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Mapping of 11 genes by FISH to BTA2, BBU2q, OAR2q and CHI2, and comparison with HSA2q

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Description/source: Comparative maps provide insight into the evolution of closely related species and are useful for studying the evolution of chromosome breakpoints and for identifying gene orthologues.[1] Assignment of loci to metaphase chromosomes using fluorescence *in situ* hybridization (FISH) results in low-resolution maps but allows anchoring of linkage and radiation hybrid (RH) maps to specific chromosome regions. Herein, we report the FISH localization of 11 type I loci to BTA2. Nine of these loci were mapped by FISH to R-banded chromosomes of river buffalo (BBU2q), sheep (OAR2q) and goat (CHI2), all of which are evolutionary homologues of BTA2. Six and seven of these loci were mapped for the first time to specific chromosomal regions of BTA2 and CHI2 respectively. Furthermore, eight of the genes are candidates for quantitative trait loci (QTL) influencing growth traits, based in part on an examination of the human–bovine comparative map.[1] A direct comparison between the cytogenetic maps of BTA2 and HSA2q revealed at least nine conserved chromosomal segments that arose from complex chromosomal rearrangements.

BAC library screening and selection of candidate genes: Primers used to screen genes from the bovine and ovine BAC libraries[2–4] are listed in Table S1. DNA sequencing of polymerase chain reaction (PCR) products amplified from the BAC clones verified the presence of each gene with at least 90% nucleotide identity to the corresponding human sequence. Because growth-related QTL have been previously identified on BTA2,[5] candidate genes for QTL (*GDF8*, *GCG*, *NEB*, *TTN*, *IGFBP2 MYL1*, *FN1* and *ACADL*) were selected from the corresponding region of the long arm of human chromosome 2.

Cell cultures and FISH analysis: Fibroblast cell cultures for cattle and peripheral blood cultures for river buffalo, sheep and goat were established. Cultures were treated for early (cattle) or late (river buffalo, sheep and goat) BrdU incorporation to obtain G/Q and R-banding patterns respectively. Meiotic preparations were made from a bull testicle collected at the time of slaughter. Single- or two-colour FISH was used to map the BAC clones to bovine mitotic or pachytene chromosomes, respectively, while single-colour FISH was used for river buffalo, sheep and goat. At least 20 metaphases were studied for all probes and species.

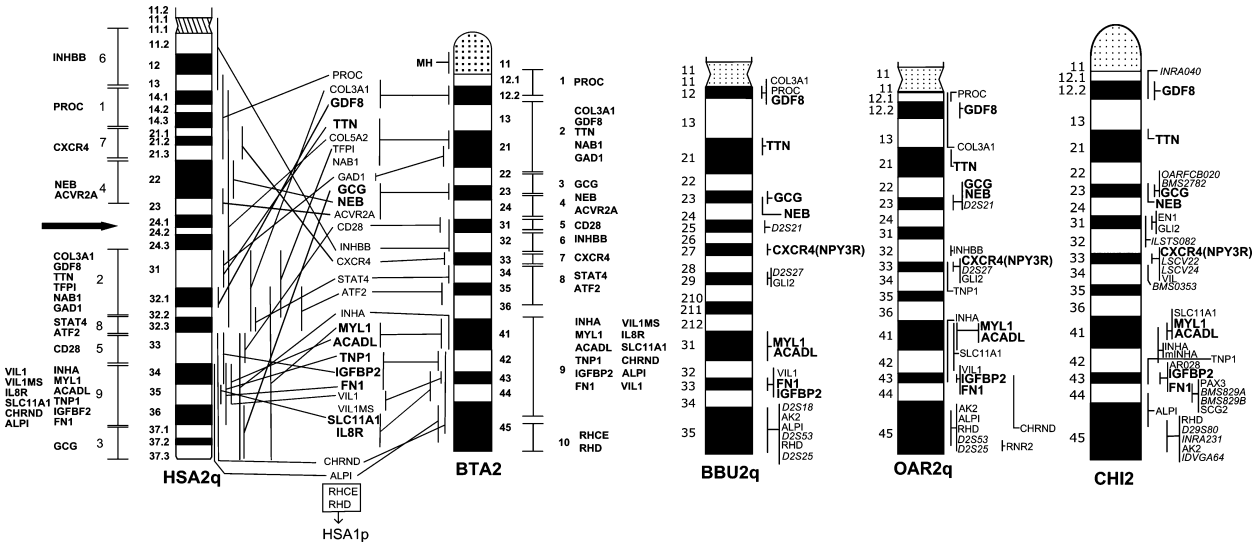

Figure 1 Comparative cytogenetic maps of BTA2, BBU2q, OAR2q and CHI2, as well as a comparison between the BTA2 and HSA2q cytogenetic maps. Type I and type II loci are reported in normal and italic characters respectively. Loci mapped in the present study are reported in large, bold characters. The remaining loci are from BOVMAP (http://locus.jouy.inra.fr/cgi-bin/bovmap/intro2.pl), GOATMAP (http://locus.jouy.inra.fr/cgi-bin/lgbc/mapping/common/intro2.pl?BASE= goat), and SheepBase (http://www.thearkdb.org/browser?species=sheep), as well as from earlier publications.[8–11] To facilitate the comparison of locus order and to visualize conserved syntenies between BTA2 and HSA2q, conserved chromosomal segments between the two species are numbered on the left (HSA2q) and right (BTA2) sides of the banded ideograms. Ten conserved syntenies were identified on BTA2 (nine from HSA2q and one from HSA1p). No loci on HSA2q24.1–q24.3 (arrow) have been mapped on BTA2 until now.

