et al., 2000; Lee et al., 2006; Reutershan et al., 2009; Schnabel et al., 2010; Reiner et al., 2011), resides at 99 Mb on SSC4 in pig genome. We detected a neutrophil QTL at 106 Mb on the same chromosome (Table 3). For the same trait, Reiner et al. (2008) reported two QTL in a Meishan×Pietrain cross, which were located at 8 cM on SSC4 and at 23 cM on SSC11, respectively. In the similar regions, two QTL were detected at 17 Mb and 18 Mb in the respective

**Table 2.** The numbers of available SNPs and average interval distance between flanking SNPs across all 18 autosomes (SSC) in the KNP×YK F<sub>2</sub> population

SSC	Number of SNPs	Average interval size (kb)	SD (kb)	Total distance (bp)
1	6,622	44.6	133.5	295,554,054
2	2,677	57.3	169.2	153,324,765
3	2,178	67.1	201.1	146,204,919
4	3,693	39.1	90.2	144,452,577
5	2,257	45.9	141.2	103,518,423
6	1,967	87.3	236.3	171,673,133
7	3,554	37.5	34.9	133,307,931
8	2,224	67.5	168.1	149,995,098
9	2,638	58.5	189.8	154,365,393
10	1,363	57.0	182.5	77,744,768
11	1,862	45.2	111.3	84,223,424
12	1,098	62.2	170.1	68,252,608
13	3,633	59.9	168.9	217,637,564
14	4,056	36.7	28.6	148,678,088
15	2,659	63.7	204.8	169,455,966
16	1,574	55.5	168.4	87,281,785
17	1,672	35.6	56.9	59,523,835
18	1,138	51.8	129.2	58,905,756
Total	46,865	Average 54.0	Average 143.6	Total 2,424,100,087

**Table 3.** Identities, positions, and effects of the SNPs associated with blood component traits in a KNP×YK cross population

