

Quantitative trait loci for baseline white blood cell count, platelet count, and mean platelet volume

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Abstract

A substantial genetic contribution to baseline peripheral blood counts has been established. We performed quantitative trait locus/loci (QTL) analyses to identify chromosome (Chr) regions harboring genes influencing the baseline white blood cell (WBC) count, platelet (Plt) count, and mean platelet volume (MPV) in F₂ intercrosses between NZW/LacJ, SM/J, and C57BLKS/J inbred mice. We identified six significant WBC QTL: *Wbcq1* (peak LOD score at 38 cM, Chr 1), *Wbcq2* (42 cM, Chr 3), *Wbcq3* (0 cM, Chr 15), *Wbcq4* (58 cM, Chr 1), *Wbcq5* (82 cM, Chr 1), and *Wbcq6* (8 cM, Chr 14). Three significant Plt QTL were identified: *Pltq1* (24 cM, Chr 2), *Pltq2* (36 cM, Chr 7), and *Pltq3* (10 cM, Chr 12). Two significant MPV QTL were identified, *Mpvq1* (62 cM, Chr 15) and *Mpvq2* (44 cM, Chr 8). In total, the WBC QTL accounted for up to 31% of the total variance in baseline WBC count, while the Plt and MPV QTL accounted for up to 30% and 49% of the total variance, respectively. These analyses underscore the genetic complexity underlying these traits in normal populations and provide the basis for future studies to identify novel genes involved in the regulation of mammalian hematopoiesis.

The influence of genetics on baseline peripheral blood cell parameters is firmly established. Heritability estimates derived from twin studies for hemoglobin (Hgb) levels, red blood cell (RBC), white blood cell (WBC), and platelet (Plt) counts range from 0.37 to 0.89 indicating that a substantial genetic

component underlies these traits (Garner et al. 2000). Studies in baboons confirm that genetic influences account for a significant portion of the variance in these blood parameters as well as in others (e.g., MCV, mean corpuscular volume; MPV, mean platelet volume) (Mahaney et al. 2005). Variations in cell numbers are likewise under genetic control, including the number of F cells, a subset of human erythrocytes containing fetal hemoglobin, and circulating B and T lymphocytes (Chen and Harrison 2002; Hall et al. 2002).

The number of F cells persisting in adults is an important modulator of sickle cell disease and thalassemia (Lal and Vichinsky 2004; Platt et al. 1994). Baseline WBC count is a significant risk factor for early mortality in the general population and for disease severity in sickle cell disease (Castro et al. 1994; de Labry et al. 1990; Kinney et al. 1999; Miller et al. 2000; Platt et al. 1994). Both the WBC and MPV correlate with heart disease and stroke risk in the general population (Bath et al. 2004; Castro et al. 1994; de Labry et al. 1990; Martin et al. 1991, 1992; Miller et al. 2000; Platt et al. 1994). Hence, identifying the primary genetic determinants of peripheral blood indices will not only enhance our understanding of hematopoiesis but also provide novel therapeutic targets for hematologic and cardiovascular pathologies.

Peripheral blood counts are continuous (vs. discrete) traits and thus lend themselves to the application of powerful quantitative trait locus/loci (QTL) analysis methods to identify interacting gene networks. QTL analysis provides an unbiased approach to the identification of novel genes and their functions and can serve as an entry point into previously unrecognized regulatory pathways. Identifying QTL by linkage analysis in humans is often difficult because of environmental influences, genetic diversity,

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and small population (linkage group) size. Inbred mice, however, provide powerful tools for complex trait analysis. Furthermore, as concordance between QTL in the mouse and human has been demonstrated for multiple complex traits and diseases, QTL analysis in the mouse is of significant biomedical relevance (Paigen 2002; Sugiyama et al. 2001a; Wang and Paigen 2005a, b).

An examination of the Mouse Phenome Database (MPD, <http://www.jax.org/phenome>) provides clear evidence of heritability for baseline peripheral blood values in mice, as substantial variation is seen among 42 inbred strains for 20 distinct hematologic traits despite exposure to an identical environment (Peters and Barker 2004). We are using the inbred strains to identify QTL for baseline hematologic parameters as a first step in elucidating the genes regulating these traits in normal populations. In this article we report the identification of QTL for WBC count, Plt count, and MPV. We have identified six significant WBC QTL, three significant Plt QTL, and two significant MPV QTL in single-locus genome-wide scans. Moreover, multiple regression analysis reveals additional significant loci for these traits. Our analyses confirm that multiple genes contribute to baseline peripheral blood counts and are a significant first step in identifying those genes.

Materials and methods

Animals. Mice were housed in humidity- and temperature-controlled rooms with a 12-h light cycle and free access to acidified water and chow (NIH 5K52). All protocols were approved by The Jackson Laboratory Animal Care and Use Committee. The Jackson Laboratory is fully accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC).

Complete blood counts. Whole blood (275 μ l) from 8-week-old adult mice was drawn from the retro-orbital sinus through EDTA-coated microhematocrit tubes directly into Eppendorf tubes containing 30 μ l 20% EDTA in murine PBS, as described (Peters et al. 2004). The additional anticoagulant is critical in preventing microclot formation. Complete blood counts were determined immediately after obtaining the samples using an Advia 120 Multi-species whole blood analyzer (Bayer Corporation, Tarrytown, NY).

Statistical analysis. To identify QTL, two F_2 intercrosses were established between F_1 hybrids of NZW/LacJ \times SM/J (NZSM cross) and C57BLKS/J \times

SM/J mice (KSSM cross). Complete blood counts in 186 F_2 progeny from each cross were performed as described above. Approximately equal numbers of males and females were obtained in the F_2 progeny from each cross; females accounted for 57% and 49% of the NZSM and KSSM F_2 progeny, respectively. Simple sequence length polymorphic (SSLP) markers spaced at 10–30 cM intervals throughout the genome were typed by the PCR using multiplexed fluorescent markers and ABI 3700 instrumentation (Applied Biosystems, Foster City, CA). Primers were purchased from Research Genetics (MapPairs, Huntsville, AL). A total of 88 markers were typed in each of the two crosses (marker information available upon request). After identification of WBC QTL, 29 additional SSLP and single nucleotide polymorphic (SNP) markers on Chr 1 were added to each cross to refine the Chr 1 QTL intervals. Also, the number of F_2 progeny was increased to a total of 279 for each cross. In this expanded population, females accounted for 54% and 49% of the NZSM and KSSM F_2 progeny, respectively. SNP genotyping was performed by KBiosciences (Hoddesdon Herts, UK).

Genome-wide scans were performed using WBC count, Plt count, and MPV as quantitative traits. All values were log transformed before analysis to approximate the normal distribution. Results were analyzed in three stages, as described (Sen and Churchill 2001). First, single loci associated with each trait (main effect QTL) were detected in genome-wide scans with sex and body weight as additive covariates (Cordell et al. 1998) using Pseudomarker software (source codes available at www.jax.org/research/churchill). Significance thresholds were determined by permutation testing ($n = 1000$ permutations) (Churchill and Doerge 1994). A LOD (logarithm of the odds ratio) score meeting or exceeding the 95th percentile ($p < 0.05$) of the permutation distribution was considered significant. QTL were considered suggestive if they met or exceeded the 37th percentile ($p < 0.63$), as suggested by Lander and Kruglyak (Lander and Kruglyak 1995). Confidence intervals (CI) were determined by computing the region of the posterior probability density curve containing 95% of the total area (Sen and Churchill 2001). The posterior probability density is proportional to 10^{LOD} and gives results that are often very similar to the 1.5 "LOD support interval" but it is better justified on theoretical grounds (Sen and Churchill 2001).

