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Quantitative trait loci for porcine baseline erythroid traits at three growth ages in a White Duroc × Erhualian F_2 resource population

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Abstract Baseline erythroid indices are increasingly involved as risk factors for common complex diseases in humans. However, little is known about the genetic architecture of baseline erythroid traits in pigs. In this study, hematocrit (Hct), hemoglobin (Hgb), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), red blood cell (RBC), and red cell distribution width (RDW) were measured in 1420 (day 18), 1410 (day 46), and 1033 (day 240) F_2 pigs from a White Duroc × Erhualian intercross resource population. The entire resource population was genotyped for 183 microsatellite loci across the pig genome, and the quantitative trait loci (QTL) analysis was performed for all erythroid-related traits measured with QTL Express based on a least-squares method. A total of 101 QTL, including 46 genome-wide significant QTL and 55 chromosome-wide significant QTL, regulating erythroid traits were found on all pig chromosomes (SSC) except for SSC15 and SSC18. The genome-wide significant QTL were mainly localized on SSC1, 7, 8, 10, and X. These results confirmed most of QTL previously identified in the swine. More importantly, this study detected age-specific QTL for baseline erythroid traits in pigs for the first time. Notably, the QTL for MCV and MCH on day 18 on SSC8 with small intervals of 3 and 4 cM, respectively, provided a good starting point for identifying causal genes underlying MCV and MCH in the future.

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Introduction

Complete blood count tests are performed routinely in medical examinations. Increasingly, baseline blood cell count phenotypes are emerging as important indicators for disease and disease severity. Recently, hematocrit (Hct) (Gagnon et al. 1994; Lippi et al. 2002), hemoglobin (Hgb) (Ohene-Frempong et al. 1998), mean corpuscular volume (MCV) (Haltmayer et al. 2002; Mueller et al. 2001, 2002), and red blood cell count (RBC) (Puddu et al. 2002) as potential markers of cardiovascular events have been recognized in epidemiologic studies. Epidemiologic and genetic studies have indicated that both environmental and genetic factors play roles in regulating these peripheral blood traits (Evans et al. 1999). Although genetic influences on these baseline erythroid traits are firmly established, the genetic architecture of these traits is still poorly understood. Thus, identifying causal genes underlying these traits will provide novel targets for risk assessment.

The domestic pig is being increasingly exploited as a model for human medicine. To identify quantitative trait loci (QTL) and causal genes for monogenic and complex traits, a variety of genomic resources have been developed in recent years (Bidanel and Rothschild 2002). Since the first QTL mapping study in pigs (Andersson et al. 1994), more than 1831 QTL have been mapped for more than 316 traits (http://www.animalgenome.org/QTLdb/pig.html).

However, only a few QTL for hematologic parameters have been identified in pigs (Edfors-Lilja et al. 1998; Reiner et al. 2007, 2008; Wattrang et al. 2005), and no studies concerning QTL for baseline erythroid traits at different ages in swine have been reported. The aim of our study was to identify QTL for baseline erythroid traits at three growth ages (days 18, 46, and 240) in pigs using a large-scale White Duroc × Erhualian intercross resource population.

Materials and methods

Animals and phenotypic measurements

A three-generation White Duroc × Erhualian intercross resource population was created and managed as described previously (Ren et al. 2008). Briefly, two White Duroc boars were mated to 17 Erhualian sows. Nine F_1 boars and 59 F_1 sows were then chosen and mated, avoiding full-sib mating, to produce a total of 1912 F_2 animals in six batches. All F_2 animals were kept under standard indoor conditions at the experimental farm of Jiangxi Agricultural University (China). All piglets were weaned at 46 days, and the male were castrated at 90 days. A total of 1420 F_2 animals at 18 days, 1410 at 46 days, and 1033 at 240 days were measured for baseline erythroid traits. Blood samples of 5 ml were collected from the cranial vena cava through disposable syringes and directly injected into eppendorf tubes containing 30 μl of 20% EDTA in phosphate-buffered saline (PBS). Complete blood counts were determined immediately after sample collection using a CD1700 whole-blood analyzer (Abbott, Chicago, IL, USA), and a standard set of cellular hematologic data was recorded, including RBC, Hct, Hgb, MCV, mean corpuscular hemoglobin (MCH, the ratio of Hb to RBC), mean corpuscular hemoglobin concentration (MCHC, the ratio of Hb to Hct), and red cell distribution width (RDW, calculated as [standard deviation of red cell volume × 100] ÷ MCV). The hematologic test was performed at The First Affiliated Hospital of Nanchang University, China.