Trait/SNP marker <sup>a</sup>	SNP <sup>b</sup>	SSC	Kb <sup>c</sup>	MAF <sup>d</sup>	$-\log_{10}P^e$	Estimate <sup>f</sup>	SE <sup>g</sup>	% $\sigma_p^2$ <sup>h</sup>
Neutrophil								42.09
INRA0001776	[G/A]	1	30,834	0.09	3.24	10.49	1.68	9.54
ALGA0023828	[T/C]	4	17,218	0.28	4.15	-4.94	1.07	5.21
ASGA0021917	[T/C]	4	106,241	0.06	3.53	7.20	1.94	3.36
ALGA0047025	[G/A]	8	10,470	0.13	3.02	-4.91	1.47	2.86
ASGA0050232	[C/T]	11	17,682	0.37	3.46	3.28	0.95	3.05
DRGA0011174	[G/A]	11	31,631	0.06	3.51	7.30	1.91	3.56
ALGA0063379	[A/G]	11	55,046	0.41	3.03	3.27	0.99	2.64
ALGA0067602	[A/G]	13	3,570	0.50	4.65	2.81	0.99	2.13
M1GA0019807	[G/A]	14	145,639	0.26	3.64	-3.64	1.19	2.35
H3GA0045535	[G/A]	15	93,065	0.33	4.11	5.36	0.99	7.40
Lymphocyte								28.91
ALGA0003271	[T/C]	1	42,786	0.10	5.57	7.73	1.82	5.85
ALGA0023828	[T/C]	4	17,218	0.28	4.52	4.93	1.21	5.39
H3GA0032363	[C/T]	11	55,220	0.30	3.05	-3.59	1.19	3.08
ALGA0067602	[A/G]	13	3,570	0.50	4.80	-5.02	1.11	7.09
M1GA0019807	[G/A]	14	145,639	0.26	3.14	4.28	1.31	3.37
ASGA0079719	[A/G]	18	24,680	0.21	3.31	4.67	1.32	4.14
Monocyte								26.65
ALGA0024578	[C/T]	4	33,041	0.47	3.32	0.87	0.22	6.29
ASGA0023933	[C/A]	5	2,659	0.06	3.70	-1.71	0.44	5.59
ALGA0037265	[G/A]	6	38,645	0.20	3.64	1.26	0.26	9.26
H3GA0055101	[C/T]	10	194	0.42	3.65	0.83	0.22	5.50
Eosinophil								24.98
DRGA0003276	[C/T]	2	52,733	0.07	3.85	-0.63	0.15	6.55
M1GA0006491	[A/G]	4	114,767	0.25	4.19	0.34	0.09	5.98
MARC0001377	[G/A]	11	39,551	0.39	3.56	-0.33	0.08	7.11
ASGA0070239	[C/G]	15	70,085	0.08	3.41	0.52	0.15	5.34
Basophil								26.82
INRA0004984	[G/A]	1	140,664	0.29	3.08	-0.08	0.03	3.72
MARC0055620	[C/T]	2	69,303	0.06	5.84	-0.22	0.05	5.80
ALGA0067658	[G/A]	13	4,270	0.10	3.21	-0.17	0.04	6.45
ASGA0060375	[T/G]	14	865	0.33	3.02	0.08	0.03	4.40
MARC0052349	[A/G]	16	42,368	0.10	3.00	0.17	0.04	6.45
Atypical Lymph								29.47
DIAS0003279	[A/G]	4	65,959	0.31	4.25	-0.51	0.11	9.08
ALGA0029239	[T/G]	4	120,981	0.27	4.77	0.46	0.10	7.16
ALGA0033066	[A/C]	5	56,650	0.43	3.30	0.42	0.09	7.81
MARC0057515	[G/A]	7	108,793	0.13	3.78	-0.51	0.13	5.42
Immuno globulin								25.37
ASGA0030288	[A/G]	6	44,238	0.15	3.61	-0.20	0.05	5.34
H3GA0021269	[A/G]	7	44,938	0.26	4.59	0.14	0.04	4.35
CASI0008911	[G/A]	13	129,859	0.17	3.58	-0.20	0.05	5.52
ASGA0070402	[A/G]	15	76,723	0.40	3.02	-0.12	0.04	3.98
ASGA0076724	[A/C]	17	32,707	0.48	4.17	-0.15	0.03	6.18

chromosomes (Table 3). In the proximal region of SSC11, Uddin et al. (2011) reported a QTL (14 cM) for cytokines interleukin 2 (*IL2*) in a Duroc×Pietrain cross population, when the individuals were induced by *Mycoplasma hypopneumoniae* vaccine. They also found two QTL at 35

cM and 50 cM on SSC11 for *IFNG* (interferon-gamma) influencing monocyte stimulation, and two *TLR9* QTL at 28 cM on SSC11 and at 142 cM on SSC14, respectively. In the similar regions, we detected two neutrophil QTL (Table 3).

For lymphocyte, Reiner et al. (2008) found three QTL at

**Table 3.** Identities, positions, and effects of the SNPs associated with blood component traits in a KNP×YK cross population (Continued)

