Genet. Sel. Evol. 34 (2002) 255–267

DOI: 10.1051/gse:2002007

Original article

A higher resolution radiation hybrid map of bovine chromosome 13

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(Received 25 June 2001; accepted 7 December 2001)

Abstract – In this paper, we present a radiation hybrid framework map of BTA13 composed of nine microsatellite loci, six genes and one EST. The map has been developed using a recently constructed 12′000 rad bovine-hamster whole-genome radiation hybrid panel. Moreover, we present a comprehensive map of BTA13 comprising 72 loci, of which 45 are microsatellites, 20 are genes and seven are ESTs. The map has an estimated length of 2694.7 $cR_{12'000}$. The proposed order is in general agreement with published maps of BTA13. Our results only partially support previously published information of five blocks of conserved gene order between cattle and man. We found no evidence for the existence of an HSA20 homologous segment of coding DNA on BTA13 located centromeric of a confirmed HSA10 homologous region. The present map increases the marker density and the marker resolution on BTA13 and enables further insight into the evolutionary development of the chromosome as compared to man.

bovine chromosome 13 / radiation hybrid / gene mapping / 12′000 rad / comparative mapping

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1. INTRODUCTION

Whole-genome radiation hybrid (WG-RH) mapping (*e.g.* [24]) has become the method of choice for the merging of type I markers such as conserved genes or expressed sequence tags (ESTs) with highly polymorphic type II markers (*i.e.* microsatellites) from recombination based marker maps. Womack and coworkers [25] reported a bovine WG-RH panel, created using 5′000 rad of gamma rays ($RH_{5'000}$). This panel has been extensively used for the construction of single chromosome specific framework maps (*e.g.* [2, 14]) and a map of the entire cattle genome [3].

A total of 99 loci are assigned to BTA13, of which 41 are of type I and 58 are microsatellites[1]. The $RH_{5'000}$ map for BTA13 [18] as well as the whole genome map [3] are limited in the number of markers included. The BTA13 specific $RH_{5'000}$ map [18] includes 27 markers of which 11 are type I loci, while the bovine whole genome RH map contains a total of 37 markers on BTA13, comprising 29 type I and eight type II markers [3]. Thus, although the RH map of Band and coworkers [3] locates a considerable number of genes and ESTs on BTA13, these type I are poorly embedded in a microsatellite framework, whereas in the map by Schläpfer and coworkers [18] the microsatellite framework is sound but the type I content should be improved.

BTA13 is of particular interest, since the bovine prion protein gene (*PRNP*) resides on this chromosome [16]. *PRNP* potentially plays a key role in the development of bovine spongiforme encephalopathy (BSE). The physical position of *PRNP* has been localized on the BTA13 band q17 [19] by fluorescent *in situ* hybridization (FISH).

Bovine/human ZOO-fluorescence *in situ* hybridization (ZOO-FISH) studies have identified homologous chromosome segments between cattle and man (*e.g.* [22]). BTA13 has been reported to be homologous to parts of the human chromosomes 10 and 20 (HSA10 and HSA20), in that BTA13 seems to be composed of an HSA10 segment sandwiched by centromeric and telomeric HSA20 regions. The HSA10 homologous segment on BTA13 is represented by the interleukin 2 receptor alpha (*IL2RA*) and vimentin (*VIM*).

The goal of the present study was to refine the resolution of the BTA13 map using a 12′000 rad bovine-hamster WG-RH panel [15] and to order as many microsatellites and type I markers as possible. This would allow to define the breakpoints between HSA10 and HSA20 and to evaluate and expand previous findings of five blocks of conserved locus orders on BTA13 [18].

2. MATERIALS AND METHODS

The construction and characterization of the 12′000 rad whole genome-radiation hybrid (WG-RH) panel has previously been described [15].