Marker genotyping

Genomic DNA was extracted from ear tissue or blood of experimental pigs according to a standard phenol/chloroform method. The quality and concentration of DNA samples were guaranteed for the subsequent PCR using a DU®640 Nucleic Acid and Protein Analyzer (Beckman Coulter, Fullerton, CA, USA). Markers were initially selected from the USDA-MARC porcine reference map (http://www.marc.usda.gov/) and were then analyzed for their informativeness using DNA from the F_1 individuals. A final set of 183 informative microsatellite markers distributed among 19 pig chromosomes was genotyped across the entire White Duroc × Erhualian intercross resource population. Briefly, fluorescence labeled PCR primers were used to amplify genomic DNA on PTC-200 thermal cyclers (MJ Research, Waltham, MA, USA) and PCR products were separated and recorded in a 3130XL Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The number of markers on each pig chromosome (SSC) varied from 5 (SSC18) to 24 (SSC13), with an average interval of 13.40 cM.

Statistical analysis

The comprehensive linkage map was constructed using Crimap ver. 2.4 (Lander and Green 1987) as described in Guo et al. (2008). Recombination units were transformed to map distances using the Haldane mapping function. Mean values and standard deviations of baseline erythroid traits were analyzed by SAS ver. 9.0 (SAS Institute Inc., Cary, NC, USA). Values were tested for approximate Gaussian distribution. The PROC GLM procedure of SAS was used to determine the fixed effects and covariates in the following QTL model. A QTL interval-mapping analysis was performed using the web-accessible program QTL EXPRESS (http://qtl.cap.ed.ac.uk/) (Seaton et al. 2002) and was based on a least-squares method (Haley et al. 1994). Gender and batch were considered fixed effects and live weight was considered a covariate in the QTL model. Significance thresholds were derived empirically from 1000 permutation data (Churchill and Doerge 1994). The 95% confidence intervals (CI_{95}) of QTL were calculated using a bootstrap method with 2000 iterations (Visscher et al. 1996). To confirm the assumption that marker genotypes associated with multiple QTL were uncorrelated, the QTL analysis was carried out sequentially (Holl et al. 2004) and the largest QTL effects were placed sequentially in the model as background effects.

Results and discussion

Erythroid phenotypes of the F_2 pigs are presented in Table 1. Mean RBC, Hgb, and Hct increased significantly with age ($P < 0.0001$). In contrast, mean RDW declined significantly with age ($P < 0.0001$). No significant age-dependent change was observed for mean MCH, MCHC, and MCV.

The genome-wide significant QTL for baseline erythroid traits are shown in Table 2 and suggestive (5% chromosome-wide significant) QTL are given in Supplementary Table 1. The statistic F values of the QTL on SSC7 and SSC8 are depicted in Fig. 1. A total of 101 QTL, including 46 at genome-wide significant levels and 55 at the

Table 1 Mean values and standard deviations of erythroid traits at four growth ages in the White Duroc × Erhualian intercross

