Genetic analysis of litter size in mice

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ABSTRACT. We performed quantitative trait locus (QTL) mapping analysis for litter size (total number of pups born and/or number of pups born alive) in 255 backcross mice derived from C57BL/6J and RR/Sgn inbred mice. We identified one significant QTL on chromosome 7 and 4 suggestive QTLs on chromosomes 3, 5, 10 and 13. In addition, two suggestive QTLs were identified on chromosomes 1 and 4 for the number of stillbirth. These results suggested that both litter size and number of stillbirth were heritable traits, although they were controlled by distinct genes. The RR allele was associated with reduced litter size and increased stillbirth at all QTLs. Therefore, RR mothers were observed to have reduced prolificacy in this particular genetic cross.

KEYWORDS: litter size, quantitative trait locus (QTL), RR/Sgn mouse, stillbirth

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The number of pups, or litter size, is a representative reproductive quantitative trait in female animals [3]. Although the heritability of litter size is generally low [8], identifying genes responsible for litter size would be beneficial for livestock improvement.

During the course of our experiments on female reproductive performance in backcross (hereafter BC) mice produced by mating C57BL/6J (hereafter B6) and RR/Sgn (hereafter RR) inbred mice, we noted large variations in litter size. The total number of pups born per dam ranged from 1 to 16, with an average of 8.5. We considered that these BC mice would be useful for identifying genes that controlled litter size. Thus, in this study, we performed quantitative trait locus (QTL) mapping analyses for litter size.

The inbred mouse RR strain was purchased from the Riken BioResource Center (Tsukuba, Japan), and the inbred mouse B6 strain was purchased from the Clea Japan Inc. (Tokyo, Japan). B6 females were crossed with RR males to produce B6 × RR F1 mice. F1 females were crossed with RR males to produce (B6 × RR) × RR BC mice. 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 provided ad libitum throughout the experimental period. All animal experiments were approved by the Institutional Animal Care and Use Committee of the National Institute of Agrobiological Sciences.

Throughout the study, we crossed nulliparous BC females, and data for only primiparous females were analyzed. BC mice were weaned at 4 weeks of age. At 8–10 weeks of age, 1 or 2 BC males were housed with 4 or 5 BC females. Subsequently, pregnant BC females were housed individually. On the day of parturition, the number of newborn offspring was scored once a day between 7:00 to 14:00. We defined the total number of pups born as TNB, and the number of pups born alive as NBA. The number of stillbirth was also scored (defined as NSB). TNB was also referred to as “litter size.” Although it is not sufficiently detailed, information on litter size for the parental strains and F1 mice are available. TNB in B6 strain was 6.7 according to the information retrieved from the web site of Clea Japan Inc. (http://www.clea-japan.com/en/animals/animal_f/f_11.html). According to the breeding data compiled in authors’ laboratory, NBA in RR strain was 6.7 (data based on 83 litters) [25]. Probably, due to hybrid vigor, NBA in B6 × RR F1 mice was 8.5 (based on 20 litters).

Genomic DNA isolation and genotyping of microsatellite markers were performed as described previously [25]. QTL analysis was conducted using R/qtl version 1.33-7 [4, 5]. 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 [18]. For statistically 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 [4]. We initially genotyped 165 BC mice that had extreme phenotypes with regard to litter size 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,
We also genotyped eight additional microsatellite markers (D7Mit162, D7Mit220) on chromosome 7 for the fine mapping. Reported genetic map positions were retrieved from the Mouse Genome Informatics database (http://www.informatics.jax.org). Because the locations of 3 microsatellite marker loci (D5Mit267, D13Mit110 and D19Mit6) were not available, their locations relative to adjacent markers were calculated on the basis of our own linkage map. Once suggestive linkages were identified for a trait, then the remaining 90 BC mice were genotyped for all markers on relevant chromosomes.

