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Mapping of Quantitative Trait Loci for Hematological Traits on Pig Chromosome 10

[1]Shaoqian Cai, [2]Yang Liu, [1]Chenhua Zhang, [2]Weixuan Fu, [2]Yuanfang Gong, [2]Xin Lu, [2]Qin Zhang and [1]Zongjun Yin

[1]College of Animal Science and Technology, Anhui Agricultural University, Hefei 230036, China
[2]Key Laboratory of Animal Genetics and Breeding of The Ministry of Agricultural, College of Animal Science and Technology, China Agricultural University, Beijing 100193, China

Corresponding Author: Yin Zongjun, Department of Animal Science, College of Animal Science and Technology, Anhui Agricultural University, Hefei 230036, China Tel: 0086-551-5785180 Fax: 0086-551-5785027

ABSTRACT

This study was aimed to identify Quantitative Trait Loci (QTL) for haematological traits in pig chromosome 10 and facilitate the cloning of candidate genes underlying the QTLs. Hematological traits are essential parameters for evaluating the health status of animals and play a extremely important role in disease resistance. In this study, three main components, leukocyte traits, erythrocyte traits and platelet traits were measured in a composite pig population consisting of 445 pigs of three breeds (Landrace, Large White and Songhao Black Pig) distributed in 16 boar families, before and after vaccination with modified live CSF (classical swine fever) vaccine. A partial genome scan for mapping Quantitative Trait Loci (QTL) for these traits was performed by genotyping 13 microsatellite markers on chromosome 10. Through a linear mixed model and the permutation for empirical threshold values, 4 significant QTLs on chromosome 10 were identified affecting hematocrit (HCT), hemoglobin (HGB), Mean Corpuscular Volume (MCV) and blood platelet counts (PLT) ($p<0.05$), respectively. Our results confirms that haematological traits variation differs between the three pig breeds and variations of HCT, HGB, MCV, PLT are associated with the 81~133 cM region in chromosomal 10.

Key words: Pig, microsatellite, QTL mapping, chromosome, hematological traits, likelihood ratio

INTRODUCTION

Haematological traits and their immune function are essential parameters and biochemical indicators for evaluating the health status of individuals. Interpretation of haematological values is complicated by the pronounced variability caused by non-systematic factors. Differences in haematological traits among breeds and populations provide evidence of genetic control (Reiner *et al*., 2007, 2008). With the development of molecular biology and improvement of high-density genetic map of pig, the genetic influence on immune response and susceptibility to disease has been focused on considerable Quantitative Trait Loci (QTL) mapping research in pigs (Edfors-Lilja *et al*., 1998).

Since, the first QTL mapping project in pigs (Andersson *et al*., 1994), most QTL studies have focused on growth, carcass and meat quality and reproduction traits (Campos *et al*., 2009; Gholizadeh *et al*., 2008; Hu *et al*., 2005, 2007), QTL for immune capacity and disease resistance

is very limited, mainly due to these traits are complex traits and difficult to determine. Edfors-Lilja *et al.* (1994) identified a QTL on pig chromosome 1 (SSC1) with significant effect on circulating leucocyte numbers and also identified chromosomal regions harbouring genes for 'stress' induced alterations in porcine leukocyte numbers and functions and the most prominent QTL located on chromosome 8 (SSC8) influencing 'transport stress'-induced alterations in numbers of neutrophil in a F2 population of wild boar (W) × Swedish Yorkshire (Y) (Edfors-Lilja *et al.*, 2000). Another farther study confirmed QTL with influence leukocyte count, blood parameters and leukocyte function were on pig chromosome 1 and 8 (Wattrang *et al.*, 2005). Reiner *et al.* (2007) detected 43 QTL affecting red blood cell traits (HCT, HB, RBC and MCHC) in a F2 hybrid population (n = 139) of Meishan pigs and Pietrain pigs, which distributed on 16 chromosomes and 12 QTL showed significant effects on genome-wide level and 31 QTL showed significant effects on chromosome level.

In this study, we report the identification of QTL for hematological traits in pig chromosome 10. For this purpose, 13 highly polymorphic microsatellite markers spaced at an average distance of 10 cm throughout pig chromosome 10 according to the latest linkage map on NCBI were selected. A composite resource population with three breeds (Landrace, Large White, Songliao Black pig) distributed in 16 boar families was used for QTL mapping.