Trait	Symbol	18 days	46 days	240 days
Hematocrit (100%)	Hct	0.30 ± 0.07^{a}	0.30 ± 0.07^{a}	0.41 ± 0.05^{c}
Hemoglobin (g/l)	Hgb	95.5 ± 26.5^{a}	100.6 ± 18.7^{b}	137.2 ± 16.1^{c}
Mean corpuscular hemoglobin (pg)	MCH	19.4 ± 3.8^{a}	18.2 ± 4.3^{b}	19.3 ± 1.5^{a}
Mean corpuscular hemoglobin content (g/l)	MCHC	319.7 ± 32.5^{a}	340.9 ± 49.8^{b}	337.4 ± 15.9^{c}
Mean corpuscular volume (fl)	MCV	60.7 ± 9.7^{a}	52.7 ± 8.2^{b}	57.2 ± 4.3^{c}
Red blood cell count ($\times 10^{12}$)	RBC	4.93 ± 0.90^{a}	5.67 ± 1.12^{b}	7.41 ± 0.84^{c}
Red cell distribution width (100%)	RDW	0.25 ± 0.05^{a}	0.24 ± 0.06^{b}	0.19 ± 0.03^{c}

Values with different superscript letters are significantly different ($P < 0.0001$)

suggestive level, for the seven traits measured were identified on all pig chromosomes except for SSC15 and 18. The genome-wide significant QTL were localized mainly on SSC1, 7, 8, 10, and X. The phenotypic variance explained by the QTL and CI_{95} of the QTL are generally smaller than previously reported (Reiner et al. 2007). This could be due to the larger experimental animals used in the present study, which enhanced the QTL detection power. Approximately three-fourths of the QTL detected have not been described previously, including eight genome-wide significant QTL. Moreover, to our knowledge, this is the first time that QTL for MCV, MCH, and RDW and QTL for baseline erythroid traits at different ages in the pig have been reported.

Eleven, 16, 19, 19, 7, 14, and 15 QTL were associated with RBC, Hgb, MCV, MCH, MCHC, RDW, and Hct at the three growth ages, respectively. For a given age, a total of 47 QTL at 240 days were detected, including 22 genome-wide significant QTL. Moreover, 26 and 30 QTL were identified at 18 and 46 days, respectively. Most of the QTL for a given erythroid trait at the three growth ages were detected in the same genomic regions, while some age-specific QTL were found on SSC1, 2, 7, 8, and 10. A majority of the QTL for MCV and MCH were detected in the same positions. The two traits were highly correlated ($P < 0.0001$), so it is conceivable that QTL for the two traits were detected in the same regions. Favorable QTL alleles were inherited from both Duroc founder boars and Erhualian founder sows. This suggests that modern selection in Western commercial breeds such as Duroc did not contribute to a significant difference in erythroid traits between the two founder breeds.

Seven QTL for Hct, Hgb, MCHC, MCV, and RDW were detected on SSC1, including two 1% genome-wide significant QTL for Hct and Hgb at 240 days (Table 2). To our knowledge, the QTL for Hgb at 240 days on this chromosome has not been identified in previous studies. The QTL for Hct at 240 days confirmed the previous QTL reported by Reiner et al. (2007). Both peaks of the two QTL were located between *SW781* and *S0313* (data not shown). This region is syntenic with the QTL for MCV and RBC in mice (Peters et al. 2006). A 5% genome-wide significant QTL for RDW at 240 days was detected at a different position on this chromosome.

The QTL for MCH at 18 and 46 days on SSC2 were located in the same region (Table 2), suggesting a common genetic architecture regulating MCH at the two stages. No QTL for MCH at 240 days was found on this chromosome, indicating that the QTL had a certain early-acting effect on MCH values. Another 1% genome-wide significant QTL on SSC2 for RDW at 240 days was detected in a region close to the QTL for Hct, Hgb, and RBC previously identified in pigs (Reiner et al. 2007) and the Hct QTL in mice (Johannes et al. 2006).