Despite a bell-shaped distribution (Fig. 1A), litter size was not normally distributed and could not be normalized using a Box-Cox transformation. Therefore, we performed QTL mapping analyses using a nonparametric method. LOD score plots for TNB and NBA are shown in Fig. 1B. For TNB, we identified one significant QTL on chromosome 7 and four suggestive QTLs on chromosomes 3, 5, 10 and 13 (Thereafter, the QTL on chromosome 7 was further mapped with eight additional microsatellite markers. See Fig. 2) (Table 1). For NBA, 4 suggestive QTLs were identified on chromosomes 3, 7, 10 and 13. Plots for both traits were very similar, and we expected that the significant QTL for TNB and the suggestive QTL for NBA on chromosome 7 were the same locus. Therefore, we designated this QTL on chromosome 7 as Litter size QTL 1 (Lsq1). The RR allele was associated with a smaller litter size at all loci (Fig. 1C). We searched MGI database with the term “abnormal litter size” and found 13 candidate genes within 95% CI for Lsq1 (Table 2). Of 13 candidate genes within 95% CI for Lsq1 (Table 2). Of 13
candidate genes, 6 genes (Aurkc, Trim28, Chst8, Oca2p-25H, Ube3a and Chrna7) are unlikely to be causative of Lsq1, because the abnormal reproductive phenotypes are also identified in mutant males [2, 12, 14, 15, 19, 21, 22, 24]. B6 and RR strain males are fully fertile without gloss abnormalities in terms of the reproductive system and function. Likewise, Dll3pu-J, Cebpa and Magel2 may be eliminated from the candidates for Lsq1, because Dll3pu-J (MGI) is accompanied by a number of severe skeletal malformations, and Cebpa [10] and Magel2 [17] are concerned with the viability of postpartum and postnatal pups. Also, Myod1 is not a suitable candidate gene, because Myod1 influences litter size only when it is with Fgf6 deficiency [11]. Myod1 is located on chromosome 7, and Fgf6 is located on chromosome 6; however, our pairwise scan does not identify any evidence of interaction between these chromosomes. Thus, remaining candidate genes, Ppp5c [1], Ccna2l10 [9] and Egln2 [27], are the most appropriate candidate genes at the present time. These genes are concerned with the embryonic viability. Therefore, we expect that the Lsq1 plays a role in the embryonic survival/lethality, thereby controlling the litter size.

We previously analyzed nurturing ability and NBA in KK × RR F2 mice [25, 26], but we could not identify even suggestive QTLs. To address whether Lsq1 had an effect on NBA in the KK × RR F2 mouse population, we examined the effect of D7Mit232 (located on 33.06 cM) on NBA using a point-wise threshold rather than a genome-wide threshold. This showed that D7Mit232 had a significant effect on NBA (P<0.03). The RR allele was associated with reduced NBA. The mean ± SE NBA of mice with the KK/KK genotype was

| Trait | Chromosome | Locationa | 95% CI(b) | Max LODe | Nearest marker | High allelef | Nameg |
|-------|------------|------------|-----------|----------|----------------|-------------|-------|
| TNB   | 3          | 79         | 2–79      | 1.40     | D3Mit162       | B6          |       |
|       | 5          | 74         | 19–77     | 1.61     | D5Mit221       | B6          |       |
|       | 7          | 27         | 3–36      | 2.67*    | D7Mit228       | B6          | Lsq1  |
|       | 10         | 6          | 6–72      | 1.49     | D10Mit188      | B6          |       |
|       | 13         | 51         | 33–67     | 1.98     | D13Mit110      | B6          |       |
| NBA   | 3          | 79         | 2–79      | 1.63     | D3Mit162       | B6          |       |
|       | 7          | 27         | 3–36      | 2.13     | D7Mit228       | B6          |       |
|       | 10         | 6          | 6–72      | 1.58     | D10Mit188      | B6          |       |
|       | 13         | 51         | 34–67     | 1.91     | D13Mit110      | B6          |       |
| NSB   | 1          | 42         | 16–67     | 2.24     | D1Mit49        | RR          |       |
|       | 4          | 4          | 4–78      | 1.83     | D4Mit235       | RR          |       |

a) Location indicates a chromosomal position showing a peak logarithm of odds (LOD) score in cM. b) 95% confidence interval (CI) was defined by a 1.5-LOD support interval. c) Maximum LOD score for a QTL. Significant QTL is indicated by an asterisk. d) Alleles associated with higher trait values. e) Name was assigned only to significant QTL.

