NOTE *Laboratory Animal Science*

Genetic analysis of litter size in mice

Jun-ichi SUTO[1)]*

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

(Received 6 July 2014/Accepted 7 November 2014/Published online in J-STAGE 26 November 2014)

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.

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

doi: 10.1292/jvms.14-0350; *J. Vet. Med. Sci.* 77(3): 353–358, 2015

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 F_1 mice. F_1 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 F_1 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 F_1 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*,

---

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

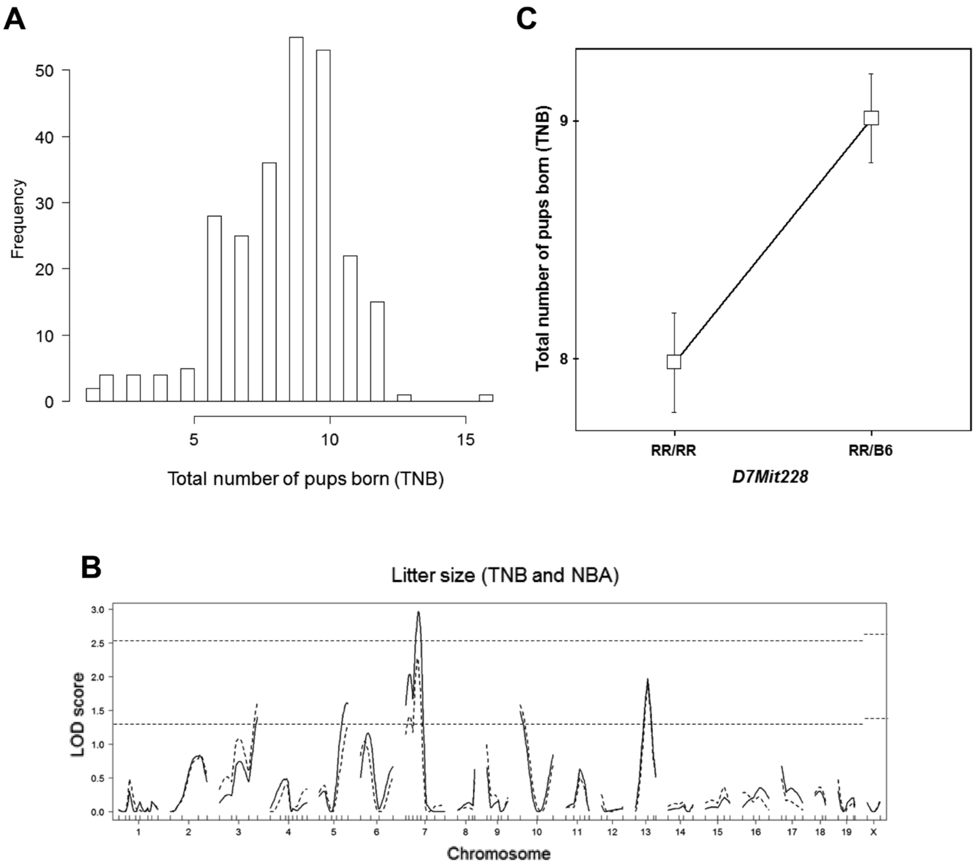

Fig. 1. (A) A histogram showing the distribution of total number of pups born (TNB). (B) Genome-wide LOD score plots for TNB (solid lines) and NBA (broken lines). The horizontal dashed lines indicate significant and suggestive threshold LOD scores determined from 1,000 permutations. For TNB, threshold LOD scores for significant and suggestive linkages were 2.51 and 1.31 for autosomes and 2.65 and 1.42 for the X chromosome, respectively. For NBA, threshold LOD scores for significant and suggestive linkages were 2.62 and 1.33 for autosomes and 2.56 and 1.40 for the X chromosome, respectively. (C) Allele effects of Litter size QTL 1 (*Lsq1*; *D7Mit228*) on TNB. Squares indicate the mean TNB, and error bars indicate standard errors.