[1] http://locus.jouy.inra.fr/cgi-bin/lgbc/mapping/common/Req_select.operl?BASE=cattle

2.1. Loci, PCR primers and conditions

Forty-five of the 72 markers were microsatellites, 20 were genes and seven ESTs. Where available, published primer sequences and PCR conditions were used. Primer pairs for *GHRH*, *PRMS* and *PRND* were designed *de novo*, using the OLIGO 5.0 program package (National Biosciences, Plymouth, MN, USA). The PCR fragments were 350, 323 and 235 base pairs (bp) long respectively. The sense and antisense primer sequences for *GHRH* were 5′ CTGCCTTCCCAAGCCTCTCA and 5′ AGCTGGCCCAGAACCTTCC, for *PRMS* 5′ ATGTTGGAAAATTGCTGGTG and 5′ CGGTCTGATTTTTGTCATCA and for *PRND* 5′ GGAGTGGAGGGCAATAGGT and 5′ AGTCACAGTGCTTGGTGGAG. For these three markers, a touch-down PCR program [7] was implemented. Standard PCR were carried out in 12 μL containing 2 μL of WG-RH DNA, 2.5 pmol of each primer, 0.25 mM of each dNTP 1× PCR buffer with 1.5 mM $MgCl_2$ (Appligene, Gaithersburg MD, USA) and 0.35 unit *Taq* polymerase (Appligene).

2.2. Genotyping

A set of 180 HAT-resistant WH-RH clones was available for typing. All 72 markers were analyzed in duplicate for the presence or absence of a bovine-specific PCR product. Markers were retyped whenever the results were ambiguous. Generally, typing was performed on 8% denaturing polyacrylamide gels using a LI–COR DNA sequencer model 4200 (LI–COR, Lincoln, NE, USA). For the type I markers *CSNK2A1*, *HCK*, *VIM*, *PLC–II/PLCG1* and *THBD* amplification products were electrophoresed on 2% Sea Kem, HGT agarose (FMC Bio Products, Rockland, ME, USA), ethidium bromide stained and then photographed.

2.3. Statistical analysis and generation of the RH map

Cell lines that retained none of the 72 BTA13 markers tested, were excluded from the subsequent analyses. Only one of the cell lines showing identical retention patterns for all 72 doubly typed loci was included in the analyses. The RHMAP statistical software program package version 3.00 [6, 10] was used for data analysis. First, the RH2PT option was used to estimate marker retention frequencies and to identify markers with identical retention patterns. The same option was used for the two-point analysis with lod score thresholds of 3.0 and 5.0.

The RHMAXLIK option was used for the development of the framework as well as the comprehensive map. Due to the large number of markers, the stepwise loci ordering strategy was implemented. Data were analyzed under the equal retention model with a SAVMAX setting of 9.00. Initially, all loci

were used for the development of the framework map ordered with a relative likelihood of 1000:1 (ADDMIN 3.00). The loci ordered with this level of support were then forced in this order and the program was rerun to add loci with a level of support of 100:1 (ADDMIN 2.00). Again, loci ordered this way were forced into the most likely order. As many as possible additional rounds of adding loci at a given ADDMIN setting and forcing them into the most likely order were performed before decreasing the level of the support. The support level steps applied were 3.00, 2.00, 1.00, 0.50 and 0.00.

In parallel, all markers forming a linkage group of at least three loci at lod score level 5.0 based on the two-point analysis (RH2PT), were ordered with a relative likelihood of 1000:1 (ADDMIN 3.00).

3. RESULTS

Following the removal of uninformative hybrids, a total number of 134 hybrids were used in the analysis. The two genes- oxytocin (*OXT)* and arginine-vasopressin (*AVP*), and the two microsatellite markers, *BMC1222* and *HEL7*, showed identical retention patterns. The average marker retention frequency in the hybrids was 18.8% with the lowest for the *PRMS* microsatellite in the *PRNP* gene (10.4%) and the highest for the EST *BE217542* (32.1%). Retention frequencies for all the loci are given in Figure 2. One hundred and thirty-four unique retention patterns were detected for the 72 loci tested. At lod 5.0, the 72 loci were divided into ten linkage groups (Tab. I).

