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Investigation of single nucleotide polymorphisms in porcine candidate genes for blood component traits in pigs

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Abstract

This study used 209 public single nucleotide polymorphism (SNP) arrays for 151 candidate genes of pigs to analyse their association with nine blood component traits (insulin-like growth factor-I, insulin, immuno globulin, lymphocyte, monocyte, eosinophil, basophil, neutrophil and atypical lymph) in 209 Korean native pigs and Yorkshire F_2 hybrids. Of these, 52 SNPs in 49 candidate genes showed significant association with one or more blood component traits. Nineteen of these SNPs were found to be present in blood component QTL regions. The 49 candidate genes corresponding to 52 SNPs with significant effects were detected and used for gene ontology analysis to understand the function of the candidate genes at molecular level. Based on functional classification (biological process, cellular components, and molecular function) of annotated candidates, 34 candidate genes (11 genes of IGF-1, 9 of IS, 9 of IG, 6 of NP, and 3 of EP) were detected. Additionally, eight genes (*PSMB4, PSME3, MAPKAPK3, CTLA4, CUL7, GGT1, IDH3B,* and *RXRB)* interacting with four immune pathways (immune system, adaptive immune system, Class I MHC-mediated antigen processing and presentation, and antigen processing: ubiquitination and proteasome degradation) were found through pathway and network analyses. The eight candidate genes identified in this study are included in class I MHC-mediated antigen pathway, which is an important factor that determines the success of organ transplantation in addition to the improvement of diseases and immunity of pigs. Therefore, these genes can potentially be used in heterogeneous organ research in future research.

Keywords: Association analysis, gene network, pathway, SNP, swine

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Introduction

For the success of the swine industry, it is important to produce safe high-quality pigs and prevent or reduce the incidence of infectious diseases in pigs. Although the development of livestock breeding has been greatly improved through many studies on growth and meat quality domestically and abroad, there have been insufficient numbers of recent studies on the improvement of traits related to diseases and immunity worldwide. In particular, modern pig farming is a large and intensive production system, and the pigs are easily exposed to pathogens that cause infectious diseases and pathologies. As the incidence of diseases worldwide increases, studies are being conducted to improve the capacity of the pig immune system (Schroyen & Tuggle, 2015). In addition, pigs are useful as human biomedical models and in genetic studies that involve the immune system, because they are highly comparable with humans in terms of their anatomy, physiology, genetics and genomics (Lunney, 2007).

Single nucleotide polymorphisms (SNPs) are simple genetic characters that are widely distributed and are the most abundant type of variation in the whole genome. SNPs have the advantages of relatively high availability and stability compared with microsatellites and other DNA polymorphism markers. Therefore, SNPs are important markers that can be applied in various fields (e.g. evolution and disease) of the study of livestock (Kijas *et al*., 2009).

Previous studies related to pig disease that used SNP markers have shown that the WUR10000125 single-base mutation in the *GBP1* (guanylate binding protein 1) gene located on pig chromosome 4, and c.2509 G>C, c.2638 G>A, and c.3534 C>T mutations in the *CD163* (cluster of differentiation 163) gene on

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chromosome 5 significantly affect viremia and weight gain in serum when pigs are infected with porcine reproductive and respiratory syndrome virus (PRRSV) (Boddicker *et al*., 2012; Lim *et al*., 2018). In addition, the ALGA1150315 single-base mutation located on pig chromosome 9 and the ALGA0122080 and MARC0001766 mutations located on chromosome 12 have been reported to have significant effects on the same traits in pigs that are infected with porcine circovirus (PCV) (McKnite *et al*., 2014).

Recent research using these methods has identified critical candidate genes through gene network and pathway analysis in immune response experiments related to PRRSV resistance and gram-negative *Bacillus* resistance in pigs (Wysocki *et al*., 2012; Zhao *et al*., 2012).

This study was implemented to carry out an association analysis of blood component traits to 209 public SNP arrays of 151 candidate genes in Korean native pigs (KNP) and Yorkshire F_2 hybrid populations. The authors also carried out a gene ontology (GO) analysis to identify the potential role of these candidate genes in metabolism and network and pathway analyses of gene interactions to detect candidate genes that are related to the immune function in pigs.