![Fig. 2. Comparison of LOD score plots for litter size on chromosome 7 between two independent QTL mapping analyses. (Left) LOD score plots for TNB in (B6 × RR) × RR BC mice. The horizontal dashed lines indicate significant and suggestive threshold LOD scores determined from 1,000 permutations. (Right) LOD score plots for NBA in KK × RR F2 mice. Solid and broken lines indicate the results of nonparametric and parametric methods, respectively. Localization of several microsatellite markers is shown on the X-axis.](image-url)
Fig. 3. (A) A histogram showing the distribution of number of stillbirth (NSB). (B) Genome-wide LOD score plots for NSB by nonparametric (solid lines) and binary trait (broken lines) methods. The horizontal dashed lines indicate significant and suggestive threshold LOD scores determined from 1,000 permutations. With the nonparametric method, threshold LOD scores for significant and suggestive linkages were 2.52 and 1.34 for autosomes and 2.67 and 1.42 for the X chromosome, respectively. With the binary trait method, threshold LOD scores for significant and suggestive linkages were 2.73 and 1.37 for autosomes and 2.75 and 1.44 for the X chromosome, respectively.

Table 2. Candidate genes for Lsq1 on chromosome 7

| Symbol | Gene Name | Location | Reference |
|--------|-----------|----------|-----------|
| Aurkc  | aurora kinase C | 4.06 7.00 | [15] |
| Trim28 | tripartite motif-containing 28 | 7.73 13.02 | [2, 12] |
| Ppp5c  | protein phosphatase 5, catalytic subunit | 9.15 17.00 | [1] |
| Ceacam10| carcinoembryonic antigen-related cell adhesion molecule 10 | 12.78 24.78 | [9] |
| Egln2  | egl-9 family hypoxia-inducible factor 2 | 15.83 27.16 | [27] |
| DI3my-3 | delta-like 3 (Drosophila); pudgy Jackson | 16.67 28.30 | MGI |
| Chst8  | carbohydrate (N-acetylgalactosamine 4-0) sulfotransferase 8 | 20.53 34.67 | [21] |
| Cebpa  | CCAAT/enhancer binding protein (C/EBP), alpha | 21.02 35.12 | [10] |
| Myod1  | myogenic differentiation 1 | 30.03 46.38 | [11] |
| Oca2p-25H | oculocutaneous albinism II; pink-eyed dilution 25 Harwell | 33.44 56.24 | [14, 19] |
| Ube3a  | ubiquitin protein ligase E3A | 33.95 59.23 | [24] |
| Magel2 | melanoma antigen, family L, 2 | 34.37 62.38 | [17] |
| Chrna7 | cholinergic receptor, nicotinic, alpha polypeptide 7 | 34.47 63.10 | [22] |

Data are retrieved from MGI (September 17, 2014). Candidate genes within 95% CI for Lsq1 are sorted in the order of chromosomal location.
9.88 ± 0.46, that with the KK/RR genotype was 8.70 ± 0.29, and that with the RR/RR genotype was 8.13 ± 0.46. Based on the Tukey–Kramer HSD test, mice with the KK/KK genotype had a significantly higher litter size than mice with the RR/RR genotype. Because the chromosomal localization of Lst1 and the locus identified in KK × RR F₂ mice was very similar (Fig. 2), we expected that both loci were allelic. Reed et al. also identified a litter size QTL on chromosome 7 in a chromosome substitution mouse strain [23]. These results further substantiated the possibility of the presence of a litter size QTL on mouse chromosome 7.

In addition, we analyzed NSB for 39 litters (Fig. 3A). When NSB was analyzed using the nonparametric method, two suggestive QTLs were identified on chromosomes 1 and 4 (Fig. 3B). At both loci, the RR allele was associated with an increase in NSB. When this trait was analyzed using a binary method (whether or not each litter included stillbirth), suggestive QTLs were again identified on chromosomes 1 and 4. Of the 39 dams, 29 were homozygous for the RR allele (RR/RR) at D1Mit49.

NSB may be a fairly unreliable trait, because the offspring could have died after parturition or have been eaten by their mother. Therefore, it was surprising that NSB was genetically controlled. However, NSB has been recognized as a heritable trait in pigs [7, 16], and QTLs for NSB have been identified [6, 13, 20]. This strongly suggested that NSB was also a heritable trait in mice. In addition, in this study, NSB was controlled by gene loci that were distinct from those that controlled TNB and NBA. This was in accordance with the results of pig studies, in which most QTLs for NSB were identified on chromosomes that differed from those for NBA [6, 13, 20].

Finally, the RR allele was associated with reduced litter size and increased NSB at all QTLs. Thus, the RR mothers were observed to have reduced prolificacy in this particular genetic cross.

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