A framework map consisting of sixteen loci ordered with odds greater than 1000:1 was developed (Fig. 1). The framework map was oriented relatived to the centromere being based on published, FISH derived physical assignments of *PRNP* and the microsatellite *BL42* [19]. The linear locus order of the framework map is: centromere – *BE217508* – *URB058* – *BMS1669* – *PRNP* – *PRMS* – *CHGB* – *BMS9248* – *ETH7* – *BL42* – *PCK1* – *BMS1226* – *RM327* – *CSNK2A1* – *HCK* – *BMS1784* – *AHCY*.

Figure 1. Comprehensive radiation hybrid map of BTA13 comprising 72 loci. Type I loci are in italics. Two-point analysis of all loci, calculated with the option RH2PT of the RHMAP program package defined ten linkage groups at lod score 5.0. The loci of linkage groups 1, 3, 4, 5, 6, 7, 8 and 9 were ordered within the respective linkage group (middle axis). The level of support for the orders of selected markers within the linkage groups are indicated at the far right side. Markers that connect ordered linkage groups protrude on the right side of the middle axis. Loci ordered within the linkage groups with lower levels of support stick out on the left side of the middle axis. The markers are color coded for the over all lod scores of the comprehensive map. Loci in red are ordered with lod $\geq$ 3.0, loci in blue are ordered with lod $\geq$ 2.0, loci in green are ordered with lod $\geq$ 1.0, loci in pink are ordered with lod $\geq$ 0.5 and loci in black are ordered with lod 0.0.

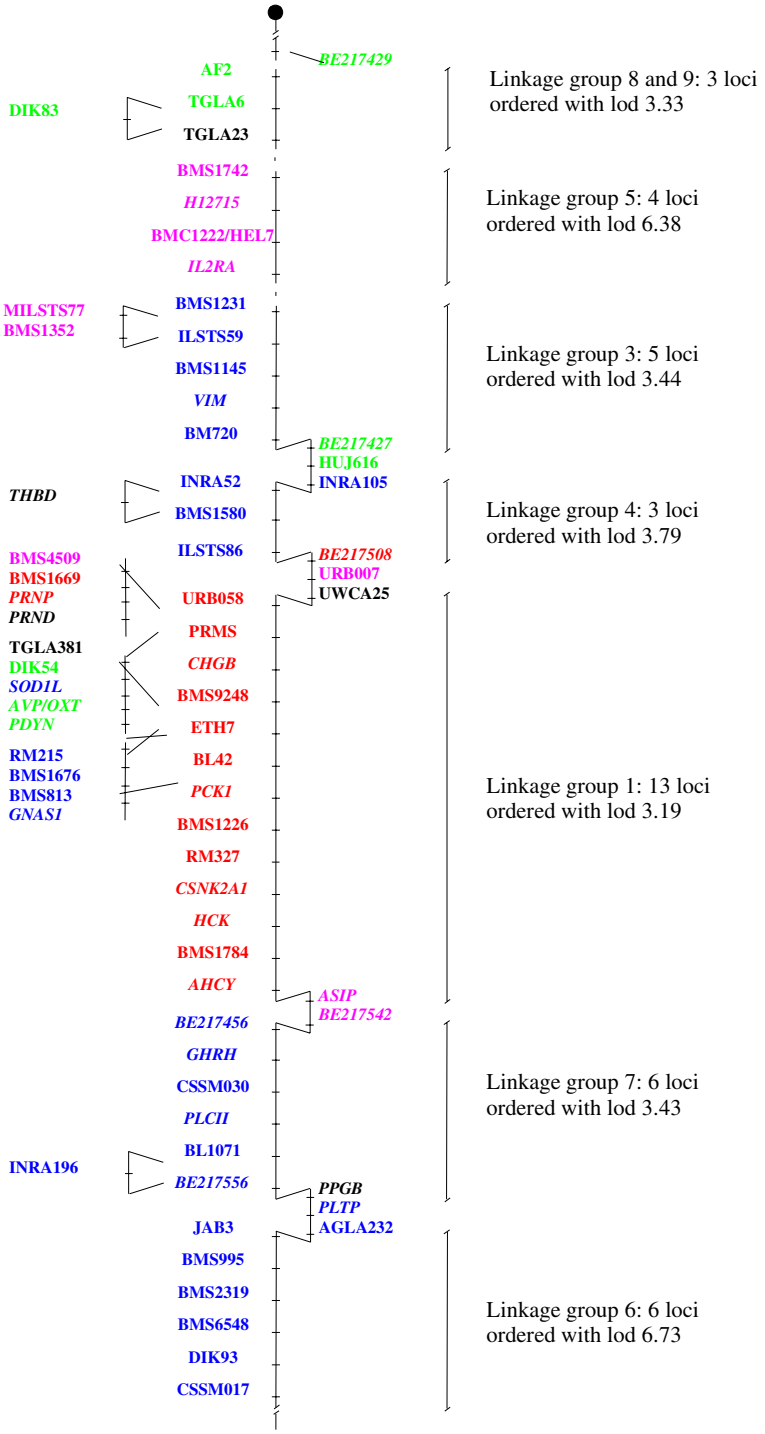
BE217429
AF2
DIK83
TGLA6
TGLA23
Linkage group 8 and 9: 3 loci ordered with lod 3.33
BMS1742
H12715
BMC1222/HEL7
IL2RA
Linkage group 5: 4 loci ordered with lod 6.38
MILSTS77
BMS1352
BMS1231
ILSTS59
BMS1145
VIM
BM720
Linkage group 3: 5 loci ordered with lod 3.44
BE217427
HUJ616
INRA105
THBD
INRA52
BMS1580
Linkage group 4: 3 loci ordered with lod 3.79
ILSTS86
BMS4509
BMS1669
PRNP
PRND
BE217508
URB007
UWCA25
URB058
TGLA381
DIK54
SOD1L
AVP/OXT
PDYN
PRMS
CHGB
BMS9248
ETH7
RM215
BMS1676
BMS813
GNAS1
BL42
PCK1
BMS1226
RM327
CSNK2A1
HCK
BMS1784
AHCY
Linkage group 1: 13 loci ordered with lod 3.19
ASIP
BE217542
BE217456
GHRH
CSSM030
PLCII
BL1071
INRA196
BE217556
Linkage group 7: 6 loci ordered with lod 3.43
PPGB
PLTP
AGLA232
JAB3
BMS995
BMS2319
BMS6548
DIK93
CSSM017
Linkage group 6: 6 loci ordered with lod 6.73