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*. We also genotyped eight additional microsatellite markers (*D7Mit306*, *D7Mit308*, *D7Mit225*, *D7Mit247*, *D7Mit229*, *D7Mit195*, *D7Mit162* and *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

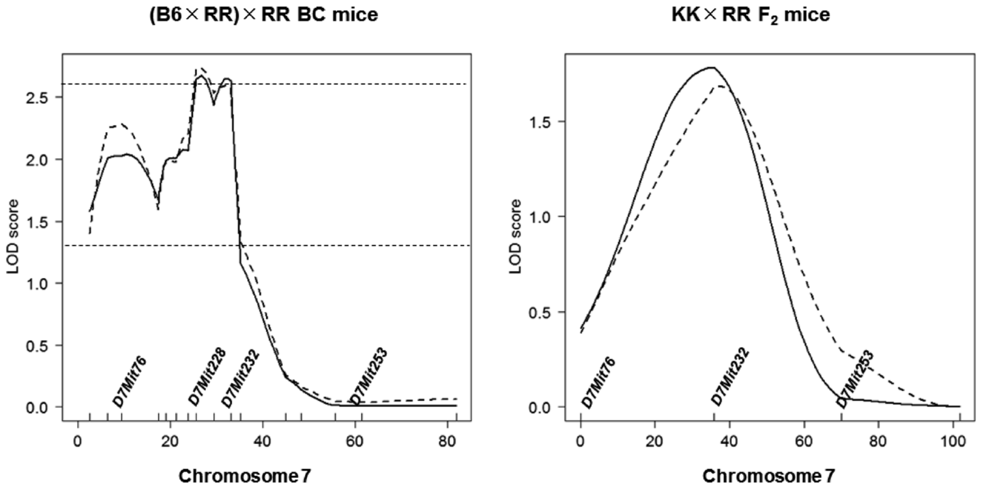

Fig. 2. Comparison of LOD score plots for litter size on chromosome 7 between two independent QTL mapping analyses. (Left) LOD score plots for TNB in (B6 × RR) × RR BC mice. The horizontal dashed lines indicate significant and suggestive threshold LOD scores determined from 1,000 permutations. (Right) LOD score plots for NBA in KK × RR F_2 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.

Table 1. Identification of quantitative trait loci (QTLs) for litter size-related traits

Trait	Chromosome	Location[a)]	95% CI[b)]	Max LOD[c)]	Nearest marker	High allele[d)]	Name[e)]
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.

candidate genes, 6 genes (*Aurkc*, *Trim28*, *Chst8*, *Oca2*$^{p\text{-}25H}$, *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, *Dll3*$^{pu\text{-}J}$, *Cebpa* and *Magel2* may be eliminated from the candidates for *Lsq1*, because *Dll3*$^{pu\text{-}J}$ (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], *Ceacam10* [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 F_2 mice [25, 26], but we could not identify even suggestive QTLs. To address whether *Lsq1* had an effect on NBA in the KK × RR F_2 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

Table 2. Candidate genes for *Lsq1* on chromosome 7

Gene		Location		Reference
Symbol	Name	cM	Mbp	
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]
Dll3$^{pu\text{-}J}$	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]
Oca2$^{p\text{-}25H}$	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.