MATERIALS AND METHODS

Animal composite resource population: The animals consisted of 445 pigs which are 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), respectively. All pigs were vaccinated with live CSF vaccine at 21 days of age and were weaned at 35 days of age. All pigs were raised in 2007, 2008 and under standard indoor conditions at the experimental farm of the Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China.

Collection and measurement of blood samples: Blood samples were collected from each piglet one day before the vaccination (day 20) and two weeks after the vaccination (day 35), respectively. The samples were directly injected into VACUETTE® Serum Clot Activator tubes.

Eighteen parameters include 7 leukocyte traits White Blood Cell count (WBC), neutrophilic granulocyte count (GRAN) and its percentage (GR%), lymphocyte count (LYMF) and its percentage (LY%), monocytes count (MONO) and its percentage (MO%), 7 erythrocyte traits (red blood cell (RBC), hemoglobin (HGB), hematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC) and red blood cell volume distribution width (RDW), 4 platelet traits (blood platelet counts (PLT), Mean Platelet Volume (MPV), platelet distribution width (PDW) and plateletocrit (PCT). All these blood routine parameters were measured by MEK-6318K type full automatic Hematology Analyzer (Nihon Kohden, Japan).

Microsatellite markers: According to number of alleles (>3) and average distance of marker is about 10 cm, 13 μL (Table 1) on pig chromosome 10 were selected based on the latest linkage map of NCBI (http://www.ncbi.nlm.nih.gov/genemap).

PCR amplification and genotype determination: Genomic DNA was isolated from the ear tissue sample using phenol/chloroform extraction and ethanol precipitation (Sambrook *et al.*, 1989).

Table 1: Marker names and their relative positions of the 13 microsatellites used in this research

Microsatellite	Relative position/cM	Fragment size (bp)	Microsatellite	Relative position/cM	Fragment size (bp)
SW830	0.0	177-192	SWR334	67.5	181-222
SWR136	7.6	191-229	SW1991	79.4	196-212
SW249	17.3	235	PIP5K2	91.0	162-193
SW1894	23.2	196-208	SW951	101.0	121-136
SW2491	43.0	136-168	SY30	108.0	174-192
SWC19	50.5	168-190	SW2067	128.0	108-136
S0070	62.3	261-293			

The PCR was carried out in a total volume of 20 μL including 50 ng of template DNA 1 μL, 10×buffer (containing 15 mmol L^{-1} $MgCl_2$) 2 μL, 10 mmol L^{-1} dNTPs 1.6 μL, 10 μmol L^{-1} of each primers 0.4 μL, 5 U μL^{-1} of Taq polymerase 0.1 μL. After PCR reaction, mixed PCR products of 3-4 markers, adds in 6~8 μL deionized formamide and ROXTM-350 internal standard reagent (100:1) to 95°C denaturation for 5 min, then detected by ABI377 DNA sequencer. Finally, GeneScan3.7 software was used to genotype determination.

Statistical analysis and QTL mapping: Mapping of QTL was performed by QTL-express software (Seaton *et al.*, 2002) using half-sibling analysis in the online website (http://latte.cap.ed.ac.uk), which is based on a linear model as follows:

$$Y_{ijklm} = \mu + b_i + F_{ij} + M_{ijk} + H_l + q_{ijklt}Q_{ijklt} + e_{ijkltm}$$

where, Y is a vector of the phenotypic value for haematological parameters, μ is the overall mean, b is a vector of breed effect, F is a vector of boar effect, M is a vector of sow effect, H is a vector of fixed effect of the year and season, q is a vector of residual polygenic effects, Q is a vector of QTL allelic effects, e is a vector of random residuals.

The QTL analysis was scanned in 1 cM steps within the region covered by the 13 markers. The least square method was used to estimate the variance components in the model and the Likelihood Ratio (LR) statistics were calculated for scanning particular location on pig chromosome 10.

$$LR = n \times \ln(SSE_{reduced} / SSE_{full})$$

where, $SSE_{reduced}$ and SSE_{full} are the least square functions corresponding to the null hypothesis (there is no QTL) and the alternative hypothesis (there is a QTL), respectively, n is the samples size.

