Genome-Wide Scan and Fine-Mapping of QTL for Respiratory Disease in Landrace Purebred Swine

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Introduction

Respiratory disease in pigs, which induces poor production, is the most important health concern for swine producers today. Vaccination, medication and sanitation can reduce the disease effects, but such approaches are not reliably effective. Therefore, development of livestock with genetically increased resistance to respiratory disease is desired. Because heritability is low and measuring costs for disease resistance are generally high, identification of genes or markers linked to genes for disease resistance facilitates the improvement of pig health. Attempts to find quantitative traits loci (QTL) for immune response in pigs were first made in F2 animals from a pedigree based on an intercross between the European Wild Boar and Swedish Yorkshire pig (Edfors-Lilja *et al*. 1998). Subsequently, some QTLs for disease resistance were identified. However, these analyses used an F2 resource population created by mating two genetically distinct breeds. Information about these QTLs would be difficult to use directly for improvement of purebred populations because their genetic backgrounds differ. Results of several studies show that some detected QTLs are segregating in purebred populations (Nagamine *et al*. 2003, Evan *et al*. 2003). Especially, Uemoto *et al*. (2008) reported QTL for meat production, meat quality, and carcass traits within a Duroc purebred population with a complex multigenerational pedigree structure. The Landrace pigs had been selected over five generations at Miyagi Prefecture Animal Industry Experiment Station. Using that population, we conducted genome-wide scan and fine-mapping of QTLs for respiratory disease.

Material and methods

Experimental Animals

The Landrace pigs used in this experiment had been selected over five generations during 2003 -2008 at the Miyagi Prefecture Animal Industry Experiment Station. All experimental pigs were provided ad libitum access to a commercial diet in testing periods, during which their body weight increased from 30 to 105 kg. Pigs had free access to water. We divided them into two environments: a normal pen (group A) and a susceptible pen (group B). The

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group A pigs were reared in a concrete-floored building with daily wash, while group B pigs were reared in a vinyl-film-covered piggery. We divided sib-tested pigs into different environments because we predicted that the selection for disease resistance lowered their risk of disease. Boars used for full-sib tests were subsequently castrated. In all, 1,399 pigs from the first to fifth generation were used for QTL analyses.

Measurement of morbid of AR and MPS

Gross lesion score of Atrophic rhinitis (AR)

At slaughter, the snouts were cut transversely between the cuspid and first premolar. After gently cleaning the turbinates with water, each snout was visually scored independently. Normal turbinates received a grade of 0. Slight, but readily apparent atrophy of the dorsal turbinate was graded as 1.0. Moderate atrophy of the turbinates, especially the dorsal and ventral scrolls,was graded as 2.0. Severe atrophy of the dorsal scrolls and noted atrophy of the ventral scrolls was graded as 3.0. Severe atrophy of both dorsal and ventral scrolls was graded as 4.0. To avoid deviation to 0 or 1.0, we graded the turbinates with 0.5-grade increments from 0 to 2.0. We expressed AR as the score.

Gross lesion score of Mycoplasmal pneumonia of swine (MPS)

At slaughter, the lungs of all invested pigs were assessed for the presence of lung lesions typical of mycoplasmal pneumonia of swine as modified Goodwin *et al*. (1979). MPS was expressed from 0 to 100% based on the proportion of lung tissue involved in the pneumonic process, suggestive of *Mycoplasma hyopneumoniae* infection (discoloration and consolidation). Briefly, 10 points were allocated to each apical and cardiac lobe, 5 points to the intermediate lobe and 5 points to each leading edge of the diaphragmatic lobes. The scores from each lobe were then added together, divided by 55(if all these areas were totally consolidated the score would be 55 points) and expressed in percentage to determine final visual scores for each pig, ranging from 0% to 100%.

AR and MPS were log-transformed to approximate the normal distribution.

Genetic marker and linkage map

For genome-wide scanning, we used 109 informative microsatellite (MS) markers distributed over all autosomes and genotyped all experimental animal. Data from all genotyped animals and CRI-MAP mapping software (Green *et al*., 1990) were used to estimate the distance between markers. The average distance between markers using the Haldane map function was 21.48 cM in genome-wide scan. For fine-mapping analysis, 19 MS markers and 8 SNP markers located with chromosome 2. Because of the use of pre-established marker panels, these MS markers included 9 markers that had been included previously in the genome-wide scan. The average distance was 5.12 cM in fine-mapping.

QTL analysis

For this study, QTL analysis was performed using maximum likelihood techniques as implemented in SOLAR, based on a multipoint variance components analysis (Amos, 1994; Almasy and Blangero 1998) as described in a previous report (Uemoto *et al*. 2008). The QTL analysis, which necessitates correction of the phenotypic value using the best linear unbiased estimator for generation, sex, and pen, was conducted using the VCE4.25 program (Neumaier and Groeneveld 1998). Because the SOLAR program cannot estimate IBD scores for a complicated multigeneration pedigree, multipoint IBD scores were estimated using

LOKI (Heath 1997). All multipoint variance-component approaches were donducted according to the procedures outlined in SOLAR (Almasy and Blangero, 1998). Variance component analysis was performed at 1-cM to 5-cM intervals along every candidate region. Significance tests were based on a likelihood ratio, as obtained by multiplying the LOD score by 4.605. Tests were conducted for each chromosome represented in the set of markers, and significance was assessed using Bonferroni's correction for a chromosome-wise type 1 error rate of 0.05. For instance, when significance is assessed for six markers on *Sus scrofa* chromosomes (SSCs) 1, the overall α=0.05 has a threshold for each comparison P<0.05/6, and the threshold of P=0.05/6 of the $\chi 2$ test with 1 df is an LOD score of 1.511 (6.960/4.605) and 2.079 (9.575/4.605) with 2 df.