Table I. Linkage groups and their loci at lod score level 5.0 derived from two point analysis.

Linkage group	Lod score 5.0
1	*ETH7*, *BL42*, *TGLA381*, *BM9248*, *UWCA25*, *PRMS*, *BMS1669*, *BM4509*, *PRNP*, *BMS1676*, *BMS1226*, *BMS813*, *RM215*, *RM327*, *URB007*, *AHCY*, *ASIP*, *PDYN*, *BMS1784*, *CHGB*, *DIK54*, *GNAS1*, *OXT/AVP*, *SOD1L*, *URB058*, *PCK1*, *CSNK2A1*, *HCK*, *BE217542*, *PRND*
2	*HUJ616*, *INRA05*
3	*MILSTS77*, *BM720*, *BMS1352*, *BMS1145*, *BMS1231*, *ILSTS59*, *VIM*, *BE217427*
4	*BMS1580*, *ILSTS86*, *INRA52*, *THBD*, *BE217508*
5	*BMC122/HEL7*, *BMS1742*, *IL2RA*, *AF213840/H12715*
6	*JAB3*, *AGLA232*, *BMS6548*, *PLTP*, *PPGB*, *BMS2319*, *BMS995*, *CSSM17*, *DIK93*
7	*CSSM30*, *BL1071*, *INRA196*, *GHRH*, *PLC-II*, *BE217456*, *BE217556*
8	*AF2*, *TGLA6*
9	*DIK83*, *TGLA23*
10	*BE217429*

In a parallel approach, the markers in the linkage groups consisting of more than three loci at lod 5.0 were ordered separately. The microsatellites *DIK83* and *TGLA23* as well as *AF2* and *TGLA6* fell into separate linkage groups at a lod 5.0 (Tab. I), but merged into one at a lod 3.0 (data not shown). The ordering of markers within linkage groups led to seven subsets of loci, each ordered with odds greater than 1000:1 (Fig. 1) and thus each representing a framework map of the linkage group.

