FULL PAPER *Laboratory Animal Science*

Genetic analysis of low survival rate of pups in RR/Sgn inbred mice

Jun-ichi SUTO[1)]*

[1)]*Agrogenomics Research Center, National Institute of Agrobiological Sciences, Tsukuba, Ibaraki 305–8634, Japan*

(Received 11 July 2014/Accepted 20 February 2015/Published online in J-STAGE 5 March 2015)

ABSTRACT. Newborn offspring of the inbred mouse RR/Sgn strain have a low survival rate prior to weaning. We hypothesized that this is a consequence of an inferior nurturing ability of RR/Sgn mothers and that RR/Sgn mothers have a tendency to lose their pups. We performed quantitative trait locus (QTL) mapping for inferior nurturing ability and tendency to lose pups in RR/Sgn mothers. The number of pups was adjusted to 6 per dam on the day of delivery, and the number of surviving pups and their total weight (litter weight) were scored at 12 days after birth. Nurturing ability was evaluated by litter weight, and tendency to lose pups was evaluated by scoring whether or not the mothers lost their pups. For litter weight, we identified one significant QTL on chromosome 4 and three suggestive QTLs on chromosomes 7, 9 and 17. The RR/Sgn allele was associated with lower litter weight at all loci. For the tendency to lose pups, we identified three suggestive QTLs on chromosomes 4, 9 and 16. The RR/Sgn allele was associated with an increased tendency to lose pups at all loci. These results supported our hypothesis that the low survival rate phenotype was attributable, at least in part, to a phenotype whereby mothers display inferior nurturing ability and a tendency to lose pups. Thus, it suggests that these two traits share genetic basis.

KEY WORDS: inferior nurturing ability, low survival rate, quantitative trait locus (QTL) mapping, RR/Sgn mouse, tendency to lose pups

doi: 10.1292/jvms.14-0361; *J. Vet. Med. Sci.* 77(7): 809–813, 2015

Females of the inbred mouse RR/Sgn strain (hereafter referred to as RR) mate normally, and their pregnancies continue to term; however, their newborn offspring have a low survival rate prior to weaning (the weaning rate was 64.5% with respect to the number of offspring, and 50% of dams lost pups) [11]. There are several possible explanations for low survival rate of RR pups: (1) an inferior nurturing ability of RR mothers, specifically, low lactational yield and/or poor milk quality leading to malnourished pups, (2) abnormal nursing behaviors of RR mothers and (3) inherent suckling defect in RR pups. At present, we cannot specify the reason for the low survival rate, because the survival rate as well as pup growth varies considerably between litters. It is possible that the low survival rate is a complex trait caused by plural etiologies.

Under such conditions, we postulated that the low survival rate of RR pups was at least in part due to the inferior nurturing ability of RR mothers, because there are large variations in female nurturing ability among inbred mouse strains [4, 8]. Based on this hypothesis, we previously performed genetic analyses of the inferior nurturing ability of RR mothers based on the litter weight of six pups from an F_2 intercross between RR and KK/Ta (hereafter referred to as KK) strains [11, 12]. Using total litter weight at 12 days after birth as a metric, we identified one significant and one suggestive QTL on chromosomes 5 (*Naq1*) and 9, respectively. At both loci, the RR allele was associated with lower litter weight. Litter weight at 12 days after birth has been shown to reflect maternal lactational yield [3, 6, 7]. Therefore, it was possible that the low survival rate of RR pups was in part a consequence of poor lactational yield of RR mothers.

However, the above consideration was based on specific parameters that were set forth regarding optimal pup growth. Alternatively, we can evaluate the low survival rate phenotype with regard to the number of surviving pups based on the fact that RR mothers frequently lose their pups during rearing. In this study, we examined the hypotheses that the low survival rate is a consequence of inferior nurturing ability of RR/Sgn mothers and that these mothers have a tendency to lose their pups.

MATERIALS AND METHODS

Mice: The inbred mouse RR strain was purchased from Riken BioResource Center (Tsukuba, Japan), and the inbred mouse C57BL/6J (hereafter B6) strain was purchased from Clea Japan Inc. (Tokyo, Japan).