Six QTL on SSC3 were associated with Hct, Hgb, MCH, MCHC, and MCV, including two novel significant QTL each for MCH and Hgb at 240 days (Table 2). On SSC4, QTL for all erythroid traits except for MCH were found, while only the QTL for Hct at 46 days reached the genome-wide significance level (Table 2). It should be noted that no QTL for these traits have been previously reported on this chromosome in the pig. The QTL region affecting RBC, Hgb, MCHC, and Hct at 240 days corresponds to the QTL region for Hct in the mouse (Peters et al. 2005). No genome-wide significant QTL were identified on SSC5. A 1% genome-wide significant QTL for MCV at 240 days was detected on SSC6 (Table 2), which overlapped with the porcine Hct QTL described in Reiner et al. (2007).

In this study, a high proportion of QTL for erythroid traits were identified on SSC7 and 8 (Table 2). Age-specific QTL were observed on the two chromosomes. On SSC7, the QTL for RBC has a significant effect only at 240 days (Fig. 1A) and can therefore be viewed as a late-acting QTL. At 18 days, genome-wide significant QTL were detected only for MCH and MCHC (Supplementary Fig. 1A). Moreover, prominent QTL were not detected for RBC and RDW at 46 days (Supplementary Fig. 1B). This suggests that there might be different genes influencing erythroid traits at different growth ages in pigs. On SSC7, we detected a 1% genome-wide significant QTL for MCV

Table 2 Genome-wide significant quantitative trait loci for baseline erythroid traits at three growth ages in the White Duroc × Erhualian intercross[a]