The most likely comprehensive $RH_{12'000}$ map, based on two-point analysis and data analysis using the program option RHMAXLIK, is depicted in Figure 1. Figure 2 shows the relative distance of the 70 loci in the comprehensive map indicated in $cR_{12'000}$ as estimated by the multi-point analysis program.

4. DISCUSSION

Due to the identical retention patterns for the two microsatellites *BMC1222* and *HEL7*, as well as the two genes *AVP* and *OXT*, the four loci were treated as two marker pairs (*BMC1222/HEL7* and *AVP/OXT*), reducing the number of loci in the final map to 70. An identical retention pattern of two loci can either be due to the loci being identical or the loci being too close together to resolve their relative position. The typing results of *AVP* and *OXT* are in agreement

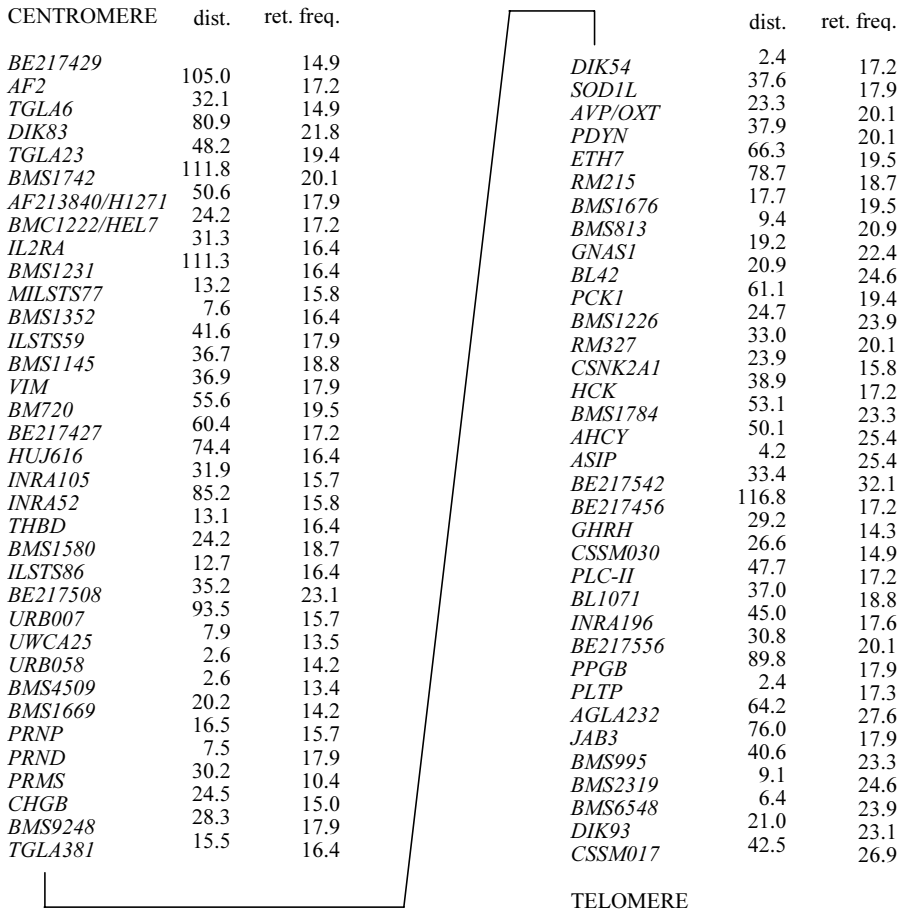

Figure 2. Linear marker order of the comprehensive map. The relative distance between the loci indicated in $cR_{12'000}$ was estimated according to the RHMAXLIK multi-point analysis program option of the RHMAP package. The total map length is 2694.7 $cR_{12'000}$. The program option RH2PT was used to determine the retention frequencies in percent for all loci tested.