Materials and Methods

The pig population used in this study was a sample of 209 KNP and Yorkshire F_2 hybrid groups, which had been used for predecessor QTL discovery, SNP association, and candidate gene identification studies (Kim *et al*., 2011; Lee *et al*., 2012). In the F_2 group, levels of nine blood components that were related to immunity were measured in serum: insulin-like growth factor-1 (IGF-1), insulin (IS), immuno globulin (IG), lymphocyte (LC), monocyte (MC), eosinophil (EP), basophil (BP), neutrophil (NP) and atypical lymph (AL). Table 1 shows the mean values and their standard deviation for each of these blood component traits.

Table 1 Summary statistics for observations on blood component traits of pigs

Trait	Abb	N	Mean	SD	Min	Max	CV
Insulin-like growth factor-1 (ng/mL)	IGF-1	193	207.47	74.91	75.09	534.31	36.11
Insulin (μIU/mL)	IS	193	7.82	6.74	1.57	40.66	86.15
Immuno globulin (g/dL)	IG	193	2.17	0.42	1.16	3.48	19.50
Lymphocyte (mg/dL)	LC	184	44.76	12.12	14.00	88.00	27.08
Monocyte (mg/dL)	MC	184	3.50	2.20	0.00	12.00	62.87
Neutrophil (mg/dL)	NP	184	50.44	12.70	7.00	82.00	25.17
Eosinophil (mg/dL)	EP	184	0.46	0.94	0.00	8.00	203.50
Basophil (mg/dL)	BP	184	0.08	0.30	0.00	2.00	399.81
Atypical lymph (mg/dL)	AL	184	0.75	1.25	0.00	6.00	166.25

Abb: abbreviation
SD: standard deviation
CV: coefficient of variation: 100 x SD/mean

The SNPs used in this experiment were 209 confirmed SNPs, from which 22 breed-specific SNPs were removed. Thirty SNPs among the 261 SNPs selected by Li *et al*. (2011) were also excluded, which were not mapped to pig genome build 11 using the NCBI blast tool. The genotypes of the confirmed SNPs were analysed by a MassARRAY method using the MassARRAY Design Software (Sequenom Inc, San Diego, USA) (Li *et al*., 2011). Amplification primers were designed using Spectro DESIGNER 1.3.4 (Sequenom Inc, San Diego, USA) and SNPs were multiplexed in 14 assays using the manufacturer's instructions for the iPLEX system (Sequenom Inc, San Diego, USA).

Quality control (QC) of the 209 SNP markers that were analysed by genotyping was carried out using the PLINK 1.07 programme. An SNP was removed based on two criteria, minor allele frequency (MAF) <0.05 and call rate <50% (Purcell *et al*., 2007). To estimate the linkage disequilibrium (LD) between mutations in genes on the same chromosome, analysis was performed using the Haploview software package (Barrett *et al*., 2004) and the values of D′ and r^2 were estimated using the methods of Stephens *et al*. (2001). Mutations with $r^2 \geq 0.8$ and LD values within the range of those of chromosomal single-base mutations were selected, and then used in the association analysis (Barrett *et al*., 2004).

To evaluate the genotype effects of the selected SNP markers by QC and LD analyses, association

analysis was performed using the generalized linear model (GLM) function of SAS 9.4 software package. The model used for the analysis was as follows:

$$Y_{ijklm} = \mu + S_i + G_j + b_1 A_k + e_{ijkl},$$

where: Y_{ijklm} is the observed value of blood component traits
μ is the mean of the samples
S_i is the effect of sex
G_j is the effect of genotype
A_l is the covariate of slaughter age (days)
b_1 is the regression coefficient of slaughter age (days
e_{ijkl} is the random error

The candidate genes containing SNPs that have significant effects on blood component traits were selected through association and GO analyses using the functional annotation tool provided by the database for annotation, visualization, and integrated discovery (DAVID). In the GO analysis, the relevance of candidate genes was determined by classifying them in three functional groups (biological process, cellular components and molecular function), based on their associations.

Gene pathway and network analyses provide crucial insight into the genetic structure behind complex polygenic traits (Kadarmideen *et al*., 2006). Therefore, gene pathway and network analyses were performed in the GeneMANIA and KEGGParser tool plugins, respectively, supported by Cytoscape software 3.6.1 for candidate genes based on blood component traits-related GO term results (Shannon *et al*., 2003).