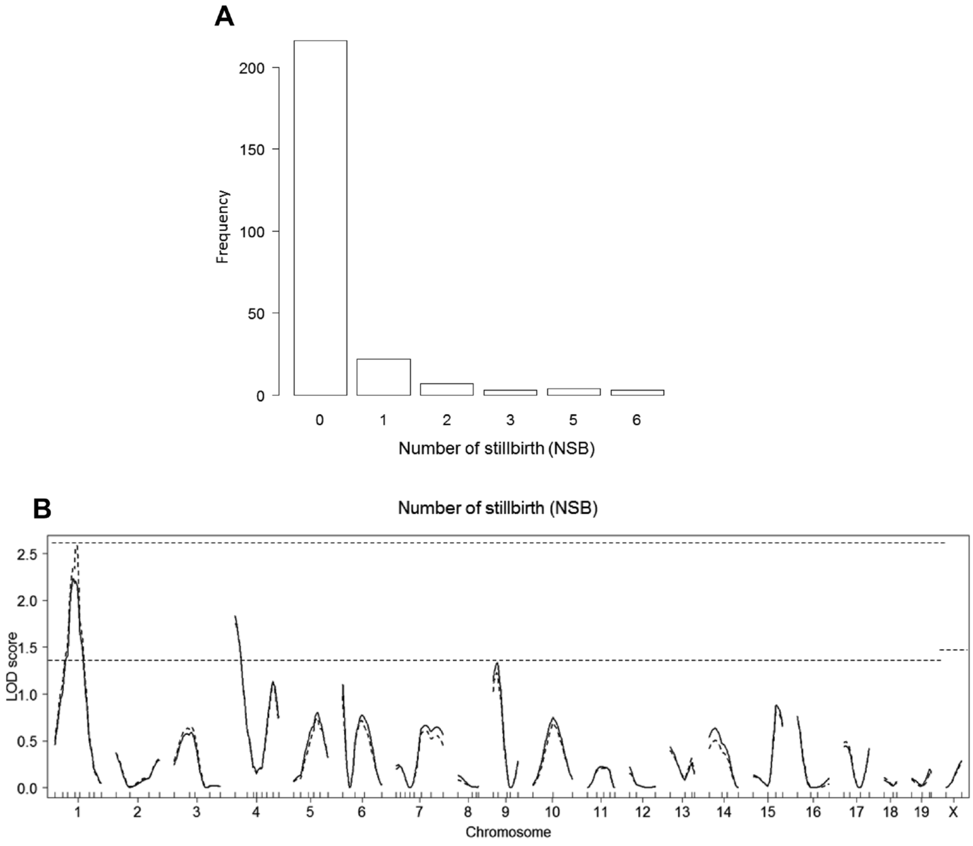

Fig. 3. (A) A histogram showing the distribution of number of stillbirth (NSB). (B) Genome-wide LOD score plots for NSB by nonparametric (solid lines) and binary trait (broken lines) methods. The horizontal dashed lines indicate significant and suggestive threshold LOD scores determined from 1,000 permutations. 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.

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 *Lsq1* and the locus identified in KK × RR F_2 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.

ACKNOWLEDGMENT. This work was supported by an institutional grant from the National Institute of Agrobiological Sciences.

REFERENCES

1. Amable, L., Grankvist, N., Largen, J. W., Ortsater, H., Sjoholm, A. and Honkanen, R. E. 2011. Disruption of serine/threonine protein phosphatase 5 (PP5:PPP5c) in mice reveals a novel role for PP5 in the regulation of ultraviolet light-induced phosphorylation of serine/threonine protein kinase Chk1 (CHEK1). *J. Biol. Chem.* **286**: 40413–40422. [Medline] [CrossRef]
2. Ashe, A., Morgan, D. K., Whitelaw, N. C., Bruxner, T. J., Vickaryous, N. K., Cox, L. L., Butterfield, N. C., Wicking, C., Blewitt, M. E., Wilkins, S. J., Anderson, G. J., Cox, T. C. and Whitelaw, E. 2008. A genome-wide screen for modifiers of transgene variegation identifies genes with critical roles in development. *Genome Biol.* **9**: R182. [Medline] [CrossRef]
3. Bidanel, J. P. 2011. Biology and genetics of reproduction. pp. 218–241. *In*: The Genetics of the Pig, 2nd ed. (Rothschild, M. F. and Ruvinsky, A. eds.), CAB International, Oxfordshire.
4. Broman, K. W. and Sen, Ś. 2009. A Guide to QTL Mapping with R/qtl. Springer, New York.
5. 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]
6. Cassady, J. P., Johnson, R. K., Pomp, D., Rohrer, G. A., Van Vleck, L. D., Spiegel, E. K. and Gilson, K. M. 2001. Identification of quantitative trait loci affecting reproduction in pigs. *J. Anim. Sci.* **79**: 623–633. [Medline]
7. Chen, C. Y., Misztal, I., Tsuruta, S., Herring, W. O., Holl, J. and Culbertson, M. 2010. Genetic analyses of stillbirth in relation to litter size using random regression models. *J. Anim. Sci.* **88**: 3800–3808. [Medline] [CrossRef]
8. Falconer, D. S. and Mackay, T. F. C. 1996. Introduction to Quantitative Genetics, 4th ed., Longman, Harlow, Essex.
9. Finkenzeller, D., Fischer, B., Lutz, S., Schrewe, H., Shimizu, T. and Zimmermann, W. 2003. Carcinoembryonic antigen-related cell adhesion molecule 10 expressed specifically early in pregnancy in the decidua is dispensable for normal murine development. *Mol. Cell. Biol.* **23**: 272–279. [Medline] [CrossRef]
10. Flodby, P., Barlow, C., Kylefjord, H., Ahrlund-Richter, L. and Xanthopoulos, K. G. 1996. Increased hepatic cell proliferation and lung abnormalities in mice deficient in CCAAT/enhancer binding protein alpha. *J. Biol. Chem.* **271**: 24753–24760. [Medline] [CrossRef]
11. Floss, T., Arnold, H. H. and Braun, T. 1997. A role for FGF-6 in skeletal muscle regeneration. *Genes Dev.* **11**: 2040–2051. [Medline] [CrossRef]
12. Herzog, M., Wendling, O., Guillou, F., Chambon, P., Mark, M., Losson, R. and Cammas, F. 2011. TIF1β association with HP1 is essential for post-gastrulation development, but not for Sertoli cell functions during spermatogenesis. *Dev. Biol.* **350**: 548–558. [Medline] [CrossRef]
13. Holl, J. W., Cassady, J. P., Pomp, D. and Johnson, R. K. 2004. A genome scan for quantitative trait loci and imprinted regions affecting reproduction in pigs. *J. Anim. Sci.* **82**: 3421–3429. [Medline]
14. Hunt, D. M. and Johnson, D. R. 1971. Abnormal spermiogenesis in two pink-eyed sterile mutants in the mouse. *J. Embryol. Exp. Morphol.* **26**: 111–121. [Medline]
15. Kimmins, S., Crosio, C., Kotaja, N., Hirayama, J., Monaco, L., Hoog, C., van Duin, M., Gossen, J. A. and Sassone-Corsi, P. 2007. Differential functions of the Aurora-B and Aurora-C kinases in mammalian spermatogenesis. *Mol. Endocrinol.* **21**: 726–739. [Medline] [CrossRef]
16. Knol, E. E., Leenhouwers, J. I. and van der Lande, T. 2002. Genetic aspects of piglet survival. *Livest. Prod. Sci.* **78**: 47–55. [CrossRef]
17. Kozlov, S. V., Bogenpohl, J. W., Howell, M. P., Wevrick, R., Panda, S., Hogenesch, J. B., Muglia, L. J., Van Gelder, R. N., Herzog, E. D. and Stewart, C. L. 2007. The imprinted gene Magel2 regulates normal circadian output. *Nat. Genet.* **39**: 1266–1272. [Medline] [CrossRef]
18. 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]
19. Lehman, A. L., Nakatsu, Y., Ching, A., Bronson, R. T., Oakey, R. J., Keiper-Hrynko, N., Finger, J. N., Durham-Pierre, D., Horton, D. B., Newton, J. M., Lyon, M. F. and Brilliant, M. H. 1998. A very large protein with diverse functional motifs is deficient in rjs (runty, jerky, sterile) mice. *Proc. Natl. Acad. Sci. U.S.A.* **95**: 9436–9441. [Medline] [CrossRef]
20. Li, K., Ren, J., Xing, Y., Zhang, Z., Ma, J., Guo, Y. and Huang, L. 2009. Quantitative trait loci for litter size and prenatal loss in