with previous findings [18,19], which showed tight linkage of the two genes in cattle. In humans, Sausville and coworkers [17] found *AVP* and *OXT* to be closely linked, and Marini *et al.* [12] found an intergenic sequence of 3.5 kb separating *AVP* and *OXT* in the mouse. Sequence comparisons of database entries of 351 nucleotides of the microsatellite *BMC1222* (EMBL: G19106) and 516 nucleotides of *HEL7* (EMBL: X65210) did not reveal evidence for the identity of the two markers (data not shown). The distance between both marker pairs *AVP/OXT* and *BMC1222/HEL7* is therefore below the resolution of the $RH_{12'000}$ mapping panel.

Rexroad and coworkers [15] found an average retention frequency of 30.6% for 18 BTA1 specific markers tested in 88 $RH_{12'000}$ hybrids, whereas our analyses of 72 BTA13 specific markers in 134 hybrids indicate a lower retention frequency of 18.8%. We selected the 134 RH cell lines in order to reach a maximal number of unique retention patterns for the tested loci. The retention frequency of the 27 markers in the $RH_{5'000}$ panel was 26.4% [18], as compared to 17.8% of the same set of markers in the $RH_{12'000}$ panel. Since the bovine $RH_{12'000}$ panel [15] has not been used for a chromosome wide mapping effort yet, we wanted to type all available hybrids. The reduction to 134 hybrids in the analysis was based on the retention of BTA13 specific markers and the exclusion of identical clones. For practical reasons it would be advantageous to reduce the number of hybrids to 90 to use a single 96-well PCR plate for amplification. Selecting clones based on high retention frequencies would allow to set up such a panel for the use on other bovine chromosomes.

Apart from the *BMS1669* microsatellite, the linear locus order of the 16 framework markers is in agreement with four linkage maps [4,9,20,23], the two $RH_{5'000}$ maps [3,18] as well as findings from physical assignments [8, 19]. Except for EST *BE217508*, all loci that constitute the $RH_{12'000}$ framework map are members of linkage group 1 at a lod score level of 5.0 (Tab. I). Markers in this linkage group are located in the mid-chromosomal region of BTA13. This location is characterized by a relatively high density of markers, that leads to a higher number of informative typing results. This in turn seems to result in an optimal data structure, that facilitates marker ordering as compared to loci in a more telomeric or centromeric position.

In contrast to the $RH_{5'000}$ map [18], where the *GNAS1* locus served as a framework marker between *BL42* and *HCK*, *GNAS1* could not be included in the $RH_{12'000}$ map in this function. Construction of the comprehensive map located *GNAS1* between *ETH7* and *BL42* with odds of $\geq$ 100:1. When using the loci that form linkage group 1, without the microsatellites *ETH7* and *BMS9248*, the order *BL42 – GNAS1 – BMS813 – BMS1676 – RM215* had a lod score of 4.06 (data not shown). It is likely that the typing information of additional markers in this chromosomal region improves the ordering capabilities of a given mapping panel, leading to the revision of previous findings.

The loci within the seven linkage groups, that are ordered with odds greater than 1000:1 (Fig. 1) show no discrepancy to the orders of framework markers found in other marker maps. However, the strong support of a given locus order does not allow for the prediction of the orientation of a set of markers relative to the chromosomal endpoints.

In the comprehensive $RH_{12'000}$ map, several mostly minor discrepancies to some of the other published maps are apparent (Fig. 1). In most cases the orientation of groups of two or three markers are inverted relative to the chromosomal endpoints.

Our study has produced the most extensive marker map of BTA13 to date, ordering 72 loci and covering 2694.7 $cR_{12'000}$ of the chromosome. The present map is approximately four times larger than the BTA13 map produced by the RH_{5000} panel [18]. Inflation of the map length is likely due to the adding of markers, since this phenomenon is also known in recombination based linkage maps.