To compare nurturing ability and tendency to lose pups between RR and B6 mothers, we performed test crosses reciprocally; i.e., ♀RR × ♂B6 and ♀B6 × ♂RR. Nurturing ability was evaluated by litter weight, and tendency to lose pups was evaluated by scoring whether or not the mothers lost their F_1 pups (for details, see below "Phenotyping" section). For QTL analysis, B6 females were crossed with RR males to produce B6 × RR F_1 mice. F_1 females were crossed with RR males to produce (B6 × RR) × RR backcross (hereafter BC) progeny.

All mice were maintained in a specific pathogen-free facility with a regular light cycle and controlled temperature and humidity. Food (CRF-1; Oriental Yeast Co., Ltd., Tokyo, Japan) and water were freely available throughout the experimental period. All animal experiments were approved by the Institutional Animal Care and Use Committee of the

*CORRESPONDENCE TO: SUTO, J., Agrogenomics Research Center, National Institute of Agrobiological Sciences, Tsukuba, Ibaraki 305–8634, Japan. e-mail: jsuto@affrc.go.jp

Table 1. Comparison of nurturing ability and tendency to lose pups between RR and B6 mothers

Test cross		No. of litters	Average litter size[a)] (Range)	No. of dams that lost all or some of F_1 pups (No. of dams that lost all F_1 pups)	No. of surviving pups	Survival rate (%)	Average litter weight (g) (Range)	Average body weight of surviving pup (g)
Dam	Sire							
RR	B6	13	5.6[b)] (3–9)	11 (10)	13	16.7	5.47 (0–34.41)	5.47
B6	RR	7	9.1 (7–12)	0 (0)	42	100	42.55 (37.56–49.08)	7.09

The number of newborn F_1 offspring was culled to 6 per dam at the day of parturition. When the number of pups was less than 6, pups from other strain litters with the same birthday were added to constitute 6 pups per dam. The number of surviving pups and the litter weight were scored at Day 12. a) Litter size indicates the total number of live pups. b) Pups were supplemented with those from other strains (RR × B6 F_1, B6, DDD/Sgn and/or ICR; DDD/Sgn and ICR have also been maintained at our facility) in 7 litters.

National Institute of Agrobiological Sciences.

Phenotyping: Throughout the study, we crossed nulliparous BC females, and only data on primiparous females were analyzed. BC mice were weaned at 4 weeks of age. At the age of 8–10 weeks, BC males and females were housed together. Thereafter, pregnant BC females were housed individually. At the day of parturition, the number of newborn offspring was culled to 6 per dam. When the number of pups was less than 6, pups from other litters with the same birthday were added to constitute 6 pups per dam. The number of surviving pups and the total weight of the surviving pups (litter weight) were scored at 12 days after birth (Day 12). Nurturing ability was evaluated by litter weight, and tendency to lose pups was evaluated by scoring whether or not the mothers lost their pups.

QTL mapping: QTL analysis was conducted using R/qtl [1, 2]. Threshold logarithm of odds (LOD) scores for suggestive (P<0.63) and significant (P<0.05) linkages was determined by performing 1,000 permutations for each trait [5]. For significant QTLs, a 95% confidence interval (CI) was defined by a decline of 1.5 LOD. After single QTL scans, we performed pairwise evaluations for potential interactions between loci. At this stage, threshold LOD scores were strictly based on those recommended by Broman and Sen [1].