Results

Sixty-five SNPs of the 209 public SNPs examined were filtered out by QC and LD, leaving 144 SNPs for further analyses. Forty-one of the 65 filtered SNPs were removed through further QC. Twenty-four SNPs had a MAF lower than 0.05 and 18 had a call rate lower than 50%. The remaining 24 SNPs of these 65 were eliminated during LD analysis because their r^2 values were not higher than 0.8. Finally, the association of the 144 SNPs filtered through QC and LD with blood component traits was analysed.

An association analysis was performed between the 144 filtered SNPs and nine blood component trait (IGF-1, IS, IG, LC, MC, NP, EP, BP, and AL), and significantly associated SNPs were detected (Table 2; Table 3). A total of 52 SNPs showed significant associations with one or more of the blood component traits. The SNPs identified in the association analysis were classified as 2 missense variants, 9 synonymous variants, 1 UTR variant and 40 intron variants. Among these, 4 SNPs – including the SNP rs318932969 (associated with IS, LC, NP, EP, and AL) located in the *SEMA6D* gene, rs45432355 (associated with IGF-1, IS, LC, and NP) in the *DECR1* gene, rs45433852 (associated with IG, LC, and NP) in the *GRM7* gene, and rs10720292 (associated with IS, LC, and NP) in the *RRP9* gene – were found to be significantly associated with three or more blood component traits. Based on the blood component traits, 11 SNPs were significantly associated with IGF-1, 12 with IS, 12 with IG, 13 with LC, 6 with MC, 13 with NP, 5 with EP, and 7 with AL trait (all $P < 0.05$).

Genes containing those SNPs that showed significant associations with blood component traits were selected as candidate genes and used for GO analysis. A total of 38 candidate genes were detected, 11 (*CUL7*, *VIM*, *VLDLR*, *PSMB4*, *MAPKAPK3*, *CTLA4*, *EPRS*, *F5*, *NDUFV1*, *DECR1* and *RXRB*) of which were related with IGF-1; 9 (*PSMB4*, *RBM42*, *SEMA6D*, *ILK*, *PSME3*, *NEFL*, *CGN*, *LIFR* and *RRP9*) with IS; 9 (*TRIM29*, *GRM7*, *CNOT1*, *KARS*, *PRPF6*, *ATG9A*, *IDH3B*, *CXADR* and *ABCF3*) with IG; 6 (*GRM7*, *BTBD2*, *AP3D1*, *BAP1*, *RRP9* and *CBX8*) with NP; and 3 (*SEMA6D*, *GGT1* and *ETV4*) were related with EP. Four (PSMB4, SEMA6D, GRM7 and RRP9) of these genes overlapped between two traits. The 11 candidate genes related with IGF-1 trait were detected in 14 GO terms (9 biological processes, 3 cellular components and 2 molecular functions), 9 genes of IS were detected in 3 GO terms (2 biological processes and 1 cellular component), 9 genes of IG were detected in 5 GO terms (1 biological process, 3 cellular components and 1 molecular function), 6 genes of NP were detected in 1 GO term (a cellular component), and 3 genes of EP were detected in 1 GO term (a biological process) (Table 4).

Pathway searches were performed on selected candidate genes using the GeneMANIA and KEGGParser analysis tools, and the direct interactions between candidate genes were also investigated. Eight genes (*PSMB4*, *PSME3*, *MAPKAPK3*, *CTLA4*, *CUL7*, *GGT1*, *IDH3B*, and *RXRB*) had integrated interactions in four blood component trait-related immune pathways (immune system, adaptive immune system, class I MHC-mediated antigen processing and presentation, and antigen processing: ubiquitination and proteasome degradation), and genetic interactions among candidate genes were also observed (Figure 1).