We also carried out single-locus scans that included sex as an interactive covariate to identify sex-specific QTL (Korstanje et al. 2004b). The difference in LOD scores between the two scans is a test for sex by QTL interaction. In the second stage of analysis, a

Table 1. WBC count, Plt count, and MPV in parental strains (8 weeks)

	<i>NZW/LacJ</i>		<i>C57BLKS/J</i>		<i>SM/J</i>	
	<i>Male (7)</i>	<i>Female (13)</i>	<i>Male (17)</i>	<i>Female (17)</i>	<i>Male (12)</i>	<i>Female (21)</i>
WBC ($\times 10^3/\mu\text{l}$)	13.9 \pm 4.5	12.6 \pm 1.9	9.9 \pm 2.0	11.2 \pm 2.8	4.2 \pm 1.0	4.8 \pm 1.1
Plt ($\times 10^3/\mu\text{l}$)	1227 \pm 171	1131 \pm 304	883 \pm 188	791 \pm 150	667 \pm 96	639 \pm 124
MPV (fl)	6.5 \pm 4.2	7.2 \pm 4.7	3.9 \pm 0.1	4.0 \pm 0.2*	5.0 \pm 0.5	4.4 \pm 0.3*

All values X \pm SD.

* $p < 0.05$ vs. males of the same strain.

simultaneous search for pairs to detect epistatic interactions was performed using a two-way ANOVA model, as described (Sen and Churchill 2001; Sugiyama et al. 2001a). Pairwise genome scans can detect QTL that affect a phenotype by interacting with another QTL even when neither QTL alone reaches the significance threshold. Finally, to determine the combined effects of all QTL on each trait, multiple regression analysis was performed that included all significant and suggestive QTL for that trait and all possible interactions of the significant and suggestive QTL. Possible sex-by-QTL interactions were also included in the multiple regression analysis. Terms that failed to meet significance levels in the analysis were eliminated one at a time and the analysis was repeated until all remaining QTL were significant, resulting in the final model. The percent variance reported for each QTL derives from this analysis.

To refine the final regression model for WBC count QTL on Chr 1, data from both crosses were combined and analyzed as described (Li et al. 2005). Combining data from two or more crosses can narrow CIs and better resolve linked QTL.

Other statistical analyses were performed using JMP 5.1.2 software. Between-group comparisons were analyzed by the Tukey HSD test to determine statistical significance.

Allele effects. Allele effects were determined by calculating the phenotypic mean at the peak marker for each of the three possible genotypes (Lyons et al. 2003b). We then determined which strain contributed the allele that increased the trait and whether it acted in a dominant, additive (codominant), or recessive manner.

Results

Selection of strains. The greater the difference in the trait of interest between parental strains, the more robust the QTL analysis, thereby enhancing the likelihood of successful QTL identification. Examination of the Mouse Phenome Database revealed markedly divergent WBC and Plt counts, our primary focus initially, in strains NZW/LacJ (NZ), C57BLKS/J (KS), and SM/J (SM) (Peters and Barker 2004). Our analysis of peripheral blood cell counts at eight weeks of age confirmed that these strains differ substantially for the traits of interest and that the general trends were in agreement with MPD data obtained at ten weeks of age. Males and females did not differ in WBC or Plt counts in any of the three strains; MPV values showed minor, but significant, differences between males and females in strains KS and SM (Table 1).

We established reciprocal crosses between strains NZ and SM and strains KS and SM to generate F₁ offspring. Reciprocal F₁ offspring at eight weeks of age showed counts similar to those of the parental strains, indicating lack of maternal effects, sex linkage, and imprinting. Because SM females are not always the best breeders, we set up NZ \times SM and KS \times SM crosses to generate the F₂ progeny for analysis. Data for these F₁s are given in Table 2. With the exception of female NZSM Plt counts, all traits for F₁ animals (Table 2) differed significantly ($p < 0.05$) from those of the corresponding parental strains (Table 1). Values for WBC counts in both NZSM and KSSM F₁ animals were intermediate to those of the parental strains suggesting the influence of recessive and/or additive genes in the high-WBC count strains. Interestingly, in the case of Plt counts

Table 2. WBC count, Plt count, and MPV in reciprocal F₁s (8 weeks)

	<i>NZSM</i>		<i>KSSM</i>	
	<i>Male (9)</i>	<i>Female (9)</i>	<i>Male (8)</i>	<i>Female (13)</i>
WBC ($\times 10^3/\mu\text{l}$)	8.1 \pm 1.1	7.9 \pm 1.4	7.3 \pm 2.0	6.7 \pm 1.0
Plt ($\times 10^3/\mu\text{l}$)	1545 \pm 157	1148 \pm 134*	1103 \pm 165	975 \pm 147
MPV (fl)	14.4 \pm 2.5	11.6 \pm 3.6*	10.8 \pm 5.0	9.8 \pm 4.5

All values X \pm SD.

* $p < 0.05$ vs. males of the same strain.