We initially genotyped 165 F_2 mice with the following 92 microsatellite markers: *D1Mit211*, *D1Mit236*, *D1Mit303*, *D1Mit49*, *D1Mit217*, *D1Mit33*, *D1Mit36*, *D1Mit291*, *D2Mit312*, *D2Mit297*, *D2Mit274*, *D2Mit285*, *D3Mit60*, *D3Mit25*, *D3Mit230*, *D3Mit254*, *D3Mit162*, *D4Mit235*, *D4Mit214*, *D4Mit178*, *D4Mit327*, *D4Mit306*, *D4Mit279*, *D4Mit69*, *D4Mit232*, *D5Mit267*, *D5Mit184*, *D5Mit259*, *D5Mit240*, *D5Mit95*, *D5Mit221*, *D6Mit116*, *D6Mit188*, *D6Mit149*, *D6Mit14*, *D7Mit340*, *D7Mit76*, *D7Mit246*, *D7Mit228*, *D7Mit232*, *D7Mit250*, *D7Mit253*, *D7Mit12*, *D8Mit191*, *D8Mit248*, *D8Mit211*, *D8Mit113*, *D9Mit90*, *D9Mit191*, *D9Mit107*, *D9Mit196*, *D9Mit212*, *D10Mit188*, *D10Mit42*, *D10Mit297*, *D11Mit229*, *D11Mit86*, *D11Mit219*, *D11Mit212*, *D11Mit124*, *D12Mit109*, *D12Mit201*, *D12Nds2*, *D13Mit139*, *D13Mit110*, *D13Mit230*, *D13Mit35*, *D14Mit11*, *D14Mit64*, *D14Mit193*, *D14Mit165*, *D15Mit175*, *D15Mit63*, *D15Mit159*, *D15Mit193*, *D16Mit131*, *D16Mit4*, *D16Mit139*, *D16Mit71*, *D17Mit16*, *D17Mit139*, *D17Mit93*, *D17Mit123*, *D18Mit21*, *D18Mit149*, *D18Mit123*, *D19Mit40*, *D19Mit53*, *D19Mit35*, *D19Mit6*, *DXMit64* and *DXMit121*. The reported genetic map positions were retrieved from the Mouse Genome Informatics database (MGI, http://www.informatics.jax.org). Because the locations of three microsatellite marker loci (*D5Mit267*, *D13Mit110* and *D19Mit6*) were not available, their locations from adjacent markers were calculated on the basis of our own linkage map.

Binomial test: For BC females that lost their pups, we used a binomial test to assess the statistical significance of a deviation from the expected 1:1 ratio of homozygosity to heterozygosity for the RR allele (RR/RR and RR/B6, respectively) genotypes [10]. The probability (p) that x of the n BC females that lost pups had an RR/RR genotype was calculated using the following binomial formula:

$$P\ (X = x) = \left(\frac{n}{x}\right) p^x (1 - p)^{n-x},\ \text{where}\ \left(\frac{n}{x}\right) = {}_nC_x = \frac{n!}{x!(n-x)!}$$

Under the null hypothesis of no linkage, the probability (p) that BC females that lost their pups have an RR/RR genotype was 0.5. P<0.0001 was considered to indicate significant linkage, and P<0.0034 was considered to indicate suggestive linkage [5]. We also assessed the strength of the linkage on the basis of the Bonferroni correction. In this case, because additional 7 microsatellite markers (*D9Mit144*, *D9Mit303*, *D9Mit289*, *D9Mit263*, *D9Mit343*, *D16Mit165* and *D16Mit152*) were genotyped for the binomial test (giving a total of 99 markers), the significant threshold P value was 0.00051 (0.05/99).

RESULTS

Nurturing ability and tendency to lose pups between RR and B6 mothers: Nurturing ability was apparently inferior in RR mothers, and tendency to lose pups was substantially higher in RR mothers (Table 1).

Reproduction in F_1 mice: Nurturing ability was assessed in 20 (♀B6 × ♂RR) F_1 females. Twenty F_1 females gave rise to 169 pups, yielding an average litter size of 8.45 (range 3–12). In the F_1 study, we did not cull pups, irrespective of litter size, and all pups were successfully weaned in all litters. The tendency to lose pups was thus suggested to be inherited as a recessive trait. Therefore, we used BC females of a cross of ♀ (♀B6 × ♂RR) F_1 × ♂RR for subsequent analyses.

QTL mapping analyses in BC mice: Among 255 litters, litter weight was not determined for 33. Therefore, litter

