A TARGETED COMPARATIVE MAP OF THE REGION SURROUNDING THE BOVINE SPINAL DYSMYELINATION LOCUS

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INTRODUCTION

The identification of unknown trait genes in livestock has hitherto often relied on the candidate gene approach and on the positional candidate gene approach, in which information on the position of the gene locus is combined with comparative maps primarily between the livestock species and human. In a number of cases this approach has proved efficient when genes with a biological function matching the phenotype of interest could be identified. However, for traits without a clear human/mouse counterpart mapped to the region of interest, the success relies on the resolution of the comparative maps. For cattle a step towards a high-resolution comparative map was taken with the cattle-human whole genome comparative radiation hybrid map (Band *et al*., 2000). This map showed what was invisible in the chromosome paints previously used to define blocks of conserved synteny between human and cattle (e.g. Hayes, 1995), namely numerous intra-chromosomal rearrangements.

The locus for the recessively inherited neurological disorder bovine spinal dysmyelination (BSD) was recently mapped to bovine chromosome 11 (BTA11) flanked by microsatellite markers *BP38* and *BMS1953* (Nissen *et al*., 2001). From the whole genome comparative radiation hybrid (RH) map by Band and colleagues (2000) the block of conserved synteny between BTA11 and human chromosome 2 (HSA2) previously demonstrated by chromosome paints is verified. However, specifically the proximal part of BTA11 seems to be rearranged intensively as compared to HSA2, thus making it difficult to point out positional candidate genes for BSD based on comparative map information. The work presented here takes a step towards a comparative map targeted to the region containing the *BSD* gene.

MATERIALS AND METHODS

BAC screening and sequencing. BAC clones were isolated from the RPCI-42 BAC library using a pooling scheme and subsequent screening with PCR. Two clones were isolated with *BMS1953* primers and four clones using *BP38* primers. Primer sequences were obtained from the USDA MARC database (http://www.marc.usda.gov/). PCR conditions for these primers were as described previously (Nissen *et al*., 2001). BAC DNA was extracted with the Qiagen large construct kit as recommended by the manufacturer. DNA from individual BAC clones was subjected to cyclesequencing with T7, SP6 and the reverse primer of the microsatellite marker, using BigDye terminator chemistry (PE Applied Biosystems). Sequencing reactions were precipitated and redissolved in 3 µl formamide loading buffer (80% formamide, 20% loading buffer). The reactions were denatured for 3 minutes and 1µl were separated on Long Ranger Singel pack gels (Biowhittaker Molecular Applications) on an ABI 377 sequencer (PE Applied Biosystems).

Subcloning. Four BAC clones isolated using *BP38* primers were pooled and digested with MspI. After 2 hours incubation at 37°C the reactions were separated on a 1% agarose gel and fragments ranging from 1-5kb were cut out and purified with the Qiaquick kit (Qiagen). The purified DNA was cloned in the ClaI site of pTrueBlue (GenomicsOne) and transformed in *E.Coli* XLI-blue. After overnight incubation at 37°C on IPTG/X-Gal containing LB plates, 96 recombinant clones were inoculated in 2ml LB/ampicillin. Plasmid was prepared using 96 well MultiScreen system (Millipore) and sequenced with the T7 primer as described above.

Sequence analysis. Sequences were searched for similarity to the draft human genome sequence with BLASTn (Altschul *et al*., 1997) through the ENSEMBL server (http://www.ensembl.org/perl/blastview).

RH mapping of candidate genes. For each candidate gene the public EST database (http://www3.ncbi.nlm.nih.gov/BLAST/) under NCBI was searched with the corresponding human mRNA sequence using BLAST. In some cases, if more than one significant hit of bovine EST sequence was obtained, sequences were assembled using the fragment assembly system of the GCG package (Genetics Computer Group). ESTs or consensus sequences were aligned against the draft human genome sequence to investigate the intron-exon structure, and primers were designed to avoid potentially large introns, assuming that the intron-exon structure and size is similar in cattle and human. An *HNK-1ST* cDNA (clone 243G13) was isolated from a bovine brain cDNA library. The clone was partially sequenced and primers were designed from this sequence. In a few cases the PCR products were sequenced to verify the identity of the PCR product. Each candidate gene was mapped in a commercially available radiation hybrid panel (Research Genetics). Furthermore, microsatellites *BP38*, *BMS2569*, *BMS1953* and *BM2818* (Kappes *et al*., 1997) were mapped in the panel.

RH map construction. Construction of the radiation hybrid map was done with the RHMAP package. Initially two point LOD scores were calculated using RH2PT. Markers producing a single linkage group under a LOD score criterion of 6 were used subsequently to construct the RH map. The best order of markers was calculated using the branch and bound option of RHMINBRK, and finally the map was constructed with RHMAXLIK using an equal retention model.

RESULTS AND DISCUSSION

In order to produce a targeted comparative map of the region containing the *BSD* gene, BAC clones was isolated with the microsatellite markers *BP38* and *BMS1953*. BLAST analysis of the BAC sequences against the draft human genome sequence resulted in significant hits on chromosome 2 (Table 1). Sequence from BAC 410J6 produced two hits on HSA2. Both hits aligned to ENSEMBL contig AC067950 positioned at approximately 75.75 megabases (Mb). This position corresponds to band p13.1. A hit with a sequence from BAC 164F21 gave a BLAST hit to ENSEMBL contig AC068695 positioned at approximately 34.4 Mb, corresponding to band p22.3 on HSA2. Since the latter hit gave a lower probability than the first and was located more than 40 Mb from the BAC 410J6 hit, *BP38* positive BACs were subcloned and sequenced at random. One BLAST hit aligned to ENSEMBL contig AC009499

neighbouring AC068695 with a P value of $6.8e^{-40}$ (table 1), supporting the hit from BAC 164F21.