Trait/SNP marker <sup>a</sup>	SNP <sup>b</sup>	SSC	Kb <sup>c</sup>	MAF <sup>d</sup>	$-\log_{10}P^e$	Estimate <sup>f</sup>	SE <sup>g</sup>	% $\sigma_p^2$ <sup>h</sup>
Insulin								40.66
DRGA0000138	[C/T]	1	11,545	0.08	4.68	-3.67	0.74	6.49
ALGA0001580	[C/T]	1	16,659	0.23	3.22	-1.74	0.50	3.34
ALGA0019935	[G/A]	3	35,523	0.23	6.43	-2.07	0.51	4.38
ALGA0023517	[C/T]	4	13,640	0.46	3.90	-2.05	0.48	5.32
M1GA0007246	[T/C]	5	1,569	0.47	3.16	1.39	0.40	3.43
ALGA0046513	[T/G]	8	4,535	0.46	3.98	-1.94	0.41	5.94
MARC0091595	[T/C]	8	65,895	0.28	3.02	1.67	0.44	3.91
MARC0038074	[A/G]	9	1,979	0.38	4.07	-1.63	0.47	3.51
ALGA0109641	[T/C]	11	28,168	0.15	3.02	1.35	0.65	1.26
ASGA0077323	[T/C]	17	42,488	0.49	3.16	1.40	0.40	3.07
Insulin-like growth factor-I								28.09
ASGA0001642	[C/T]	1	18,567	0.32	3.17	-20.94	6.81	3.67
ASGA0006375	[A/G]	1	213,459	0.49	5.46	-24.79	6.51	5.49
ASGA0024563	[A/G]	5	9,250	0.06	3.46	47.87	12.62	5.36
ALGA0053141	[C/T]	9	29,004	0.06	3.84	-41.32	11.04	4.02
MARC0020510	[G/A]	16	18,000	0.45	3.05	-21.97	6.53	4.01
M1GA0023311	[T/C]	18	29,832	0.20	6.87	29.67	7.18	5.54

<sup>a,c</sup> SNP marker annotations and their positions were referred from the probes of the Illumina porcine 60 k chip that was mapped on the *Sus scrofa* 9.0.

<sup>b</sup> Nucleotides of substitution. <sup>d</sup> Minor allele frequency. The first letter of the SNP indicates minor allele.

<sup>e</sup> Negative logarithm of the comparison-wise p value of the test statistic against the null hypothesis of no SNP effect at the SNP position.

<sup>f</sup> Estimate is for the allele substitution effect of replacing the latter with the former allele (nucleotide) in the SNP column. <sup>g</sup> Standard error.

<sup>h</sup> Proportion of phenotypic variance explained by the SNP. The values on the rows of trait names are the sum of the %  $\sigma_p^2$  values across all SNPs for the trait.

62 cM on SSC1, at 138 Mb near SW761 on SSC14, and at 29 cM on SSC18, respectively, in a Meishan×Pietrain cross population. In the similar regions (43 Mb, 146 Mb, and 25 Mb), we detected QTL for lymphocyte (Table 3). Watrang et al. (2005) reported a QTL for CD2+/CD8+ leukocyte in the S0008 marker region (43.5 to 89.2 cM in *PigQTLdb*) on SSC1 in the European Wild Boar, Landrace and Yorkshire crosses, while a lymphocyte QTL was detected at 43 Mb in this study. Li et al. (2010) found one QTL for lymphocytes at 29.8 cM on SSC1 in Landrace pigs, while we detected a lymphocyte QTL at 43 Mb on the same chromosome (Table 3).

A few QTL for monocytes were detected in this study. Lu et al. (2011) reported a QTL for CD+/CD8+ at 1 cM on SSC5 in Landrace, white boar and Songliao Black pig families, while a QTL for monocyte was detected at 2.6 Mb in the KNP x Landrace cross (Table 3). Uddin et al. (2011) reported a QTL for *IL10* at 73 cM on SSC6, which was flanked by SW1067 and SW193 (35.8 to 44.2 Mb in Ensembl). In the region, a QTL for monocytes was detected at 39 Mb in this study.

For eosinophil, which acts mainly against parasites, four QTL were detected in this study (Table 3). Reiner et al. (2007) reported an eosinophil QTL at 69 cM on SSC11 flanked by S0182 and SW903 (33 to 77 cM) in a European Pietrain×Chinese Meishan cross, in which we detected a

QTL for the trait (40 Mb). In the similar region, Uddin et al. (2011) also found a QTL for *TLR2* at 38 cM (SW2008-S0071 between 14 and 42 Mb, Ensembl).