SSC	Trait	Age (days)	Position (cM)	*F* value[b]	Add ± SE[c]	Dom ± SE[d]	CI_{95}[e] (cM-cM)	Var%[f]
1	Hct	240	87	11.3***	9.70 ± 2.10	4.60 ± 3.50	53.5–108.0	1.81
	Hgb	240	80	10.8***	3.17 ± 0.68	0.28 ± 1.13	38.0–142.0	1.69
	RDW	240	13	9.4**	−2.33 ± 0.86	−4.96 ± 1.50	5.0–80.0	1.59
2	MCH	18	21	8.7**	−0.33 ± 0.08	−0.18 ± 0.14	0.0–118.0	1.02
		46	25	11.3***	−0.38 ± 0.08	0.05 ± 0.13	0.0–80.5	1.50
	RDW	240	72	10.9***	−3.37 ± 0.73	1.00 ± 1.13	18.0–82.0	1.86
3	Hgb	240	74	8.2**	2.65 ± 0.66	0.50 ± 1.01	25.5–106.0	1.25
	MCH	240	76	11.4***	0.29 ± 0.06	−0.03 ± 0.09	28.0–103.0	1.60
4	Hct	46	65	9.4**	−8.80 ± 2.15	−4.10 ± 3.16	32.0–84.0	1.24
6	MCV	240	99	11.0***	0.75 ± 0.19	−0.79 ± 0.31	21.0–188.0	1.54
7	Hct	46	60	11.7***	9.50 ± 2.13	−4.90 ± 3.09	22.5–89.0	1.58
		240	57	41.7***	16.20 ± 1.82	−5.50 ± 2.54	49.0–70.0	7.14
	Hgb	46	60	17.0***	3.03 ± 0.60	−1.92 ± 0.88	53.0–72.0	2.18
		240	57	41.1***	5.10 ± 0.58	−1.80 ± 0.81	57.0–67.0	6.93
	MCH	18	46	11.3***	0.34 ± 0.08	−0.19 ± 0.11	36.0–123.5	1.37
		46	58	12.8***	0.33 ± 0.07	−0.22 ± 0.11	31.0–72.0	1.72
		240	59	22.5***	0.35 ± 0.06	−0.25 ± 0.08	52.5–59.0	3.32
		240	92	15.9***	−0.32 ± 0.06	−0.12 ± 0.08	60.0–99.0	2.30
	MCHC	46	1	9.1**	−5.30 ± 1.25	0.66 ± 1.79	0.0–90.5	1.21
	MCV	18	47	9.7**	1.14 ± 0.31	−1.07 ± 0.44	18.0–147.0	1.18
		46	59	10.1**	1.12 ± 0.28	−0.74 ± 0.40	2.0–112.0	1.32
		240	57	21.4***	1.07 ± 0.19	−0.80 ± 0.24	53.0–59.0	3.17
		240	92	13.4***	−0.91 ± 0.19	−0.44 ± 0.25	52.0–96.0	1.92
	RBC	240	71	26.2***	0.23 ± 0.03	0.04 ± 0.05	64.0–90.5	4.41
8	Hgb	46	64	8.1**	−2.32 ± 0.59	−0.63 ± 0.84	20.5–100.0	1.02
	MCH	18	74	37.2***	0.64 ± 0.07	0.12 ± 0.11	73.0–77.0	4.83
		46	74	14.2***	0.40 ± 0.07	0.04 ± 0.11	73.0–89.0	1.90
		240	74	83.5***	0.65 ± 0.05	0.01 ± 0.07	70.0–76.0	12.72
	MCV	18	77	39.0***	2.41 ± 0.27	0.15 ± 0.39	74.0–77.0	1.07
		46	75	11.6***	1.37 ± 0.29	0.29 ± 0.42	72.0–140.0	1.55
		240	74	79.7***	2.01 ± 0.16	−0.28 ± 0.24	69.0–76.0	12.16
	RBC	18	73	16.6***	−0.17 ± 0.03	−0.04 ± 0.04	47.0–86.5	2.22
		46	71	22.6***	−0.21 ± 0.03	−0.04 ± 0.05	66.0–74.0	3.20
		240	75	38.8***	−0.27 ± 0.03	−0.03 ± 0.05	65.0–77.0	6.63
	RDW	18	73	10.5***	−6.04 ± 1.35	−1.93 ± 1.97	13.5–81.0	1.33
9	Hgb	46	96	9.0**	−2.40 ± 0.61	1.20 ± 0.89	8.0–104.0	1.10
10	Hct	240	69	13.5***	9.50 ± 1.89	3.40 ± 2.71	18.5–89.0	2.19
	Hgb	240	71	16.3***	3.26 ± 0.6	1.38 ± 0.85	13.0–108.0	2.64
	MCH	240	77	17.6***	0.33 ± 0.05	−0.03 ± 0.08	57.0–82.0	2.57
	MCV	240	78	14.0***	0.92 ± 0.17	−0.04 ± 0.25	43.0–82.0	2.03
13	Hct	240	38	8.3**	8.80 ± 2.16	1.40 ± 3.96	0.0–112.0	1.29
	Hgb	240	38	8.5**	2.79 ± 0.69	0.88 ± 1.26	0.0–114.0	1.29
X	MCH	18	54	9.9**	−0.34 ± 0.08	0.05 ± 0.11	6.0–67.5	1.18
		46	52	14.5***	−0.58 ± 0.11	0.18 ± 0.11	43.0–66.0	1.95
	MCV	18	54	9.9**	−1.20 ± 0.28	0.40 ± 0.40	8.5–87.0	1.20
		46	53	18.5***	−2.54 ± 0.42	0.17 ± 0.42	50.0–56.0	2.56

[a] The Add ± SE and Dom ± SE of Hct and RDW are shown by × 1000

[b] Significance level: ** 5% genome-wide significant; *** 1% genome-wide significant