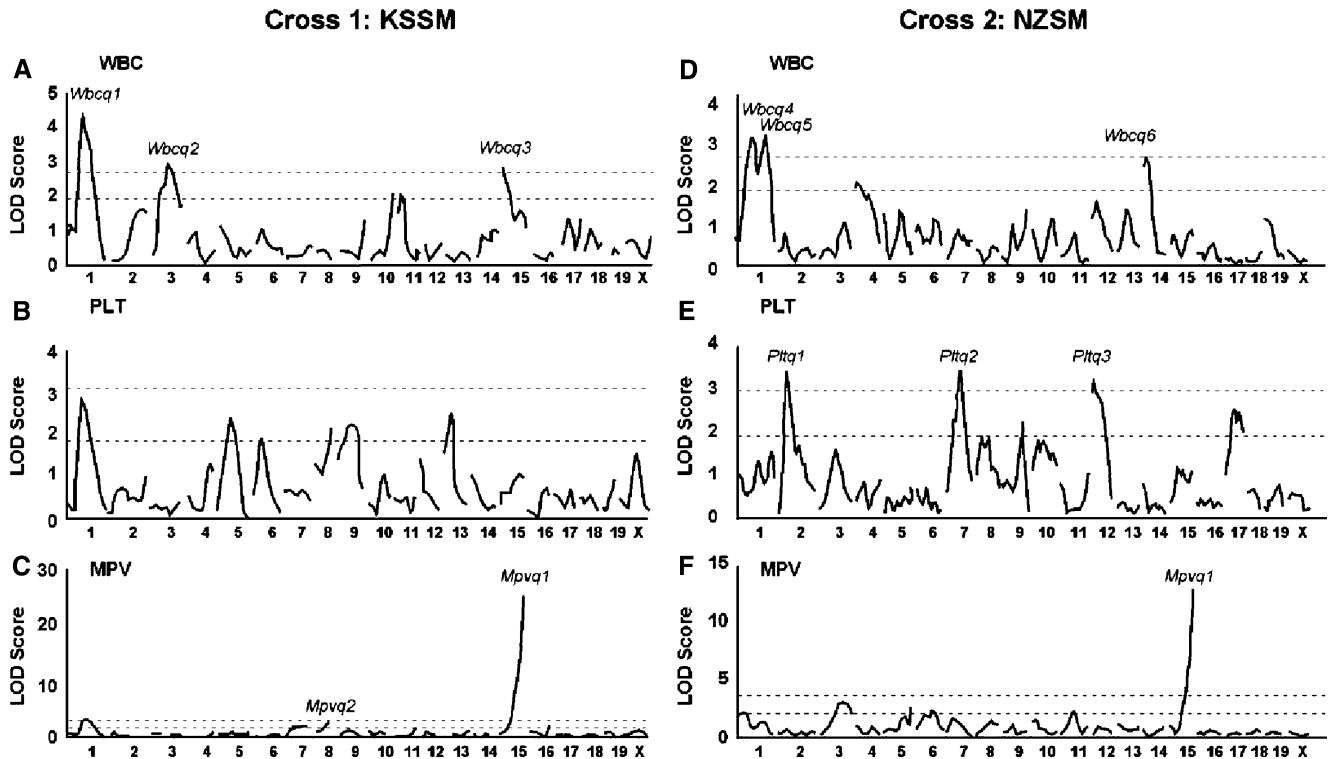


Fig. 1. Genome-wide scans for WBC count, Plt count, and MPV QTL. Genome-wide scans for single QTL underlying WBC count (A, D), Plt count (B, E), and MPV (C, F) in the KSSM and NZSM crosses. Significant ($p = 0.05$) and suggestive ($p = 0.63$) LOD scores are indicated by the upper and lower dotted lines, respectively, above significant QTL. Chromosomes 1 through X are displayed on the ordinates with the relative spacing representative of the relative length of each chromosome. LOD scores are given on the Y axis.

and MPV, the F_1 animals showed values comparable to or even greater than the parental strains. This suggests the possibility that both strains contributed alleles that increased these Plt parameters. Alternatively, alleles contributing to lower Plt counts and MPV may be recessively inherited and thus not apparent in heterozygous F_1 animals.

Identification of QTL. F_2 mice from both crosses were phenotyped at eight weeks of age. Genome-wide scans for single QTL are shown in Fig. 1. Centimorgan (cM) positions are from the Mouse Genome Informatics database (www.informatics.jax.org). For each cross 186 F_2 animals were analyzed using 88 SSLP markers. The data for all significant QTL detected in this study are summarized in Table 3.

QTL for baseline WBC count. Analysis for single-locus QTL in both crosses using sex and body weight as additive covariates revealed significant loci for WBC count on chromosomes (Chrs) 1, 3, 14, and 15 (Fig. 1). Three loci were identified on Chr 1. The peak LOD score and the posterior probability density curves used to define the 95% confidence

intervals (Cis) for all WBC QTL are shown in Fig. 2 and summarized in Table 3. No influence of sex was detected for any of the WBC QTL, as determined by repeating the analysis with sex as an interacting covariate (no significant differences in LOD scores were seen between the two scans). A simultaneous search to detect interacting gene pairs (epistasis) was negative. In accordance with conventions established by the International Committee on Standardized Genetic Nomenclature for Mice (<http://www.informatics.jax.org/mgihome/nomen>) and the Complex Traits Consortium (Abiola et al. 2003), these QTL were named *Wbcq1* (white blood cell quantitative locus 1) – *Wbcq6*. Three suggestive QTL for WBC count were also detected (Chrs 4, 10, and 11) (Fig. 1). Suggestive QTL await confirmation before naming, also by convention.

In the NZSM cross, the genome-wide scan suggests that two Chr 1 loci are present, while the KSSM scan reveals a single Chr 1 peak (Fig. 1). To further explore the number of WBC QTL on Chr 1, we added additional F_2 animals (to a total of 279) and 29 additional SSLP/SNP Chr 1 markers to each cross to increase statistical power and repeated the analyses for Chr 1. In the KSSM cross, a single QTL,

Table 3. QTL for baseline WBC count, Plt count, and MPV

Name	Trait	Chr	Peak, cM (Mb ^c)	95% CI (cM)	Human location ^d	High Allele	Inheritance	Peak marker	LOD
<i>Wbcq1</i> ^a	WBC	1	38 (71)	36–52	2q37	C57BLKS/J	Dominant	<i>D1MIT282</i>	7.0
<i>Wbcq2</i>	WBC	3	42 (89)	24–74	1q21	SM/J	Recessive	<i>D3MIT142</i>	3.2
<i>Wbcq3</i>	WBC	15	0 (0)	0–52	5p13	SM/J	Dominant	<i>D15MIT13</i>	2.8
<i>Wbcq4</i> ^a	WBC	1	58 (94)	50–72	2q37	NZW/LacJ	Additive	<i>D1MIT306</i>	4.3
<i>Wbcq5</i> ^a	WBC	1	82 (156)	76–90	1q25	NZW/LacJ	Recessive	<i>D1MIT227</i>	4.2
<i>Wbcq6</i>	WBC	14	8 (24)	0–20	3p14	NZW/LacJ and SM/J	Heterozygotes low ^b	<i>D14MIT98</i>	2.8
<i>Pltq1</i>	Plt	2	24 (35)	14–42	9q33-q34	SM/J	Recessive	<i>D2MIT296</i>	3.5
<i>Pltq2</i>	Plt	7	36 (52)	24–52	11p15	NZW/LacJ	Dominant	<i>D7MIT30</i>	3.3
<i>Pltq3</i>	Plt	12	10 (23)	0–31	2p25	NZW/LacJ	Additive	<i>D12MIT182</i>	3.2
<i>Mpvq1</i>	MPV	15	62 (104)	60–62	12q12-q13	SM/J	Additive	<i>D15MIT16</i>	14.2
			62 (104)	58–62		SM/J	Recessive	<i>D15MIT16</i>	24.2
<i>Mpvq2</i>	MPV	8	44 (92)	24–49	16q13-p21	SM/J	Recessive	<i>D8MIT211</i>	2.7

^aData derived from an initial genome-wide scan using 186 F₂ animals with the exception of *Wbcq1*, *Wbcq4*, and *Wbcq5* (279 animals).

^bFor QTL for which heterozygotes are low, we hypothesize that there may be closely linked QTL with effects in the opposite direction.

^cEstimated peak positions in Mb determined using Mouse Genome Informatics linkage maps (http://www.informatics.jax.org/searches/linkmap_form.shtml) and Ensemble Mouse Genome Browser (Build 33) (http://www.ensembl.org/Mus_musculus/).

^dHuman chromosome region conserved with peak QTL position.

Wbcq1, with a peak LOD score at 38 cM was identified (Fig. 2A). In the NZSM cross, two Chr 1 QTL, *Wbcq4* and *Wbcq5*, were detected with peak LOD scores at 58 and 82 cM, respectively (Fig. 2D). The presence of two closely linked QTL often skews their relative positions. For this reason, the position of *Wbcq4* may be altered by the presence of *Wbcq5* in the NZSM cross. *Wbcq1* (38 cM) and *Wbcq4* (58 cM) might, therefore, represent a single-QTL locus on Chr 1. To test this possibility, we computed the LOD scores for multitrait analysis using various two- and three-QTL models with the QTL located at 38, 58, and 82 cM (Almasy et al. 1997; Jiang and Zeng 1995). Analysis of multilocus models using combined data from the two crosses (Li et al. 2005) provides support for a two-QTL model on Chr 1. A final model of QTL38 and QTL82 is the most significant and accounts for the largest percentage of the variance (Table 4), suggesting that QTL38 and QTL58 are the same locus. However, the allele effects at QTL 38 and QTL 58 differ (see below), casting some doubt on this conclusion. Clearly, additional data will be required to definitively resolve this issue. The two loci will be considered separate QTL pending further study.