Table 2 Single nucleotide polymorphisms (SNPs) associated with hormone traits of blood in pigs

Trait	Chr	Gene	"rs" number	Ma	MAF	SNP effect (SE)	*P*-value	Variant
IGF-1	1	VLDLR	rs45434583	G	0.07	-34.17 (13.60)	0.0128	Intron
	2	NDUFV1	rs45431005	C	0.18	20.79 (9.85)	0.0361	Synonymous
	4	DECR1	rs45432355	G	0.45	-17.43 (8.61)	0.0445	Intron
	4	F5	rs45432640	C	0.48	-16.08 (7.77)	0.0400	Intron
	4	PSMB4	rs45435360	C	0.24	-22.53 (8.97)	0.0129	Intron
	7	RXRB	rs45430980	A	0.50	-18.45 (7.56)	0.0156	Missense
	7	CUL7	rs45432828	C	0.39	22.29 (9.71)	0.0230	Intron
	10	EPRS	rs45431069	T	0.43	-19.24 (9.68)	0.0484	Intron
	10	VIM	rs696126535	G	0.25	-20.23 (8.32)	0.0160	Intron
	13	MAPKAPK3	rs45432062	A	0.07	52.93 (16.44)	0.0016	Intron
	15	CTLA4	rs45432756	G	0.19	25.18 (11.09)	0.0243	Intron
IS	1	SEMA6D	rs318932969	T	0.06	3.65 (1.64)	0.0271	Intron
	4	DECR1	rs45432355	G	0.45	-2.59 (0.78)	0.0010	Intron
	4	CGN	rs45433976	G	0.38	-1.91 (0.94)	0.0450	Intron
	4	PSMB4	rs45435360	C	0.24	-2.00 (0.81)	0.0152	Intron
	6	KARS	rs45431702	C	0.17	-2.07 (1.02)	0.0445	Synonymous
	6	RBM42	rs10720248	G	0.41	-1.75 (0.79)	0.0276	Intron
	9	ILK	rs45430734	A	0.19	3.24 (0.93)	0.0006	Intron
	12	PSME3	rs45434514	A	0.29	1.61 (0.79)	0.0424	Intron
	13	RRP9	rs10720292	C	0.13	2.13 (0.92)	0.0218	Synonymous
	14	NEFL	rs45433391	T	0.18	-1.90 (0.91)	0.0397	Synonymous
	14	MMRN2	rs698012877	G	0.10	3.24 (1.13)	0.0045	Synonymous
	16	LIFR	rs45434001	T	0.43	1.60 (0.71)	0.0248	Intron
IG	6	KARS	rs45431702	C	0.17	0.13 (0.06)	0.0428	Synonymous
	6	CNOT1	rs45432668	A	0.47	-0.14 (0.05)	0.0089	Intron
	7	CMTR1	rs45431209	A	0.48	-0.11 (0.05)	0.0133	Intron
	9	TRIM29	rs45432745	A	0.35	0.15 (0.07)	0.0236	Intron
	12	FKBP10	rs45432349	T	0.29	0.09 (0.04)	0.0337	Intron
	13	GRM7	rs45433852	T	0.27	-0.11 (0.05)	0.0288	Synonymous
	13	RASA2	rs321506146	A	0.31	0.14 (0.05)	0.0081	Intron
	13	ABCF3	rs45432345	T	0.19	0.13 (0.06)	0.0319	Intron
	13	CXADR	rs328132771	C	0.09	0.19 (0.08)	0.0165	Intron
	15	ATG9A	rs45435354	T	0.48	-0.40 (0.16)	0.0150	Intron
	17	IDH3B	rs10720906	C	0.24	0.17 (0.07)	0.0187	3' UTR
	17	PRPF6	rs1108981338	G	0.22	0.15 (0.06)	0.0089	Intron

IGF-1: insulin-like growth factor-1; IS: insulin; IG: immuno globulin; MA: minor allele; MAF: minor allele frequency

Table 3 Single nucleotide polymorphisms (SNPs) associated with white blood cell traits of blood in pigs

