Appendix S1 This annotation presents putative human orthologues to porcine sequences incorporated in the Affymetrix Porcine genome array. The basis and bit score of each entry is provided to allow the choice of an annotation confidence threshold appropriate to the intended application.

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Sequence variation and evaluation of *inhibin α-subunit* and *steroidogenic acute regulatory protein* for reproductive traits in swine

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Source/description: Ovulation rate is an important trait influencing litter size in swine and contributes to reproductive success in pig production. Several quantitative trait loci (QTL) for ovulation rate have been identified in the pig. One region on chromosome 15 was associated with ovulation rate in a Meishan cross and in lines selected for components of litter size.[1–2] The porcine *inhibin α-subunit* (*INHA*) and *steroidogenic acute regulatory protein* (*STAR*) genes were previously mapped to this region by polymerase chain reaction-restriction fragment length polymorphism.[3–4] Inhibin α-subunit is a gonadal glycoprotein that binds to the inhibin βA and βB subunits to form heterodimer complexes known as inhibin A or inhibin B respectively; it also regulates the secretion of pituitary follicle stimulating hormone (FSH).[5] Plasma inhibin is correlated with higher ovulation rates in pigs.[6] The transportation of cholesterol from the outer to the inner mitochondrial membrane is the rate-limiting step for steroidogenesis,[7] is conducted by STAR in testis, ovary and adrenal[8] and is regulated by FSH and insulin-like growth factor 1 (IGF1).[9] Therefore, we considered *INHA* and *STAR* as positional candidates for this QTL and evaluated their sequence for potential causative genetic variation.

Polymorphism detection: Primers were designed in exons to amplify fragments of the entire *INHA* and *STAR* genes based on porcine cDNA sequences (X03265 and U53020[8] respectively) and sequences generated from the current study (Table S1). The gene organization of pig *INHA* was similar to human and contained an intron of 966 bp (DQ356013). Six SNPs were identified in the gene (Fig. 1a). The complete *STAR* gene was amplified using genomic DNA from the USMARC reference population[10] and consists of seven exons and six introns spanning approximately 9 kb (Fig. 1b). The genomic organization of *STAR* was similar to human except for intron 5, which was much larger in the pig than human (1500 vs. 644 bp respectively). A total of 21 SNPs in the *STAR* gene were identified in the eight parents of the MARC swine mapping family. Two contiguous SNPs (G>C and G>T) in exon 4 of *STAR* (bases 498 and 499 of U53020 respectively) were found, where the G>T polymorphism changed amino acid 126 from valine to leucine (V126L). Valine at this position is conserved in human, cattle, sheep, horse and mouse, while rat has leucine at this position.

SNP genotyping and linkage mapping: Assays were designed to genotype the *INHA* polymorphisms at nucleotides X03265:c.572G>A and X03265:c.1172G>A, as well as at the *STAR* polymorphism U53020:c.499G>T, using primer exten-

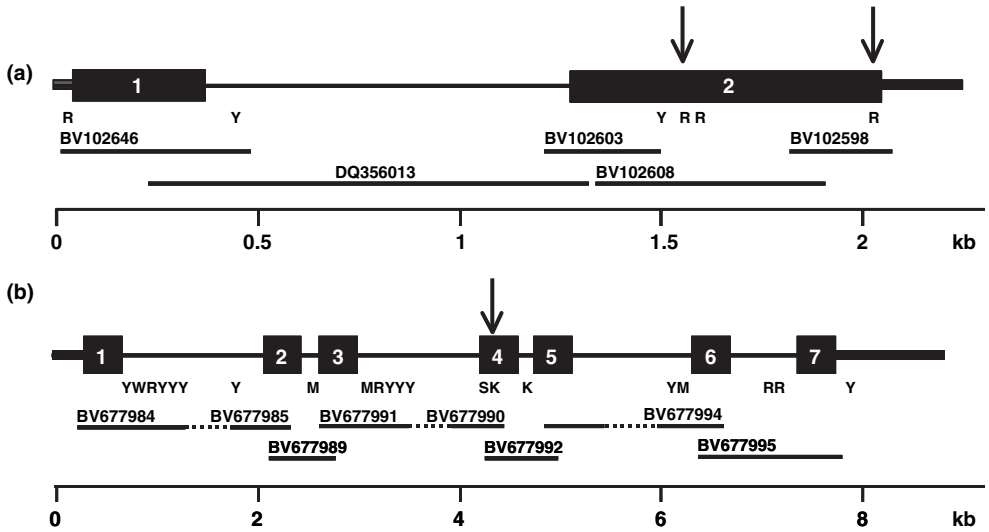

Figure 1 Genomic organization of the porcine *inhibin α-subunit* (*INHA*) (a) and *steroidogenic acute regulatory protein* (*STAR*) (b) genes. Exons are numbered as shown in boxes, and single nucleotide polymorphisms are identified in IUB code below the diagram. Arrows show the positions of the G>A polymorphisms in INHA (bases 572 and 1127 of X03265) and the G>T polymorphism (base 499 of U53020) in STAR, which were genotyped. The sequenced fragments are shown with GenBank accession numbers, and dotted lines indicate regions where sequences were not determined. Approximate sizes (in Kb) are shown below.