Considering the distributions of our measuring traits, significance threshold was established by the permutation approach to obtain the empirical distribution of LR statistics (Churchill and Doerge, 1994). For each trait, 1000 permutations of the phenotypes were performed to generate the empirical distributions of the LR-values and then the thresholds for inferring the existence of significant QTL were obtained.

RESULTS AND DISCUSSION

Detected results of microsatellites PCR products: Fluorescent PCR products in the AB1377 sequencer on agarose gel electrophoresis were showed in Fig. 1. The same lane on the two different

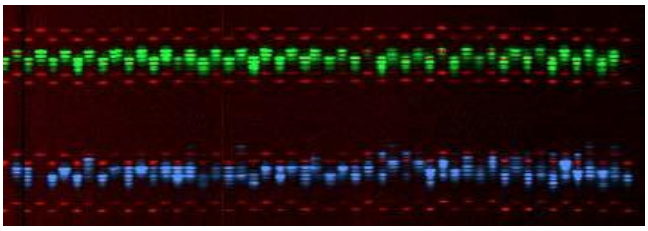

Fig. 1: Detection of productions of microsatellites marker SW830 PCR labeled by fluorescence on ABI377 sequencer

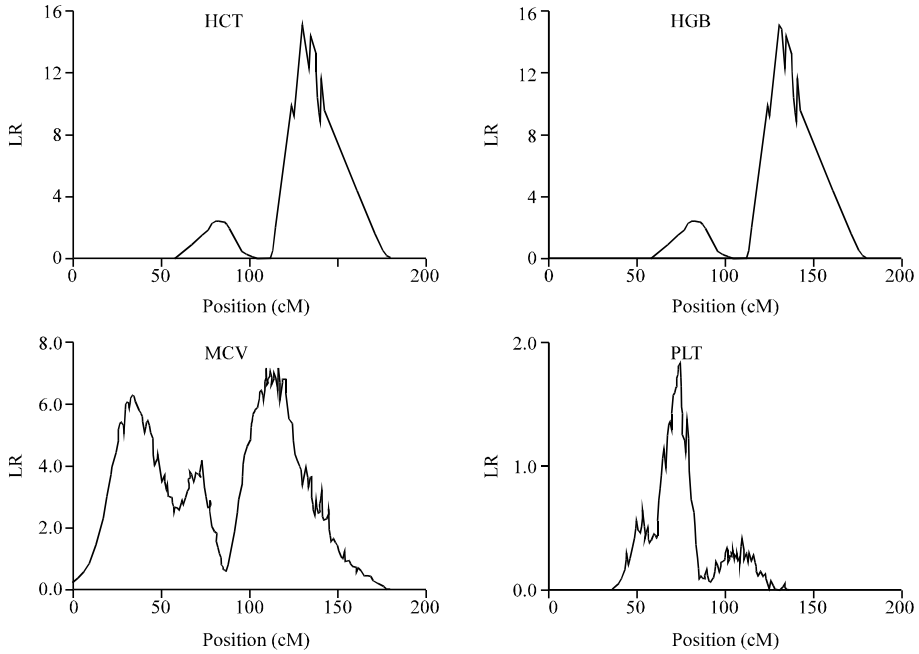

Fig. 2: Likelihood Ratios (LR) profile for linkage mapping of QTL for HCT, HGB, MCV, PLT (35 day) level in blood on SSC10

individuals with different microsatellite was visible. Fluorescence intensity of products of each primer meets with the requirement of detection.

QTL for haematological traits: Four QTL with p-value <0.05 are identified in chromosome 10 (Table 2) through the permutation test, in which one QTL was found with significant effect on blood platelet counts (PLT) at p<0.05. The most significant QTL (p<0.01) was found with effect on hematocrit (HCT), hemoglobin (HGB), Mean Corpuscular Volume (MCV) (Fig. 2). Those QTLs are mainly concentrated in the 81~133 cM region of SSC10 and close to the microsatellite markers SW249, SWR136, S0070 and SW1894.