The main scan suggests that in addition to *Wbcq3*, at least one additional WBC QTL exists on Chr 15 (Fig. 2C). Further analyses using markers saturating the entire length of Chr 15 and additional F₂ progeny will be required to resolve the number of QTL on this chromosome.

Allele effects. In the KSSM cross, a dominant KS allele linked to the peak marker for *Wbcq1* increases WBC count (Fig. 3A). In the case of *Wbcq2* and *Wbcq3*, recessive and dominant SM alleles, respec-

tively, increase WBC count (Fig. 3B, C). Hence, a QTL inherited from the parental strain with the low WBC count, SM, increases WBC count compared with the allele inherited from the high WBC count strain, KS, at these loci. This phenomenon, known as transgressive segregation, is common in QTL crosses and indicates that both parental strains carry alleles that influence the trait in both directions (Cheung et al. 2004; Moore and Nagle 2000).

An additive NZ allele at *Wbcq4* increases the WBC count (Fig. 3D), contrasting with the dominant KS allele effect at *Wbcq1* (Fig. 3A), as discussed above. A recessive NZ allele at *Wbcq5* increases WBC count (Fig. 3E). *Wbcq6* showed complicated allele effects; both homozygotes had higher WBC counts than did the heterozygotes. Here, it is likely that other alleles not detected in this analysis are influencing the phenotype.

Combined effects of WBC QTL. Single-locus genome scans as displayed in Fig. 1 do not consider other QTL. Multiple regression models, however, allow a QTL effect to be assessed in the context of all other QTL. We performed multiple regression analysis integrating all suggestive and significant WBC QTL and all possible WBC QTL interactions in order to determine the contribution of each QTL to the total variance. The combined effects of the QTL accounted for 31% of the variance in WBC count in the KSSM cross and 21% in the NZSM cross (Table 5). The regression analysis predicts that the Chr 4 QTL, which was suggestive only in the main scan, is significant in the final model for the NZSM cross. Moreover, although a simultaneous search to detect interacting pairs (epistasis) was negative, the multiple regression model predicts an interaction

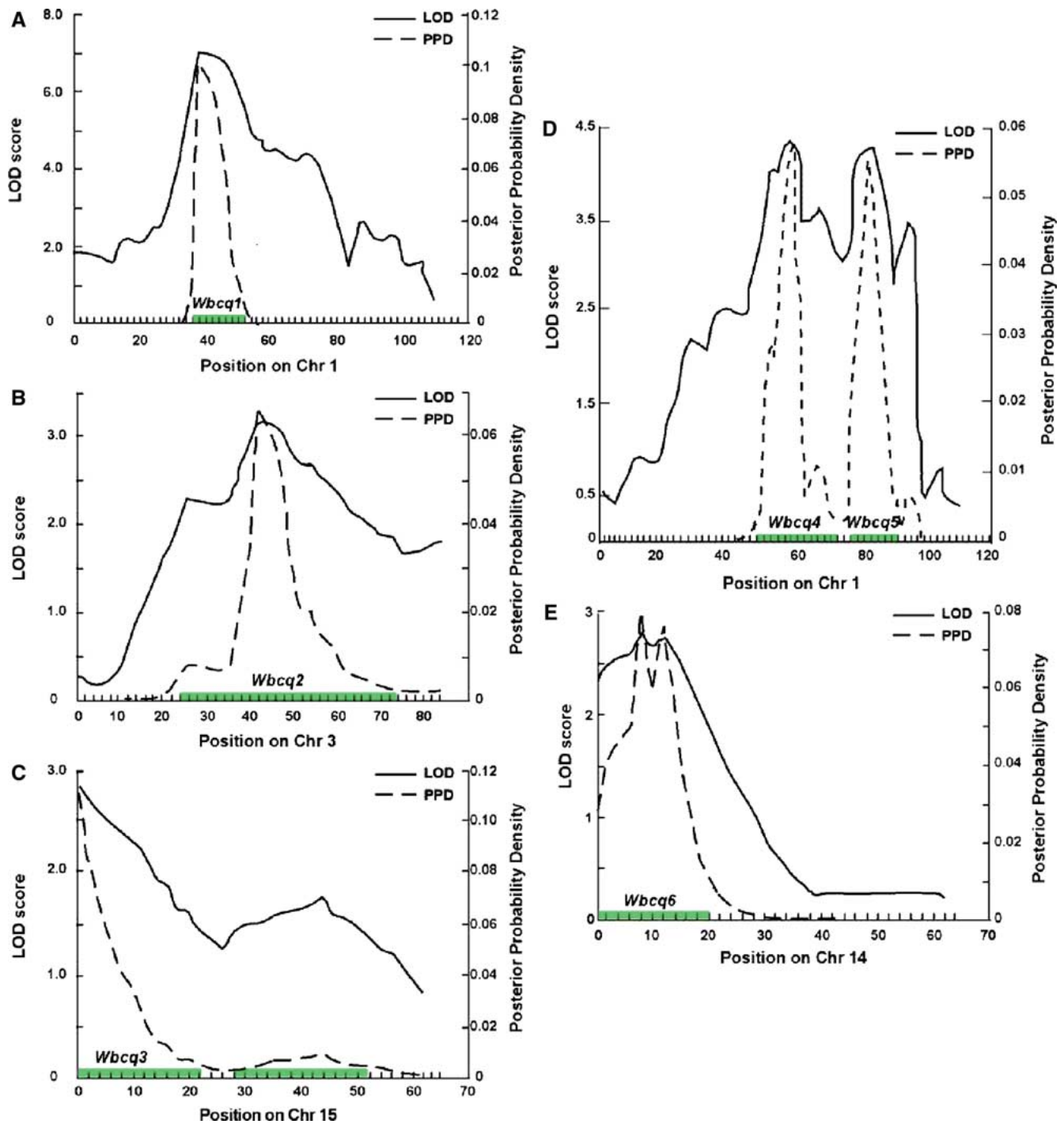


Fig. 2. Genome-wide scans (solid lines) and posterior probability densities (dashed lines) for significant WBC QTL. (A) *Wbcq1*, (B) *Wbcq2*, (C) *Wbcq3*, (D) *Wbcq4*, *Wbcq5*, (E) *Wbcq6*. Posterior probability density is the likelihood statistic that is used to compute the 95% confidence interval, indicated by the gray bars. Position along the chromosome is shown on the ordinates. The Y axis shows LOD scores. Results are based on single locus scans of 186 F_2 animals (panels B, C, E) or 279 F_2 animals (panels A, D).

between *Wbcq2* and *Wbcq3* in the KSSM cross. When the *Wbcq3* genotype is homozygous and to a lesser extent heterozygous for the KS allele, the presence of one or two SM alleles at *Wbcq2* acts to further increase the WBC count (Fig. 4). Finally, no

interaction effect between sex and any of the WBC QTL was found in the regression analysis, confirming that the QTL effects on baseline WBC counts are independent of sex. The same is true for Plt and MPV QTL as well.