Table 1. Data from BLAST similarity searches against the draft human genome sequence of sequences from BAC clones

Clone (microsatellite)	Identities[A]	Probability	Ensembl location contig/(Mb)	Cytogenetic location
410J6 (*BMS1953*)	143/181 + 192/272	P=4.5e-37 + P=4.5e-37	AC067950 (72.75Mb)	2p13.1
164F21 (*BP38*)	173/247	P=8.5e-14	AC068695 (34.4Mb)	2p22.3
subclone85 (*BP38*)	303/400	P=6.8e-40	AC009499 (34.51Mb)	2p22.3

[A]Number of identities/number of bases in hit

To verify the alignment results, we screened an RH panel and constructed a map with four microsatellite markers covering a total of approximately 9 cM (Kappes *et al*., 1997) and seven genes. Four microsatellite markers from the *BSD* critical interval (Nissen *et al*., 2001) were mapped in the RH panel. Four genes were picked from the draft human genome sequence based on the position of the BLAST hits from the BAC sequences. Seven genes were chosen from HSA2 based on known or inferred biological function.
Under the most stringent LOD score criterion (LOD>8) a linkage group consisting of *BP38*, *BMS1953*, *BMS2569*, *EGR4*, *SPG4*, *HSA242910* and *DYSF* was produced. Lowering the LOD score criterion to LOD>6 expanded the linkage group with *BM2818*, *CRIM1*, *MAL* and *HNK-1ST*. In figure 1 the radiation hybrid map based on multipoint mapping of the LOD>6 linkage group is compared with a map derived from the draft human genome sequence. This map supports the findings from the BLAST similarity searches using BAC sequences, and suggests that the interval between *BMS1953* and *BP38* in the bovine genome harbour an evolutionary breakpoint. Thus, this linkage group is divided in three blocks of conserved synteny on human chromosome 2. The block containing *EGR4*, *DYSF* and *HSA242910* has been inverted in the bovine compared to the human genome.

CONCLUSION

The results presented here demonstrates a new intra-chromosomal rearrangement between HSA2 and BTA11 occurring in the *BSD* critical region, and three genes are mapped within the *BSD* interval between *BP38* and *BMS1953*. The study indicates that a combination of partial sequencing of BAC clones similarity searches against the human genome sequence and finally radiation hybrid mapping of putative orthologs, is a powerful method to target a comparative map to a specific region. These data will serve as a prerequisite for the identification of the causative mutation for bovine spinal dysmyelination.

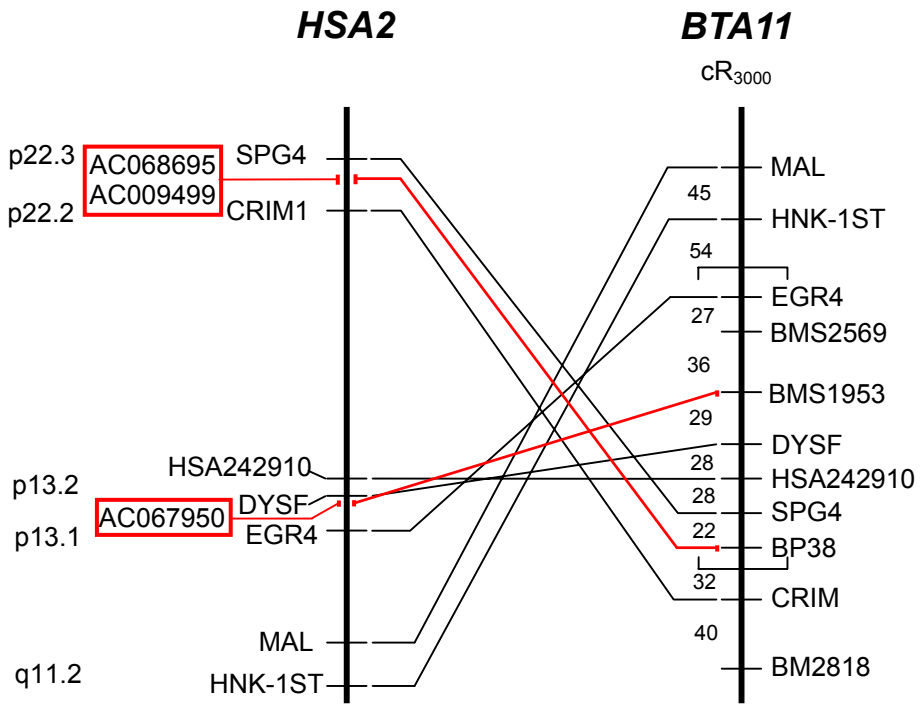

Figure 1. Radiation hybrid map of the proximal region of BTA11, compared to the map positions of the corresponding human genes, derived from the draft human genome sequence. The BLAST hits obtained from the similarity search with the BAC sequences against the draft human genome sequence is indicated with red lines

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