Chromosome identification and banding followed the latest standard nomenclatures.[6,7]

Clones containing QTL candidate genes (*GDF8*, *GCG*, *NEB*, *TTN*, *IGFBP2*, *MYL1*, *FN1* and *ACADL*) and three additional genes (*IL8RA*, *SLC11A1* and *CXCR4*) were assigned to BTA2 by both single and dual-colour FISH. Nine of these loci (*GDF8*, *TTN*, *GCG*, *NEB*, *CXCR4*, *MYL1*, *ACADL*, *FN1* and *IGFBP2*) were subsequently mapped on BBU2, OAR2 and CHI2 (Fig. 1, Table S1). Five loci (*GDF8*, *TTN*, *GCG*, *NEB* and *SLC11A1*), which were previously mapped by FISH to BTA2 (BOVMAP, http://locus.jouy.inra.fr/cgi-bin/bovmap/intro2.pl), were more precisely assigned in this study. Four loci (*GCG*, *GDF8*, *NEB* and *SLC11A1*), which were previously assigned to CHI2 (GOAT-MAP, http://locus.jouy.inra.fr/cgi-bin/lgbc/mapping/common/intro2.pl?BASE=goat), were assigned to alternative locations on CHI2 in this study.

The locus order on BTA2 was centromere-*GDF8-TTN–GCG–NEB-CXCR4*-(*MYL1*, *ACADL*)–(*FN1-IGFBP2*)-(*IL8RA*, *SLC11A1*) (Fig. 1). These results anchor scaffolds 8348 and 77611, which harbour *MYL1*, and correct the March, 2005 bovine sequence assembly (http://genome.ucsc.edu), which erroneously assigned *CXCR4* to BTA22. Loci were located on homologous chromosome bands among the four bovids, confirming the high degree of banding homologies among these species. These cytogenetic maps allow detailed comparisons not only among bovids but also between bovid and human chromosomes. Indeed, a comparison of the BTA2 and HSA2q comparative map (Fig. 1) revealed at least nine conserved chromosome segments in addition to the conserved synteny involving the telomere of BTA2 and HSA1p.[8] These data are consistent with the RH map, although comparative cow–human RH maps reveal only five conserved syntenies between BTA2 and HSA2q (four syntenic segments) and HSA1p (one syntenic segment).[1]

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Supplementary Material

The following supplementary material is available online at http://www.blackwell-synergy.com:

Table S1 Information for type I loci mapped by FISH to BTA2 (11 loci), CHI2/OAR2 and BBU2 (nine loci).

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Analysis of the *MC1R*, *KIT* and *ASIP* loci in Chinese and European pigs

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Source/description: In this study, three coat colour candidate genes (*KIT*, *MC1R* and *ASIP*) were compared in Chinese and European pig breeds. A total of 158 unrelated individuals from five Chinese native pig breeds and four European pig breeds with characterized coat colour phenotypes[1,2] were included in the study (Table 1).

Polymorphism analysis: Primer pairs for *MC1R*,[3,4] *ASIP*[5] and *KIT*[6,7] are presented in Table S1. Polymerase chain reaction (PCR) products were genotyped by single-strand conformational polymorphism (SSCP)[8] with the exception of the *KIT* intron 17 polymorphism, which was genotyped by restriction fragment length polymorphism (RFLP) using the *Nla*III restriction enzyme[6] (New England Biolabs, Inc., Beverly, MA, USA). The wild-type allele was characterized by 130- and 45-bp fragments, whereas the mutant allele contained 130-, 80-, 50- and 45-bp fragments. One or two individuals of each homozygous class were randomly selected and sequenced at least twice after cloning their amplicons into vectors.

Allele frequencies: The allele frequencies for each variant identified in the three genes are shown in Table 1 (AY308992–AY308994, AY308996–AY308998 and AY916519–AY916524). A deletion mutation (AGTT) in intron 18 of *KIT* and a substitution mutation (G>A) in intron 17 of *KIT* were found only in Yorkshire and Landrace pigs, both showing dominant white colour. SSCP analysis of these pigs indicated that the duplication of *KIT*[6,7] was present with dominant white coat colour. Genotype frequencies were determined using the quantitative PCR-RFLP test described by Marklund *et al.*,[6] and the results are shown in Table 1. In the *MC1R* gene, the synonymous variant c.730G>A was polymorphic in different Chinese pig breeds, and the seven other allelic variants had allele frequencies of 1.0. The 67_68dupCC allele was found in all Yorkshire, Landrace and Pietrain pigs. Variants c.284G>A, c.309T>C and c.364T>C were found in all Chinese native pigs with allele frequencies of 1.0. Variants c.492C>T and c.728G>A were found in all Duroc pigs. For the *ASIP* gene, c.−61G>A and c.−59T>G were detected in the 5′-untranslated region of exon 1; c.−61G>A had a frequency of 0.93 in Jinhua pigs; and c.−59T>G had a frequency of 0.09 in Wuzhishan pigs. A missense mutation (c.165G>A) was found in several Landrace and Duroc pigs, with frequencies of 0.07 and 0.37 respectively.

Comments: In this study, mutations related to coat colour formation were examined in Chinese and European pigs.