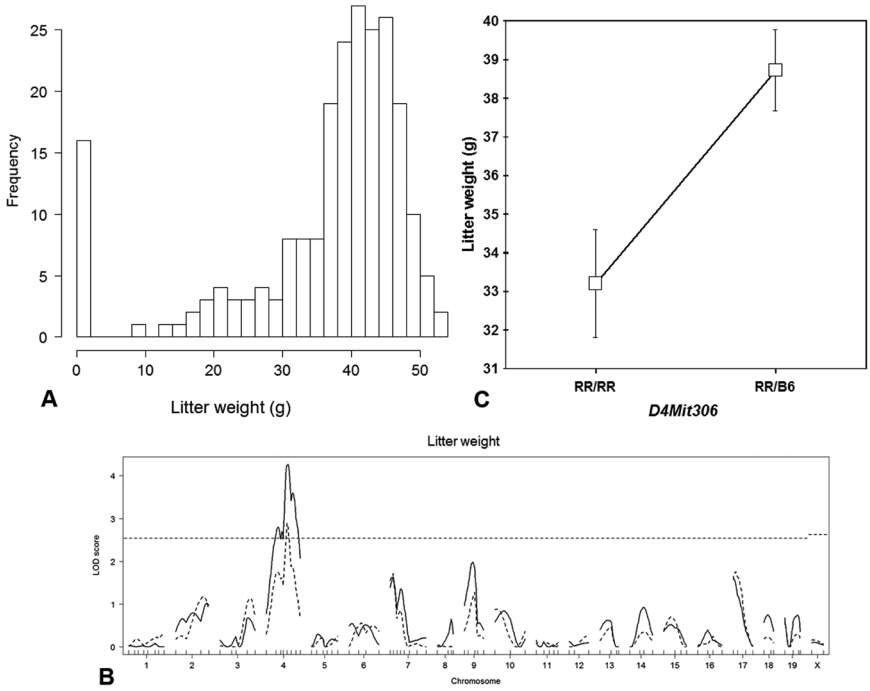

Fig. 1. QTL mapping for inferior nurturing ability. (A) A histogram showing the distribution of litter weight. (B) Genome-wide LOD score plots for litter weight. Solid and broken lines indicate the results obtained using nonparametric and parametric methods, respectively. For the parametric method, PL1 was included as an additive covariate. The horizontal dashed lines indicate the significant threshold LOD scores as determined by 1,000 permutations. Threshold LOD scores for significant and suggestive linkages were 2.42 and 1.31 for autosomes and 2.45 and 1.37 for the X chromosome, respectively. (C) Allele effects of *Naq2* (*D4Mit306*) on litter weight. Marker indicates the mean trait value. Error bars indicate the standard error.

Table 2. Identification of QTLs for prolificacy-associated traits

Trait[a)]	Chromosome	Location[b)]	95% CI[c)]	Max LOD[d)]	Nearest marker	High allele[e)]	Name[f)]
Litter weight	4	51	27–73	4.27*	*D4Mit306*	B6	*Naq2*
	7	9	3–82	1.65	*D7Mit76*	B6	
	9	36	18–57	1.98	*D9Mit107*	B6	
	17	18	18–56	1.61	*D17Mit16*	B6	
PL1	16	25	12–40	2.47	*D16Mit4*	RR	
PL2	4	49	4–78	1.35	*D4Mit306*	RR	
	9	38	18–57	1.56	*D9Mit107*	RR	
	16	25	6–48	1.82	*D16Mit4*	RR	

a) PL1 (Whether BC females lost their entire litter or not) and PL2 (Whether BC females lost all or some of their pups) were analyzed not only as the covariate but also as independent binary traits. b) Location indicates a chromosomal position showing a peak LOD score in cM. c) 95% CI is defined by a 1.5-LOD support interval. d) Maximum LOD score for QTL. Significant QTLs are indicated by an asterisk. e) Allele associated with higher trait values. f) Assignment of the QTL name is limited to significant QTLs.

weight was analyzed in the remaining 222 litters. Overall, 13 of 255 BC females lost all pups, and whether BC females lost their entire litter or not was included as the covariate PL1 (BC females that lost pups were labeled as 1, whereas those that did not were labeled as 0). Similarly, 36 of 255 BC females lost all or some of their pups, and this was included as the covariate PL2. PL1 and PL2 were also analyzed as independent binary traits.