[c] Additive effects of the QTL and their standard deviation

[d] Dominance effects of the QTL and their standard deviation

[e] 95% confidence interval

[f] Percentage of the phenotypic variance explained by the QTL

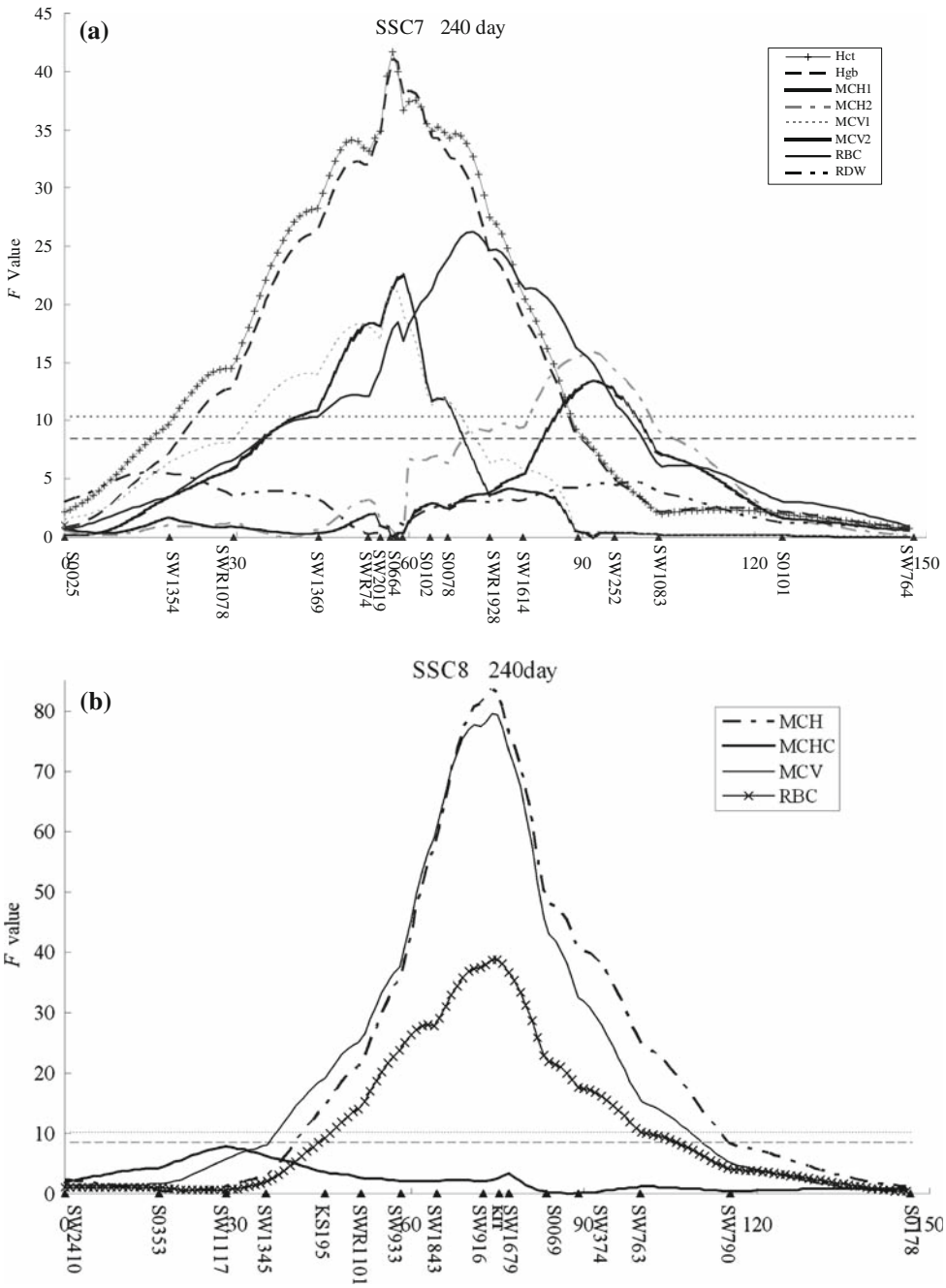

Fig. 1 Statistical *F* values indicating QTL for erythroid trait at day 240 on SSC7 (**A**) and SSC8 (**B**). Markers and distance in cM are given on the *x*-axis, and *F* ratios are indicated on the left *y*-axis. The 1% (dotted line) and 5% (dashed line) genome-wide significant levels are indicated by two horizontal lines. Hct, hematocrit; Hgb, hemoglobin; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin content; MCV, mean corpuscular volume; RBC, red blood cell count; RDW, red cell distribution width