Haematological traits include white blood cells related traits, red blood cells related traits and platelets traits, which are important components of the animal immune system. Edfors-Lilja *et al.* (1998) reported a QTL for WBC on SSC1 in a F2 population of wild boar (W) × Swedish Yorkshire (Y). In another study, a QTL with effect on WBC was also identified on SSC1 in boar families of Landrace × Yorkshire (Wattrang *et al.*, 2005). The White Blood Cell count (WBC) is a powerful

Table 2: Results of QTL mapping for blood biochemical parameter on SSC10

			Flanking markers	
Trait	Position (cM)	LR value	Left	Right
GR%	83	1.7652	SW249	SWR136
GRAN	94	1.8720	SWR136	S0070
HCT	136	15.0423**	S0070	SW1894
HGB	133	16.3014**	S0070	SW1894
LY%	76	0.2621	SW249	SWR136
LYMF	114	1.5013	S0070	SW1894
MCH	81	1.7420	SW249	SWR136
MCHC	122	1.2300	SWC19	SW1991
MCV	106	6.9441**	SWR136	S0070
MO%	2	0.3005	SW830	SW2491
MONO	110	0.4723	SWR136	S0070
MPV	102	0.2500	SWR136	S0070
PCT	47	1.5125	SW2491	SW249
PDW	38	1.8204	SW2491	SW249
RBC	52	0.7110	SW2491	SW249
RDW	110	0.5520	SWR136	S0070
WBC	7	0.5642	SW830	SW2491
PLT	81	1.9232*	SW249	SW136

*Indicate significant level of chromosome ($p<0.05$); **Indicate significant level of chromosome ($p<0.01$)

indicator for infectious and inflammatory disease, leukaemia, lymphoma and bone marrow disorders (Kannel *et al*., 1992). No QTL for white blood cells related traits was found in the present study, which suggests that white blood cells related traits are not controlled by QTL region on SSC10.

Red blood cells are important innate immune cells in blood circulation and they can recognize antigen, kill antigen, clear and circulating immune complexes, they are also involved in immune regulation and have a complete self-regulation system. In our study, 2 QTL for HGB, PCT were found on SSC10, respectively and a QTL for MCV was also identified between the markers SWR136 and S0070 on SSC10. Reiner *et al*. (2007) mapped QTL for HGB on SSC2 and SSC7 in a F2 population of Meishan pigs and Pietrain pigs. These disagreements may be due to a resource population by different building design, different molecular genetic markers and different QTL mapping method.

Platelet-related traits are essentially important in the medical tests for a number of complex diseases. Mean Platelet Volume (MPV) and Platelet Distribution Width (PDW) are strongly associated with increased risk of heart disease (Bath *et al*., 2004) and thrombocytosis (Syed *et al*., 2007), respectively. In this study, no QTL for MPV and PDW were identified but the QTL for platelet count (PLT) was mapped between marker SW249 and SW136 on SSC10. PLT is highly heritable, with genetic factor accounting for 80% of variance in humans (Evans *et al*., 2004). Platelet count (PLT) has been correlated with blood clotting time and may be a risk factor in the development of thrombosis and atherosclerosis (Mustard *et al*., 1977). Therefore, fine mapping for platelets in our population will be the next interesting point in farther research.

At present, there have different results of QTL mapping on immune capacity of pig and they are also having not strictly comparison. However, comparing with the results of these studies, we can initially understand the relationship between them. Our analysis is also the first step for

indentifying significant regions on chromosome 10 controlling haematological traits which are important immunomodulation agents. The present results increase our understanding of genetic control of haematological traits, in order to reveal the QTL of immune capacity of pigs completely, farther whole genomic scan and candidate gene cloning by QTL position are necessary to identify the functional genes, then provide the molecular marker genes for marker-assisted selection in breeding of disease resistance.

CONCLUSION

A partial genome scan for mapping quantitative trait loci (QTL) for these traits was performed using 13 microsatellite markers on chromosome 10. 4 QTL were identified on chromosome 10, of which 3 QTL are very significant affecting HCT, HGB, MCV ($p<0.01$) and 1 QTL significant affecting PLT ($p<0.05$). These QTL are associated with the 81~133 cM region in chromosome 10 and close to microsatellite SW249, SWR136, S0070 and SW1894.