a White Duroc × Chinese Erhualian resource population. *Anim. Genet.* **40**: 963–966. [Medline] [CrossRef]

21. Mi, Y., Fiete, D. and Baenziger, J. U. 2008. Ablation of GalNAc-4-sulfotransferase-1 enhances reproduction by altering the carbohydrate structures of luteinizing hormone in mice. *J. Clin. Invest.* **118**: 1815–1824. [Medline]
22. Morley, B. J. and Rodriguez-Sierra, J. F. 2004. A phenotype for the alpha 7 nicotinic acetylcholine receptor null mutant. *Brain Res.* **1023**: 41–47. [Medline] [CrossRef]
23. Reed, D. R., McDaniel, A. H., Avigdor, M. and Bachmanov, A. A. 2008. QTL for body composition on chromosome 7 detected using a chromosome substitution mouse strain. *Obesity (Silver Spring)* **16**: 483–487. [Medline] [CrossRef]
24. Smith, C. L., DeVera, D. G., Lamb, D. J., Nawaz, Z., Jiang, Y. H., Beaudet, A. L. and O'Malley, B. W. 2002. Genetic ablation of the steroid receptor coactivator-ubiquitin ligase, E6-AP, results in tissue-selective steroid hormone resistance and defects in reproduction. *Mol. Cell. Biol.* **22**: 525–535. [Medline] [CrossRef]
25. Suto, J., Yamanaka, H. and Sekikawa, K. 2002. Genetic analysis of inferior nurturing ability in RR mice. *Reproduction* **123**: 52–58. [Medline] [CrossRef]
26. 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]
27. Takeda, K., Aguila, H. L., Parikh, N. S., Li, X., Lamothe, K., Duan, L. J., Takeda, H., Lee, F. S. and Fong, G. H. 2008. Regulation of adult erythropoiesis by prolyl hydroxylase domain proteins. *Blood* **111**: 3229–3235. [Medline] [CrossRef]