Three of the 255 dams did not have any living pups, and 13 of the 255 dams lost all their pups; thus, litter weights of these 16 were 0 g (Fig. 1A). The distribution of litter weight was not normal and could not be normalized. Accordingly, we analyzed the litter weight trait using a nonparametric method. We identified one significant QTL on chromosome 4 and 3 suggestive QTLs on chromosomes 7, 9 and 17 (Table 2 and Fig. 1B). At all loci, the RR allele was associated with lower litter weight (Fig. 1C). We named the significant QTL on chromosome 4 *Naq2* (nurturing ability QTL 2). When the parametric method was applied, no significant QTLs were identified (data not shown). However, when PL1 was

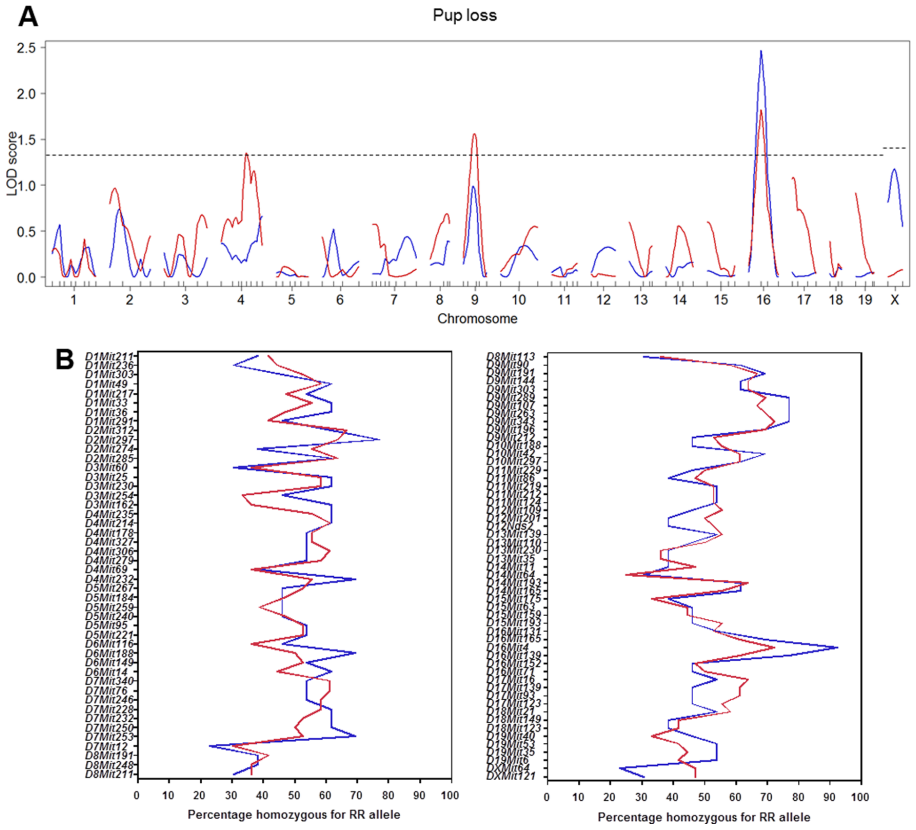

Fig. 2. QTL mapping and binomial test for analysis of tendency to lose pups. (A) Genome-wide LOD score plots for PL1 (blue lines) and PL2 (red lines). Horizontal dashed lines indicate the suggestive threshold LOD scores as determined by 1,000 permutations. For PL1, threshold LOD scores for significant and suggestive linkages were 2.78 and 1.49 for autosomes and 2.92 and 1.59 for the X chromosome, respectively. For PL2, threshold LOD scores for significant and suggestive linkages were 2.63 and 1.34 for autosomes and 2.78 and 1.44 for the X chromosome, respectively. (B) Plots of percentages of mice homozygous for RR alleles at each locus among 99 microsatellite marker loci. Blue and red lines are plots for PL1 and PL2, respectively.

included as an additive covariate, one significant QTL was again identified on chromosome 4 (Fig. 1B).

We analyzed PL1 and PL2 as binary traits and identified one suggestive QTL for PL1 on chromosome 16 and 3 suggestive QTLs for PL2 on chromosomes 4, 9 and 16 (Table 2 and Fig. 2A). Because 13 of the 255 dams lost their entire litter (PL1) and 36 of the 255 dams lost part of or entire litter (PL2), we next analyzed these small fractions of mice by binomial tests. After genotyping 7 additional microsatellite markers (*D9Mit144*, *D9Mit303*, *D9Mit289*, *D9Mit263*, *D9Mit343*, *D16Mit165* and *D16Mit152*), we evaluated the percentage of mice that were homozygous for the RR allele. We observed that a locus on chromosome 16 near *D16Mit4* showed evidence of an association with regard to PL1 (Fig. 2B). Overall, 12 of 13 (92.3%) mice were homozygous for the RR allele at this locus. Although the percentage was very high (P=0.0016), the association was not significant; however, it was suggestive when the genome-wide threshold was applied. Finally, we investigated whether or not *D16Mit4* had effects on litter weight. Although the RR allele was associated with lower litter weight (mean ± SE litter weight in RR/RR and RR/B6 was 34.99 ± 1.32 g and 37.97 ± 1.07 g, respectively), the difference was not significant.