Yang et al. (2009) reported a QTL for WBC at 63.8 cM on SSC2 in a White Duroc×Erhualian cross. We detected one QTL for basophil, which is an anti-parasitic and anti-coagulant component, at 69 Mb on the same chromosome. Uddin et al. (2010; 2011) reported QTL for *TLR9*, an immunity related element in gut, at 0 to 47 cM and for *IFNG* at 47 to 93 cM on SSC16 in a Duroc×Pietrain cross, where the QTL for basophil was also detected at 42 Mb in this study (Table 3).

Reiner et al. (2002) reported a QTL for a pseudo-rabies resistance/susceptibility QTL at 42 cM on SSC6 in Large White Boars and Meishan sows. We detected one QTL for immunoglobulin (Ig) at 44 Mb on the same chromosome (Table 3). In the similar region, Edfors-Lilja et al. (1998) found a QTL for anti-O149 *E. coli*, i.e. at 67 cM on SSC6, in a wild boar and Yorkshire cross. Uddin et al. (2010) found *Mycoplasma hyopneumoniae* antibody titer change due to two QTL on SSC7 (30.2 cM) and SSC15 (83.2 cM) in a Duroc×Pietrain cross, while two QTL for Ig were detected at 45 Mb and 77 Mb at the respective chromosomes in this study (Table 3). Ji et al. (2011) reported a QTL for enterotoxigenic *E. Coli* susceptibility at 89.9 cM on SSC13 (~140 Mb; *PigQTLdb*) in a White

Duroc×Erhualian F<sub>2</sub> cross, while an Ig QTL was located at 130 Mb in this study (Table 3).

Lu et al. (2011) reported two QTL for CD4<sup>+</sup>/CD8<sup>+</sup> lymphocytes at 73 cM and 124 cM on SSC4 and SSC7, respectively, in Landrace, white boar and Songliao Black pig families. We detected two QTL for atypical lymph at 66 Mb and 109 Mb on the respective chromosomes (Table 3). Reiner et al. (2007) reported one QTL for platelets at 36 cM on SSC3 in an European Pietrain×Chinese Meishan cross, while we detected one QTL for insulin at 35 Mb on the same chromosome (Table 3).

We detected three QTL for Ig at 32 Mb on SSC17, for insulin at 42 Mb on SSC17, and for monocyte at 33 Mb on SSC4, while Reiner et al. (2007) reported two eosinophil QTLs (at 26 cM and 41 cM) and one IgG1 QTL in the similar regions of this study. Lu et al. (2011) also found QTL for lymphocytes nearby the insulin like growth factor and for Insulin on SSC16 (18 Mb) and SSC8 (65.9 Mb), respectively. The lymphocytes QTL in Li et al. (2010) was located at 29.8 cM on SSC1, which was closely located to the lymphocyte QTL in this study (Table 3).

Cytokines and TLRs influence each other and it is no wonder of a pleiotropic effect since cytokines and TLRs interact in complex networks (Uddin et al., 2011). TLR9 and TLR2 signaling together account for MyD88-dependent control of parasitemia (Baffica et al., 2006). Therefore, closer locations of TLRs and IL2 QTLs along with WBC QTLs may be an active region, which is involved in execution of a large dependent complex mechanism to build up the innate and acquired immunity of an individual.

However, few QTL for immune components were confirmed in this study, which were previously reported at the same or similar chromosomal regions in other studies on immunity QTL. This may be partly due to breed differences, as the baseline immune response and performance traits are affected by breeds (Sutherland et al., 2005). Further QTL studies are, thus, needed to verify the detected QTL for immune response and to identify causal genes to apply to pig health breeding programs with greater efficiency and accuracy.

## CONCLUSION

Selection program for immunity has not been extensively implemented in pig industry. In this study, 54 QTL (SNPs) were detected for nine blood component traits with significant contributions to the phenotypic variances. These QTL would provide a set of genetic markers to implement marker-assisted selection (MAS), which would enable genetic improvement on immunity through pig health breeding programs.

## ACKNOWLEDGEMENT

This work was supported by grants from the Next-Generation BioGreen 21 Program (No. PJ008089).

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