sion assays on the Sequenom MassArray™ system (San Diego, CA, USA). Analysis of the USMARC swine mapping family resulted in a map assignment of *INHA* to chromosome 15 at position 88 cM and generated 67 informative meioses in seven families (86 progeny). The *STAR* marker generated 43 informative meioses and mapped to chromosome 15 at position 61 cM, the same position as microsatellite marker *SW1401* on the MARC swine linkage map (http://www.marc.usda.gov/). The *T* allele at nucleotide 499 was found only in Meishan with a frequency of 0.8.

Association of SNPs with ovulation rate or age of puberty: One-quarter of Meishan F_8 gilts ($n = 150$) that were descendants of the MARC resource population[1] with age at puberty and ovulation rate data were genotyped for the *INHA* 572G>A and *STAR* 499G>T polymorphisms. Data were analysed using the GLM procedure of SAS in which sire and maternal grandsire were fitted as fixed effects for age at puberty. The model for ovulation rate also included a fixed effect for method of measuring ovulation rate (laparoscopy versus slaughtered). The frequencies of the rare alleles were 0.32 (X03265:c.572G>A of *INHA*) and 0.19 (U53020:c.499G>T of *STAR*) respectively. The *INHA* and *STAR* genotypes were not associated with ovulation rate ($P = 0.84$ and $P = 0.95$ respectively) or age at puberty ($P = 0.32$ and $P = 0.75$ respectively) in this data set. Due to a lack of association of these single nucleotide polymorphism (SNPs) with ovulation rate, we suggest that the linkage disequilibrium between the causative mutation and these genes has been disrupted by recombination in this population, and the gene responsible for the QTL effects on porcine chromosome 15 probably resides some distance away from *INHA* and *STAR*.

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Supplementary Material

This following supplementary material is available as part of the online article from http://www.blackwell-synergy.com:
Table S1 PCR primers for STSs in porcine *INHA* and *STAR*.

OnlineOpen: This article is available free online at www.blackwell-synergy.com

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Polymorphisms of the *prion protein* gene (*PRNP*) in Alaskan moose (*Alces alces gigas*)

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Source/description: The *prion protein* (*PRNP*) gene of mammals encodes a prion protein (PrP), which is expressed in many tissues including the brain. Misfolded PrP conformers are responsible for neurodegenerative diseases known as spongiform encephalopathies. Transmissible spongiform encephalopathies (TSEs) include bovine spongiform encephalopathy, ovine scrapie, human Creuzfeldt–Jakob disease and chronic wasting disease (CWD)[1,2] in mule deer (*Odocoileus hemionus*), white-tailed deer (*Odocoileus virginianus*) and Rocky Mountain elk (*Cervus elaphus*). First found in Colorado, CWD has now been identified in the eastern USA, as far south as New Mexico and as far north as west-central Canada.[3]

Polymorphisms of *PRNP* appear to be linked to susceptibility to TSE in numerous species including free-ranging white-tailed deer[4] and mule deer.[5] In mule deer, the *SS* genotype at residue 225 is associated with a higher incidence of CWD.[5] Differences in PrP amino acid sequence are believed to be species barriers to disease transmission.[6] However, Wyoming moose sequences that were previously deposited in GenBank (AY225484 and AY225485) are similar to the sequence of *Odocoileus*. CWD has not been observed in Rocky Mountain moose (*Alces alces shirasi*) or in caribou at higher latitudes (*Rangifer tarandus*), yet both species overlap the geographical range of *Odocoileus* species. We report here the *PRNP* sequences for 44 Alaskan moose (*Alces alces gigas*).

Polymerase chain reaction conditions and sequence analysis: Genomic DNA was purified from blood samples of 44 moose (*Alces alces gigas*) that were sampled from eight locations across Alaska (Fig. S1). DNA purification protocols, primers, amplification conditions and sequence analysis methods are provided in Appendix S1.

Polymorphisms: Two unique sequences (i.e. alleles) were found in the sequences of 44 individual moose (DQ154297 and DQ154298); these differed only at codon 209. The allele encoding methionine was present with a frequency of 0.45, and the allele encoding isoleucine was present with a frequency of 0.55. The diploid genotypes did not depart significantly from Hardy–Weinberg predictions ($\chi^2 = 0.4$, $P < 0.01$).

Comments: The conservation of amino acid sequences in the PrP of moose, caribou and deer is striking (Table 1) and consistent with the fact that all three genera are in the subfamily