Table 4. Multilocus models for Chr 1 WBC QTL

QTL	df	% variance	F value	P(f)
A. Multiple regression for combined crosses with 3-QTL model ^a				
Chr1@38	4	2.171	3.387	0.009446 (<i>p</i> < 0.01)
Chr1@58	4	0.420	0.655	(ns)
Chr1@82	4	2.092	3.264	0.011643 (<i>p</i> < 0.05)
B. Multiple regression for combined crosses with 2-QTL model (QTL58 and QTL82) ^b				
Chr1@58	4	3.263	5.003	0.000575 (<i>p</i> < 0.001)
Chr1@82	4	2.003	3.071	0.016121 (<i>p</i> < 0.05)
C. Multiple regression for combined crosses with 2-QTL model (QTL38 and QTL58) ^c				
Chr1@38	4	2.082	3.195	0.013081 (<i>p</i> < 0.05)
Chr1@58	4	1.427	2.190	0.068905 (ns)
D. Multiple regression for combined crosses with 2-QTL model (QTL38 and QTL82) ^d				
Chr1@38	4	5.015	7.844	3.76×10^{-6} (<i>p</i> < 0.001)
Chr1@82	4	3.100	4.848	0.000755 (<i>p</i> < 0.001)

ns = not significant.

^aQTL58 is not significant and therefore can be excluded.

^bBoth are significant; information lost by excluding QTL38 is contained in QTL58.

^cQTL58 is not significant.

^dBoth are highly significant and account for the biggest percentage of the variance.

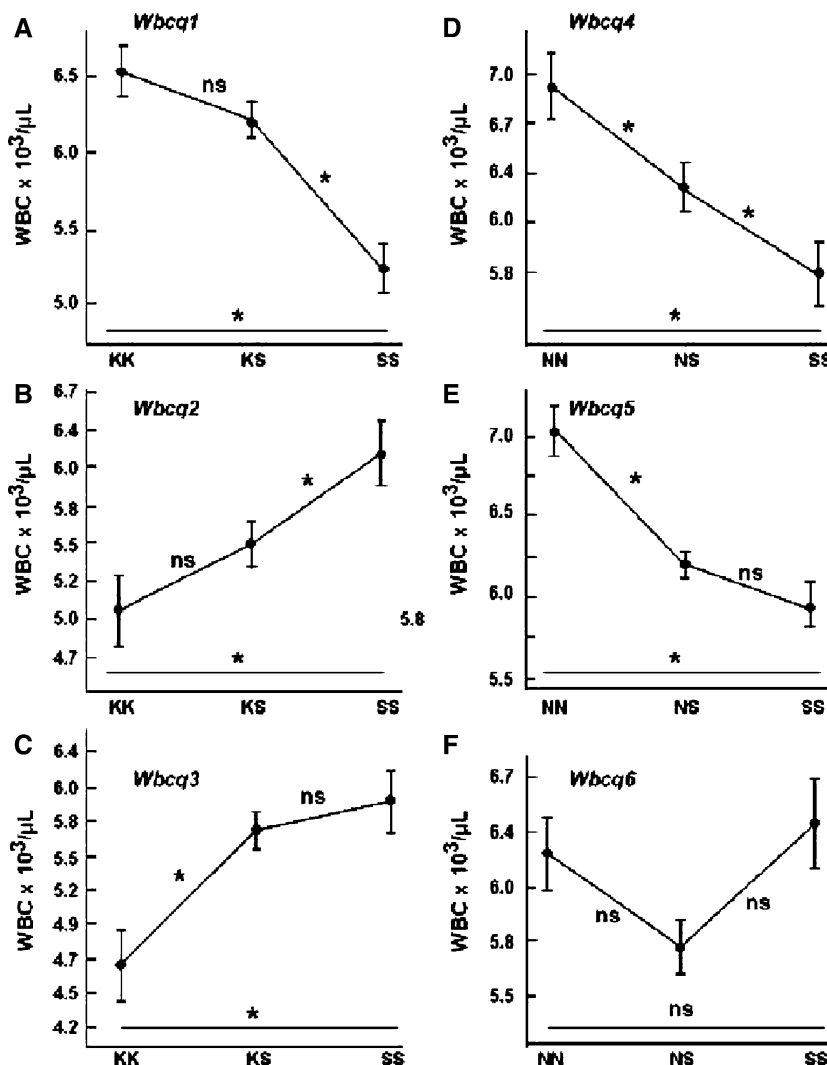


Fig. 3. Effect plots for WBC QTL. Allelic contributions determined at the peak marker for (A) *Wbcq1*, (B) *Wbcq2*, (C) *Wbcq3*, (D) *Wbcq4*, (E) *Wbcq5*, and (F) *Wbcq6*. The three possible genotypes are indicated on each ordinate. K, C57BLKS/J allele; N, NZW/LacJ allele; S, SM/J allele. The Y axis represents WBC counts. Error bars represent SEM. **p* < 0.05, ns = not significant.

Table 5. Multiple regression analysis of WBC QTL

	<i>df</i>	% variance ^b	<i>F</i> value	<i>P</i> (<i>f</i>)
KSSM cross				
Sex	1	0.25	0.625	0.430341 (ns)
<i>Wbcq1</i>	2	9.5	11.950	1.37×10^{-5} ($p < 0.001$)
<i>Wbcq2</i>	6	11.6	4.860	0.000129 ($p < 0.001$)
<i>Wbcq3</i>	6	13.3	5.578	2.59×10^{-5} ($p < 0.001$)
<i>Wbcq2:Wbcq3</i>	4	5.8	3.648	0.007004 ($p < 0.01$)
Total		31		
NZSM cross				
Sex	1	1.7	3.710	0.055729 (ns)
<i>Wbcq4</i> and <i>Wbcq5</i> ^a	4	8.7	4.8123	0.001049 ($p < 0.01$)
Chr 4@8 cM	2	3.6	3.985	0.020322 ($p < 0.05$)
<i>Wbcq6</i>	2	3.5	3.854	0.023029 ($p < 0.05$)
Total		21		

^aBecause of close correlation resulting from genetic linkage on Chr 1, these QTL must be considered together in the regression model (Sokal and Rohlf 1981).

^bPercent variances are based on type III sums of squares, also called adjusted sums of squares (SS). The adjusted SS is used to compute the % variance and takes into account other major QTLs in the population and thus does not necessarily sum up to the total variance explained. A discussion can be found in Sokal and Rohlf (Sokal and Rohlf 1981).

Identification of QTL for Plt count and MPV. Genome-wide scans revealed three significant QTL for baseline Plt count, *Pltq1* (Platelet quantitative locus-1) on Chr 2, *Pltq2* on Chr 7, and *Pltq3* on Chr 12 (Figs. 1 and 5). These loci were detected in the NZSM cross in which the differences in the platelet counts between the parental strains were the greatest. No significant QTL were detected in the KSSM cross where differences between the parental strains were not as great. However, multiple suggestive loci were detected in the KSSM cross (Chrs 1, 5, 6, 8, 9, and 13) and one in the NZSM cross (Chr 17) (Fig. 1). No influence of sex was detected for any of these QTL, and a simultaneous search to detect interacting pairs was negative. *Pltq1* demonstrates transgressive segregation. A recessive SM allele at *Pltq1* increases platelet count. At *Pltq2* and *Pltq3*, dominant and co-dominant NZ alleles, respectively, increase platelet count (Fig. 5).

The KSSM suggestive QTL on Chrs 5, 8, 9, and 13 are significant in the multiple regression model for the KSSM cross and account for 30% of the variance (Table 6). Likewise, the Chr 17 suggestive QTL is significant in the final model in the NZSM cross and, with the three significant main effect QTL, account for 29% of the variance (Table 6).