Results and discussion

The genome scan results showed evidence for significant QTL affecting AR on SSC 3 and 4, and MPS on SSC2 and 12 (Table 1). This is the first report in the relevant literature to describe evidence of QTL affecting respiratory disease. Especially, we mapped a QTL for MPS on SSC2 between *SW1650* (17.2 cM) and *SW240* (38.3 cM) (Fig. 1a). Moreover, the QTL had a high LOD score and the estimated proportion of the polygenic heritability was explained by the fact that QTL was large (Table 1).

Table 1: LOD score and QTL position for respiratory disease

Trait	*N*	SSC	Position†	LOD score‡	h_g^2§	h_q^2¶	Marker range	
AR	639	3	99	1.56*	0.19	0.10	*SW314*	*SW349*
AR	639	4	0	1.56*	0.21	0.08	*SW489*	
MPS	630	2	31	3.03**	0.05	0.18	*SW1650*	*SW240*
MPS	630	12	92	1.48*	0.13	0.10	*S0106*	*SW2180*

†Map position in Haldane cM
‡Levels of significance (chromosome level: *df=1 (P<0.05), **df=2 (P<0.05)
§Residual polygenic heritability
¶QTL genotypic heritability

Therefore, to restrict the candidate region, the same family as the genome-wide scan was reanalyzed using MS and SNP markers distributed densely throughout the QTL region. We defined the QTL within the 7-cM interval (Fig. 1b) and the QTL region shows synteny with human chromosome 11 (HSA11p13→p11.12). A list comprising only 44 loci was obtained from the NCBI Map viewer (build 37.1) and was subsequently submitted to functional annotation tool while particularly addressing Gene Ontology biological pathway terms covering genes of immunological relevance. For the QTL regions, three genes were functionally annotated to the term 'immune system process' (GO0002376). For one of these genes, functional association is known to exist with the inflammation response, which is involved in lung lesions.

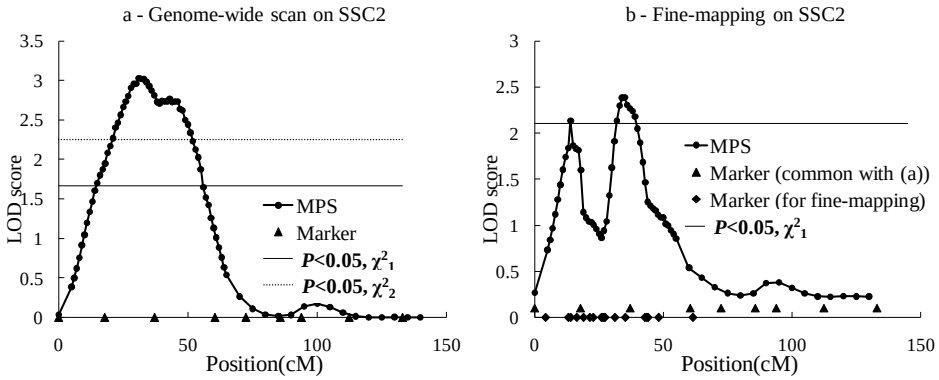

Figure 1: Genome-wide scan (a) and Fine-mapping (b) on SSC2

Conclusion

We detected four QTLs for the lesion score of respiratory disease in Landrace purebred swine for the first time. Results suggested there is a gene involved in inflammation response could exist on the region of QTL for the gross lesion score of Mycoplasmal pneumonia of swine. We plan to investigate a connection between the lung lesions and polymorphism and expression of the gene.

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References

Almasy, L. Blangero, J. (1998) *Am. J. Hum. Genet.*, 62:1198-1211.

Blangero, J. Williams, J. T. Almasy, L. (2000). *Genet. Epidemiol.*, 19:8-14.

Edfors-Lilja, I. Wattrang, E. Marklund, *et al.* . (2000) *J. Immunol.*, 161: 829-835.

Evans, G. J. Giuffra, E. Sanchez, *et al.* . (2003) *Genetics*, 164: 621-627.

Green, P. Falls, K. Crooks, S. (1990) *Washington Univ School of Medicine, St. Louis, MO*

Heath, S. C. (1997) *Am. J. Hum. Genet.*, 1997, 61, 748-760.

Nagamine, Y. Haley, C. S. Sewalem, A. *et al.*, . (2003) *Genetics*, 164: 629-635

Neumaier, A. Groeneveld, E. (1998) *Genet. Sel. Evol.*, 30: 3-26.

Uemoto, Y. Nagamine, Y. Kobayashi, E., *et al*. (2008) *J. Anim. Sci.*, 97:193-202

R. F. W. Goodwin and P. Whittlestone. (1973) *Br. Vet. J.*, 129:456-462