Looking at the $RH_{12'000}$ map in a comparative genetic context provides no evidence for the existence of the postulated region homologous to HSA20 on BTA13, located centromeric to a confirmed HSA10 homologous region. Independent ZOO-FISH experiments (*e.g.* [22]) had suggested such an HSA20 region, but both the RH_{5000} map presented by Schläpfer *et al.* [18] and Band *et al.* [3] failed to confirm these results. The present $RH_{12'000}$ map locates the two ESTs *BE217429* and *AF213840/H12715* centromeric to *IL2RA*. A sequence database search (BLASTN) [1], using the EST *BE217249* and *AF213840/H12715* as query sequences identified human DNA sequences (EMBL accession number AL161799 and AL390294) from clones assigned to HSA 10 as the most likely homologues (data not shown). Considering that the genes which were included in this present map are relatively evenly distributed over both arms of HSA20, and the fact, that the ordering of six newly assigned genes on BTA13 [21] has not identified the postulated HSA20 homologous segment, might point towards a reevaluation of the ZOO-FISH findings. However, the postulated HSA20 homologous segment located close to the centromere on BTA13, could represent an evolutionarily conserved block of non-coding DNA.

The $RH_{5'000}$ map [18] has described five blocks of conserved gene order between cattle and man. The results of the $RH_{12'000}$ mapping effort partially support this proposition. In its revised form, block 1 consists of the HSA10 homologues, *IL2RA* and *VIM*. All loci in the remaining four blocks are HSA20 homologues. Block 2 consists of *THBD*, *PRNP*, *PRND*, *CHGB*, *SOD1L*, *AVP/OXT* and *PDYN*; block 3 contains *GNAS1* and *PCK1*; and block 4 consists solely of *CSNK2A1*. *HCK*, *AHCY*, *ASIP*, *GHRH*, *PLC–II*, *PPGB* and *PLTP* make up block 5. As compared to the findings presented in the $RH_{5'000}$ map [18], blocks 2 and 5 have been significantly extended. At the given marker density, no subdivision of the conserved gene order in block 5 was identified. The situation in block 2 is somewhat more complicated, since internal rearrangements led to a division of the block into four subunits (block 2a-2d). Block 2 is of special interest, since it contains *PRNP*, the microsatellite *PRMS* in intron 2 of *PRNP*, as well as the *PRNP*-like gene *PRND* [13]. *PDYN* has not been included in the latest HSA20 map [5], however the gene has been physically assigned to 20pter-p12.2 [11], which is located in the vicinity of *AVP/OXT* and *CSNK2A1*. We assume *PDYN* to be located between *AVP/OXT* and *CSNK2A1* (Fig. 3). The comparison of HSA20 and BTA13 shows a rearranged order of the loci