Trait	Chr	Gene	"rs" number	Minor Allele	Major Allele Frequency	SNP effect (SE)	*P*-value	Variant
Lymphocyte	1	SEMA6D	rs318932969	T	0.06	8.71 (2.79)	0.0021	Intron
	2	AP3D1	rs10719760	T	0.43	2.99 (1.31)	0.0236	Intron
	2	BTBD2	rs45430365	A	0.33	3.69 (1.34)	0.0067	Intron
	4	WDYHV1	rs337607838	C	0.30	4.58 (1.51)	0.0027	Intron
	4	DECR1	rs45432355	G	0.45	-4.48 (1.36)	0.0013	Intron
	5	SLC25A3	rs10719816	G	0.48	2.46 (1.08)	0.0238	Intron
	6	PTPRM	rs45431026	T	0.29	5.12 (2.27)	0.0261	Intron
	12	CBX8	rs45434014	C	0.13	7.39 (2.34)	0.0020	Intron
	12	SUPT6H	rs10720118	A	0.27	3.60 (1.59)	0.0245	Intron
	12	SUPT6H	rs10720117	G	0.16	-6.07 (1.91)	0.0018	Intron
	13	RRP9	rs10720292	C	0.13	5.68 (1.59)	0.0005	Synonymous
	13	GRM7	rs45433852	T	0.27	3.56 (1.51)	0.0195	Synonymous
	15	NGEF	rs45434870	C	0.50	26.28 (11.99)	0.0298	Intron
Monocyte	2	SSRP1	rs45433934	T	0.37	-0.64 (0.29)	0.0268	Intron
	5	SLC25A3	rs10719816	G	0.48	-0.42 (0.19)	0.0319	Intron
	6	HSPG2	rs45432189	A	0.21	0.78 (0.31)	0.0119	Intron
	7	RXRB	rs45430980	A	0.50	-0.48 (0.23)	0.0354	Missense
	13	MAPKAPK3	rs45432062	A	0.07	-1.08 (0.49)	0.0306	Intron
	15	DIS3L2	rs710273415	A	0.40	1.03 (0.44)	0.0192	Missense
Neutrophil	1	SEMA6D	rs318932969	T	0.06	-10.26 (2.90)	0.0005	Intron
	2	AP3D1	rs10719760	T	0.43	-3.21 (1.38)	0.0217	Intron
	2	BTBD2	rs45430365	A	0.33	-3.89 (1.41)	0.0063	Intron
	4	WDYHV1	rs337607838	C	0.30	-4.38 (1.59)	0.0066	Intron
	4	DECR1	rs45432355	G	0.45	4.62 (1.44)	0.0015	Intron
	12	CBX8	rs45434014	C	0.13	-7.79 (2.44)	0.0018	Intron
	12	SUPT6H	rs10720118	A	0.27	-4.05 (1.66)	0.0160	Intron
	12	SUPT6H	rs10720117	G	0.16	6.37 (2.01)	0.0018	Intron
	12	P2RX1	rs45433973	T	0.41	2.55 (1.25)	0.0420	Intron
	13	BAP1	rs10720225	C	0.45	-3.69 (1.69)	0.0306	Synonymous
	13	RRP9	rs10720292	C	0.13	-5.86 (1.67)	0.0005	Synonymous
	13	GRM7	rs45433852	T	0.27	-4.37 (1.57)	0.0060	Synonymous
	15	NGEF	rs45434870	C	0.50	-28.08 (12.48)	0.0257	Intron
Eosinophil	1	SEMA6D	rs318932969	T	0.06	0.46 (0.23)	0.0445	Intron
	2	DPF2	rs45430343	T	0.40	-0.35 (0.18)	0.0500	Intron
	2	SSRP1	rs45433934	T	0.37	-0.27 (0.12)	0.0338	Intron
	12	ETV4	rs10719800	C	0.50	-0.75 (0.36)	0.0401	Intron
	14	GGT1	rs45432035	G	0.49	-0.95 (0.32)	0.0032	Intron
Atypical lymph	1	SEMA6D	rs318932969	T	0.06	0.60 (0.29)	0.0396	Intron
	2	AP3D1	rs10719761	A	0.42	0.28 (0.13)	0.0388	Synonymous
	6	DHX38	rs10721016	A	0.40	-0.40 (0.17)	0.0212	Synonymous
	14	NEFL	rs45433391	T	0.18	-0.35 (0.17)	0.0436	Synonymous
	15	ATG9A	rs45435354	T	0.48	-1.38 (0.46)	0.0029	Intron
	17	IDH3B	rs10720906	C	0.24	0.45 (0.19)	0.0206	3' UTR
	17	PRPF6	rs45431912	C	0.26	-0.33 (0.15)	0.0336	Intron