Two significant MPV QTL were detected, *Mpvq1* (mean platelet volume quantitative locus-1) on Chr 15 and *Mpvq2* on Chr 8 (Fig. 6). *Mpvq1*, which is highly significant, was detected in both crosses. An SM/J allele at *Mpvq1* increases Plt count in an additive (KSSM cross) or a recessive (NZSM cross) manner. Given the complete overlap of the 95% CIs between the two crosses, a single locus is likely and is given the same name, *Mpvq1*, by convention. A recessive SM/J allele at *Mpvq2* increases Plt count (Fig. 6).

In the multiple regression analysis for the KSSM cross, *Mpvq1* and *Mpvq2* account for 49% of the variance (Table 7). Notably, a locus on Chr 1 was significant in the main scan (Fig. 1). However, in the final regression model, this QTL is not significant. In the NZSM regression analysis a suggestive QTL on Chr 3 is significant in the final model and, with *Mpvq1*, accounts for 34% of the variance.

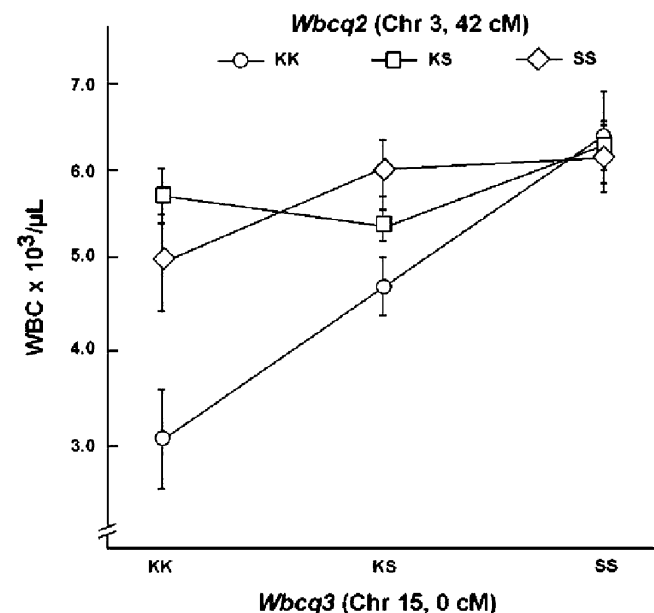


Fig. 4. Interaction between *Wbcq2* and *Wbcq3*. The effects of gene interaction predicted by multiple regression analysis are shown. The three possible genotypes for *Wbcq3* are indicated on the ordinate. *Wbcq2* genotypes are indicated by the circles, boxes, and diamonds. K, C57BLKS/J allele; S, SM/J allele. The Y axis represents WBC counts. Error bars represent SEM.

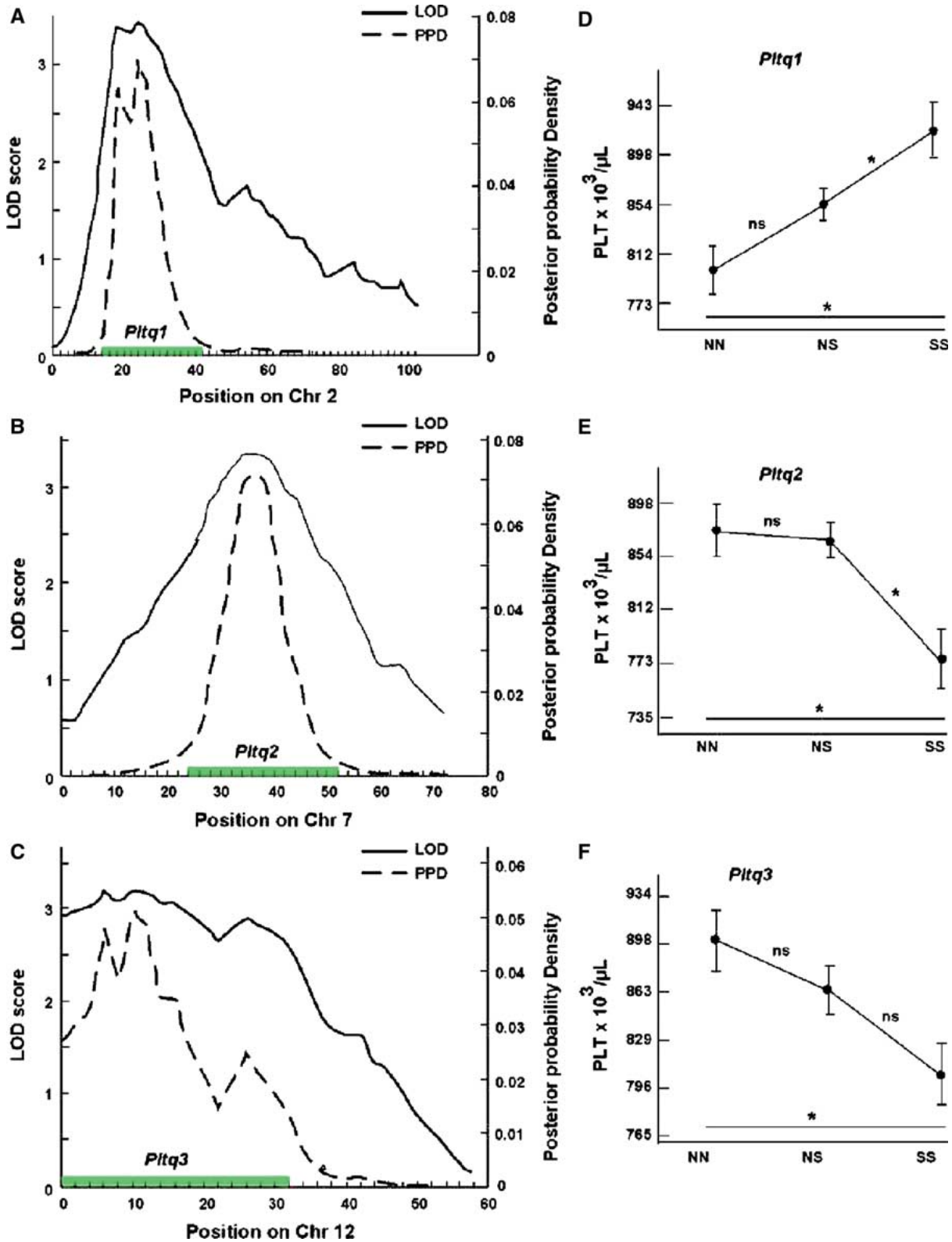


Fig. 5. Genome-wide scans (solid lines), posterior probability densities (dashed lines), and effect plots for significant PLT QTL. Posterior probability density is the likelihood statistic that is used to compute the 95% confidence interval, indicated by the gray bars for (A) *Pltq1*, (B) *Pltq2*, and (C) *Pltq3*. Position along the chromosome is shown on the ordinates. The Y axis shows LOD scores. Results are based on single-locus scans of 186 F₂ animals. (D-F) Effect plots. The three possible genotypes are indicated on each ordinate. K, C57BLKS/J allele; N, NZW/LacJ allele; S, SM/J allele. The Y axis represents Plt counts. Error bars represent SEM. **p* < 0.05, ns = not significant.