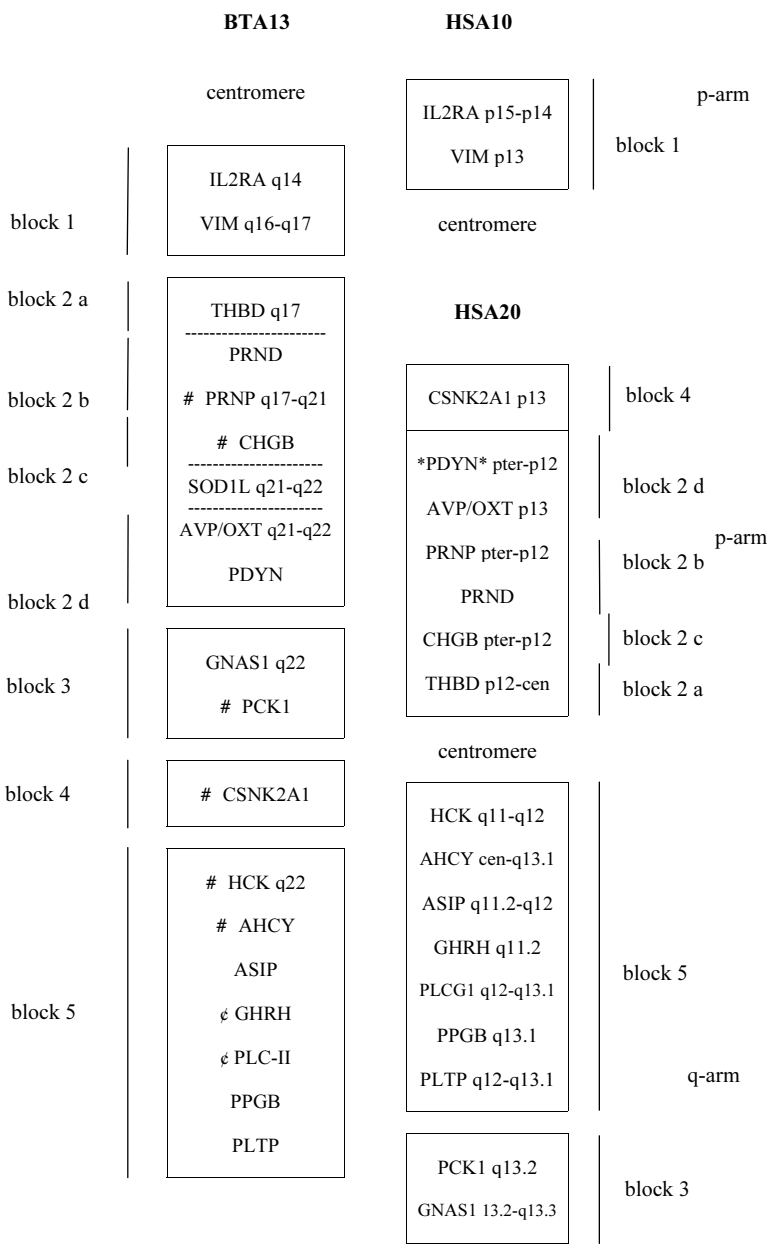

Figure 3. Revised blocks of conserved gene order in cattle and man. The bovine *PLC-II* gene is homologous to the human *PLCG1* locus. No gene homologous to the cattle *SOD1L* is known in man. * indicates the assumed location of *PDYN* between *AVP/OXT* and *CSNK2A1* in man. # indicates framework markers. ¢ indicates markers ordered with a lod score of at least 3 within the linkage groups. Where known, the physical location in cattle [8, 18] and man are indicated.

PRND and *PRNP* (block 2b) and *CHGB* (block 2c), in cattle, relative to the centromeric *THBD* (block 2a) and *AVP/OXT* (block 2d). In humans, no gene homologous to bovine *SOD1L* has been identified. We therefore did not assign the locus to a given block (Fig. 3). The linear locus order centromere – *PRNP* – *PRMS* – *CHGB* has odds greater than 1000:1, since the three loci are framework markers (Fig. 1). In contrast to this high level of support, the comprehensive $RH_{12'000}$ position of *PRND* (lod score $\geq$ 0.0) in the locus order centromere – *PRNP* – *PRND* – *PRMS* is questionable, since *PRND* has been found to be located 16 kb downstream of *PRNP* in the mouse [13]. The microsatellite *PRMS* however, is approximately 21′500 bp upstream of the *PRNP* coding region. Given the proposed internal rearrangement of block 2b and 2c in cattle compared to man, the locus order centromere – *PRND* – *PRNP* – *PRMS* – *CHGB* is most likely. However these are preliminary results that have to be confirmed, for example by mapping of additional markers in the vicinity or by fiber FISH experiments.

Despite these limitations, the present $RH_{12'000}$ map provides further insight into the evolutionary development of BTA13 compared to HSA10 and HSA20. The WG-$RH_{12'000}$ mapping panel has been shown to supplement the $RH_{5'000}$ panel in the development of marker maps featuring a higher resolution. The finding within block 2 in cattle provides further evidence that, although synteny between HSA10/HSA20 and BTA13 is conserved, gene order within the homologous segments has been significantly altered by intrachromosomal rearrangements.

ACKNOWLEDGEMENTS

We would like to thank Elaine Owens for her cell culture work, Jan Johnson for the large-scale DNA extractions, Ursula Sattler and Brigitta Colomb for their genotyping and Etienne Bäumle and Cyril Gaillard for the computer support. This work was founded by the Swiss Federal Office for Education and Research (BBW grant no. 97.0579-1) and the Swiss Federal Veterinary Office.

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