DISCUSSION

Because the results of reciprocal test crosses strongly suggested that the low survival rate of RR pups was attributable to the defects in mothers rather than to those in pups, we examined the low survival rate of RR pups by analyzing the following: (1) inferior nurturing ability of RR mothers based on the litter weight of surviving pups and (2) tendency to lose pups by scoring whether or not RR mother lost their pups. On the basis of the analysis of inferior nurturing ability, we identified one significant QTL on chromosome 4 (*Naq2*) and three suggestive QTLs on chromosomes 7, 9 and 17. Using a similar analysis, we had previously identified one significant and one suggestive QTL for litter weight on chromosomes 5 (*Naq1*) and 9 in KK × RR F_2 mice [10, 11]. *Naq1* and *Naq2* are suggested to be related to lactational yield, because the total weight of 6 pups at 12 days after birth has been known to reflect maternal lactational yield [3, 6, 7]. It was important to note that the RR allele was associated with lower litter weight in all QTLs. Although significant

Table 3. Candidate genes for *Naq2* on chromosome 4

Gene/Locus		Location		Reference
Symbol	Name	cM	Mbp	
vc	vacillans	syntenic	57.19	MGI
Ambp	alpha 1 microglobulin/ bikunin	33.96	63.14	[13]
Whrn	whirlin	33.97	63.41	MGI
Khdrbs1	KH domain containing, RNA binding, signal transduction associated 1	63.34	129.70	[9]

Data are retrieved from MGI (December 16, 2014). Candidate genes within 95% CI for *Naq2* are sorted in the order of chromosomal location.

QTLs for litter weight differed between the two studies, we considered this to be due to the fact that a different counterpart strain was used in the two analyses. On the other hand, on the basis of the analysis of tendency to lose pups, we identified suggestive QTLs on chromosomes 4, 9 and 16. It was again important to note that the RR allele was associated with an increased tendency to lose pups at all QTLs. Of these QTLs, those on chromosomes 4 and 9 may be allelic with those identified in the analysis of inferior nurturing ability according to their chromosomal locations. Therefore, *Naq2* on chromosome 4 had significant and suggestive effects on litter weight and tendency of losing pups, respectively.

We submitted the term "abnormal maternal nurturing" as a query to the MGI database, which then retrieved 161 genotypes with 188 annotations. Of 8 gene loci that were located on chromosome 4, 4 gene loci (*Ambp*, *Khdrbs1*, *vc* and *Whrn*) were located within 95% CI for *Naq2* as candidate genes (Table 3). Of these candidates, 2 genes, i.e., *vc* and *Whrn*, were unlikely to be causative of *Naq2*, because homozygous mutants also displayed behavioral abnormalities, including tremors and swaying gait (*vc*), and circling and head-shaking (*Whrn*). *Ambp*-deficient males are fully fertile; however, mutant females show severe infertility [13]. *Ambp*-deficient females normally copulate with males, although more than half of these individuals do not become pregnant. Although some *Ambp*-deficient females produce young, litter size was very small (1.6 in average). The neonatal pups usually die within 2 days after birth when fostered by homozygous mutant ($Ambp^{-/-}$) mothers; however, these survive when fostered by heterozygous mutant ($Ambp^{+/-}$) mothers. In contrast to the *Ambp*-deficient females, most of the RR females become pregnant after copulation. The litter size of RR strain was not significantly smaller. The RR mothers predominantly tend to lose their pups; the time of pup loss considerably varies among litters. On the other hand, *Khdrbs1*-deficient females rarely provide adequate care to their young, and many of $Khdrbs1^{-/-}$ pups die at birth because of unknown causes [9]. Based on these findings, *Khdrbs1* mutants resemble the RR mice. However, one conclusive difference between the *Khdrbs1* mutants and RR mice is that the males of the former strain were sterile (RR strain males are fully fertile). Thus, both *Ambp* and *Khdrbs1* were unlikely to be causative of *Naq2*.