Table 6. Multiple regression analysis of Plt QTL

	<i>df</i>	% variance	<i>F</i> value	<i>P</i> (<i>f</i>)
KSSM cross				
Sex	1	6.8	17.059	5.59×10^{-5} ($p < 0.001$)
Chr5@36	2	5.2	6.524	0.001848 ($p < 0.01$)
Chr8@42	2	4.8	5.993	0.003034 ($p < 0.01$)
Chr9@46	2	5.8	7.244	0.000948 ($p < 0.001$)
Chr13@20	2	5.3	6.632	0.001671 ($p < 0.01$)
Total		30		
NZSM cross				
Sex	1	5.9	14.459	0.000198 ($p < 0.001$)
<i>Pltq1</i>	2	4.4	5.377	0.005423 ($p < 0.01$)
<i>Pltq2</i>	2	2.9	3.497	0.032423 ($p < 0.05$)
<i>Pltq3</i>	2	6.4	7.815	0.000562 ($p < 0.001$)
Chr17@24	2	6.6	8.107	0.000430 ($p < 0.001$)
Total		29		

Discussion

QTL analysis in mice is a powerful approach to the identification of novel genes and pathways influencing complex phenotypes. However, identification of QTL for baseline blood counts has received little attention. A recent study identified loci associated with baseline Plt counts in mice (Cheung et al. 2004), but none have been reported for steady-state WBC count and MPV, important risk factors for heart disease and stroke. The identification of QTL underlying hematologic parameters holds the promise to both enhance our understanding of the regulation of hematopoiesis and identify therapeutic targets for human disorders, including sickle cell disease. Moreover, as a result of conservation of chromosome regions between human and mouse, identification of QTL in the mouse can predict the location of corresponding human QTL (Table 3). QTL analyses in mice over the past decade have been instrumental in identifying the genetic determinants involved in obesity (Estrada-Smith et al. 2004; Ishimori et al. 2004b; Rocha et al. 2004), HDL cholesterol levels (Ishimori et al. 2004a,b; Korstanje et al. 2004b; Lyons et al. 2003a, 2004a,b; Pitman et al. 2002; Wang et al. 2003; Wang and Paigen 2005a,b), blood pressure control (DiPetrillo et al. 2004; Sugiyama et al. 2001a,b, 2002; Tsukahara et al. 2004), gallstone formation (cholelithiasis) (Lammert et al. 2001, 2002; Lyons et al. 2003b,c,d; Paigen et al. 2000; van Erpecum et al. 2001; Wittenburg et al. 2002, 2003a,b), and susceptibility to atherosclerosis (Korstanje et al. 2004a; Mu et al. 1999; Phelan et al. 2002; Purcell et al. 2001; Wang and Paigen 2002). All these considerations make a compelling case for mobilizing mouse resources to study hematology as a complex phenotype.

In the current study using two crosses between inbred strains of mice, we have identified QTL

influencing baseline WBC and Plt counts and MPV. Six significant QTL influencing WBC counts were identified. Three localized to Chr 1 (*Wbcq1*, *Wbcq4* and *Wbcq5*). The data are conflicting as to whether *Wbcq1* and *Wbcq4* are distinct loci. Multilocus testing suggests they represent one locus, while allele effects and the largely nonoverlapping CIs suggest that they are two closely linked, but distinct loci. Notably, two QTL for peripheral blood B cell percentage were previously detected on Chr 1 at 48 and 63 cM (Chen and Harrison 2002), close to the peak positions of our WBC QTL. Determining whether any of these loci are the same will require further analyses.

Additional significant WBC QTL were identified on Chr 3 (*Wbcq2*), Chr 15 (*Wbcq3*), and Chr 14 (*Wbcq6*). In multiple regression analyses, which examines each QTL in the context of all other QTL, a seventh significant QTL emerged located on proximal Chr 4 (8 cM). This locus was only suggestive on single-locus main scans. Together, these loci accounted for 21%–31% of the variance in baseline WBC count. An interaction between *Wbcq2* and *Wbcq3* was also detected in regression analyses. Examination of allele effects, several of which showed transgressive segregation, suggest that additional WBC QTL await identification. Clearly, a complex regulatory gene network influences baseline WBC count.

We identified three significant QTL for baseline platelet count—*Pltq1* on Chr 2, *Pltq2* on Chr 7, and *Pltq3* on Chr 12. None of these colocalize with the Plt QTL identified in a recent separate study on Chr 1 (94 cM) and Chr 11 (45 cM) (Cheung et al. 2004). Notably, loci on Chrs 5, 8, 9, 13, and 17 emerged as highly significant in the final regression models. These loci accounted for approximately 30% of the variance in baseline Plt count.

