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Characterization of porcine *autism susceptibility candidate 2* as a candidate gene for the number of corpora lutea in pigs

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ABSTRACT

In a previous study, we mapped two quantitative trait loci (QTL) approximately 50 cM apart, both influencing the number of corpora lutea in pigs on chromosome 3. One locus included functional candidate genes for proteins related to specific aspects of fertility, such as the follicle-stimulating hormone receptor and the luteinizing hormone/choriogonadotropin receptor. However, specific genes related to the second locus have not yet been identified. This study aims to identify another candidate gene influencing the number of corpora lutea in pigs. Using 12 polymorphic markers, we fine-mapped a narrow region of pig chromosome 3 that had been shown to contain a QTL for corpora lutea. In the critical region, only 1 gene, *autism susceptibility candidate 2 (AUTS2)*, was identified as a positional candidate. Our results demonstrate that the porcine *AUTS2* gene consists of 19 exons with a complete open reading frame of 3768 bp encoding an *AUTS2* protein of 1256 amino acids. We screened the whole coding sequence and parts of the untranslated region for polymorphisms in an F_2 population of Duroc × Meishan crosses. We found 1 ins/del and 7 single nucleotide polymorphisms (SNP), including 2 nonsynonymous variants, c.943C>T in exon 7 and c.2828C>T in exon 19, resulting in P315S and A943V, respectively. The SNP c.943C>T within a proline-rich domain was genotyped in several breeds; the C allele occurred in all breeds, whereas the T allele occurred only in Meishan pigs. Using *in situ* hybridization, the mRNA expression of the *AUTS2* gene was observed on granulosa cells in the porcine ovary and thus may be associated with hormone sensitivity.

1. Introduction

Understanding the genetic basis of reproductive performance is an important research area. In swine, litter size at birth is the net result of numerous component traits expressed by the embryo and the dam, including ovulation rate, potential embryonic viability, and uterine capacity (Bennett and Leymaster, 1989). However, there is a negative relationship between ovulation rate and prenatal survival ($r = -0.37$) and a low correlation between ovulation rate and litter size ($r = 0.19$; Blasco et al., 1996). Moreover, in a previous report (Sato et al., 2006), we mapped quantitative trait loci (QTL) for the number of corpora lutea (CL) and found no support for a correlation between CL and litter size. Fecundity in multiparous animals, especially in pigs, is one of the most complex and difficult traits to evaluate because of its low heritability and long generation interval, as well as the polygenic nature of reproductive traits and strong environmental influences on reproduction processes (Buske et al., 2006). Improvements in fecundity may be facilitated by the identification of genetic markers associated with DNA variants affecting these traits.

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Ovulation rate, which is commonly estimated from the number of CL on the surfaces of ovaries and is important to breeders seeking to provide stable production, has been studied in mammals such as ovine, bovine, swine, rats, and mice. In mice, growth differentiation factor (GDF-9) was the first oocyte-derived growth factor found to be required for somatic cell function (Dong et al., 1996). In ovine, bone morphogenetic protein 15 (BMP15; also known as growth differentiation factor 9B) and the maternally imprinted gene *FecX2*, located on chromosome X, were found to be associated with increased ovulation rate and an infertility phenotype (Galloway et al., 2000; Davis et al., 2001). Moreover, bone morphogenetic protein IB receptor (BMPR-IB, also known as ALK-6), which is located on OAR 6, was expressed in oocytes and granulose cells in the sheep ovary; the expression pattern was consistent with the receptor playing an important role in oocyte and granulose cell development within the ovary (Mulsant et al., 2001; Wilson et al., 2001).

A suggestive QTL for ovulation rate was identified near microsatellite marker *SW72* on the p-arm of the *Sus scrofa* chromosome 3 (SSC3) in a multigenerational Meishan × White composite population (Rohrer et al., 1999). A previous report from our laboratory also mapped a QTL for CL near *SW72* in a Duroc × Meishan population (Sato et al., 2006). Radiation-hybrid comparative mapping suggests that the region around *SW72* on SSC3 is orthologous to human chromosome 7 (Meyers et al., 2005; Mousel et al., 2006). The refined maximum likelihood location of the QTL was directly over the *autism-susceptibility candidate 2* (*AUTS2*) gene, which was physically assigned to this region by Sultana et al. (2002) and radiation-hybrid mapped by Mousel et al. (2006). *AUTS2*, the function of which is unknown, is associated with autism and mental retardation in humans (Sultana et al., 2002).