Table 4 Functional annotation of candidate genes within immune-related trait

Trait	Category	Term	Genes	*P*-value
IGF-1	BP	GO:0010975 (regulation of neuron projection development)	CUL7, VIM, VLDLR	0.0120
		GO:0006955 (immune response)	PSMB4, MAPKAPK3, CTLA4, EPRS	0.0200
		GO:0031344 (regulation of cell projection organization)	CUL7, VIM, VLDLR	0.0221
		GO:0045664 (regulation of neuron differentiation)	CUL7, VIM, VLDLR	0.0239
		GO:0050776 (regulation of immune response)	PSMB4, MAPKAPK3, CTLA4	0.0308
		GO:0045595 (regulation of cell differentiation)	CUL7, VIM, CTLA4, VLDLR	0.0340
		GO:0050767 (regulation of neurogenesis)	CUL7, VIM, VLDLR	0.0359
		GO:2000026 (regulation of multicellular organismal development)	CUL7, VIM, CTLA4, VLDLR	0.0437
		GO:0051960 (regulation of nervous system development)	CUL7, VIM, VLDLR	0.0474
	CC	GO:0005737 (cytoplasm)	PSMB4, CUL7, F5, NDUFV1, VIM, MAPKAPK3, CTLA4, EPRS, DECR1	0.0308
		GO:0043231 (intracellular membrane-bounded organelle)	PSMB4, CUL7, F5, RXRB, NDUFV1, VIM, MAPKAPK3, CTLA4, DECR1	0.0483
		GO:0031988 (membrane-bounded vesicle)	PSMB4, F5, VIM, CTLA4, DECR1	0.0498
	MF	GO:0097367 (carbohydrate derivative binding)	PSMB4, NDUFV1, VIM, MAPKAPK3, EPRS, VLDLR	0.0017
		GO:0005488 (binding)	PSMB4, F5, RXRB, NDUFV1, VIM, MAPKAPK3, EPRS, DECR1, VLDLR	0.0237
IS	BP	GO:0048519 (negative regulation of biological process)	PSMB4, RBM42, SEMA6D, ILK, PSME3, NEFL	0.0293
		GO:0000904 (cell morphogenesis involved in differentiation)	SEMA6D, ILK, NEFL	0.0415
	CC	GO:0032991 (macromolecular complex)	PSMB4, CGN, LIFR, PSME3, RRP9, NEFL	0.0472
IG	BP	GO:0044271 (cellular nitrogen compound biosynthetic process)	TRIM29, GRM7, CNOT1, KARS, PRPF6	0.0480
	CC	GO:0005737 (cytoplasm)	ATG9A, TRIM29, GRM7, IDH3B, CNOT1, CXADR, KARS	0.0445
		GO:0044424 (intracellular part)	ATG9A, TRIM29, GRM7, IDH3B, CNOT1, CXADR, KARS, PRPF6	0.0492
		GO:0044444 (cytoplasmic part)	ATG9A, GRM7, IDH3B, CNOT1, CXADR, KARS	0.0488
	MF	GO:0005488 (binding)	ABCF3, TRIM29, GRM7, IDH3B, CNOT1, CXADR, KARS, PRPF6	0.0379
NP	CC	GO:0032991 (macromolecular complex)	GRM7, BTBD2, AP3D1, BAP1, RRP9, CBX8	0.0472
EP	BP	GO:0009605 (response to external stimulus)	SEMA6D, GGT1, ETV4	0.0101

IGF-1: insulin-like growth factor-1; IS: insulin; IG: immuno globulin; NP: neutrophil; EP: eosinophil; BP: biological process CC: cellular component; MF: molecular function