at 240 days. After this QTL was used as the context of all other QTL, another 1% genome-wide significant QTL for this trait was found. We observed that the confidence intervals of the two QTL are distinct (Fig. 1A), indicating that there are at least two different causal genes that underlie MCV on SSC7. Notably, the CI_{95} of the QTL for MCV and MCH at 240 days on SSC7 were only 6 and 7 cM, respectively. It is thus feasible for us to select positional candidate genes in this region, such as cyclin D3. Peterson et al. (2005) showed that cyclin D3 was able to inhibit the hematopoietic transcription factor *AML1* (*RUNX1*) function, and that the interaction between *AML1* and cyclin D proteins regulated cell cycle and cell differentiation during hematopoiesis.

On SSC8, 14 genome-wide significant QTL for erythroid traits were detected, including the most significant QTL detected in this study ($F = 83.48$): the QTL for MCH at 240 days (Fig. 1B). This QTL accounted for 12.72% of the phenotypic variance, with $CI_{95} = 6$ cM, and also had a significant effect on MCH at days 18 and 46. It is likely that two prominent QTL had a consistent effect on MCV and RBC at days 18, 46, and 240 (Supplementary Fig. 1A, B) in the same region, and the QTL at 240 days explained up to 6.63% of phenotypic variance. This QTL not only

confirmed the results of the previous study (Reiner et al. 2007), it also narrowed the CI_{95} into a 8-cM region compared with that of 45 cM reported by Reiner et al. (2007). The observation of common QTL for MCV, MCH, and RBC at the three growth ages suggested that the corresponding causal gene(s) in the QTL region have a pleiotropic effect on the whole life process of the animal. Strikingly, the CI_{95} of QTL for MCV at 18 days on SSC8 was only 3 cM. A strong candidate gene in this interval is the stem cell growth factor receptor (*KIT*) gene, which is essential for normal hematopoiesis, melanogenesis, and germ cell development (Russell 1979). Several mutations in *KIT* have significant influence in RBC in the mouse (Jackson et al. 1994). Moreover, Johansson et al. (2005) showed strong associations of *KIT* variants with erythroid traits in pigs. A QTL for RDW at 18 days also was located in the *KIT* region. However, no QTL for RDW were detected at days 46 and 240, indicating that another closely linked locus with *KIT* regulated RDW only at an early stage.

A 5% genome-wide significant QTL for Hgb at 46 days was detected on SSC9 (Table 2). To our knowledge, this QTL has not been reported in either pigs or mice. We mapped four significant QTL for Hct, Hgb, MCH, and MCV on SSC10 (Table 2) in a region where the QTL for Hgb and Hct in the pig had been detected (Reiner et al. 2007). The colocalization of these QTL suggested that the underlying gene had a pleiotropic effect on these traits. Moreover, these QTL were associated with these traits only at 240 days, indicating that the causal gene affects erythropoiesis at a late stage. On SSC13, a 5% genome-wide significant QTL for Hgb was detected. This QTL overlapped with the previously reported QTL (Reiner et al. 2007).

All QTL for baseline erythroid traits on SSCX were located in the same region (52–55 cM, Table 2), so it is probably true that a single QTL controlled these baseline erythroid traits. The QTL has not been mapped before. Notably, the CI_{95} of the QTL for MCV at 46 days was within a 6-cM interval. Therefore, it is worthwhile that future work refine and identify the causal gene in that small interval.

In conclusion, we detected a total of 101 QTL for baseline erythroid traits at days 18, 46, and 240 in the White Duroc × Erhualian intercross. The results not only confirmed those of previous reports, but also showed numerous novel and age-associated QTL. The age-specific QTL offer new insights into the biological regulation of these erythroid traits throughout the life of the pig. Future work will be directed toward the fine mapping of QTL and ultimately the identification of casual genes. This will not only increase our understanding of hematopoiesis biology but also suggest novel therapeutic possibilities in the treatment of blood diseases.

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