In the present study, fine mapping of CL QTL regions was performed to narrow the QTL intervals and thus reduce the number of positional candidate genes. Novel porcine *AUTS2* was then sequenced in Duroc × Meishan pigs to determine if genetic variation in the coding region was associated with variation in CL in the F_2 population. We showed that *AUTS2* expressed in the ovaries might contribute to a portion of the genetic variation for CL in pigs.

2. Materials and methods

2.1. Population

An F_2 resource population was constructed, and phenotypes were measured at the Ibaraki and Miyazaki Branches of the National Livestock Breeding Center. The F_1 generation (27 males and 25 females) was produced by crossing a Meishan sow with a Duroc boar. A total of 865 F_2 progeny (450 males and 415 female) were obtained in 6 farrowings from 24 F_1 females mated to 4 F_1 males; each F_1 sow always was crossed with the same F_1 boar (Sato et al., 2006). The total number born of 306 F_2 sows, after they were mated to 18 Large White boars, was counted to obtain first parity litter size data. Subsequently, 248 of these 306 F_2 sows were mated again to 18 Large White boars, slaughtered at week 4, and the number of CL at second parity was recorded. The CL of nonpregnant F_2 sows was not counted; therefore, the number of CL was determined in 234 F_2 sows. Allele frequencies were determined from a sampling of 226 animals from 4 pig breeds: Meishan ($n = 47$), Large White ($n = 46$), Landrace ($n = 94$), and Duroc ($n = 39$). Their DNA was extracted from tissue samples collected from each animal.

2.2. Map construction and QTL mapping

Although a linkage map was constructed previously (Sato et al., in press), we constructed a new linkage map for SSC3 that included 12 additional microsatellite markers in the CL QTL region, using CRI-MAP 2.4 software (Green et al., 1990). Based on this linkage map of SSC3, we reanalyzed QTL for CL using multilocus least-squares analysis, a method developed by Haley et al. (1994). The *F* ratio was used as a test statistic for detecting QTL, and the chromosome-wise threshold was obtained using a permutation test (Churchill and Doerge, 1994) of 5000 repetitions. The *F* ratio threshold was regarded as the value corresponding to the empirical 0.1% chromosome-wise significance level, which was given as 12.87.

2.3. Cloning the porcine AUTS2 gene

To clone the porcine *AUTS2* gene, total RNA was isolated from a Duroc and Meishan pig ovary with Trizol (Invitrogen, Carlsbad, CA, USA) according to the protocol provided by the manufacturer. Then, 15 μg of total RNA was used as a template for first-strand cDNA synthesis and primed for reverse transcription (RT) using oligo$(dT)_{12–18}$ primer and random hexamers. First-strand buffer (1×), dNTPs (0.5 mM), dithiothreitol (10 mM), and superscript II reverse transcriptase (400 U; Invitrogen) were added to a final volume of 50 μL, and the samples were incubated at 42 °C for 50 min. The samples were then heated to 70 °C for 15 min, and a 2-μL aliquot of the resulting product was used as a template for polymerase chain reaction (PCR) as described below.

RT-PCR involving 3 primer pairs designed from the human *AUTS2* sequence (GenBank accession no. NM_015570.1) were used to amplify porcine *AUTS2* cDNA. The sequences of the PCR primers were as follows:

pA1, between 5′UTR and exon 9 amplicon of 1.7 kb.
Forward 5′-tgaaggggagggagggctcggtgtcaatttttttt-3′ and
Reverse 5′-tgctggtgctggtggaactcggtccgcaggtaggg-3′;
pA2, between exon 9 and 15 amplicon of 0.7 kb.
Forward 5′-agcaagacatcttgcgacag-3′ and
Reverse 5′-ttcaggccaaagtccagctt-3′;
pA3, between exon 15 and 3′UTR of 1.8 kb.
Forward 5′-aaacagatgcagtcagaccca-3′ and
Reverse 5′-ctgctcttgcgctttcgaga-3′.

Each PCR reaction (total volume, 50 μL) contained 2 μL of first-strand cDNA, 15 pmol of each primer, 0.4 mM of each dNTP, 1× PCR buffer, and 1.0 U KOD FX (ToYoBo, Osaka, Japan). Reaction conditions were 94 °C for 2 min; 35 cycles at 98 °C for 10 s and 68 °C for 2 min; and 68 °C for 5 min. The amplified product was purified with kit