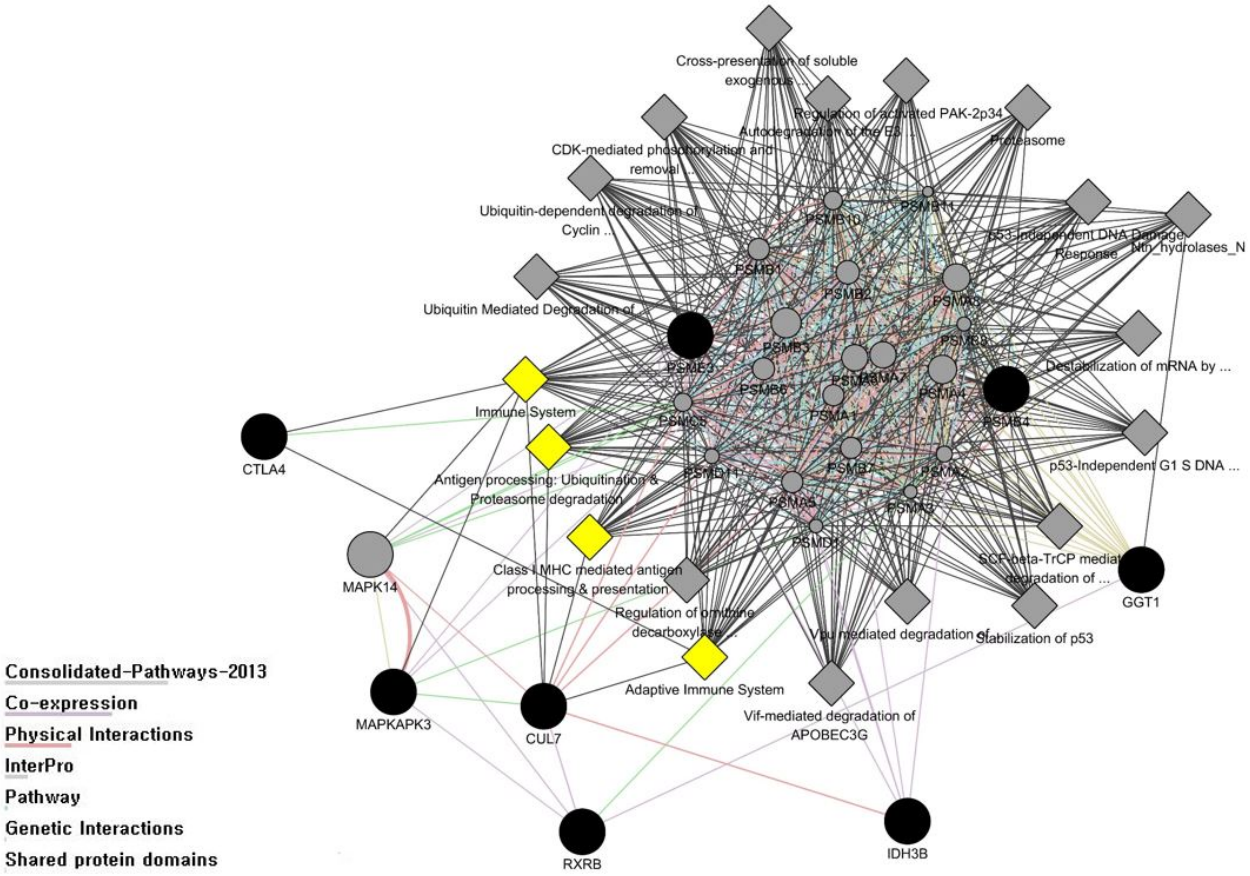

Figure 1 Pathway and network analysis of the candidate genes
It shows direct interactions between blood component trait-related immune candidate genes and their regulatory relationships. Black and yellow highlighted genes and pathway indicate candidate genes from gene network and pathway analysis

Discussion

This study was conducted to investigate blood component traits-related immune candidate genes using 209 publicly available SNPs of 151 genes in KNP and Yorkshire F_2 hybrid pig populations.

The genotypes of 209 SNPs were analysed using the Sequenom MassARRAY system method, which is the method that the authors used in a previous study (Li *et al*., 2011). Using 144 SNPs filtered through QC and LD analyses, they performed association analysis on nine blood component traits (IGF-1, IS, IG, LC, MC, EP, BP, NP and AL) and hormones.

Table 2 and Table 3 show that 52 SNPs among the 144 SNPs were effectively associated with 9 blood component traits, and 19 SNPs were included in blood component traits-related immune QTL regions. In previous reports, the SNPs in the QTL region of genes related to PRRSV susceptibility (123,334–124,643 kb) and PRRS viral load (32,901–33,123 kb) were *SEMA6D* (rs318932969), *IDH3B* (rs10720906), and *HSPG2* (rs45432189) (Boddicker *et al*., 2012; Waide *et al*., 2017). In the QTL regions (609–21,136 kb) of the interleukin 10 level and Toll-like receptor 9 level genes that affect the resistance to viral disease (Uddin *et al*., 2011), the SNPs *NDUFV1*, *DPF2*, and *SSRP1,* were found on chromosome 2. In addition, the CSFV antibody level and CD4-positive/CD8-positive leukocyte ratio QTL regions (80,511–106,510 kb) related to classical swine fever (CSF) virus infection were found to contain the *CGN* and *PSMB4* SNPs located on chromosome 4. Further, the CD4-positive/CD8-positive leukocyte ratio QTL region (57,165–127,880 kb) was also reported to be present on chromosome 15, and SNPs were observed as *CTLA4* (rs45432756) and *ATG9A* (rs45436465) in the same region (Lu *et al*., 2011; Lu *et al*., 2014). Moreover, the CD4-negative and CD8-positive leukocyte percentage QTLs, which are related to the disease resistance of pigs (Lu *et al*., 2011), were found on chromosome 13 (27,210–58,927 kb), and *MAPKAPK3* (rs45432062), *RRP9* (rs10720292), and *BAP1* (rs10720225) SNPs were present in the same QTL region. The QTL region of genes that are associated with traits for *Salmonella* count in the liver, and in the liver and spleen, which were