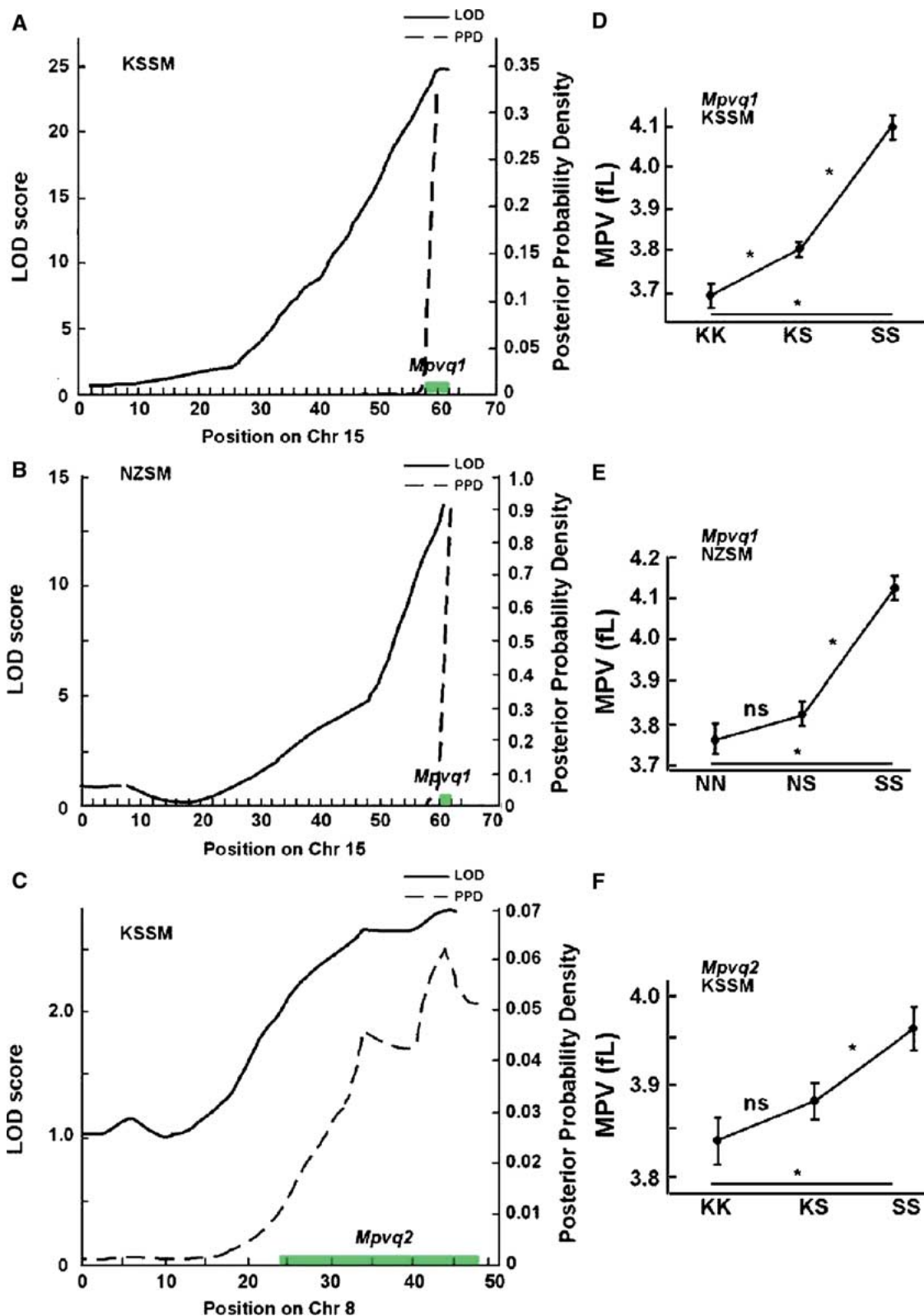


Fig. 6. Genome-wide scans (solid lines), posterior probability densities (dashed lines), and effect plots for significant MPV QTL. Posterior probability density is the likelihood statistic that is used to compute the 95% confidence interval, indicated by the gray bars for (A) *Mpvq1* in the KSSM cross, (B) *Mpvq1* in the NZSM cross, and (C) and *Mpvq2*. Position along the chromosome is shown on the ordinates. The Y axis shows LOD scores. Results are based on single-locus scans of 186 F₂ animals. (D-F) Effect plots. The three possible genotypes are indicated on each ordinate. K, C57BLKS/J allele; N, NZW/LacJ allele; S, SM/J allele. The Y axis represents MPV values. Error bars represent SEM. **p* < 0.05, ns = not significant.

Table 7. Multiple regression analysis of MPV QTL

	<i>df</i>	% variance	<i>F</i> value	<i>P</i> (<i>f</i>)	
KSSM cross					
Sex	1	0.016	0.055	0.815081	(ns)
<i>Mpvq2</i>	2	3.538	6.224	0.002434	(<i>p</i> < 0.01)
<i>Mpvq1</i>	2	42.075	74.016	0.0	(<i>p</i> < 0.001)
Total		49			
NZSM cross					
Sex	1	0.176	0.479	0.489697	(ns)
Chr3@66	2	4.386	5.958	0.003129	(<i>p</i> < 0.01)
<i>Mpvq1</i>	2	27.750	37.698	2.2×10^{-14}	(<i>p</i> < 0.001)
Total		34			

Two significant QTL for MPV were identified in single-locus scans, *Mpvq1* on Chr 15 and *Mpvq2* on Chr 8. *Mpvq1* is an exceptionally robust locus (LOD score = 24.2 in the KSSM cross). A third locus on Chr 3 became significant in the regression analysis. Conversely, the existence of a locus on Chr 1 that was significant in the genome-wide scan was not supported by the regression analysis.

In baboons, a perfect genetic correlation between MPV and WBC was found, suggesting that the same genes modulate both MPV and WBC count (pleiotropy) (Mahaney et al. 2005). Here, the phenotypic correlation between MPV and WBC for KSSM and NZSM were only 0.11 and 0.17, respectively. Furthermore, genome-wide scans for both crosses did not show any significant shared QTL for these two traits. However, we have examined only three inbred mouse strains and the baboon study included a limited number of families. Clearly, this intriguing association warrants further study.

The CIs for our QTL are quite large, as expected in a "first pass" analysis. Moreover, the smallest interval, that for *Mpvq1*, is extremely gene-rich. Hence, examination of potential candidate genes is currently unwieldy at best. However, some candidates near the QTL peak markers deserve comment. The physical location of each marker was obtained through the Sanger Institute public database (Ensembl, <http://www.ensembl.org/>). All candidates show appropriate expression patterns according to publicly available gene array databases (<http://symatlas.gnf.org/SymAtlas/>) (e.g., expression in bone marrow, hematopoietic precursors cells, lymphoid cell lines, myeloid cells). The *Igfbp2* (insulin-like growth factor binding protein-2) and *Igfbp5* genes are near the *Wbcq1* peak. These binding proteins modulate the interactions of IGF-1 (insulin-like growth factor-1) with its receptors and have been shown to have effects on apoptosis and cell proliferation. For example, increased IGFBP5 correlates with increased cell death in Purkinje cells of the weaver (*wv/wv*) neurologic mouse mutant and in developing limb

buds in the hypodactyl (*Hd*) mouse (Allan et al. 2000; Lee et al. 1995). Overexpression of IGFBP2 inhibits IGF-1-stimulated cell proliferation and DNA synthesis in zebrafish and cultured CHO cells (Duan et al. 1999). In IGFBP2 knockout mice, spleen weights and numbers of total lymphocytes are decreased (Wood et al. 2000). The *Wbcq5* interval includes the *Creg1* (cellular repressor of E1A-stimulated genes 1) gene, which is also involved in proliferation (Di Bacco and Gill 2003). The gene encoding the alpha receptor of IL-3 (*Il3ra*) is near the peak marker for *Wbcq6*. IL-3 stimulates colony formation of multiple hematopoietic lineages (Mangi and Newland 1999). Several inbred strains of mice carry a mutation in *Il3ra*, and their bone marrow cells do not respond to IL-3 (Hara et al. 1995). Interestingly, strain SM/J, the low WBC count strain in both our crosses, carries the defective gene, while NZW/LacJ, the high WBC count strain, does not.

The lack of colocalization of any of the significant and suggestive Plt QTL with *Thpo* (thrombopoietin, Chr 16) or with *Mpl* (thrombopoietin receptor, Chr 4) is noteworthy. Three genes stand out in the *Mpvq1* interval, *Tuba2* (alpha 2 tubulin), *Tuba6* (alpha 6 tubulin), and *Nfe2* (nuclear factor, erythroid derived 2) encoding the 45 kDa subunit of the heterodimeric transcription factor NF-E2 (Lecine et al. 1998; Shivdasani et al. 1995). The tubulins are intriguing in light of the sliding microtubule theory of platelet formation (Hartwig and Italiano 2003; Italiano and Shivdasani 2003). The transcription factor p45 NF-E2 is required for platelet formation (de Sauvage et al. 1998; Lecine et al. 1998, 2000; Shivdasani et al. 1995). Downstream targets include both platelet- and megakaryocyte-specific β 1 tubulin and Rab27b (Lecine et al. 2000; Tiwari et al. 2003). Notably, hypoprenylation of Rab27b in the gunmetal (*gm*) mouse mutation due to deficiency of the α subunit of Rab geranylgeranyltransferase is associated with a macrothrombocytopenia phenotype (Tiwari et al. 2003).

We are investigating these and other genes as potential candidates using criteria established by the

Complex Trait Consortium (Abiola et al. 2003). These criteria include identification of polymorphisms in coding or regulatory sequences, changes in expression level in high vs. low strains, correlation of sequence/expression changes in F₂ offspring, and *in vitro* functional testing where possible. However, we are also establishing additional crosses to further explore QTL for baseline hematologic parameters. Identifying QTL for any given trait in more than one cross allows the application of powerful new statistical methods, such as combined cross analysis (Li et al. 2005) and haplotype analysis, to significantly narrow QTL intervals and facilitate identification of the underlying genes (Wang et al. 2004) Ultimately, such studies will provide critical focal points for clinical interventions.

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