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REFERENCES

Andersson, L., C.S. Haley, H. Ellegren, S.A. Knott and M. Johansson *et al.*, 1994. Genetic mapping of quantitative trait loci for growth and fatness in pigs. Science, 263: 1771-1774.

Bath, P., C. Algert, N. Chapman and B. Neal, 2004. Association of mean platelet volume with risk of stroke among 3134 individuals with history of cerebrovascular disease. Storke, 35: 622-626.

Campos, R.L.R., M. Ambo, M.F. Rosario, A.S.A.M.T. Moura and C. Boschiero *et al.*, 2009. Potential association between microsatellite markers on chicken chromosomes 6, 7 and 8 and body weight. Int. J. Poult. Sci., 8: 696-699.

Churchill, G.A. and R.W. Doerge, 1994. Empirical threshold values for quantitative trait mapping. Genetics, 138: 963-971.

Edfors-Lilja, I., E. Wattrang, U. Magnusson and C. Fossum, 1994. Genetic variation in parameters reflecting immune competence of swine. Vet. Immunol. Immunopathol., 40: 1-16.

Edfors-Lilja, I., E. Wattrang, L. Marklund, M. Moller, L.A. Eklund, L. Andersson and C. Fossum, 1998. Mapping quantitative trait loci for immune capacity in the pig. J. Immunol., 160: 829-835.

Edfors-Lilja, I., E. Wattrang, L. Andersson and C. Fossum, 2000. Mapping quantitative trait loci for stress induced alterations in porcine leukocyte numbers and functions. Anim. Genet., 31: 186-193.

Evans, D.M., G. Zhu, D.L. Duffy, G.W. Montgomery, I.H. Frazer and N.G. Martin, 2004. Multivariate QTL linkage analysis suggests a QTL for platelet count on chromosome 19q. Eur. J. Hum. Genet., 12: 835-842.

Gholizadeh, M., G.R. Mianji and H.S. Zadeh, 2008. Potential use of molecular markers in the genetic improvement of livestock. AJAVA., 3: 120-128.

Hu, Z.L., S. Dracheva, W. Jang, D. Maglott, J. Bastiaansen, M.F. Rothschild and J.M. Reecy, 2005. A QTL resource and comparison tool for pigs: PigQTLDB. Mamm. Genome, 16: 792-800.

Hu, Z.L., E.R. Fritz and J.M. Reecy, 2007. AnimalQTLdb: A livestock QTL database tool set for positional QTL information mining and beyond. Nucleic. Acids. Res., 35: D604-D609.

Kannel, W.B., K. Anderson and P.W. Wilson, 1992. White blood cell count and cardiovascular disease. Insights from the framingham study. J. Am. Med. Assoc., 267: 1253-1256.

Mustard, J.F., S. Moore, M.A. Packham and R.I. Kinlough-Rathbone, 1977. Platelets, thrombosis and atherosclerosis. Prog. Biochem. Pharmacol., 13: 312-325.

Reiner, G., R. Fischer, S. Hepp, T. Berge, F. Kcehler and H. Willems, 2007. Quantitative trait loci for red blood cell numbers in swine. Anim. Genet., 38: 447-452.

Reiner, G., R. Fischer, S. Hepp, T. Berge, F. Kohler and H. Willems, 2008. Quantitative trait loci for white blood cell numbers in swine. Anim. Genet., 39: 163-168.

Sambrook, J., E.F. Fritsch and T.A. Maniatis, 1989. Molecular Cloning: A Laboratory Manual. 2nd Edn., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, USA., ISBN-13: 9780879695774, pp: 21-51.

Seaton, G., C.S. Haley, S.A. Knott, M. Kearsey and P.M. Visscher, 2002. QTL express: Mapping quantitative trait loci in simple and complex pedigrees. Bioinformatics, 18: 339-340.

Syed, N.N., M. Usman and M. Khurshid, 2007. Thrombocytosis: Age dependent aetiology and analysis of platelet indices for differential diagnosis. Indian J. Pathol. Microbiol., 50: 628-633.

Wattrang, E., M. Almavist, A. Johansson, C. Fossum and P. Wallgren *et al.*, 2005. Conformation of QTL on porcine chromosome 1 and 8 influencing Leukocyte numbers, haematological parameters and leukocyte function. Anim. Genet., 36: 337-345.