reported to be associated with porcine salmonellosis susceptibility, showed that *AP3D1* (rs10719761) and *BTBD2* (rs45430365) SNPs on chromosome 2 (75,040–121,864 kb) were located within the QTL regions (Galina-Pantoja *et al*., 2009). In addition, the SNPs *PTPRM* (rs45431026) located on chromosome 6, *SUPT6H* (rs10720118) on chromosome 12, and *PRPF6* (rs1108981338 and rs45431912) on chromosome 17 were found in the white blood cell count QTL (SSC6: 79,653–104,254 kb; SSC12: 38,822–47,927 kb) and lymphocyte number QTL (48,731–66,928 kb) regions reported in previous studies (Reiner *et al*., 2008; Okamura *et al*., 2012). However, because there is a dearth of QTL studies related to the immune system of pigs and the QTL region is wide, high-density QTL fine mapping and additional studies should be performed to better evaluate the genes that were analysed in this study as positional candidate genes.

The authors performed functional annotation of 49 genes that were detected to be significantly associated with blood component traits using tools on the DAVID website and detected 24 GO terms in all three categories (biological process, cellular component, and molecular function). Pathway searches were performed using the GeneMANIA and KEGGParser tools and integrated interactions between candidate genes were also investigated. Only eight genes (*PSMB4*, *PSME3*, *MAPKAPK3*, *CTLA4*, *CUL7*, *GGT1*, *IDH3B*, and *RXRB*) of the 49 candidate genes were found to interact with each other, and four pathways (immune system, adaptive immune system, class I MHC-mediated antigen processing and presentation and antigen processing: ubiquitination & proteasome degradation) related to the total immune system were identified (Figure 1). In particular, the *PSMB4* and *PSME3* genes were actively networked with the other pathways and genes, which not only serve in cell cycle progression, proliferation, signal transduction, and degradation of abnormal proteins, and defective enzymes in metabolic regulation by the ubiquitin-proteasome pathway, but also have mechanisms involved in genetic diseases. For example, PA28 activators, which are among the two known activator types of the 26S proteasome (PA28 and PA700), are known to contain three genes, namely *PSME1*, *PSME2* and *PSME3* (Husom *et al*., 2004; McNaught *et al*., 2003; Matsushita *et al*., 2004). In addition, *PSMB4* (proteasome subunit beta 4) is a gene encoding 20S proteasome subunit beta-7, which is known to regulate protease assembly in humans (Hirano *et al*., 2008; Murata *et al*., 2009). *PSMB4* regulation is also known to be associated with carcinogenesis of various malignant types of tumour, including hepatocellular carcinomas and gliomas (Cui *et al*., 2006; Thaker *et al*., 2009). However, research on the molecular genetic function of the pig's immune system is still insufficient.

Conclusion

The results of this study suggest that the six remaining candidate genes also interact directly with the blood component traits-related immune pathways, which may be helpful in pig immunization and disease studies. Moreover, the class I MHC-mediated antigens that are expressed in all somatic cells are the most important antigens to determine the success of skin and organ transplantation in relation to immune rejection. Because the eight candidate genes that the authors found are included in the pathway related to this complex, additional studies should be performed on porcine heterologous organs. Further studies should be also conducted to provide data on the practical applications of pig production.

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Authors' Contributions

BL and SK contributed equally to this work.

Conflict of Interest Declaration

The authors declare that they have no competing interests.

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