Although the association was not significant, the suggestive association of chromosome 16 with PL1 was noteworthy. According to this sample size, a significant association was accomplished only when all 13 mice were homozygous for the RR allele. When statistical evaluation was confined to the portion of chromosome 16 that was identified to contain suggestive QTLs for both PL1 and PL2 by the preceding genome-wide QTL scan, the association was significant. Thus, we expect that there would be a locus concerning the tendency to lose pups on chromosome 16. In contrast to *Naq2* on chromosome 4, the locus on chromosome 16 had a suggestive effect only on the tendency to lose pups.

Taken together, these results support our hypothesis that the low survival rate phenotype was attributable, at least in part, to the phenotypes of inferior nurturing ability and tendency to lose pups. Thus, it suggests that these two traits share genetic basis.

ACKNOWLEDGMENT. This work was supported in part by a grant from the Kieikai Research Foundation.

REFERENCES

1. Broman, K. W. and Sen, Ś. 2009. A guide to QTL mapping with R/qtl. Springer, New York.
2. Broman, K. W., Wu, H., Sen, Ś. and Churchill, G. A. 2003. R/qtl: QTL mapping in experimental crosses. *Bioinformatics* **19**: 889–890. [Medline] [CrossRef]
3. Falconer, D. S. 1947. Milk production in mice. *J. Agric. Sci.* **37**: 224–235. [CrossRef]
4. Hadsell, D. L., Wei, J., Olea, W., Hadsell, L. A., Renwick, A., Thomson, P. C., Shariflou, M. and Williamson, P. 2012. In silico QTL mapping of maternal nurturing ability with the mouse diversity panel. *Physiol. Genomics* **44**: 787–798. [Medline] [CrossRef]
5. Lander, E. and Kruglyak, L. 1995. Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. *Nat. Genet.* **11**: 241–247. [Medline] [CrossRef]
6. Nagai, J. 1975. Sources of variation in preweaning weight of mice. *Lab. Anim. Sci.* **25**: 450–453. [Medline]
7. Nagai, J., Eisen, E. J., Emsley, J. A. B. and Mcallister, A. J. 1978. Selection for nursing ability and adult weight in mice. *Genetics* **88**: 761–780. [Medline]
8. Peripato, A. C., de Brito, R. A., Vaughn, T. T., Pletscher, L. S., Matioli, S. R. and Cheverud, J. M. 2002. Quantitative trait loci for maternal performance for offspring survival in mice. *Genetics* **162**: 1341–1353. [Medline]
9. Richard, S., Torabi, N., Franco, G. V., Tremblay, G. A., Chen, T., Vogel, G., Morel, M., Cléroux, P., Forget-Richard, A., Komarova, S., Tremblay, M. L., Li, W., Li, A., Gao, Y. J. and Henderson, J. E. 2005. Ablation of the Sam68 RNA binding protein protects mice from age-related bone loss. *PLoS Genet.* **1**: e74. [Medline] [CrossRef]
10. Sokal, R. R. and Rohlf, F. J. 1995. Biometry. 3rd ed., W.H. Freeman and Company, New York.
11. Suto, J., Yamanaka, H. and Sekikawa, K. 2002. Genetic analysis of inferior nurturing ability in RR mice. *Reproduction* **123**: 52–58. [Medline] [CrossRef]
12. Suto, J. and Sekikawa, K. 2004. Further mapping and characterization of *Naq1*, a quantitative trait locus responsible for maternal inferior nurturing ability in RR mice. *J. Vet. Med. Sci.* **66**: 1033–1038. [Medline] [CrossRef]
13. Zhuo, L., Yoneda, M., Zhao, M., Yingsung, W., Yoshida, N., Kitagawa, Y., Kawamura, K., Suzuki, T. and Kojima, K. 2001. Defect in SHAP-hyaluronan complex causes severe female infertility. *J. Biol. Chem.* **276**: 7693–7696. [Medline] [CrossRef]